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Risk factors in autism: Thinking outside the brain

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Abstract

Autism spectrum disorders (ASD) are complex neurodevelopmental conditions that have been rising markedly in prevalence for the past 30 years, now thought to affect 1 in 68 in the United States. This has prompted the search for possible explanations, and has even resulted in some controversy regarding the "true" prevalence of autism. ASD are influenced by a variety of genetic, environmental, and possibly immunological factors that act during critical periods to alter key developmental processes. This can affect multiple systems and manifests as the social and behavioral deficits that define these disorders. The interaction of environmental exposures in the context of an individual's genetic susceptibilities manifests differently in each case, leading to heterogeneous phenotypes and varied comorbid symptoms within the disorder. This has also made it very difficult to elucidate underlying genes and exposure profiles, but progress is being made in this area. Some pharmaceutical drugs, toxicants, and metabolic and nutritional factors have been identified in epidemiological studies as increasing autism risk, especially during the prenatal period. Immunologic risk factors, including maternal infection during pregnancy, autoantibodies to fetal brain proteins, and familial autoimmune disease, have consistently been observed across multiple studies, as have immune abnormalities in individuals with ASD. Mechanistic research using animal models and patient-derived stem cells will help researchers to understand the complex etiology of these neurodevelopmental disorders, which will lead to more effective therapies and preventative strategies. Proposed therapies that need more investigation include special diets, probiotics, immune modulation, oxytocin, and personalized pharmacogenomic targets. The ongoing search for biomarkers and better treatments will result in earlier identification of ASD and provide much needed help and relief for afflicted families.

Keywords

Autism; Gene-environment; Immune dysfunction; Risk; Maternal factors

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1. Introduction

Autism is a spectrum of neurodevelopmental disorders that manifest in early childhood. The core features of autism spectrum disorders (ASD) include persistent difficulties in verbal and nonverbal communication, along with restricted, repetitive patterns of behavior, interests, or activities [1]. There has been much debate and inquiry surrounding the nature of the increase in prevalence of ASD, and recent reports certainly paint a picture of concern. In the United States (US), these heterogeneous neurodevelopmental disorders are currently estimated to affect 1 in 68 (or 147 in 10,000, reported in 2010 for birth year 2002), which is 30% higher than the previous (2008) estimate of 1 in 88 [2]. This is but a glimpse into the overall trend that has been ongoing for over 20 years in the US, which appears to extend globally. Available reports from other countries show variable rates of autism prevalence with definite increases over time, but these estimates are mostly limited to high-income countries [3]. Other countries reporting high rates of autistic disorder (AD) include Sweden (72.6 cases per 10,000 in 1999) and South Korea (94 cases per 10,000 in 2011), and high rates of pervasive developmental disorders (PDD) have also been reported in South Korea (189 cases per 10,000 in 2011) as well as the United Kingdom (116.1 cases per 10,000 in 2006) and Japan (181.1 cases per 10,000 in 2008) [3]. *But is this observed rise in prevalence real (due to increasing cases/incidence), or is it due to heightened awareness and changes in diagnostic criteria (reviewed in Ref.* [4]*)?* There is evidence to support both positions.

Earlier this year, a population-based study of a large Danish cohort found that 60% of the increase in documented autism prevalence could be attributed to changes in reporting practices, including changes in diagnostic criteria [5]. Similarly, a recent report from Sweden concluded that autism symptom phenotype prevalence was actually stable during the study period, and administrative changes were responsible for the apparent increase in official registered prevalence [6]. A slightly older report using data from the US suggests that greater than 70% of the tracked rise in autism since the late 1980s represents a true increase in the disorder rather than a constant-prevalence condition that was underdiagnosed in the past [7]. In 2009, another investigation concluded that while several factors, including changes in diagnostic criteria, an earlier age of diagnosis, and the inclusion of milder cases, did contribute to the observed rise in autism, these could not account for the full extent of the increase [8]. This is clearly a complex and difficult metric to capture, and despite great efforts to standardize diagnostic criteria across regions and countries, we may never know the actual prevalence of ASD. However, the majority of reports and findings from the US seem to support the frequency of ASD being on the rise.

