Prenatal factors associated with autism spectrum disorder (ASD)

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Abstract

Autism spectrum disorder (ASD) affecting about 1% of all children is associated, in addition to complex genetic factors, with a variety of prenatal, perinatal and postnatal etiologies. We discuss the known associated prenatal factors affecting the fetus throughout pregnancy; whenever relevant, also summarize some animal data. Among the maternal diseases in pregnancy associated with ASD are pregestational and/or gestational diabetes mellitus (PGDM, GDM), maternal infections (i.e. rubella, cytomegalovirus (CMV)), prolonged fever and maternal inflammation, which cause changes in a variety of inflammatory cytokines. Among the drugs are valproic acid, thalidomide, and possibly misoprostol and serotonin reuptake inhibitors (SSRIs). Associations were described with ethanol, and possibly cocaine, heavy metals heavy smoking and Folic acid deficiency. Heavy exposure to pesticides and air pollution during pregnancy was recently associated with ASD. We need more epidemiologic data to establish many of these associations; if proven, they might be promising avenues for prevention.

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1. Introduction

1.1. Definition of ASD

ASD is defined by the Diagnostic Statistical Manual of Mental Disorders 5 (DSM 5) which is a book published by the American Psychiatric Association and contains the clinical definition of all mental and neurobehavioral disorders, as a neurobehavioral disorder manifested by persistent deficits in social and communication interaction, deficits in developing, understanding and maintaining relationships, as well as abnormal and fixed interests and repetitive behavior [1]. Symptoms must be present at early childhood and interfere with daily function.

ASD is 4–5 times more prevalent in males than in females. It is now one of the most common childhood morbidities presenting in various degrees of severity. Mental impairment is more common in the children with severe presentations of symptoms. The etiology is diverse, largely unknown, and seems to be the result of genetic and environmental interaction [2,3].

Environmental exposures are increasingly being recognized as potential risk factors for ASD, and the possibility that the prenatal environment affects fetal programming is a promising direction for research. Prenatal environment includes maternal use of medication, maternal infection and inflammations, and exposure to various substances such as alcohol and heavy smoking during pregnancy.

1.2. Epidemiology of ASD

The reported prevalence of autism has increased dramatically over time, from 4 to 5 cases per 10,000 in 1966 to approximately 100/10,000 (1%) today [2–4]. The increase is thought to result from a mixture of factors that include increased public awareness and changed diagnostic standards. It is still unclear what proportion of the increase can be explained by these factors and what proportion is due to a true increase in risk.

In the US, for example, the autism and developmental disabilities monitoring network (ADDM network) found in children aged 8 years in the 2008 monitoring at 14 sites in the US an increase from 67/10,000 (0.67%) in 2000 to 114/10,000 (1.14%) in 2008 [5]. Moreover, the same network found in the 2010 monitoring of 8 years old children at 11 sites in the US a further increase of ASD prevalence to 147 per 10,000 children, raising the rate to 1.47% of children. They also found different prevalence of ASD among the different participating sites, ranging from 57 to 219 per ten thousand children! [6]. Significant ethnic differences were also found, with the highest rate among non-Hispanic white children and the lowest among Hispanic children. The reason for this wide geographic and ethnic variation in the rate of ASD is not clear, but could be explained by ascertainment variability due to different diagnostic practices, socioeconomic disparities and differences in the access to medical services. Another study, looking at the prevalence of ASD in metropolitan New Jersey in 2006 in comparison to 2002, found an increase from 106/10,000 in 2002 to 174/10,000 in 2006, with boys outnumbering girls by nearly 5:1 [7].

In Israel, judging from the number of children who received childhood disability benefits by the Israeli Insurance Institute because of ASD, the cumulative incidence at 8 years of age at 2011 has increased 10 folds from 1991 and reached 0.49% with a ratio of males/females of about 5 [8]. In a different study on children that receive medical services from Maccabi health services the rate in 2010 was found to be 0.48% [9]: of 423,524 children aged 1–12 years, 2034 children were diagnosed as having ASD, with a ratio of males to females of 4.9.

The more recent global prevalence of autism was estimated to be 0.62% [5,10]. In spite of a wide variation in prevalence between the studies, the authors conclude that there is no evidence of a significant impact of ethnic or socioeconomic factors on the rate of ASD.

The increasing rate of ASD is mostly attributed to the changes in the clinical definition in both the Diagnostic Statistical Manual of Mental Disorders (DSM) and International Classification of Diseases (ICD) which is the internationally used classification of all diseases, congenital malformations and syndromes. The DSM 4 definition of ASD was more general, including additional clinical symptoms compared to earlier versions of the DSM. Moreover, the increase in awareness, increase in available services for children with ASD and the decrease in the age of diagnosis are very important contributors to the elevated prevalence of ASD [5]. It was also shown that many of the children and adults who were previously diagnosed as having mental impairment have severe presentations of ASD according to the diagnostic criteria of DSM 4 [10,11]. The wide variation in the prevalence of ASD in the different US States that have similar populations [5,6] is an additional proof that the increased prevalence of ASD mainly stems from better ascertainment following the clinical definitions of ASD [5]. Hence, it is expected that the changes in the definition of ASD symptoms, as recently defined in the DSM 5, will have some influence on the prevalence of ASD, although exact figures demonstrating these changes are still lacking [12,13]. Indeed, from an analysis of 14 studies where ASD was diagnosed on the basis of DSM 4 criteria, applying the DSM 5 criteria significantly reduced the rate of ASD, especially of the formerly group of Pervasive Developmental Disorder not Otherwise Specified (PDD NOS) [14].
In spite of the fact that most of the increase in the prevalence of ASD can be explained by better ascertainment, studies on the incidence (new cases per year) of ASD also showed, in spite of only few changes in the available services, a steady increase in the last years, implying that some of the heightened prevalence may result from a true increase in ASD rate. For example, in the UK in 1988–1992 the incidence was 0.40/10,000 person years and it raised to 2.98/10,000 person years in 2000–2001 [15]. The authors attributed this increase in incidence to better ascertainment but could not exclude a real increased incidence. An increased incidence was also reported in Israel where diagnostic and treatment methods are well developed for the last 10–15 years [9]. If this is correct, it may largely result from prenatal environmental causes because genetic, ethnic, socioeconomic and geographic factors did not change significantly during that time.

1.3. Etiology of ASD

The main cause of ASD is genetic. Twin studies have shown a high concordance among homzygous twins which is much lower in discordant twins [3]. However, in search for specific genes responsible for ASD it became obvious that there are numerous candidate genes on many chromosomes, and only rare cases of ASD can be related to specific genes. Thus, ASD seems to result from the interaction of genetic factors and the prenatal and postnatal environment [3].

The prenatal causes of ASD can be divided into environmental chemicals (i.e. drugs such as valproic acid, thalidomide, misoprostol; alcohol, cocaine and toxic metals taken by the mother during pregnancy), exposure to particulate matter (PM) air pollution of up to 2.5 μm in diameter (PM2.5), maternal infections during pregnancy (i.e. rubella, CMV), maternal and fetal inflammation [16] and maternal diseases (i.e. diabetes mellitus), including allergic diseases such as asthma [17]. Advanced maternal and paternal age was also found to increase the risk of ASD [18,19]. An association of ASD with prematurity was also reported by several investigators [20]. In addition, maternal depression and/or emotional strain also seem to play a role. Many studies pointed to different additional possibilities, but some of these possibilities are rare and difficult to prove, and some have no biological plausibility [3,19,21–24].

