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## Environmental Risk Factors for Autism: Do They Help Cause *De Novo* Genetic Mutations That Contribute to the Disorder?

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### Abstract

Recent research has discovered that a number of genetic risk factors for autism are *de novo* mutations. Advanced parental age at the time of conception is associated with increased risk for both autism and *de novo* mutations. We investigated the hypothesis that *other* environmental factors associated with increased risk for autism might also be mutagenic and contribute to autism by causing *de novo* mutations. A survey of the research literature identified 9 environmental factors for which increased pre-conceptual exposure appears to be associated with increased risk for autism. Five of these factors – mercury, cadmium, nickel, trichloroethylene, and vinyl chloride – are established mutagens. Another four – including residence in regions that are urbanized, located at higher latitudes, or experience high levels of precipitation – are associated with decreased sun exposure and increased risk for vitamin D deficiency. Vitamin D plays important roles in repairing DNA damage and protecting against oxidative stress – a key cause of DNA damage. Factors associated with vitamin D deficiency will thus contribute to higher mutation rates and impaired repair of DNA. We note how *de novo* mutations may also help explain why the concordance rate for autism is so markedly higher in monozygotic than dizygotic twins. *De novo* mutations may also explain in part why the prevalence of autism is so remarkably high, given the evidence for a strong role of genetic factors and the low fertility of individuals with autism – and resultant selection pressure against autism susceptibility genes. These several lines of evidence provide support for the hypothesis, and warrant new research approaches – which we suggest – to address limitations in existing studies. The hypothesis has implications for understanding possible etiologic roles of *de novo* mutations in autism, and it suggests possible approaches to primary prevention of the disorder, such as addressing widespread vitamin D deficiency and exposure to known mutagens.

### Keywords

Autism; Autism Spectrum Disorder; De Novo; Mutation; Genetic; Environment; Risk Factor

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Conflicts of interest Statement

None Declared

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## INTRODUCTION

Many lines of research, including family and twin studies, as well as genetic linkage and association studies, indicate that genetic factors are important in the etiology of autism [1,2, 3]. Recent research has discovered that a number of the genetic risk factors for autism are *de novo* mutations [3,4,5,6,7]. This research includes, for example, multiple reports of *de novo* mutations in genes that either code for, or regulate expression of, genes involved in the structure or function of synapses [6,8]. Smith et al. [3] review evidence for mitochondrial as well as nuclear genomic instability in autism, and they note the importance of investigating whether environmental factors, such as reactive oxygen species that can cause mutations, may play a role in producing this instability.

If *de novo* mutations are indeed important contributing factors in autism, it could help to explain several puzzling facts about the disorder. Thus, for example, autism is surprisingly common for a disorder that is so disabling and is associated with such low rates of marriage and fertility, yet is estimated to have an extremely high heritability – over 90% based on twin concordance rates [9]. *De novo* mutations could help explain this puzzle if a constant influx of such new mutations into a population helps offset the continual elimination of autism susceptibility genes from the population because of low average fertility rates in individuals with autism. If *de novo* mutations play a significant role in a disorder, it will also tend to produce a much higher concordance rate in monozygotic than dizygotic twins, because the same *de novo* mutation will typically be inherited by both members of a monozygotic twin pair, but only very rarely by both members of a dizygotic twin pair. It is notable that this is the pattern of twin concordance rates that is found in autism [1,5,10]. Rutter and Simonoff [2] reviewed the results of the three twin studies of autism that used samples representative of the general population; the pairwise concordance rates for monozygotic twins in these three studies [9,11,12] were, respectively, 36%, 69%, and 91%, whereas in each study the concordance rate for dizygotic twins was 0%.

Another striking finding on autism is the strong tendency for autism risk to increase with the age of the parents – particularly the father – at the time of conception. For example, Croen et al. [13] found the relative risks of autism associated with advanced maternal and paternal age to be 1.18 and 1.34, respectively. Reichenberg et al. [14] found that risk for autism spectrum disorders (ASD) was 5.75 times higher if individuals were born to fathers  $\geq 40$  years of age or older, rather than 30 years or younger.

