

Environmental risk factors for autism

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Autism is a devastating childhood condition that has emerged as an increasing social concern just as it has increased in prevalence in recent decades. Autism and the broader category of autism spectrum disorders are among the increasingly seen examples in which there is a fetal basis for later disease or disorder. Environmental, genetic, and epigenetic factors all play a role in determining the risk of autism and some of these effects appear to be transgenerational. Identification of the most critical windows of developmental vulnerability is paramount to understanding when and under what circumstances a child is at elevated risk for autism. No single environmental factor explains the increased prevalence of autism. While a handful of environmental risk factors have been suggested based on data from human studies and animal research, it is clear that many more, and perhaps the most significant risk factors, remain to be identified. The most promising risk factors identified to date fall within the categories of drugs, environmental chemicals, infectious agents, dietary factors, and other physical/psychological stressors. However, the rate at which environmental risk factors for autism have been identified via research and safety testing has not kept pace with the emerging health threat posed by this condition. For the way forward, it seems clear that additional focused research is needed. But more importantly, successful risk reduction strategies for autism will require more extensive and relevant developmental safety testing of drugs and chemicals.

Keywords: autism; autism spectrum disorders; drugs; environmental chemicals; infections; vaccinations; stressors; critical windows of development; developmental neurotoxicity testing

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For the purpose of this review, autism, autism disorder, and autism spectrum disorder (ASD) will be referred to simply as autism. Autism has three primary features that underscore its relationship with environmental risk factors. First, the prevalence of autism has increased in recent decades at an alarming rate. Second, as with most neurodevelopmental (or immunological) disorders, autism is chronic in nature and the ramifications of the dysfunction are borne across a lifetime. Finally, autism is but one of a disturbingly large cadre of chronic conditions (e.g. asthma, type 1 diabetes, metabolic syndrome, schizophrenia, sleep disorders) that have followed similar paths of increased prevalence. These conditions all have early life origins (1), appear to be connected to grids or patterns of additional comorbidities, (2, 3) and have environmental risk factors that, at least in part, are yet to be identified. An effective environmental risk reduction strategy for one of these diseases or conditions is likely to have a broader relevance that could encompass other developmentally based diseases.

Research into the etiology of autism has intensified to meet the goals of: (1) providing fundamental information

concerning the nature of autism needed for improving quality of life strategies, and (2) aiding the identification of causative risk factors that are targets for public health risk reduction efforts. We agree with other recent reviews on this subject (4) that additional targeted research is needed to support these goals. But increased research usually follows epidemics like autism, childhood asthma, and metabolic syndrome rather than preceding and preventing them. Instead, developmental toxicity safety testing of drugs and chemicals (e.g. neurological and immunological) is the frontline process used to identify environmental risk factors, reduce risks to children and pregnant women, and prevent these epidemics. Based on the outcomes of safety testing relative to autism, asthma, and metabolic syndrome, this public health protection strategy has been inadequate at best. As will be discussed in the following sections, no single environmental factor is sufficient to explain the current autism epidemic (5) and more risk factors are likely to be identified in coming years. The different categories of factors that determine the overall risk of autism and the roles played by environmental risk factors are illustrated in Fig. 1.

