

# Persistent Inflammation Initiated by TORCH Infections and Dysbiotic Microbiome in Autism Spectrum Disorders: A Prospect for Future Interventions

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## Abstract

Autism spectrum disorders (ASD) are a range of neurodevelopmental conditions that are clinically present early in childhood with the symptoms of social withdrawal and repetitive behavior. Despite an extensive research on ASD, no commonly accepted theory on the disease etiology exists. Hence, we reviewed several scientific publications, including reviews, preclinical and clinical investigations, and published hypotheses to analyze various opinions on the nature and cause of the disorder. Many studies suggest that infections and inflammation during pregnancy play a significant role in genetic and epigenetic changes in the developing fetus, resulting in an autistic phenotype in a child. Still, there is a lack of comprehensive literature about the multitude of autism inducing factors. Therefore, this article reviews and discusses available scientific evidence on the roles of viral, bacterial, fungal, and parasitic infections, overactivation of the immune system, and intestinal microflora in the pathogenesis and clinical manifestation of ASD. The overview of the scientific publications, including our own studies, suggests that TORCH infections, imbalanced microbiome, and persistent inflammation are significantly

associated with the disruption of the social domain in ASD children. The ASD-related changes begin prenatally as maternal-to-fetal immune activation triggered by infection. It results in continuous low-grade inflammation and oxidative stress in a fetus, causing germline and somatic genetic changes in the developing brain and the establishment of the dysregulated immune system. These changes and dysregulations result in central and peripheral nervous systems dysfunctions as well as other comorbid conditions found in autistic children.

## Keywords

Autism spectrum disorder, inflammation, TORCH infections, microbiome, wall-free pathogens

## Introduction

Autism Spectrum Disorder (ASD) is a complex neuropsychiatric developmental disorder with a wide range of neurological and psychiatric symptoms including language impairment, repetitive and restricted behavior patterns, limited interests, and disruption of social function (Black and Andreasen 2020). The first attempt to define and classify this disorder was made by Leo Kanner. He published his article “Autistic disturbances of affective contact” in 1943 in *The Nervous Child* (Harris 2016). He described two main symptoms in his patients: the lack of social interest and resistance to change (Kana 2022). ASD was also initially recognized as a childhood form of schizophrenia. But this viewpoint was revised in the 1980s when it was reclassified as a pervasive developmental disorder (Harris 2016).

The current diagnostic statistical manual-5 (DSM-5) incorporated a range of developmental disorders under an umbrella ASD term (Black and Andreasen 2020). For many years, autism has been considered a rare disease. However, in recent years there has been an explosive increase in ASD cases among children. The current data showed that in the United States, the prevalence was 1 case per 54 children in 2016 (Kana 2022). On December 2, 2021, the Centers for Disease Control’s Network for Monitoring Autism and Developmental Disorders published a report on the surveillance of ASD, which notes that 1 in 44 children in the U.S. is on the autism spectrum. While 50 years ago it was almost 60 times rarer than today, with only 1 case in 2500 (Rutter 2005). Currently, the treatment approaches are focused on behavioral therapies and special education. The two most common approaches that have been used for a long time are the Applied Behavioral Approach (ABA) and Treatment and Education of Autistic and Related Communication Handicapped Children (TEACCH) (Huang et al. 2021, Rieske 2019). Other methods include speech therapy, occupational therapy, dietary assessment and correction, and physical therapy (Rieske 2019). Pharmacologic interventions are limited and involve using antidepressant and antipsychotic medications to alleviate some psychiatric symptoms of autism (Huang et al. 2021). The symptoms of anxiety, compulsion, and depression are attempted to treat using selective serotonin reuptake inhibitors and oxytocin (Black and

Andreasen 2020, Huang et al. 2021). Since some studies demonstrated the role of microbiome composition in modulating behavioral problems (Neufeld et al. 2011), probiotic administration (Tan et al. 2021) showed a positive effect in autistic children. Although multiple hypotheses have been proposed to date about the possible causes of this disorder, a complete understanding is lacking. Hence, ASD is still considered incurable despite all the existing supportive therapies that focus on alleviating symptoms but do not target the actual cause of the disease, which remains elusive to date. Therefore, this article focuses on the pathology of ASD and existing evidence about the etiology and pathogenesis of ASD. Specifically, we focus on establishing autistic genotypes in the prenatal period and developing autistic phenotype postnatally. We show sequential pathoanatomic and pathophysiologic changes resulting from the infectious and inflammatory nature of inciting events and the triggering factors that play a role in this disease. Indeed, viral, bacterial, fungal, and parasitic infections during the prenatal period cause systemic inflammation that disrupts the fetus's normal brain formation and contributes to genetic mutations, epigenetic changes, and immune dysfunction.

## Clinical presentation of ASD

Before diving into the etiopathogenesis of autism, it is crucial to characterize the clinical picture of ASD patients, which includes not only behavioral abnormalities but also various somatic disorders. Comorbidities range from otitis media (Wimberley et al. 2018), oral (Somma et al. 2010), and urogenital infections to gastrointestinal (GI) and endocrine disturbances (Autism Society, n.d.). Table 1 shows comorbid conditions and health problems often found in ASD patients. In addition to having central nervous system (CNS) problems, these patients develop peripheral nervous system (PNS) issues.

Table 1.

Comorbidities in ASD

Disease	Clinical presentation
GI disturbance	Diarrhea, constipation, dyspepsia, indigestion, chronic flatulence, intestinal ulcers, etc.
Allergies	Asthma, food allergies, atopic dermatitis, etc.
Autoimmune disorders	Antibodies against various proteins in the brain
Endocrine and metabolic problems	Hypothyroidism, folate and vitamin D deficiencies
Neurologic	Disturbed hearing, vision, olfaction, proprioception, etc.
Persistent infections	Otitis media, skin infections, pharyngitis, conjunctivitis, etc.