1.4. Pathogenesis of ASD

Numerous mechanisms for ASD have been offered, based on human studies as well as experimental animal models. In addition to complex genetic susceptibility, epigenetic changes have also been proposed [2]. In the recent years it is proposed that ASD is a result from general immune and metabolic disturbances that affect the brain. One of the mechanisms is immune dysregulation that includes abnormal levels of cytokines and growth factors, fetal and maternal antibodies to brain tissue [16,17], microglial activation, abnormal numbers of CD4 and CD8 cells and a variety of antibodies [21]. Additional proposed mechanisms are increased oxidative stress, with or without mitochondrial dysfunction. Frye et al. found [24] that at least one third of children with ASD meet partial or full criteria for mitochondrial dysfunction as a sign of a systemic metabolic problem in ASD. These children also have a variety of markers for increased oxidative stress, and many also have specific mitochondrial DNA abnormalities.

Other mechanisms, more related to the brain, include abnormal white matter connectivity and altered synapses, as demonstrated by different imaging techniques [25]. For example, by using Diffusion Tensor Imaging – an acceptable method for the demonstration of white matter structure, in 22 young children with ASD, Billeci et al. [25] found increased fiber length in some areas of the corpus callosum, increased fractional anisotropy of the fibers and a variety of connectivity modifications. Some investigators found abnormalities in brain seratonin, zinc deficiency, or alterations in the gut microbiome–brain–interactions, which might lead to anxiety, reduced sociability and social cognition deficits as demonstrated in mice [21,23,26].

The purpose of the present review is to summarize the data associating antenatal exposure to different injurious agents in the etiology of ASD. In addition, some animal studies will also be presented as they often support the findings in humans (e.g. prenatal exposure to valproic acid in rats and mice, and exposure of zebrafish embryo/larva). It should be emphasized that the possibility to reproduce in pregnant animals the data observed in human strengthens the epidemiological and pathogenetic data. However, the lack of capability to reproduce the human data in animals does not necessarily decrease the relevance of the human studies.

2. Maternal diseases and ASD in the offspring:

2.1. ASD in offspring of diabetic mothers

Diabetes mellitus, during pregnancy, has been associated with an increased rate of ASD in the offspring. Pregestational diabetes mellitus (PGDM) and gestational diabetes (GDM) are associated with a large number of pregnancy complications. PGDM may increase the rate of congenital anomalies in the offspring, affect fetal well-being and growth. GDM, which usually develops in the second half of pregnancy, is mainly associated with disturbed fetal growth and increased rate of a variety of pregnancy complications. Both PGDM and GDM are associated with slight disturbances in postnatal growth and development, also affecting fine and gross motor development and increasing the rate of learning difficulties and of attention deficit hyperactivity disorder (ADHD), a common comorbidity neurobehavioral problem in ASD [27].

The negative effects of maternal diabetes on the brain may result from intrauterine increased fetal oxidative stress, epigenetic changes in the expression of several genes and other unknown causes. It was shown repeatedly that good control of diabetes in pregnancy may reduce these complications but apparently will not completely prevent them.

It is not surprising that in the search of antenatal factors which might be associated with ASD, maternal diabetes, because of its rising incidence, was an obvious candidate. Lyall et al. [28] assessed the possible association of maternal diabetes and ASD in 793 children with ASD from a cohort of 66,445 pregnancies. The highest association was found with maternal GDM, as the odds ratio (OR) of ASD among children born to mothers with GD was 1.76. This is in slight contrast to other studies that found that the risk for ASD is higher among offspring of mothers with PGDM compared to mothers with GDM. Gardener et al. [19] assessed the possible association of a variety of antenatal maternal factors with ASD in the offspring. The highest association was with advanced parental age but maternal diabetes was among the other leading factors associated with ASD in 793 children with ASD from a cohort of 66,445 pregnancies. 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twelve; three cohort and nine case control studies. For the cohort studies, the pooled risk of maternal diabetes resulted in a pooled OD of 1.48 (1.25–1.75, 95% CI p < 0.001), and for the case–control studies, the pooled OD was 1.72 (1.24–2.41, 95% CI p = 0.001). The OR for offspring of mothers with GDM was generally lower than for that of mothers with PGDM. The mechanism for producing this association is unknown, although one of the offered mechanisms was increased oxidative stress. Thus, additional investigations are certainly warranted concerning the type of diabetes the mother has, and the severity and glycemic control of the diabetes during pregnancy. We should also remember that the increased risk found may be related to other pregnancy complications that are common in diabetes, or to effects on fetal growth rather than to complications of hyperglycemia. It is also unknown whether optimal control of diabetes will further decrease this association.

3. The infectious and inflammatory origin of ASD

3.1. Infection and inflammation

Adverse intrauterine environment resulting from maternal bacterial and viral infections during pregnancy represents a significant risk factor for several neuropsychiatric disorders including ASD [33]. The association between intrauterine inflammation, infection and ASD is based on both epidemiological studies and case reports. The time that passes from the pregnancy until the diagnosis of ASD and the uncertainty considering maternal morbidity that is based many times on maternal memory, makes the association between intrauterine infection and ASD hard to prove. However, many studies, as specified below, associate maternal infection with ASD [31–39]. Additionally, some studies found that the season or month of birth was significantly related to the risk of ASD. Gardner et al. [34] found in a meta-analysis that although the seasonal trends varied across studies, March and August were both suggested as birth months associated with an elevated risk of ASD and hypothesized that a relationship may be caused by seasonal variation in viral or other infections.

3.2. Population studies

Population studies are based on birth cohorts, ASD registries and data considering maternal infection during pregnancy. Two large epidemiological studies from Scandinavia found increased risk of ASD following maternal hospitalization due to infection. Since infections treated in the community by a general practitioner were not included, there is most probably an under estimation of the infectious rate. Collier et al. [35] reported from the National Perinatal Study (CHAPS) and 2075 matched controls. They found that 3.7% of ASD cases and 2.6% of non-cases had mothers that were hospitalized with a diagnosis of infection during pregnancy. Maternal inpatient diagnosis of infection during pregnancy was associated with increased OR of ASD of 1.37 regardless of whether the infection was bacterial, viral, or other/unknown. In analysis of specific infections, only respiratory infections were statistically significant. The higher risk of ASD was observed during all trimesters: the 1st trimester (OR = 1.24), 2nd trimester (OR = 1.38), and 3rd trimester (OR = 1.36). In a subsample of the total Swedish population, the Stockholm Youth Cohort, the OR for ASD without intellectual disability for inpatient diagnosis of infection was 1.19 but the OR for ASD with intellectual disability increased to 1.50. The association of inpatient diagnosis of infection and ASD with intellectual disability was not related to the type of infection.

Contradictory to their findings, Dadds et al. [39] found among 129,733 children born in Nova Scotia between 1990 and 2002, of which 924 had ASD, that maternal infection during pregnancy did not increase the rate of ASD.

In a meta-analysis of 40 papers published until 2007 Gardner et al. [19] evaluated the relationships between autism and pregnancy-related factors. Combined together there was no statistical increase in ASD: Maternal infection during pregnancy – OR 1.18 (95% CI 0.76–1.73), p = 0.09, Maternal Rubella – OR 1.66 (95% CI 0.84–3.29), p = 0.02, genital infections – OR 0.99 (95% CI 0.22–1.09), p = 0.36, maternal fever – OR 1.24 (95% CI 0.76–2.04), p = 0.27. However when the analysis was limited to the four studies that controlled for multiple covariates or used sibling controls exposure to intra-uterine infections, a significant increase in risk for autism was observed with an OR of 1.82 (95% CI 1.01–3.30).

3.2.1. Case control studies that found associations between maternal infection and ASD

Wilkinson et al. [40] compared 183 autistic children and 209 controls whose mothers completed the Maternal Perinatal Scale (MPS), a maternal self-report that surveys complications of pregnancies and medical conditions of the mother. They found that maternal viral infection was, in addition to several other factors, associated with ASD.

