Contributions of the environment and environmentally vulnerable physiology to autism spectrum disorders

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Purpose of review

This review presents a rationale and evidence for contributions of environmental influences and environmentally vulnerable physiology to autism spectrum disorders (ASDs).

Recent findings

Recent studies suggest a substantial increase in ASD prevalence above earlier Centers for Disease Control figures of one in 150, only partly explicable by data artifacts, underscoring the possibility of environmental contributors to increased prevalence. Some gene variants in ASD confer altered vulnerability to environmental stressors and exposures. De-novo mutations and advanced parental age as a risk factor for ASD also suggest a role for environment. Systemic and central nervous system pathophysiology, including oxidative stress, neuroinflammation, and mitochondrial dysfunction can be consistent with a role for environmental influence (e.g. from air pollution, organophosphates, heavy metals) in ASD, and some of the underlying biochemical disturbances (such as abnormalities in glutathione, a critical antioxidant and detoxifier) can be reversed by targeted nutritional interventions. Dietary factors and food contaminants may contribute risk. Improvement and loss of diagnosis in some with ASD suggest brain circuitry amenable to environmental modulation.

Summary

Prevalence, genetic, exposure, and pathophysiological evidence all suggest a role for environmental factors in the inception and lifelong modulation of ASD. This supports the need for seeking targets for early and ongoing medical prevention and treatment of ASD.

Keywords

autism, dynamic encephalopathy, environment, glutathione, oxidative stress, pathophysiology

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Introduction

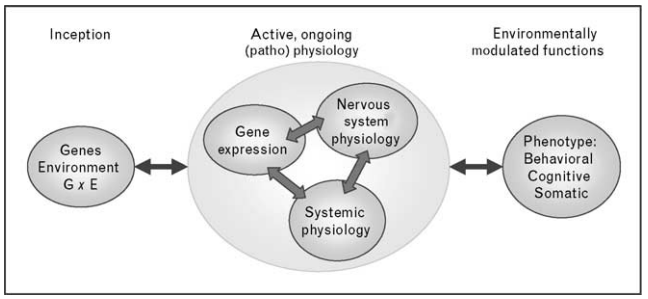
The present review will consider recent documentation of increasing prevalence rates in autism spectrum disorders (ASDs) and what may be contributing to these prevalence rates. It will explore different interpretations of the significance of these reports, gene–environment interactions, and vulnerabilities in physiology in ASD that may be targets of various environmental factors and thereby may be contributing to those portions of the increases in reported prevalence that are not due to other factors, such as broadening of diagnostic criteria and greater awareness of ASD.

Overview: environment and anomalies

Once thought to be rare, ASDs are now reportedly on the rise and are the subject of daily media attention. In addition, there is less consensus than in the past about neural systems being the primary loci of dysfunction in ASD and a growing sense of whole body systems involvement in autism wherein the brain may be impacted in parallel with other systems (Fig. 1) [1,2]. There is beginning to emerge a train of inquiry looking more seriously at potential environmental causes of and mechanisms for rising numbers and systemic features in autism [3,4]. Only in the past few years have funding opportunities started to become available to support these types of inquiries, and it is at least in part for this reason that the relatively small share of the literature devoted to these questions includes studies of varying quality and often with small sample sizes.

Even so, a vantage point with some consistent themes and perspectives is beginning to emerge. We are seeing growing attention to indications of environmental contribution beyond early notice of autism incidence in association with in-utero valproic acid [5] or congenital rubella [6]. Now on the radar screen, we find other evidence for a role of environmental factors in the rising

Figure 1 The figure schematizes the inputs from genes, environment, and gene–environment interactions as they impact the organism's gene expression and physiological activities



These activities in turn shape the various levels of phenotype, including behavior, cognitive functioning, and somatic/medical domains.

numbers, such as incomplete monozygotic concordance, differences related to geography, occupation and time of birth; gene–environment interactions; environmental toxins; investigation of genetic and physiological features of the ASD phenotype that may be unusually vulnerable to environmental exposures and stressors; and also unanticipated evidence of plasticity and improvement in response to environmental modulation. Proceedings from a 2007 Institute of Medicine workshop on Autism and the Environment [7•] and a workshop summary [8•] were published. Along with an increased number of articles, there have appeared two books [9••,10••] and one dedicated journal issue [11••,12] focusing centrally on metabolic, immune, and environmental issues in ASD. This review will present a view of this emerging perspective with an emphasis on the linkages being investigated between environment and vulnerable physiology.

