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The plausibility of a role for mercury in the etiology of autism: a cellular perspective

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Autism is defined by a behavioral set of stereotypic and repetitious behavioral patterns in combination with social and communication deficits. There is emerging evidence supporting the hypothesis that autism may result from a combination of genetic susceptibility and exposure to environmental toxins at critical moments in development. Mercury (Hg) is recognized as a ubiquitous environmental neurotoxin and there is mounting evidence linking it to neurodevelopmental disorders, including autism. Of course, the evidence is not derived from experimental trials with humans but rather from methods focusing on biomarkers of Hg damage, measurements of Hg exposure, epidemiological data, and animal studies. For ethical reasons, controlled Hg exposure in humans will never be conducted. Therefore, to properly evaluate the Hg-autism etiological hypothesis, it is essential to first establish the biological plausibility of the hypothesis. This review examines the plausibility of Hg as the primary etiological agent driving the cellular mechanisms by which Hg-induced neurotoxicity may result in the physiological attributes of autism. Key areas of focus include: (1) route and cellular mechanisms of Hg exposure in autism; (2) current research and examples of possible genetic variables that are linked to both Hg sensitivity and autism; (3) the role Hg may play as an environmental toxin fueling the oxidative stress found in autism; (4) role of mitochondrial dysfunction; and (5) possible role of Hg in abnormal neuroexcitory and excitotoxity that may play a role in the immune dysregulation found in autism. Future research directions that would assist in addressing the gaps in our knowledge are proposed.

Keywords: autism; mercury; cellular; oxidative stress; mitochondrial; immune dysfunction

Introduction

Rather than critically examining the extensive literature relating to the possible role of Hg in the etiology of autism, this review focused specifically on the biological plausibility of the hypothesis from a cellular perspective. This is an essential prerequisite for furthering our understanding of Hg's possible role in autism because, for ethical reasons, one cannot conduct experimental Hg trials on humans. Instead, one needs to rely on the broad discipline of epidemiology to bring together disparate lines of inquiry and methodologies that inform the area. Independent and *a priori* of this, it is of fundamental importance to evaluate the biological plausibility of the hypothesis. A body of epidemiological evidence

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supporting a role for Hg in autism's etiology would be substantially compromised in the event that biological plausibility could not be established.

Autism is a behaviorally defined neurodevelopmental disorder first characterized in the early 1940s by psychiatrist Kanner (1943). Autism is defined by a set of key features that include social interaction and communication impairments plus the presence of repetitive and stereotypic patterns of behavior (American Psychiatric Association [APA] 1994). In addition, autism is associated with symptoms such as mental retardation, seizures, gastrointestinal problems, and autoimmune dysfunctions (Rao et al. 2009).

For the first few decades following the seminal paper by Kanner (1943), autism appeared to be a rare mental disorder. However, epidemiological data gathered over the past couple of decades indicate that the prevalence of autism is on the rise (Newschaffer et al. 2007). Current estimates of autism prevalence range from 1 in 150 to 1 in 91 children in the USA (Kogan et al. 2009; Rice 2009). Although the cause of this change in prevalence is somewhat unclear, it is reasonable to assume that it is to some degree due to a broadening in diagnostic criteria and increased public awareness. Nevertheless, several studies have not been able to fully explain the rise in prevalence by these factors alone, which suggests the possible involvement of environmental factors (Grether et al. 2009; Hertz-Picciotto and Delwiche 2009; King and Bearman 2009). The progression of autism from a rare condition to a widespread public health concern has led to an increase in scientific research focused on biological and etiological theories of the condition. Although the etiology of autism remains uncertain, there is emerging evidence that exposure to environmental toxins at critical moments in prenatal and postnatal periods plays an important role in the development of autism (Austin 2008; Deth et al. 2008; Herbert 2010).

Mercury has been known for its potent neurotoxicity for centuries, yet is often used in a variety of medicinal, commercial, and industrial practices (Clarkson 2002). Mercury is classified by three specific species: elemental (Hg^0), inorganic salts (e.g., $HgCl_2$), and organic (methyl and ethyl mercury). As a ubiquitous natural element, Hg cycles from the air as vapor to the water or soil, where upon it is either vaporized or converted to an organic form. Hg vapor disseminates into the air from various sources both natural and industrial, such as in volcanic eruptions, gold refining processes, and coal burning. Environmental Hg spreads far from the original source and eventually enters the biosphere (Agency for Toxic Substances and Disease Registry 1999; Clarkson 2002). Apart from environmental Hg, additional sources of exposure in humans include the vaccine preservative thimerosal (or thiomersal), lab accidents, and other accidental exposures such as those of old thermometer breakages (Zahir et al. 2005). Dental amalgams may be a significant source of inorganic Hg entering the blood stream *via* inhalation of Hg vapor produced from actions such as chewing. Amalgams are of concern as they contribute to the Hg burden of adults as well as the embryo and fetus in the presence of maternal amalgam fillings (Mutter 2007).

Several recent studies profiled the prevalence of autism and its relation to environmental factors such as proximity to coal-burning power plants, atmospheric pollution (Windham et al. 2006; DeSoto 2009), as well as more specifically to Hg (Austin 2008; Rose et al. 2008; Palmer et al. 2009; Schweikert et al. 2009). However, while these studies do not support causation, they do support a plausibility argument that Hg may be involved and warrants further investigation.

The topic of much heated debate in the broader autism area has been vaccinations; more specifically, the inclusion of thimerosal as a preservative in vaccines given to children and pregnant women (Blaxill et al. 2004; DeSoto and Hitlan 2010; Schultz 2010). Thimerosal is a long-chain alkyl Hg compound which is approximately 50% Hg by weight.

Its mostly stable ethyl-mercury bond allows facilitated transfer across membranes such as the blood–brain barrier, after which it is broken down into inorganic Hg (Hoffman et al. 2007). It seems logical to make the assumption that vaccination is a cause of autism due to the simular temporal period in which the first signs of autism occur and modern vaccination schedules are administered. However, numerous epidemiological studies reviewed by Schultz (2010) are not able to support such an association and those that do have some severe limitations. Another more recent study was not able to support a clear association between increased risk of autism and Hg-containing vaccinations (Price et al. 2010).

Despite the lack of a clear association based on epidemiological studies, a recent pilot study examining Hg-containing vaccines in infant Rhesus monkeys found effects on parts of the brain that are linked to autism (Hewitson, Lopresti, et al. 2010). Another recent study by Hewitson, Houser, et al. (2010) examined the effect of thimerosal-containing Hepatitis-B vaccines in infant Rhesus macaques who received the vaccination within 24 h of birth. The study found that infants who received the Hg-containing vaccine suffered neurodevelopmental delays with gestational age and birthweight being contributing factors. This is interesting, especially in light of another recent study in which an increased risk of autism was associated with boys who had received the Hepatitis-B vaccine in the first month of life (Gallagher and Goodman 2010). Vaccinations are not the only source of Hg exposure in children (Counter et al. 2002; Counter and Buchanan 2004) and, indeed, the pathophysiology of Hg-induced toxicity is still not understood enough, especially in developing fetuses and newborns, to dismiss the hypothesis on the basis of vaccine studies.

Mercury: evident exposure in children

Mercury is toxic mainly due to its high affinity for sulfur groups present in numerous cellular proteins, which remains true for all species of Hg (Clarkson 2002). Differences in symptoms from intoxication of the various Hg species are postulated to be due to differences in metabolism and transportation to the target tissues (Castoldi et al. 2001). The central nervous system (CNS) is one of the major targets of Hg and both high- and low-dose exposures often produce significant and long-term neurological damage (Zahir et al. 2005; Johansson et al. 2007).

The clinical symptoms of the various species of Hg depend greatly on the dose, pattern, timing and route of exposure, individual excretion rates, and idiosyncratic sensitivity to Hg (Clarkson 1997; Blaxill et al. 2004). The neurological symptoms attributed to Hg intoxication are usually most evident and include ataxia, deafness, psychosis, loss of speech, erethism, and constriction of the visual field (Ceccatelli et al. 2010). There have been several studies examining the effects of Hg on children (Counter et al. 2002; Counter and Buchanan 2004). Studies consist mainly of the large exposures to methyl-mercury (MeHg) in Japan and Iraq (Matsumoto et al. 1965; Bakir et al. 1973; Amin-Zaki et al., 1974; Harada 1978; Harada et al. 1999), smaller but widespread exposures to inorganic Hg which produced pink disease or acrodynia (Dally 1997) and coastal communities which consume high amounts of seafood which contain MeHg (Kjellstrom et al. 1986, 1989; Myers, Davidson, et al. 1995; Myers, Marsh, et al. 1995; Grandjean et al. 1997, 1998).

One of the major factors contributing to MeHg toxicity is its ability to accumulate in biological organisms. This bioaccumulation occurs when Hg from environmental sources is methylated by microbes and then consumed or absorbed by both plants and animals, especially aquatic animals such as fish and crustaceans (Morel et al. 1998). The presence of

initially low amounts of organic Hg in the tissue of these animals is magnified in predatory animals such as birds and mammals with a diet high in seafood through a process termed biomagnification.

Even in communities where fish consumption is not high, the level of organic Hg was shown to be higher in pregnant women with a diet of local or commercially bought fish than in pregnant women who had not consumed any fish during pregnancy (Morrissette et al. 2004). In a recent study, the levels of Hg decreased over the course of the pregnancy in both mothers who had consumed fish and those that had not. However, despite this fall in the mother, the levels of Hg in the umbilical cord blood at birth was double than that in the mother (Morrissette et al. 2004; Schoeman et al. 2010). This phenomenon whereby the placenta not only fails to protect the fetus from Hg exposure but, rather, facilitates preferential movement of Hg to the fetus, was also a noted feature of the Minamata disease outbreak in Japan (Sakamoto et al. 2010). As a well-founded cautionary measure, it has been recommended that, during pregnancy, the consumption of fish be restricted to two meals per week of low Hg-containing fish and the strict avoidance of high Hg-containing fish such as Shark, Swordfish, and King Mackerel (U.S. Food and Drug Administration 2004; Koren and Bend 2010).

