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Review article

Environmental factors in the development of autism spectrum disorders

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ABSTRACT

Autism spectrum disorders (ASD) are highly heterogeneous developmental conditions characterized by deficits in social interaction, verbal and nonverbal communication, and obsessive/stereotyped patterns of behavior and repetitive movements. Social interaction impairments are the most characteristic deficits in ASD. There is also evidence of impoverished language and empathy, a profound inability to use standard nonverbal behaviors (eye contact, affective expression) to regulate social interactions with others, difficulties in showing empathy, failure to share enjoyment, interests and achievements with others, and a lack of social and emotional reciprocity. In developed countries, it is now reported that 1%–1.5% of children have ASD, and in the US 2015 CDC reports that approximately one in 45 children suffer from ASD. Despite the intense research focus on ASD in the last decade, the underlying etiology remains unknown. Genetic research involving twins and family studies strongly supports a significant contribution of environmental factors in addition to genetic factors in ASD etiology. A comprehensive literature search has implicated several environmental factors associated with the development of ASD. These include pesticides, phthalates, polychlorinated biphenyls, solvents, air pollutants, fragrances, glyphosate and heavy metals, especially aluminum used in vaccines as adjuvant. Importantly, the majority of these toxicants are some of the most common ingredients in cosmetics and herbicides to which almost all of us are regularly exposed to in the form of fragrances, face makeup, cologne, air fresheners, food flavors, detergents, insecticides and herbicides. In this review we describe various scientific data to show the role of environmental factors in ASD.

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1. Introduction

Autistic individuals typically have restricted capacity for communication, a compromised ability to interact with others, and unusually repetitive (stereotypic) behaviors that often make social interactions challenging, hurt job potential, and make numerous other problems more likely. The American Psychiatric Association categorizes autism spectrum disorder (ASD) among many other neurodevelopmental disorders, which include Tourette's Disorder, Tic Disorder, and Social Communication Disorder (Neurodevelopmental Disorders. DSM-5 Development (Geschwind, 2009; Lichtenstein et al., 2010). In developed countries, it is now reported that 1% to 1.5% of the children have autism (Geschwind, 2009; Lichtenstein et al., 2010; Zablotsky et al., 2015). Genetic predisposition may explain around 10% of cases. There are genetic diseases, for example Angelman syndrome, Rett syndrome, fragile X syndrome, Cohen syndrome, and Down's syndrome, which greatly increase the probability of developing autism (Fombonne, 2003; Geschwind, 2009; Lichtenstein et al., 2010; Zablotsky et al., 2015). Monozygotic twin concordance has been discovered as reaching 70%–90% for just autistic behavioral traits and it is generally assumed that ASD is due to inherited genetic defects (Geschwind, 2009). However, this conclusion is based on misinterpretations of existing data and bias reporting (see below) and this concordance could be due to exposure to environmental agents that are neurotoxic to the developing twins' brains (Chamak, 2010). Of note, a significant percent of monozygotic twins (MZ) are discordant (Hallmayer et al., 2011). In dizygotic twins, it has not been proven to be higher than in their isolated brothers (Chamak, 2010; Hallmayer et al., 2011) and there is strong evidence that environmental factors play as much of a role in development of autism as the genetic factors (Hallmayer et al., 2011). The number of children who have ASD has increased considerably since the early 1980s (Tammimies et al., 2015). ASD varies significantly in presentation among those affected (Geschwind, 2009; Lichtenstein et al., 2010). It is, therefore, not surprising that the etiology of ASD is thought to be similarly heterogeneous and multifaceted in nature. The broad spectrum in the definition of ASD suggests that the disease may result from exposure to certain environmental agents instead of primarily a genetic disorder (Landrigan, 2010; Ortega García et al., 2007; Tammimies et al., 2015). This rather controversial view is explained in detail in this review.

2. Autism: overestimation of the inherited genetic origins

For over a century, the belief is held that autism is a genetic and heritable disease (Chamak, 2010; Hallmayer et al., 2011; Landrigan, 2010; Tammimies et al., 2015). A thorough inspection of outcomes and claims that support a strong genetic source of autism demonstrates incorrect interpretations, methodological biases, and flawed approximations, not to mention overstated media reports. Recently, Hallmayer et al. (2011) carried one of the largest twin-pair studies. They completed 192 twin-pair studies and reported that a large degree of risk for ASD in MZ was due to environmental factors and a smaller risk was due to heritability or genetic. Of note, they have not considered the *de novo* mutations and single copy number variations (CNV) as we describe below.

3. More than 1000 genetic and genomic disorders and still counting

ASD is considered a highly heritable disorder; yet genome-wide association studies, copy number variation, and candidate gene association have found no single genetic factor accounting for over 90% of ASD cases. Interestingly, trio (the parents and the affected children) exome sequencing analyses, where exons or the expressed genes are sequences, have revealed genes with recurrent *de novo* loss-of-function variants in the infants, where such mutations are not found in the parents (Tammimies et al., 2015). There more than 1000 genes that are predicted to play a role in ASD. In an attempt to collate all genes and recurrent genomic imbalances that have been implicated in the etiology of ASD, the recent exhaustive review of the clinical genetics and research genetics literature has shown that there may be 103 disease genes and 44 genomic loci reported in subjects with ASD or autistic behavior (Betancur, 2011; Tammimies et al., 2015). These genes and loci have all been causally implicated in intellectual disability, indicating that these two neurodevelopmental disorders share common genetic bases.

Prenatal exposure to harmful chemicals, or occurrence of an infection during the primary gestation period of a maturing fetus, can detrimentally affect the development of the immune system (Landrigan, 2010). The occurrence and exposure to harmful chemicals or infection can increase the incidence of autoimmune deficiencies in young children. Thus, research has linked autoimmune deficiencies as one of the many trademarks of autism spectrum disorder. There are various examples of autoimmune abnormalities observed in autistic children, such as a higher influx in pro-inflammatory cytokines, a decrease in plasma IL-23, and an elevated count of plasma leptin levels (Hertz-Picciotto et al., 2008). This strong correlation of exemplifying irregular immune system devolvement in autism demonstrates that damaging events are possibly occurring during certain stages of the gestation period. During specific stages of the gestation period, critical stages of the immune system development take place. In theory, if certain parts of the immune system development are altered, adverse life lasting effects may persist. There is assumption that during prenatal and initial postnatal stages of fetal development that neurodevelopment and immune system developments have an association to one another. Research suggests that during development of the fetus there is an influx of the TH1 response, which aids in pro-inflammatory response (Hertz-Picciotto et al., 2008). Thus the association of the occurrence of an infection can induce certain immunological changes, such as an inflammatory response (Hertz-Picciotto et al., 2008).