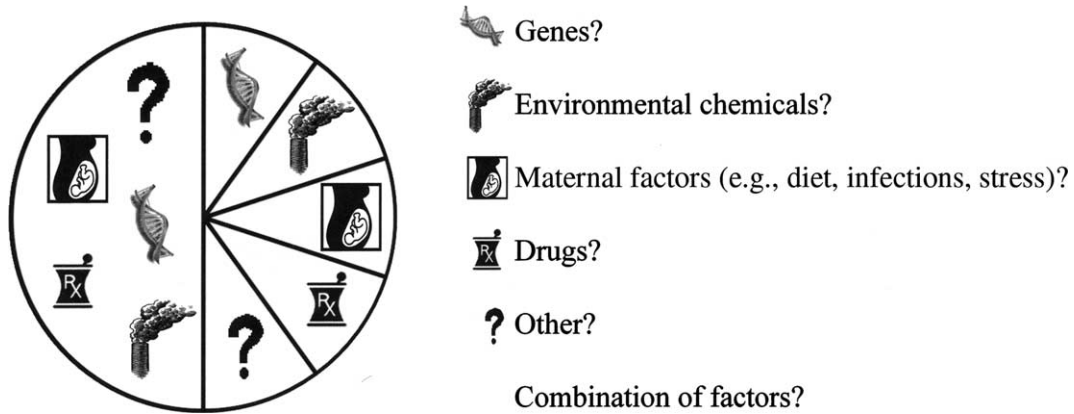


Fig. 1. The pie chart illustrates the most likely risk factors for autism. At issue is the way in which these factors act either independently and/or through various interactions to produce autism. To date, much of this remains to be determined.

The landscape of autism

Description

Autism is a moderately to highly heritable condition (6, 7) that favors the male gender (8). It is considered to be a Pervasive Developmental Disorder, which means that developmental milestones are not met in a timely manner over a broad spectrum of assessment points. In general, individuals with autism are markedly to severely impaired for the necessary components that would allow for appropriate social interaction. These include impaired non-verbal language skills in which they do not look at the eyes of others, cannot read body language, do not have the sense of others as being separate from themselves, and do not have the internal neural mirror capacity for empathy to the point of having no concept of the needs of others or knowing when another is in distress. They often lack verbal language skills as well (9). Along with communication and social skills impairments, individuals with autism often have extremely narrow interests, preferring to focus on one or two interests obsessively. They often rely on repetitive motor mannerisms (e.g. hand flapping, spinning in circles, etc.) as a means of self-calming or self-stimulation (9).

Diagnosis

A diagnosis of autism is only considered after two other disorders have been ruled out: Rett's Disorder, which includes severe mental retardation and Childhood Disintegrative Disorder, in which the child regresses after 2 years of normal development not just in language and social skills but often also in the areas of bladder and bowel control. The delays or abnormal functioning must be present in the child before they reach 3 years of age (9). For diagnosis, children must exhibit at least two impairments of social interaction. For diagnosis, children must exhibit a total of six or more symptoms from among three discrete sets. At last two symptoms of social impairment, at least one language-based symptom and

at least two symptoms showing repetitive, stereotypical patterns related to behavior, interest, and activities must be evident as well (9).

Prevalence

Autism was originally identified in 1943 (10) and was more formally recognized by the American Psychiatric Association in 1980 (11). Several decades ago, a diagnosis was made for every 1 in 1,000 children, but over the last decade the prevalence rate for the diagnosis of autism has exploded. Now approximately 1 in 110 children or 1% of children in the United States are diagnosed with autism (12). A portion of the increased prevalence is attributable to better awareness by physicians and parents, changed criteria for diagnosis, and improved diagnostic tools. However, a recent study of autism in California estimated that these diagnostic variables across the decades could only account for a quarter of the recent increase in prevalence (13). The remainder of this alarming trend must come from early life environmental or physical conditions or environment-gene interactions that impact the development of children both in utero and in early childhood. The epidemic nature of these changes is most easily quantified by the flow of autistic individuals through the school systems. Between 1991 and early 2001, the rate of children in US schools with autism increased by 1,700% (14). Ironically, while the increased prevalence appears to be an actual epidemic, a recent study of the current autism records-based surveillance system concluded that recent estimates of the prevalence of autism are likely to be conservative (15).

Parallel epidemics and our safety testing standards

Autism is one of several childhood illness epidemics (e.g. childhood asthma, metabolic syndrome) that have emerged together in recent decades. This co-emergence of childhood epidemic patterns may provide a clue to those changes in environmental exposures that are of greatest concern for pregnant women and children. Other

researchers have noted this parallel and commented on these possible associations for autism with asthma (16, 17) and metabolic syndrome (18). These conditions affect an alarming percentage of children, particularly in countries like the United States and United Kingdom. The existence of multiple major childhood epidemic patterns should be sufficient cause for us to abandon outdated and largely ineffective safety testing approaches that have produced these results over the past 30 years. As discussed later, more effective approaches for protecting the health of children are needed if not significantly overdue (19).