That parental age may contribute to increased autism risk by causing such *de novo* mutations is suggested by evidence that advanced parental age contributes significantly to the frequency of *de novo* mutations [15,16], as well as to risk for autism. This is particularly true in the male germline, as the lifelong production of sperm cells offers significantly more opportunities for mutations than is the case for ova. Paternal age effects and paternally linked mutations have been reported in a number of genetic disorders, including achondroplasia, Apert syndrome, Crouzon syndrome, and Pfeiffer syndrome [16].

These findings suggested to us the hypothesis that *other* environmental factors may also be associated with increased risk for autism, at least in part, because they contribute to *de novo* mutations. As an initial test of this hypothesis, we surveyed the research literature to identify environmental factors for which increased pre-conceptual exposure appears to be associated with increased risk for autism. We then examined whether these risk factors for autism are likely to contribute to higher rates of *de novo* mutations.

## FACTORS FOR WHICH PRECONCEPTUAL EXPOSURE INCREASES RISK FOR AUTISM

Table 1 displays 9 environmental factors for which a computer-assisted review of the research literature found that an association of pre-conceptual exposure with increased risk for autism was suggested by at least one ecological or epidemiological study. The table groups these factors according to the kind of evidence that suggests they contribute to de novo mutations. Windham et al. [17] identified several pollutants associated with increased prevalence of autism spectrum disorders by comparing U.S. Environmental Protection Agency data on pollutant exposure with demographic data on 284 children with ASD and 657 controls in the San Francisco Bay Area. Children from areas exposed to higher concentrations of three heavy metals and two chlorinated solvents had significantly higher rates of ASD than children residing in areas with low exposure. The substances for which higher exposure was most strongly correlated with increased ASD risk were mercury, cadmium, nickel, trichloroethylene, and vinyl chloride. In a complementary ecological study, Palmer et al. [18] investigated autism prevalence as a function of proximity to industrial and power plant sources of environmental mercury in 1,040 Texas school districts. After controlling for socioeconomic factors and urbanicity, Palmer et al. found that autism prevalence increased 2.6% for every 1,000 pounds of mercury released in the vicinity of the geographical center of a given district, and 3.7% with nearby power plant emissions.

Ecological studies have also identified several other environmental factors, listed in Table 1, for which exposure early in development was associated with increased risk for autism. Exposure to these factors appears to have included the period before conception. A recent meta-analysis of prevalence studies around the world found that significantly increased risk for autism is associated with urban vs. rural residence (odds ratio – OR – of 2.44) [19]. Several studies have also reported that a significantly higher autism prevalence is associated with residence in geographic regions at higher latitudes. For example, two studies conducted by the Centers for Disease Control [20] found that when prevalence rates of ASD were compared across different U.S. states that had used the same ascertainment procedures, the autism prevalence was significantly higher in New Jersey than in any of the 9 other, more southern states. In a second comparison, involving 4 states that used a different ascertainment procedure from the first set of states, autism prevalence was significantly lower in Alabama than in any of 3 other, more northern states. Higher prevalence has also been found to be significantly associated with infants' and toddlers' residence in counties with high levels of precipitation; this was found to hold in each of three different states, even after controlling for income and ethnicity [21]. Waldman et al. found a significantly higher prevalence in counties with increased access to cable television [22]. Waldman et al. suggest that their data are consistent with early childhood television watching as a contributor to autism risk. However, their data are also consistent with autism risk being associated with more time spent inside watching television by *parents* – and thus with decreased exposure to sunlight before the children were conceived.

There are also several reports that in geographic regions at higher latitudes, such as Sweden and Minnesota, there is an extremely high incidence of autism among children of dark-skinned immigrants from African countries such as Somalia [23,24,25,26]. In Minnesota, for example, the Department of Health recently released a study estimating the prevalence of autism among Somali children to be between 2 and 7 times greater than the prevalence among non-Somali children [26]. Although these studies of immigrants involved modest sample sizes, the increases in autism risk are so large that they merit attention.