## Sensation and empathy

The idea that people with autism are insensitive to pain is now largely a myth. For example, in an experimental study by (Carissa Cascio et al. 2008), researchers subjected

people with ASD to a mild electrical shock, pressure, heat, or cold. The results showed that individuals with autism have normal pain thresholds or may even be more sensitive to pain. Another myth surrounding ASD patients is that they lack empathy. Two imaging studies (Bird et al. 2010, Fan et al. 2014) revealed that the insula and anterior cingulate gyrus, brain regions responsible for empathetic feelings for other people's suffering, are equally activated in autistic people and healthy controls. However, individuals with ASD have difficulty displaying their emotions, which was associated with lower intensity of signals in the same brain areas.

## **Neurological problems**

Autism is a clinically complex disease in which, in addition to classic neuropsychiatric symptoms and signs, many sensory and motor pathologies are described (Bhat et al. 2011). The variety of these clinical manifestations includes auditory, visual, olfactory, tactile, vestibular, and other sensory disorders (Crasta et al. 2020, Jorquera-Cabrera et al. 2017, McCormick et al. 2016, Suarez 2012). Furthermore, many children with autism have different types of motor impairments, including fine and gross motor problems, in the form of poorly coordinated gait, difficulty manipulating objects, and troubles with writing (Bhat et al. 2011). Some have challenges coordinating movements between the left and right body sides (Thompson et al. 2017), which points to abnormalities in the corpus callosum that will be discussed in the next section. Others may have reduced muscle tone and hence problems maintaining posture, balance, or hand-eye coordination. Such a variety of pathologies can indicate both disorders in CNS and PNS, including afferent and efferent pathways.

Since ASD is primarily a neurodevelopmental disorder, it is intuitive to assume that autistic patients have more brain, spinal cord, and PNS problems. Examples of such issues include various types of headaches, including migraines, as well as epilepsy (Kursun et al. 2021, Lukmanji et al. 2019, Pan et al. 2020, Vetri 2020). Interestingly, the review by (Vezzani et al. 2015) described a variety of infectious and inflammatory conditions that could result in the development of epilepsy. Since infections and inflammation are almost universally present in ASD, as will be discussed later in the paper, epileptic seizures should not be surprising in these patients.

## **Gastrointestinal issues**

Understanding the possible etiological factors and pathogenetic mechanisms may contribute to recognizing and treating various medical diseases currently associated with autism. For example, children with autism suffer from frequent pain syndrome and such GI symptoms as abdominal pain, bloating, belching, diarrhea or constipation, flatulence, nausea, and reflux (Berry et al. 2015, Zeratsky 2019). Proper medical treatment of these diseases can improve affected patients' quality of life, well-being, and behavior.

## Endocrine and metabolic problems

Endocrine issues are often underdiagnosed in ASD patients due to a multitude of other problems. Additionally, persistent inflammation, abnormal microflora, and restrictive nutrition contribute to the disruption of endocrine health (Loyacono et al. 2020). In fact, thyroid diseases are among the most frequently found in autistic individuals (Fini and Demeneix 2019). Since thyroid hormones are essential for the normal metabolism of various organs and tissues, including the nervous system, it is crucial to recognize and treat these problems in ASD patients to prevent further exacerbation of their symptoms. Interestingly, the study by (Petrov AM et al. 2017) found an association between the disruption of cholesterol metabolism and such neurodegenerative diseases as Alzheimer's and Parkinson's as well as ASD. Such metabolic disorder as type 2 diabetes mellitus was found to exacerbate ASD symptoms, likely due to the tissue damage from high blood glucose levels (Alhowikan et al. 2019). Moreover, dietary and environmental factors like folic acid and vitamin D deficiency were found to play a significant role in developing autistic phenotype (Alhowikan et al. 2019). Overall, it is evident that a broad spectrum of health problems seen in ASD patients, behavioral and somatic, should have a complex but common etiology, which we suggest to be various infections and persistent systemic inflammation.

## Autistic brain: structural, cellular and molecular changes

Autopsies of ASD patients and brain imaging showed that these children often have substantial disturbances in their brain anatomy compared to neurotypical individuals. For example, it was reported that during the first year of life, autistic children have an enlargement of their brains, which disappears by adolescence, but it is explained by shrinkage of various brain regions rather than return to normal (Galvez-Contreras et al. 2020).

There is no doubt that the factors mediating future ASD develop either in early postnatal period or even prenatally. For example, neuroimaging study of 106 infants at high familial risk of ASD and 42 low-risk infants showed that hyper-expansion of the brain's cortical surface area between 6 and 12 months of age precedes brain volume overgrowth observed from 12 to 24 months (Hazlett et al. 2017). Some other studies have shown that children with ASD have a significant decrease in the number of Purkinje cells, formation of which is initiated only during the prenatal period, and hence it indicates that ASD-related changes in the brain have a prenatal origin (Fatemi 2016). Several studies examining behavioral and skill pathologies of autistic children show that autistic children have numerous differences in many regions of the brain which can result in behavioral and skills disturbances. The differences have been found in the cerebral cortex (Herbert et al. 2002), temporal cortex, and cerebellum (Ladd-Acosta et al. 2014) which are responsible for the impaired skills found in children with autistic behavior. Several studies show that pathological changes in the brain occur because of constant exposure to inflammatory and oxidative factors. For example, ASD patients are believed to have abnormal axonal connectivity due to persistent inflammation and oxidative stress that results in glial

activation and abnormal myelination (Galvez-Contreras et al. 2020). Chronic inflammation is caused by a number of factors, including viral, bacterial, fungal, and parasitic infections, the influence of abnormal microflora, and autoimmune or endocrine maternal pathologies during pregnancy – all of which will be discussed later in the paper. Sustained neuroinflammation that results from these inciting events is characterized by overactive microglia, resident macrophages of the brain that, when chronically activated, continue producing pro-inflammatory cytokines such as IL-8 and TNF-alpha (Liao et al. 2020, Pangrazzi et al. 2020). In turn, IL-8 recruit neutrophils, resulting in persistent acute inflammation that causes oxidative stress. Since the brain contains a high amount of lipids, reactive oxygen species can produce extensive damage to the CNS (Pangrazzi et al. 2020). Even when acute inflammation is resolved, in the case of some infectious agents, chronic inflammation may persist, producing more pro-inflammatory mediators and reactive oxygen species that may damage tissues and organs.