Socioeconomic impact

Each new pediatric diagnosis of autism carries with it specific implications for family, community, and national socioeconomic systems. These extend from the child or autistic adult to the immediate family but then rapidly encompass the health care financing system, physicians' perceptions, educational systems, the need for long-term half-way houses, adult employment for the disabled, and the economic trajectory for the autistic individual and immediate family members. Knapp et al. (20) used a combination of autism prevalence information, services provided, and lost productivity in the UK to estimate that the cost of supporting children with autism was 2.7 billion pounds per annum, while the cost for adults was 25 billion pounds per annum.

Children with autism and their families have a significantly reduced quality of life that affects many different domains (e.g. economic, social, educational, psychological) (21). Parents of children with autism were seven times more likely than parents of typically developing children to state that child care problems had affected their employment (22). One US study found that direct impact on family annual income was a loss of approximately 14% (23). Not surprisingly, raising an autistic child presents additional stress and psychological challenges for the parents (24). Children with autism often have a greater prevalence of sleep problems than typically developing children (25–27). A recent study suggested that parents of autistic children themselves also have a greater prevalence of sleep problems than did parents of typically developing children (28).

Remarkably, Järbrink (29) estimated that in Sweden, the societal cost was approximately 50,000 euros per annum per child and that parents of autistic children devoted an extra 1,000 hours of time (in support of their children's needs) compared with parents of typically developing children. In a US study of the vocational rehabilitation system, Cimera and Cowan (30) reported that adults with autism supported by these services increased by more than 121% from 2002 to 2006. Additionally, this group of adults was among the most costly within the system.

The levels of medical care costs are unlikely to improve significantly in the near future. Training of broader segments of the medical care community for the specialized considerations of autistic patients has lagged behind the epidemic itself. For example, a recent survey of primary care physicians found that a greater proportion of this front-line group of health care providers lacked self-confidence in their ability to care for autistic children (31).

Gene-environment interactions including epigenetic considerations

Studies examining gene-environment interactions are underway and are encouraging as they involve the prospective collection of samples and genetic information among a large unselected birth cohort. These types of human studies have the potential to narrow the landscape of likely environmental risk factors (32). One of the concerns in precisely identifying the relationship between the beginning of the autism epidemic and a modified environment is the potential environmental epigenomic influences. Environmentally mediated changes in fetal programming can be limited to an individual's prenatal period of development or alternatively, may have multi-generational effects where alteration in control of gene expression may appear in later generations after the introduction of an environmental risk factor. Such multi-generational effects have been reported between tobacco smoking and childhood asthma (33). However, it is apparent that epigenetic effects also extend to autism (34, 35).

Critical developmental windows for environmental risk of autism

Autism is a developmental disorder meaning that alteration or disruption of specific maturational steps in the brain is thought to be prerequisite for developing the condition. But precisely when does this disruption occur? Clearly, with autism now routinely diagnosed before 2 years of age, sensitive windows of developmental vulnerability must occur during the prenatal and/or early postnatal periods of development. Within that overall period of development, there are likely to be narrower windows of greater risk for environmental exposures and conditions. Some possible critical windows were discussed in a prior review of autism (36).

It seems clear that the prenatal period should be a primary focus for risk of autism (37, 38). Early mid-gestational exposure of rats to valproic acid (VPA) produced autism-like characteristics in the offspring (39). The association of preterm birth with autism suggests that prenatal windows were particularly important in risk of autism (40). Using meta-analysis, Gardener et al. (5) concluded that pregnancy complications (based on maternal prenatal medication) were associated with an

increased risk of autism; however, no single prenatal factor was identified. These findings are also supported by other striking examples where the fetal environment significantly impacts later-life neurological status (41). The information from these studies supports the existence of important prenatal windows. However, it does not exclude the potential for significant postnatal exposure effects in risk of autism.