While most studies looked at the effects of high dosage, acute toxicity, comparatively few have examined low-dose chronic Hg toxicity. This inequity is especially egregious considering that this latter exposure pattern is the pattern that the vast majority is exposed to (Zahir et al. 2005). Furthermore, this issue is particularly important to children at both prenatal and postnatal stages of development as they are particularly vulnerable to Hg (Counter and Buchanan 2004). There have been several major studies which have examined the neurological outcomes of children in coastal communities in which high-seafood diets may have led to a higher then average prenatal and postnatal MeHg exposures (Kjellstrom et al. 1986, 1989; Myers, Davidson, et al. 1995; Myers, Marsh, et al. 1995; Grandjean et al. 1997, 1998; Oken et al. 2008). Overall, the conclusions from these studies on the effect of this low-level MeHg exposure on the neurological outcomes of the children have been mixed. It is most likely that the data from these studies are confounded by the dietary benefits of seafood consumption. Confounding factors beneficial to neurodevelopment, such as long-chain polyunsaturated fatty acids and selenium, are found in fish and are likely to cause an underestimation of MeHg-induced toxicity (Choi, Cordier, et al. 2008).

The pink disease outbreak of the twentieth century highlights the potential severity of the reaction (including death) that some children may have to a low level of Hg exposure (Black 1999), and it is this variable, idiosyncratic, and unpredictable individual susceptibility to Hg that determines the reaction. In the early 1900s, the increased availability of new pharmaceuticals led to the exposure of infants to inorganic Hg present as calomel in teething powder (Black 1999). Calomel contained Hg levels low enough to not be poisonous to the average infant. However, for a small subset of the population highly susceptible to Hg, the levels were sufficient to be detrimental and produce symptoms which would eventually be classified as a disease entity: pink disease or acrodynia (Dally 1997). This disease highlights the importance of understanding the pathophysiology of Hg in children, which would enable identification of those most at risk to environmental Hg. Indeed, in a recent study examining the prevalence of developmental disorders amongst grand-children of survivors of pink disease, a 7-fold higher rate of autism was found compared to the general population, suggesting a genetic (or at least hereditary) basis for the possible role of mercury in the etiology of autism (Shandley and Austin in press).

Mercury exposure in autism

Bernard et al. (2001) published an article reviewing the similarities between the symptoms of Hg intoxication and the symptoms of autism. The claimed neurological similarity between autism and Hg poisoning was debated by Nelson and Bauman (2003), a paper which in turn was disputed by Blaxill et al. (2004). A short section in the Nelson and Bauman's (2003) article contends that the lack of any scientific papers describing the presence of abnormal Hg body burdens within autistic children does not lend support to a Hg–autism association. However, this is not exactly the case. Outlined in the recent reviews by Schultz (2010) and Geier et al. (2008), there have been several studies examining potential Hg exposure and Hg body burdens in autistic children (Bradstreet et al. 2003; Holmes et al. 2003; Fido and Al-Saad 2005; Adams, Romdalvik, et al. 2007; Adams et al. 2008; DeSoto and Hitlan 2007; Kern et al. 2007; Williams et al. 2008; Geier et al. 2009c; Hertz-Picciotto et al. 2010). These studies employed various methods and looked at a range of Hg sources (e.g., fish consumption, dental amalgams, and vaccinations) in a range of children within the autism spectrum. Some studies (Adams et al. 2006; Kern et al. 2007) examined a wide array of toxic heavy metals from hair samples obtained from children diagnosed with autism and neurotypical children but found no significant difference in the Hg concentrations between them. However, studies do describe a somewhat impaired ability to excrete heavy metals in general.

Numerous studies (Holmes et al. 2003; Fido and Al-Saad 2005; Adams, Romdalvik, et al. 2007; Adams et al. 2008; Kern et al. 2007) analyzed the Hg concentrations within hair and teeth samples from autistic children and then matched them against neurotypical controls. The studies which examined samples from older autistic children (>4 years old) found a higher concentration of Hg in autistic children when compared to the neurotypical children. However, the studies which analyzed samples taken at the time of the baby's first haircut or teeth taken from autistic children aged 12–24 months found that Hg concentrations were lower than neurotypical children. This relationship between age and Hg levels is also a major finding in a recent study (Majewska et al. 2010) in which the levels of hair Hg between two age groups (group 1 aged 3–4 and group 2 aged 7–9) of autistic children were compared. The results indicated that compared to the control children, the younger autistic group had significantly lower hair Hg concentrations while the older autistic group had significantly higher hair Hg concentrations. It was suggested that this discrepancy in Hg body burdens was due to an impaired ability to excrete Hg in younger autistic children.

In further support of an abnormal Hg body burden in autistic children, Holmes et al. (2003) found that an increase in autism severity correlated to a lower concentration of Hg in the hair samples taken at the time of their first haircut (around 11–24 months). This was despite an estimated increase in Hg exposure in autistic children than in neurotypical children. A similar conclusion was reached by Geier et al. (2009c), who found that a rise in autism severity correlated with an elevation in the number of dental amalgams in the mother during pregnancy, and thus an increased risk of fetal Hg exposure. In a study by Bradstreet et al. (2003), looking at 221 autistic children aged between 3 and 15 years of age, a rise in urine Hg concentrations was found after 3 days of heavy metal chelation therapy, again supporting the hypothesis that autistic children do not eliminate Hg efficiently as normal children. The data from these studies support the plausibility that autistic children have an elevated risk of sustaining damage from an exposure to Hg due to this reduced capacity to effectively excrete Hg compared to non-autistic children.

The above studies are not without limitations including small sample sizes and a lack of accuracy in determining the dose of Hg exposure in autistic and normal controls. In addition, there are also studies which do not support the hypothesis. Williams et al. (2008) released a study comparing 15 autistic children of age 2–6 years and their gender- and age-matched neurotypical sibling controls. Williams et al. (2008) found no significant difference between the Hg concentrations in the hair samples obtained from these two groups. In a recent study (Hertz-Picciotto et al. 2010), no significant difference was noted in a comparison between blood Hg concentrations of 249 autistic children (around 2–5 years of age) and 143 neurotypical children. However, this study recognized that because the half-life of inorganic Hg and MeHg is around 60–90 days (Clarkson 1997) and this was a measurement of a single-time event, it could not evaluate any possible differences in Hg excretion rates between autistic and neurotypical groups. While it is clear that more research is needed to bring more clarity to the hypothesis that an abnormal Hg body burden exists within autistic children, the above studies do imply that autistic children may be more susceptible to Hg damage than non-autistic children.

Recently, biomarkers of Hg damage have been explored by looking at urinary porphyrins (Nataf et al. 2006; Austin and Shandley 2008). Porphyrins are a necessary intermediate in heme production and have a well-established metabolism and excretion pathway. This pathway is affected by exposure to specific heavy metals interfering with the enzymes uroporphyrin decarboxylase and coproporphyrinogen oxidase. Mercury exposure was shown to elevate levels of pentacarboxyl (5Cxp), precoproporphyrin (PrCp), and coproporphyrin detectable in urine (Woods et al. 1991). There are an increasing number of studies finding an elevation in these porphyrins in autistic children (Geier and Geier 2006b; Nataf et al. 2006; Austin and Shandley 2008). Recently, in a group of 65 autistic children, Youn et al. (2010) found elevated levels of both 5Cxp and PrCp, which were in concordance with previous studies. These findings are further supported in a recent study by Woods et al. (2010), in which samples from 218 children aged 2–12 were examined.

This latter study is interesting because, first, it shows increased levels of PrCp in normal children, which suggests a degree of elevation of this porphyrin is a natural occurrence in young children. However, the levels of PrCp and 5Cxp were still significantly higher in children with autism compared to age-matched controls which is concordant with the aforementioned studies. Woods et al. (2010) attempted to measure the background Hg exposure levels of these children by surveying potential Hg risk factors the child and mother may have been exposed to. The resulting survey data revealed no significantly different level of Hg exposure occurred between the two groups. This seems to be consistent with a genetically mediated pathogenesis of autism whereby, given equal Hg exposure, only a small minority of particularly Hg-sensitive individuals will manifest autism. Woods et al. (2010) concluded that porphyrin disruption may be an important characteristic in autism, suggesting that genetic polymorphisms in the enzyme coproporphyrinogen oxidase (CPOX4) could be associated with increased autism risk. Interestingly, polymorphisms in CPOX4 have also been associated with an increase in sensitively to Hg neurotoxicity (Echeverria et al. 2006). If this is the case, it will be important to monitor the levels of exposure, identify susceptible persons in the population, and to understand the actual mechanisms that may link the neurotoxicity of Hg and the etiology of autism.

Oxidative stress and mercury lead to metabolic dysfunction in autism

Electrons that "leak" from the electron transport chain within the mitochondria are accepted by O_2 and create a whole range of reactive oxygen species (ROS; Halliwell 1992). The build-up of ROS leads to a state of oxidative stress resulting in: (1) oxidation of lipid membranes, (2) the deleterious alteration of proteins, and (3) activation of apoptotic mechanisms (Valko et al. 2007). The potentially damaging oxidative environment is controlled by a collection of reducing elements such as superoxidase dismutase, glutathione peroxidase (GPx), and catalase, as well as small molecules such as glutathione (GSH) and vitamin E (Schulz et al. 2000; Oktyabrsky and Smirnova 2007). Glutathione is a tripeptide (γ-glutamyl-cysteinylglycine) consisting of a glutamate and disulfide that allows it to bind to free radicals and donate an electron. It is considered to be the major antioxidant molecule in the body due to the numerous roles it plays in maintaining a redox balance, high abundance, and endogenous production (Townsend et al. 2003).

The antioxidant mechanisms of GSH involve the action of two enzymes: GPx and glutathione reductase (GR). GPx is the enzyme responsible for the reduction of peroxide and other oxidized lipid molecules. The reduction of these toxic molecules requires GPx to oxidize GSH, which in turn results in the production of oxidized GSH (GSSG; glutathione disulfide; Dringen 2000; Franco et al. 2009). The fate of GSSG is either to be removed from the cell or to be reverted back to GSH by the enzymatic action of GR with the addition of NADPH. Thus, maintenance of the necessary levels of GSH is both dependent on *de novo* synthesis and the levels of GR (Dringen et al. 2000). The ratio of GSH/GSSG has been shown to be important in the activation of enzymes in catabolic and metabolic pathways (Fratelli et al. 2002). In the mitochondria, however, the levels of GSH are dependent on the functional levels of GR and NADPH in order to reduce GSSG and maintain the GSH : GSSG ratio (Lash 2006; Hu et al. 2008). The reduction of GSSG to GSH inside the mitochondria is important for two reasons: (1) the production of ROS as stated above and (2) GSSG can form disulfide bonds with complex I of the electron transport chain in the mitochondria resulting in the increased production of superoxides, which can lead to mitochondrial dysfunction (Taylor 2003).