Deykin and MacMahon [41] evaluated 163 cases of autism and their 335 unaffected siblings for intra-uterine or early infancy viral infection. Data was obtained from medical records and parental interviews. When clinical illness was combined with household exposure to infection, ASD cases had increased rate of maternal measles, rubella and mumps during gestation and mumps and chickenpox during infancy.

Zerbo et al. [42] evaluated 407 cases from the Childhood Autism Perinatal Study (CHAPS) and 2075 matched controls. They found that nearly 50% of both case and control mothers had at least one infection diagnosed as an outpatient or inpatient at anytime during pregnancy. The frequency of maternal infection anytime during pregnancy for each trimester was similar for cases and controls. Viral infections were not associated with increased ASD risk but bacterial infections during the second and third trimester were associated with moderately increased risk of ASD: OR 1.31 (95% CI 0.96–1.80). However when women that were hospitalized were evaluated, bacterial infections accounted for the majority of infections diagnosed during the hospital admission, and were associated with increased risk of ASD: OR 1.58 (95% CI 1.06–2.37). The majority of bacterial infections were genitourinary infections diagnosed in the third trimester. Mothers of ASD children were more likely than mothers of controls to have two or more infections during...
Pregnancy (OR 1.36, 95% CI 1.05–1.78). Two or more infections diagnosed in the third trimester of pregnancy increased the risk of ASD (OR 1.76, 95% CI 1.25–2.48).

3.2.2. Studies that did not find increased ASD rate

Juu-Dam et al. [43] compared the pre and perinatal data of 66 autistic children (autism, N = 53, PDD, N = 13) with the US population as reported in the Report of final natality statistics, 1995. Data on pre-, peri-, and neonatal risk factors were obtained from parental interviews and a review of all available obstetric and neonatal records. Maternal infection was not associated with ASD, only incidence of second- or third-trimester uterine bleeding and rhesus incompatibility occurred at significantly higher rates in autistic participants compared with the general population.

Langridge et al. [44] cross matched the data from West Australia on the maternal conditions and perinatal characteristics of 1179 cases of ASD, categorized according to association with intellectual disability: (ASD with intellectual disability, N = 727; ASD without intellectual disability, N = 452 and the 4576 cases of intellectual disabilities without ASD also categorized by level of intellectual disability: mild, N = 4339; severe, N = 237), and compared the data with the remainder of the children born in west Australia between 1984 and 1999, and not identified as having intellectual disability or an ASD (N = 376,539). Maternal urinary tract infection (UTI) was associated with 25% increased risk for mild developmental defect but not for ASD.

The families of 61 patients with autism filled a written questionnaire and were compared with 46 healthy controls with no family history of autism. Maternal flu, UTI and vaginal bleeding had an OR of 2.1 but it did not reach statistical significance \( p = 0.079 \) [45].

In summary: many studies have shown a slight to moderate association of maternal infections with ASD, but several did not find such an association. The controversy might be related to the fact that only several specific maternal infections (subgroups of women with infection during pregnancy) are associated with ASD.

3.3. Congenital rubella

The association between rubella and ASD is based on a few population studies and case reports. The rubella epidemic was stopped due to universal vaccination. Berger et al. [46] calculated that the rubella vaccination prevented an estimated number of congenital rubella cases that ranged from 8300 to 62,250 and that the corresponding ASD prevention estimates ranged from 614 to 4607 cases, depending on the infectious rate and prevalence of ASD among offspring of infected mothers. Since rubella became a rare disease, most of the studies use old definitions and do not entitle the children with the term ASD even when they describe the typical clinical picture.

The largest study associating congenital rubella with ASD was reported in 1978 by Chess et al. [47] who evaluated 243 children with congenital rubella of which 205 were followed until 9 years of age. Eighteen (7.4%) had autism compared to expected 0.07 (0.035%) in the general population at that time. All the children with autism had some other anomalies including cardiac, neurologic, audiologic and visual. Desmond et al. [48] reported behavioral disturbances among 45% of 29 non-retarded children with congenital rubella at the age of 9–12 years. Symptoms were probably recognized; however, at that time autism was not yet recognized as a spectrum of diseases. Ames et al. [49] described 118 congenital rubella children with hearing problems from whom 30 had central auditory interception: they had normal hearing tests but abnormal reaction to noise and failure to develop speech. Those children might have suffered from autism which was less diagnosed in 1970. Carvill et al. [50] described 12 young males with congenital Rubella who suffered from sensory imbalance and ASD. Associated morbidities included: visual impairment, N = 2, hearing impairment, N = 11 and seizures, N = 3. Feldman et al. [51] evaluated 12 children with autism or autistic traits between 1970 and 1971 and compared them with 25 children with a variety of psychiatric diagnoses, 21 children with language delay and 26 normal controls. None had a history of rubella. Seropositivity for rubella was found to be significantly higher among the children with language delay 8/21, and higher among children with autism 3/12, compared to the other groups and the general population in Montreal that time. All the mothers of the positive children were positive as well. Other reported cases of congenital rubella also described ASD children who suffered from hearing and visual impairment, iris hypoplasia, heart malformations, retinopathy and seizures [52,53]. See Table 1 for more detail. It can be summarized that maternal rubella infection in pregnancy is indeed associated with an increased rate of ASD in the offspring, mainly in those that have other manifestations of congenital rubella.

3.4. CMV infection

Since there is no universal screening for CMV, the association of maternal CMV infection and ASD in the offspring is based mostly on case reports. The diagnosis of maternal disease is based on medical history or late diagnosis by preserved blood, mostly from Guthrie cards or dried umbilical cord. Koyano et al. [54] demonstrated the feasibility of using dried umbilical cords for retrospective diagnosis of congenital CMV infection in Japan where obstetric hospitals customarily provide dried umbilical cord to every parent as a symbol of the bond between mother and child. The accuracy of retrospective diagnosis by dried umbilical cord or Guthrie paper was also evaluated by Sakamoto [55] that evaluated children with positive urine and found that 4/10 with positive urine had negative blood
test and concluded that dried blood or umbilicus underestimates the number of infants with congenital CMV.

We found no population studies that proved association between congenital CMV and ASD. Townsend et al. [56] evaluated 176 infants born in Malmö, Sweden, and London, United Kingdom between 1977 and 1986 and diagnosed with congenital CMV and followed them until at least 5 years, none was diagnosed with ASD. No specific neurological findings among children with congenital CMV were associated with ASD. Neuroimaging studies did not vary between those who developed or did not develop autism when seven infants with congenital CMV were evaluated. Three had sub-ependymal cysts and two had calcifications. All had mental retardation [57]. However, when children with ASD were assessed, those seropositive for CMV tended to test worse in the major severity scales than the seronegative ones [58]. Additionally, Yamazaki et al. found that ASD cases with cochlear implants had lower language associated social function than other children with corresponding developmental quotients [59]. Some studies showed that CMV DNA was detected among ASD cases in a higher prevalence than in the general population; however the numbers are small: 2/27 by Sakamoto et al., compared to incidence of ASD in Nagasaki which was 0.31%, p = 0.004 [55]; 3/76 by Stubbs et al. even though two had multifactorial prenatal causes for autism [60]; 4/26 by Engman et al. among children with cerebral cortical malformations, two of the CMV cases had ASD [61].

