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AUTISM SPECTRUM DISORDER, MATERNAL IMMUNE ACTIVATION AND HUMAN INDUCED PLURIPOTENT STEM CELLS

What can research using hiPSC models tell us about maternal immune activation as a cause to autism spectrum disorders?

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Abstract

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by a wide range of symptoms and varying degrees of severity. Understanding the underlying mechanisms of ASD is crucial, as it affects approximately 1% of the population and has a strong genetic component. Recently, environmental factors like maternal immune activation (MIA) during pregnancy have been linked to a higher risk of ASD. While animal models have limitations, the advent of human induced pluripotent stem cells (hiPSCs) and their differentiation into human brain organoids (hBO), neurons and glial cells has enabled researchers to study human brain cells in vitro, offering insights into human-specific responses and the interaction of MIA. This review summarizes studies utilizing iPSC-derived models, primarily from healthy individuals but also ASD patients, exposed to inflammatory cytokines or heat shock. The result indicates that there is a connection between immune activation and cellular and molecular changes in brain cells, including changes in ASD-related genes, neuroinflammation, structural brain alterations, and potential synaptic dysfunction, potentially leading to the development of ASD. This is supported by previous studies suggesting that MIA triggers immune responses and genetic alterations in the fetal brain, resulting in brain development and behavioral abnormalities in offspring. The utilization of models like hBO derived from hiPSCs of individuals with ASD holds promise for advancing diagnostic capabilities and developing targeted interventions. For instance, it may pave the way for implementing anti-inflammatory treatments during pregnancy to mitigate ASD risk factors. However, further investigation is necessary to fully understand the cellular and molecular mechanisms associated with MIA and identify key pathophysiological changes in ASD progression.

Content

Introduction

Autism spectrum disorders (ASD) are a collective term of neurodevelopmental disorders with a strong genetic vulnerability (1). Autism was initially described by Leo Kanner in 1943, who characterized it as an inability to establish typical, biologically endowed connections with others (2, 3). In the same year, Hans Asperger described children exhibiting restricted social abilities, peculiar interests, and atypical language development, employing the term "autistic psychopathy" (4, 5). More recently, several hundred genes have been associated to ASD. In addition, there are also several environmental factors that can influence the development of the brain in the fetus, such as the use of medications, exposure to toxins, complications during pregnancy or childbirth, or infections. Several lines of evidence have suggested maternal immune activation (MIA) may increase the risk of ASD during an infection by increasing inflammatory markers and antibodies in both mother and fetus (6). There are primarily two hypotheses regarding how MIA affects the fetus: one posits that cytokines are transmitted via the placenta, leading to immune and gene dysregulation in the fetus, while the second hypothesis suggests that maternal immune activation triggers inflammation and cytokine production within the placenta itself (7). However, fever in the mother has also been suggestion increase the risk of autism in children (8). A large amount of research has been done on ASD since Kanner and Asperger first described autism and up to today, both epidemiological, cohort studies, animal studies and studies using induced pluripotent stem cells (iPSC), but there are still questions to be answered related to the mechanisms of the etiology and pathology of ASD. Animal models have been the primary *in vivo* research method for years. The most popular method is the mouse model, but scientists have also used zebrafish (*Danio rerio)*, fruit flies (*Drosophila melanogaster)*, chicken embryos, and monkeys (*rhesus macaque)* for research on neurodevelopment disorders and other conditions in the brain (9). Problematically, the use of animals like mice as models in brain research gives a limitation related to brain size, function, and complexity, which results in an incomplete picture of the disease pathology (9, 10). Another challenge related to brain research on humans is the limited access to brain tissue, earlier the only available brain tissue from ASD patients have been post-mortem tissue. Human induced pluripotent stem cells (hiPSC) and human brain organoids (hBO) have made it possible to do more extended research on a number of neurological and neuropsychiatric diseases, e.g. autism (11, 12), but also other

conditions such as schizophrenia (12, 13), Alzheimer's disease (14) and bipolar disorder (12, 15). It has given opportunities for disease modeling, drug research and discovery, cell therapy development, and regenerative medicine (16, 17).

In this review, we will look at how induced pluripotent stem cells (iPSC) and human brain organoids (hBO) can be used in research to study environmental factors as causes of autism spectrum disorders, specifically maternal immune activation (MIA). We will first describe what ASD, iPSC, and MIA are, and then present previous research on these areas regarding autism and MIA, before we look at the result presenting the newest research on MIA as a cause to autism using hiPSC-derivate models.

Autism spectrum disorder

Classification

Today we use the International Classification of Diseases eleventh revision (ICD-11) and The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) to define the diagnostic criteria of mental disorders, such as ASD. Persons with ASD are characterized by a reduced ability to initiate and sustain reciprocal social interactions, abilities, and communication, and at the same time, they have unusual interests and/or behavioral changes. It is a heterogeneous group of disorders and symptoms normally appear early in childhood, but they can also occur later. Earlier, autism spectrum disorders were divided into various subgroups, such as Asperger's syndrome and childhood autism, but in ICD-11 and DSM-5 the subgroups have been removed, and it is now called autism spectrum disorder with a spectrum of severity, from well-functional individuals to non-functional (1, 4, 18).

Epidemiology

The prevalence of autism have increased from approximately 5 per 10 000 (19) in the 1960´, to around 1/100 in 2022 (1, 20). Males have a three to four times higher risk of developing ASD than females, a reason could be that girls have a higher risk of not being diagnosed (4). There are several possible hypotheses, but the real reason for this difference remains largely unknown (6, 21).

Comorbidity

ASD has a high comorbidity; evidence show that 72% of children with autism has at least one comorbid condition. The most common comorbid conditions are anxiety disorders and ADHD. Other comorbid conditions are obsessive-compulsive disorders, behavioral disorders, epilepsy, mental retardation, and schizophrenia. Of somatic comorbidity, gastrointestinal complaints such as stomach pain, constipation and diarrhea occur in 50% of children with ASD. Studies have also shown that children with autism have a higher incidence of gender identity problems than the rest of the population (4). ASD can occur as a part of a genetic syndrome, e.g. Rett syndrome, Fragile X syndrome, Angelman syndrome, and Cohens syndrome, it's then called “syndromic autism” and 10-25% have this. Idiopathic autism which is called “non-syndromic autism” and has no associated symptoms or signs of a syndrome, there is not a monogenetic cause (4, 22, 23).

Symptoms

Typical symptoms in early childhood may be that the affected children do not raise their arms, show emotion, or respond to their names. Children with autism are often not very engaged in imaginative play, do little imitation of others, and make poor eye contact. They may also have poor or late-developed language and will not be able to contribute to a conversation with others in contrast to other children. It can be particularly difficult in various social situations, as the child can struggle to adapt and participate in the social interactions. Many have special fields of interest that they know a lot about, this could be e.g., dinosaurs, trucks, or mathematics. Some can also be more compulsive and create rigid routines at home. It has also been shown that several people with autism meet the criteria for having exceptional cognitive abilities, and a research article described that among 137 people with autism, almost 30% met these requirements. (4)

Affected brain regions

Magnetic Resonance Imaging (MRI) is an imaging method that have made it possible to visualize and study the brain non-invasively, and it is possible to study the anatomical structures of the brain (structural MRI (sMRI)) and the metabolic activation (functional MRI(fMRI)) during tasks (Task-based fMRI) or in rest (resting-state fMRI) (24). MRI studies have identified unusual features in both gray and white matter structures within the social brain network, irregularities in intrinsic activity and connectivity within the social brain

regions, and deviations in activity and connectivity within the social brain regions when processing social stimuli in individuals with ASD (25). This is also supported by another review, where fMRI studies show different degrees of activation, in contrast to typically developing children, when performing tasks such as facial recognition, rewards and social cognition. The frontal lobe (FL), superior temporal gyrus (STG) and the amygdala are the most frequently observed to be abnormal in these studies, and that these changes, especially enlargement of the FL and STG, already occurs in early stages of the disease. (26, 27) In addition, reduced grey matter volume in cerebellum (28), enlarged hippocampus and amygdala has been seen in children with autism, interestingly there is no difference in amygdala size in adults with and without autism (27, 29, 30). Furthermore, there is also suggestions of affected orbitofrontal cortex, temporoparietal cortex and insula, and that abnormalities in these regions, including amygdala, could result in disturbances and symptoms from strongly interconnected brain regions (31). Moreover, a metanalysis showed enlarged head circumference (macrocephaly) in 16% of ASD patients and enlarged total brain volume was found in 9% (32).

Etiology ASD

There are many factors that could cause the onset of ASD, and the disease has a complex, multifactorial etiology involving genetic, epigenetic and environmental factors (4, 23)

Genetic factors in ASD

Genetics play an important role and hundreds of genes have been implicated in the disorder, both inherited and de-novo mutations (23). ASD presents a strong genetic vulnerability, with an estimated heritability of about 0.9 (1, 6). A study involving more than two million children born between 1982 and 2006 in Sweden, concluded that ASD has an inheritability of approximately 50% (33), and a meta-analysis involving thirteen studies on twins showing a heritability between 64-91% (34). Thus, ASD is one of the psychiatric diagnoses with the highest heritability (35).