Figure 2 illustrates the potential windows of developmental vulnerability that have been described to date. At present and based on the best examples of known human risk factors, the first trimester of pregnancy is one probable critical window for some categories of environmental agents (42–45). But some studies suggest there are likely to be other critical windows that exist later in pregnancy and/or in the newborn (46).

Routes to autism

Autism exhibits phenotypic heterogeneity, which suggests that a mechanistic route does not encompass a single pathway but includes disturbances to myriad pathways or processes that culminate in an autism diagnosis (Fig. 1). Although multiple factors contribute to autism etiology, including genes and environmental agents, potential mechanisms appear common to autistic patients and indicate that a mechanistic route to autism is a gene-environment interaction. Therefore, the challenge in describing autism etiology requires identifying candidate genes and likely environmental factors and the

downstream effects that arise from environmentally induced disturbances to genes and gene products. While several different mechanisms of developmental disruption have been suggested to contribute to autism, a detailed discussion of these is beyond the scope of the present review. Briefly, Herbert et al. (47) identified candidate genes for autism that are influenced by environmental factors but that have been overlooked because they do not have an explicit linkage to the central nervous system; therefore, candidate genes may be numerous. Although the brain may be altered by environmental factors directly, autism may also result from downstream effects of environmentally modulated changes to other systems (47). Herbert (48) also reported that mechanistic routes to autism occur via developmental disturbances to three main pathways/processes: neurotransmission pathways, redox and methylation pathways, and increases in *de novo* copy number variants. Effects on other systems, such as the immune and endocrine systems, also, may be indirect downstream effects that lead to changes in neurological development. Current evidence indicates that a proportion of autism cases result from the interaction of multiple susceptible genetic loci (49) and that environmental interactions with susceptible genetic loci produce myriad routes that may culminate in an autistic phenotype.

Environmental risk factors

In the search for the environmental risk factors of autism, the data are derived from both human and animal

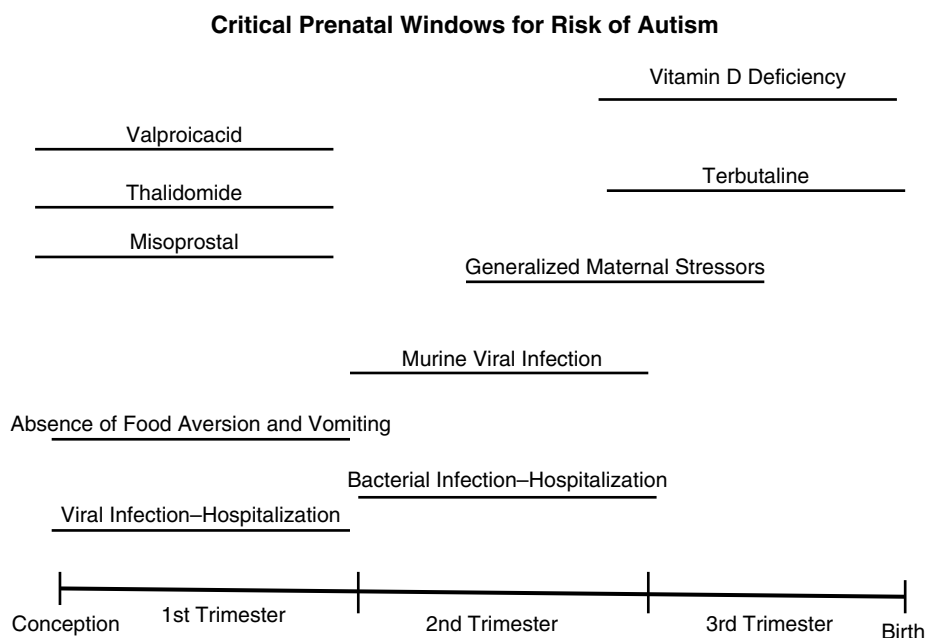


Fig. 2. Putative critical windows of developmental vulnerability for autism are shown based on reports involving potential environmental risk factors. Information is included from human studies as well as from animal data. References for each risk factor and window are as follows: Viral Infection—Hospitalization (92), Absence of Food Aversion and Vomiting (44), Misoprostal (70), Thalidomide (109), Valproic Acid (37), Bacterial Infection—Hospitalization (92), Murine Viral Infection (110), Generalized Maternal Stressors (102), Terbutaline (111), and Vitamin D Deficiency (77).