Nevertheless, research has shown that postnatal inflammation that occurs during the primary stages of fetal development can result in irreversible effects in the cerebral and peripheral regions of the Central Nervous System (Hertz-Picciotto et al., 2008). This occurrence of brief inflammation and influx of cytokine release can promote predisposition to seizures in adult life (Hertz-Picciotto et al., 2008). For example, a study to investigate if exposing certain harmful endotoxins during critical developmental stages can induce seizures. This study was done with male Sprague Dawley rats where bacterial endotoxin lipopolysaccharide LPS were injected into the rats at a certain developmental stage at Postnatal day (P) 7 to P14. The end results at six to eight weeks post infection showed that there was, indeed, an increase in predisposition to seizures and also an increase in cytokine release and heightened activity in the hippocampus, such as neuronal degradation (Galic et al., 2009; Hertz-Picciotto et al., 2008). In addition, the results also showed that exposing the LPS only at certain stages induce seizures, and when exposing rats to the LPS before P1 and after P20 there was no significant predisposition to seizure occurrence (Hertz-Picciotto et al., 2008). In conclusion both studies conclude that exposure to harmful chemicals and induced endotoxins such as LPS, to a developing fetus during vital

postnatal development can bring about permanent damaging effects, such as the development precursors of autism and susceptibility to seizures. However, we caution that this rodent model of LPS exposure at early postnatal period can be easily translated into human condition. As wisely noted by the authors "Currently, there is limited clinical evidence to suggest that peripheral childhood infection contributes to the etiology of adult epilepsies" (Hertz-Picciotto et al., 2008). Of note, a genetic overlap between ASD and epilepsy is also apparent in many cases and the observation noted by Galic et al. (Galic et al., 2009) may be factor in the mini-epileptic seizures founds in many ASD children (Geschwind, 2009; Lichtenstein et al., 2010). Taken together, these findings clearly show that autism is not a single clinical entity but a behavioral manifestation of tens or perhaps hundreds of genetic or epigenomic disorders (Kubota et al., 2015; Tammimies et al., 2015). If one explores these exhaustive data and analyses it becomes evident that so many genetic mutations are most likely not the sole cause of ASD, but perhaps secondary effects of mutagenic agents that a developing fetus is exposed to during gestation (Bagasra et al., 2013; Sealey et al., 2015). We have shown that all the 98 fragrances we tested by the Ames test induced mutations in fetal brain cell lines even at 1:50,000 dilutions (Bagasra et al., 2013) suggesting that even a seemingly innocuous exposure to synthetic chemicals may induce significant mutations in a developing fetus. Most recently, have explored the molecular genesis of why ASD is more prevalent in male than female children (Sealey et al., 2015). We have discovered that the fetal brain cell lines derived male are highly susceptible to fragrances and they dramatically lose their oxytocin and arginine-vasopressin positive receptors as oppose to the female fetal neuronal cell lines (Sealey et al., 2015).

4. Exome sequencing (ES) of the ASD parent and the affected child show *de novo* mutation

A recent development in exome sequencing has unveiled several important aspects of ASD. The exome consists of exons, or coding units, of genes, which is made up of around 30 million base pairs, or 1% of the entire genome. Exome sequencing is done by selecting the exons with the use of one of many new array or solution-based methods. Then, the selected cDNA is organized by massive parallel sequencing, and single nucleotide polymorphisms (SNPs) are found by comparison against the reference genome. (Hoischen et al., 2010; Krumm et al., 2015; Mefford et al., 2013; Miller et al., 2009; Ng et al., 2010).

The ES data analyses of trios (the parents and the affected children) have shown *de novo* mutations in the ASD children (again likely due to exposure to the in utero environmental factors) but absent in the parents' exome (Hoischen et al., 2010; Krumm et al., 2015; Mefford et al., 2013; Miller et al., 2009; Ng et al., 2010). By contrast, targeted and genome-wide microarray studies revealed that large *de novo* copy number variants (CNVs) were significantly enriched among probands when compared to unaffected siblings and/or controls (Miller et al., 2009). Both initial and subsequent higher-resolution studies estimate that 8% of sporadic ASD cases carried a *de novo* CNV, as compared with only 2% of unaffected siblings (Krumm et al., 2015; Miller et al., 2009). Furthermore, among children with general developmental delay (DD) and ASD, rare large *de novo* CNVs are thought to account for up to 15% of disease burden (Cooper et al., 2011; Mefford et al., 2013).

4.1. Possible etiologies of autism

Accurate scientific data shows that in the industrialized areas of the world, where fragrance and other environmental synthetic chemical use are most common, there are serious mutagenic effects (Bagasra et al., 2013: Fig. 1). We maintain that these mutations increase the probability of ASD. Causation commonly involves multiple factors including the time period of fetal development when pregnant mothers are exposed to potential environmental insults that may interfere with the developing fetal brain. Generally, the 4–18 weeks of gestation are considered to be the most susceptible period (Landrigan, 2010; Ortega García et al., 2007). Previously, we have shown that all of the 98 fragrances we examined have significant mutagenic effects (Bagasra et al., 2013). It should be noted that various environmental factors can induce not only changes in the DNA directly, but can induce epigenetic modifications which can change the DNA codes indirectly by interfering with gene regulation (Kubota et al., 2015).

5. Older age of mother and increased risk

During the investigation on the birth of children born in California during the 1990s, it has been concluded that the risks for having ASD are much higher for mothers who are older when they give birth (Shelton et al., 2010). Research suggests that it was 51% more likely for mothers older than age 40 to give birth to an autistic child, than mothers between the ages of 25 and 29 (Fig. 2).

Shelton et al. (2010) also found that while a higher maternal age consistently increases the risk for autism in children, paternal age only has a negative impact when the mother is younger than 30 and the father is older. The reason that this is caused in older parents has yet to be solved, but genetic, immunological, environmental, and endocrine explanations are potential suggestions.

We suggest that the repeated, cumulative exposure to chemicals that have teratogenic and/or mutagenic effects over a long period of time will increase the probability of parents to pass on accumulated germ cell mutations to the fetus.

6. Monozygotic (MZ) twins discordance in ASD

Interestingly, one of the most telling aspects of a potential role of environmental factors in ASD may be the evidence of discordant MZ twins at genetic levels (Hallmayer et al., 2011). As one expects MZ twins to be genetically identical, each of them should be equally vulnerable to ASD. Current evidence suggests the phenotype of autism spectrum disorder to be driven by a complex interaction of genetic and environmental factors impacting brain maturation, synaptic function, and cortical networks. On the basis of the idea that MZ twins can exhibit genetic differences, one could hypothesize that *disease discordance* in MZ twins can also derive from acquired genetic differences, or varied epigenetic influences. The discordance for the autosomal dominant disease neurofibromatosis 1 (NF1) in an MZ twins has been explained by the presence of an *NF1* mutation; the affected twin carried the *de novo* *NF1* mutation in all investigated cells, while the unaffected twin was mosaic (Kaplan et al., 2010). Therefore, one way to explain these observations is that the environmental factors induced genetic mutations, which would not be equally distributed due to slight variations in maternal blood delivery to the twins. The unequal blood flow in MZ is well documented (Chalouhi et al., 2010; Chang et al., 2009; Lewi et al., 2008; van Gemert and Sterenborg, 1998). This may result in unequal genetic mutations to each twin (Dolcetti et al., 2013; Dumas and Sikela, 2009; Fraga et al., 2005; Shur, 2009). Since, certain environmental chemicals can interfere in neurodevelopment there will be discordant autism symptoms in some MZ twins (Chalouhi et al., 2010; Chang et al., 2009; Lewi et al., 2008). This phenomenon may partially explain the paradox of discordant MZ twins, but also shed light on our hypothesis that even a small (perhaps a few molecules of a harmful chemical reaching a fetal brain) could deplete specific neurons and induce ASD. We also confirmed the relative resistance of female NBC against certain fragrances with regards to oxytocin receptor positive (OXYR) and (arginine-vasopressin receptor positive (AVPR+) neuron depletion (Sealey et al., 2015).