Different outcome in association with CMV stigmata was described by Kitajima [62] in a trichorionic triamniotic triplet born at 31 weeks after maternal febrile illness at 24 weeks. Two girls were asymptomatic; the third, a boy, had lower birth weight, increased IGM titer, thrombocytopenia, hepatosplenomegaly, retinitis, brain calcifications and compared to his normal sisters suffered from ASD. The triamniotic−trichorionic placentas had no fusion or structural abnormalities. Examination of the placenta of the third−born triplet showed that it was paler than the other two and had more severe villitis.

Other cases reported also had associated anomalies including brain calcifications and choriorretinitis [60,63–69] and Table 1.

In summary: The association between maternal CMV and increased rate of ASD may be an indirect multifactorial phenomenon related to the various symptoms of congenital CMV. Most associations were indeed found mainly in the children who suffer from other manifestations of congenital CMV.

3.5. Influenza

Studies associating maternal infection with influenza and ASD are based on maternal reports. Since diagnosis is based on clinical data, it is not clear whether the impact of maternal influenza is due to specific viruses or inflammation as maternal fever per se is associated with neurological perturbation. Atladottir et al. [37] found self-reported maternal infection with influenza to be associated with increased risk for infantile autism (adjusted HR: 2.3 [95% CI: 1.0–5.3]). The highest association was found with prolonged episodes of fever.

Zerbo et al. [70] used maternal telephone interview to evaluate cases and controls in the Childhood Autism Risk from Genetics and Environment (CHARGE) Study. When data from 538 children with ASD, 163 with developmental delay and 421 normal controls were associated with maternal influenza or fever during pregnancy, weighted odds ratio (OR) between mothers of ASD children and those of children with normal development was 1.26, (95% CI 0.73, 2.19). However, in the analysis of associations between fever and ASD, the odds that mothers of children with ASD reported fever during pregnancy doubled that of mothers of normal controls [OR = 2.12, (95% CI 1.17, 3.84)]. They also found that the OR for ASD was 2.55, (95% CI 1.30, 4.99) for children whose mothers reported fever but did not take anti-pyretic medication. For women who reported fever and took anti-pyretic medications, the OR for ASD was only 1.30, (95% CI 0.59, 2.84).

In summary there is no evidence for a direct effect of the influenza virus on the occurrence of ASD; it seems that fever may be the trigger for the increased ASD rate following maternal influenza infection.

3.6. Toxoplasma

No clinical studies have specifically linked ASD to toxoplasmosis, however T. gondii interactome genes were significantly enriched in the susceptible gene datasets for autism OR, 1.22, p = 0.013 [71]. Recently, Prandota [72] suggested that patients with autism should be tested for toxoplasmosis. These recommendations are based on the hypothesis that the development of ASD is triggered by several maternal and/or environmental factors and associated with chronic neuroinflammation, increased lipid peroxidation and oxidative stress. Accordingly, the neuropathological changes and clinical features of autism are similar to those reported in congenital and chronic cerebral toxoplasmosis; hence, the development of autism may be due to the reactivation of latent toxoplasmosis.

3.7. Parvovirus

No association between parvovirus infection and ASD was found by Anlar et al. [73] who measured levels of IgG and IgM from 22 ASD children, 50 children with cerebral palsy, mental retardation or epilepsy and 26 controls. Six of the 22 mothers of the ASD children reported flu like symptoms during the pregnancy. There was no statistical difference between the groups.

3.8. Tic borne infections

The association between tic borne disease and ASD is based on clinical practice. Bransfield et al. [74] claimed in a review article that there is an association between Lyme disease and other tick-borne infections during fetal life and ASD if mothers are not treated with antibiotics. Other studies describing Lyme disease during pregnancy do not describe ASD in the offspring. In a literature review, Walsh et al. [75] reviewed the English-language articles published between 1977 and 2005 and concluded that from the available evidence, it seems reasonable to conclude that no distinct pattern of teratogenicity from Lyme infection has been described.

3.9. Other viral infections in pregnancy and ASD

There seems to be no data on other maternal viral infections in pregnancy in association with ASD. However, in a series of recent studies conducted by Gentile et al. [58,76,77] who studied the levels of antibodies to Varicella–Zoster, CMV, Epstein–Barr virus, and herpes virus types 1 and 2 in children with ASD compared to controls. The significance of these findings in relation to pregnancy is unknown.

4. Inflammation

Despite the association of ASD with certain viruses, especially rubella and CMV, it seems that maternal inflammation per se may harm the embryo/fetus. Maternal immune activation and cytokine dysregulation may be a mediator in the neuropsychological behavior observed in autism. To evaluate the role of maternal inflammation in the development of ASD, maternal immune system and animal models are used to evaluate the role of cytokines in brain dysregulation. In vitro studies are carried out...
to evaluate the association between inflammatory mediators and neurological components.

4.1. Studies evaluating maternal immune system

Increased levels of inflammatory mediators obtained from stored samples were found in large population studies. Abdallah et al. [78,79] used a pool of samples from more than 100,000 pregnant women in Denmark who underwent screening or diagnostic amniocentesis and/or phlebotomy and had their biologic samples stored from 1980 to 2004, and matched 421 singleton ASD cases born between 1982 and 2000 for gender and birth year with 820 controls. Levels of Monocyte Chemotactic Protein-1 (MCP-1), were significantly elevated among ASD cases compared to controls. MCP-1 is believed to play an important role in the maturation of cerebellar Purkinje cells, and may serve as a useful marker of abnormal neuronal development. Using the same cohort, samples of amniotic fluid from 331 ASD cases had significantly elevated levels of IL-4, IL-10, TNF-α and TNF-β when compared with 698 controls. Increased levels of IL-4, IL-5 and IFN-gamma were found by Goines et al. [80] in banked serum collected from women at 15–19 weeks of gestation who gave birth to a child ultimately diagnosed with ASD (N = 84). They studied serum concentrations of eotaxin, granulocyte macrophage colony-stimulating factor (GM-CSF), IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, IFN-γ-induced protein 10, macrophage inflammatory protein (MIP)-1α, MIP-1β, RANTES and TNF-α. Grether et al. [81] found by examining screening filter paper blood specimens from the California Genetic Disease Screening Program of 213 ASD cases and 265 controls that children with ASD had lower total IgG and that the median value, for HSV1 IgG was significantly lower compared to controls.

The association between maternal immune system and behavioral disturbances was evaluated by Braunschweig et al. [82] who found reactivity to two protein bands against fetal brain at approximately 73 kDa and 37 kDa in plasma from 7 of 61 (11.5%) mothers of children with autism, which was not found in controls. Reactivity to the two bands correlated with the diagnosis of behavioral regression in the child and individual reactivity to the 37 kDa band was observed significantly more often in the ASD cases. They assumed that the presence of specific fetal anti-brain antibodies in the circulation of mothers during pregnancy might be a potential trigger to induce a downstream effect on neurodevelopment that may lead to autism. To further elucidate the mechanism of maternal immune activity Mazina et al. [83] investigated the gene–environment interaction by evaluating the interactive effects of maternal infection in pregnancy and the presence of copy number variants which are considered likely to play a contributing role in symptoms of ASD. Participants included 1971 children (1711 males, 260 females; 79% white, 89% non-Hispanic) with ASD between the ages of 4 and 18 years with complete genetic, maternal pregnancy history, and phenotypic information. A statistically significant interactive effect of the presence of copy number variants and maternal infection on autistic symptomatology was observed.

4.2. In vitro studies of inflammation

To further elucidate the association between maternal infection and ASD, Lucchese et al. evaluated [84] the potential role of the maternal immune response to viral infection in determining fetal brain injuries that increase the risk of neurological disorders in the adult. By using influenza infection as a disease model they demonstrated that humoral and/or cellular immune responses following influenza A H1N1 infection in humans had the potential to cross-react with human axon guidance molecules.

