

What causes autism? Exploring the environmental contribution

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Purpose of review

Autism is a biologically based disorder of brain development. Genetic factors – mutations, deletions, and copy number variants – are clearly implicated in causation of autism. However, they account for only a small fraction of cases, and do not easily explain key clinical and epidemiological features. This suggests that early environmental exposures also contribute. This review explores this hypothesis.

Recent findings

Indirect evidence for an environmental contribution to autism comes from studies demonstrating the sensitivity of the developing brain to external exposures such as lead, ethyl alcohol and methyl mercury. But the most powerful proof-of-concept evidence derives from studies specifically linking autism to exposures in early pregnancy – thalidomide, misoprostol, and valproic acid; maternal rubella infection; and the organophosphate insecticide, chlorpyrifos. There is no credible evidence that vaccines cause autism.

Summary

Expanded research is needed into environmental causation of autism. Children today are surrounded by thousands of synthetic chemicals. Two hundred of them are neurotoxic in adult humans, and 1000 more in laboratory models. Yet fewer than 20% of high-volume chemicals have been tested for neurodevelopmental toxicity. I propose a targeted discovery strategy focused on suspect chemicals, which combines expanded toxicological screening, neurobiological research and prospective epidemiological studies.

Keywords

autism, developmental neurotoxicity, national children's study, toxicity testing, vaccines

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Introduction

Autism is a complex, serious, biologically based disorder of brain development first described in 1943 by Kanner [1]. Social deficits, abnormalities in communication, repetitive behaviors, and cognitive inflexibility are the characteristic features [2]. There is no specific biochemical indicator or distinct neuroanatomical abnormality that defines autism, and the diagnosis is based on clinical and behavioral assessment.

Cases of autism vary from mild to profound and in the relative prominence of particular features and comorbidities. Approximately 50% of autistic children have intellectual disability, some have abnormally increased brain size, one-third have had at least two epileptic seizures by late adolescence, and about half have severely impaired speech [3]. Yet some children with autism, notably those with Asperger's syndrome, have highly developed intellectual skills, sometimes in specific areas such as mathematics. Because of this heterogeneity, the term 'autism spectrum disorder' (ASD) has come into use. ASD

encompasses autistic disorder (DSM 299.00), Asperger's syndrome (DSM 299.80) and pervasive developmental disorder – not otherwise specified (PDD-NOS) (DSM 299.80).

The causation of autism is the subject of intense inquiry [4–7,8*]. Genetic factors are clearly important. Gene mutations, gene deletions, copy number variants (CNVs) and other genetic anomalies are all persuasively linked to autism [9]. But none accounts for more than a relatively small fraction of cases. The hypothesis therefore arises that early environmental exposures may also contribute to causation, perhaps acting in concert with genetic susceptibilities. It may further be hypothesized that variation in the interplay between different environmental exposures and inherited vulnerabilities may account for the observed heterogeneity in the autism phenotype.

The article explores the possible contribution of early environmental exposures to causation of autism, with particular focus on the possible role of toxic chemicals.

It proposes a strategy for discovery of currently unrecognized and potentially preventable causes of autism.

Epidemiology of autism

The prevalence of autism currently reported in the US is 6–7 cases per 1000 children [10]. This reported prevalence is substantially higher than that of a decade earlier. Similar increases have been noted in the UK, Europe and Japan [11,12]. The CDC survey that established the current US rate found no significant difference between Caucasian and African–American children. It confirmed previous reports that ASD is 3–5 times more common in boys [13].

The reported increase in prevalence of autism has triggered vigorous debate as to whether the trend reflects a true increase in incidence, or is merely a consequence of expansion in the definition of ASD and greater awareness, improved diagnosis and better reporting [11]. This highly controversial question is not yet settled [14]. A recent critical analysis concludes that increases in recognition, changed diagnostic criteria, and changing public attitudes about autism have played a major role in catalyzing the upward trend in reported prevalence. This analysis observes, however, that the possibility of a true rise in incidence cannot be excluded [12].

Genetic factors in autism

Genetic and familial factors are unquestionably involved in causation of autism [4]. Families with multiple cases have been described. Autism has repeatedly been seen in sibs and twin pairs. Concordance in monozygotic twins is reported to be as high as 70% [15], and, when the broader phenotype of autism is considered, concordance in monozygotic twins approaches 90%. Concordance rates for autism in dizygotic twins appear no higher than among singleton siblings. Families with autistic children may contain members with ‘autistic traits’ such as social isolation or tendency toward repetitive behavior [13]. Autism occurs in a number of genetic conditions, among them Fragile X syndrome, Down syndrome, Cohen syndrome, Angelman syndrome [16] and Rett syndrome [17].