But what makes it so difficult to accept these statistics, and why do we keep searching for alternate explanations? Is it too alarming or frightening to consider that autism rates could really be increasing this quickly? Are the data not compelling enough in either direction? Whether or not the increase is “real” does not change the fact that a large proportion of individuals with ASD are severely impaired, and a report from California earlier this year indicates that the number of substantially disabled cases is also increasing dramatically [9]. The majority of individuals with ASD also suffer from one or more serious medical comorbidities such as gastrointestinal (GI) distress, immune system dysfunction, seizures, sleep disorders, and psychiatric problems, in addition to the core social and behavioral

features that define this disorder [10]. On the more extreme end, patients with autism can be completely nonverbal, exhibit aggressive or self-injurious behavior, intellectual disability, a personality disorder, or some combination. Those individuals that fall on the more drastically impaired end of the spectrum will require lifelong care, which can have negative psychological consequences for caregivers, usually immediate family members, in addition to creating substantial economic costs and demand for services [11].

Researchers and the scientific community recognize the need to continue their focus on deciphering the etiology of ASD in order to identify risk factors, prevent adverse outcomes, provide early identification, and better treat patients. Great progress has already been made in this area, with increasing evidence for and recognition of the important roles the environment and immune system play in ASD, and how this information can be used to improve lives.

2. More than genetics: environmental factors

Although ASD has been shown to be highly heritable, there is more to the story than can be solely explained by genetics. Several genetic syndromes are known to have significant associations with autism (for review, see Ref. [12]), but these account for a minority of cases. And while the exact ranges from different studies vary, monozygotic twin concordance rates of autism and associated conditions consistently amount to less than 100%, which strongly suggests a role for environmental factors [13]. Indeed, epidemiological studies have identified numerous correlations between non-genetic influences and ASD, opening the doors for further studies to investigate mechanisms, establish causation, and in some instances promote regulatory actions [14].

Maternal treatment with pharmaceutical drugs such as the valproic acid, thalidomide, and antidepressants (specifically selective serotonin reuptake inhibitors), especially during the first trimester of pregnancy, has been associated with an increased risk of ASD in the child [15,16]. However, it can be difficult to decipher medication-related effects from those of the mother’s underlying condition that may also influence autism risk [17]. Exposure to various toxicants including pesticides, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs), can have detrimental consequences on developmental processes, particularly for genetically susceptible individuals. Not only do PCBs and PBDEs have endocrine-disrupting and neurotoxic effects, but both also persist in the environment and bioaccumulate up the food chain [18]. In addition, many neurotoxic compounds are suspected to interfere with neurotransmitter systems also implicated in ASD [19]. Maternal residential proximity to agricultural applications of pesticides during pregnancy has been associated with an increased risk of ASD, but this scenario may represent abnormally high exposure levels [20]. These chemicals have the additional potential to cause immunotoxicity, which may lead to altered cytokine production frequently observed in ASD [21].

Although advanced maternal and paternal age are well established risk factors for autism, a recent, large international study reaffirmed this and also found that ASD risk is increased when parents are disparately aged [14,22]. It is thought that advanced parental age contributes to methylation defects in gametes, which can be induced through increased

oxidative stress causing DNA damage and fragmentation [23]. Maternal metabolic and nutritional risk factors include obesity, diabetes, and folic acid deficiency, while zinc deficiency has been noted in autistic children and may contribute to pathophysiology [14,17,20]. Air pollution has been linked to an increased risk of ASD, including prenatal exposure to heavy metals, chlorinated solvents, ozone, diesel, small particulate matter, and residential proximity to freeways [17,20]. As humans are introduced to numerous external environmental insults that may adversely impact fetal development, establishing relevant exposure profiles will be difficult yet essential to promote preventative measures.