4.3. Animal models of inflammation

Animal models are used to associate maternal infection/inflammation during pregnancy and abnormal histological and laboratory findings in the offspring and correlate the finding with behavioral impairment resembling autistic traits. The validity of animal models for human ASD is uncertain. Inflammation is triggered by injection of immunogens during pregnancy and the offspring is evaluated. The models evaluate the critical time period of neuronal toxicity and the mechanisms involved which can influence brain function including epigenetic modifications of CNS genes, the long-term changes in structure or function of specific neuronal pathways and permanent dysregulation of the neurological system.

Boksa [85] reviewed animal studies published until 2010 and found that different models demonstrated that maternal prenatal immune activation was associated with changes in various interleukins in the fetal brain mostly including: IL1, IL6 and TNF-α. Both increased and decreased levels were found, depending on the pregnancy time of injection of the immunogen and the time of evaluation. Various morphological changes were described, mostly in the hippocampus and cerebral cortex. However, changes were noted in other areas as well. Intraperitoneal lipopolysaccharide (LPS) from *Escherichia coli* was used by Carpenter et al. [86] in a murine model showing that mild inflammatory signaling was sufficient to elicit vasodilation, hemorrhage, and tissue necrosis in the placenta. Concurrent with defects in placental function, hypoperfusion of the fetus and reduced neural progenitor cell mitotic index was observed in the developing cortex. The maternal immune system was activated by an inflammatory stimulus producing cytokines that acted on the placenta and fetal brain Polyriboinosinic: polyribocytidylic acid (poly I:C) system, a double-stranded RNA, mimicking viral infection is used as a model for viral infection and was found by Ohkawara et al. [87] to cause a significant decrease in hippocampal serotonin levels in the murine adult offspring which may cause behavioral and cognitive abnormalities. They also found increased expression of cytokine genes such as interferon-ε (IFN-ε), IL-1β, IL-6, and TNF-α. They claimed that elevated cytokine levels and activated microglia and astrocytes are present in postmortem brains of autistic people aged 5–44 years and elevation of cytokine levels are observed in the cerebral spinal fluid of autistic children which suggest that immune activation induced by poly I:C may cause ASD in offspring. The rate of ASD is much higher among males. Xuan and Hampson [88] found in a murine model that male and female offspring from mothers injected with LPS or Poly I:C during mid-gestation displayed disparate behaviors on tests of motor activity, social interactions, and repetitive or stereotyped behaviors. Maternal immune activation induced selective induction of stereotyped, repetitive behavior in male mouse offspring but not female offspring.

4.4. Conclusion

It seems that the association of ASD with specific infections like Rubella and CMV may result from the pervasive insult including vision and auditory problems. Additionally, many of these children have brain malformations suggesting that ASD may be secondary to the primary morbidity. Large population studies did not find a specific infection but rather increased rate of ASD, especially when maternal infection was more severe and necessitated hospitalization. The association between maternal infection during pregnancy and ASD is apparently related to maternal inflammatory process; hence, maternal immune activation may play a role in neuro-developmental perturbation.
5. Exposure to drugs and chemicals during pregnancy and ASD in the offspring

5.1. Exposure to Selective serotonin reuptake inhibitors (SSRIs)

Between 9 and 14 percent of pregnant women occasionally show at least one symptom of depression [89, 90]. Untreated maternal depression is associated with poor health outcomes for both mothers and children [91]. Therefore, in recent years, more women were using anti-depressant medication during pregnancy. Selective serotonin reuptake inhibitors, which increase extracellular serotonin, are recommended for first-line pharmacological management of depression because they are considered safer and better tolerated than other types of antidepressants. SSRIs and other antidepressant medications cross the placenta and are secreted in breast milk, thus raising concerns about possible adverse effects from fetal and infant exposure. Several studies show a possible connection between SSRIs exposure during pregnancy and increased risk of ASD in children [92–95]. Four out of six recently published case–control studies reported a significantly positive association between SSRI exposure during pregnancy and ASD in children. Croen et al. [92] found in a group of 298 children with ASD and 1507 randomly selected control children, a 2-fold increased risk of ASD associated with SSRI treatment by the mother during the year before delivery (OR, 2.2; 95% CI, 1.2–4.3), with the strongest effect associated with treatment during the first trimester of pregnancy (OR, 3.8; 95% CI, 1.8–7.8). No increase in risk was found for children exposed to maternal mental health treatment during pregnancy, in the absence of SSRIs.

Rai et al. [93] found in a Swedish population case control study of 4429 children with ASD and 43,277 control children, that SSRI use during pregnancy was associated with an increased risk of ASD, particularly without intellectual disability (OR, 2.34; 95% CI, 1.09–5.06).

Gidaya et al. [94] studied a group of 5215 children diagnosed with ASD and 52,150 controls, all born in Denmark between 1997 and 2006. They found that the OR for ASD doubles among children born to mothers who used SSRIs at any time during pregnancy.

Harrington et al. [95] examined 492 pairs of mothers and ASD children and 320 pairs of mothers and children with typical development. They found that among boys, prenatal SSRI exposure was nearly 3 times as likely in children with ASD relative to normal children (OR, 2.91; 95% CI, 1.07–7.93). In this study, too, the strongest association occurred with first-trimester exposure (OR, 3.22; 95% CI, 1.17–8.84).

In contrast to these studies, Hviid et al. [96] found no connection between the use of SSRIs during pregnancy and the risk for ASD (OR, 1.20; 95% CI, 0.90–1.61). Sorensen et al. [97] found that children exposed to SSRIs during pregnancy have a 50% higher risk for ASD compared with unexposed children (95% CI, 1.2–1.9). The association was found for high as well for low dose levels, and risk estimates were comparable regardless of timing of exposure. However, after controlling for important confounding factors such as maternal history of affective disorder and familial risk factors, the researchers concluded that they did not detect a significant association between maternal use of SSRI’s during pregnancy and ASD in the offspring. Both researchers used data from the Danish civil registration system and Danish health registry systems about children born in Denmark between 01.01.96 and 31.12.2005 (Hviid) and until 31.12.2006 (Sorensen).

In summary: Many of the studies indicate an increased risk of ASD in children exposed to SSRIs during pregnancy. A recently published meta-analysis by Man et al. [98] supports these findings. However, we should remember that maternal depression itself, or other causes, might also be responsible or contribute to the association.

5.2. Exposure to valporic acid (VPA)

More than half of antiepileptic-drug prescriptions are written for pain management and psychiatric indications [99] therefore, antiepileptic drugs are among the most common teratogens prescribed to women of childbearing age. Prenatal exposure to antiepileptic drugs (AEDs) is associated with an increased risk of major congenital malformations and delayed cognitive development among the offspring, in a dose dependent manner [100–102]. Knowledge of antiepileptic drug teratogenicity has increased in recent years, including a concern that valporic acid, which has been identified as the most teratogenic AED [103], is associated with impaired cognitive outcomes [99]. VPA is used for treatment of epilepsy, bipolar disorders and migraine headaches. Many reports demonstrate that prenatal exposure to VPA is associated with reduced cognitive functioning and high risk for ASD in the prenatally exposed child [104–108].

Christianson et al. [104] were apparently the first ones to draw to attention of a possible association between intrauterine exposure to VPA and ASD. They described four children exposed to VPA during pregnancy. All children showed developmental delay and one of them had ASD.

Several years later, Williams at al [105] examined five children with ASD. They found that VPA was used alone during pregnancy in two of the children and in combination with another anticonvulsant in three children.