studies. Direct human exposures usually involve drugs, environmental chemicals, medical procedures, dietary factors or stress involving the mother (and sometimes the father) shortly before conception, the pregnant woman, the nursing mother, and/or the young child. A majority of epidemiology studies are examining the associations between exposures and/or body burden estimates and autistic outcomes, and several large scale studies are ongoing at present. In some cases these human studies are buttressed by animal studies where biological plausibility of the link between environmental exposure and altered neurological development is established. The precision of the representation of some animal models of autism for the human condition has been open to considerable discussion. But most researchers agree that environmental factors contributing to human autism are likely to produce some detectable developmental brain alterations in relevant animal models. This has been an important concept in focusing the search for the most important risk factors of autism.

Environmental chemicals

Myriad environmental chemicals have been implicated as contributory factors to autism development. In a scientific consensus statement by the Collaborative on Health and the Environment's Learning and Developmental Disabilities Initiative (50), several environmental agents were identified as strong contributors to learning and developmental disabilities in humans. These included arsenic, lead, manganese, mercury, pesticides, polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and solvents. However, as previously mentioned in the 'routes to autism' section, a direct effect on the developing nervous system is not the only route to autism. Environmental agents that affect other systems during development may lead to downstream effects on the developing brain. Limiting research to just those agents that directly impact brain development may exclude a multitude of triggers. In addition, no single agent or specific combination of agents has been identified as a trigger. It is more likely that certain individuals with enhanced susceptibility to environmental chemicals and with certain genetic predispositions are at increased risk for autism after exposure to a variety of environmental triggers including chemicals (51). It would be nearly impossible to describe every single study examining links between environmental chemicals and autism. Therefore, this section will highlight chemicals with the strongest associative evidence.

Heavy metals

Heavy metal contamination is ubiquitous and children are generally more susceptible to heavy metal toxicity than are adults, but heavy metal exposure received during

early development is difficult to discern after an autism diagnosis. Regardless, several studies have evaluated associations between autism and biomarkers of heavy metal exposure and effects. The evidence that heavy metal exposure leads to an autistic phenotype is increasingly convincing. Sulfhydryl-reactive metals including arsenic, cadmium, lead, and mercury are the metals most commonly reported as being associated with autism prevalence and risk. Kern et al. (52) evaluated hair samples from autistic and control children and found that levels of sulfhydryl-reactive metals were lower in autistic children relative to control children, which suggests that subgroups of autistic children may have increased susceptibility to certain metals because they are poor excretors or detoxifiers. Other metals may also increase risk; Adams et al. (53) reported that severity of autistic symptoms was influenced by body burdens of aluminum, antimony, lead, and mercury as measured by urinary excretion. Additional research into heavy metal exposure and autism risk is warranted not only to identify metals most commonly associated with increased risk, but to determine the most appropriate biomarkers for assessing risk.

Pesticides

A 2007 study of maternal exposures to agricultural pesticides and autism incidence found that risk for autism was consistently associated with exposure to organochlorine pesticide applications during the period of nervous system organogenesis (54). In a recent review, Landrigan (4) indicated that a strong link existed between exposure to chlorpyrifos, an organophosphate insecticide, and autism. Several classes of pesticides act by dysregulating the nervous systems of target species (i.e. organochlorine, organophosphate, and carbamate insecticides), and non-target species can be similarly affected by environmental exposures. However, additional epidemiological evidence is necessary to strengthen the link between pesticide exposure and increased autism risk.