As Cannell [27] has noted, each of these latter five risk factors is consistent with an etiologic role for vitamin D deficiency in autism. The action of UV rays in sunlight on the skin is the

most powerful natural source of vitamin D, and factors that reduce the amount and intensity of sunlight to which skin is exposed significantly increase the risk of vitamin D deficiency. Vitamin D deficiency is common in populations at higher latitudes [28], especially those with darker skin, because more darkly pigmented skin reduces penetration of UV rays to the skin layers that synthesize vitamin D. Higher rates of precipitation are associated with less sunshine. Precipitation, like television watching, also encourages people to spend more time inside, reducing their exposure to sunlight and increasing their risk of vitamin deficiency.

Vitamin D plays an important role in dozens of different biochemical processes, in addition to its well-known role in bone metabolism [29,30,31,32]. As Cannell [27] pointed out, prenatal vitamin D deficiency can disrupt normal brain development. A complementary effect of vitamin D deficiency in families, however, will be – as we will discuss later – to increase *de novo* mutations in offspring.

## EVIDENCE FOR MUTAGENICITY OF AUTISM RISK FACTORS

Several lines of evidence indicate that oxidative stress induces mutagenesis [33,34]. Oxidation reactions produce deleterious effects on DNA by a variety of mechanisms, depending on the type of affected nucleotide [33]. Indeed, the threat of oxidation reactions to DNA is so prevalent that most genetic material would be altered by reactive oxygen species (ROS), were it not for the cell's natural defenses and capacity for DNA repair [33]. Sperm cells appear to be more vulnerable to the mutagenic effects of oxidative stress than oocytes. Several features of sperm cells, including the unique membrane structure crucial to fertilization, provide greater opportunities for the production of ROS [35,36,37]. In contrast, in oocytes there are multiple pathways to repair DNA damage caused by ROS [38].

Mercury, nickel, and cadmium, like vinyl chloride and trichloroethylene, have been identified as significant mutagens by a variety of studies. These substances appear to exert their mutagenic effects in at least two ways. First, they contribute to oxidative stress, leading to oxidative DNA damage by free radicals [39]. Second, they tend to inhibit DNA repair systems, thereby leading to the accumulation of mutations [40,41]. To maintain intracellular redox balance and protect against oxidative stress, cells produce reducers such as glutathione and nicotinamide adenine dinucleotide phosphate (NADPH). Mercury, cadmium, and nickel become toxic to cells by depleting intracellular levels of glutathione and binding to sulfhydryl groups of proteins [39], leaving DNA vulnerable to the mutagenic effects of ROS.

For each of the heavy metals and industrial solvents discussed below, there has been extensive research involving dozens of studies confirming their mutagenic effects, in both *in vitro* and *in vivo* studies, and in research from different species, including humans. We briefly summarize examples of studies on each of the five factors; more comprehensive summaries of research on each factor are available online at the website of the Agency for Toxic Substances and Disease Registry (ATSDR), part of the U.S. Center for Disease Control.

For *mercury*, there is evidence for mutagenicity from several dozen studies; many of these are summarized at the ATSDR [42] webpage. For example, Ariza and Williams [43] found low concentrations of mercury to be mutagenic in mammalian cells; the investigators found significantly more mutations in cells exposed to mercury acetate, even at levels too dilute to cause cytotoxicity. In a follow-up study, Ariza & Williams [44] found a dose-dependent effect on type of mutation after mercury exposure; that is, at concentrations of 0.4 or fewer nanomoles, mercury tended to induce *point mutations*, while higher concentrations mostly induced partial and complete *deletions* in chromosomes. Silva-Pereira et al. [45] found low concentrations of organic mercury compounds to have a significant mutagenic effect on chromosomes in cultured human lymphocytes.

