COACTION

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*

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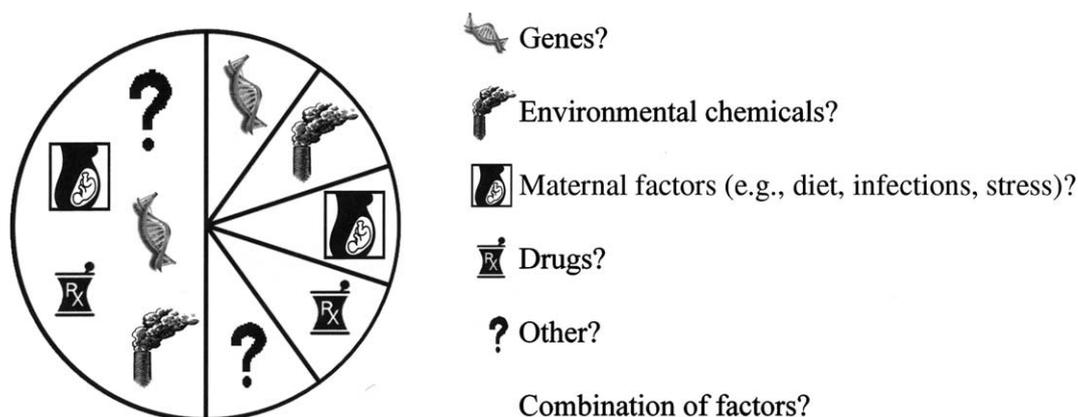
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Fig. 1. The pie chart illustrates the most likely risk factors for autism. At issue is the way in which these factors act either independently and/or though various interactions to produce autism. To date, much of this remains to be determined.

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 manner-isms (e.g. hand flapping, spinning in circles, etc.) as a means of self-calming or self-stimulation (9).

Diagonosis
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 Disin-tegrative 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 impair-ments 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 multigenerational effects where alteration in control of gene expression may appear in later generations after the introduction of an environmental risk factor. Such multigenerational 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

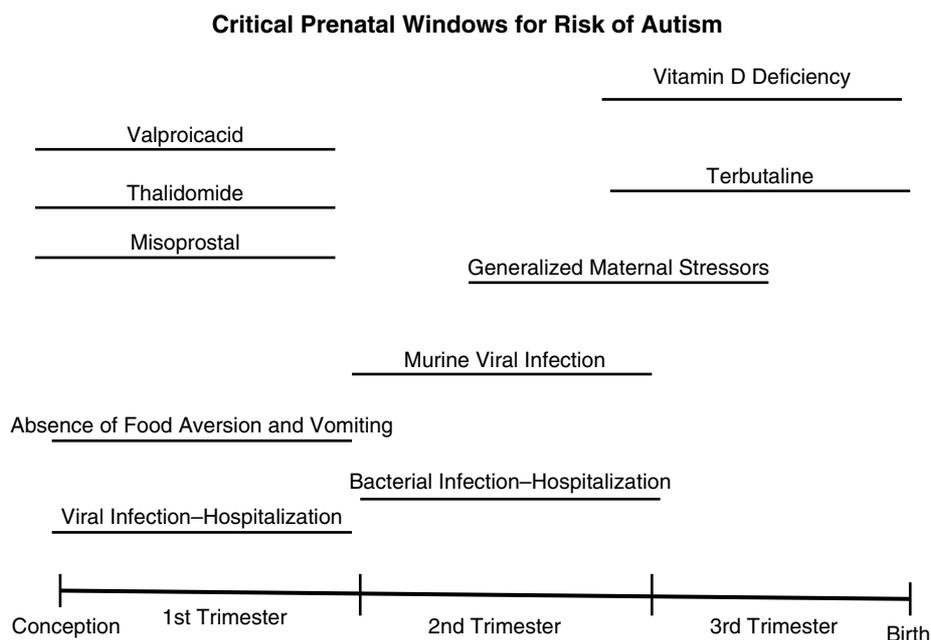**Critical Prenatal Windows for Risk of Autism**

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 adrengeneric 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 ibruprofen 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 thimeresol) 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.