7. Evidence of environmental causation

ASD is considered to be among the most heritable mental disorders, a notion based on surprisingly sparse data from small clinical studies

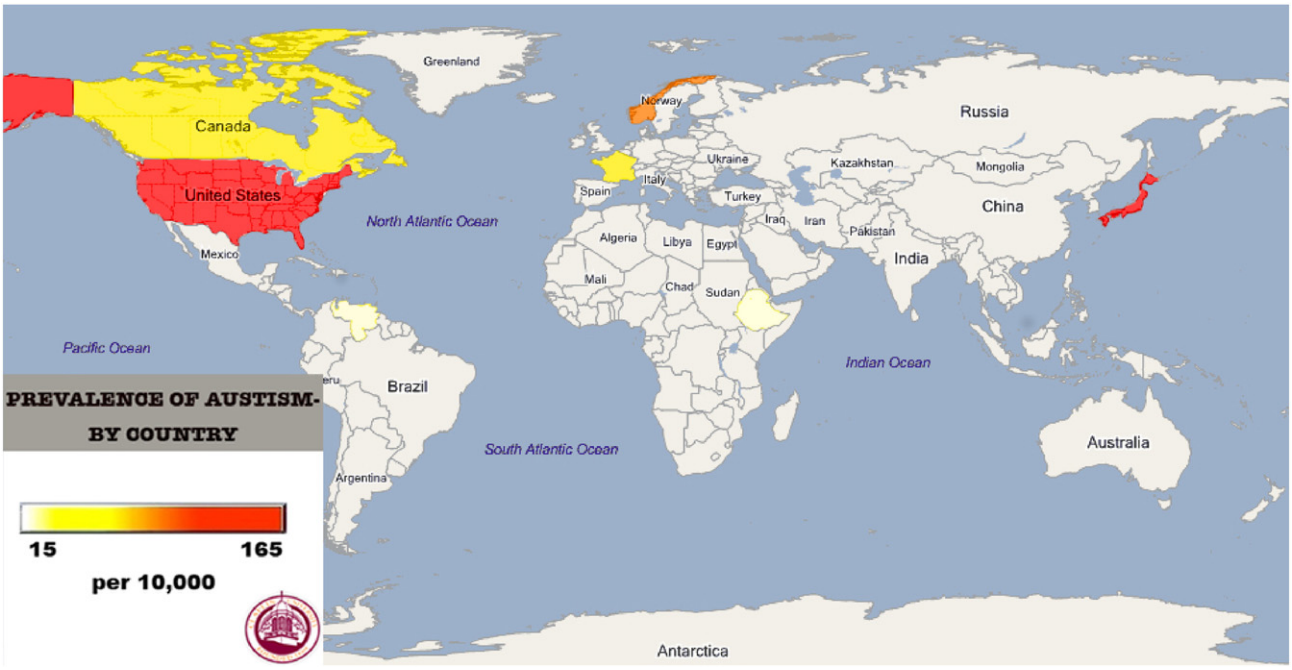

Fig. 1. A global picture of ASD, showing the highest prevalence of ASD in the areas of the globe with largest perfume using population (Japan and the United States) and the lowest in the world where use of perfumes is forbidden by religious decree (Saudi Arabia). Adopted from: http://www.targetmap.com/viewer.aspx?reportId=15821.

(Chamak, 2010). Population-based studies of the heritability of other neuropsychiatric disorders and comorbidities among them have also been sparse. We sought to address both of these issues. The main reason of its heritability notion is an assumed by misinterpreted significant concordance in the MZ twins. Since the genes in MZ are exactly the same, it was assumed that autism is inherited (Hallmayer et al., 2011; Tammimies et al., 2015). Of note, when Leo Kanner first described the syndrome he assumed that it was a genetic illness. However, he noted that there was a common thread found in how each autistic child was raised, allowing him to suspect environmental contributions. Environmental contributions in ASD is well regarded in some cases (i.e. exposure to valproic acid) and thoroughly debunked in other cases (Betthelheim's "refrigerated mother" theory).

In contrast to the single nucleotide mutational disorders like Parkinson's, Huntington disease, Phenylketonuria, Caustic fibrosis, Neurofibromatosis, Muscular dystrophy, Sickle cell anemia, etc., or syndromes that are defined by a single microdeletion or several recurrent microdeletions and duplications (Mefford et al., 2013; Tammimies et al., 2015), ASD has been associated with a wide range of phenotypic features and severity. Deletions of 1q21.1 have been associated with variable degrees of intellectual disability (Geschwind, 2009; Lichtenstein et al., 2010; Mefford et al., 2013). Some patients have one

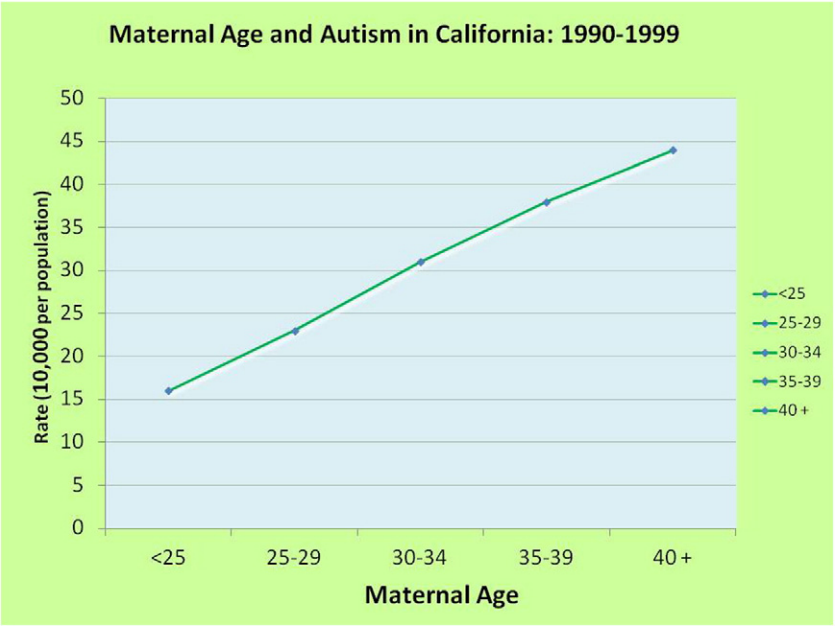

Fig. 2. Advanced maternal age is linked to a significantly elevated risk of having a child with autism, regardless of the father's age, according to an exhaustive study of all births in California during the 1990s by UC Davis Health System researchers. Advanced paternal age is associated with elevated autism risk only when the father is older and the mother is under 30, the study found (Adopted from Shelton et al., 2010).

or more congenital anomalies, including cataracts and congenital heart disease (Castillo-Fernandez et al., 2014; Dolcetti et al., 2013; Dumas and Sikela, 2009; Mefford et al., 2013). The deletion is quite often inherited from one of the patient's parents, who may be only mildly affected or unaffected. Deletions of this region have also been associated with schizophrenia (Dolcetti et al., 2013; Dumas and Sikela, 2009; Fraga et al., 2005; Sealey et al., 2015; Shur, 2009). Duplications in the same region are also associated with mild-to-moderate intellectual disability and autistic features in some patients (Hallmayer et al., 2011; Tammimies et al., 2015). Although dysmorphic features have been reported in many patients, there is no characteristic constellation of features in the majority of patients. A study involving patients with congenital heart disease suggests an increased frequency of the 1q21.1 duplication in this population as well (Dolcetti et al., 2013; Fraga et al., 2005; Happé et al., 2006; Kim and State, 2014; Kubota et al., 2015; Mefford et al., 2013). Another example of a copy-number change with highly variable outcomes is the 16p11.2 deletion. Deletions of 16p11.2 were first identified in patients with autism (Kim and State, 2014) and are present in up to 1% of those with ASD, but it is now clear that such deletions are also associated with intellectual disability without autistic features (Berg and Geschwind, 2012). The 16p11.2 deletion is associated with dysmorphic features, but like the 1q21.1 rearrangement, it is not associated with a recognizable constellation of clinical features (Berg and Geschwind, 2012; Griswold et al., 2015).