Other environmental chemicals

Strong evidence linking environmental neurotoxicants and autism is scant; the majority of research papers concerning these chemicals focus on neurotoxicological endpoints in laboratory models. Regardless, neurotoxicants are autism risk factors for two main reasons: (1) many are known to disrupt neural development and (2) environmental concentrations of several neurotoxicants have increased over the past decades, somewhat tracking with increases in autism prevalence. Messer (55) suggested PBDEs as risk factors for autism not only because during the period of time that autism prevalence has increased, levels of brominated flame retardants have increased immensely, but because PBDEs are analogs of thyroid hormone. Binding of PBDEs to thyroid hormone

transporters and receptors during critical periods of brain development may alter the course of development toward an autistic phenotype (55). Similarly, PCBs are developmental neurotoxicants that have been environmental pollutants for decades. Certain PCB congeners, notably those that disrupt thyroid hormone, may inhibit or alter thyroid hormone-regulated gene expression and push brain development toward an autistic phenotype (56, 57). Other environmental pollutants, especially those that disrupt endocrine and immune system signaling, may also increase autism risk; however, data solidifying the link between increased exposure and increased risk are limited.

Drugs

Exposure to pharmacological agents particularly during pregnancy represents a highly relevant environmental concern relative to the risk of autism. Some changing trends in availability and use of certain categories of prescription and over the counter drugs parallel the changing prevalence of autism (17, 58). Additionally, prenatal exposure to a handful of specific drugs such as thalidomide and VPA are among the best examples to date of known environmental risk factors. Finally, as will be discussed later, the disconnection between the routine drug safety screening usually performed and evaluation of the risk of autism (e.g. via relevant developmental neurotoxicity testing) means that the potential for some categories of drugs to promote autism remains relatively undetermined.

Valproic acid (VPA)

VPA is an antiepileptic drug and mood stabilizer in addition to being a known human teratogen (59). When the drug is taken during pregnancy, it can result in children having a well-defined syndrome of abnormalities affecting several systems known as the valproate syndrome (60). In children from women who have taken this medication during pregnancy, autism spectrum disorders are increased several fold (61). The dose and timing of exposure may be key to the extent of the risk.

Results supporting human data have been seen in animal models. In utero exposure of mice to VPA produced autism-like signs in the offspring (62). The VPA exposure produces developmental delays, deficits in motor performance and social behavior, and alterations in postnatal growth and development (63–66). As in humans, alterations of postsynaptic cell-adhesion molecules appear associated with the condition (e.g. neuroligin gene transcription) (62). The VPA is thought to alter gene expression and increase oxidative stress (to which the brain is very susceptible during critical windows of development) (60).

Thalidomide

Thalidomide is a known human teratogen that, among its actions, binds to cereblom and inhibits ubiquitin ligase activity (67). Two studies have reported increased incidences of autism in children following prenatal exposure to thalidomide (68, 69). As discussed by Landrigan (4), the pattern of malformation noted has suggested that the critical window of exposure is in the first trimester.

Misoprostol

Misoprostol is a prostaglandin analog drug that is prescribed for the prevention and treatment of gastric ulcers. Because of its action on the uterus, it has also been used for medical abortion. In a study in Brazil, Bandim et al. (70) reported the association between misoprostol during the first trimester of pregnancy and autism.

Beta 2 adrenergic agonist drugs

Beta 2 adrenergic agonists such as terbutaline are used in the treatment of asthma. At least one research group reported that exposure in prenatal developmental is associated with an elevated risk of autism spectrum disorders as well as other neurophysiological outcomes (71). While human reports describing this potential association are limited, studies in animals provide biological plausibility for this interaction (72).