Studies have identified a total of over 800 genes that increase the risk of ASD and it's been discovered multiple single nucleotide polymorphisms (SNPs), single nucleotide variants (SNVs), and copy number variants (CNVs) associated with ASD. These mutations can affect genes encoding for cell adhesion molecules (neurexins (NRXN), neuroligins (NLGN),

contactins (CNTN), neuronal cell adhesion molecule (NCAM) and Cadherins (CDH)), scaffolding proteins (SHANK gene family and CAPG gene), cytoskeletal proteins, signaling pathways (PTEN and mTOR/PI3K/MAPK/ERK), ion channels (L-type and T-type calcium channels, sodium channels, potassium channels, possibly the voltage-dependent anion channel (VDAC)), and cell signaling molecules, which are involved in chromatin remodeling and transcriptional regulation, cell proliferation and synaptic formation, plasticity and transmission. Some well-known mutations that could predispose for ASD are the FMR1 gene in Fragile X syndrome, the SHANK gene family and the CNV with duplication in loci 15q13. (6, 23)

Problematically, a genetic variant can only explain some cases, generating the so-called "missing heritability" to explain ASD. This indicates that there is still much to uncover to understand the causes of autism spectrum disorders, including gaps in the knowledge about gene variants, gene-to-gene interactions, epigenetics and the environmental and developmental aspects of the disease. (1, 6).

Environmental factors

There are some environmental factors that are more likely to contribute to ASD than others. Environmental factors can cause ASD through epigenetically changes due to exposure during pregnancy, which can affect the development of the brain. One established risk factor is increased parental age in one or both parents. Other contributing factors during pregnancy such as environmental toxins, use of medications, alcohol and cigarettes, pregnancy and birth complications also statistically increased the risk of ASD (Figure 1). Maternal infections have also been associated with ASD (34), suggesting matneral immune activation (MIA) leading to an increase in inflammatory markers and antibodies as a mechanism for the disease (4, 6). Epigenetic changes driven by environmental factors such as MIA, lead to DNA methylation and histone modifications that are thought to be important in the predisposition of ASD, and can cause abnormal brain development (23, 36).

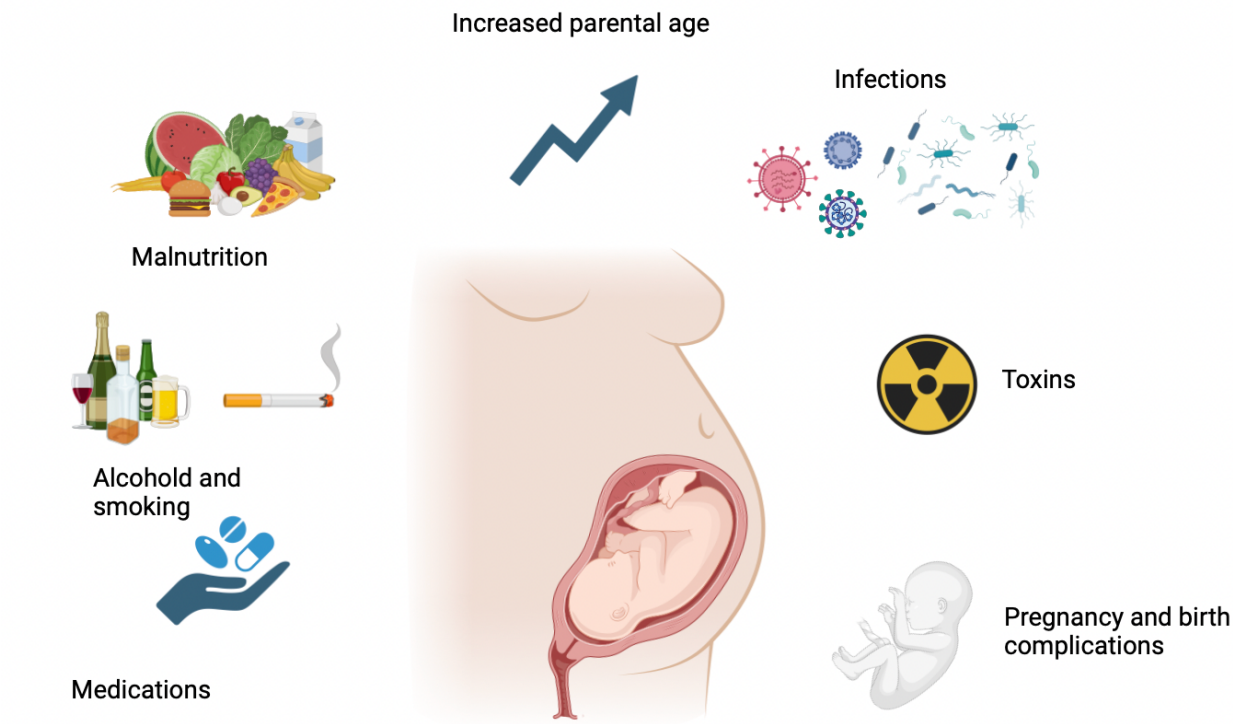


Figure 1: Overview of environmental factors in ASD.

Maternal Immune Activation (MIA)

One of the first infections linked to ASD was congenital rubella infection (37). This is an example of a virus that can cross the placenta and directly affect the fetus. However, the fetus can also be affected indirectly by immune activation in the mother, called maternal immune activation (MIA). The risk of developing neurodevelopmental disorders such as autism is not necessarily directly linked to the bacteria or virus, but to the immune activation in the mother. A meta-analysis from 2021 concludes that there is a significant association between maternal infection and fever during pregnancy, and autism spectrum disorder in the offspring (38).

MIA and ASD

Epidemiological studies, in conjunction with animal and 2D in vitro models of MIA, have identified increased pro-inflammatory mediators such as interferons (IFN), interleukins (IL), and tumor necrosis factor (TNF), including IL-1β, IL-6, IL-17α, TNF-α, and IFN-γ, that could play a crucial role in driving the adverse neurodevelopmental outcomes associated with MIA (39-42) and contribute to the atypical neurodevelopment seen in ASD (7, 43, 44). In addition,

increasing maternal c-reactive protein (CRP) levels is associated with a 43% increase in the risk of autism in the offspring (45), and there are also findings of increased cytokines in the cerebrospinal fluid (CSF) in ASD patients (46). Other findings show significantly higher levels of inflammatory mediators such as TNF-α, TNF-β, IL-4 and IL-10 in amnion fluid samples from individuals with ASD relative to controls (47). Moreover, viral infections in the first trimester and bacterial infections in the second trimester during pregnancy have been associated with an increased risk of ASD (48), especially if the mother is hospitalized or has a severe infection (49). A cohort study from Sweden including 2 371 403 participants showed that all types of hospitalized maternal infections increased the risk of ASD by 30% (50). Additionally, there is also an indirect effect during MIA, originate from fever in the mother increasing the risk of autism (8, 51, 52).

The pro-inflammatory cytokines can possibly cross the placenta, enter the fetal circulation, and cross the fetal blood–brain barrier (BBB) to affect the fetal brain (42). Scientists are uncertain of how cytokines reach the fetus during MIA, suggesting that cytokines may be transferred via the placenta or produced in the placenta itself (47). In addition, considering the elevated permeability of the fetal BBB during development, maternal pro-inflammatory cytokines can enter and affect the brain (53).
Furthermore, MIA can induce changes in the neuroanatomy such as increased migration of neurons, altered cortical lamination and enlarged ventricles (54) and behavioral dysfunction (55). Utilizing mouse models of MIA resulted in offspring with autistic-like behavior, such as alternations in sociability, increased anxiety and reduced motor control (44, 56-58). A systematic review concluded that MIA could result in changes like decreased proliferation of neuroepithelial cells and increased neuronal differentiation, leading to changes in cortical lamination, particularly in the density of deep layer neurons (59).

In addition, findings from recent studies using a mouse model revealed cellular pathways implicated in inflammation during fetal development, resulting in an increased risk for ASD. One study identified dysregulation of immune and metabolism-related biological pathways, as well as alterations in gene expression and methylation (36). Another study revealed that endogenous activation of a cellular pathway, called NLRP3-IL-1β, via stimulation of P2X7 receptors, resulted in ASD features in male offspring, and that these features could effectively be reduced with a P2X7 receptor antagonist (60). A third study showed remarkably significant

neuroanatomical changes in the brain, especially when the mice were exposed to MIA late compared to early in pregnancy (61).

IL-6 associations to ASD

and IL-6 is believed to have a key role in causing abnormalities in the brain development in the presence of MIA, and experiments on mice injected with IL-6 had offspring exhibiting defects in inhibitory neuronal signaling associated with autism, (39, 62). High levels of IL-6 have been associated to lower cognitive ability, social, learning and behavior deficits in mice offspring(39, 63-65). A systematic review on the same topic additionally highlights that IL-6 contributes to amygdala enlargement and increased connectivity between the amygdala and brain regions involved in learning and memory processing, increase excitatory synapse formation and decreasing inhibitory synapses and reduced neurogenesis in hippocampus, to mention some of the findings (66).