8. Gender bias towards males and male hormones during fetal development

There is an inexplicable bias towards males in classical autism by a ratio of ~4:1 (Geschwind, 2009; Lichtenstein et al., 2010; Teatero and Netley, 2013; Zablotsky et al., 2015), and ~10:1 in Asperger's syndrome (Geschwind, 2009; Lichtenstein et al., 2010; Teatero and Netley, 2013; Zablotsky et al., 2015). Besides the common dogma that genetic components are the basis for ASD, there is not a single responsible gene(s) that has been definitively linked to ASD as the causative factor (Berg and Geschwind, 2012; Griswold et al., 2015; Happé et al., 2006; Tammimies et al., 2015). As a matter of fact more than 1000 genes or SNPs have been linked to ASD (Berg and Geschwind, 2012; Griswold et al., 2015; Happé et al., 2006; Tammimies et al., 2015). However, there are several epigenetic factors that have been identified to be possible co-factors in ASD etiology (Dumas and Sikela, 2009; Fraga et al., 2005; Kim and State, 2014; Tammimies et al., 2015). It should be noted that many environmental factors could induce genetic mutations, closing the circle between the gene and the environment (Knickmeyer and Baron-Cohen, 2006; Mefford et al., 2013; Sealey et al., 2015; Tammimies et al., 2015).

It has been suggested that perhaps fetal or perinatal exposure to higher levels of male hormones could increase susceptibility towards autism (Knickmeyer and Baron-Cohen, 2006; Teatero and Netley, 2013). The extreme male brain (EMB) theory of ASD suggests that fetal testosterone (FT) exposure may underlie sex differences in autistic traits. A link between the organizational effects of FT on the brain and ASD is often drawn based on research using digit ratio (2D:4D), a putative biomarker. The extreme EMB theory is the most popular hypothesis put forward to explain the sex difference in ASD (Knickmeyer and Baron-Cohen, 2006). This theory postulates that children with ASD exhibit an enhanced form of the male cognitive profile, and proposes that gestation exposure to testosterone causes biological effects of autism. Some evidence from animal studies suggests that testosterone may mediate cognitive differences between the sexes via organizational effects on the brain. Recent evidence supporting the EMB theory found sex steroid levels in amniocentesis samples to be correlated with the diagnosis of ASDs. The index to ring finger ratio (second-to-fourth digit ratio, 2D:4D) has been widely used as a proxy for fetal testosterone exposure in autism research. An observation supporting a causal association between 2D:4D and fetal testosterone is that 2D:4D has been shown to be sexually dimorphic, with males generally having lower 2D:4D (ie, a relatively shorter index finger (2D) compared with their ring finger (4D) although this is not a unanimously reported finding. This sexual dimorphism is apparent from the first trimester of pregnancy, and appears to be largely static after birth, with most, but not all studies finding that it is unaffected by pubertal androgen. 2D:4D has also been shown to be sexually dimorphic in endocrine models of elevated (congenital adrenal hyperplasia) and reduced fetal testosterone exposure (complete androgen insensitivity syndrome).

Accordingly, there appears to be a strong evidence to link elevated fetal levels of testosterone in amniotic fluid to autistic symptomatology, as well as an increase in rightward asymmetry of the corpus callosum (Baron-Cohen et al., 2015; Jamnadass et al., 2015). One of the most interesting studies is elevated fetal male hormones and development of autism by various groups led by Baron-Cohen (Baron-Cohen et al., 2015). By utilizing the Danish Historic Birth Cohort and Danish Psychiatric Central Register, this team has analyzed 128 amniotic fluid samples of males born between 1993 and 1999 who later received ICD-10 (International Classification of Diseases, 10th Revision) diagnoses of autism, Asperger syndrome or PDD-NOS (pervasive developmental disorder not otherwise specified), then compared them with matched typically developing controls. Concentration levels of four sex steroids hormones (i.e. progesterone, 17α-hydroxy-progesterone, androstenedione and testosterone) and cortisol were measured with liquid chromatography tandem mass spectrometry. The autism group showed elevations across all hormones on this latent generalized steroidogenic factor and this elevation was uniform across the ICD-10 diagnostic label. These results have provided the first direct evidence of elevated fetal steroidogenic activity in autism. Such elevations may be important as epigenetic fetal programming mechanisms and may interact with other important pathophysiological factors in autism (Baron-Cohen et al., 2015). Previous studies that have examined the relationships between amniotic measurements of fetal testosterone levels and autistic traits employed Q-CHAT (Quantitative Checklist for Autism in Toddlers). Sex differences were observed, with boys scoring higher on the Q-CHAT than girls and there was a correlation between fetal testosterone and autistic traits (Baron-Cohen et al., 2015). These studies point towards higher levels of fetal testosterone but fail to explain why there is a significant increasing incidence of autism in the last three decades as compared to several decades ago. More recently, several studies have analyzed the Baron-Cohen hypothesis and found no evidence of digital ratio. Therefore, Guyatt et al. (2015) analyzed 6015 ASD children and controls and found that 2D:4D was not associated with ASDs in males or females.

9. Are there synthetic chemicals that humans are not evolutionarily exposed to?

Over the last 3 decades, a substantial increase in the prevalence of autism has been reported, from 4 to 5 per 100,000 in the 1960s to around one in 45 children today (Zablotsky et al., 2015). We maintain that the alarming 10-fold increase in autism in recent years is due to exposure of the human population to an increasingly diverse set of synthetic chemicals including fragrances, many of which have steroidogenic (male and female hormone-like chemicals) activity (Sarantis et al., 2010). According to published laboratory and epidemiological studies, undisclosed chemicals in fragrances, such as those that lead to different scents, increased shelf life, control time release of fragrances and improve stability, have endocrine-disrupting properties (Abramsson-Zetterberg and Slanina, 2002; Gomez et al., 2005; Inui et al., 2003; Kunz et al., 2006; Liu et al., 2009; Reiner et al., 2007; Roy et al., 2009; Sarantis et al., 2010; Schmutzler et al., 2004; Spencer et al., 1979; Suzuki et al., 2009; Swan, 2008; Furuhashi et al., 1994). These disruptors have been linked to increased risks of cancer (Sarantis et al., 2010), adverse effects on developing fetuses (Inui et al., 2003), and metabolic diseases. For example, chemicals that have been shown to increase human estrogen receptor expressions include