Ongoing research into the genetics of autism has employed the following three main strategies [18]:

- (1) Family-based and case–control evaluations of candidate genes [19,20]. These studies have identified numerous candidate loci, most consistently on chromosomes 7q, 15q and 2q [19,20]. They have also identified specific mutations associated with ASD, notably in *SHANK3*, a gene that encodes a synaptic scaffolding protein; in *NLGN 3/4*, also involved in synapse formation; and in *PTEN* [16].

- (2) Cytogenetic studies. Cytogenetic studies have identified abnormalities on chromosome 15q [19].
- (3) Genome-wide association screens [9,21]. These studies – the most recent generation of genetic investigations into the causation of autism – have identified large-scale genetic duplications, deletions and CNVs associated with ASD. These include CNVs in *CNTN4*, a gene involved in development of neuronal networks; in *NRXN1*, involved in synaptogenesis [21]; and a recurrent microdeletion on chromosome 16p [22,23*]. Each of these microdeletions accounts for approximately 1% of cases of ASD.

At the present time, genetic factors are thought to account for 7–8% of autism cases, but this fraction will likely increase as genetic research advances and additional genetic causes are discovered.

Despite rapid advances in understanding the genetic contribution to autism, a purely genetic explanation for causation has difficulty in explaining certain clinical and epidemiological aspects of autism, among them the occurrence of sporadic cases, wide heterogeneity in clinical presentation, discordant development in monozygotic twins, and occurrence within families of members with fully developed autism side by side with others who manifest only ‘autistic traits’ [7,20]. This situation therefore raises the possibility that environmental exposures could also play a role in causation of autism [7,20]. These factors could act in concert with inherited susceptibilities or through inducing epigenetic changes [24*].

Plausibility for an environmental contribution to causation of autism

Support for the possibility that there is an environmental contribution to causation of autism comes from the following two sources:

- (1) Current understanding of the exquisite vulnerability of the developing human brain to toxic exposures in the environment [25]; and
- (2) Historically important, proof-of-concept studies that specifically link autism to environmental exposures experienced prenatally.

Vulnerability of the developing human brain to toxic exposures

Long and tragic experience that began with studies of lead [26] and methylmercury [27] has documented that toxic chemicals can damage the developing human brain to produce a spectrum of neurodevelopmental disorders ranging from overt toxicity at high levels of exposure down to subclinical dysfunction [28–31].

The developing human brain is understood today to be exquisitely susceptible to injury caused by toxic chemicals in the environment [32]. This vulnerability is greatest during embryonic and fetal life, and may be especially great in the first trimester of pregnancy [33–35]. There exist windows of susceptibility in early development that have no counterpart in the mature brain [36].

A growing list of chemicals is now implicated in causation of neurodevelopmental disabilities, including:

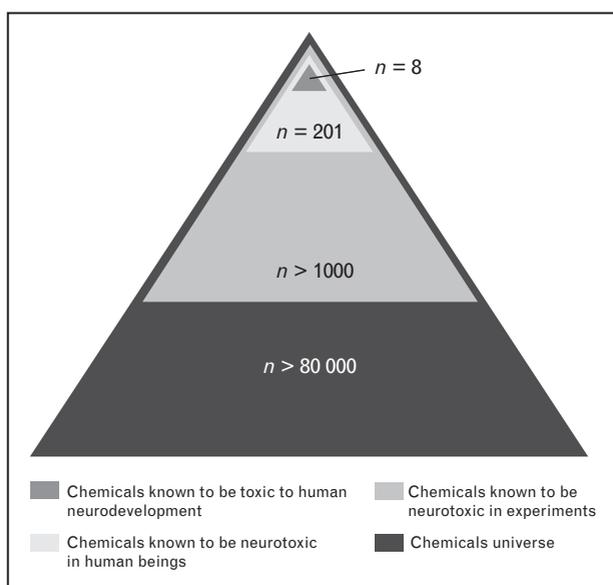
- (1) Lead [26,28–30];
- (2) Methylmercury [27,31];
- (3) Polychlorinated biphenyls (PCBs) [37,38];
- (4) Arsenic [39,40];
- (5) Manganese [41];
- (6) Organophosphate insecticides [42–44,45**,46];
- (7) DDT [47];
- (8) Ethyl alcohol [48].

Can other chemicals cause developmental neurotoxicity?

This short list of chemicals currently identified as human developmental neurotoxicants may be only the currently visible tip of a potentially much larger problem (Fig. 1).

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. HPV chemicals are found in a wide array of consumer goods, cosmetics, medications, motor fuels and building materials. They are common in hazardous waste

Figure 1 The iceberg (triangle) of developmental neurotoxicants



Reproduced with permission from [33].

sites [49]. They 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 [50]. Fewer than 20% of HPV chemicals have been tested for potential to cause neurodevelopmental toxicity [51].