MHC molecules and ASD

Another important part of the immune system that may also be associated with ASD is the Major Histocompatibility Complex (MHC) (42, 67). There are two types of MHC molecules: the MHC Class I (MHC-I), which is found on most cells in the body, and displays peptides from inside the cell, enabling other immune cells to (e.g. T cells) identify cell status regards being infected by a virus, damaged or healthy. The MHC Class II (MHC-II) is exclusively found on antigen-presenting cells (APC), such as macrophages and natural killer cells, which can phagocytize and break down pathogens, and present the antigens to helper T cells (68). In humans MHC-molecules are called human leukocyte antigen (HLA) and are expressed in all cells in the brain, including glial cells, and they are present during brain development where they regulate connectivity and plasticity (67). A systematic review from 2022 highlights the MHC-I molecule´s function the brain, where they are expressed in axons and dendrites pre- and post-synaptic, controlling axonal and dendritic outgrowth and regulating the initial establishment of connections in the CNS modulate, in addition to contribute to the synaptic transmission in neurons by impacting the balance of excitatory and inhibitory signals. The same reviews suggest a hypothesis that mutations and/or immune dysregulation leading to altered MHC expression in the developing brain might contribute to the characteristic changes in brain connectivity and function in ASD individuals (42, 67). Furthermore, there are also

findings of both increased MHC-II and active neuroinflammation in brain tissue from patients with autism (46).

Glial cells, neuroinflammation and ASD

Evidence of neuroinflammation have been found in individuals with ASD, characterized by the reactivity of microglia and astrocytes, the activation of reactive oxygen species (ROS) and induced nitric oxide synthase (iNOS), and the increased release of proinflammatory cytokines and chemokines (46, 69). Microglia and astrocytes are two of the main types of glial cells in the central nervous system (CNS), together with oligodendrocytes. During embryonic development, glial cells create a cellular framework that facilitates the development of the nervous system and regulates neuronal survival and differentiation (70).

The astrocyte, also known as astroglia, plays a crucial role in maintaining homeostasis and defending the CNS. It participates in metabolic processes and facilitates communication among other CNS cells, including neurons, oligodendrocytes, and microglia (71, 72). Recent research suggests that astrocytes release neurotransmitters such as glutamate and GABA, influencing the activity of projection neurons, interneurons, and other astrocytes, thereby affecting synaptic modification through effects on neuronal depression and potentiation (73). Additionally, there are findings of increased astrocyte activation in ASD, suggesting that disrupted astrocyte function may impair proper neurotransmitter metabolism, synaptogenesis, and contribute to brain inflammation, potentially leading to connectivity impairments observed in ASD (74).

Microglia originate from yolk sac stem cells known as erythromyeloid progenitor (EMP) cells and is an important component of the immune system in the CNS, where it functions as the brain´s macrophage, affecting brain homeostasis, tissue repair and development (75, 76). Progenitor microglia settles in the brain early in development, from post conception week (pcw) 4, and acquire immune functions by the 9th pcw (76, 77). During different stages of brain development microglia undergo several states of maturation and differentiation, where the stages have different morphology and function (75, 77). In response to activation, due to e.g. damaged cells or immune activation, microglia produce and stimulate to increased proinflammatory mediators and cytokines and increase the release of ROS and iNOS. This is

followed by a transition to an anti-inflammatory phenotype to clear debris and aid in tissue repair (23, 78).

A review summarizes microglia's multiple areas of influence in the developing human brain, including neurogenesis, oligodendrogenesis, astrogliogenesis, axonal myelination, programmed cell death, apoptotic cell clearance, neuronal migration, white matter tract formation, and specialized interactions with both neuronal and non-neuronal cells, contributing to synapse formation, maturation, and pruning, and cortical folding (77). There are findings of activated microglia and neuroinflammation in the brains of patients with ASD, possibly contributing to the pathophysiology of the disease (46, 79-82). Moreover, there is also shown higher density of glial cells in the brain of mice modelling autism(61), where they can affect synaptic function, pruning and plasticity and causing behavioral changes associated with ASD (23).

Fever and ASD

Last, fever have been associated with developing ASD. A case-control study determine that there is an increased risk of ASD with maternal infection and fever in the second trimester (51), another study from Norway had the same findings (8). Moreover, a population-based cohort study consisting of 96 736 children found no increased risk of autism after mild infections, but that prolonged episodes of fever and maternal influenza infection increased the risk of ASD (52).

Induced pluripotent stem cells (iPSC)

A pluripotent stem cell is a stem cell with the capacity to differentiate into cells of any of the three germ layers (endoderm, mesoderm or ectoderm). The fate of a pluripotent stem cell is highly dependent on different types of tissue-specific transcriptional factors that stimulate the progenitor cell (17). Takahashi and Yamanaka were the first to induce pluripotent stem cells from mouse embryos (ES-cells) and adult fibroblasts in 2006 (83). Takahashi and Yamanaka identified specific genes, called embryonic stem cell-associated transcripts (ECAT) that could induce self-renewal and potentially function as reprogramming factors. They started with 24 ECATs, but narrowed it down to 4 and called them OSKM (OCT3/4, SOX2, KLF4, MYC) – the Yamanaka factors. They also demonstrated that these factors could generate human induced pluripotent stem cells (hiPSC), resulting in the first reports on hiPSC in 2007. (16,

17). Thus, OSKM were used to generate the first hiPSC, despite it was incomplete and the process presented low reprogramming efficiency. Later, there have been identified several reprogramming factors that could facilitate the reprogramming of somatic cells to generate hiPSC, such as enhancers that affect pluripotent-associated genes, cell-cycle regulating genes e.g. GTP-binding protein REM2 and cyclin D1, and epigenetic modifiers such as upregulation or downregulation of enzymes that regulate posttranscriptional modification of histones, histone composition and demethylation of DNA. These additional reprogramming enhancers increase the efficiency of iPSC generation and, in some cases, can replace the Yamanaka factors (17).

Today hiPSC models can be reprogrammed from fully differentiated cells, such as β-cells in the pancreas, hepatocytes, skin fibroblasts, T- and B lymphocytes and other blood cells (16, 17). The cells are first reprogrammed into induced pluripotent stem cells (iPSC) and then into other differentiated cells, such as neurons or cardiomyocytes (16). Concerning the brain, it is possible to produce all kinds of neurons and glial cells. Since nowadays there are several protocols to differentiate hiPSC into several cell types (12), somatic cells of patients with different disorders have been reprogrammed into hiPSC and further on differentiated into monolayer cultures of cell types relevant of those diseases. Then, these cell cultures have been used in research to model human diseases in a dish.

The two-dimensional models have gradually replaced the use of malignant cell lines and primary cell cultures from specific organs of rodents and other animals for research purposes, especially to study brain disorders, given the differences of brain cells between humans and other mammals. It has made it possible to do research on pathophysiology in many neurological and neuropsychiatric diseases, e.g. autism, schizophrenia and bipolar disorder (12, 15). It has also given opportunities for drug research and discovery, cell therapy development, and regenerative medicine (16, 17). However, 2D culture models lack the complexity, heterogeneity, and neuronal connectivity of the human brain (9), therefore demanding more complex models based on three-dimensional brain structures which gives a more accurate disease modelling of ASD (84)

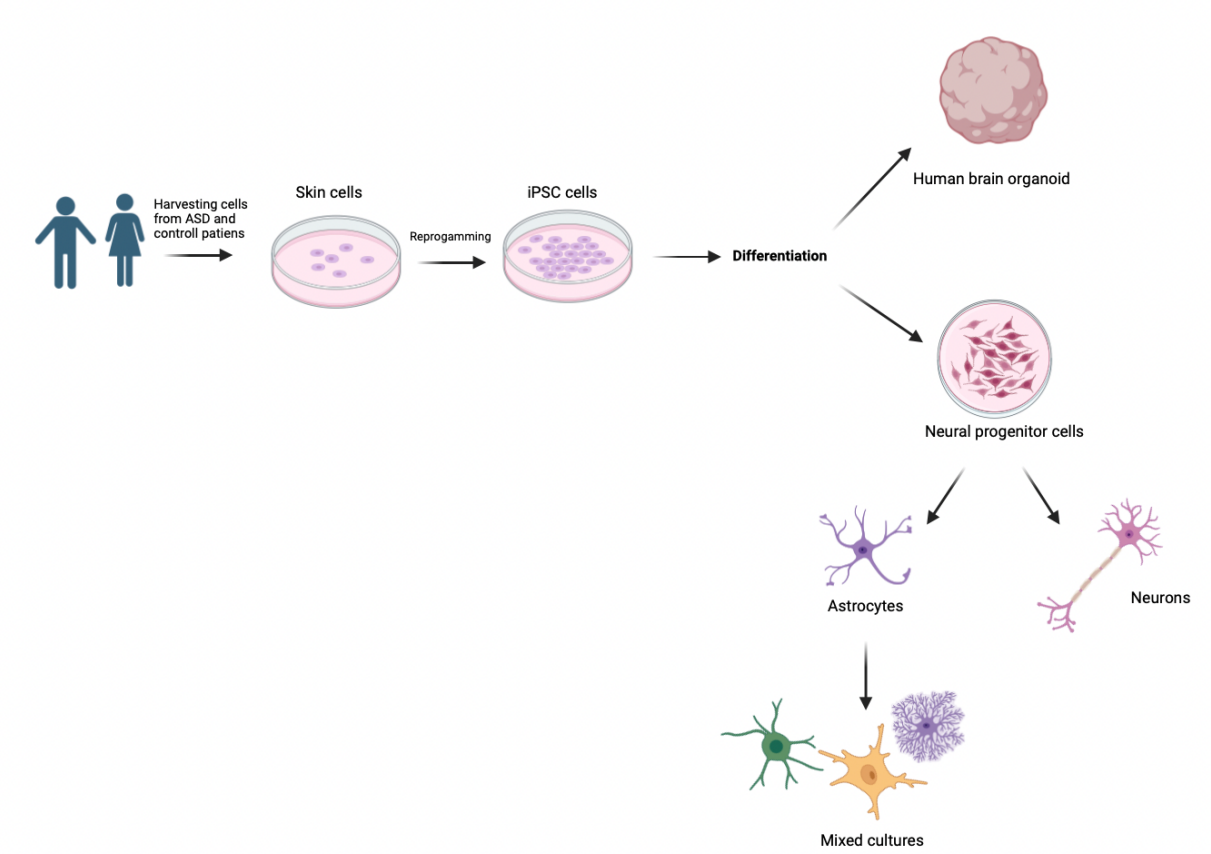


Figure 2: hiPSC-derivate models in brain research.