Conflict of interest and funding

References

1. Hanson MA, Gluckman PD. Developmental origins of health and disease: New insights. Basic Clin Pharmacol Toxicol. 2008;102:90–3.
2. Dietert RR, Zelikoff JT. Pediatric immune dysfunction and health risks following early life immune insult. Curr Pediatr Rev. 2009;5:36–51.
3. Dietert RR, DeWitt J, Germolec DR, Zelikoff JT. Breaking patterns of environmentally-influenced disease for health risk reduction: Immune perspectives. Environ Health Perspect. 2010;188:1091–9.
4. Landrigan PJ. What causes autism? Exploring the environmental contribution. Curr Opin Pediatr. 2010;22:219–25.
5. Gardener H, Spiegelman D, Buka SL. Prenatal risk factors for autism: Comprehensive meta-analysis. Br J Psychiatry. 2009; 195:7–14.
6. Freitag CM, Staal W, Klauck SM, Duketis E, Waltes R. Genetics of autistic disorders: Review and clinical implications. Eur Child Adolesc Psychiatry. 2010;19:169–78.
7. Stilp RL, Gernsbacher MA, Schweigert EK, Arneson CL, Goldsmith HH. Genetic variance for autism screening items in an unselected sample of toddler-age twins. J Am Acad Child Adolesc Psychiatry. 2010;49:267–76.
8. Kogan MD, Blumberg SJ, Schieve LA, Boyle CA, Perrin JM, Ghandour RM, et al. Prevalence of parent-reported diagnosis of autism spectrum disorder among children in the US, 2007. Pediatrics. 2009;124:1395–403.

9. DSM-IV. Diagnostic and statistical manual of mental disorders. 4th ed. Arlington, VA: American Psychiatric Association; 2000. 943 p.

10. Kanner L. Autistic disturbances of affective contact. Nervous Child. 1943;2:217–50.

11. DSM-III. Diagnostic and statistical manual of mental disorders. 3rd ed. Arlington, VA: American Psychiatric Association; 1980. 567 p.

12. Centers for Disease Control and Prevention (CDC). Autism and Developmental Disabilities Monitoring Network surveillance year 2006. Principal investigators. Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, United States, 2006. MMWR Surveill Summ. 2009;58(10):1–20.

13. King M, Bearman P. Diagnostic change and the increased prevalence of autism. Int J Epidemiol. 2009;38:1224–34.

14. Yazbak FE. Autism in the United States: A perspective. J Am Physicians Surg. 2003;8:103–7.

15. Avchen RN, Wiggins LD, Devine O, Van Naarden Braun K, Rice C, Hobson NC, et al. Evaluation of a records-review surveillance system used to determine the prevalence of autism spectrum disorders. J Autism Dev Disord. 2011;41:227–36.

16. Larsson M, Weiss B, Janson S, Sundell J, Bornehag CG. Associations between indoor environmental factors and parental-reported autistic spectrum disorders in children 6–8 years of age. Neurotoxicology. 2009;30:822–31.

17. Becker KG, Schultz ST. Similarities in features of autism and asthma and a possible link to acetaminophen use. Med Hypotheses. 2010;74:7–11.

18. Curtin C, Anderson SE, Must A, Bandini L. The prevalence of obesity in children with autism: A secondary data analysis using nationally representative data from the National Survey of Children's Health. BMC Pediatr. 2010;10:11.

19. Dietert RR, Dietert JM. Strategies for protecting your child's immune system. Singapore: World Scientific Publications; 2010.

20. Knapp M, Romeo R, Beecham J. Economic cost of autism in the UK. Autism. 2009;13:317–36.

21. Lee LC, Harrington RA, Louie BB, Newschaffer CJ. Children with autism: Quality of life and parental concerns. J Autism Dev Disord. 2008;38:1147–60.

22. Montes G, Halterman JS. Child care problems and employment among families with preschool-aged children with autism in the United States. Pediatrics. 2008;122:e202–8.

23. Montes G, Halterman JS. Association of childhood autism spectrum disorders and loss of family income. Pediatrics. 2008;121:e821–6.

24. Estes A, Munson J, Dawson G, Koehler E, Zhou XH, Abbott R. Parenting stress and psychological functioning among mothers of preschool children with autism and developmental delay. Autism. 2009;13:375–87.

25. Goldman SE, Surdyka K, Cuevas R, Adkins K, Wang L, Malow BA. Defining the sleep phenotype in children with autism. Dev Neuropsychol. 2009;34:560–73.

26. Souders MC, Mason TB, Valladares O, Bucan M, Levy SE, Mandell DS, et al. Sleep behaviors and sleep quality in children with autism spectrum disorders. Sleep. 2009;32:1566–78.