Moore et al. [106] found among 57 children affected by antiepileptic drugs that 4 had autistic syndrome and 2 had Asperger syndrome. Five of these six children were exposed to VPA alone or combined with another AED. The ASD rate was 10.8% of 46 exposed to VPA. However, it is difficult to assess from this data the increase in the rate of ASD in children of VPA-treated compared with the general population.

Rasalam et al. [107] evaluated the clinical features and frequency of autistic disorder or Asperger syndrome in 260 children exposed to anticonvulsant medication (VPA or Carbamazepine) during pregnancy, by using the DSM 4 criteria. They found that Sodium valproate was the drug most commonly associated with autistic disorder. The prevalence of ASD in children exposed to sodium valproate alone, was 5 of 56 (8.9%, 95% CI 1.3–16.5) and 9 of 77 (11.7%, 95% CI, 4.4–19) among children exposed to sodium valproate in combination with other AEDs, while among those exposed to Carbamazepine alone or in combination, five of 110 (4.5%, 95CI 0.5–8.5) had ASD. They concluded that the prevalence of ASD in children with prenatal exposure to AEDs is 8–18 times higher than the rate in the general population.

Bromley et al. [108] examined 296 children of epileptic mothers and 336 children born to mothers without epilepsy. Out of the 632 children from both groups 10 have been diagnosed with ASD, 7 of them were exposed to AEDs. Of those seven children, four were exposed to VPA (4/64 6.3%). Of the remaining three children, one was exposed to VPA in combination with Lamotrigine (1/51 2%), one child was exposed to Lamotrigine alone (1/44 2%), and one child to penytoin (1/91 1%).

Christensen et al. [109] in a much larger Danish population based study reviewed the National Population Register with prescription data, psychiatric register and birth records. Records were collated for 655,615 eligible children, with 508 exposed to VPA and 2136 to other anticonvulsants. An increased risk of ASD (Hazard Ratio – HR = 2.9, 95% CI, 1.7–4.9) was found in children exposed to VPA during pregnancy. The risk of ASD was elevated compared to non-exposure when VPA was taken in the first trimester as well as in children whose mothers filled prescriptions only after the first trimester.

Supporting data to the human studies have been seen in rodent models. Exposure of mice and rats to VPA produced autism-like
behaviors in the offspring [110,111]. Maternal exposure to VPA induced developmental delay, lifelong deficits in motor performance and social behavior, anxiety-like behavior and alterations in postnatal growth and development in the offspring [110,112,113]. As in humans, anatomical alterations such as reduced number of cerebellar Purkinje cells, damage to cranial nerve nuclei have been described [114,115] as well as enhanced synaptic plasticity of the prefrontal cortex [116].

Moreover, Beker van Woudenberg et al. [117] used a multiple-endpoints strategy, including morphology, motor activity (MA), histopathology and kinetics, to improve the predictability of the zebrafish embryotoxicity test (ZET) for developmental (neuro)toxicity screening. The model compounds used were different antiepileptic drugs (AEDs) including VPA. They found that these AEDs could be ranked in terms of their developmental toxicity/neurotoxicity potency with valproic acid being the most potent neurotoxic drug. This AEDs ranking appeared to be in good agreement with finding by others, both in animals and in man, further demonstrating the strong neurotoxic potency of VPA.

In conclusion: Maternal treatment in pregnancy with VPA is associated with an increased rate of ASD in the offspring. Hence, VPA should not be used as a first-line antiepileptic drug in pregnant women or, since data indicate that half of pregnancies are unplanned, in women of childbearing age.

5.3. Exposure to thalidomide

Thalidomide is a well-known human teratogen originally used to treat morning sickness in pregnant women; it caused, however, multiple birth defects such as limb reduction defects, ocular and cardiovascular anomalies. Today, thalidomide is still used in the treatment of leprosy and multiple myeloma [118], Strömland et al. [119] have reported an increased number of children with ASD following prenatal exposure to thalidomide. They examined 100 thalidomide embryopathy patients between 27 and 30 years of age. At least four of the patients met full criteria for DSM 3 R autistic disorder – much higher prevalence than in the general population. The critical window of exposure to thalidomide was in the first trimester, mainly days 20–35 post fertilization.

No further data has found regarding this possible association. Hence, on the current data it is difficult to accept or deny this possible association.

5.4. Exposure to cocaine

Cocaine use during pregnancy has many deleterious effects on both the mother and the fetus, including preterm delivery, intrauterine growth retardation, placental abruption and neonatal death [120,121]. Cocaine crosses the placenta and the fetal blood–brain barrier readily, and may affect the fetal central nervous system [122]. Several studies reported a possible association between the use of cocaine during pregnancy and the development of ASD in the offspring.

Davis et al. [123] examined 70 children with a history of prenatal cocaine exposure, born between 01.01.87 and 31.01.89 in Harlem, New-York. They reported presence of DMS 3 R criteria for autistic disorder in nine of the children – 11.4%, much higher than the national average at that time.

Harris et al. [124] examined three, 25–36 month old children with prenatal exposure to cocaine, alcohol and other illicit drugs. They reported that all three children showed a notable pattern of autistic-like behaviors.

We could not find other studies on the association between prenatal cocaine exposure with ASD. Nevertheless, although available information is poor and conclusions must be taken with caution, the studies so far point in the direction of a positive association between prenatal cocaine exposure and ASD.

5.5. Exposure to ethanol

Fetal alcohol syndrome (FAS) is a pattern of physical and neurodevelopmental abnormalities which develop in some of the children exposed to high levels of alcohol during pregnancy. Several studies have shown a connection between FAS and an increased risk of ASD.

Nanson [125] described six children at ages ranging from 6 to 15 years, who showed the physical phenotype of fetal alcohol syndrome and had a history of maternal alcohol abuse during pregnancy. These children had a very different behavioral phenotype, typical of autistic children (they had low IQ scores, difficulty relating to people, resistance to changes in daily routine and language disabilities), and were also characterized by more significant retardation of both their cognitive and social skills.

Aronson et al. [126] examined 24 children born to mothers who used high doses of alcohol during pregnancy (from moderate to severe alcohol abuse). Ten of the children had attention deficit hyperactivity disorder (ADHD), Two had Asperger syndrome and one had an autistic-like condition. There was a clear correlation between the occurrence and severity of the neuropsychiatric disorder and the degree of alcohol exposure in utero.

In a Swedish population-based study, Landgren et al. [127] assessed 71 children adopted from Eastern Europe between 4.8 and 10.5 years of age. They reported FAS in 37 (52%) children and autism in 6 (9%). Although only 2 children had both FAS and autism the authors reported that the main reason for leaving a child in an orphanage is maternal alcohol abuse, therefore it is likely that a larger proportion of the cohort was exposed to various amounts of alcohol in utero suggesting a positive association between prenatal alcohol consumption and ASD.

In contrast to these studies, in a much larger population-based cohort study Eliasen et al. [128] evaluated a total of 80,522 mother–child pairs. Almost half of the women reported an average weekly intake of at least half a standard alcoholic drink (45%), and approximately one-quarter of the women reported at least one episode of binge drinking during pregnancy. They found no positive associations between ASD and average alcohol consumption, number or timing of binge episodes during pregnancy. It is worth noting that in this study the mothers were exposed to moderate or low doses of ethanol.

In summary: Although alcohol consumption in pregnancy and the risk for ASD in children (especially those with FAS) is well established, the effects of lower alcohol amounts are not yet determined.