Antipyretics

Fever suppression strategies and their possible implication for risk of autism has been an emerging topic of consideration. Torres (58) suggested that antipyretic use in early life should be examined as a possible link to the risk of autism and other neurodevelopmental disorders. These associations have not been firmly established but are biologically plausible. Innate immune response differences in toll-like receptor responses have been reported among autistic versus non-autistic children (73). Such immune cell response differences would be key to potential differences in fever responses. Additionally, Becker and Schultz (17) pointed out that early life acetaminophen use is a risk factor for asthma, and that a similar concern may exist for some populations of children relative to risk of autism. In a preliminary study using parental surveys, Schultz et al. (74) found that acetaminophen use, but not ibuprofen use, was associated with an elevated risk of autistic disorder.

Dietary factors

Diet-influenced factors are part of the landscape of potential environmental risk factors for autism that have been largely overlooked until recently. Levels of two diet-related factors that also can be affected via pharmacological (supplements) or physical routes

(sunlight) have taken on new importance in the search for the environment-autism connection.

Vitamin D

Low prenatal levels of vitamin D have been suggested to play a role in the risk of autism (75, 76). Some studies have found that mothers of autistic children versus those with non-autistic children have reduced serum vitamin D levels (77, 78). Vitamin D can influence many different biological processes and appears to act as an anti-inflammatory agent on brain tissue (79) as well as to exert effects on DNA repair processes (80).

Folate

Folate availability has also been suggested as a possible factor in risk of autism (81). The interest in folate partly stems from its known role in affecting neurodevelopment (82, 83). In general, it is thought that folate levels and their impact on the metabolic pathway for methionine could play a role in autism risk. Deficiency in this metabolic pathway has been suggested as a possible causative factor in autism. Both genetic polymorphisms for the pathway and folate receptor autoimmunity (84) have been suggested as possible routes to pathway alteration. A recent review of this topic suggested that the heterogeneity of the studies conducted to date on folate and autism make definitive conclusions problematic (85). In particular, the capacity to examine the potential interactions between genetic background, folate levels, and gene expression varied widely among the studies.

Infections and vaccinations

Infections and vaccinations are among the categories of possible risk factors for autism that have garnered the most attention. The idea that maternal and/or early infant infections could influence neurodevelopment and contribute to adverse outcomes is not restricted to autism (86). The association of maternal infection and elevated risk of schizophrenia is well supported by human and animal research (87) with increased inflammatory cytokine levels in the brain as a likely route to altered neurodevelopment (88). Among the most well-established infections associated with a purported increased risk of autism is congenital rubella infection (89, 90). In a more limited study, Yamashita et al. (46) reported possible association between congenital infection with cytomegalovirus and occurrence of autism.

Both the stage of the pregnancy and the nature of the infectious agent appear to be important in the likely neurological outcome (91). The proposed maternal infection-autism connection is supported by more general data as well. Atladóttir et al. (92) found evidence that maternal infection resulting in hospitalization elevated the risk of autism in the offspring. Some evidence from animals suggests that maternal inflammation in response

to infectious agents may be an important factor in brain development (93). In this study, an IL-1 receptor antagonist was able to protect against the prenatal neurodevelopmental deficits.

If maternal infections represent a potential risk factor where there has been recent scientific agreement, the same cannot be said of vaccinations. Vaccinations, by their design, are intended to modulate the immune system by inducing production of a protective and highly specific immunological response. At issue has been the question of when and under what circumstances unintended immunomodulation occurs and whether unintended adverse outcomes associated with vaccinations could affect the risk of autism. Additionally, among the potential types of adverse outcomes to vaccinations is the question of whether concentrations of toxicants (e.g. mercury-containing thimerosal) and the developmental timing of exposure are sufficient to produce adverse outcomes such as autism. It is of note that in many cases when postnatal childhood exposure to infectious agents elevates risk of chronic disease, the infectious challenge serves as a triggering event in children previously made susceptible (reviewed in Dietert (94)). This is one reason why childhood triggers promoting the onset of diseases such as asthma are often more obvious than are the actual causative prenatal environmental conditions that established susceptibility in the child.