### **Oxidative damage and abnormal myelination**

Abnormal myelination in ASD occurs because oligodendrocytes, the cell responsible for nerve myelination in the CNS, are particularly susceptible to oxidative damage due to insufficient amounts of glutathione and abundance of sphingolipids (Thorburne and Juurlink 1996). In fact, the report by (Galvez-Contreras et al. 2020) describes multiple regions of the brain that have abnormal myelination. These regions include the orbitofrontal cortex, lateral prefrontal cortex, anterior cingulate gyrus, arcuate fasciculus, amygdala, medial lemniscus column, thalamus, temporoparietal junctions, and some others (Galvez-Contreras et al. 2020). Notably, magnetic resonance imaging (MRI) revealed asymmetry in these regions of the brain in a cohort of 411 ASD children (Richards et al. 2020). The state of persistent inflammation caused by various factors in ASD children made it inevitable for them to develop neuronal and glial damage from the byproducts of inflammatory process.

### **Molecular abnormalities in ASD brains**

Apart from gross changes in the brains of autistic patient, disruptions of molecular pathways were also examined. The study by (Silva et al. 2019) showed that microdeletions in the 15q11.2 chromosomal region resulted in an increase in the white matter volume but a reduction in the size of the corpus callosum. At the same time, the disturbance of epidermal growth factor (EGF) or its receptor that are involved in promoting the proliferation of oligodendrocytes was also associated with ASD symptoms. Specifically, low levels of EGF were found in adult patients with high-functioning autism (Suzuki et al. 2007). The review by (Chen et al. 2014) demonstrated that the disruption of IGF-1/PI3K/AKT/mTOR signaling pathways was found to play a role in various neurodevelopmental disorders (NDDs), including ASD, because this pathway impacts such vital cellular processes as metabolism, response to hypoxia, and, in case of neurons, neurotransmitter synthesis (Pagani et al. 2021). Moreover, research by (Aida et al. 2015) showed that the GLT1 receptor knockout mouse models demonstrated an elevated ratio of excitation-inhibition in astrocytes, resulting in repetitive patterns of behavior. It may suggest that glucose transport disruption may be present in the glial cells of autistic children. Overall,

these studies indicate that ASD pathophysiology and abnormal wiring in the brain are not caused by one change or several similar disruptions but by various factors that affect balanced myelination, oligodendrocyte proliferation, apoptosis, cellular metabolism, response to inflammatory stress, and abnormal neurotransmitter production. All these disruptions in the normal functioning of the CNS are caused by genetic and epigenetic changes that result in altered transcriptional regulation, which in turn contributes to the gross changes described earlier. Moreover, infectious agents and inflammatory mediators play an important role in these changes as well, making them as important potential targets for a curative treatment of ASD patients.

## The role of brain inflammation in ASD

As seen from the information presented above, inflammation of the CNS is uniformly present in children with autism. Although the exact mechanism of how inflammatory mediators result in ASD is yet to be found, one of the reasons why persistent inflammation induces the development of autistic phenotype is that it alters the morphology of astrocytes and microglia in the hippocampus and the frontal cortex, two essential regions for memory formation, higher-order cognition, and behavior (Matta et al. 2019). Microglia and astrocytes play a critical role in synaptic plasticity under a normal physiologic state; however, when they are constantly exposed to infectious agents and pro-inflammatory cytokines, their morphology changes, affecting their normal functioning (Matta et al. 2019). As discussed later in the paper, these children are exposed to maternal infections and inflammation *in utero*, which contributes to abnormal brain development. Since the inflammatory state persists, glial cells remain in their abnormal shapes, unable to perform synaptic modification and pruning.

## Genetics, epigenetics, and transcriptomics of ASD

### Genetics

Autism is a multifactorial disorder in which genetic and external risk factors interact to trigger its development. If the search is performed on the SFARI GENE database (<https://gene.sfari.org>), the results show approximately 700 genes associated with ASD, including copy number variations (CNVs), while some papers report about 1000 candidate autism-related genes (Ramaswami et al. 2020). And amongst them, hundreds of genes have been identified as contributors to deficits in social communication, cognition, and behavioral problems of ASD patients (Cheroni et al. 2020). Still, none of these mutations were found to explain more than a small subset of autism cases. The candidate genes, potentially involved in ASD development, are known to participate in the formation of fetal brain architecture and some other organs and tissues (Masini et al. 2020). Examples of the genes include MECP2, CHD2, CHD7, FMR1, NF1, PTEN, CDKL5, and many others (Masini et al. 2020). Notably, genes that were found to be associated with ASD had also been described to be involved in other pathologies. For instance, germline mutations in the PTEN gene have been identified in up to 20% of children with ASD, macrocephaly, and

white matter abnormalities (Busch et al. 2019). At the same time, this gene mutation is associated with a high risk of various malignancies and multiple hamartomas (Yehia et al. 2020). As another example, CHD2 mutations relate to various NDDs characterized by onset epileptic encephalopathy with refractory seizures and progressive cognitive decline associated with it (Carvill and Mefford 2015). CHD2-related disorders are autosomal dominant, caused by a de novo pathogenic variant, which means these mutations are sporadic (Carvill and Mefford 2015). As important as they may seem, genetic studies play a minor role in understanding this disease. Instead, epigenetic and transcriptomics research was found to be crucial to identifying the abnormal molecular pathways and mechanisms as future therapeutic targets.