For *cadmium*, there is also evidence for mutagenicity from dozens of studies [46]. For example, Filipic et al. [40] found that low concentrations of cadmium induced oxidative DNA damage and also impaired the capacity of the cell for DNA repair. Coen et al [47] demonstrated that exposure to cadmium can induce delayed effects in the progeny of exposed cells even after removal of the toxic substance. The investigators found significant increases of chromosomal aberrations in human lung tissue cells eight generations removed from the originally exposed ancestor cells.

*Nickel* has also been found to be mutagenic in many studies [48]. Thus, for example, point mutations and chromosomal deletions have been detected in a number of mammalian cell lines exposed to nickel [48], and DNA damage has been detected in the lymphocytes of nickel refinery workers [49]. Nickel has been shown to have deleterious effects on DNA through several processes, such as potentiation of the effect of other mutagens [50], production of ROS [51], and inhibition of DNA repair [41].

*Trichloroethylene* is a volatile industrial solvent and metal-cleaning agent. Again, many studies have indicated that it is mutagenic [52]. Hu et al. [53], for example, found that trichloroethylene induced DNA damage in human liver cells in a dose-dependent manner. The mutagenic effects were exacerbated in cells with depleted levels of the antioxidant glutathione, suggesting that trichloroethylene causes DNA damage via its application of oxidative stress to the cell.

*Vinyl chloride* is a known mutagen [54] that is metabolized in the cell to products that react directly with DNA to create various DNA adducts, which then in turn have their own mutagenic effects, including, for example, chromosomal deletions and transversions [55].

## VITAMIN D, DNA REPAIR, AND PROTECTION AGAINST OXIDATIVE STRESS

As noted earlier, Cannell [27] identified several risk factors for autism that are also associated with vitamin D deficiency. Several lines of evidence suggest that vitamin D in its active form – $1\alpha, 25$ -dihydroxyvitamin  $D_3$  (1,25 VD) – has significant antioxidant properties. For example, Chatterjee [56] demonstrated that rat liver cells treated with vitamin D four weeks before the introduction of a mutagenic agent showed significantly fewer chromosomal aberrations. Bao et al. [34] found that 1,25 VD protected human prostate cells against oxidative stress induced by a peroxide solution; 1,25 VD increased levels of a key enzyme in the cell's natural antioxidative defenses. Vitamin D plays an important role in promoting DNA synthesis and repair [57,58]. The power of active vitamin D to promote expression of glutathione may be particularly important in cells exposed to heavy metals such as mercury, nickel, and cadmium, because the mutagenicity of these agents is directly related to their propensity to deplete intracellular levels of glutathione.

In summary, two of vitamin D's roles are to help protect against oxidative stress and promote DNA repair. Thus, the five risk factors associated with vitamin D deficiency – like the other risk factors of mercury, nickel, chromium, vinyl chloride, and trichloroethylene – should tend to both increase oxidative damage to DNA and inhibit repair of that damage.

## DISCUSSION

In conclusion, growing evidence indicates that increased risk for autism is significantly associated with de novo mutations. A literature review found several factors for which increased preconceptional exposure appears to be associated with increased risk for autism. These factors are either substances that are themselves mutagenic (mercury, cadmium, nickel, vinyl chloride, and trichloroethylene) or are associated with increased risk of vitamin D deficiency (four environmental factors associated with decreased sunlight exposure, plus darker skin, which reduces penetration of sunlight to skin levels where vitamin D synthesis

occurs), which increases mutation rates. For each of these risk factors, the available evidence appears consistent with the hypothesis that preconceptual exposure will increase de novo mutations. However, the research to date has important limitations. One limitation concerns lack of specificity regarding the timing of exposure. In the studies to date, the focus was usually on exposure during gestation or infancy, and separate sets of data on exposure were not available for the preconceptual, gestational, and childhood periods. It is likely that in the studies reviewed here, individuals who had elevated exposure to a mutagenic factor during one of those developmental periods would also have had higher exposure during the other two periods. Another limitation of previous research is that a number of studies used ecological designs – that is, exposure data were obtained at a group level, rather than a more accurate, individual one.