The brain is more susceptible to oxidative stress than other tissues in the body due to its high oxygen demands as well as containing a high degree of polyunsaturated fats, which are sensitive to ROS attacks. The brain also has limited protective mechanisms against ROS build-up, as well as higher % mitochondria due to brain energy requirements (Halliwell 1992; Singh et al. 2004). The presence of high oxidative stress is a characteristic of autism (James et al. 2004; Kern and Jones 2006; Deth et al. 2008) as well as neurodegenerative disorders such as Parkinson's and Alzheimer's diseases (Kidd 2005).

Mercury readily crosses the blood brain and placental barriers, where upon it enters the cell and binds to its numerous targeted thiol groups (Taber and Hurley 2008; Ceccatelli et al. 2010). *In vitro*, Hg has the ability to damage cell membranes and deoxyribonucleic acid (DNA) at micromolar concentrations particularly in neuronal cells, which were shown to be more sensitive than other cell types (Baskin et al. 2003). Methyl-mercury is considered to be highly neurotoxic as it has been shown to enter the brain more effectively than other organic Hg compounds (Magos 2003; Harry et al. 2004). The reason for this is due to the sterospecific transport of cysteine–MeHg compounds into astrocytes *via* neutral amino acid transport systems. While this ubiquitous amino acid transport system has been identified as one of the transport mechanisms in the brain, the particular mechanisms by which MeHg actively enters the cell is still unclear (Heggland et al. 2009). At present, a complete understanding of the mechanisms underlying Hg neurotoxicity remains

unknown. However, it is clear that Hg induces ROS production and oxidative stress (Sarafian et al. 1994; Shenker et al. 2002) while also decreasing the levels of GSH which is an important factor in protecting against the cytotoxicity of Hg (Woods and Ellis 1995; Olivieri et al. 2000; James et al. 2005).

The essential nutrient selenium has also been identified as important in redox mechanisms (in the form of GPx and selenoproteins) as well as being potentially protective against the neurotoxicity of Hg (Whanger 2001). The protective mechanisms of selenoproteins are thought to be due to both their high affinity for binding Hg and their role in counteracting Hg-induced ROS damage (Chen et al. 2006). However, the relationship between selenoproteins and Hg in terms of neurological and neurodevelopment disease is not clear and requires further research (Choi, Budtz-Jørgensen, et al. 2008; Battin and Brumaghim 2009).

There have been numerous studies that examined oxidative stress in autism by measuring biomarkers indicative of oxidative stress. Although it seems clear that at least a subset of autistic children suffer from elevated oxidative stress, it is not clear what the cause of this redox imbalance is. Several studies examined autism as a metabolic disease (Sogut et al. 2003; James et al. 2004, 2008, 2009, 2010; Geier and Geier 2006a; Kern and Jones 2006; Suh et al. 2008; Adams et al. 2009; Al-Gadani et al. 2009; Geier et al. 2009a, 2009b). Among these studies, an examination of trans-sulfuration pathways is common. This typically involves measurement of various metabolites from venous blood which include cysteine, cystine, GSH, and GSSG as well as components of the methionine pathway (methionine, S-adenosylmethionine, and S-adenosylhomocysteine).

At present, most studies examining metabolic factors in children with autism have used blood plasma to test for biomarkers such as levels of carnitine, the S-adenosylmethionine/S-adenosylhomocysteine ratio or the GSH : GSSG ratio. However, a recent study by James et al. (2009) used autistic cell lines to examine the cell ability to maintain a redox balance under stress conditions. The study examined both the cytosolic and mitochondrial GSH : GSSG ratios as a measurement of the redox potential. James et al. (2009) found that both mitochondrial and cytosolic redox states were imbalanced containing higher levels of GSSG then normal. However, interestingly the mitochondria had a significantly lower GSH : GSSG ratio compared to that in the cytosol, suggesting that mitochondrial function may be particularly impaired in autism.

In the same study, the Hg-based vaccine preservative thimerosal did not appear to significantly decrease the amount of GSH in autism cell lines over the control cells. However, it was suggested that as autism cells already have a compromised redox imbalance, any further reduction by external factors, such as environmental insults from Hg, will be less obvious. This also concurs with the evidence that Hg diminishes antioxidant protection and increases ROS production (Sarafian and Verity 1991; Ou et al. 1999; Allen et al. 2001). MeHg was shown to impair GPx activity in rats (Dringen et al. 2000), decrease glutamine uptake, and increase mitochondrial membrane permeability (Yin et al. 2007).

Autism has been reported to affect more males than females in a ratio of 4:1 (Yeargin-Allsopp et al. 2003). This is an interesting phenomenon in relation to the toxicology of Hg. Studies in rats showed male rats to be significantly more susceptible to the adverse effects of inorganic Hg (Ekstrand et al. 2010) and also, more recently, to thimerosal (Branch 2009). The mechanisms underlying this gender disparity seem to be linked to differences in testosterone and estrogen levels, as testosterone was found to increase the toxicity of Hg, while estrogen was reported to be protective against Hg damage (Muraoka and Itoh 1980).

Estrogen has also been shown to be protective against Hg-induced oxidative stress in neurons (Olivieri et al. 2002; Nilsen 2008).

The consistent finding in autistic children is the decrease in cystine, cysteine, GSH, as well as an elevation in oxidized GSSG, all of which are indicative of oxidative stress. There are also reports indicating an abnormal decrease in S-adenosylmethionine/S-adenosylhomocysteine ratio (James et al. 2004). This is important in the methylation of protein and DNA, in which a fall is likely to also reduce protein synthesis as well as affect levels of GSH and cysteine. There is concordance among the research that although the underlying mechanisms of oxidative stress in autism are not definitively confirmed, the possibility that environmental toxins (especially Hg) fuel oxidative stress is plausible.

Mercury and mitochondrial dysfunction

As mitochondria is the cellular powerhouse and also one of the major targets of oxidative stress, having a compromised redox balance is more likely to be detrimental to mitochondria. This is because in mitochondria, GSSG is not transported rapidly across mitochondrial membranes under conditions of oxidative stress like in the cytosol but is dependent on NADPH and GR reducing it to GSH (Olafsdottir and Reed 1988; Garcia et al. 2010). Mitochondrial dysfunction was shown to play a role in producing oxidative stress within a cell. Mitochondrial dysfunction is also suspected to play a role in autism as studies identify reduced activity of electron transport chain enzymes (especially ETC complexes 1, 3, and 4) and other biomarkers of mitochondrial dysfunction in autistic children (Oliveira et al. 2005; Weissman et al. 2008; Ezugha et al. 2010). In a recent review (Gargus and Imtiaz 2008), the mechanisms and mitochondrial mutations that might be involved in autism are discussed in detail.

In a recent study by Monroe and Halvorsen (2009), environmental toxins (Hg, cadmium, and the pesticide rotenone) were used to examine the mechanisms by which oxidative stress produces damage in neuronal cells compared to non-neuronal cells. It was found that the generation of ROS and the dysfunction of the mitochondria resulted in the inhibition of the Janus kinase (JaK) and signal transducer and activator of transcription (STAT) pathways. However, this only occurred in the neuronal cells despite comparable levels of oxidative stress, toxins, and equal mitochondrial sensitivity to the loss of membrane potential. It was suggested that there exist differing mechanisms of dealing with ROS build-up and oxidative stress between neuronal cells and non-neuronal cells. JaK and STAT are involved in a wide range of cytokine signaling processes as well as in playing an important role in glial cells (gliogenesis) (Rodig et al. 1998; Aringer et al. 1999; Zhao et al. 2007). The inhibition of neurotropic factors just as JaK–STAT by Hg is thought to be one of the ways Hg induces neurological damage (Monroe and Halvorsen 2006).

In a study by Nishimura (2007) using lymphoblastoid cell lines derived from autistic patients, it was shown that an important protein in the JaK pathway (JaK and microtubule-interacting protein 1) was dysregulated (Steindler et al. 2004; Bill and Geschwind 2009). It was suggested that this might be involved in the disruption of glutamate signaling in neurons as well as serve as a possible biomarker for autism, although the mechanisms for its irregular expression were not clear. This finding combined with the known effects of Hg-impairing microtubule action (Crespo-Lopez et al. 2009) and the JaK pathway mentioned above provides compelling evidence that the combination of Hg exposure during critical periods of brain development and underlying vulnerability may play a role in autism. However, it is also clear that more research is needed to further

elucidate the mechanisms at work as, while the link may be present, it is not strong enough to support a Hg-autism etiology alone.

Underlying genetic susceptibility to environmental toxins

It is likely that there are genetic predispositions to autism, given the high concordance between monozygotic twins (Ritvo et al. 1985) and a greater chance of autism recurring within affected families (Bailey et al. 1995). Thus, there has been extensive research into the identification of autism-associated or autism-susceptibility genes (Cook 1998; Muhle et al. 2004; Freitag et al. 2010). While the concordance among twins studies is high, it is not 100%, which strongly suggests other factors playing a role, such as environmental factors (Deth et al. 2008) and differences in gene expression as a result of epigenetic factors (Hu et al. 2006). This has led to research targeting genes associated with environmental sensitivity and metabolism that might also be involved in autism.

The family of enzymes known as glutathione-S-transferase (GST) play an important role in the detoxification of the products of oxidative stress and mercury damage such as hydrogen peroxide (Hayes et al. 2005). Polymorphisms in GST genes have been linked to an increased sensitivity to thimerosal and are also associated with autism to varying degrees (Buyske et al. 2006; James et al. 2006). The deletion of GSTM1 was also shown to affect the neuronal growth of the hippocampus and cerebellum in mice after oxidative stress was induced (Yochum et al. 2010). Polymorphisms associated with reduced GST function have also been associated with Hg susceptibility in a number of studies (Westphal et al. 2000; Gundacker et al. 2007; Lee et al. 2010). In a recent study by Willams et al. (2007), functional polymorphisms in the GST-P1 genotype was examined among 49 families containing at least one child diagnosed with autism. It was found that a specific haplotype (313A, 313C) was more frequently transmitted above any others in the study. While the role this had was not made clear, it was suggested to have a possible teratogenic effect (Williams et al. 2007).

The abnormal metabolism present in autism suggests the involvement of genes related to the maintenance and functioning of the methylation and trans-sulfuration pathways (James et al. 2006). The methionine transmethylation pathway is important in maintaining the correct functioning of cellular processes *via* the methylation of DNA, proteins, and phospholipids (Mato et al. 2002). In this pathway, homocysteine is converted to methionine *via* methylation by methionine synthase in a B12-dependent reaction with 5-methyltetrahydrofolate (5-MTHF) acting as the methyl donor. Maintaining the availability of methionine is essential and there have been several studies looking at the polymorphisms which may affect this process in autism (James et al. 2004; Mohammad et al. 2009). The polymorphisms of most concern have been in the MTHF reductase gene, which reduces 5, 10-methylene-THF to 5-MTHF. However, there are conflicting results with some studies finding significant associations (Boris et al. 2004; Mohammad et al. 2009; Pasca et al. 2009) and others finding no association with autism (James et al. 2004; Adams, Lucock, et al. 2007a).