Human brain organoids (hBO)

Human brain organoids (hBO) are self-assembling three-dimensional *in vitro* aggregates of the brain tissue derived from hiPSC or human embryonic stem cells (hiss) to form an organized architecture that model the human brain microenvironment. It resembles and develops like fetal human brain tissue mimicking the *in vivo* sequence of events, and it is composed of multiple cell types, such as neural progenitors, several types of neurons, glial cells, such as astrocytes, oligodendrocytes and microglia (54, 85). Unlike the hips 2D models cultures, hiPSC-derived hBO are more like the human brain tissue on a molecular, cellular, and structural level. This gives an unprecedented opportunity to further explore diseases, development, and evolution of the brain. However, there are still some brain features that are still not fully recapitulated, such as the formation of gyrification and distinct cortical neuronal layers, and of the complex neuronal circulation (85).

There are two different main types of methods that can be used to generate brain organoids: unguided methods and guided methods. In the unguided method, the hiPCS aggregates will

spontaneously initiate morphogenesis and differentiation, and in guided method, several external factors must be added to develop specific and wanted cell lines. Which and how many external factors are used varies, depending on desired results (85).

hBO and ASD

hBO have made it possible to get important insights into various neurological diseases. The first neurodevelopmental disorder to be detected in an hBO was microcephaly (86), and further on other neurodevelopmental disorders as autism and epilepsy have been modeled using hBO (16, 87).

Recent systematic reviews have summarized main findings that hBO derived from patients with autism. These changes include cortical growth, ventricular enlargement, reduced ventricular wall thickness, increased calretinin expression, and early shifts towards neural cell maturation. Other findings are increased excitatory and GABAergic synapses, and altered gene expression, especially of genes related to GABAergic interneuron differentiation. Additional findings suggest alterations and/or dysregulation in neural progenitor cells (NPCs) and premature cortical formation, neuronal migration and maturation, and in the cortical layer formation. Moreover, abnormalities in neural stem cell proliferation and neuronal maturation, and defects in the neurogenesis and neuronal morphology changes are also observed. Furthermore, alternations in gene expression of specific neurotransmitter markers, leading to an increase of transmitters such as cholinergic, GABAergic, and glutamatergic signaling is also seen in several studies. Last, there is observed an affection of the calcium and potassium signaling pathways, in addition to dysregulation of neural development, synaptic transmission and synaptic processes, and intrinsic and excitatory synaptic deficits, reduced synaptic puncta, increased neuronal excitability and defects in tube formation. (54, 88, 89)

Method

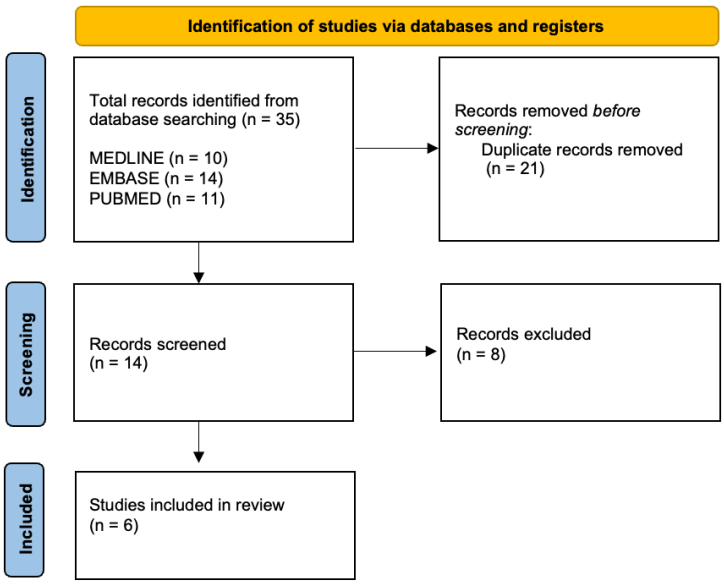
My initial question was how maternal immune activation during pregnancy could lead to ASD in the child. Based on this, I used the PICO (Population, Intervention, Comparison, Outcome) model as the basis for my research question. This resulted in the following: What can iPSC models tell us (P) about maternal immune activation (I) as a cause to autism spectrum disorders (O)? Since this is such a new and unexplored research area, there is expected to be significant heterogeneity in trends in the results. My goal is therefore to summarize these in a

descriptive manner. Based on this, I will highlight the most promising areas for further research.

There was performed a systematic literature search 30.06.23 in EMBASE, MEDLINE and PubMed using three combined search themes, MIA, ASD and iPSC. No additional restrictions were added. The keywords and subject headings were as following:

1. (maternal infection or maternal viral infection or maternal bacterial infection or maternal immune activation or MIA).kw,ti,ab.
2. exp induced pluripotent stem cell/
3. (autism or autism spectrum disorder or asperger).kw,ti,ab.
4. (induced pluripotent stem cell* or iPSC or hiPSC or brain organoid*).kw,ti,ab.
5. exp autism/
6. 2 or 4
7. 3 or 5
8. 1 and 6 and 7

After removing duplicates, papers were initially screened by title, abstract and full-text by one person. Additionally, the articles were excluded by following criteria: (1) written in another language than English or Norwegian, (2) not available for full text assessment and (3) not research articles and. Only studies using hiPSC-derivates as research model was included. The selection process is illustrated in figure 3.



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

Figure 3: PRISMA flow diagram of literature search and study selection.

Full-text reading was conducted for all articles, and data were extracted using a table. Emphasis was placed on findings highlighted in the discussion and abstract, as these was considered most significant. A structural quality and risk of bias assessment was not conducted. This decision was influenced by the novelty of the research and the considerable uncertainty in the findings, highlighting the need for additional research to determine generalizability.

Results

My systematic search identified 35 studies. Following the removal of duplications and screening, a total of 6 research articles were included in the review (refer to Figure 3). The studies employed in vitro hiPSC-derived models, encompassing both 2-dimensional and 3-dimensional formats. Several studies utilized the same pro-inflammatory mediator to induce immune activation within the models. Specifically, two studies employed IL-6 on dorsal brain organoids and neurons, while two other studies exposed IFN-γ to NPCs, neurons, and immature glutamatergic neurons. The remaining two studies utilized IL-17a and heat shock on NPCs and 3-dimensional neural aggregates, respectively. Notably, the IL-17a study was the sole research article to employ hiPSC derivatives from both ASD patients and healthy controls, while the remainder solely used samples from healthy individuals. Collectively, the results underscore the intricate interplay among cytokines, gene expression, and brain development. They suggest a correlation between immune activation and cellular and molecular changes in brain cells, including alterations in ASD-related genes, neuroinflammation, structural brain changes, and potential synaptic dysfunction. The studies uniformly reported a possible association between autism and MIA. The findings are summarized in figure 4.

Exogenic exposure	HiPSC-derivate model	Cell line used	Observations	Reference
IL-6	Dorsal forebrain organoid	Healthy male subjects	- Activation of STAT3 pathways - Long-term abnormal cortical layering - Upregulation of MHC-I - Alternations in gene expression of NR2F1 - Small increase in radial glial cells - Radial glial cells show the highest number of differentially expressed genes, such as downregulation of genes related to protein translation in the cytoplasm and proteins targeting ER and plasma membrane	(90)
IL-6	Neurons	Healthy subjects	- Increased activation of STAT3 pathway - Increased ratio of astrocytes - Reduced ratio of early-born neurons	(91)
IFN-γ	NPCs and neurons	Healthy subjects	- Upregulation of MHC-I mediated by PML nuclear bodies - Neurite outgrowth measurable in both length and branch number, which required both MHC-I and PML - Findings of common genetic mutations and variants in SZ and ASD such as PTEN, TCF4, SHANK2, NLGN3, and NRXN3	(92)
IFN-γ	Immature glutamatergic neurons	Healthy subjects	- Activation of STAT1 pathway - Increased MHC-I (HLA-B) expression - Decreased levels of synaptic vesicles and synpasin I/II proteins - Increased expression of C4A - No increase of IFN-γ receptor	(93)
IL-17a	NPCs	ASD subjects (n=7) and healthy controls (n=5)	- Activation of ERK1/2 pathway and inhibition of mTORC1 pathway - Increased differentiation; increased neurite outgrowth and levels of synpasin I, synaptophysin and MAP2, but no significant difference between ASD and control NPCs - No increase of IL-17a receptor - No effect on proliferation and migration	(94)
Fever	3-dimensional neural aggregates	Healthy subjects	- Changes in genes encoding HSP and NGFR, and SZ and ASD candidate genes such as SMARCA2, DPP10, ARNT2, AHI1, and ZNF804A	(95)

Figure 4: Overview of the results.