Autism prevalence

In the past year, a number of reports suggest that the prevalence of ASD is greater than the one in 150 that was reported by the Centers for Disease Control (CDC) in 2007, using 2000 and 2002 data [13,14]. Baron-Cohen *et al.* [15] generated prevalence estimates in the UK of 94 per 10 000 using the Special Educational Needs register and 99 per 10 000 using a diagnosis survey of children aged 5–9 years in participating schools; when adjusted for a ratio of known:unknown cases of about 3 : 2, their final prevalence estimate was 157 per 10 000, or one in 64. A report from the US Department of Health and Human Services utilizing telephone interview data from the 2007 National Survey of Children's Health found a weighted point prevalence of 110 per 10 000 in the United States, though this study's telephone interview was limited and could have skewed the estimate [16].

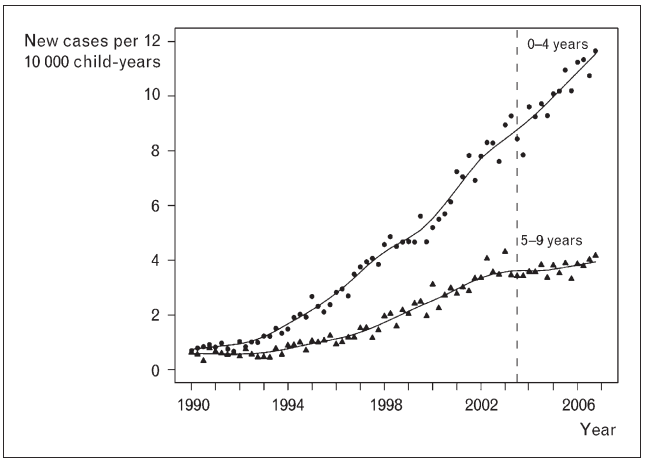
Concerns have been raised about how much of the increases in prevalence represents an actual growth in numbers. Diagnostic substitution – labeling people autistic who previously would have been diagnosed with something else – is one consideration [17–19]. Shortcomings of various data sources, such as administrative data or clinician diagnosis, are also an issue [20–22].

To quantify the extent to which reported increases may be explained by factors other than a true increase in incidence, Hertz-Picciotto and Delwiche [23•] investigated cases in the California Department of Developmental Services databases from 1990 to 2006, during which a 600% increase in incidence rate was observed (Fig. 2). Out of this 600% increase, 24% could be explained by earlier diagnosis, an increase of possibly 56% could have been due to inclusion of milder cases, and based on data from a Finnish study, 120% was considered attributable to changes in the diagnostic criteria; the other 2/3 could not be accounted for by such factors. This analysis minimized the influence of the limitations of administrative data by examining files of individual cases and linking to state birth records, by supplementing with clinical confirmation of diagnoses, and by considering 'age at diagnosis' as a metric that may lag variably behind actual age of onset [24]. The authors acknowledge considerable remaining uncertainty, but nevertheless argue that given the gap between the observed increase and the proportion explained by major factors such as diagnostic substitution, the possibility of a true increase in incidence deserves serious consideration. Two further studies have also shown that diagnostic substitution can only partially account for increased prevalence numbers [25,26].

Genes and environment

Gene–environment interactions could contribute to prevalence increases. In many cases, genes and environment

Figure 2 Annual incidence rates of autism based on the administrative database of the California Department of Developmental Services, 1990–2006



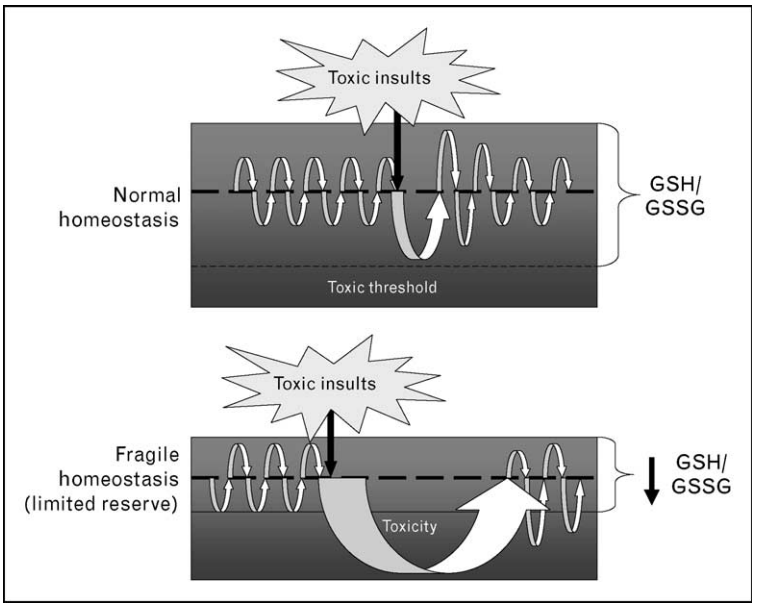
Reproduced with permission from [23•].