## Epigenetics and transcriptomics

Gene expression studies have been instrumental in comparing groups of people with ASD and control samples to measure which genes are dysregulated in ASD. Transcriptomic studies are essential as a key link between measuring protein levels and analyzing genetic information. In this field, research concentrates on epigenetic and transcriptional alterations in ASD development. For instance, hypermethylation of specific regions of MECP2 were associated with the behavioral changes observed in ASD (Masini et al. 2020). Moreover, the study by (Hughes et al. 2018) showed that activation of TLR2 and TLR4 genes with LPS exposure in monocytes from ASD patients resulted in consistently elevated inflammatory pathways. In contrast, similar activation in healthy controls ended with a gradual decline of these pathways. It means that these children are born with aberrant epigenetic changes in their immune system, causing a persistent inflammatory state. In fact, gene expression studies have shown that there is a significant upregulation of genes associated with the KEGG inflammatory pathway in ASD patients (He et al. 2019, Hughes et al. 2022). Moreover, transcriptome analysis studies revealed substantial abnormalities in gene expression patterns in microglia, astroglia, and cortical neurons of autistic children (Gupta et al. 2014, Velmeshev et al. 2020). Transcriptional regulations were also found to play a significant role in ASD phenotype. Specifically, a number of microRNAs (miRNAs), small non-coding RNA involved in transcriptional regulation, were reported to be downregulated (miR-23a-3p, miR-628-5p, miR-32-5p, and miR-140-39) or upregulated (miR-2467-5p and miR-7-5p) in these patients (Sehovic et al. 2020). These miRNAs are essential for regulating various processes during cell differentiation and proliferation, targeting messenger RNAs of the genes that were earlier found to be involved in ASD.

To summarize the genetics, epigenetics, and transcriptomics data presented above, although above 100 ASD-susceptibility genes have been found, the utility of this information remains elusive. On the other hand, gene expression studies allow to understand thresholds for differential expression of genes, which in turn helps to identify pathologic pathways (Ansel et al. 2017). Approximately 12,000 genes were differentially expressed between ASD compared to controls in gene expression studies since 2011. Among the 12,000 differentially expressed genes in various studies over the past decade three main sources were recognized: brain (about 3500 dysregulated genes), GI (approximately 2600 dysregulated genes), and lymphoblastoid cell lines (5600



dysregulated genes) (Ansel et al. 2017). Notably, these ASD gene expression studies allowed to find various pathways that lead to abnormalities in cell cycle, immune function, neurogenesis, and GI diseases in autistic patients. It suggests that targeting the causes of these dysregulations may lead to improvement of behavioral and somatic symptoms in children with ASD.

## Parental conditions

Parental well-being is an essential regulator in the future child's health not only in terms of inheritance but also in the sense of the autoimmune, metabolic, and inflammatory effects of, especially, maternal health issues on the developing fetus. ASD is not an exception because various endocrine and infectious diseases of the mothers had a strong correlation with autism in their children. For example, the systematic review by (Han et al. 2021) assessed 26 studies and 32 meta-analyses to identify the relation between maternal diseases during pregnancy and NDDs in the offspring. The authors report that pre-eclampsia, obesity, gestational diabetes, autoimmune conditions, stress, depression, and infection had a statistically significant association with ASD in children (Table 2). Furthermore, prenatal stress was found to make male infants more susceptible to the development of ASD, while female infants were more prone to develop affective disorders later in life (Wilson et al. 2021). In the case of pre-eclampsia, high blood pressure in a mother leads to impaired blood flow to the placenta, leading to hypoxia in a fetus, which damages the developing brain in the first place. The elevated glucose level in gestational diabetes is harmful to the organs and tissues of a future child due to the damage from advanced glycation end (AGE) products, resulting in a range of health problems, including NDD. Hypothyroidism, regardless of its origin but mostly of inflammatory and autoimmune nature, was reported to be linked to autism development (Wilson et al. 2021). Indeed, thyroid hormones play a critical role in CNS formation; hence, it is not surprising that women with severe disbalance of thyroid function are at greater risk of giving birth to children with NDDs.

Summarizing the information presented in Table 2, it is important to state that all these conditions, despite the difference in clinical manifestations, are united by one important condition – chronic immune activation and systemic inflammation (Abell et al. 2015, Ellulu et al. 2017, Harmon et al. 2016, Liu et al. 2017, Shi et al. 2021). It is known that chronic infectious pathologies are inducers of chronic inflammation (Pahwa et al., 2021). However, the etiologic relationship between infection and inflammation can be bidirectional since chronic low-gradient inflammation can be a modulator of susceptibility to infections (Aguilera and Lenz 2020, Kaspersen et al. 2016). That is, it cannot be ruled out that, despite the difference in the nosology of maternal pathologies listed in Table 2, infections and inflammation can jointly play a role in inducing and promoting autism.

Table 2.

The effect of maternal diseases on the developing fetus (Han et al. 2021, Wilson et al. 2021)

Maternal condition during pregnancy	Effect on fetus
Pre-eclampsia	Impaired blood flow to placenta is harmful for fetal brain
Gestational diabetes	AGEs damage various organs and tissues
Obesity	High pro-inflammatory profile
Hypothyroidism	Low level of thyroid hormones causes abnormal brain development and growth retardation
Autoimmune diseases	High inflammatory profile
Chronic stress	Exposure to cortisol at the wrong time during intrauterine development may disrupt normal brain formation
Infections	Persistent inflammatory state and toxic metabolites damage the developing brain and other organs

## Maternal infections and inflammation in ASD

Various maternal infections during pregnancy were found to have a positive correlation with the clinical manifestation of ASD. A 2019 publication in the journal *JAMA Psychiatry* showed the involvement of maternal infection in the development of ASD. The article presented the results of a study in which about 1.8 million children born in Sweden were observed from birth for over 40 years (Al-Haddad et al. 2019). The results of this study suggest that maternal infection requiring hospitalization increased the risk of autism and depression over the lifetime of observed children (Al-Haddad et al. 2019). In a Danish study that lasted from 1980 to 2005, it was found that children born to mothers who were hospitalized due to a viral infection in the first trimester and a bacterial infection in the second trimester had a higher risk of being diagnosed with ASD (Atladóttir et al. 2010). Another Swedish study (Lee et al. 2015) examined the association between maternal infection during pregnancy and the risk of ASD in the nationwide register-based birth cohort from 1984 to 2007. The total sample consisted of over 2 million people with 24,414 cases of ASD (Lee et al. 2015). The results showed that the odds ratio of developing the disease increased by approximately 30% in children born to mothers who had an infection at any point during their pregnancies. Remarkably, in the subsample analysis, ASD with mental retardation had a higher association with the term infections.