IL-6

There were two studies exposing IL-6 to hiPSC derivates. In the first article, they used a hBO model and the IL-6 dose was corresponding to serum concentration of a septic infection resulted in both cellular and molecular changes. Firstly, there was an upregulation of genes that code for MHC-I and beta-2-microglobulin (B2M). They also discovered a small increase in radial glial cells (90), which are a non-neural cell in the developing CNS that support migrating neurons and are neuronal precursors that can differentiate into other cell types, including neurons and astrocytes (96). Additionally, there was a downregulated genes related protein translation in the radial glial cells, especially cytoplasmic translation and proteins targeting the endoplasmic reticulum (ER) and plasma membrane. Furthermore, they detected long-term abnormal cortical layering indicating a migration or maturation defect induced by excessive IL-6 (90).

In the other IL-6-study, they used hiPSC- derived neural cells models to investigate whether luteolin, found in edible plants such as fruits and vegetables, could prevent MIA-induced abnormalities. Injection of IL-6 significantly increased the of activation of STAT3, increased the area ratio of astrocytes and decreased the area ratio of early-born neurons relative to controls (91).

IFN-γ

Interferon-γ (IFN-γ) is a cytokine that is mainly produced by helper T cells and natural killer cells. IFN-γ main task is to activate the immune system by prime and activate macrophages. When activating a cell this leads to a production of various cytokines, growth factors and molecular patterns recognition receptors (PAMPs), such as TNF-α, IL4, IFN-β- and -α. Other effects of IFN-γ are inhibiting cellular proliferation, affecting apoptosis, up-regulating antigen presentation on the cell surface and increasing leukocyte recruitment (68, 97).

IFN-γ-exposure resulted an increase in neurite outgrowth were both neurite length and branch number were increased, and an upregulation of MHC-I genes. Neurite outgrowth represents a foundational phase in neuronal maturation, during which neural progenitors extend processes that may eventually develop into axons or dendrites. The upregulation of MHC-I was facilitated by the presence of IFN-γ-induced promyelocytic leukemia protein (PML) nuclear bodies (92). PML nuclear bodies are chromatin-associated structures that modulates

transcription and are associated with chromosomal loci for MHC-I, additionally, PML are involved in and regulate brain development and in particular affect stem cell function (98). Importantly, the IFN-γ-induced neurite outgrowth was dependent on the simultaneous presence of both PML and MHC-I. The absence of MHC-I at the cell surface prevented IFN-γ from inducing the neurite outgrowth. Warne-Cornish suggested that these findings prove the involvement of MHC-I proteins in IFN-γ-induced neurite outgrowth (92).

Furthermore, there was not found overlap between genes responsive to IFN-γ and common ASD risk variants identified through genome-wide association studies (GWAS). Nonetheless, the SFARI database, which encompasses rare single gene mutations, detected multiple ASD risk genes, such as PTEN, TCF4, SHANK2, NLGN3, and NRXN3 (92).

Based on Warne-Cornish´ work it was conducted a study that wanted to see if IFN-γ could impact the development of immature glutamatergic neurons using hiPSC and made an induced neuronal cellular system. Acute exposure to IFN-γ activated STAT1 pathway, increased the MHC-I expression in immature neurons, decreased synapsin I/II levels, and increased complement component 4A (C4A) gene expression (93).

IL-17a

Interleukin-17 (IL-17) is a cytokine primarily associated with the immune response against certain infections and inflammatory conditions, but excessive levels can contribute to chronic inflammation and autoimmune diseases (99). Gomes and his team did a study where they exposed NPCs derivates from ASD-individuals with known and unknown genetic cause as well as from neurotypical controls, for exogenic interleukin-17a (IL-17a). This did not induce abnormal migration or proliferation of neurons, but it did positively affect the differentiation increasing the expression of synaptic and neuronal polarity proteins, more specific synaptophysin-1, synapsin-I and MAP2. ASD and control cells had the similar response to exogenic IL-17a. It was concluded that IL- 17a may disturb normal neuronal and synaptic development and may be involved with MIA-induced brain and behavioral changes, but that it might be necessary to have more inflammatory molecules to trigger gene-environment interactions, and a larger sample size to have sufficient statistical power and conclusions (94).

Fever

A study conducted in 2014 investigated the potential effects of fever on neurodevelopment and neurogenesis by examining heat shock (HS)-regulated cellular stress pathways. The researchers used 3-dimensional neuronal aggregates, derived from hiPSCs from healthy individuals, resembling telencephalic structures equivalent to the first trimester of gestation as a model. These organoids were exposed to a temperature of 39 degrees Celsius for 24 hours, with a control hBO maintained normal body temperature. The findings revealed induced genetic alterations, including heat shock proteins (HSP), neuronal growth factor receptor (NGFR), and several ASD candidate genes such as SMARCA2, DPP10, ARNT2, AHI1, and ZNF804A (95).

Discussion

ASD is a complex neurodevelopmental disorder characterized by a diverse range of symptoms and challenges of varying severity (1). Its prevalence is increasing, affecting approximately 1% of the global population (1, 4, 20) . ASD arises from a combination of genetic, epigenetic, and environmental factors (4, 23), with a substantial genetic heritability estimated at 64-91% based on twin studies (34). However, the precise etiology of ASD, particularly idiopathic cases, and the influence of environmental factors remain incompletely understood (1, 6). Historically, limitations in research models have hindered the clarification of ASD pathophysiology. Nonetheless, the advent of hiPSC and hBO has opened new avenues for investigating neurodevelopmental disorders like ASD (9, 12, 16, 17). While numerous studies have leveraged hiPSC and hBO to explore development of disorders such as autism (54, 84, 88, 89), investigations often overlook environmental influences, despite their potential to interact with genetic predispositions and contribute to ASD phenotypes.

MIA has emerged as a significant, albeit poorly understood, risk factor for ASD, implicated in altered fetal brain development and behavioral abnormalities (39-42, 44, 45, 47). Research linking MIA to ASD pathogenesis draws support from cohort (34), case-control (49), epidemiological studies (48, 50), and animal models (36, 44, 54-56, 58, 59). Yet, the precise mechanistic of MIA-induced ASD remain elusive.

In this systematic review we aimed to provide an overview of MIA as a causative factor for ASD by examining studies that utilized hiPSC and hBO as research models, as these are

currently the research models most closely resembling the human brain. The result indicates that there is a connection between immune activation and cellular and molecular changes in the brain, including changes in ASD-related genes, neuroinflammation, neurite outgrowth and potential synaptic dysfunction, and alterations in brain structure. This is a very young field of study, and there is little research done on this specific topic. Therefore, I will focus on findings that several articles converge on. Additionally, I highlight a couple of results that I believe may also be important to further investigate.

Alternations in ASD relevant genes

The result suggest that MIA induces alternation is risk genes associated with ASD, especially through IFN-γ (92) and heat shock (95), potentially contributing to the development of autism. The mutations identified in genes such as PTEN, SHANK2, NLGN3, NRXN3, AHI1, ARNT2, and ZNF804A are known to be associated with ASD (6, 23, 100-105), and may be part of the underlying pathophysiology in cases where MIA plays a role in ASD development. These findings suggest that MIA may affect genetic mechanisms relevant to autism development, underscoring the importance of understanding how environmental factors can impact genetic vulnerability.

MIA mediated neuroinflammation: glial cells and MHC molecules

First, the increased presence of astrocytes, a type of glial cell, following exposure to IL-6 (91) suggests a key role of astrocytes in the neuroinflammatory response triggered by MIA. Increasing glial cells and neuroinflammation in ASD patients have been observed in both postmortem studies, mouse studies, and human studies (23, 46, 61, 69, 72, 77, 79-82). Astrocytes do not only participate in the inflammatory process but also modulate neurotransmitter release and synaptic modifications (69, 73), and findings of increased astrocyte activation in ASD suggest a disrupted function that potentially leading to connectivity impairments observed in ASD (74). The consistent presence of elevated glial cells and neuroinflammation in ASD patients across studies highlights the potential association between MIA-induced neuroinflammation and ASD development.

Additional evidence from our results supports the hypothesis that MIA induced neuroinflammation plays a role in the development of ASD. This includes the increase of MHC molecules. Exposure to both IL-6 and IFN-γ resulted in an increase in MHC-I

expression (90, 92, 93). In the studies using IFN-γ, this resulted in neurite outgrowth, commented in the next paragraph. Previous studies have linked both MHC-I and MHC-II to autism (42, 46, 67). These findings further support the involvement of neuroinflammation and immune dysregulation in ASD pathogenesis. More research is needed to explore how MHC molecules and immune pathways contribute to ASD following MIA exposure, enhancing our understanding of the disorder's underlying pathophysiology.