A number of other environmental factors have also been reported to increase risk for autism, e.g., pregnancy and birth complications [59] or pesticides [60]. However, for those other factors, risk for autism appears to be increased by gestational or perinatal, rather than preconceptual, exposure. The present hypothesis does not exclude a role for gestational and childhood exposure to toxic factors in the etiology of autism, as oxidative stress and mutagenic effects are also likely to have adverse effects on mitosis and development. Rather, the hypothesis proposes that preconceptual factors that produce de novo mutations in parental germ cell lines may also be significant etiologic factors.

Thus, one issue that needs to be addressed in future research is distinguishing the effects of preconceptual vs. gestational and childhood exposures to mutagens and their respective effects on risk for autism. Another important test of our hypothesis would be to examine, for each of the autism risk factors noted in this paper, whether exposure is especially elevated in individuals with autism if they have no family history of ASDs and/or have de novo mutations associated with increased risk of ASD.

Our hypothesis has potentially significant public health implications for understanding and preventing autism. Prospective research is warranted on the role of vitamin D in the prevention of oxidative stress-related mutagenesis and risk for autism. For example, vitamin D supplementation could potentially offer a rather safe, acceptable, and inexpensive measure for reducing de novo mutations in populations that are deficient in vitamin D. As noted earlier, clinically significant vitamin D deficiency is quite common in the U.S. and Northern Europe [28]. Randomized clinical trials that administer vitamin supplements to samples of adults who are particularly likely both to conceive children in a few years and to be deficient in vitamin D could provide a way to test the hypothesis experimentally. Such research deserves serious consideration, because the unusually great economic and human costs of autism mean that effective measures of primary prevention could yield enormous savings in financial, medical, and human resources.

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**Table 1**

**Environmental Exposures and Other Risk Factors for Autism – Association with Mutagenesis**

<b>Risk Factors for Autism – Evidence for Link to De Novo Mutations</b>	<b>Evidence That Factor Increases Risk for Autism</b>
<p><b>Exposure to Established Mutagens (Multiple in vitro and in vivo studies indicate the factors are mutagens)</b>  <i>Mercury</i>  <i>Cadmium, Nickel, Trichloroethylene, Vinyl Chloride</i></p>	<p>Increased prevalence in vicinity of mercury release (+2.6% per 1,000 lbs. Hg), power plant emissions (+3.7%) [18].                      Increased risk in California children with higher mercury exposure: OR = 1.92 [17].                      Respective ORs in California children with higher exposure: 1.54, 1.46, 1.47, 1.75. [17].</p>
<p><b>Exposure to Factors Linked to Vitamin D Deficiency (This deficiency weakens natural defenses against mutations)</b>  <i>Regions at Higher Latitudes</i> (At such latitudes, risk is especially high for individuals with dark skin)  <i>Urban Residence</i>  <i>Residence in Counties With Increased Precipitation</i>  <i>Residence in Counties With More Cable TV Consumption</i> (Likely associated with more TV watching and time spent indoors)</p>	<p>Several studies in the U.S. and in Sweden [20,23,24,25,26]                      OR = 2.44, from a meta-analysis of 23 studies around world [29].                      Support from data in each of 3 U.S. states [21].                      Support from cross-sectional and longitudinal analyses of data from California and Pennsylvania [22].</p>
<p><b>Other Factors Linked to De Novo Mutations</b>  <i>Advanced Paternal Age</i>                      Several lines of evidence [15,16]  <i>Monozygotic Co-twin Status</i> (Both monozygotic co-twins typically inherit the same de novo mutation, but this rarely occurs in dizygotic co-twins)</p>	<p>Risk was 5.75 times higher in offspring of fathers over 40 years of age vs. fathers under 30 years of age [14].                      Adjusted RR for paternal age modeled as a continuous variable = 1.34 [13].                      36–91% autism concordance for MZ twins, but 0% for DZ twins, in the three representative twin samples [9,11,12]</p>