5.6. Exposure to misoprostol and Möbius sequence

Misoprostol is a prostaglandin analog drug commonly prescribed for the prevention and treatment of gastric ulcers and in several countries widely used to induce medical abortions [129]. Möbius sequence is a rare congenital disorder, characterized by uni- or bilateral eye-face palsy due to damage to cranial nerve nuclei, associated with muscle or skeletal malformations in the upper or lower limbs, with an estimated prevalence of 1:50,000 cases in the general population [130,131]. Among the etiological factors responsible for Möbius sequence there are genetic factors, fetal vascular insult and intrauterine exposure to teratogens including misoprostol (during failed abortion attempts with the use of misoprostol) [132,133].

Johansson et al. [134] analyzed 25 Swedish individuals with Möbius sequence. Six patients met all diagnostic DSM 3 R criteria for autism; a much higher frequency than that of the general population. In another research, that had been done using the same 25
Swedish individuals, Strömland et al. [131] identified six patients with an autistic syndrome, one with an autistic-like condition. All these patients also had mental retardation. These findings suggest a strong association between Möbius sequence and ASD.

Bandim et al. [133] reported a positive association between the exposure to misoprostol during pregnancy and ASD. They examined 23 Brazilian patients with the age range of 1–11 years that had been diagnosed as having Möbius sequence based on clinical findings. They identified seven children with ASD of whom four (57.1%) had a positive history of prenatal exposure to misoprostol during the first trimester of pregnancy. They concluded that the prevalence of ASD found in Möbius patients is around 26.1%, much higher than the general population, thus suggesting a strong association linking the two pathologies.

The correlation between misoprostol exposure during pregnancy and ASD is suggested on the basis of reports published in the literature, suggesting that the use of misoprostol during pregnancy may give rise to Möbius sequence in about 1% of exposed children [135,136]. However, since apparently all affected children also had Möbius syndrome, it is difficult to directly associate misoprostol exposure to ASD. We found no reports on an association of ASD to misoprostol exposure in exposed children that do not present the symptoms of Möbius syndrome. It should be borne in mind that the experimental models of VPA–induced ASD in rats and mice have revealed damage to the cranial nerve nuclei, similar to the damage observed in Möbius syndrome [114–116]. So, it seems plausible to conclude that a direct association between misoprostol exposure during pregnancy and ASD is not likely to exist, but ASD is associated with Möbius syndrome.

5.7. Exposure to cigarette smoking

Maternal cigarette smoking during pregnancy is associated with an increased rate of spontaneous abortions, preterm delivery, reduced birth weight, immune system difficulties such as asthma and allergies and developing learning disabilities, attention deficit disorders, and mental impairment later in life [137–139]. Several recently published population based studies have examined whether prenatal exposure to tobacco smoke is also associated with ASD but the findings are inconsistent.

A Swedish study by Hultman et al. [30] found a mild association between smoking during pregnancy and the risk of childhood autism (OR, 1.4; 95% CI, 1.1–1.8). Positive association between maternal smoking and ASD was also found by Larsson et al. [140] in a study that included 72 cases of ASD and 4707 controls (OR, 2.09; 95% CI, 1.08–4.03). However, most of the studies did not report similar associations between tobacco exposure and ASD. In a large registry based Swedish case–control study, that included 3958 ASD cases and 38,983 controls, Lee et al. [141] found that maternal smoking during pregnancy is not associated with increased risk of ASD after adjustments for parental education, occupation, and income. Tran et al. [142] examined 4019 ASD cases and 16,123 controls, in a nested case–control study based on the Finnish Prenatal Study of Autism (FIPS-A). They did not find any connection between maternal smoking and childhood autism or Asperger syndrome, but found a slight association with pervasive developmental disorder (PDD) (OR, 1.2, 95% CI, 1.0–1.5). Smoking exposure limited to the first trimester of pregnancy was not associated with ASD or PDD.

Kalkbrenner et al. [143] estimated the association between maternal smoking during pregnancy and ASD among 3315 children with ASD and 630,674 control children, all were 8 years old. They reported no association between gestational tobacco exposure and ASD after accounting for the potential of under-ascertainment bias.

Several additional studies [144,145] and a literature meta-analysis by Rosen et al. [146], also reported no significant association between maternal smoking during pregnancy and ASD.

It can be summarized that the etiology of ASD is most probably unrelated to maternal smoking.

5.8. Exposure to air pollution

In recent years a growing body of research examined the effects of air pollution exposure during pregnancy on the risk of ASD in children.

One of the first studies was conducted in San Francisco Bay area in 2006 by Windham et al. [147] who examined the effects of prenatal exposure to 19 hazardous air pollutants in 284 children with ASD and 657 controls. Elevated risk for ASD was found in adjusted analyses of the top quartile of exposure to chlorinated solvents and heavy metals [95% CI, 1.1–2.1]. The problem of this study is that the exposure was estimated two years after birth and may be a little different from the exact exposure during pregnancy. Moreover, it might be attributed to postnatal effects of these pollutants.

Kalkbrenner et al. [148] examined prenatal exposure to 35 hazardous air pollutants in 383 children with ASD and 2829 control children with speech and language impairment. Exposure to ambient concentrations of metal, particulate, and volatile organic air pollutants in the census tract of the child’s birth residence were assigned from the National Air Toxics Assessment. They found that prenatal exposure to hazardous air pollutants including methylene chloride (OR, 1.4; 95% CI, 0.7–2.5), quinoline (OR, 1.4; 95% CI, 1.0–2.2), and styrene (OR, 1.8; 95% CI, 1.0–3.1) were associated with a higher risk for ASD.

Volk et al. [149] investigated the association between autism and proximity of residence to freeways and major roadways during pregnancy and near the time of delivery, in a group of 304 children with ASD and 259 controls, as a surrogate for air pollution. They reported that maternal residence during the third trimester (OR, 2.22; 95% CI, 1.16–4.42) and at the time of delivery (OR, 1.86; 95% CI, 1.04–3.45) were more likely to be near a freeway (<309 m) for the ASD children than for the controls.

Becerra et al. [150] studied the influence of exposures to traffic-related air pollution during pregnancy on the development of autism in a group of 7603 children with ASD and 10 controls per case, matched by sex, birth year, and gestational age. They mapped children’s birth addresses and linked it to the nearest air monitoring station. They reported a 12–15% relative increase, per interquartile range, in odds of autism for ozone (OR, 1.12; 95% CI, 1.06–1.19; per 11.54 ppb increase) and particulate matter ≤2.5 μm (OR = 1.15; 95% CI, 1.06–1.24; per 4.68 μg/m³ increase) when mutually adjusting for both pollutants. However, the additional risk for ASD is small.

In a later population-based case-control study, Volk et al. [151] examined 279 children with ASD and 245 control children with typical development. They found that, during gestation, children with ASD were more likely to live at residences that had the highest quartile of exposure to traffic-related air pollution (OR, 1.98; 95% CI, 1.20–3.31) compared with control children. Regional exposure during pregnancy with nitrogen dioxide and particulate matter less than 2.5 and 10 μm in diameter (PM2.5 and PM10) were also associated with autism (exposure to nitrogen dioxide: OR, 1.81; 95% CI, 1.37–3.09; exposure to PM2.5: OR, 2.08; 95% CI, 1.93–2.25; exposure to PM10: OR, 2.17; 95% CI, 1.49–3.160).

Roberts et al. [152] estimated the association between levels of hazardous air pollutants at the time and place of birth and ASD in 325 children with ASD and 22,101 controls. They reported that prenatal exposures to the highest quintile of air pollutants, such as diesel, lead, manganese, mercury and methylene chloride, were significantly associated with ASD, with odds ratios ranging from 1.5 (for overall metals measure) to 2.0 (for diesel and mercury). For most pollutants, associations were stronger for boys (279 cases)
than for girls (46 cases) suggesting a significant difference according to sex.