Neurite outgrowth and synapsin I

There were also observations of increased neurite outgrowth and alternations in levels of synapsin-I protein. IFN-γ exposure induced morphological alterations in neurons, including increased neurite length and branch number (92). Prior investigations have reported similar findings (106, 107). The IFN-γ-induced outgrowth was depended on both MHC-I and PML present, suggesting that MHC-I expression may not only be associated with neuroinflammation but that they also are involved in with neurite outgrowth (92). There were also observations of increased neurite outgrowth and differentiation in IL-17a exposed NPCs, together with elevated levels of synapsin I (94). In contrast, IFN-γ exposure demonstrated reduced levels of synapsin I/II proteins, and the authors hypothesized that this was associated with MHC molecule upregulation (93). On the other hand, this could also mean that exposure to IFN-γ and IL-17a has different effects on the development of the brain and its nerve cells. Recent studies suggest that aberrant neurite outgrowth and migration, influencing brain structure and function, (108), and defects in dendrite morphogenesis may contribute to autism (109). In addition, this highlights a third of the MHC molecule´s possible functions due to MIA, that they contribute to synaptic alternations as well. In fact, MHC molecules are known to control axonal and dendritic outgrowth and modulate synaptic transmission in neurons by impacting the balance of excitatory and inhibitory signals (42, 67). The result suggests that these alternations could have significant consequences for brain development and its function, indicating complex mechanisms involved in regulating neurological development, and underscore the intricate relationship and complexity between immune responses, neurodevelopment, and ASD pathogenesis, necessitating further investigation.

Fever as a contribution in ASD

Besides the cytokine elevation, fever during the second trimester has been linked to autism (8, 51). Our findings revealed an upregulation of HSP genes in hiPSC-derived neural aggregates

following exposure to heat shock (95). HSP function as molecular chaperons in cell, contributing in folding of newly synthesized polypeptides, refolding metastable proteins, protein complex assembly, dissociating protein aggregate dissociation, degradation of misfolded proteins, and assisting in signaling transduction, cell cycle, and apoptosis regulation (110). HSP can be induced by not only hypothermia, but also various stressors akin to environmental factors implicated in autism, including hypoxia, malnutrition, nutrient deficiency, inflammation, infection, exposure to heavy metals, and toxins (95), which is similar to the environmental factors believed contributing in ASD. This upregulation of HSP potentially influences specific pathways involved in differentiation, neurite outgrowth, cell migration, and angiogenesis (111), and can possibly affect the structural and functional aspects of the brain and have long-term consequences for the cognitive and behavioral development. Heat shock also induced upregulation of genes encoding NGFR. NGF, a ligand for NGFR, is essential for CNS, and functions as a neuronal trophic factor that has various important tasks in the brain and nerve cells. Studies have shown that individuals with autism have higher levels of neuronal NGF in their blood compared to children without ASD, with levels reported to be 50% higher in one study (95, 112). However, this area requires further research to understand the exact mechanisms and effects of fever and heat shock in brain development under such conditions.

Structural changes

IL-6 were observed led to alternations in the cortical layers of the hBO (90), indicating a potential migration or maturation defect, suggesting that IL-6 and MIA may induce structural changes in the brain as part of ASD development. This finding aligns with other reviews and hBO studies in individuals with ASD, which have reported alternations in cortical layers in addition to cortical growth, ventricular enlargement, and reduced ventricular wall thickness (54, 59, 88, 89).

Radial glial cells; a new finding

Last, exposure to IL-6 resulted in a slight increase in radial glial cells. Additionally, these cells exhibited the highest occurrence of genetic alterations, especially in genes encoding protein translation and proteins in cytoplasm, ER, and cell membrane (90). Radial glial cells, non-neuronal cells in the developing central nervous system, support migrating neurons and serve as neuronal precursors capable of differentiating into various cell types, including

neurons and astrocytes (96). Conversely, a study from 2022 investigating the role of TRIM32 in cortical development in autism found reduced radial glial cells (113). Further investigation into the role of radial glial cells in autism is necessary to determine the significance of these alterations in ASD development.

Strengths and limitations

Strengths

First, previous studies on MIA and autism have primarily utilized animal models, which exhibit distinct differences in brain development and function compared to humans. Employing hiPSC-derived models enhances accuracy by reflecting human biology and mimicking various aspects of human brain development. This approach offers insights into disease mechanisms previously inaccessible. Additionally, hBO models present a more physiologically relevant model compared to traditional cell culture methods. Second, to our knowledge, this review represents the first overview of the relationship between maternal immune activation and autism using hiPSC-derived models. Moreover, it offers a valuable descriptive overview of potential cellular and molecular trends associated with immune activation and autism risk. This serves as a crucial starting point for further investigation into this intricate relationship, aiding in identifying areas necessitating additional research and comprehension.

Limitations

Firstly, previous studies on MIA and autism have primarily relied on animal models, which differ significantly in brain development and function compared to humans. While hiPSC-derived models offer enhanced accuracy by reflecting human biology and various aspects of brain development, they may not fully replicate the complexity of living organisms. Additionally, while hBO models are more physiologically relevant than traditional cell culture methods, they still have limitations in capturing the entirety of biological and environmental factors influencing human conditions. Methodological limitations and the presence of unknown or unidentified confounding factors can further contribute to the challenges associated with these models. Secondly, limited observations of the long-term effects of MIA exist. This temporal constraint hampers a comprehensive understanding of the sustained impact of MIA on offspring. Thirdly, the scarcity of studies utilizing hBO models and hiPSC derivatives from ASD patients alongside healthy controls is a notable weakness. Such

limitations may result in overlooking critical cellular and molecular mechanisms, potentially affecting the comprehensiveness of the findings.

Moreover, difficulty in quantifying or summarizing results due to limited data poses challenges in conducting meta-analyses and drawing concrete conclusions. This limitation may obscure the overall understanding of the relationship between MIA and autism. In addition, the reliance on associative findings between MIA and autism in the studies underscores the necessity for randomized controlled trials to confirm any associations. Establishing a causal relationship and distinguishing between causes and random effects remain challenging tasks.

Lastly, general ethical concerns regarding the source of cells and cell manipulation represent overarching weaknesses of these models, underscoring the need for ethical considerations in research practices.

Future implications

The utilization of hBO is expected to provide further significant insights. While several studies have utilized hiPSC, they may lack critical interactions prevalent in hBO models. Nonetheless, using these research models promises enhanced understanding of human brain function given their complexity, and as these models improve, they could offer further insights into complex neurodevelopmental disorders such as autism. It would be interesting to test how simultaneous MIA-effects affect the brain, as different cytokines may have opposing or varying effects on brain development. Furthermore, it would be intriguing to observe if there is a difference between individuals with predisposing gene mutations or healthy individuals, as only one of the studies in the results examined this aspect. Last, I believe further exploration of complement activation's contribution to MIA and autism should be pursued, as this was identified as an unexpected finding in one of the articles and may be relevant. There is also no doubt that more studies are needed to confirm the above findings, as only six studies have investigated MIA as a cause of autism using hiPSC and hBO models. Further research into the mechanisms underlying MIA-induced neuroinflammation and its consequences for neurodevelopment would provide valuable insights for the development of diagnostic, preventive and therapeutic strategies in ASD. Hopefully, this is not far in the future.

Clinical implications

The clinical implications of MIA for autism are complex and still under research. Here are some potential clinical implications:

(1) Early identification and intervention; Identifying risk factors associated with MIA can aid in early diagnosis and intervention for autism. This may involve early screening of children born to mothers exposed to immune activation during pregnancy. In addition, the possibility of early interventions for pregnant women with infections, such as anti-inflammatory treatment or antibiotics, could potentially prevent critical non-reversible alterations in fetal neurodevelopment. A 2021 meta-analysis suggests that preventing infections and administering early treatment may decrease the occurrence of autism (38), and a recent systematic review highlights the potential benefits of anti-inflammatory treatment during pregnancy, such as decreased inflammatory response in the brain (114). However, limited research exists in this field (115) and is yet to be explored further.

(2) Treatments: Understanding the mechanisms behind MIA-associated autism can help develop targeted treatment strategies. This may include pharmacological interventions targeting the immune system or neurological mechanisms, possibly reducing symptoms, such as anti-inflammatory treatment, but other potential drug targets could be specific cytokines that induce critical processes in ASD development during MIA, akin to TNF-alpha inhibitors, but the feasibility and efficacy of this approach are yet to be determined.

(3) Diagnostics: Measuring inflammatory mediators or other biomarkers elevated due to neuroinflammation or other alternations in the brain, in early in childhood, may perhaps contribute to earlier and faster diagnostics of autism. Notably, such measurements will probably not sufficient alone for making a correct diagnosis.

(4) Preventative measures: Identifying risk factors associated with MIA and information about the link between MIA and autism can aid in early interventions and support to affected families.

Conclusion

Overall, the findings in this report highlight the importance of understanding the impact of MIA on fetal brain development and its potential role in the etiology of ASD, and collectively

underscore the complex interplay between cytokines, gene expression, and brain development. It appears to be a connection between immune activation and cellular and molecular changes in brain cells, including changes in ASD-related genes, neuroinflammation, structural brain alterations, and potential synaptic dysfunction. Moreover, understanding human-specific responses, and exploring the synergistic effects of MIA and its interaction with genetic factors will be important for a comprehensive understanding of the pathophysiology of neurological disorders. There is no doubt that the use of hBOs and other hiPSC-derivates models will be crucial in this matter. In the future, these insights may contribute to the development of diagnostics, and preventive and symptomatic treatment.