could both be necessary but neither alone sufficient to cause autism. Pessah and Lein [27•] review how low-level chemical exposure can influence some of the same molecular, cellular, and behavioral outcomes that are also influenced by genetics. They focus particularly on environmental agents that interfere with three neurotransmitters and pathways [gamma-aminobutyric acid (GABA), acetylcholine, and calcium signaling pathways and calcium-dependent effectors] already at risk in some individuals with ASD for genetic reasons. The existence of inborn genetic vulnerabilities in such pathways may lower the threshold at which the influence of environmental factors may be felt, leading to an impact of environment that differs across the population based on genetic substrate [28•].

Vulnerabilities are being identified in ASD in a growing number of environmentally responsive or sensitive genes and pathways. The Environmental Genome Project of the National Institute of Environmental Health Sciences (NIEHS) has been investigating such genes and haplotypes [29,30], and informatics resources such as the Comparative Toxicogenomics Database [31] are becoming increasingly detailed and valuable. Aberrant metabolism in environmentally sensitive pathways in individuals with ASD who have no known neurometabolic disease is of growing interest, particularly abnormalities in redox and methylation, given the known impact of toxins on these processes [32•]. Glutathione may be particularly important because, as a critical antioxidant as well as an important endogenous detoxifier, it plays a central role in how the organism handles many types of exposures and stressors (Fig. 3) [33••]. Significant transmission disequilibrium in transmission of three alleles of a human glutathione peroxidase (GPX1) repeat was noted in 103 trios (probands and parents) of autism disorder with undertransmission of ALA6, suggesting a possible protective effect of this allele [34]. An increased frequency of the ALAD2 variant of delta aminolevulinic acid dehydratase in ASD, conferring vulnerability to lead exposure along with a decreased frequency of CPOX (coproporphyrin oxidase) variants associated with vulnerability to lead suggests that ALAD2, particularly in combination with lower glutathione levels, may contribute to lead toxicity as an autism risk factor [35]. Paraoxonase 1 (PON1), which is associated with organophosphate hydrolysis and which has low activity in childhood leading to increased vulnerability in young children [36], was found to be associated with autism in a White-American cohort from the United States where organophosphates have been in more recent use but not in an Italian cohort with less organophosphate exposure [37]. The bioavailability and the catalytic activity of PON1 were significantly impaired in a cohort of 50 children with ASD, despite no association with polymorphisms in the *PON1* gene and a normal distribution of the PON1 phenotype [38], suggesting possible environmental targeting of these functions.

The observation of more frequent de-novo copy number variants in sporadic autism than in cases with affected

Figure 3 Greater cytoxicity from exposures can occur in the setting of impaired glutathione-dependent redox reserves



With a low reserve, there is a fragile homeostasis that shows more vulnerability and lower resilience. Glutathione depletion will increase sensitivity to environmental toxins. With robust glutathione reserves, toxic insults are buffered and may never reach toxic threshold. Depleted glutathione reserve leads to a fragile homeostasis in which a similar toxic insult will lead to toxicity and disorder. Many individuals with autism spectrum disorder (ASD) have reduced glutathione reserve that may render them more sensitive to pro-oxidant environmental exposures. Adapted with permission from [33••].

first-degree relatives or in controls [39], as well as the presence of hundreds of distinct variants seen only once [40], suggest a potential role for environment. The possibility of de-novo mutations is also raised by the documentation of advanced parental age as a risk factor for autism [41], which could explain some acquired germline mutations. Possible environmental causes were discussed in a literature survey that identified nine pre-conception environmental exposures associated with increased risk for autism and noted that five of these factors (mercury, cadmium, nickel, trichloroethylene, and vinyl chloride) are 'established mutagens' [42]. The impact of such xenobiotics might be amplified by vitamin D deficiency [43] due to the importance of this substance in DNA repair mechanisms [42].