Raz et al. [153] examined the association between maternal exposure to particulate matter (PM) air pollution during pregnancy and the risk of ASD in children. They used a group of 245 children with ASD and 1522 children without ASD and found that PM2.5 exposure during pregnancy was associated with increased risk of ASD, with an adjusted odds ratio for ASD per interquartile range higher PM2.5 (4.42 μg/m³) of 1.57 (95% CI: 1.22, 2.03). The association between ASD and PM2.5 was stronger for exposure during the third trimester (OR, 1.42 per inter-quartile range increase in PM2.5, 95% CI, 1.09–1.86).

Collectively, these case control studies suggest that there might be a positive association between maternal exposure during pregnancy to air pollution, mainly to small PM and ASD in children, especially following exposure in the third trimester. However, direct measurements are generally missing, and there might be other factors in the air pollutants that are associated and were not measured.

5.9. Exposure to heavy metals

Most studies regarding the outcome of maternal exposure to heavy metals (i.e. lead, mercury, cadmium etc.) show a rise in neurodevelopmental disorders including mental retardation and inattention [154]. There are only few studies addressing the possible association between ASD and exposure to heavy metals in pregnancy. They are inconsistent and do not address this issue adequately. There is more data on mercury, but the available data is controversial, suggesting that maternal exposure to mercury does not play an important role in the development of ASD. Moreover, several of the studies were carried out on the affected children and not on their mothers, hence making the association with pregnancy an unproven possibility [155].

Geier et al. [156] examined the association between maternal dental amalgams (containing 50% mercury) and the severity of ASD diagnosis in a group of 100 autistic children. They found that a rise in autism severity correlated with an elevation in the number of dental amalgams in the mother during pregnancy.

In contrast to this study, Yau et al. [157] investigated the association between ASD and levels of total mercury measured in maternal serum from mid-pregnancy and infant blood shortly after birth in 84 children with ASD and 159 control children. They found no significant associations between ASD and mercury levels in maternal serum samples (OR, 0.96; 95% CI, 0.49–1.90) or in newborn blood samples (OR, 1.18; 95% CI, 0.71–1.95).

Similarly, van Wijngaarden et al. [158] examined prenatal exposure to methylmercury in 1784 children and young adults, by measuring maternal hair samples collected at or near the time of birth. They found no significant association between ASD and prenatal methylmercury exposure.

5.10. Exposure to pesticides and insecticides

Few studies have examined the effects of in utero exposure to pesticides such as organophosphates and carbamates on the neurobehavioral development and the risk of ASD in children. Each study, however, reported an association with ASD.

Robert et al. [159] examined the association between maternal residence near agricultural pesticide applications during key periods of gestation and ASD in children. They reported that ASD risk was higher in children whose mothers lived near agricultural applications of organochlorines during the first trimester of pregnancy and decreased with distance from field sites (OR, 6.1; 95% CI, 2.4–15.3).

Shelton et al. [160] evaluated whether residential proximity to agricultural pesticides during pregnancy is associated with ASD. They found that residence near organophosphates at some point during gestation was associated with a 60% increased risk for ASD, higher for third-trimester exposures (OR, 2.0; 95% CI, 1.1–3.6), and second-trimester chlorpyrifos applications (OR, 3.3; 95% CI, 1.5, 7.4). Children of mothers residing near pyrethroid insecticide applications just before conception or during the third trimester were at greater risk for ASD with ORs ranging from 1.7 to 2.0. More studies with accurate measurements of the pesticides/insecticides are needed to establish such a possible association.

5.11. Vitamin D deficiency

Vitamin D deficiency during pregnancy is known to be associated with multiple adverse health outcomes in the offspring, including intrauterine growth restriction, reduced bone mineral accrual, diabetes and recurrent wheeze [161,162]. Because Vitamin D insufficiency or deficiency is common in children with ASD [163–165] recent studies proposed that maternal vitamin D deficiency during pregnancy may also increase the risk for ASD in children [166].

Fernel at al [167] examined the values of 25-hydroxyvitamin D in 40 women with at least one autistic child and 40 women without a child with autism. They found no significant difference in the 25-hydroxyvitamin D levels between the groups.

Whitehouse et al [168] found that maternal Serum 25(OH)-vitamin D concentrations at 18 weeks of pregnancy were unrelated to offspring scores on the majority of autism-spectrum Quotient scales. But the sample in this study was small and included only three children with ASD (from a total of 406 children). So far, there is no evidence for possible association of maternal vitamin D deficiency and ASD.

5.12. Folic acid deficiency

Folic acid deficiency has also been proposed as a possible risk factor for ASD. In a population based case control study, Schmidt et al. [169] were the first to report that mothers of children with autism were less likely, than those of typically developing children, to report having taken prenatal vitamins, including folic acid, during the three months before pregnancy or the first month of pregnancy (OR, 0.62; 95% CI, 0.42–0.93).

In a later study, Schmidt et al. [170] examined the effect of maternal folic acid intake on the ASD risk in a group of 429 children with ASD and 278 children with typical development. They reported a significantly greater mean folic acid intake during the first month of pregnancy for mothers of typically developing children than for mothers of children with ASD. A mean daily folic acid intake of ≥600 μg (compared with <600 μg) during the first month of pregnancy was associated with reduced ASD risk (OR, 0.62; 95% CI, 0.42–0.92; P = 0.02), and risk estimates decreased with increased folic acid (P-trend = 0.001). The association between folic acid and reduced ASD risk was strongest for mothers and children with methylenetetrahydrofolate reductase (MTHFR) 677C>T variant genotypes.

In a prospective Norwegian cohort study, Surén et al. [171] followed up 85,176 children of whom 270 had been diagnosed with ASD. They reported a significantly higher rate of autistic disorder in children not exposed to folic acid (0.21%) compared to children of mothers who took 400 μg or more folic acid (0.10%) during a month before and two month after the start of pregnancy (OR, 0.6; 95% CI, 0.41–0.90). No association was found with Asperger syndrome or PDD-NOS.

More studies are needed to elucidate the possible association of maternal folic acid deficiency with ASD. However, the above data
supports the recommendations to ensure adequate folic acid intake before and during pregnancy.

6. Conclusions

It is yet unknown whether the increase in the incidence of ASD in the last decades is mainly due to better ascertainment or is a reflection of a real increase in the occurrence of ASD. If the latter is true, it is reasonable to assume that it stems mainly from pre-natal causes as a result of significant changes in our surrounding environment and life habits. This is apparently why so many studies trying to relate ASD to prenatal insults were published in the last 20 years. Generally, the search for prenatal etiology did not reveal too many environmental factors to be blamed for the rise in ASD. Evidence from animal studies may further support the apparent associations observed in humans, but they are not too common. Despite the increasing efforts in recent years, we still seem to be in a stage where the etiology of ASD is largely unknown and the associations described in the literature are for many agents inadequately proven. More research is needed to unravel environmental contribution to the genetic etiology of ASD; nevertheless, based on the findings reviewed here we may conclude – with caution – that: (1) No association with ASD was found for many maternal infections in pregnancy (i.e. herpes viruses, Epstein Barr virus, parvovirus), smoking, exposure to heavy metals or vitamin D deficiency. (2) A possible association was suggested with maternal influenza or toxoplasmosis in pregnancy, exposure to pesticides and insecticides, exposure to misoprostol, thalidomide SSRIs or folic acid toxoplasmosis in pregnancy, exposure to pesticides and insecticides, exposure to heavy metals or vitamin D deficiency. (3) A probable association was found for maternal fever, diabetes, and air pollution. (4) Finally, an association of ASD with maternal Rubella and CMV infection in pregnancy, maternal inflammation and immune activation, or exposure in pregnancy to VPA, cocaine and high levels of ethanol seems very likely.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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