27. Glickman G. Circadian rhythms and sleep in children with autism. Neurosci Biobehav Rev. 2010;34:755–68.

28. Lopez-Wagner MC, Hoffman CD, Sweeney DP, Hodge D, Gilliam JE. Sleep problems of parents of typically developing children and parents of children with autism. J Genet Psychol. 2008;169:245–59.

29. Järbrink K. The economic consequences of autistic spectrum disorder among children in a Swedish municipality. Autism. 2007;11:453–63.

30. Cimera RE, Cowan RJ. The costs of services and employment outcomes achieved by adults with autism in the US. Autism. 2009;13:285–302.

31. Golnik A, Ireland M, Borowsky IW. Medical homes for children with autism: A physician survey. Pediatrics. 2009;123:966–71.

32. Stoltenberg C, Schjølberg S, Bresnahan M, Hornig M, Hirtz D, Dahl C, et al. ABC Study Group. The Autism Birth Cohort: A paradigm for gene-environment-timing research. Mol Psychiatry. 2010;15:676–80.

33. Li YF, Langholz B, Salam MT, Gilliland FD. Maternal and grandmaternal smoking patterns are associated with early childhood asthma. Chest. 2005;127:1232–41.

34. Schanen NC. Epigenetic of autism spectrum disorders. Hum Mol Genet. 2006;15:R138–50.

35. Nagarajan RP, Patzel KA, Martin M, Yasui DH, Swanberg SE, Hertz-Picciotto I, et al. MECP2 promoter methylation and X chromosome inactivation in autism. Autism Res. 2008;1:169–78.

36. Dietert RR, Dietert JM. Potential for early-life immune insult including developmental immunotoxicity in autism and autism spectrum disorders: Focus on critical windows of immune vulnerability. J Toxicol Environ Health B Crit Rev. 2008;11:660–80.

37. Arndt TL, Stodgell CJ, Rodier PM. The teratology of autism. Int J Dev Neurosci. 2005;23:189–99.

38. Bilder D, Pinborough-Zimmerman J, Miller J, McMahon W. Prenatal, perinatal, and neonatal factors associated with autism spectrum disorders. Pediatrics. 2009;123:1293–300.

39. Dufour-Rainfray D, Vourc'h P, Le Guisquet AM, Garreau L, Ternant D, Bodard S, et al. Behavior and serotonergic disorders in rats exposed prenatally to valproate: A model for autism. Neurosci Lett. 2010;470:55–9.

40. Buchmayer S, Johansson S, Johansson A, Hultman CM, Sparén P, Cnattingius S. Can association between preterm birth and autism be explained by maternal or neonatal morbidity? Pediatrics. 2009;124:e817–25.

41. Schlotz W, Phillips DI. Fetal origins of mental health: Evidence and mechanisms. Brain Behav Immun. 2009;23:905–16.

42. Johansson M, Wentz E, Fernell E, Strömland K, Miller MT, Gillberg C. Autistic spectrum disorders in Möbius sequence: A comprehensive study of 25 individuals. Dev Med Child Neurol. 2001;43:338–45.

43. Rinaldi T, Kulangara K, Antoniello K, Markram H. Elevated NMDA receptor levels and enhanced postsynaptic long-term potentiation induced by prenatal exposure to valproic acid. Proc Natl Acad Sci USA. 2007;104:13501–6.

44. Brown GE, Jones SD, MacKewn AS, Plank EJ. An exploration of possible pre- and postnatal correlates of autism: A pilot survey. Psychol Rep. 2008;102:273–82.

45. Ploeger A, Raijmakers ME, van der Maas HL, Galis F. The association between autism and errors in early embryogenesis: What is the causal mechanism? Biol Psychiatry. 2010;67:602–7.

46. Yamashita Y, Fujimoto C, Nakajima E, Isagai T, Matsuishi T. Possible association between congenital cytomegalovirus infection and autistic disorder. J Autism Dev Disord. 2003;33:455–9.

47. Herbert MR, Russo JP, Yang S, Roohi J, Blaxill M, Kahler SG, et al. Autism and environmental genomics. Neurotoxicology. 2006;27:671–84.

48. Herbert MR. Contributions of the environment and environmentally vulnerable physiology to autism spectrum disorders. Curr Opin Neurol. 2010;23:103–10.