octinoxate, oxybenzone, benzophenone-1, benzophenone-2, benzyl salicylate, benzyl benzoate, butylphenyl methylpropional, and synthetic musks (galaxolide, tonalide, and musk ketone). Of these, oxybenzone, benzophenone-1, galaxolide, and tonalide also affect androgens, while butylated hydroxytoluene, benzophenone-2, and octinoxate have been linked to thyroid hormone disruption (Abramsson-Zetterberg and Slanina, 2002; Furuhashi et al., 1994; Gomez et al., 2005; Inui et al., 2003; Kunz et al., 2006; Roy et al., 2009). Even at very low concentrations, fragrances that contain these chemicals can be mutagenic and carcinogenic (Kubota et al., 2015; Sealey et al., 2015). Of note, one of the fragrance ingredients, Acetyl Ethyl Tetramethyl Tetralin (AETT) was used for 22 years in fragrances, colognes, soaps, detergents and cosmetics (Furuhashi et al., 1994). It resulted in behavioral changes and degeneration of the white matter of the brain including "*widespread demyelination and scattered axonal degeneration in the central peripheral nervous systems*." It was voluntarily discontinued in 1978. Along with this, the fragrance ingredient musk ambrette (a fixative recently banned in the European Union but still allowed here in the U.S.) has been found to also cause degeneration of the myelin sheath and distal axonal degeneration (Furuhashi et al., 1994). Even if one seriously considers high fetal testosterone levels during the early stages of gestation, this data does not explain why the incidence of ASD is steadily going up, except that synthetic chemicals are behaving like hormones and can reach the fetal blood circulation (Baron-Cohen et al., 2015; Betts, 2014; Schmutzler et al., 2004; Suzuki et al., 2009). We believe that many synthetic fragrances contain testosterone-like hormones (Bagasra et al., 2013; Sarantis et al., 2010). Chlordane was a termite preventative chemical used in U.S. homes in the 1960's, 70's and 80's. It was banned for use inside homes in 1979 and underneath homes as a soil treatment in 1983 (Bidleman et al., 2002; Byard et al., 2015). Due to concerns about damage to the environment and harm to human health, the EPA banned all use of chlordane in 1988 (Rogan and Ragan, 1994).

A question that remains is why female neuronal cells are relatively more protected than their male counterparts. Some investigators propose that the male bias for ASD may be partially due to the human female's ability to mask some of the symptoms of ASD or under diagnosis of autism in female (Creswell and Skuse, 1999; Skuse et al., 1997). Even so, these investigators agree that male bias is observed in ASD. A leading hypothesis to explain potential mechanisms is that X chromosome gene dosage could play a role in sex ratios if the non-silenced genes were protective. One of the hypotheses involves "genomic imprinting," the process by which genetic effects are influenced by the father (X_f) or the mother (X_m). Skuse et al. (1997) suggested that an imprinted X-locus could explain sex differences in social and communication skills. His theory was inspired by the finding that in individuals with Turner's syndrome (TS), the rate of social difficulties varied according to whether their single X chromosome was (X_f) or (X_m) (Bidleman et al., 2002; Rogan and Ragan, 1994). Typical females inherit an X chromosome from both parents (X_fX_m), but typical males have only a maternal X (X_mY). Skuse hypothesized that a gene expressed on the maternal X_m acts as a protective factor against the social problems seen in TS and, by extrapolation, as a protective factor against ASD (Liu et al., 2009). However, recent data from Creswell and Skuse (1999) who have reported five cases of ASD that were from an unselected sample of 150 subjects with TS, showed that all cases were X_m. Also, given that 77% of TS females are X_mO, while only 23% are X_f (Rogan and Ragan, 1994), then, by chance one would expect to find ASD more often associated with X_m than with X_f, making the X_m hypothesis unlikely.

Recently, we have shown that one of the factors that ASD is more common in boys than girls may be due to the preferential depletion of OXYR+ and AVPR+ neurons in the developing fetal brains (Sealey et al., 2015) Utilizing neuroblastoma cell lines derived from both genders which were exposed to femtomolar concentrations of most commonly used fragrances, we determine that both types of the neurons are significantly depleted from the male neuroblastoma cells as compared to the females (Sealey et al., 2015). This finding needs further confirmation with primary fetal brain cell lines.

10. The smell of autism

Many modern companies do not disclose the industrial secrets in many of their fragrances that are, in reality, a complex concoction of synthetic chemicals and natural essences, which often have been found to be petrochemicals. Laboratory testing that took place under the direction of the Campaign for Safe Cosmetics, and that later was analyzed by the Environmental Working Group, disclosed 38 hidden chemicals in 17 name-brand fragrance products (Environmental Working Group (EWG), 2005). Topping the list was American Eagle's Seventy Seven, which contained 24 undisclosed chemicals, followed by Chanel Coco with 18, and Britney Spears Curious and Giorgio Armani Acqua Di Gio with 17 secret chemicals each (Fig. 3).

Among those are chemicals, such as musk ketone and diethyl phthalate, which are responsible for allergic reactions and hormone disruption (Sarantis et al., 2010; Roy et al., 2009; Gomez et al., 2005; Inui et al., 2003; Kunz et al., 2006; Furuhashi et al., 1994; Betts, 2014; Environmental Working Group (EWG), 2005). Although these chemicals have been found to accumulate in human tissues, they have not yet been adequately analyzed for safety in products used by unsuspecting humans. As a result of a giant loophole in the Federal Fair Packaging and Labeling Act of 1973, which explicitly exempts fragrance producers from having to disclose cosmetic ingredients on product labels, fragrance concealment is not illegal and is often used by the industry to hide from the public the full list of ingredients, even substances that can cause grave health problems (Environmental Working Group (EWG), 2005). It is a common practice for businesses to list the chemicals as simply "fragrance," which may mean that the majority of the ingredients are never revealed to buyers. Even worse, people who use cologne, fragrances, body spray, and other scented cosmetics are blindly exposed to dangerous chemicals since the Food and Drug Administration lacks authority to control mandates to manufacturers that require testing of all fragrances for safety, before being released to the public. When applied on the skin or sprayed, many chemicals from fragrances are either designed to be absorbed or inhaled, and many of the chemicals damage the body before birth until the end of life. Research by the Environmental Working Group (EWG) found tonalide and galaxolide, two man-made musks, in the cord blood of newborns (Environmental Working Group (EWG), 2005). Although toxic to the endocrine system, musks were discovered in most of the 17 fragrances tested (Sarantis et al., 2010; Environmental Working Group (EWG), 2005). Also, during pregnancy, the use of fragrances and other cosmetics may actually expose the developing fetus to diethyl phthalate (DEP), a common fragrance solvent that can cause abnormal development of reproductive organs in infant males, Attention Deficit Disorder in children, and sperm damage in adults (Reiner et al., 2007; Spencer et al., 1979; Swan, 2008; Schmutzler et al., 2004; Liu et al., 2009; Suzuki et al., 2009; Abramsson-Zetterberg and Slanina, 2002; Roy et al., 2009; Gomez et al., 2005; Inui et al., 2003; Kunz et al., 2006; Furuhashi et al., 1994; Betts, 2014).

11. Environmental factors of autism

Support for the possibility that there is an environmental contribution to causation of autism has come from two major sources: (1) Current understanding of the exquisite vulnerability of the developing human brain to toxic exposures in the environment; and (2) historically important, proof-of-concept studies that specifically link autism to environmental exposures experienced prenatally (Landrigan, 2010). There are numerous excellent review articles that have covered these subjects (Landrigan, 2010), and we will just point out the names of these agents that include: lead; methylmercury; polychlorinated biphenyls (PCBs);