It is likely that these discrepancies are due to the heterogeneous nature of autism and/or the sample sizes used in the study (Main et al. 2010). However, there are numerous other genes related to the methionine transmethylation pathway that have been associated with autism such as reduced folate carrier (James et al. 2010), Transcobalamin II (James et al. 2006), and dihydrofolate reductase (Adams, Lucock, et al. 2007). This pathway is also interconnected with the trans-sulfuration pathway, which competes for the

availability of homocysteine in order to synthesize glutathione (Persa et al. 2004). Therefore, as this process was shown to be important in maintaining both proper methylation and providing protection against oxidative stress, any dysfunctions due to underlying genetic traits would make these children susceptible to environmental toxic insults such as that from Hg (Deth et al. 2008).

A recent review by Gundacker et al. (2010), details what is currently known about important genetic factors involved in Hg susceptibility, and it also highlights how an individual genetic background reflects their ability to metabolize Hg. The uptake of Hg by the amino acid transporter LAT1, encoded by the gene SLC7A5, was shown to affect the uptake of MeHg in mice, increasing uptake when over-expressed and decreasing when knocked down (Yin et al. 2008; Nicklin et al. 2009). This gene has also been linked to autism (Anderson et al. 2009).

Genetic research into autism, while not providing the ultimate break-through that was hoped for, has at least provided some clues as to the etiology of this complex disorder. Numerous genes that are important in the metabolism of environmental toxins (but not limited to Hg) such as a polymorphisms of glutathione peroxidase (GPX1), paraoxonase 1, and a delta-aminolevulinate dehydratase variant (ALAD2) have also been linked to autism (Herbert 2010). There is a broad concordance across the disparate lines of inquiry, suggesting that an individual's genetic background may influence susceptibility to environmental insult. The heterogeneity of autism most likely reflects a broad spectrum of susceptibility, in which specific genotypes may differ between individuals. This has made establishing the specific autism susceptibility genes so difficult to identify. In addition, the genetic components which convey sensitivity are still not well established (Gundacker et al. 2010). This makes establishing strong links with autism difficult and is an area of much needed research. The apparent overlap between the gene pathways known to influence Hg defense and their presence in autistic populations supports the plausibility of the Hg-autism hypothesis.

Glial cells: the central component of excitotoxicity and immunotoxicity

Glial cells are non-neuronal cells that, until recently, were thought only to be involved in neuronal health and maintenance. However, new insights revealed glial cells to play a much larger role in information processing and early neurodevelopment (Fields and Stevens-Graham 2002). There is an indication that the complex mechanisms and interactions of these non-neuronal cells are important in brain function; however, they are still not completely understood (Fields 2008). Glial cells do, however, play important roles in excitotoxicity and immunotoxicity, which are two key aspects involved in the developmental neurotoxicity of autism.

Astrocytes have been established to be integral to the health and function of neurons (Fallon 1985). Their association with the node of Ranvier, the synapse, and capillaries is indicative of the role they play in signal conduction and mediating the permeability of the blood–brain barrier. In summary, astrocytes remove excitatory amino acids (EAAs) such as glutamate, release neurotrophic factors, and metabolize neurotransmitters (Fields and Stevens-Graham 2002; Koehler et al. 2009). As mentioned earlier, astrocytes are also necessary for maintaining the redox homeostasis of neurons by releasing GSH and its precursor cysteine for uptake by neurons, which is important as cysteine is the rate-limiting component of the GSH synthesis pathway (Sagara et al. 1993; O'Connor et al. 1995; Dringen et al. 1999).

Glial cells also play a role in MeHg neurotoxicity due to a number of factors involving their aforementioned role in the brain. While MeHg affects numerous cell types, Aschner et al. (2000) showed MeHg preferentially accumulates in astrocytes and to a lesser extent microglial cells. MeHg is thought to affect the function of astrocytes and produce neuronal damage in numerous ways. These include the induction of swelling in astrocytes that results in the release of EAA, the production of ROS, interfering with redox homeostasis and disturbing the uptake of glutamate, the result of which is the induction of oxidative stress and excitotoxicity in neurons (Aschner et al. 2000). This suggests a plausible mechanism for the presence of oxidative stress in autism.

Excitotoxicity is defined as being the chronic and abnormal excitatory stimulation of susceptible neurons by EAA (Blaylock and Strunecka 2009). The common neurotransmitter glutamate is controlled due to its high toxicity. If the levels of glutamate in the synaptic cleft are allowed to get too high, then this leads to chronic activation which ultimately results in the excessive influx of Ca^{2+} into the cytoplasm. This activates two problematic enzymatic pathways, the protein kinase C pathway and the inducible nitric oxide synthase pathway, both of which eventually lead to the generation of ROS and thus damages both mitochondria and cellular function (Blaylock and Strunecka 2009).

There is growing evidence that excitotoxicity during the early vulnerable periods of neural development is involved in the etiology of neurodevelopmental disorders such as autism (Blaylock and Strunecka 2009). A recent study by Ferraro et al. (2009) examined the effect on rat pups of prenatal exposure to a single dose of MeHg on neurochemistry and long-term cognitive function. Data showed a significant effect on microtubule production, resulting in the disruption of the developing neuronal network. The role of glutamate excitotoxicity was suggested as possibly playing a role in producing cell death among neuronal cell cultures. It was also suggested that the neural damage from prenatal exposure to MeHg impaired long-term adult memory evidenced by the significant deficiencies in retention performance in adult pups. This study suggests that prenatal exposure to Hg may exert long lasting effects on brain development in animals that are broadly analogous to autism in humans.

Immunotoxicity involves the overactivation of the innate immune system resulting in inflammation and autoimmunity (Schwenk et al. 2009). Microglial cells respond to neuronal activity and are able to produce inflammatory immune responses as well as remove cellular debris and apoptotic cells (Fields and Stevens-Graham 2002). The overproduction of cytokines and recruitment of the innate immune system by early and chronic activation of microglial cells and astrocytes was suggested to be one of the underlying mechanisms of immunotoxicity in autism (Dietert and Dietert 2008). Recently, Hung et al. (2010) showed that normal brain activity supresses microglial cells and stimulation of neurons activates inflammatory responses from microglial cells. This not only demonstrates that the interaction of glial cells with the brain is able to produce immune responses, but also presents the possiblity that abnormal exicitation and excitotoxicity might play a role in immune dysfunction such as that found in autism (Pardo et al. 2006; Blaylock and Strunecka 2009).

As outlined by Schwenk et al. (2009), Hg was shown to actively increase the reactivity of the immune system in rodents. This immunostimulation led to the development of immunotoxic problems such as allergeric responses and autoimmune disease. A new study (Kempuraj et al. 2010) found that Hg induces release of inflammatory cytokines from human mast cells that may interfer with the blood–brain barrier and produce neuroinflamination. Data indicated that Hg-related immunotoxic repsonse may be one of the underlying mechanisms in the etiology of autism. In fact, increased activation of

microglial and astroglial cells was reported in post-mortem austitic brain tissue (Vargas et al. 2005). This activation was proposed to result in neuroinflamination due to the up-regulation of inflamatory cytokines resulting in changes to neuronal and synpatic functions. Markers of increased immune activation and autoimmunity (e.g., cytokine interleukin-12 and anti-neuronal antibodies) were also linked to autism in a several studies (Singh 1996; Singh et al. 1997) suggesting a possible role of immuntoxicity in the pathophysiology of autism. It is reasonable to presume that Hg may be involved in this suggested immunological pathology or, at the very least, in the abnormal cellular mechanisms observed in autistic children.

Conclusions

From a cellular perspective, it would appear that the existing scientific literature supports the biological plausibility of a Hg-based autism pathogenesis. Hg has well-known effects relating to the disruption of sulfur chemistry leading to elevated oxidative stress which, in turn, results into broader physiological/organ affects, particularly to the CNS. Oxidative stress was consistently elevated in autism. Although this is not unique to autism (as many disease states are associated with this biochemical characteristic), it does suggest that autism is more than just a neurological disease but also a disease which reflects dysfunction at various metabolic levels. Nevertheless, research studies identifying Hg's effects on glial cells and mitochondria that are consistent with findings in autistic patients, lend further support to the Hg-autism hypothesis.

To understand the case for plausibility, it is essential to recognize that a critical variable (or unpredictable confound as the case may be) is the idiosyncratic sensitivity of any given individual to Hg, which likely has some genetic basis. As experienced through the pink disease epidemic, this variable is unpredictable *a priori* of exposure, and so the only way to end the pink disease epidemic was to minimize Hg exposure to *all* children. If Hg is indeed an etiological agent in autism, it is likely to require a similar global response as one cannot identify "at risk" children. This population level approach to reducing Hg exposure has not, as of yet, been comprehensively introduced, with the exception of partial Hg reduction in the vaccine schedule, and warning the public (especially pregnant and nursing mothers) regarding the consumption of seafood.

Despite the biological plausibility of Hg as an etiological agent in autism, there remains a great deal of work to do in order to better understand how Hg acts in any pathogenic process. Obviously, there is a need to establish the broad range of Hg affects across various exposure models. For example, future studies are urgently required utilizing both animal and *in vitro* cellular methods that manipulate Hg species, dose frequency, and mode of administration (e.g., oral and parenteral), as well as individual difference variables such as age of animal (including varying stages of gestational age) and known genetic variants. Outcome variables need to include not just behavioral analogs of autism but a wide range of physiological variables known to be associated with Hg damage including urinary PrCP, markers of oxidative stress, and genetic variables.

References

Adams, J.B., E. Geis, J. Mitchell, J. Ingram, A. Hensley, I. Zappia, S. Newmark, et al. 2009. The severity of autism is associated with toxic metal body burden and red blood cell glutathione levels. *Journal of Toxicology* 9: 1–7.

Adams, J.B., C.E. Holloway, F. George, and D. Quig. 2006. Analyses of toxic metals and essential minerals in the hair of Arizona children with autism and associated conditions, and their mothers. *Biological Trace Element Research* 110: 193–209.