49. Grice DE, Buxbaum JD. The genetics of autism spectrum disorders. Neuromolecular Med. 2006;8:451–60.

50. Gilbert SG (Institute of Neurotoxicology and Neurological Disorders). Scientific consensus statement on environmental agents associated with neurodevelopmental disorders. Collaborative on Health and the Environment's Learning and Developmental Disabilities Initiative; 2008 July 1 [accessed 2010 July 6]. Available from: http://www.iceh.org/pdfs/LDDI/LDDIStatement.pdf

51. Daniels JL. Autism and the environment. Environ Health Perspect. 2006;114:A396.

52. Kern JK, Grannemann BD, Trivedi MH, Adams JB. Sulfhydryl-reactive metals in autism. J Toxicol Environ Health A. 2007;70:715–21.

53. Adams JB, Baral M, Geis E, Mitchell J, Ingram J, Hensley A, et al. The severity of autism is associated with toxic metal body burden and red blood cell glutathione levels. J Toxicol. 2009: 532–640.

54. Roberts EM, English PB, Grether JK, Windham GC, Somber L, Wolff C. Maternal residence near agriculture pesticide applications and autism spectrum disorders among children in the California central valley. Environ Health Perspect. 2007;115:1482–9.

55. Messer A. Mini-review: Polybrominated dipenyl ether (PBDE) flame retardants as potential autism risk factors. Physiol Behav. 2010;100:245–9.

56. Kuroda Y. Effects of endocrine disruptors on brain development—Development of novel assay systems for risk assessment. Environ Sci. 2003;10Suppl:23–33.

57. Kimura-Kuroda J, Nagata I, Kuroda Y. Disrupting effects of hydroxyl-polychlorinated biphenyl (PCB) congeners on neuronal development of cerebellar Purkinje cells: A possible causal factor for developmental brain disorders? Chemosphere. 2007;67:S412–20.

58. Torres AR. Is fever suppression involved in the etiology of autism and neurodevelopmental disorders? BMC Pediatr. 2003;3:9.

59. Alsdorf R, Wyszynski DF. Teratogenicity of sodium valproate. Expert Opin Drug Saf. 2005;4:345–53.

60. Ornoy A. Valproic acid in pregnancy: How much are we endangering the embryo and fetus? Reprod Toxicol. 2009; 28:1–10.

61. Bromley RL, Mawer G, Clayton-Smith J, Baker GA; Liverpool and Manchester Neurodevelopment Group. Autism spectrum disorders following in utero exposure to antiepileptic drugs. Neurology. 2008;71:1923–4.

62. Kolozsi E, Mackenzie RN, Roullet FI, deCatanzaro D, Foster JA. Prenatal exposure to valproic acid leads to reduced expression of synaptic adhesion molecule neuroligin 3 in mice. Neuroscience. 2009;163:1201–10.

63. Narita M, Oyabu A, Imura Y, Kamada N, Yokoyama T, Tano K, et al. Nonexploratory movement and behavioral alterations in a thalidomide or valproic acid-induced autism model rat. Neurosci Res. 2010;66:2–6.

64. Schneider T, Przewłocki R. Behavioral alterations in rats prenatally exposed to valproic acid: Animal model of autism. Neuropsychopharmacology. 2005;30:80–9.

65. Schneider T, Ziòłkowska B, Gieryk A, Tyminska A, Przewłocki R. Prenatal exposure to valproic acid disturbs the enkephalinergic system functioning, basal hedonic tone, and emotional responses in an animal model of autism. Psychopharmacology (Berl). 2007;193:547–55.

66. Schneider T, Roman A, Basta-Kaim A, Kubera M, Budziszewska B, Schneider K, et al. Gender-specific behavioral and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid. Psychoneuroendocrinology. 2008;33:728–40.

67. Ito T, Ando H, Suzuki T, Ogura T, Hotta K, Imamura Y, et al. Identification of a primary target of thalidomide teratogenicity. Science. 2010;327:1345–50.

68. Strömland K, Nordin V, Miller M, Akerström B, Gillberg C. Autism in thalidomide embryopathy: A population study. Dev Med Child Neurol. 1994;36:351–6.

69. Rodier PM. Converging evidence for brain stem injury in autism. Dev Psychopathol. 2002;14:537–57.

70. Bandim JM, Ventura LO, Miller MT, Almeida HC, Costa AE. Autism and Möbius sequence: An exploratory study of children in northeastern Brazil. Arq Neuropsiquiatr. 2003;61: 181–5.