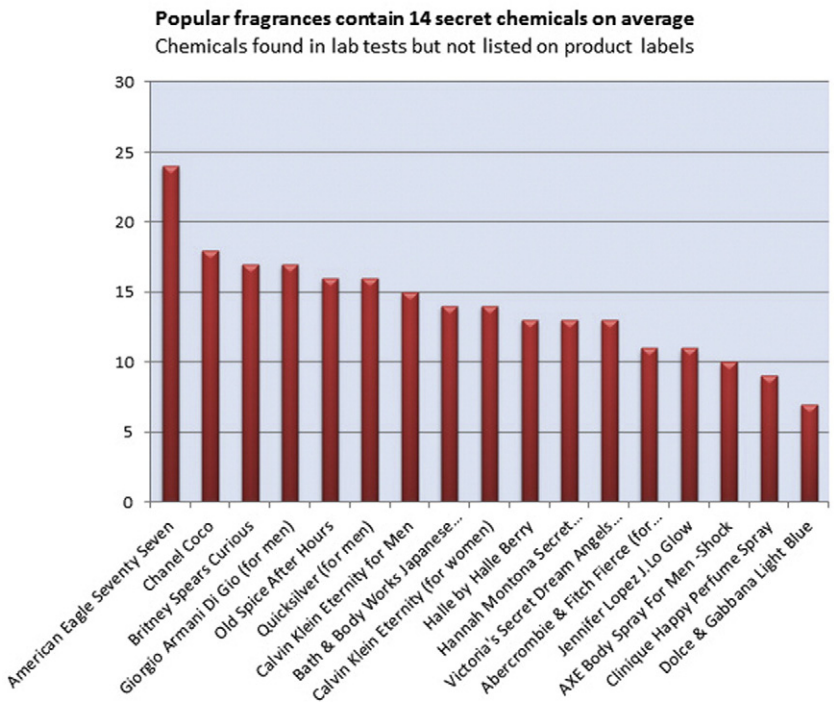

Fig. 3. Most popular fragrances and their number of secret ingredients (adopted from Environmental Working Group (EWG), 2005).

arsenic; manganese; organophosphate insecticides; (DDT); and ethyl alcohol.

11.1. Neurotoxins

This short list of chemicals currently identified as human developmental neurotoxicants may only be the tip of a potentially much larger iceberg (Landrigan, 2010). Children today are at risk of exposure to 3000 synthetic chemicals produced in quantities of more than 1 million pounds per year, termed high-production-volume (HPV) chemicals. High-production volume chemicals are found in a wide array of consumer goods, cosmetics, medications, motor fuels and building materials. Also, they are common in hazardous waste sites and are routinely detected in air, food and drinking water. Measurable quantities of several hundred HPV chemicals are found in the blood and urine of nearly all Americans, as well as in human breast milk and the cord blood of newborn infants (Reiner et al., 2007). Fewer than 20% of HPV chemicals have been tested for potentially causing neurodevelopmental toxicity. Some of these neurotoxicants include thalidomide, valproic acid, misoprostol, chlorpyrifos and various infectious teratogenic agents (most common among them are cytomegalovirus, rubella, toxoplasmosis, herpes simplex virus syphilis and numerous others) (Landrigan, 2010).

12. Vaccines and autism

Childhood immunization is a factor that has received much scrutiny as a potential environmental cause of autism. Claims of a link between vaccines and autism first arose in the late 1990s in the UK, the US and other countries and were triggered by clinical observation of onset of autism in the days immediately following vaccination (Gerber and Offit, 2009; Landrigan, 2010). In the UK, these claims focused on the measles–mumps–rubella (MMR) vaccine (Poland and Jacobson, 2011; Thompson et al., 2007). In the US, they focused on thimerosal, a preservative that contains ethyl mercury, which was added to multidose vials of many vaccines to prevent microbial contamination (Landrigan, 2010; Poland and Jacobson, 2011; Thompson et al., 2007). Thimerosal has been removed from the vaccines since then (Landrigan et al., 2003).

Exposure to mercury during childhood is associated with neurophysiological abnormalities in language, attention and memory. Studies have shown that hippocampal cell death, reductions in neurogenesis, and severe learning deficits are induced from acute exposure to methyl mercury during development (Landrigan et al., 2003). In humans, such exposure levels can come from eating whale blubber, as in people who live in the Faroe Islands, or from living in an environment contaminated by mercury (Landrigan et al., 2003).

Thimerosal is a mercury-containing organic compound (an organomercurial). It was widely used since the 1930s as a preservative in a number of biological and drug products, including many vaccines, to help prevent microbial contamination. Many parents have suspected ethyl mercury contained in thimerosal to be linked with neurodevelopmental outcomes including autism (Landrigan et al., 2003; Slotkin et al., 2007). The FDA determined that children who received multiple vaccines at a young age were at risk for exceeding Environmental Protections Agency's (EPA) safety limits for methyl mercury levels. This has led to debates since 1999 about the association between exposure to thimerosal containing vaccines and developmental abnormalities (Landrigan, 2010; Thompson et al., 2007). Epidemiological studies have shown both significant and non-significant associations on whether childhood vaccines containing thimerosal results in neurodevelopmental disorders (Landrigan et al., 2006; Slotkin et al., 2007).

To address the issue to definitely determine the role of thimerosal in ASD, a series of studies were undertaken in the US, the UK, Europe and Japan. None of these studies have found any credible evidence for a link between vaccines and autism. Key findings are (reviewed in Landrigan, 2010 and Landrigan et al., 2003):

(1) In the UK, there was a steady year-to-year increase in the reported number of cases of autism from the 1980s into the late 1990s. There was no evidence of a change in this trend following introduction of Measles, Mumps, and Rubella (MMR) vaccination in 1988. In a British series of 498 cases of autism, there was no difference in age at diagnosis of autism between vaccinated children and children never vaccinated. There was no temporal association between MMR vaccination and onset of autism.

(2) In California, continuous increase in the rate of diagnosed autism occurred from the 1980s into the 1990s, but did not correlate with immunization patterns. Thus, autism cases increased from 44 per 100,000 live births in 1980 to 208 per 100,000 live births in 1994 (a 373% increase). Whereas, in the same time period MMR coverage increased from only 72 to 82%. In Denmark, a comparison of autism rates in 44,655 immunized children versus 96,648 unimmunized children in the years 1991–1998 found no differences in incidence or prevalence between the two groups. There was no association between age at immunization or season at immunization and rate of autism (Landrigan, 2010).

Recently, there were reports that show exposure to high levels of aluminum and silica in drinking water (Rondeau et al., 2009). Aluminum and silica in drinking water and the risk of Alzheimer's disease or cognitive decline: findings from 15-year follow-up of the PAQUID cohort determine that a clear association was not found (Rondeau et al., 2009). Lujan et al. (Lujan et al., 2013) have described a severe neurodegenerative syndrome in commercial sheep, linked to the repetitive inoculation of aluminum-containing vaccines. In particular, the "sheep syndrome" appears to be similar to some human diseases also linked to the effect of multiple vaccinations (Lujan et al., 2013). Notably, the adverse phase of this syndrome affected 50–70% of flocks and up to 100% of animals within a flock. It was characterized by severe neurobehavioral outcomes (restlessness, generalized weakness, muscle tremors, loss of response to stimuli, ataxia, tetraplegia, stupor, coma and death), inflammatory lesions in the brain and the presence of aluminum in central nervous system tissues (Lujan et al., 2013). However, the study by Lujan et al. describes development of an acute illness not resembling ASD in specific flocks of sheep. It is generally accepted that the genetic background of animals may play a crucial role in manifestation of certain neurological diseases and, in this particular study, an acute illness may reflect this problem. Aluminum in drinking water and in the environment appears to show different outcomes, as shown by Rondeau et al. (2009). Therefore, in the 15 year-long study they showed that aluminum in drinking water was associated with dementia but there was no such association if exposed via environmental (Rondeau et al., 2009).