Adams, M., M. Lucock, J. Stuart, S. Fardell, K. Baker, and X. Ng. 2007. Preliminary evidence for involvement of the folate gene polymorphism 19 bp deletion-DHFR in occurrence of autism. *Neuroscience Letters* 422: 24–9.

Adams, J.B., J. Romdalvik, K.E. Levine, and L.W. Hu. 2008. Mercury in first cut baby hair of children with autism vs typically-developing children. *Environmental Toxicology and Chemistry* 90: 739–53.

Adams, J.B., J. Romdalvik, V.M. Ramanujam, and M.S. Legator. 2007. Mercury, lead, and zinc in baby teeth of children with autism versus controls. *Journal of Toxicology and Environmental Health Part A* 71: 1046–51.

Agency for Toxic Substances and Disease. 1999. *Registry toxicological profile for mercury*. Washington, DC, USA: Department of Health and Human Services.

Al-Gadani, Y., A. Ei-Ansary, O. Attas, and L. Al-Ayadhi. 2009. Metabolic biomarkers related to oxidative stress and antioxidant status in Saudi autistic children. *Clinical Biochemistry* 42: 1032–40.

Allen, J.W., G. Shanker, and M. Aschner. 2001. Methylmercury inhibits the in vitro uptake of the glutathione precursor, cystine, in astrocytes, but not in neurons. *Brain Research* 894: 131–40.

Amin-Zaki, L., M.A. Majeed, S. Elhassani, T.W. Clarkson, R.A. Doherty, and M. Greenwood. 1974. Intra-uterine methylmercury poisoning in Iraq. *Pediatrics* 54: 587–95.

American Psychiatric Association (APA). 1994. *Diagnostic and statistical manual of mental disorders*. 4th ed. Washington, DC: American Psychiatric Association.

Anderson, B.M., N.C. Schnetz-Boutaud, J. Bartlett, A.M. Wotawa, H.H. Wright, R.K. Abramson, M.L. Cuccaro, J.R. Gilbert, M.A. Pericak-Vance, and J.L. Haines. 2009. Examination of association of genes in the serotonin system to autism. *Neurogenetics* 10: 209–16.

Aringer, M., A. Cheng, J.W. Nelson, M. Chen, C. Sudarshan, Y.J. Zhou, and J.J. O'Shea. 1999. Janus kinases and their role in growth and disease. *Life Science* 64: 2173–86.

Aschner, M., C.P. Yao, J.W. Allen, and K.H. Tan. 2000. Methylmercury alters glutamate transport in astrocytes. *Neurochemistry International* 37: 199–206.

Austin, D.W. 2008. An epidemiological analysis of the 'autism as mercury poisoning' hypothesis. *International Journal of Risk and Safety in Medicine* 20: 135–42.

Austin, D.W., and K. Shandley. 2008. An investigation of porphyrinuria in Australian children with autism. *Journal of Toxicology and Environmental Health Part A* 71: 1349–51.

Bailey, A., A. Lecouteur, I. Gottesman, P. Bolton, E. Simonoff, E. Yuzda, and M. Rutter. 1995. Autism as a strongly genetic disorder – evidence from a British twin study. *Psychological Medicine* 25: 63–77.

Bakir, F., S.F. Damluji, L. Amin-Zaki, M. Murtadha, A. Khalidi, N.Y. Al Rawi, S. Tikriti, et al. 1973. Methylmercury poisoning in Iraq. *Science* 181: 230–41.

Baskin, D.S., N. Hop, and V.D. Vladimir. 2003. Thimerosal induces DNA breaks, caspase-3 activation, membrane damage, and cell death in cultured human neurons and fibroblasts. *Toxicological Science* 74: 361–8.

Battin, E., and J. Brumaghim. 2009. Antioxidant activity of sulfur and selenium: A review of reactive oxygen species scavenging, glutathione peroxidase, and metal-binding antioxidant mechanisms. *Cell Biochemistry and Biophysics* 55: 1–23.

Bernard, S., A. Enayati, L. Redwood, H. Roger, and T. Binstock. 2001. Autism: A novel form of mercury poisoning. *Medical Hypotheses* 56: 462–71.

Bill, B.R., and D.H. Geschwind. 2009. Genetic advances in autism: Heterogeneity and convergence on shared pathways. *Current Opinion in Genetics and Development* 19: 271–8.

Black, J. 1999. The puzzle of pink disease. *Journal of the Royal Society of Medicine* 92: 478–81.

Blaxill, M.F., L. Redwood, and S. Bernard. 2004. Thimerosal and autism? A plausible hypothesis that should not be dismissed. *Medical Hypotheses* 62: 788–94.

Blaylock, R.L., and A. Strunecka. 2009. Immune-glutamatergic dysfunction as a central mechanism of the autism spectrum disorders. *Current Medicinal Chemistry* 16: 157–70.

Boris, M., A. Goldblatt, J. Galanko, and S. Jill James. 2004. Association of MTHFR gene variants with autism. *Journal of American Physicians and Surgeons* 9: 106–8.

Bradstreet, J., D.A. Geier, J.J. Kartzinel, J.B. Adams, and M.R. Geier. 2003. A case-control study of mercury burden in children with autistic spectrum disorders. *Journal of American Physicians and Surgeons* 8: 76–9.

Branch, D.R. 2009. Gender-selective toxicity of thimerosal. *Experimental and Toxicologic Pathology* 61: 133–6.

Buyske, S., T.A. Williams, A.E. Mars, E.S. Stenroos, S.X. Ming, R. Wang, M. Sreenath, et al. 2006. Analysis of case-parent trios at a locus with a deletion allele: Association of GSTM1 with autism. *BMC Genetics* 7: 8.

Castoldi, A.F., T. Coccini, S. Ceccatelli, and L. Manzo. 2001. Neurotoxicity and molecular effects of methylmercury. *Brain Research Bulletin* 55: 197–203.

Ceccatelli, S., E. Daré, and M. Moors. 2010. Methylmercury-induced neurotoxicity and apoptosis. *Chemico-Biological Interactions* 188: 301–8.

Chen, C.Y., H.W. Yu, J.J. Zhao, B. Li, L.Y. Qu, S.P. Liu, P.Q. Zhang, and Z.F. Chai. 2006. The roles of serum selenium and selenoproteins on mercury toxicity in environmental and occupational exposure. *Environmental Health Perspectives* 114: 297–301.

Choi, A.L., E. Budtz-Jørgensen, P.J. Jørgensen, U. Steuerwald, F. Debes, P. Weihe, and P. Grandjean. 2008. Selenium as a potential protective factor against mercury developmental neurotoxicity. *Environmental Research* 107: 45–52.

Choi, A.L., S. Cordier, P. Weihe, and P. Grandjean. 2008. Negative confounding in the evaluation of toxicity: The case of methylmercury in fish and seafood. *Critical Reviews in Toxicology* 38: 877–93.

Clarkson, T.W. 1997. The toxicology of mercury. *Critical Reviews in Clinical Laboratory Sciences* 34: 369–403.

Clarkson, T.W. 2002. The three modern faces of mercury. *Neurotoxicology and Teratology* 28: 425–6.

Cook, E.H. 1998. Genetics of autism. *Mental Retardation and Developmental Disabilities Research Reviews* 4: 113–20.

Counter, S.A., and L.H. Buchanan. 2004. Mercury exposure in children: A review. *Toxicology and Applied Pharmacology* 198: 209–30.

Counter, S.A., L.H. Buchanan, F. Ortega, and G. Laurell. 2002. Elevated blood mercury and neuro-otological observations in children of the Ecudorian gold mines. *Journal of Toxicology and Environmental Health Part A* 65: 149–63.

Crespo-Lopez, M.E., G.L. Macedo, S.I. Pereira, G.P. Arrifano, D.L. Picanco-Diniz, J.L. Nascimento, and A.M. Herculano. 2009. Mercury and human genotoxicity: Critical considerations and possible molecular mechanisms. *Pharmacological Research* 60: 212–20.

Dally, A. 1997. The rise and fall of pink disease. *Social History of Medicine* 10: 291–304.

DeSoto, M.C. 2009. Ockham's razor and autism: The case for developmental neurotoxins contributing to a disease of neurodevelopment. *Neurotoxicology* 30: 331–7.

DeSoto, M.C., and R.T. Hitlan. 2007. Blood levels of mercury are related to diagnosis of autism: A reanalysis of an important data set. *Journal of Child Neurology* 22: 1308–11.

DeSoto, M.C., and R.T. Hitlan. 2010. Sorting out the spinning of autism: Heavy metals and the question of incidence. *Acta Neurobiologiae Expermentalis* 70: 165–76.

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

Dietert, R.R., and J.M. Dietert. 2008. Potential for early-life immune insult including developmental immunotoxicity in autism and autism spectrum disorders: Focus on critical windows of immune vulnerability. *Journal of Toxicology and Environmental Health Part B* 11: 660–80.

Dringen, R. 2000. Metabolism and functions of glutathione in brain. *Progress in Neurobiology* 62: 649–71.

Dringen, R., J.M. Gutterer, and J. Hirrlinger. 2000. Glutathione metabolism in brain – metabolic interaction between astrocytes and neurons in the defence against reactive oxygen species. *European Journal of Biochemistry* 267: 4912–6.

Dringen, R., B. Pfeiffer, and B. Hamprecht. 1999. Synthesis of the antioxidant glutathione in neurons: Supply by astrocytes of CysGly as precursor for neuronal glutathione. *Journal of Neuroscience* 19: 562–9.

Echeverria, D., J.S. Woods, N.J. Heyer, D. Rohlman, F.M. Farin, T. Li, and C.E. Garabedian. 2006. The association between a genetic polymorphism of coproporphyrinogen oxidase, dental mercury exposure and neurobehavioral response in humans. *Neurotoxicology and Teratology* 28: 39–48.

Ekstrand, J., J.B. Nielsen, S. Havarinasab, R.K. Zalups, P. Soderkvist, and P. Hultman. 2010. Mercury toxicokinetics-dependency on strain and gender. *Toxicology and Applied Pharmacology* 243: 283–91.

Ezugha, H., M. Goldenthal, I. Valencia, C.E. Anderson, A. Legido, and H. Marks. 2010. 5q14.3 Deletion manifesting as mitochondrial disease and autism: Case report. *Journal of Child Neurology* 25: 1232–5.

Fallon, J.R. 1985. Preferential outgrowth of central nervous system neurites on astrocytes and Schwann cells as compared with nonglial cells in vitro. *Journal of Cell Biology* 100: 198–207.