Other clinical and epidemiological studies also confirmed maternal infection's role. In the study by (Fang et al. 2015), the data about 4184 children diagnosed with ASD and 16,734 non-autistic controls between 2000 and 2007 were retrieved from the National Health Insurance Research Database. Specifically, information about the maternal infection, medical history, and sociodemographic data was obtained and analyzed. The study found that there was a slight increase in the risk of ASD development in a child if a mother had two or more hospital visits for vaginal infections during pregnancy (Fang et al. 2015). The meta-analysis by (Tioleco et al. 2021) found a correlation between maternal infection and

autism risk across 36 studies, assessing children with the definitive diagnosis of ASD and their prenatal exposure to infections. In fact, even adjustment for publication bias only mildly reduced the statistical significance. The prospective multicenter study by (Joseph et al. 2017) assessed the past medical records of 889 children with ASD and intellectual disability for prenatal exposure during 23-27 weeks of pregnancy from 2002 to 2004. The authors report that evaluation of the interviews obtained from the mothers showed that vaginal infections during the early stages of gestation seemed to have an increased association with the diagnosis of ASD with intellectual disability. Another meta-analysis by (Jiang et al. 2016) found that among the 15 studies assessed, any maternal infection during pregnancy increased the odds of ASD progression in a child by 12%. The authors suggest two possible mechanisms of involvement of maternal infections in the formation of autistic phenotype in children: direct exposure of a fetus to a pathogen and fetal brain damage from the maternal immune activation in response to infection.

### Wall-free pathogens (L-Forms)

One of the most interesting discoveries about the role of a mother's illnesses in children's autistic phenotype was a wall-free pathogen, also known as L-form. This phenomenon was found both in the intestines of autistic patients and their mothers (Markova 2017, Markova 2019). The pathogenic microflora was presented by *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Morganella morganii*, *Chryseobacterium indologenes*, *Proteus mirabilis*, *Candida parapsilosis*, *Cryptococcus albidus*, *Rhodotorula mucilaginosa*, *Cryptococcus albidus*, *Aspergillus fumigatus* and several others (Markova 2019). Interestingly, almost all autistic children had wall-deficient forms of *Aspergillus fumigatus* in their guts as well as the circulation, but it was not detected in healthy controls (Markova 2019). Moreover, *Candida parapsilosis* and *Rhodotorula mucilaginosa*, as well as cultures of opportunistic bacteria, often found as causes of urogenital infections were isolated from the blood of children with ASD. The increase in the specific characteristics of IgG, IgM, and IgA, together with the typical growth of mold, was a decisive argument in favor of the proven presence of *Aspergillus fumigatus* in almost all autistic children. A fungal infection like *Aspergillus fumigatus* or *Candida albicans* may suppress the immune system and damage the normal development of the nervous system through the production of toxic metabolites (Markova 2019). Notably, L-forms are capable of transforming into a fully-functional reproducing cell (Markova 2019). The theory is that infection may occur through vertical transmission from a mother to a fetus during pregnancy since L-forms were demonstrated to be capable of crossing the placenta (Markova 2019). This finding explains why no therapy used for the treatment of ASD is effective when used alone since improving the state of microbiota or exclusively targeting neurotransmitters only improves some symptoms. However, the fragmented approach is not curative because the chronic systemic inflammation present in autistic children is multifactorial in nature. Hence, evaluating pregnant women for prenatal infections and treating them accordingly may help reduce the incidence of ASD in children.

## **TORCH infections and immune dysregulation**

TORCH infections are a group of congenital infections that may cause severe abnormalities in the developing fetus. TORCH stands for Toxoplasmosis, Other (Syphilis, Parvovirus B19, HIV, HBV, and Varicella Zoster), Rubella, CMV, and HSV (Morsy et al. 2022). These pathogens are considered teratogens because they cause substantial developmental anomalies in the infected fetuses (Jash and Sharma 2022). The clinical presentation of TORCH infections at birth is described with such symptoms as meningoencephalitis, cerebral palsy, thrombocytopenia, anemia, sensorineural deafness, visual deficit, seizures, intracranial calcifications, growth retardation, and substantial delay in attaining developmental milestones (Hon et al. 2020). Apart from harming an infant's physical well-being, TORCH infections play a significant role in disturbing normal brain development and leading to ASD.

These infections negatively impact brain development in the prenatal period, during which about 57% of crucial differential gene expression in the neocortex occurs (Kang et al. 2011). The proposed mechanism, in this case, is that TORCH or other infections can cause the intrauterine cytokine storm, which alters fetal brain transcriptome (Jash and Sharma 2022). Specifically, it was reported that the level of IL-17 was elevated in the amniotic fluid of these mothers, as well as in the serum of many autistic patients (Jash and Sharma 2022). The literature shows that IL-17 plays an essential role in bacterial, fungal, viral, and parasitic infectious processes, autoimmune diseases, and even cancers (Ge et al. 2020). It interacts with such mediators as NF- $\kappa$ B, MAPK, GM-CSF, IL-22, and IFN- $\gamma$  to produce a pro-inflammatory state, particularly in the spleen, heart, and brain (Ge et al. 2020). TORCH, as well as other pathogens, cause a substantial immune reaction that damages the host, resulting in tremendous developmental problems seen in ASD patients.

## **Formation and pathogenic effects of abnormal microbiome ASD**

Severe disorders caused by TORCH infections in the form of a “dysfunctional or even paralyzed immune system” also trigger the process of forming a pathogenic resident microflora in almost all organs and systems of ASD children. The microbiota plays important roles in digestion, nutrient assimilation, vitamin production, and metabolism (Jandhyala et al. 2015, van de Wouw et al. 2017). In addition, it has recently been shown to have a significant influence on the bi-directional signaling, which occurs between the intestinal microflora and the nervous system, termed the microbiota-gut-brain axis (Cryan and Dinan 2012).