Shaw et al. (2014a) and Shaw et al. (2014b) claim that neurodevelopmental disorders, such as autism, are mediated by abnormal immune signaling activity during fetal development, which is possibly caused by exposure to xenobiotics (Shaw et al., 2014a). Furthermore, they suggest that pregnant women and infants are universally exposed to xenobiotics, such as mercury and aluminum, through pediatric vaccinations (Shaw et al., 2014b). Aluminum is used as an adjuvant in vaccines and is considered to be both immuno- and neuro-toxic according to previous animal and human studies. In recent experiments, aluminum adjuvant compounds have also been found to have the ability to cross the blood–brain and blood–cerebrospinal fluids barriers (Shaw et al., 2014b). Therefore, these authors speculate that a typical child in a western country may receive up to 4.225 mg of elemental aluminum by 12 months of age, which has been reported to be the dosage that is within the US Agency for Toxic Substances and Disease Registry's minimum risk levels for infants (Shaw et al., 2014b). They explain that the majority of the vaccinations given to children are during the first 2 years after birth, which is when important aspects of brain development, including synaptogenesis, occur (Shaw et al., 2014a). However, since the symptoms of ASD begin very early in a child's life, before they are old enough to receive vaccinations; it is highly speculative that pediatric vaccinations are a causative factor in autism. Furthermore, the majority of the investigators believe that ASD process begins at 8–14 weeks of gestation (Lai et al., 2014), not postnatal, as suggested by these authors (Shaw et al., 2014a; Shaw et al., 2014b). Even though the information unveiled by Shaw et al. is interesting, they still remain speculative regarding the role of aluminum. Children in many parts of world that live in rural areas are exposed to considerable amount of aluminum and numerous other xenobiotics, but do not appear to suffer from ASD at the rate that is observed in the industrial world (Barton, 2008).

Immune dysfunction has also been a topic of concern in the prenatal development of ASDs. During gestation, the fetus may be exposed to xenobiotics such as heavy metals, pesticides, alcohol, and maternal smoking (Landrigan, 2010). These immunotoxicants may affect inflammatory cell responses during the critical windows of fetal brain development. Dietert (2008) and Dietert and Dietert (2008) coined the phrase early-life-induced immune insult (ELII), including developmental immunotoxicity (DIT), to explain the broad category of the effects of environmental exposure of toxins on prenatal-perinatal immune system development. Neurological disorders can result from DIT from toxins affecting prenatal microglial development and inflammatory cell responses. This broad range of disorders includes ASDs, Parkinson's, and Alzheimer's, with the time of exposure during development, the type of environmental insult, and the gender of the offspring all influencing the course of immune system development. Specifically, recent research on autism, which is known as a multisystem disorder (Dietert, 2008; Dietert and Dietert, 2008), shows that immune dysfunction is an attribute common to divergent ASD cases. Furthermore, gender is also important in dysfunction of the immune system. In male and female offspring, Bunn et al. showed that in utero exposure to low levels of lead caused decreased levels of interleukin-10, which is anti-inflammatory in the case of autism (Bunn et al., 2001). As a result, it can be suggested that fetal development in males and females is not the same in response to heavy metals and other xenobiotics, which further explains the extreme male bias of ASDs, as we have shown recently (Sealey et al., 2015). Even though there are many studies on the critical developmental window of exposure to toxins, ELII, and DIT pathways, further research is needed to confirm a link to autism (Ishii and Hashimoto-Torii, 2015; Noriega and Savelkoul, 2014).

In summary, the extensive series of high-quality, peer-reviewed studies have apparently failed to show any clear association between autism and childhood immunization, especially exposure to aluminum containing vaccine adjuvant. However, we believe this area of research should be further explored and the scientific community should remain vigilant in their exploration of vaccine and ASD connection.

12.1. Glyphosate and autism

Recently, glyphosate, an active ingredient in the herbicide, Roundup, has been shown to be an important factor in the development of ASD, as proposed by MIT scholar Dr. Seneff. The use of pesticides and herbicides, most specifically Roundup, has been increasing steadily since the 1990s (Samsel and Seneff, 2013a, Fig. 4). The use of glyphosate has increased 6504% from 1991 to 2010, according to data from the USDA: National Agricultural Statistics Service (Ishii and Hashimoto-Torii, 2015; Noriega and Savelkoul, 2014). Glyphosate is the most widely used herbicide in the world, mainly because it is believed to be nontoxic to humans and is relatively inexpensive (Samsel and Seneff, 2013a). Most importantly, glyphosate is perceived to be harmless because its mechanism of action is to disrupt the shikimate pathway which is absent from human cells (Samsel and Seneff, 2013b). However, human gut bacteria do contain this pathway. The shikimate pathway is involved in the synthesis of the aromatic amino acids in both plants and microbes (Samsel and Seneff, 2013a). These amino acids are the precursor to all the monoamine neurotransmitters, including serotonin, dopamine and melatonin. Seneff claims that this disruption of the shikimate pathway in human gut microbes poses significant implications to our health (Samsel and Seneff, 2013b). Seneff further argues that glyphosate may be a key contributor to the autism epidemic in the United States (Ishii and Hashimoto-Torii, 2015; Noriega and Savelkoul, 2014; Samsel and Seneff, 2013a). Seneff explains that glyphosate kills beneficial forms of bacteria in the gut and causes an overgrowth of pathogenic bacteria,

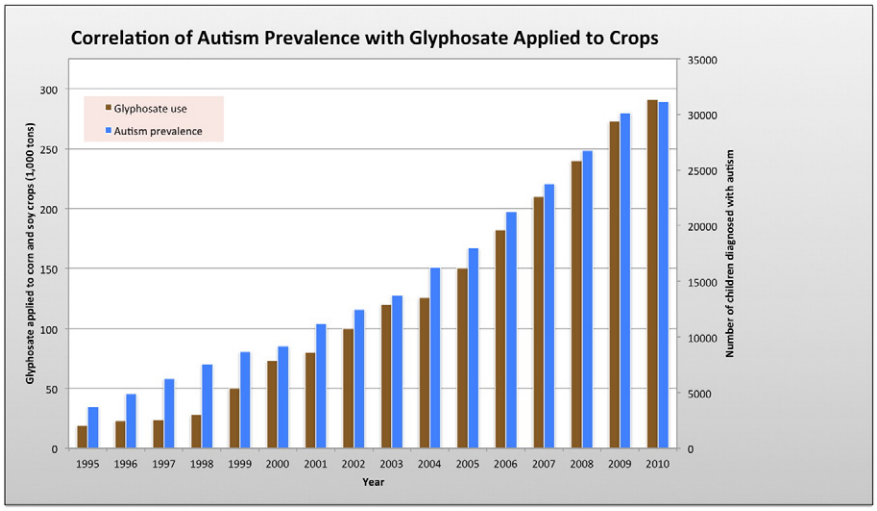

Fig. 4. The bar graph shows the number of children diagnosed with ASD versus amount of glyphosate used on corn and soy crops in the US from 1995 to 2010. Of note, this correlation does to prove causation and the correlation may be simply an unusual event (adopted from Samsel and Seneff, 2013a, 2015).

including *Bacteroides fragilis* and *Clostrdium difficile* (Samsel and Seneff, 2013a; Samsel and Seneff, 2013b; Samsel and Seneff, 2015). *Clostridium difficile* induces leaky gut syndrome and produces *p*-cresol, which is a phenolic compound and a known biomarker for autism (Noriega and Savelkoul, 2014). A higher level of *p*-cresol in the urine is associated with autism (Noriega and Savelkoul, 2014). Glyphosate exposure also disrupts sulfate synthesis, as well as sulfate transport from the gut to the liver and pancreas (Persico and Napolioni, 2013). Serum sulfate deficiency is another known biomarker for autism (Samsel and Seneff, 2013a). Defects in serotonin supply have been associated with various mood disorders. Seneff argues that glyphosate's disruption in the synthesis of serotonin can lead to a defective serotonin transporter gene, which would decrease the bioavailability of serotonin for neuronal signaling (Samsel and Seneff, 2015). This decreased supply of serotonin in the brain is a major feature of autism (Samsel and Seneff, 2013a). However, it should be noted that Seneff's hypothesis does not explain the gender bias in ASD (Noriega and Savelkoul, 2014; Samsel and Seneff, 2013a; Samsel and Seneff, 2013b; Samsel and Seneff, 2015).