A recent systematic review of the world's literature undertaken to identify chemicals potentially toxic to the developing human brain produced a list of approximately 200 industrial chemicals documented to be neurotoxic in adult humans [33]. These are primarily industrial chemicals – metals, solvents, and pesticides – and nearly half are HPV materials. This search also produced a second list of approximately 1000 chemicals that have not been examined in humans, but that are neurotoxic in experimental models.

Given current understanding of the great vulnerability of the developing brain to toxic chemicals, likelihood is high that many of the materials identified through this search have potential to cause injury to the developing brain and to produce neurodevelopmental disorders, possibly autism among them.

Direct evidence for environmental causation of autism

The most strongly positive, 'proof-of-concept' evidence to support the hypothesis that environmental factors contribute to causation of autism comes from clinical and epidemiological studies that link autism with specific environmental exposures.

- (1) Thalidomide: An increased incidence of autism is reported among children exposed prenatally to thalidomide [52]. In a population of 100 Swedish thalidomide embryopathy cases, at least four met full diagnostic criteria for autism [53]. On the basis of the pattern of concomitant somatic malformations, the time of critical exposure was calculated to be 20–24 days post conception [54].
- (2) Misoprostol: Misoprostol is a prostaglandin analogue, licensed in the US for the prevention of gastric ulcers. It is widely used in some countries as an abortifacient. A case series from Brazil describes a group of seven children with ASD, of whom four (57.1%) had prenatal exposure to misoprostol [55]. The relevant exposures were reported to have occurred in the first trimester of pregnancy following unsuccessful abortion attempts; mean exposure was in the sixth week post conception.
- (3) Valproic acid: Children exposed prenatally to the anticonvulsant valproic acid exhibit patterns of somatic malformation similar to those of thalidomide

embryopathy, but of lesser severity. These include neural tube defects, cardiac malformations, cranio-facial anomalies and limb defects. They can also develop autism [56]. Autism was reported in 11% of 57 children whose mothers took valproic acid in early pregnancy. An even larger number of these children had some autistic traits. On the basis of the pattern of somatic malformations, the time of critical vulnerability was calculated to be in the first 3–4 weeks post conception [57^{*}]. In-utero exposure of rats to valproic acid has been shown to produce behavioral abnormalities analogous to autism [57^{*}].

- (4) Prenatal rubella infection: Clinical and epidemiological studies have linked maternal rubella infection in early pregnancy with autism [58]. In these studies, autism occurred in conjunction with other anomalies typical of the congenital rubella syndrome, including eye defects, deafness, mental retardation and cardiac malformations. Risk for autism appeared greatest when infection occurred in the first 8 weeks post conception.
- (5) Chlorpyrifos: Chlorpyrifos is an organophosphate insecticide widely used until a few years ago to control insects in schools and homes in the US and still used extensively in agriculture. Chlorpyrifos was first recognized to be a developmental neurotoxicant in experimental studies, in which perinatal exposure of newborn rodents to low doses of chlorpyrifos was shown to cause reduced numbers of neurons, decreases in intelligence and persistent alterations of behavior [59].

Prospective assessments of infants exposed *in utero* to chlorpyrifos have reported exposure-related decreases in duration of gestation and in body weight at birth, as well as an exposure-related increase in the number of abnormally primitive neonatal reflexes [43,60,61]. Continuing follow-up of these children through 24–36 months demonstrated significant developmental delays [62], cognitive deficits, and increased risk for attention deficit–hyperactivity disorder (ADHD). Most recently these studies have found, on the basis of maternal report, an increased incidence of PDD-NOS [47,62].

In each of these examples the environmental exposures relevant to autism appear to have occurred prenatally, indeed very early in the first trimester of pregnancy [53,63]. These findings have substantial implications for understanding the environmental contribution to causation of autism and for the design of research that seeks to discover these causes [64^{**}].

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 [65]. In the UK, these claims focused on the measles–mumps–rubella (MMR) vaccine [66]. In the US, they focused on thimerosal, a preservative containing ethyl mercury that was added to multidose vials of many vaccines to prevent microbial contamination.

To address the issue, a series of studies was 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 [12]. Key findings are:

- (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 line following introduction of 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 [67,68].
- (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% [69].
- (3) In Yokohama, Japan, the MMR vaccination rate declined significantly between 1988 and 1992, and no MMR vaccine was administered in 1993 or thereafter. Despite declining immunizations, cumulative incidence of ASD increased significantly each year from 1988 through 1996 and rose especially dramatically beginning in 1993. Overall incidence of autism nearly doubled in those years [70].
- (4) In Denmark, a comparison of autism rates in 440 655 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 [71].
- (5) In Finland, a retrospective study in 535 544 1–7-year-old children vaccinated between November 1982 and June 1986 found no increases in incidence of autism during the 3-month period following immunization and no temporal clustering of autism hospitalizations [72].
- (6) In the UK, a prospective population-based cohort study that has followed more than 14 000 children

from birth found no evidence that early exposure to thimerosal had any deleterious effect on neurologic or psychological outcome [73].