3. Immune considerations

A great number of independent studies have now implicated a role for the immune system in ASD during both the prenatal and postnatal periods, and many research projects continue to further investigate this link [24,25]. It will be critical to establish whether these immune abnormalities are a cause or consequence of the alterations in neurodevelopment, or merely an epiphenomenon in ASD.

3.1. Maternal infection during pregnancy

Multiple epidemiological studies provide strong evidence for a connection between maternal infection or the presence of a fever during pregnancy and the development of ASD in the child, for at least a subset of individuals [26]. In 2010 a large, exploratory, population-based Danish study revealed associations between a diagnosis of ASD in the child and hospitalization of the mother for either viral infection during the first trimester for birth years 1994–2005, or bacterial infection during the second trimester for all birth years examined in the study (1980–2005) [27]. Importantly, this study did not find an overall association between all infections during the total length of pregnancy and an ASD diagnosis in the child, suggesting the vital importance of timing of infection as a risk factor. A similar conclusion regarding this overall association was reached in a separate study population drawn from members of Kaiser Permanente of Northern California in 2013 [28]. However, this study did find that women who were diagnosed with any infections, especially bacterial infections, during a hospital admission had an increased risk of having a child with ASD. This was also observed for multiple infections during pregnancy, suggesting that the severity of maternal immune activation, like timing, is an important consideration in determining ASD risk.

Cytokines, which are produced during states of infection to cause fever and activate immune cells, represent diffusible factors that can potentially be transferred from mother to fetus [29]. Since cytokines have also been strongly implicated in neurodevelopmental processes, any perturbation of these tightly controlled systems could result in pathophysiological changes in the developing brain [30,31]. Indeed, increased levels of interferon gamma (IFN-γ) and interleukins 4 and 5 (IL-4, IL-5) have been found in mid-pregnancy blood samples from mothers of children with ASD, and significantly elevated levels of tumor necrosis factors alpha and beta (TNF-α, TNF-β), IL-4, and IL-10 were found in amniotic fluid samples from individuals with ASD [32,33]. In addition, an association between maternal fever during pregnancy and ASD was revealed in a 2013 study, which also found that

treatment with anti-fever medication reduced the risk of ASD in the child [34]. Together, these data collectively showcase maternal immune exposures that have the potential to alter fetal development.

3.2. Autoimmunity

Further support of the link between activation of the maternal immune system and ASD comes from epidemiological studies of incidence of familial autoimmunity. A recently released systematic review and meta-analysis of nine case–control studies and one cohort study from around the world found that maternal autoimmune diseases developed during pregnancy increased the risk of having a child with ASD, again reinforcing that abnormal maternal immune activation can have detrimental consequences for fetal development [35]. This review also found a positive association between maternal thyroid disease and ASD, and other studies have shown associations with maternal diabetes, including type 1, psoriasis, rheumatoid arthritis, celiac disease, and systemic lupus erythematosus (SLE) [36–40]. A population-based Swedish case–control report suggests that any parental autoimmune disease results in a 50% higher risk of an autism diagnosis by age 10 [41]. Regarding autoimmunity in patients with ASD themselves, at least one retrospective study reports a higher comorbidity rate for type 1 diabetes mellitus and inflammatory bowel disease in the ASD group compared to a general hospital population, although the incidence of other autoimmune diseases was found to be no different [42].

Autoimmune conditions can be passed from mother to child, largely through maternal transfer of antibodies, as in cases of neonatal lupus erythematosus (NLE). Immunoglobulin G (IgG) antibodies are transported across the placenta via the neonatal Fc receptor (FcRn) to confer passive immunity to the fetus starting in the second trimester. However, this nondiscriminatory mechanism can potentially allow self-reactive antibodies to cross into fetal circulation, and several independent groups have reported the presence of such antibodies in mothers of children with ASD [43,44]. Notably, mothers harboring these antibodies also exhibit an increased incidence of autoimmune diseases [45]. Furthermore, a combination of maternal antibodies specific to ASD that recognize targets in the developing brain were associated with elevated stereotypical behaviors [46]. Further studies will investigate how the presence of these antibodies may interfere with neurodevelopment, as well as their clinical significance as a biomarker for a subset of ASD.