Ferraro, L., M.C. Tomasini, S. Tanganelli, R. Mazza, A. Coluccia, M.R. Carratu, S. Gaetani, V. Cuomo, and T. Antonelli. 2009. Developmental exposure to methylmercury elicits early cell death in the cerebral cortex and long-term memory deficits in the rat. *International Journal of Developmental Neuroscience* 27: 165–74.

Fido, A., and S. Al-Saad. 2005. Toxic trace elements in the hair of children with autism. *Autism* 9: 290–8.

Fields, R.D. 2008. Oligodendrocytes changing the rules: Action potentials in glia and oligodendrocytes controlling action potentials. *Neuroscientist* 14: 540–3.

Fields, R.D., and B. Stevens-Graham. 2002. Neuroscience: New insights into neuron-glia communication. *Science* 298: 556–62.

Franco, J.L., T. Posser, P.R. Dunkley, P.W. Dickson, J.J. Mattos, R. Martins, A.C. Bainy, M.R. Marques, A.L. Dafre, and M. Farina. 2009. Methylmercury neurotoxicity is associated with inhibition of the antioxidant enzyme glutathione peroxidase. *Free Radical Biology and Medicine* 47: 449–57.

Fratelli, M., H. Demol, M. Puype, S. Casagrande, I. Eberini, M. Salmona, V. Bonetto, et al. 2002. Identification by redox proteomics of glutathionylated proteins in oxidatively stressed human T lymphocytes. *Proceedings of the National Academy of Sciences U.S.A.* 99: 3505–10.

Freitag, C.M., W. Staal, S.M. Klauck, E. Duketis, and R. Waltes. 2010. Genetics of autistic disorders: Review and clinical implications. *European Child and Adolescent Psychiatry* 19: 169–78.

Gallagher, C.M., and M.S. Goodman. 2010. Hepatitis B vaccination of male neonates and autism diagnosis, NHIS 1997–2002. *Journal of Toxicology and Environmental Health Part A* 73: 1665–77.

Garcia, J., D. Han, H. Sancheti, L.-P. Yap, N. Kaplowitz, and E. Cadenas. 2010. Regulation of mitochondrial glutathione redox status and protein glutathionylation by respiratory substrates. *Journal of Biological Chemistry* 285: 39646–54.

Gargus, J.J., and F. Imtiaz. 2008. Mitochondrial energy-deficient endophenotype in autism. *American Journal of Biochemistry and Biotechnology* 4: 198–207.

Geier, D.A., and M.R. Geier. 2006a. A prospective assessment of porphyrins in autistic disorders: A potential marker for heavy metal exposure. *Neurotoxicity Research* 10: 57–63.

Geier, D.A., and M.R. Geier. 2006b. A clinical and laboratory evaluation of methionine cycle-transsulfuration and androgen pathway markers in children with autistic disorders. *Hormone Research* 66: 182–8.

Geier, D.A., J.K. Kern, C.R. Garver, J.B. Adams, T. Audhya, and M.R. Geier. 2009a. A prospective study of transsulfuration biomarkers in autistic disorders. *Neurochemical Research* 34: 386–93.

Geier, D.A., J.K. Kern, C.R. Garver, J.B. Adams, T. Audhya, R. Nataf, and M.R. Geier. 2009b. Biomarkers of environmental toxicity and susceptibility in autism. *Journal of Neurological Sciences* 280: 101–8.

Geier, D.A., J.K. Kern, and M.R. Geier. 2009c. A prospective study of prenatal mercury exposure from maternal dental amalgams and autism severity. *Acta Neurobiologiae Experimentalis* 69: 189–97.

Geier, D.A., P.G. King, L.K. Sykes, and M.R. Geier. 2008. A comprehensive review of Mercury provoked autism. *Indian Journal of Medical Research* 128: 383–411.

Grandjean, P., P. Weihe, R.F. White, and F. Debes. 1998. Cognitive performance of children prenatally exposed to "safe" levels of methylmercury. *Environmental Research Part A* 77: 165–72.

Grandjean, P., P. Weihe, R.F. White, F. Debes, S. Araki, K. Yokoyama, K. Murata, N. Sørensen, R. Dahl, and P.J. Jørgensen. 1997. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicology and Teratology* 19: 417–28.

Grether, J.K., N.J. Rosen, K.S. Smith, and L.A. Croen. 2009. Investigation of shifts in autism reporting in the California department of developmental services. *Journal of Autism and Developmental Disorders* 39: 1412–19.

Gundacker, C., M. Gencik, and M. Hengstschläger. 2010. The relevance of the individual genetic background for the toxicokinetics of two significant neurodevelopmental toxicants: Mercury and lead. *Mutatation Research/Reviews in Mutation Research* 705: 130–40.

Gundacker, C., G. Komamicki, P. Jagiello, A. Gencikova, N. Dahmen, K.J. Wittmann, and M. Gencik. 2007. Glutathione-S-transferase polymorphism, metallothionein expression, and mercury levels among students in Austria. *Science of the Total Environment* 385: 37–47.

Halliwell, B. 1992. Reactive oxygen species and the central nervous system. *Journal of Neurochemistry* 59: 1609–23.

Harada, M. 1978. Congenital Minamata disease: Intrauterine methylmercury poisoning. *Teratology* 18: 285–8.

Harada, M., H. Akagi, T. Tsuda, T. Kizaki, and H. Ohno. 1999. Methylmercury level in umbilical cords from patients with congenital Minamata disease. *Science of the Total Environment* 234: 59–62.

Harry, G.J., M.W. Harris, and L.T. Burka. 2004. Mercury concentrations in brain and kidney following ethylmercury, methylmercury and thimerosal administration to neonatal mice. *Toxicology Letters* 154: 183–9.

Hayes, J.D., J.U. Flanagan, and I.R. Jowsey. 2005. Glutathione transferases. *Annual Review of Pharmacology and Toxicology* 45: 51–88.

Heggland, I., P. Kaur, and T. Syversen. 2009. Uptake and efflux of methylmercury in vitro: Comparison of transport mechanisms in C6, B35 and RBE4 cells. *Toxicology In Vitro* 23: 1020–7.

Herbert, M.R. 2010. Contributions of the environment and environmentally vulnerable physiology to autism spectrum disorders. *Current Opinion Neurology* 23: 103–10.

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

Hertz-Picciotto, I., P.G. Green, L. Delwiche, R. Hansen, C. Walker, and I.N. Pessah. 2010. Blood mercury concentrations in CHARGE study children with and without autism. *Environmental Health Perspectives* 118: 161–6.

Hewitson, L., L.A. Houser, C. Stott, G. Sackett, J.L. Tomko, D. Atwood, L. Blue, and E.R. White. 2010. Delayed acquisition of neonatal reflexes in newborn primates receiving a thimerosal-containing hepatitis B vaccine: Influence of gestational age and birth weight. *Journal of Toxicology and Environmental Health Part A* 73: 1298–313.

Hewitson, L., B.J. Lopresti, C. Stott, N.S. Mason, and J. Tomko. 2010. Influence of pediatric vaccines on amygdala growth and opioid ligand binding in rhesus macaque infants: A pilot study. *Acta Neurobiologiae Experimentalis* 70: 147–64.

Hoffman, R.S., L.S. Nelson, M.A. Howland, N.A. Lewin, N.E. Flomenbaum, and L.R. Goldfrank. 2007. Mercury. In *Goldfrank's toxicologic emergencies*, eds. L.R. Goldfrank and N.E. Flomenbaum, 739–46. New York, NY: McGraw-Hill.

Holmes, A., M. Blaxill, and B. Haley. 2003. Reduced levels of mercury in first baby haircuts of autistic children. *International Journal of Toxicology* 22: 277–85.

Hu, J.J., L.X. Dong, and C.E. Outten. 2008. The redox environment in the mitochondrial intermembrane space is maintained separately from the cytosol and matrix. *Journal of Biological Chemistry* 283: 29126–34.

Hu, V., B. Frank, S. Heine, N. Lee, and J. Quackenbush. 2006. Gene expression profiling of lymphoblastoid cell lines from monozygotic twins discordant in severity of autism reveals differential regulation of neurologically relevant genes. *BMC Genomics* 7: 118.

Hung, J., M. Chansard, S.S. Ousman, M. Nguyen, and M.A. Colicos. 2010. Activation of microglia by neuronal activity: Results from a new in vitro paradigm based on neuronal-silicon interfacing technology. *Brain, Behavior and Immunity* 24: 31–40.

James, S.J., P. Cutler, S. Melnyk, S. Jernigan, L. Janak, D.W. Gaylor, and J.A. Neubrander. 2004. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *American Journal of Clinical Nutrition* 80: 1611–17.

James, S.J., S. Melnyk, S. Jernigan, M.A. Cleves, C.H. Halsted, D.H. Wong, P. Cutler, et al. 2006. Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 141B: 947–56.

James, S.J., S. Melnyk, S. Jernigan, A. Hubanks, S. Rose, and D.W. Gaylor. 2008. Abnormal transmethylation/transsulfuration metabolism and DNA hypomethylation among parents of children with autism. *Journal of Autism and Developmental Disorders* 38: 1966–76.

James, S.J., S. Melnyk, S. Jernigan, O. Pavliv, T. Trusty, S. Lehman, L. Seidel, D.W. Gaylor, and M.A. Cleves. 2010. A functional polymorphism in the reduced folate carrier gene and DNA hypomethylation in mothers of children with autism. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 153B: 1209–20.

James, S.J., S. Rose, S. Melnyk, S. Jernigan, S. Blossom, O. Pavliv, and D.W. Gaylor. 2009. Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism. *FASEB Journal* 23: 2374–83.

James, S.J., W. Slikker 3rd, S. Melnyk, E. New, M. Pogribna, and S. Jernigan. 2005. Thimerosal neurotoxicity is associated with glutathione depletion: Protection with glutathione precursors. *Neurotoxicology* 26: 1–8.

Johansson, C., A.F. Castoldi, N. Onishchenko, L. Manzo, M. Vahter, and S. Ceccatelli. 2007. Neurobehavioural and molecular changes induced by methylmercury exposure during development. *Neurotoxicity Research* 11: 241–60.

Kanner, L. 1943. Autistic disturbances of affective contact. *Nervous Child* 2: 217–50.

Kempuraj, D., S. Asadi, B.D. Zhang, A. Manola, J. Hogan, E. Peterson, and T.C. Theoharides. 2010. Mercury induces inflammatory mediator release from human mast cells. *Journal of Neuroinflammation* 7: 20.

Kern, J.K., B.D. Grannemann, M.H. Trivedi, and J.B. Adams. 2007. Sulfhydryl-reactive metals in autism. *Journal of Toxicology and Environmental Health Part A* 70: 715–21.