## **Clinical presentation of dysbiosis**

In autism, along with many symptoms associated with the pathology of the nervous system, children with ASD tend to have medical issues related to the GI tract (Zeratsky 2019). The problems show a dysbiosis which clinically manifests itself by a wide range of symptoms of digestive disorders, including diarrhea, cramping, constipation, bloating,

abdominal pain, nausea, and vomiting (Holingue et al. 2018). Dysbiosis in children with autism contributes to a range of other health conditions with varying degrees of severity. These include cavities, periodontal inflammation, frequent infectious lesions in the form of stomatitis, as well as fungal and bacterial oral infections, accompanied by halitosis. In addition, ASD children's stool often contains undigested food, has an unusual color and smell, and, in some cases, apparent signs of predominant fungal microflora in the form of moldy growth on the surface of feces. The abnormal intestinal microbiota is believed to be the main contributor to the manifestation of these symptoms.

## A possible root cause of dysbiosis

The establishment and development of the early gut microbiome in infancy are essential for immune maturation and metabolic programming (Lozupone et al. 2012). Many factors can disrupt the formation of a healthy microbiome. One of the examples of such a factor is the delivery mode that largely determines the early gut microbial composition in children. Infants delivered by Cesarean section (CS) miss contact with the maternal vaginal microbiome compared to infants born by vaginal delivery (VD), suggesting that CS delivery perturbs the early establishment and development of the infant gut microbiome, increasing the risk of having skin microflora (e.g., *Staphylococcus aureus*, *Streptococcus mutans*, *Propionibacterium acnes*, and other bacteria belonging to four phyla – Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria) to be predominant in the intestines of the infants born through CS (Davis 1996, Dominguez-Bello et al. 2010). The list of possible pathologies associated with the formation of intestinal dysbiosis is diverse. For most, a link between intestinal dysbiosis and other pathological processes has not been established yet, but for some, a causal relationship and the underlying mechanisms have been demonstrated. Examples of the latter include type 1 diabetes (Vatanen et al. 2016, Vatanen et al. 2018) and allergies (Chua et al. 2018, Lee et al. 2018). For example, according to the results of a longitudinal study (Rogers and Kim 2020), maternal infections during pregnancy with rubella virus and cytomegalovirus increased the odds of developing type 1 diabetes in children 12- and 4-folds, respectively. The proven involvement of the TORCH pathogens and etiologic factors damaging the normal formation of the immune system may indicate that dysbiosis may be developing due to immune dysregulation and inflammation.

## Shift to abnormal microbiota in ASD

Dysbiosis can be defined a pathological disorder of the microbiota homeostasis caused by an imbalance between the microorganisms present in the natural human microflora, especially in the intestine. For example, it was found that ASD patients have dysbiosis with the abnormal ratio of Firmicutes to Bacteroidetes, the former being reduced while the latter being significantly elevated in their guts (Finegold et al. 2010). For reasons not fully understood, Bacteroidetes were demonstrated to have a strong correlation with autistic behavior in rodent models (Sivamaruthi et al. 2020). Still, some reports suggest that this particular genus is involved in causing autoimmunity by compromising the normal function of T-helper cells and contributes to intestinal permeability through upregulation of expression of pro-inflammatory cytokine IL-6 (Al-Haddad et al. 2019, Markova 2017) . The

latter should certainly be present in small amounts in fetal brain to promote stem cell differentiation into the neuroglia, but its excess leads to the damage from the immune system overactivation (Ravaccia and Ghafourian 2020). Moreover, research shows that *Clostridium* species are also associated with dysfunction in the social domain as well as with disturbance of learning and memory (Alharthi et al. 2022). In fact, many of the species of this genus are capable of producing these problems because of their ability to secrete such neurotoxins as para-cresol beta-2-toxin (Alharthi et al. 2022, Sivamaruthi et al. 2020). The former can impair phagocytosis and leukocyte adhesion, inhibit liver cytochrome p450 system, and affect neurotransmitter synthesis, while the latter is known to cause necrotizing enterocolitis. In both cases, an impaired immune system and excessively permeable intestinal wall leads to sustained systemic and brain inflammation, producing ASD symptoms.

Gut microbiota is vital for overall intestinal health and the normal functioning of such processes as food digestion, immune system regulation, energy production, and, according to recent data, behavior. It means that microbial dysbiosis, overgrowth of potentially pathogenic microorganisms, poor diversity of the microbiome, or low levels of beneficial bacteria in ASD patients can affect their behavior. Metabolome analysis in autistic children has identified perturbations in multiple metabolic pathways that might be associated with cognitive functions. Recent studies have shown that the intestinal microbiome provides environmental signals that can modify host response to stimuli by modifying the host epigenome, affecting DNA methylation, histone modification, and non-coding RNAs. The most studied microbiota-produced epigenetic modifiers are short-chain fatty acids, although other products of intestinal microbiota might also cause epigenetic alterations in the DNA of the host (Kushak et al. 2022).

A role of abnormal microflora in autism was shown in the study that found increased *Clostridia* as well as overgrowth of other spore-forming anaerobes, microaerophilic bacteria, and several *Clostridia* clusters I/IX and *C. bolteae* (46-fold increase) within the gastric and duodenal secretions not seen in controls (Finegold et al. 2002). Another research confirmed increased *Clostridia filum*, showing that its presence highly correlated with GI issues in these children (Parracho et al. 2005). In some other studies, increased *Desulfovibrio* and *Bacteroides vulgatus* were identified in children with ASD and GI dysfunction and related to autism severity (Finegold et al. 2010, Tomova et al. 2015). It is very likely that *Desulfovibrio* can be another contributor to GI inflammation, as it is a major producer of hydrogen sulfide, which is toxic for colonic epithelial cells (Carbonero et al. 2012), and may impair working memory (Ritz et al. 2016). Moreover, in the research by (Bojović et al. 2020), the role of certain bacteria in NDDs was studied. The results of that study showed an increased incidence of potentially harmful *Clostridium* bacteria in the NDD patient group compared to the control. Notably, there was a significantly smaller diversity of common commensal bacteria in the NDD patient group. *Enterococcus faecalis*, *Enterococcus gallinarum*, *Streptococcus pasteurianus*, *Lactobacillus rhamnosus*, and *Bifidobacteria* sp. were found in fewer patients or even absent in some patients with NDD (Bojović et al. 2020). The authors suggested that such drastic changes in the microflora of

these patients were associated with the altered production of short-chain fatty acids in children with NDD.