3.3. Immune dysfunction

Aside from maternal factors *in utero*, there are also multiple reports of both innate and adaptive immune dysfunction in individuals with autism. For instance, monocytes isolated from children with ASD were shown to have differential cytokine responses upon stimulation with toll-like receptor (TLR) ligands when compared to control children [47]. A similar finding of differential cytokine production from children with ASD was made following selective stimulation of T cells, with pro-inflammatory, T_H1-skewed profiles correlating with more impaired behaviors [48]. Other immune cell anomalies found in children with autism include: increased numbers of B cells and natural killer (NK) cells, differential expression of cell surface markers, increased frequencies of myeloid dendritic cells, and decreased plasma levels of IgG and IgM (without evidence of overt B cell

dysfunction) [49–51]. In addition, accumulating evidence supports initial findings of cytokine and chemokine dysregulation in both the brains and periphery (plasma/serum) in patients with ASD, which in some cases correlates with worsening behavioral measures [52–57]. Mechanistic studies are needed to further investigate these descriptive relationships.

Recent findings from RNA transcriptomic analyses have reinforced the idea that there is a robust and detectable increase in immune activation status in the brains and leukocytes of people with ASD, and it may be possible to harness these peripheral genomic biomarkers for clinical diagnostic screenings [58–60]. Related evidence of both microglial and astrocytic activation in post-mortem brain tissues from individuals with ASD supports a role for chronic neuroinflammatory processes that could potentially alter synaptic connections and change brain connectivity, contributing to ASD pathology [61]. In the future, it will be critical to establish how these immune findings relate to the development and symptoms of ASD and how they can be utilized for clinical screening, diagnoses, and treatments.

3.4. GI dysfunction and altered microbiome

Many patients with autism report chronic comorbid GI distress, which is of interest considering that proper immune system functioning and the symbiotic microbial population are critical to GI tract health. In addition, the brain-gut-microbiome connection is increasingly recognized as playing an important role in proper neurodevelopment and also has the ability to modify behavior [62,63]. GI symptoms frequently reported in ASD include abdominal pain, constipation, diarrhea, and food allergy/intolerance, the latter of which raises concerns about proper nutrition intake and diet quality [64]. A recent study utilizing prospective maternal report from the population-based Norwegian Mother and Child Cohort found that GI symptoms are more common and persistent in infants and toddlers with ASD compared to those with developmental delay (DD) or typically developing (TD) counterparts [65]. Increased intestinal permeability, or “leaky gut”, has also been observed in patients with ASD, indicating barrier defects that can affect the mucosal immune system and lead to changes in gut flora. Indeed, alterations in the composition of microbial communities have been detected in ASD, as well as corresponding variations in levels of bacterial metabolites in feces and urine [66]. Notably, increased severity of autism symptoms has been found to correlate with an increased risk of having GI problems, but how this GI dysbiosis relates to the etiology of ASD remains to be determined [67].

4. Future directions

4.1. Gene-environment interactions

Epigenetics, especially gene-environment (G × E) interactions, have become a topic of great interest and examination in ASD research [68]. By this model, a genetically susceptible pathway receives further injury from an environmental insult, likely during a specific window of developmental vulnerability, resulting in the changes that cause pathophysiology. Genetic or heritable factors may also predispose an individual to certain environmental exposures, which helps to explain both the diversity of phenotypes as well as the wide array of genes and environmental factors implicated in ASD. It has been postulated that if these two factors converge to dysregulate the same signaling pathways that underlie a fundamental

process such as neuronal connectivity during critical periods, there is a strong possibility of creating the necessary combination of conditions to adversely alter neurodevelopment [69]. In support of this, a recent study using a large, well-characterized ASD cohort has found that individuals harboring ASD-associated copy number variants (CNVs) whose mothers experienced infection or fever during pregnancy exhibited more severe behavioral phenotypes compared to individuals with the genetic predisposition or exposure alone [70]. Identifying all of these complex interactions will be challenging, but high throughput techniques and advances in methodologies will greatly aid in future research and discovery efforts.