Environment and pathophysiology

Environmental exposures and stressors act through their impact on the organism and they can be studied using markers of exposure but also of susceptibility and effect. Effects may impact brain development, development of other organs and systems, and ongoing physiological processes. A number of clinical and research findings have been encouraging this direction of work. Prominent among these are disturbances in immune function and increases in immune vulnerability that are reviewed elsewhere in this issue. One route of immune disturbance is early life insults from the environment that include xenobiotic-induced developmental immunotoxicity [44,45], as well as prenatal infection [46], prenatal stress [47,48], and immune disruption of the gut–blood–brain barrier [49]. Although we do not have measures at this point that could be considered both sensitive and specific to ASD, it does appear that many of these findings may have clinical significance.

A growing body of literature has documented that oxidative stress, which is well known to be a potential consequence of environmental insult as well as of genetic influences, is increased in ASD [33••,50]. Abnormal sulfur amino acid metabolism consistent with oxidative stress was documented in leukocytes [51]. In one study, abnormalities in these metabolites were identified in children with autistic disorder and pervasive developmental disorder (PDD) but not in Asperger's syndrome [52]. Increased oxidation of cell membrane phosphatidylethanolamine in autism was shown to be mediated by copper and ceruloplasmin, which may thereby be contributory to oxidative stress, reduced phosphatidylethanolamine levels, and abnormal membrane function [53]. A reduction in the ratio of reduced glutathione to oxidized glutathione (GSH/GSSG) indicative of oxidative stress was measured in both cytosol and mitochodria of lymphoblast cell lines from individuals with autism [54]. Parents of children with autism were shown to share similar metabolic deficits in methylation capacity and glutathione-dependent antioxidant and detoxification capacity to those observed in many autistic children [55]. Acetominophen, an over the counter drug in very common pediatric use, especially since the early 1980s when it replaced aspirin in pediatric practice, acutely impairs glutathione metabolism, which suggests it could be a potential risk factor in autism [56]; it was shown (though with disagreement in a letter to the editor) to be associated with autistic disorder in young children with regression in development [57,58]. Treatment in an open-label trial of abnormal glutathione status with methylcobalamine and folinic acid in children with ASD showed efficacy in moving glutathione status less remote from normal, and improvement in Vineland Adaptive Behavior score was used to support a double-blind placebo-controlled trial now underway [54]. However, although these studies suggest metabolic vulnerabilities pertinent to environmental insults, for the most part they have not included direct measures of potential environmental triggers of these pathophysiological alterations; such linkages would likely require larger scale funding and more systematic support of collaboration.

Metabolic alterations consistent with vulnerability to heavy metal and xenobiotic toxicity have also been observed. A reduced zinc–copper ratio was measured in a substantial cohort of children with ASD; such a reduction may indicate inadequate metallothionein function, leading to impaired metal binding and increased vulnerability to metal toxicity [59]. Elevated porphyrins, which have been regarded as markers of xenobiotic and metal (lead, mercury) exposure, have been measured in several cohorts of children with ASD [60,61]. A number of studies have emphasized the lack of connection of vaccines or mercury in vaccines with the increased prevalence of autism [62–67]. One study showed a lack of difference in blood levels of mercury between ASD and control groups, though the authors pointed out that their measure only reflected recent exposures and their findings were not sensitive to more chronic exposures and not pertinent to questions of causation [68]. Others argue that there are legitimate concerns about the impact of even low levels of chronic mercury exposure [69] and that much divisiveness, public suspicion of the health establishment, and possibly harm could have been avoided by an early decision to reduce exposure to thimerosal in vaccinations based on prior removal of this substance from topical medications, as well as development of a more coherent environmentally responsive research agenda for autism [70].

There are also findings possibly consistent with oxidative stress and immune activation in the central nervous system. Pertinent neuropathological findings, although preliminary and in small samples, include elevated

cerebellar 3-nitrotyrosine [71], reduced neuronal density with increased glial density and lipofuscin in language-related cortex [72], and immunocytochemical detection of three markers of oxidative injury and lipid peroxidation in ASD brain tissue [73]. An earlier landmark paper documenting innate immune activation and abnormal cytokines in post-mortem brain tissue [74] received some support by independent documentation of increased innate and adaptive immune activation in ASD brain tissue [75]; such neuroimmune changes can have a relationship with environmental toxins [76] from exposures such as air pollution [77–79]. The great heterogeneity in ASD may relate at least in part to the many different types of such contributors that could contribute etiologically to autism's defining behavioral characteristics.