## Abnormal microbiome starts forming at birth

Shaping of an infant's microflora, normal or pathologic, begins when one is born either through vaginal delivery or CS. In children with ASD, this microbiome consists of higher than usual, *Staphylococci*, *Streptococci*, *Actinomyces*, pathogenic *Clostridia*, *Egertella lenta*, *Propionibacterium acnes*, as well as pathogenic fungi such as *Candida*, *Aspergillus*, and other *Micromycetes* (K. Alibek, MD, unpublished data, 2022). These microbes further contribute to the pathological state of autism by enhancing the chronic inflammation, oxidative stress, and intoxication effects produced by virulence factors or chemical toxins they secrete.

## Clostridial overgrowth

The research also shows that autistic children having an overgrowth of the spore-forming *Clostridium filum* in their intestines developed a regressive form of ASD, in addition to having severe GI symptoms (Hughes et al. 2018, Sandler et al. 2000). Although the administration of vancomycin improved communication and social behavior, ASD symptoms usually recurred soon after the removal of this treatment (Hughes et al. 2018). Another study by (Saleem et al. 2020) assesses plasma and serum levels of various amino acids, oxidants, and byproducts of biochemical reactions like ammonia. The authors report that ASD patients had a significant disruption of amino acid metabolism, high ammonia concentrations, and elevated oxidative stress (Saleem et al. 2020).

## Small intestinal bacterial overgrowth

Another GI problem often seen in ASD patients is small intestinal bacterial overgrowth (SIBO), which is presented by bloating, distention, diarrhea, and abdominal pain after eating carbohydrate-rich meals, legumes, grains, or excess fiber (Adike and DiBaise 2018, Rana and Bhardwaj 2008). The study by (Wang et al. 2018) evaluated 310 ASD children for the presence of SIBO, and the results showed a statistically significant correlation between SIBO and autistic symptoms of social domain disruption. Overall, it appears that the dysbiotic microbiome participates in the formation of the pathological state of autism by strengthening the pro-inflammatory state, chronic inflammation, oxidative stress, and intoxication produced by toxic metabolites secreted by pathogenic microflora.

## Biofilms are the main obstacle to curing dysbiosis

When discussing pathogenic microflora in ASD patients, it is crucial to elaborate on why anti-microbial therapies fail to produce the desired effect soon after the completion of these therapies. It appears that biofilms play a critical role in this problem. Biofilm is a complex structure of microbiome having different bacterial colonies or single type of cells in a group,

adhering to a surface. These cells are dispersed in extracellular polymeric substances, a matrix composed of eDNA, proteins, and polysaccharides, which showed high resistance to antibiotics (Sharma et al. 2019). About 80% of recurrent and chronic bacterial infections occur due to biofilms (Sharma et al. 2019). Microbial cells within biofilms were found to have 10–1000 times greater antibiotic resistance than the planktonic cells (Mah 2012). *Streptococcus mutans*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermis*, and *Escherichia coli* are known for their capacity to establish biofilms (Sharma et al. 2019). Another group of pathogens, including *Clostridia* spp., yeasts (*Candida*), and Gram-negative bacteria, cause chronic GI infections in the host when protected by the biofilm. Additionally, these biofilms serve as shields for the pathogens, preventing their recognition and destruction by the immune system (Kernien et al. 2018). The stability of a biofilm is explained by the matrix, which is composed of a negatively charged polysaccharide substance held together with positively charged metal ions (calcium, magnesium, and iron). The microbes in a biofilm are embedded in such a way that they are protected from UV exposure, metal toxicity, acid exposure, salinity, phagocytosis, antibiotics, antimicrobial agents, and the immune system (Anwar et al. 1990). The protection that biofilms give microbes does not allow short-term introduction of antimicrobials to overcome the problem of dysbiosis due to the predominance of pathogenic microflora. At the same time, we cannot exclude the possibility of antimicrobial resistance both in bacteria and fungi precisely because of their ability to form biofilms.

## Immune dysfunction in ASD

As discussed earlier in this paper, children with ASD struggle with immune dysfunction, among other things. Indeed, immune system dysregulation makes these children susceptible to damage from opportunistic pathogens. Furthermore, they become prone to develop autoimmune disorders that, by definition, produce harm to various tissues and organs of a child who was already born with some structural abnormalities of some organs due to the undesired prenatal exposure to toxic or infectious agents. These problems include allergic reactions, contact dermatitis, chronic inflammation, and persistent infections like otitis media, as well as GI and GU infections (Autism Society n.d.). The role of viruses that will be discussed further has not been clearly defined in ASD since many people have herpesviruses in particular. Still, it is believed that immune dysregulation in autistic children occurs due to prenatal or postnatal infection with some human herpesviruses or rubella, in addition to various factors discussed above. It appears that the persistent presence of TORCH infections as well as dysbiotic microflora prevents the immune system from functioning normally.



## Mechanisms of inducing immune dysregulation by some TORCH infections

### Cytomegalovirus

Cytomegalovirus (CMV) belongs to the family of human herpesviruses and infects approximately 60-90% of the world population (Söderberg-Nauclér 2012). Furthermore, congenital CMV infection is a leading cause of various neurologic disabilities and hearing loss in children (Boppana et al. 2013). Although the risk of developing long-term sequelae from congenital CMV is high, most infants with this infection are asymptomatic. Still, approximately 10%-15% of these children develop long-term complications (Boppana et al. 2013). Primary CMV infection commonly causes a mild illness in immunocompetent individuals, presenting with lymphopenia, lymphadenopathy, fever, transaminitis, and splenomegaly. In contrast, CMV may cause substantial harm to immunocompromised children (Bateman et al. 2021) and ASD patients are not protected from harmful effects of this virus.