Most of the focus to date involving childhood vaccination and autism has centered on MMR vaccination. Specifically, in the case of the risk of autism, several recent studies indicate that MMR vaccination does not increase the risk of autism. This includes a study where the MMR vaccine was administered for a 4-year period (1989–1993) in Japan; the incidence of autism was not different across the interval before, during, and after this MMR vaccination window (95). Baird et al. (96) reported no dose response associations between the antibody response to measles virus or the measles component of the MMR in children with autism versus two control groups of children (one control group with specific educational needs unrelated to autism and a typically developing group of children). In a very recent case-controlled study, Mrozek-Budzyn et al. (97) found no association between either MMR vaccination or single measles vaccine administration and risk of autism.

Childhood vaccinations play a critical role in reducing the risk of preventable and in some cases life-threatening illnesses. If one considers the history of preventing diseases like polio, childhood vaccinations have been remarkably effective. In a recent review of environment and autism, Landrigan (4) considered the evidence for vaccines and autism and concluded that fear of autism should not result in failure to protect children against life-threatening illnesses. We concur with this conclusion.

Are there important vaccine safety issues?

The opportunity to prevent serious childhood illnesses through the use of effective vaccination regimes is a critical public health measure. In general, vaccine safety is quite exceptional when compared with overall safety of pharmaceutical agents and medical procedures as recently reviewed by House (98). However, the lack of association with MMR vaccination and risk of autism does not mean that improved vaccine safety testing is not important in future strategies to reduce environmental health risks. There are some issues particularly in the timing of vaccinations for the developing immune system (reviewed in Dietert and Dietert (36)) where some protocols may be more beneficial than others for certain health risks (99). Additionally, immune dysfunction is a prominent feature in autistic children and has led to discussion of possible immunological links with autism (36, 100, 101).

Stress

Stress can take many forms including infectious, oxidative, physical, and psychological. Each category is worthy of examination when it comes to risk of autism; some studies have suggested that stress is a potentially fruitful area of study when it comes to environmental risk of autism. Studies differ in scope in that many considered one or two categories of stress while others examined more generalized forms of stress, particularly during the period of pregnancy. Ploeger et al. (45) argued that disruption of very early developmental events (gestational days 20–40 in humans) may be a route to infantile autism. Where a stress-based model was employed in humans, a broad period spanning mid-gestation (prior to 32 weeks of gestation) was of greatest interest (102).

Mueller and Bale (103) reported that a stress experience early in prenatal development is likely to contribute to male-oriented patterns of neurodevelopmental disorders. This is relevant to risk of autism since some neurological researchers hypothesize that the autistic brain may be a 'hyper-male' form (104), and fetal testosterone levels are thought to be important in the risk of autism (105). Using a mouse model, Jones et al. (106) suggested that prenatal stress when combined with genotypic variants in the mother such as reductions in serotonin transporter, may result in autism phenotype in the offspring. But not all studies agree on the association between stress and risk of autism. For example, Li et al. (107) found no evidence for an increased risk of autism following the prenatal stress of maternal bereavement.

Risk identification and reduction: research and safety testing

Research is an important component in the ultimate prevention of autism. However, research is most often after the fact and is reactive to emerging health threats

that have already taken a heavy societal toll. In contrast, well-conceived safety testing of chemicals and drugs is far more likely to be proactive in the prevention of developmental conditions affecting the neurological and other systems. In the case of autism, increased prevalence that has emerged over the past 30–40 years and the targeted research that has followed to date, have yet to produce a definite list of causative environmental factors. Still, safety testing today is not designed to detect the types of developmental disruptions that are likely to result in autism (or other recent childhood health threats such as asthma or type 1 diabetes). Why is the primary response to this emerging health threat only to be found in research and not reflected in purposeful and effective changes to regulations that determine the safety of our drugs and chemicals? Recently, investigators focused on the developmental origins of chronic disease called for a more forceful role of public health officials to reduce the burden of disease established early in life (108). We concur with this appeal, which will require a different level of engagement on issues in developmental toxicity such as the prevention of autism.

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