4.2. Animal models

Animal models are increasingly utilized to investigate the intricate processes and mechanisms underlying ASD behaviors and phenotypes, and they are necessary for any kind of drug or therapeutic testing [71]. Both rodent and non-human primate models of maternal immune activation (MIA) have shown that even in the absence of overt infection, activation of the maternal immune system using viral and bacterial mimics as well as the cytokine IL-6 can result in behavioral changes in the offspring [72–74]. Other measures, including fetal immune factors and gene expression patterns and morphological changes in the fetal brain, have been found to be dependent on mouse strain, MIA-inducing agent, and timing of exposure, again strongly suggesting a role for G × E interactions [75,76]. Interestingly, GI abnormalities and shifts in intestinal bacterial populations have been observed in MIA and other autism murine models, and one study saw improvement in GI and behavioral symptoms following treatment with the human commensal *Bacteroides fragilis* [77,78]. The effects of maternal autoantibody exposure have also been studied in animal models where transfer of human IgG from mothers of ASD children into pregnant females results in increased anxiety, delayed sensory and motor development, and altered sociability in mice and altered brain and social development in monkeys compared to IgG from control mothers [79–81]. A more recent study in which mouse embryos received cerebral intraventricular injections of purified IgG showed that autism-specific maternal autoantibodies bind to radial glial cells in the developing brain, hinting at a possible mechanism for these antibodies [82]. Further studies will be required to elucidate exactly how maternal autoantibodies cause pathology and alter behavior.

4.3. Patient-specific stem cells

The recent explosion of stem cell research, especially the field of induced pluripotent stem cells (iPSCs), has made it possible to produce cell types such as neurons from a patient's own cells. This recently gained access to cell types that were previously unattainable provides the unique opportunity to further model environmental effects on specific genetic backgrounds in a human system and investigate ASD mechanisms *in vitro*. iPSC-derived neurons from various ASD backgrounds show common perturbations such as synaptic deficits and altered morphologies, suggesting shared causal pathways [83]. These techniques have recently allowed researchers to model mid-fetal telencephalic development using iPSCs from patients with ASD and unaffected family members to find that ASD-derived cultures produce an abundance of inhibitory neurons caused by increased expression of the gene FOXG1 [84]. iPSC lines, normally produced from skin fibroblasts, have also been generated

from peripheral blood mononuclear cells (PBMCs) isolated from children with autism, to serve as a less invasive source of starting material [85]. This is an emerging field that holds great potential, and the optimization and standardization of differentiation protocols will allow for the rapid, efficient generation of desired cell types used for mechanistic studies and drug discovery [86].

4.4. Therapeutics

The uncertain etiology and vast clinical heterogeneity of ASD have made it difficult to provide universally effective treatments, and most medications are prescribed to treat comorbid symptoms. Currently, the best therapy for improving core deficits in ASD is intensive early behavioral intervention, which is why it has become critical to define a set of biomarkers to aid with diagnosis at a younger age, even before aberrant behaviors may be apparent [10]. Plus, it is hopeful that the identification of relevant cellular pathways and refinement of sub-groups will lead to more tailored and efficacious remedies.