CMV encodes numerous proteins and miRNAs that allow the virus to replicate and disseminate in the body, evading the immune system and resulting in detrimental health outcomes (Jackson et al. 2017). Primary CMV infection triggers a series of cell-mediated immune reactions, first by innate NK cells, then adaptive CD4+ and CD8+ T cells, and plasma cells (Jackson et al. 2011). The CMV can reactivate sporadically in these immune cells, leading to vicious cycles of stimulation of inflammatory mediators, autoimmunity, and, in some cases, cancer. The adaptive immune response to CMV, as well as to many other viruses, is characterized as a large number of effector-memory T cells, causing “memory inflation,” which is a clonal expansion of CMV-specific CD8+ T cells (O'Hara et al. 2012, Pardieck et al. 2018). Overall, CMV infection was shown to be associated with higher morbidity and mortality in immunocompromised hosts (Rahbar et al. 2013, Savva et al. 2013, Wolmer-Solberg et al. 2013).

### Epstein-Barr virus

The Epstein-Barr virus (EBV) is a highly successful human herpes virus that infects more than 90% of people globally at some point in their lives (Ressing et al. 2015). Transmission of EBV through saliva leads to infection of the epithelial cells of the oropharynx. From epithelial cells, EBV can infect B cells, which are the main reservoir for the virus. EBV can move between different types of cells, mainly B cells and epithelial cells. Systemic autoimmune diseases often occur as overlapping syndromes with symptoms and characteristic autoantibodies. Moreover, because the virus can switch between the latent and lytic life cycle, EBV can cause chronic reactivating infections (Houen and Trier 2021). EBV causes latent infection in B lymphocytes, characterized by limited expression of viral genes. To produce new viral offspring, EBV is reactivated from its latency. During the active phase, EBV uses cellular machinery to express more than 80 of its genes. Even though EBV-specific T lymphocytes can react and destroy infected cells, EBV remains in its host

for a lifetime due to the multiple immune evading and suppressing mechanisms (Ressing et al. 2015).

Although symptomatic EBV infection is rare in infants, it can sometimes cause such neurologic disorders as meningitis, encephalitis, transverse myelitis, Guillain-Barré syndrome, cerebellar ataxia, sleep disorders, and psychoses (CDC 2020). Apart from these pathologies, EBV infection can cause hematologic and immune problems. The virus can induce severe lymphocytosis, neutropenia, hemophagocytic syndrome, hypogammaglobulinemia, and lymphoproliferative disease. These immunosuppressive conditions make infected individuals prone to secondary infections (CDC 2020).

## Rubella

Congenital rubella syndrome (CRS) is an infant disease that stems from maternal infection with rubella virus (Lanzieri et al. 2020). Infection with this virus in the first trimester may lead to stillbirths, miscarriages, and severe birth defects (Lanzieri et al. 2020). Fortunately, defects are rare after the twentieth week of gestation. Although the disease is primarily asymptomatic in mothers, their babies are almost always affected, especially if the infection occurs during the first 12 weeks of pregnancy (Lanzieri et al. 2020). Early complications include deafness, cataracts, heart defects, brain disorders, bone deformities, and liver or spleen damage in newborns (Lanzieri et al. 2020). Late-onset manifestations of CRS are diabetes, thyroid dysfunction, and visual or neurological abnormalities.

## Amplification of ideas

All the examples presented above show that a large variety of pathogens can play a role in the initiation, promotion, or worsening of the course of ASD. And there is a high probability that several cases of ASD could result not from a single infectious pathology but poly-infection. Many autistic patients' post-mortem samples had a burden of infections, having 2-3 infections, including the various herpes viruses, including HHV-6 and CMV, while the samples of healthy controls had no more than one virus (Lintas et al. 2010). Based on this relatively limited study, there could be another possible conclusion, the brain tissues of individuals with autism have several different viruses, which belong to different serotypes, species, and even families, suggesting that these pathogens may be triggering the development of autistic phenotype by the maintenance of chronic inflammation, harmful for the host. Overall, it appears that ASD can be induced by viral, bacterial, and fungal maternal infections that cause aberrant genetic, epigenetic, and transcriptomic changes as well as gross changes in the brain and even some other organs of these children.

## Conclusions

Autism spectrum disorders are a range of neurodevelopmental problems that often present clinically as repetitive behavior, restricted interests, and a lack of capacity for regular social interaction. The inflammatory and infectious nature of this disease is now a predominant

theory about the etiology of this disorder, whereas other hypotheses related to unempathetic parents and heavy metals exposure are no longer valid. Inflammation in a fetus can be caused by various factors, ranging from TORCH infections to wall-free pathogens from the mother. Another critical contributor to immune, humoral, and nervous system dysfunction in ASD patients is the predominance of pathogenic microflora. All these factors trigger sustained systemic inflammation and oxidative stress that cause mutations, abnormal epigenetic alterations, and tissue necrosis in various organs, particularly in the brain. Necrosis and inflammation produce brain edema and subsequent shrinkage, resulting in irreversible changes in anatomy and physiology, producing distinct autistic phenotype. Overall, the results and findings of the studies discussed in this paper suggest that future methods for improving the symptoms can be based on prophylaxis and treatment of maternal infections during and even before pregnancy. In ASD children, complex anti-microbial and anti-inflammatory therapy not only can improve somatic symptoms but also enhance the efficiency of treatment methods that target behavioral problems in these patients.

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This article does not contain any studies with human participants or animals performed by any of the authors.

## **Author contributions**

All authors equally contributed to writing and editing this article.

## **Conflicts of interest**

The authors declare that they have no conflict of interest.

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