71. Witter FR, Zimmerman AW, Reichmann JP, Connors SL. In utero beta 2 adrenergic agonist exposure and adverse neurophysiologic and behavioral outcomes. Am J Obstet Gynecol. 2009;201:553–9.

72. Zerrate MC, Pletnikov M, Connors SL, Vargas DL, Seidler FJ, Zimmerman AW, et al. Neuroinflammation and behavioral abnormalities after neonatal terbutaline treatment in rats: Implications for autism. J Pharmacol Exp Ther. 2007;322: 16–22.

73. Enstrom AM, Onore CE, Van de Water JA, Ashwood P. Differential monocyte responses to TLR ligands in children with autism spectrum disorders. Brain Behav Immun. 2010;24: 64–71.

74. Schultz ST, Klonoff-Cohen HS, Wingard DL, Akshoomoff NA, Macera CA, Ji M. Acetaminophen (paracetamol) use, measles-mumps-rubella vaccination, and autistic disorder: The results of a parent survey. Autism. 2008;12:293–307.

75. Cannell JJ. Autism and vitamin D. Med Hypotheses. 2008;70: 750–9.

76. Currenti SA. Understanding and determining the etiology of autism. Cell Mol Neurobiol. 2010;30:161–71.

77. Grant WB, Soles CM. Epidemiologic evidence supporting the role of maternal vitamin D deficiency as a risk factor for the development of infantile autism. Dermatoendocrinology. 2009;1:223–8.

78. Fernell E, Barnevik-Olsson M, Bågenholm G, Gillberg C, Gustafsson S, Sääf M. Serum levels of 25-hydroxyvitamin D in mothers of Swedish and of Somali origin who have children with and without autism. Acta Paediatr. 2010;99:743–7.

79. Moore M, Piazza A, Nolan Y, Lynch MA. Treatment with dexamethasone and vitamin D3 attenuates neuroinflammatory age-related changes in rat hippocampus. Synapse. 2007;61: 851–61.

80. Kinney DK, Barch DH, Chayka B, Napoleon S, Munir KM. Environmental risk factors for autism: Do they help cause de novo genetic mutations that contribute to the disorder? Med Hypotheses. 2010;74:102–6.

81. James SJ, Melnyk S, Jernigan S, Pavliv O, Trusty T, Lehman S, et al. A functional polymorphism in the reduced folate carrier gene and DNA hypomethylation in mothers of children with autism. Am J Med Genet B Neuropsychiatr Genet. 2010;153B: 1209–20.

82. Greenblatt JM, Huffman LC, Reiss AL. Folic acid in neurodevelopment and child psychiatry. Prog Neuropsychopharmacol Biol Psychiatry. 1994;18:647–60.

83. Julvez J, Fortuny J, Mendez M, Torrent M, Ribas-Fitó N, Sunyer J. Maternal use of folic acid supplements during pregnancy and four-year-old neurodevelopment in a

population-based birth cohort. Paediatr Perinat Epidemiol. 2009;23:199–206.

84. Ramaekers VT, Blau N, Sequeira JM, Nassogne MC, Quadros EV. Folate receptor autoimmunity and cerebral folate deficiency in low-functioning autism with neurological deficits. Neuropediatrics. 2007;38:276–81.

85. Main PA, Angley MT, Thomas P, O'Doherty CE, Fenech M. Folate and methionine metabolism in autism: A systematic review. Am J Clin Nutr. 2010;91:1598–620.

86. Patterson PH. Immune involvement in schizophrenia and autism: Etiology, pathology and animal models. Behav Brain Res. 2009;204:313–21.

87. Brown AS, Derkits EJ. Prenatal infection and schizophrenia: A review of epidemiologic and translational studies. Am J Psychiatry. 2010;167:261–80.

88. Nawa H, Takei N. Recent progress in animal modeling of immune inflammatory processes in schizophrenia: Implication of specific cytokines. Neurosci Res. 2006;56:2–13.

89. Libbey JE, Sweeten TL, McMahon WM, Fujinami RS. Autistic disorder and viral infections. J Neurovirol. 2005;11:1–10.

90. Hwang SJ, Chen YS. Congenital rubella syndrome with autistic disorder. J Chin Med Assoc. 2010;73:104–7.

91. Fortier ME, Luheshi GN, Boksa P. Effects of prenatal infection on prepulse inhibition in the rat depend on the nature of the infectious agent and the stage of pregnancy. Behav Brain Res. 2007;181:270–7.