Various brain imaging findings can also be interpreted as consistent with central nervous system tissue disturbances such as immune activation or oxidative stress. Although the highly replicated phenomenon of early rapid brain enlargement in a substantial subset of individuals with autism [80,81] has led to the inference that this size increase would be accounted for by a greater number of neurons and myelinated axons in ASD brains, imaging findings are beginning to suggest the opposite. The strong predominance of findings in magnetic resonance spectroscopy is of reduced density of metabolites [82•]. This, along with the reduced fractional anisotropy and increased diffusivity in diffusion imaging of white matter [83,84], suggests a reduction rather than an increase in neuronal and white matter integrity, cell number or density; tissue changes that could lead to such signal could derive from oxidative stress, neuroinflammation, or edema. Such potentially environmentally mediated tissue pathophysiology might also contribute to reduced cerebral perfusion, with recent single photon emission computed tomography (SPECT) studies [85,86] supporting prior documentation of hypoperfusion in a dozen and a half earlier papers.

Dietary factors are also under consideration as environmental contributors to ASD. A several-fold reduction in the proportion of ω-3 fatty acids in lipid intake over the past few generations, and potential exacerbation of the impact of this deficiency by gastrointestinal disturbances in ASD [87], may contribute to abnormal fatty acid profiles in ASD [88] that could affect neuronal processing [89], though rigorous evidence for the efficacy of essential fatty acid supplementation in ASD is still weak [90]. Nutritional insufficiencies that may reduce the availability of substrates for neuronal metabolism and increase vulnerability to oxidative stress [91] may result from self-restriction of intake common in ASD [92], and this may be further complicated by ingestion of toxicants and heavy metals as food contaminants [91]. Earlier work documented abnormal clostridial colonization in regressive ASD as well as transient behavioral improvement with eradication of these organisms [93,94]. Based on this work and concerns more broadly about rises in clostridial infection [95], as well as the impact of gut microbiota on human health and environment on the microbiota [96], rodents were injected with propionate (a major byproduct of clostridia as well as a common food preservative) and manifested autistic-like manifestations at multiple levels, including social isolation, reduced play behavior, oxidative stress, and a brain neuroinflammatory response [97,98]; this model system may be applicable to many other environmental exposures.

The dynamic nature of autism symptoms and their severity, including both regression in early childhood and improvement or even loss of diagnosis, suggest a potential role for environmental factors in modulating the pathophysiology underlying autism well after the period of in-utero and early postnatal development [1]. A surprising report of improvements in core features of ASD in the setting of fever [99] prompted much reflection on underlying neurobiological mechanisms such as cytokine alteration or lipid membrane fluidity changes that might permit such dynamism in what had previously been considered a static encephalopathy [100,101•]. One provocative paper suggested that this phenomenon may derive from developmental dysregulation of the locus ceruleus-noradrenergic system, that is widely distributed and can cause rapid state alterations, and that may be transiently restored to normal regulation during fever [102]. The phenomenon of loss of diagnosis and 'recovery' has also been discussed, not only in widespread anecdotal reports but now also in academic literature, with a review of the implications of 'recovery' [103•] and case series documenting loss of rigorously documented diagnosis [104]. One study reported that recovery occurs in 19% of cases of early autism diagnosis, which may reflect some combination of true improvement, maturation, and overdiagnosis [105].

Conclusion

A line of inquiry is developing aimed at making sense of the increasing prevalence of ASD, the failure to fully account for this increase by factors such as diagnostic substitution, a growing body of evidence demonstrating the role of gene–environment interactions in ASD, environmentally vulnerable physiology in ASD, and a wide range of contributory environmental factors. All of these support the need to increase our attention to environment and vulnerable physiology in ASD. Environmental influences on physiology might begin *in utero* and might thereby contribute to alterations in brain and other body systems development and involve epigenetic changes. But given the documentation of potentially environmentally modulated active pathophysiology later in childhood

and into adulthood, it is reasonable to devote more attention to environmental influences that could modulate encephalopathy, that is, physiological brain function, in ASD in an ongoing and active fashion [101•,106], starting preclinically in infancy before it is possible to identify clear autism symptoms, and continuing further throughout the lifespan. Through environmental modification, including medical intervention, it may be possible to avoid or limit the triggering or aggravation of vulnerabilities and thereby to reduce both prevalence and suffering in this complex and challenging syndrome.

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