Kern, J.K., and A.M. Jones. 2006. Evidence of toxicity, oxidative stress and neuronal insult in autism. *Journal of Toxicology and Environmental Health Part B* 9: 485–99.

Kidd, P.M. 2005. Neurodegeneration from mitochondrial insufficiency: Nutrients, stem cells, growth factors, and prospects for brain rebuilding using integrative management. *Alternative Medicine Review* 10: 268–93.

King, M., and P. Bearman. 2009. Diagnostic change and the increased prevalence of autism. *International Journal of Epidemiology* 38: 1224–34.

Kjellstrom, T., P. Kennedy, S. Wallis, and C. Mantell. 1986. Physical and mental development of children with prenatal exposure to mercury from fish: Stage 1. Preliminary tests at age 4. *Natl. Swed. Environ. Board Rep* 3642.

Kjellstrom, T., P. Kennedy, S. Wallis, A. Stewart, L. Friberg, B. Lind, T. Wutherspoon, and C. Mantell. 1989. Physical and mental development of children with prenatal exposure to Mercury

from fish: Stage 2. Interviews and psychological tests at age 6. *Natl. Swed. Environ. Board Rep* 3642.

Koehler, R.C., R.J. Roman, and D.R. Harder. 2009. Astrocytes and the regulation of cerebral blood flow. *Trends in Neurosciences* 32: 160–9.

Kogan, M.D., S.J. Blumberg, L.A. Schieve, C.A. Boyle, J.M. Perrin, R.M. Ghandour, G.K. Singh, B.B. Strickland, E. Trevathan, and P.C. Van Dyck. 2009. Prevalence of parent-reported diagnosis of autism spectrum disorder among children in the US, 2007. *Pediatrics* 124: 1395–403.

Koren, G., and J.R. Bend. 2010. Fish consumption in pregnancy and fetal risks of methylmercury toxicity. *Canadian Family Physician* 56: 1001–2.

Lash, L.H. 2006. Mitochondrial glutathione transport: Physiological, pathological and toxicological implications. *Chemico-Biological Interactions* 163: 54–67.

Lee, B.E., Y.C. Hong, H. Park, M. Ha, B.S. Koo, N. Chang, Y.M. Roh, et al. 2010. Interaction between GSTM1/GSTT1 polymorphism and blood mercury on birth weight. *Environmental Health Perspectives* 118: 437–42.

Magos, L. 2003. Neurotoxic character of thimerosal and the allometric extrapolation of adult clearance half-time to infants. *Journal of Applied Toxicology* 23: 263–9.

Main, P.A.E., M.T. Angley, P. Thomas, C.E. O'Doherty, and M. Fenech. 2010. Folate and methionine metabolism in autism: A systematic review. *American Journal of Clinical Nutrition* 91: 1598–620.

Majewska, M.D., E. Urbanowicz, P. Rok-Bujko, I. Namyslowska, and P. Mierzejewski. 2010. Age-dependent lower or higher levels of hair mercury in autistic children than in healthy controls. *Acta Neurobiologiae Experimentalis* 70: 196–208.

Mato, J.M., F.J. Corrales, S.C. Lu, and M.A. Avila. 2002. S-adenosylmethionine: A control switch that regulates liver function. *FASEB Journal* 16: 15–26.

Matsumoto, H., G. Koya, and T. Takeuchi. 1965. Fetal minamata disease: A neuropathological study of two cases of intrauterine intoxication by a methyl mercury compound. *Journal of Neuropathology and Experimental Neurology* 24: 563–74.

Mohammad, N.S., J.M.N. Jain, K.P. Chintakindi, R.P. Singh, U. Naik, and R.R.D. Akella. 2009. Aberrations in folate metabolic pathway and altered susceptibility to autism. *Psychiatric Genetics* 19: 171–6.

Monroe, R.K., and S.W. Halvorsen. 2006. Mercury abolishes neurotrophic factor-stimulated Jak-STAT signaling in nerve cells by oxidative stress. *Toxicological Sciences* 94: 129–38.

Monroe, R.K., and S.W. Halvorsen. 2009. Environmental toxicants inhibit neuronal Jak tyrosine kinase by mitochondrial disruption. *Neurotoxicology* 30: 589–98.

Morel, F.M., A.M.L. Kraepiel, and M. Amyot. 1998. The chemical cycle and bioaccumulation of mercury. *Annual Review of Ecology and Systematics* 29: 543–66.

Morrissette, J., L. Takser, G. St-Amour, A. Smargiassi, J. Lafond, and D. Mergler. 2004. Temporal variation of blood and hair mercury levels in pregnancy in relation to fish consumption history in a population living along the St. *Lawrence River*. *Environmental Research* 95: 363–74.

Muhle, R., S.V. Trentacoste, and I. Rapin. 2004. The genetics of autism. *Pediatrics* 113: 472–86.

Muraoka, Y., and F. Itoh. 1980. Sex difference of mercuric chloride-induced renal tubular necrosis in rats – from the aspect of sex differences in renal mercury concentration and sulfhydryl levels. *Journal of Toxicological Sciences* 5: 203–14.

Mutter, J., J. Naumann, and C. Guethlin. 2007. Comments on the article "The toxicology of mercury and its chemical compounds" by Clarkson and Magos (2006). *Critical Reviews in Toxicology* 37: 537–49.

Myers, G.J., P.W. Davidson, C. Cox, C.F. Shamlaye, M.A. Tanner, O. Choisy, J. Sloane-Reeves, et al. 1995. Pilot neurodevelopment study of Seychellois children following in utero exposure to methylmercury from a maternal fish diet. *Neurotoxicology and Teratology* 16: 629–38.

Myers, G.J., D.O. Marsh, P.W. Davidson, C. Cox, C.F. Shamlaye, M.A. Tanner, A. Choi, E. Cernichiari, O. Choisy, and T.W. Clarkson. 1995. Main neurodevelopment study of

Seychellois children following in utero exposure to methylmercury from a maternal fish diet: Outcome at six months. *Neurotoxicology and Teratology* 16: 653–64.

Nataf, R., C. Skorupka, L. Amet, A. Lam, A. Springbett, and R. Lathe. 2006. Porphyrinuria in childhood autistic disorder: Implications for environmental toxicity. *Toxicology and Applied Pharmacology* 214: 99–108.

Nelson, K.B., and M.L. Bauman. 2003. Thimerosal and autism? *Pediatrics* 111: 674–79.

Newschaffer, C.J., L.A. Croen, J. Daniels, E. Giarelli, J.K. Grether, S.E. Levy, D.S. Mandell, et al. 2007. The epidemiology of autism spectrum disorders. *Annual Review of Public Health* 28: 235–58.

Nicklin, P., P. Bergman, B.L. Zhang, E. Triantafellow, H. Wang, B. Nyfeler, H.D. Yang, et al. 2009. Bidirectional transport of amino acids regulates mTOR and autophagy. *Cell* 136: 521–34.

Nilsen, J. 2008. Estradiol and neurodegenerative oxidative stress. *Frontiers Neuroendocrinology* 29: 463–75.

Nishimura, Y., C.L. Martin, A. Vazquez-Lopez, S.J. Spence, A.I. Alvarez-Retuerto, M. Sigman, C. Steindler, et al. 2007. Genome-wide expression profiling of lymphoblastoid cell lines distinguishes different forms of autism and reveals shared pathways. *Human Molecular Genetics* 16: 1682–98.

O'Connor, E., A. Devesa, C. García, I.R. Puertes, A. Pellín, and J.R. Vina. 1995. Biosynthesis and maintenance of GSH in primary astrocyte cultures: Role of l-cystine and ascorbate. *Brain Research* 680: 157–63.

Oken, E., J.S. Radesky, R.O. Wright, D.C. Bellinger, C. Amarasiriwardena, K.P. Kleinman, H. Hu, and M.W. Gillman. 2008. Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. *American Journal of Epidemiology* 67: 1171–81.

Oktyabrsky, O.N., and G.V. Smirnova. 2007. Redox regulation of cellular functions. *Biochemistry (Moscow)* 72: 132–45.

Olafsdottir, K., and D.J. Reed. 1988. Retention of oxidized glutathione by isolated rat liver mitochondria during hydroperoxide treatment. *Biochimica et Biophysica Acta* 964: 377–82.

Oliveira, G., L. Diogo, M. Grazina, P. Garcia, A. Ataide, C. Marques, T. Miguel, L. Borges, A.M. Vicente, and C.R. Oliveira. 2005. Mitochondrial dysfunction in autism spectrum disorders: A population-based study. *Developmental Medicine and Child Neurology* 47: 185–9.

Olivieri, G., C. Brack, F. Müller-Spahn, H.B. Stähelin, M. Herrmann, P. Renard, M. Brockhaus, and C. Hock. 2000. Mercury induces cell cytotoxicity and oxidative stress and increases β-amyloid secretion and tau phosphorylation in SHSY5Y neuroblastoma cells. *Journal of Neurochemistry* 74: 231–6.

Olivieri, G., M. Novakovic, E. Savaskan, F. Meier, G. Baysang, M. Brockhaus, and F. Muller-Spahn. 2002. The effects of beta-estradiol on SHSY5Y neuroblastoma cells during heavy metal induced oxidative stress, neurotoxicity and beta-amyloid secretion. *Neuroscience* 113: 849–55.

Ou, Y.C., C.C. White, C.M. Krejsa, R.A. Ponce, T.J. Kavanagh, and E.M. Faustman. 1999. The role of intracellular glutathione in methylmercury-induced toxicity in embryonic neuronal cells. *Neurotoxicology* 20: 793–804.

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

Pardo, C.A., D.L. Vargas, and A.W. Zimmerman. 2006. Immunity, neuroglia and neuroinflammation in autism. *International Review of Psychiatry* 17: 485–95.

Pasca, S.P., E. Dronca, T. Kaucsar, E.C. Craciun, E. Endreffy, B.K. Ferencz, F. Iftene, et al. 2009. One carbon metabolism disturbances and the C677T MTHFR gene polymorphism in children with autism spectrum disorders. *Journal of Cellular and Molecular Medicine* 13: 4229–38.

Persa, C., A. Pierce, Z. Ma, O. Kabil, and M.F. Lou. 2004. The presence of a transsulfuration pathway in the lens: A new oxidative stress defence system. *Experimental Eye Research* 79: 875–86.

Price, C.S., W.W. Thompson, B. Goodson, E.S. Weintraub, L.A. Croen, V.L. Hinrichsen, M. Marcy, et al. 2010. Prenatal and infant exposure to thimerosal from vaccines and immunoglobulins and risk of autism. *Pediatrics* 126: 656–64.