92. Atladóttir HO, Thorsen P, Ostergaard L, Schendel DE, Lemcke S, Abdallah M, et al. Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. J Autism Dev Disord. 2010;40:1423–30.

93. Girard S, Tremblay L, Lepage M, Sébire G. IL-1 receptor antagonist protects against placental and neurodevelopmental defects induced by maternal inflammation. J Immunol. 2010;184:3997–4005.

94. Dietert RR. Distinguishing environmental causes of immune dysfunction from pediatric trigger of disease. Open Pediatr Med J. 2009;3:38–44.

95. Uchiyama T, Kurosawa M, Inaba Y. MMR-vaccine and regression in autism spectrum disorders: Negative results presented from Japan. J Autism Dev Disord. 2007;37:210–7.

96. Baird G, Pickles A, Simonoff E, Charman T, Sullivan P, Chandler S, et al. Measles vaccination and antibody response in autism spectrum disorders. Arch Dis Child. 2008;93:832–7.

97. Mrozek-Budzyn D, Kiełtyka A, Majewska R. Lack of association between measles-mumps-rubella vaccination and autism in children: A case-control study. Pediatr Infect Dis J. 2010;29:397–400.

98. House RV. Fundamentals of clinical immunotoxicology. Methods Mol Biol. 2010;598:363–84.

99. McDonald KL, Huq SI, Lix LM, Becker AB, Kozyrskyj AL. Delay in diphtheria, pertussis, tetanus vaccination is associated with a reduced risk of childhood asthma. J Allergy Clin Immunol. 2008;121:626–31.

100. Cohly HH, Panja A. Immunological findings in autism. Int Rev Neurobiol. 2005;71:317–41.

101. Ashwood P, Enstrom A, Krakowiak P, Hertz-Picciotto I, Hansen RL, Croen LA, et al. Decreased transforming growth factor beta1 in autism: A potential link between immune dysregulation and impairment in clinical behavioral outcomes. J Neuroimmunol. 2008;204:149–53.

102. Beversdorf DQ, Manning SE, Hillier A, Anderson SL, Nordgren RE, Walters SE, et al. Timing of prenatal stressors and autism. J Autism Dev Disord. 2005;35:471–8.

103. Mueller BR, Bale TL. Sex-specific programming of offspring emotionality after stress early in pregnancy. J Neurosci. 2008;28:9055–65.

104. Baron-Cohen S, Knickmeyer RC, Belmonte MK. Sex differences in the brain: Implications for explaining autism. Science. 2005;310:819–23.

105. Auyeung B, Baron-Cohen S, Ashwin E, Knickmeyer R, Taylor K, Hackett G. Fetal testosterone and autistic traits. Br J Psychol. 2009;100(Pt 1):1–22.

106. Jones KL, Smith RM, Edwards KS, Givens B, Tilley MR, Beversdorf DQ. Combine effect of maternal serotonin transporter genotype and prenatal stress in modulating offspring social interaction in mice. Int J Dev Neurosci. 2010;28:529–36.

107. Li J, Vestergaard M, Obel C, Christensen J, Precht DH, Lu M, et al. A nationwide study on the risk of autism after prenatal stress exposure to maternal bereavement. Pediatrics. 2009;123:1102–7.

108. Gluckman PD, Hanson MA, Mitchell MD. Developmental origins of health and disease: Reducing the burden of chronic disease in the next generation. Genome Med. 2010;2:14.

109. Miyazaki K, Narita N, Narita M. Maternal administration of thalidomide or valproic acid causes abnormal serotonergic neurons in the offspring: Implication for pathogenesis of autism. Int J Dev Neurosci. 2005;23:287–97.

110. Fatemi SH, Folsom TD, Reutiman TJ, Huang H, Oishi K, Mori S. Prenatal viral infection of mice at E16 causes changes in gene expression in hippocampi of the offspring. Eur Neuropsychopharmacol. 2009;19:648–53.

111. Connors SL, Crowell DE, Eberhart CG, Copeland J, Newschaffer CJ, Spence SJ, et al. Beta2-adrenergic receptor activation and genetic polymorphisms in autism: Data from dizygotic twins. J Child Neurol. 2005;20:876–84.

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