13. Link between the maternal antibodies and ASD

Recently Braunschweig and Van de Water (2012) and Braunschweig et al. (2013) analyzed a variety of children with autism, along with their mothers, and uncovered another potential neuromodifying factor in autism. The analyses of maternal antibodies that were present in 10% of the pregnant women can recognize certain antigens in the developing fetal brain cells. An important aspect of the study was the understanding of the role of the blood brain barrier during fetal development. Western blot analysis showed that maternal antibodies (IgG) reacted with fetal proteins (i.e. LDH, YBX1, cypin, STIP1, CRMP1 and CRMP2) and adversely affected fetal neurodevelopment, since the blood–brain barrier is permeable to the maternal IgG throughout the gestational period (Braunschweig and Van de Water, 2012; Braunschweig et al., 2013; Fox et al., 2012). This appears to play an important role in certain cases of ASD. The research suggests that over 50% of the mothers of children with ASD showed reactivity in relation to these proteins (Samsel and Seneff, 2015). This hypothesis was further validated by utilizing the Rhesus monkey model, (Bauman et al., 2013). Therefore, infusion of these antibodies into the pregnant Rhesus monkeys showed that various combinations of passively immunized antibodies can contribute to a higher percentage of ASD and that, when it comes to assessing behavior, combinations of these proteins play a key role in behavioral activity (Braunschweig et al., 2013). Gender differences were also evident. Within various male groups, a significant growth rate difference was observed, as compared to fetal brain development in females. The unanswered question is why certain mothers developed the antibodies to fetal brain antigens in the first place? We believe that this may be the result of leakage of developing fetal brain antigens into the maternal blood, initiating humoral immune responses to these antigens. Since the brain is a privilege site in mammals, the leaked fetal brain antigens would be recognized as foreign by the maternal immune system and subsequently affect the fetal brain development.

In recent years there is a special interest in vaccination with Pandemrix-a pandemic influenza A vaccine- and development of narcolepsy. The concern emerged from reports that an increased numbers of cases of narcolepsy were apparent in non-vaccinated subjects infected with wild A (H1N1) pandemic influenza virus. This suggested a role for the viral antigen(s) in disease development (Ahmed et al., 2015). Ahmed et al. (Ahmed et al., 2015) have found that the peptide in influenza nucleopeptide A (one of the antigenic components of the Pandemrix influenza vaccine) shares protein residues with human hypocretin receptor 2, which has been linked to narcolepsy. With the declaration of a global A (H1N1) influenza pandemic in June 2009, mass vaccination campaigns using newly developed monovalent A (H1N1) pandemic vaccines were initiated in a number of countries using a range of vaccines developed with different technologies (Fox et al., 2012). Recent research by Ahmed et al. (Fox et al., 2012), reported development of narcolepsy, in rare cases of Pandemrix immunized individuals. They reported that with the declaration of a global A (H1N1) influenza pandemic in June 2009, mass vaccination campaigns were carried out using newly developed monovalent A (H1N1) pandemic vaccines in a number of countries using a range of vaccines developed with different technologies. An estimated 31 million doses of European AS03-adjuvanted A (H1N1) pandemic vaccine were used in more than 47 countries. The Canadian AS03-adjuvanted A (H1N1) pandemic vaccine was used with high coverage where an estimated 12 million doses were administered. As no similar narcolepsy association has been reported to date with the AS03-adjuvanted A (H1N1) pandemic vaccine made using the Canadian inactivation/purification protocol, this suggests that the AS03 adjuvant alone may not be responsible for the narcolepsy association. To date, no narcolepsy association has been reported with the MF59®-adjuvanted A (H1N1) pandemic vaccine. A comprehensive by Persson et al. (Persson et al., 2014) who analyzed 3347, 467 individuals vaccinated with Pandemrix and compared them to 2,497,547 nonvaccinated individuals for any neurological and immunologic diseases, including narcolepsy and concluded for a large number of selected neurological and immune-related diseases, there was neither confirmation or any causal association with Pandemrix

nor refute entirely a small excess risk. They confirmed an increased risk for a diagnosis of narcolepsy in individuals ≤ 20 years of age and observed a trend towards an increased risk also among young adults between 21 and 30 years (Persson et al., 2014). Evidently, there may be minuscule increase in narcolepsy in Pandemrix immunized cases but no evidence of ASD (Persson et al., 2014).

Another study conducted by Montana et al. (2010), involves the use of influenza vaccine that contains squalene and thimerosal. Although these are rarely used nowadays, their research focuses on these two substances being a link to brain tissues in children. The researchers reported that vaccines with these two substances in them did not show clear discernment, which can be related to the cause of any neuropsychological matters. Additionally, autism cannot be accounted for in this study, being that was their area of interest.

Furthermore, the connection between narcolepsy and the Pandemrix vaccine may be a factor in other cases where cross reactivity occurs. However, this idea in terms of autism is highly speculative. Looking back on the percentage of Americans vaccinated for influenza, focusing on child bearing women, if this does contribute to autism, then the rate of occurrence would be significantly higher. Furthermore, a blanket association with influenza vaccine and development of ASD may not be scientifically valid, since each year a new vaccine is produced directed against the predicted immunodominant influenza strain. Therefore, it is unlikely that all different strains of anti-influenza with variations in antigenic epitopes would have adverse effects on the fetal brain development. In addition, it appears that from the global H1N1 flu vaccination we have learned that certain adjuvant may be better in prevention of potential narcolepsy (Ahmed et al., 2015).

We hypothesize that the leakage of the fetal antigens into the maternal blood may be the result of certain environmental factors that are cytopathic to specific progenitor cells in the developing fetal brains, resulting in development of maternal autoantibodies. This hypothesis can be examined by injecting fetal brain antigens to the pregnant Rhesus monkeys at early gestation. Our hypothesis may explain why a small percentage of mothers give birth to multiple ASD children (Bauman et al., 2013; Braunschweig and Van de Water, 2012; Braunschweig et al., 2013; Fox et al., 2012). However, we do not discount the possibility that other antigens, after an infection with microorganisms that cross react with a fetal brain antigens may not play a role in development of ASD (Ahmed et al., 2015; Bauman et al., 2013).

14. Conclusion

The role of environmental factors like fragrances, glyphosate and other synthetic chemicals derived from petrochemicals containing carcinogenic, mutagenic, hormones disturbing and neuromodifying capabilities in the molecular and cellular pathogeneses of ASD has not been evaluated. This is partly due to the 1973 FDA decision to exempt fragrances and cosmetics from appropriate testing, which is generally required for any consumer item that enters the human body and is metabolized by human metabolic pathways. Furthermore, herbicides that appears to be harmless to humans and other mammals may have severe consequences for the developing fetus or any part of the adult brain cells that are regularly going through neurogenesis in an adult human brain (i.e. piriform cortex, hippocampus, amygdala and subventricular zone). The potential neurotoxic effects of these chemicals are warranted.

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