Rao, A.N., M. Koch, V. Suresh Kumar, S. Ghosh, and G. Shobha. 2009. Assessment of metabolic parameters for autism spectrum disorders. *Online Journal of Health Allied Sciences* 8: 1.

Rice, C. 2009. Prevalence of autism spectrum disorders: Autism and developmental disabilities monitoring network, United States, 2006. Morbidity and mortality weekly report surveillance summaries. Vol. 58. http://www.cdc.gov (accessed May 5, 2011).

Ritvo, E.R., B.J. Freeman, A. Mason-Brothers, A. Mo, and A.M. Ritvo. 1985. Concordance for the syndrome of autism in 40 pairs of afflicted twins. *American Journal of Psychiatry* 142: 74–7.

Rodig, S.J., M.A. Meraz, J.M. White, P.A. Lampe, J.K. Riley, C.D. Arthur, K.L. King, et al. 1998. Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. *Cell* 93: 373–83.

Rose, S., S. Melnyk, A. Savenka, A. Hubanks, S. Jernigan, M. Cleves, and S.J. James. 2008. The frequency of polymorphisms affecting lead and mercury toxicity among children with autism. *American Journal of Biochemistry and Biotechnology* 4: 85–94.

Sagara, J.-I., K. Miura, and S. Bannai. 1993. Maintenance of neuronal glutathione by glial cells. *Journal of Neurochemistry* 61: 1672–6.

Sakamoto, M., K. Murata, M. Kubota, K. Nakai, and H. Satoh. 2010. Mercury and heavy metal profiles of maternal and umbilical cord RBCs in Japanese population. *Ecotoxicology and Environmental Safety* 73: 1–6.

Sarafian, T.A., and M.A. Verity. 1991. Oxidative mechanisms underlying methyl mercury neurotoxicity. *International Journal of Developmental Neuroscience* 9: 147–53.

Sarafian, T.A., L. Vartavarian, D.J. Kane, D.E. Bredesen, and M.A. Verity. 1994. Bcl-2 expression decreases methyl mercury-induced free-radical generation and cell killing in a neural cell line. *Toxicology Letters* 74: 149–55.

Schoeman, K., T. Tanaka, J.R. Bend, and G. Koren. 2010. Hair mercury levels of women of reproductive age in Ontario, Canada: Implications to fetal safety and fish consumption. *Journal of Pediatrics* 57: 127–31.

Schulz, J.B., J. Lindenau, J. Seyfried, and J. Dicmercuryans. 2000. Glutathione, oxidative stress and neurodegeneration. *European Journal of Biochemistry* 267: 4904–11.

Schultz, S.T. 2010. Does thimerosal or other mercury exposure increase the risk for autism? *Acta Neurobiologiae Expermentalis* 70: 187–95.

Schweikert, C., Y.J. Li, D. Dayya, D. Yens, M. Torrents, and D.F. Hsu. 2009. Analysis of autism prevalence and neurotoxins using combinatorial fusion and association rule mining. 9th IEEE international conference on bioinformatics and bioengineering, June 22–24, in Taichung, China, 400–4.

Schwenk, M., R. Klein, and D.M. Templeton. 2009. Immunological effects of mercury (IUPAC Technical Report). *Pure and Applied Chemistry* 81: 153–67.

Shandley, K., and D.W. Austin. in press. Ancestry of pink disease (infantile mercury poisoning) identified as a risk factor for autism spectrum disorders. *Journal of Toxicology and Environmental Health: Part A*.

Shenker, B.J., L. Pankoski, A. Zekavat, and I.M. Shapiro. 2002. Mercury-induced apoptosis in human lymphocytes: Caspase activation is linked to redox status. *Antioxidants Redox Signal* 4: 379–89.

Singh, V.K. 1996. Plasma increase of interleukin-12 and interferon-gamma pathological significance in autism. *Journal of Neuroimmunology* 66: 143–5.

Singh, R.P., S. Sharad, and S. Kapur. 2004. Free radicals and oxidative stress in neurodegenerative diseases: Relevance of dietary antioxidants. *Journal of Indian Academy of Clinical Medicine* 5: 218–25.

Singh, V.K., R. Warren, R. Averett, and M. Ghaziuddin. 1997. Circulating autoantibodies to neuronal and glial filament proteins in autism. *Pediatric Neurology* 17: 88–90.

Sogut, S., S.S. Zoroglu, H. Ozyurt, H.R. Yilmaz, F. Ozugurlu, E. Sivasli, O. Yetkin, et al. 2003. Changes in nitric oxide levels and antioxidant enzyme activities may have a role in the pathophysiological mechanisms involved in autism. *Clinica Chimica Acta* 331: 111–7.

Steindler, C., Z. Li, M. Algarté, A. Alcover, V. Libri, J. Ragimbeau, and S. Pellegrini. 2004. Jamip1 (Marlin-1) defines a family of proteins interacting with janus kinases and microtubules. *Journal of Biological Chemistry* 279: 43168–77.

Suh, J.H., W.J. Walsh, W.R. McGinnis, A. Lewis, and B.N. Ames. 2008. Altered sulfur amino acid metabolism in immune cells of children diagnosed with autism. *American Journal of Biochemistry and Biotechnology* 4: 105–13.

Taber, K.H., and R.A. Hurley. 2008. Mercury exposure: Effects across the lifespan. *Journal of Neuropsychiatric and Clinical Neurosciences* 20: iv–389.

Taylor, E.R., F. Hurrell, R.J. Shannon, T.-K. Lin, J. Hirst, and M.P. Murphy. 2003. Reversible glutathionylation of complex I increases mitochondrial superoxide formation. *Journal of Biological Chemistry* 278: 19603–10.

Townsend, D.M., K.D. Tew, and H. Tapiero. 2003. The importance of glutathione in human disease. *Biomedicine and Pharmacotherapy* 57: 145–55.

U.S. Food and Drug Administration. 2004. What you need to know about mercury in fish and shellfish. http://www.fda.gov/food/foodsafety/product-specificinformation/seafood/foodbornepathogenscontaminants/methylmercury/ucm115662.htm (accessed May 5, 2011).

Valko, M., D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur, and J. Telser. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology* 39: 44–84.

Vargas, D.L., C. Nascimbene, C. Krishnan, A.W. Zimmerman, and C.A. Pardo. 2005. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Annals of Neurology* 57: 67–81.

Weissman, J.R., R.I. Kelley, M.L. Bauman, B.H. Cohen, K.F. Murray, R.L. Mitchell, R.L. Kern, and M.R. Natowicz. 2008. Mitochondrial disease in autism spectrum disorder patients: A cohort analysis. *PLoS One* 3: e3185.

Westphal, G.A., A. Schnuch, T.G. Schulz, K. Reich, W. Aberer, J. Brasch, P. Koch, et al. 2000. Homozygous gene deletions of the glutathione S-transferases M1, and T1 are associated with thimerosal sensitization. *International Archives of Occupational and Environmental Health* 73: 384–8.

Whanger, P.D. 2001. Selenium and the brain: A review. *Nutritional Neuroscience* 4: 81–97.

Williams, P.G., J.H. Hersh, A. Allard, and L.L. Sears. 2008. A controlled study of mercury levels in hair samples of children with autism as compared to their typically developing siblings. *Research in Autism Spectrum Disorders* 2: 170–5.

Williams, T.A., A.E. Mars, S.G. Buyske, E.S. Stenroos, R. Wang, M.F. Factura-Santiago, G.H. Lambert, and W.G. Johnson. 2007. Risk of autistic disorder in affected offspring of mothers with a glutathione S-transferase P1 haplotype. *Archives of Pediatrics and Adolescent Medicine* 161: 356–61.

Windham, G.C., L.X. Zhang, R. Gunier, L.A. Croen, and J.K. Grether. 2006. Autism spectrum disorders in relation to distribution of hazardous air pollutants in the San Francisco Bay area. *Environmental Health Perspectives* 114: 1438–44.

Woods, J.S., S.E. Armel, D.I. Fulton, J. Allen, K. Wessels, P.L. Simmonds, D. Granpeesheh, et al. 2010. Urinary porphyrin excretion in neurotypical and autistic children. *Environmental Health Perspectives* 118: 1450–7.

Woods, J.S., M.A. Bowers, and H.A. Davis. 1991. Urinary porphyrin profiles as biomarkers of trace-metal exposure and toxicity –studies on urinary porphyrin excretion patterns in rats during prolonged exposure to methyl mercury. *Toxicology and Applied Pharmacology* 110: 464–76.

Woods, J.S., and M.E. Ellis. 1995. Up-regulation of glutathione synthesis in rat kidney by methyl mercury: Relationship to mercury-induced oxidative stress. *Biochemical Pharmacology* 50: 1719–24.

Yeargin-Allsopp, M., C. Rice, T. Karapurkar, N. Doernberg, C. Boyle, and C. Murphy. 2003. Prevalence of autism in a US metropolitan area. *Journal of American Medical Association* 289: 49–55.

Yin, Z.B., H.Y. Jiang, T. Syversen, J.B.T. Rocha, M. Farina, and M. Aschner. 2008. The methylmercury-L-cysteine conjugate is a substrate for the L-type large neutral amino acid transporter. *Journal of Neurochemistry* 107: 1083–90.

Yin, Z., D. Milatovic, J.L. Aschner, T. Syversen, J.B.T. Rocha, D.O. Souza, M. Sidoryk, J. Albrecht, and M. Aschner. 2007. Methylmercury induces oxidative injury, alterations in permeability and glutamine transport in cultured astrocytes. *Brain Research* 1131: 1–10.

Yochum, C.L., P. Bhattacharya, L. Patti, O. Mirochnitchenko, and G.C. Wagner. 2010. Animal model of autism using GSTM1 knockout mice and early post-natal sodium valproate treatment. *Behavioural Brain Research* 210: 202–10.

Youn, S.I., S.H. Jin, S.H. Kim, and S. Lim. 2010. Porphyrinuria in Korean children with autism: Correlation with oxidative stress. *Journal of Toxicology and Environmental Health Part A* 73: 701–10.

Zahir, F., S.J. Rizwi, S.K. Haq, and R.H. Khan. 2005. Low dose mercury toxicity and human health. *Environmental Toxicology and Pharmacology* 20: 351–60.

Zhao, X.Y., C.H. Pak, R.D. Smrt, and P. Jin. 2007. Epigenetics and neural developmental disorders. *Epigenetics* 2: 126–34.