References

1. Andreassen OA, Malt UF, Malt EA, Melle I. Lærebok i psykiatri : basisversjon. 1. utgave. ed. Oslo: Gyldendal; 2020.
2. Chaste P, Leboyer M. Autism risk factors: genes, environment, and gene-environment interactions. Dialogues Clin Neurosci. 2012;14(3):281-92.
3. Harris J. Leo Kanner and autism: a 75-year perspective. Int Rev Psychiatry. 2018;30(1):3-17.
4. Berit Grøholt BW, Ida Garløv, Ruth-Kari Ramleth. Lærebok i barne- og ungdomspsykiatri2022.
5. Hosseini SA, Molla M. Asperger Syndrome. StatPearls. Treasure Island (FL): StatPearls Publishing
Copyright © 2023, StatPearls Publishing LLC.; 2023.
6. Masini E, Loi E, Vega-Benedetti AF, Carta M, Doneddu G, Fadda R, et al. An Overview of the Main Genetic, Epigenetic and Environmental Factors Involved in Autism Spectrum Disorder Focusing on Synaptic Activity. Int J Mol Sci. 2020;21(21).
7. Bölte S, Girdler S, Marschik PB. The contribution of environmental exposure to the etiology of autism spectrum disorder. Cell Mol Life Sci. 2019;76(7):1275-97.
8. Hornig M, Bresnahan MA, Che X, Schultz AF, Ukaigwe JE, Eddy ML, et al. Prenatal fever and autism risk. Mol Psychiatry. 2018;23(3):759-66.
9. Damianidou E, Mouratidou L, Kyrousi C. Research models of neurodevelopmental disorders: The right model in the right place. Front Neurosci. 2022;16:1031075.
10. Chesselet MF, Carmichael ST. Animal models of neurological disorders. Neurotherapeutics. 2012;9(2):241-4.
11. Cheffer A, Flitsch LJ, Krutenko T, Roderer P, Sokhranyaeva L, Iefremova V, et al. Human stem cell-based models for studying autism spectrum disorder-related neuronal dysfunction. Mol Autism. 2020;11(1):99.
12. Wang M, Zhang L, Gage FH. Modeling neuropsychiatric disorders using human induced pluripotent stem cells. Protein Cell. 2020;11(1):45-59.
13. Collo G, Mucci A, Giordano GM, Merlo Pich E, Galderisi S. Negative Symptoms of Schizophrenia and Dopaminergic Transmission: Translational Models and Perspectives Opened by iPSC Techniques. Front Neurosci. 2020;14:632.
14. Penney J, Ralvenius WT, Tsai LH. Modeling Alzheimer's disease with iPSC-derived brain cells. Mol Psychiatry. 2020;25(1):148-67.

15. Reis de Assis D, Szabo A, Requena Osete J, Puppo F, O'Connell KS, I AA, et al. Using iPSC Models to Understand the Role of Estrogen in Neuron-Glia Interactions in Schizophrenia and Bipolar Disorder. Cells. 2021;10(2).
16. Shi Y, Inoue H, Wu JC, Yamanaka S. Induced pluripotent stem cell technology: a decade of progress. Nat Rev Drug Discov. 2017;16(2):115-30.
17. Takahashi K, Yamanaka S. A decade of transcription factor-mediated reprogramming to pluripotency. Nat Rev Mol Cell Biol. 2016;17(3):183-93.
18. Organization WH. International Classification of Diseases Eleventh Revision (ICD-11): World Health Organization 2022 [June 2th 2023]. Available from: https://icd.who.int/browse11/l-m/en#/http://id.who.int/icd/entity/437815624.
19. Levy SE, Mandell DS, Schultz RT. Autism. Lancet. 2009;374(9701):1627-38.
20. Zeidan J, Fombonne E, Scorah J, Ibrahim A, Durkin MS, Saxena S, et al. Global prevalence of autism: A systematic review update. Autism Res. 2022;15(5):778-90.
21. Loomes R, Hull L, Mandy WPL. What Is the Male-to-Female Ratio in Autism Spectrum Disorder? A Systematic Review and Meta-Analysis. J Am Acad Child Adolesc Psychiatry. 2017;56(6):466-74.
22. Genovese A, Butler MG. Clinical Assessment, Genetics, and Treatment Approaches in Autism Spectrum Disorder (ASD). Int J Mol Sci. 2020;21(13).
23. Bhandari R, Paliwal JK, Kuhad A. Neuropsychopathology of Autism Spectrum Disorder: Complex Interplay of Genetic, Epigenetic, and Environmental Factors. Adv Neurobiol. 2020;24:97-141.
24. Kaur P, Kaur A. Review of Progress in Diagnostic Studies of Autism Spectrum Disorder Using Neuroimaging. Interdiscip Sci. 2023;15(1):111-30.
25. Sato W, Uono S. The atypical social brain network in autism: advances in structural and functional MRI studies. Curr Opin Neurol. 2019;32(4):617-21.
26. Rafiee F, Rezvani Habibabadi R, Motaghi M, Yousem DM, Yousem IJ. Brain MRI in Autism Spectrum Disorder: Narrative Review and Recent Advances. J Magn Reson Imaging. 2022;55(6):1613-24.
27. Barnea-Goraly N, Frazier TW, Piacenza L, Minshew NJ, Keshavan MS, Reiss AL, et al. A preliminary longitudinal volumetric MRI study of amygdala and hippocampal volumes in autism. Prog Neuropsychopharmacol Biol Psychiatry. 2014;48:124-8.
28. Stoodley CJ. Distinct regions of the cerebellum show gray matter decreases in autism, ADHD, and developmental dyslexia. Front Syst Neurosci. 2014;8:92.
29. Schumann CM, Hamstra J, Goodlin-Jones BL, Lotspeich LJ, Kwon H, Buonocore MH, et al. The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. J Neurosci. 2004;24(28):6392-401.
30. Nordahl CW, Scholz R, Yang X, Buonocore MH, Simon T, Rogers S, et al. Increased rate of amygdala growth in children aged 2 to 4 years with autism spectrum disorders: a longitudinal study. Arch Gen Psychiatry. 2012;69(1):53-61.
31. Weston CSE. Four Social Brain Regions, Their Dysfunctions, and Sequelae, Extensively Explain Autism Spectrum Disorder Symptomatology. Brain Sci. 2019;9(6).
32. Sacco R, Gabriele S, Persico AM. Head circumference and brain size in autism spectrum disorder: A systematic review and meta-analysis. Psychiatry Res. 2015;234(2):239-51.
33. Sandin S, Lichtenstein P, Kuja-Halkola R, Larsson H, Hultman CM, Reichenberg A. The Familial Risk of Autism. JAMA. 2014;311(17):1770-7.
34. Tick B, Bolton P, Happé F, Rutter M, Rijsdijk F. Heritability of autism spectrum disorders: a meta-analysis of twin studies. J Child Psychol Psychiatry. 2016;57(5):585-95.
35. Forsberg SL, Ilieva M, Maria Michel T. Epigenetics and cerebral organoids: promising directions in autism spectrum disorders. Transl Psychiatry. 2018;8(1):14.

36. Yin H, Wang Z, Liu J, Li Y, Liu L, Huang P, et al. Dysregulation of immune and metabolism pathways in maternal immune activation induces an increased risk of autism spectrum disorders. Life Sci. 2023;324:121734.
37. Chess S. Follow-up report on autism in congenital rubella. J Autism Child Schizophr. 1977;7(1):69-81.
38. Tioleco N, Silberman AE, Stratigos K, Banerjee-Basu S, Spann MN, Whitaker AH, et al. Prenatal maternal infection and risk for autism in offspring: A meta-analysis. Autism Res. 2021;14(6):1296-316.
39. Smith SE, Li J, Garbett K, Mirnics K, Patterson PH. Maternal immune activation alters fetal brain development through interleukin-6. J Neurosci. 2007;27(40):10695-702.
40. Kalish BT, Kim E, Finander B, Duffy EE, Kim H, Gilman CK, et al. Maternal immune activation in mice disrupts proteostasis in the fetal brain. Nat Neurosci. 2021;24(2):204-13.
41. Choi GB, Yim YS, Wong H, Kim S, Kim H, Kim SV, et al. The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. Science. 2016;351(6276):933-9.
42. Robinson-Agramonte MLA, Noris García E, Fraga Guerra J, Vega Hurtado Y, Antonucci N, Semprún-Hernández N, et al. Immune Dysregulation in Autism Spectrum Disorder: What Do We Know about It? Int J Mol Sci. 2022;23(6).
43. Estes ML, McAllister AK. Maternal immune activation: Implications for neuropsychiatric disorders. Science. 2016;353(6301):772-7.
44. Braunschweig D, Golub MS, Koenig CM, Qi L, Pessah IN, Van de Water J, et al. Maternal autism-associated IgG antibodies delay development and produce anxiety in a mouse gestational transfer model. J Neuroimmunol. 2012;252(1-2):56-65.
45. Brown AS, Sourander A, Hinkka-Yli-Salomäki S, McKeague IW, Sundvall J, Surcel HM. Elevated maternal C-reactive protein and autism in a national birth cohort. Mol Psychiatry. 2014;19(2):259-64.
46. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol. 2005;57(1):67-81.
47. Abdallah MW, Larsen N, Grove J, Nørgaard-Pedersen B, Thorsen P, Mortensen EL, et al. Amniotic fluid inflammatory cytokines: potential markers of immunologic dysfunction in autism spectrum disorders. World J Biol Psychiatry. 2013;14(7):528-38.
48. Atladóttir HO, Thorsen P, Østergaard L, Schendel DE, Lemcke S, Abdallah M, et al. Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. J Autism Dev Disord. 2010;40(12):1423-30.
49. Zerbo O, Qian Y, Yoshida C, Grether JK, Van de Water J, Croen LA. Maternal Infection During Pregnancy and Autism Spectrum Disorders. J Autism Dev Disord. 2015;45(12):4015-25.
50. Lee BK, Magnusson C, Gardner RM, Blomström Å, Newschaffer CJ, Burstyn I, et al. Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. Brain Behav Immun. 2015;44:100-5.
51. Croen LA, Qian Y, Ashwood P, Zerbo O, Schendel D, Pinto-Martin J, et al. Infection and Fever in Pregnancy and Autism Spectrum Disorders: Findings from the Study to Explore Early Development. Autism Res. 2019;12(10):1551-61.
52. Atladóttir H, Henriksen TB, Schendel DE, Parner ET. Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. Pediatrics. 2012;130(6):e1447-54.
53. Langen UH, Ayloo S, Gu C. Development and Cell Biology of the Blood-Brain Barrier. Annu Rev Cell Dev Biol. 2019;35:591-613.