Because of the frequent GI comorbidities and altered brain-gut-microbiome axis experienced in ASD, therapies such as dietary interventions and probiotics have been proposed to help at least a subset of patients. In particular, a gluten-free and/or casein-free diet might be recommended to combat leaky gut and intestinal inflammation, and although some studies suggest behavioral improvements following the diet, the scientific evidence regarding the effectiveness of this alternative treatment is still very limited [87,88]. The immune system represents another appealing potential therapeutic target for both mitigating the effects of fever or inflammation during pregnancy, as well as addressing immune abnormalities in patients with autism. To this end, immune-modulating and anti-inflammatory drugs such as corticosteroids have shown some behavioral improvements in open-label trials and case studies of patients with ASD, but these types of treatments should be more targeted toward specific subtypes and validated in larger, more rigorous trials [89]. Another emerging, individualized treatment approach is pharmacogenomics, which aims to use personal genetic information to guide pharmacologic interventions that maximize therapeutic benefits while minimizing side-effects [90]. Oxytocin-based therapies have also been investigated in hopes of improving the social behaviors core to ASD, but again any beneficial effects shown in previous studies need to be replicated in larger trials [91]. Overall, much more research is needed on these and other emerging and alternative therapies before they will achieve clinical significance and improve ASD outcomes.

5. Final thoughts/closing remarks

Despite mixed conclusions regarding the true prevalence of ASD, it has become apparent that there is a growing population of people severely afflicted with this disorder globally. The research community has been making great strides in the discovery of exposures, changes, and risk factors that contribute to ASD, but we are still far from a complete understanding of these complex issues. It appears to be of mixed and multifactorial etiology, likely arising *in utero*, and involves various other comorbidities that can be serious medical issues and/or possible contributing factors (Fig. 1). Advances in technologies, techniques, and model systems will greatly aid in the study of disease progression and effective

therapies, however, it remains to be seen if there will ever be a single overarching, universally effective pharmacologic drug for ASD. A much more realistic goal for the research community will be to utilize the heterogeneity of ASD to refine sub-groups and develop individualized, precise treatments based on biological markers, symptoms, and previous exposures that, while we are getting closer, have yet to be clearly defined.

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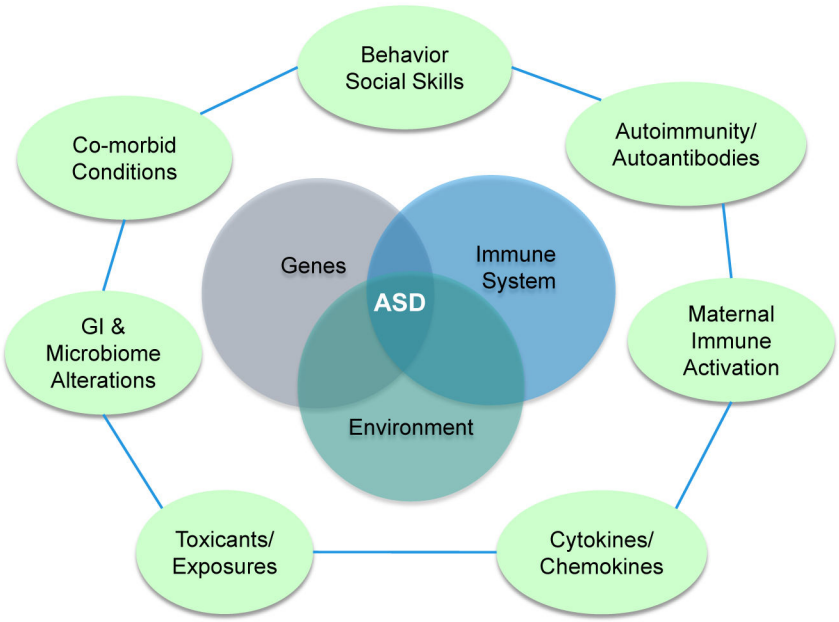

Fig. 1.

Complex relationships and multifactorial etiology lead to heterogeneous and variable phenotypes in ASD. Genes, the environment, and the immune system have all been implicated in ASD. This triad also can influence behavior, comorbid symptoms, and gut pathology. Maternal factors, such as toxicant exposure, infection, autoimmunity, and the presence of anti-fetal brain autoantibodies have the potential to alter neurodevelopment during critical periods and predispose vulnerable individuals to subsequent cytokine dysregulation.