- (7) In the US, an analysis of neuropsychological function in 1047 children found no consistent correlation between neuropsychological functioning at age 7–10 years and early exposure to thimerosal-containing vaccines [74].

Taken together, this extensive series of high-quality, peer-reviewed studies has failed to show any association between autism and childhood immunization. Fear of autism does not justify failure to vaccinate children against life-threatening diseases [75].

Need for an autism discovery strategy

Although vaccines and their components are not credible causes of autism, the possibility remains open that there exist unrecognized environmental causes of autism [33]. Most likely these are to be found among the HPV chemicals to which pregnant women and children today are routinely exposed. The rationale for seeking environmental causes of autism is that, once discovered, these causes are potentially preventable [76].

A successful strategy for discovering the environmental causes of autism will need to be highly interdisciplinary. It will need to bring together researchers from outside the traditional autism research community [12]; from a wide array of disciplines including toxicology, epidemiology, developmental psychology, developmental neurobiology, neuropathology, molecular genetics, genomics, proteomics, functional neuroimaging and medical informatics.

Three key components of a proposed autism discovery strategy are:

- (1) Toxicological studies: To identify chemicals that are developmental neurotoxicants and therefore have potential to contribute to causation of autism, a highly targeted toxicological search is urgently needed. A logical starting point for this search would be the 1200 chemicals identified as neurotoxic through the literature review described above [33]. Highest priority should be assigned to examining the chemicals on these lists that are already known to be neurotoxic in either humans or animals and that are most widely distributed in children's environments. Chemical classes that fulfil these criteria are organophosphate pesticides, organohalogenes, phthalates and phenols, such as Bisphenol A.

New, more rapid screening tools for detection of developmental neurotoxicity need to be applied in this search [77,78*]. Current testing methods are slow

and cumbersome and leave too many common chemicals untested. Also, to better detect the potential of chemicals to injure the developing brain, toxicity testing protocols need to expand to include examination of neurobehavioral function [79]. Current test protocols rely mainly on such crude parameters as brain weight and gross morphology [80,81] and are therefore relatively insensitive.

- (2) Neurobiological research: To understand the cellular and molecular mechanisms involved in environmental causation of autism, a broad range of neurobiological studies need to be undertaken. These studies will discover how toxic chemicals interact with the developing brain, and identify the ways in which chemicals interact with the genome to produce changes in brain structure and function.

A possible approach to such studies would be to expose animals in early gestation to chemicals known to cause autism, such as thalidomide or valproic acid, and then to study the cascade of genetic, molecular and cellular effects that these exposures produce in offspring. That work has the potential to identify perturbations in signaling pathways that are critical in the genesis of autism. Those pathways could then be queried in relation to other synthetic chemicals in new, high-throughput toxicological testing systems.

- (3) Prospective epidemiological studies: Large-scale, prospective epidemiological studies such as the recently launched US National Children's Study are extraordinarily powerful engines for discovery of the environmental causes of autism. The National Children's Study is the largest study of children's health ever undertaken in the US. It will follow 100 000 children, a statistically representative sample of all children born in the United States from conception to age 21 [81,82]. It is the first large-scale prospective study of children's health to specifically measure children's environmental exposures, prenatally as well as after birth, using a combination of maternal and infant biological markers and direct sampling of the ambient environment. It will collect samples for genetic analysis from each mother and child.

The National Children's Study will attempt to link children's prenatal and postnatal environmental exposures with the subsequent appearance of disease and dysfunction. It will examine gene–environment interactions. It is hypothesis-driven and will specifically seek environmental causes of autism and other neurodevelopmental disorders. Findings from the toxicological and neurobiological studies described above could inform and focus the National Children's Study by identifying particular classes of chemicals as targets for investigation. Given the currently reported prevalence of autism in the US, the study can be expected to include nearly 700

children with autism. It will provide an unparalleled opportunity to examine interactions between genetic and environmental factors in the genesis of autism.

Conclusion

Much attention in recent years has focused on understanding the genetic contribution to causation of autism. This elegant research has identified a series of genetic factors and will likely discover still more. But none of these anomalies accounts to date for more than a small fraction of cases, and there is substantial imbalance between the extensive and highly sophisticated information on the genetics of autism and the scarcity of investigation into potential environmental causes. This situation raises the possibility that unsought environmental exposures contribute to causation of autism.

To discover the undiscovered environmental causes of autism, an interdisciplinary autism discovery strategy is proposed that combines toxicological screening, neurobiological research and prospective epidemiological study. Likelihood is high that this strategy will identify new environmental causes of autism, causes that can in theory be prevented. Potential for breakthrough discovery is high.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 255).

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