54. Rabeling A, Goolam M. Cerebral organoids as an in vitro model to study autism spectrum disorders. Gene Therapy. 2023;30(9):659-69.
55. De Miranda J, Yaddanapudi K, Hornig M, Villar G, Serge R, Lipkin WI. Induction of Toll-like receptor 3-mediated immunity during gestation inhibits cortical neurogenesis and causes behavioral disturbances. mBio. 2010;1(4).
56. Dalton P, Deacon R, Blamire A, Pike M, McKinlay I, Stein J, et al. Maternal neuronal antibodies associated with autism and a language disorder. Ann Neurol. 2003;53(4):533-7.
57. Singer HS, Morris C, Gause C, Pollard M, Zimmerman AW, Pletnikov M. Prenatal exposure to antibodies from mothers of children with autism produces neurobehavioral alterations: A pregnant dam mouse model. J Neuroimmunol. 2009;211(1-2):39-48.
58. Camacho J, Jones K, Miller E, Ariza J, Noctor S, de Water JV, et al. Embryonic intraventricular exposure to autism-specific maternal autoantibodies produces alterations in autistic-like stereotypical behaviors in offspring mice. Behav Brain Res. 2014;266:46-51.
59. McEwan F, Glazier JD, Hager R. The impact of maternal immune activation on embryonic brain development. Front Neurosci. 2023;17:1146710.
60. Szabó D, Tod P, Gölöncsér F, Román V, Lendvai B, Otrokocsi L, et al. Maternal P2X7 receptor inhibition prevents autism-like phenotype in male mouse offspring through the NLRP3-IL-1β pathway. Brain Behav Immun. 2022;101:318-32.
61. Guma E, Bordeleau M, González Ibáñez F, Picard K, Snook E, Desrosiers-Grégoire G, et al. Differential effects of early or late exposure to prenatal maternal immune activation on mouse embryonic neurodevelopment. Proc Natl Acad Sci U S A. 2022;119(12):e2114545119.
62. Ben-Reuven L, Reiner O. Dynamics of cortical progenitors and production of subcerebral neurons are altered in embryos of a maternal inflammation model for autism. Mol Psychiatry. 2021;26(5):1535-50.
63. Rasmussen JM, Graham AM, Entringer S, Gilmore JH, Styner M, Fair DA, et al. Maternal Interleukin-6 concentration during pregnancy is associated with variation in frontolimbic white matter and cognitive development in early life. Neuroimage. 2019;185:825-35.
64. Bauman MD, Iosif AM, Ashwood P, Braunschweig D, Lee A, Schumann CM, et al. Maternal antibodies from mothers of children with autism alter brain growth and social behavior development in the rhesus monkey. Transl Psychiatry. 2013;3(7):e278.
65. Wei H, Chadman KK, McCloskey DP, Sheikh AM, Malik M, Brown WT, et al. Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors. Biochim Biophys Acta. 2012;1822(6):831-42.
66. Elgueta D, Murgas P, Riquelme E, Yang G, Cancino GI. Consequences of Viral Infection and Cytokine Production During Pregnancy on Brain Development in Offspring. Frontiers in Immunology. 2022;13:816619.
67. Needleman LA, McAllister AK. The major histocompatibility complex and autism spectrum disorder. Dev Neurobiol. 2012;72(10):1288-301.
68. Sompayrac L. How the Immune System Works2019.
69. Kern JK, Geier DA, Sykes LK, Geier MR. Relevance of Neuroinflammation and Encephalitis in Autism. Front Cell Neurosci. 2015;9:519.
70. Jessen KR. Glial cells. Int J Biochem Cell Biol. 2004;36(10):1861-7.
71. Verkhratsky A, Nedergaard M. Physiology of Astroglia. Physiol Rev. 2018;98(1):239-389.
72. Gzielo K, Nikiforuk A. Astroglia in Autism Spectrum Disorder. Int J Mol Sci. 2021;22(21).
73. Cuellar-Santoyo AO, Ruiz-Rodríguez VM, Mares-Barbosa TB, Patrón-Soberano A, Howe AG, Portales-Pérez DP, et al. Revealing the contribution of astrocytes to glutamatergic neuronal transmission. Frontiers in Cellular Neuroscience. 2023;16.

74. Vakilzadeh G, Martinez-Cerdeño V. Pathology and Astrocytes in Autism. Neuropsychiatr Dis Treat. 2023;19:841-50.
75. Oschwald A, Petry P, Kierdorf K, Erny D. CNS Macrophages and Infant Infections. Frontiers in Immunology. 2020;11 (no pagination).
76. Schafer ST, Mansour AA, Schlachetzki JCM, Pena M, Ghassemzadeh S, Mitchell L, et al. An in vivo neuroimmune organoid model to study human microglia phenotypes. Cell. 2023;186(10):2111-26.e20.
77. Matuleviciute R, Akinluyi ET, Muntslag TAO, Dewing JM, Long KR, Vernon AC, et al. Microglial contribution to the pathology of neurodevelopmental disorders in humans. Acta Neuropathol. 2023;146(5):663-83.
78. Carson MJ, Bilousova TV, Puntambekar SS, Melchior B, Doose JM, Ethell IM. A rose by any other name? The potential consequences of microglial heterogeneity during CNS health and disease. Neurotherapeutics. 2007;4(4):571-9.
79. Morgan JT, Chana G, Abramson I, Semendeferi K, Courchesne E, Everall IP. Abnormal microglial-neuronal spatial organization in the dorsolateral prefrontal cortex in autism. Brain Res. 2012;1456:72-81.
80. Morgan JT, Chana G, Pardo CA, Achim C, Semendeferi K, Buckwalter J, et al. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biol Psychiatry. 2010;68(4):368-76.
81. Rodriguez JI, Kern JK. Evidence of microglial activation in autism and its possible role in brain underconnectivity. Neuron Glia Biol. 2011;7(2-4):205-13.
82. Herbert MR. Large brains in autism: the challenge of pervasive abnormality. Neuroscientist. 2005;11(5):417-40.
83. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126(4):663-76.
84. Russo FB, Brito A, de Freitas AM, Castanha A, de Freitas BC, Beltrao-Braga PCB. The use of iPSC technology for modeling Autism Spectrum Disorders. Neurobiol Dis. 2019;130:104483.
85. Qian X, Song H, Ming GL. Brain organoids: advances, applications and challenges. Development. 2019;146(8).
86. Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, et al. Cerebral organoids model human brain development and microcephaly. Nature. 2013;501(7467):373-9.
87. Wang L, Owusu-Hammond C, Sievert D, Gleeson JG. Stem Cell-Based Organoid Models of Neurodevelopmental Disorders. Biol Psychiatry. 2023;93(7):622-31.
88. Acharya P, Choi NY, Shrestha S, Jeong S, Lee M-Y. Brain organoids: A revolutionary tool for modeling neurological disorders and development of therapeutics. Biotechnology and Bioengineering. 2024;121(2):489-506.
89. Santos JLS, Araújo CA, Rocha CAG, Costa-Ferro ZSM, Souza BSF. Modeling Autism Spectrum Disorders with Induced Pluripotent Stem Cell-Derived Brain Organoids. Biomolecules. 2023;13(2).
90. Sarieva K, Kagermeier T, Khakipoor S, Atay E, Yentür Z, Becker K, et al. Human brain organoid model of maternal immune activation identifies radial glia cells as selectively vulnerable. Mol Psychiatry. 2023:1-13.
91. Zuiki M, Chiyonobu T, Yoshida M, Maeda H, Yamashita S, Kidowaki S, et al. Luteolin attenuates interleukin-6-mediated astrogliosis in human iPSC-derived neural aggregates: A candidate preventive substance for maternal immune activation-induced abnormalities. Neurosci Lett. 2017;653:296-301.

92. Warre-Cornish K, Perfect L, Nagy R, Duarte RRR, Reid MJ, Raval P, et al. Interferon-gamma signaling in human iPSC-derived neurons recapitulates neurodevelopmental disorder phenotypes. Sci Adv. 2020;6(34):eaay9506.
93. Pavlinek A, Matuleviciute R, Sichlinger L, Dutan Polit L, Armeniakos N, Vernon AC, et al. Interferon-gamma exposure of human iPSC-derived neurons alters major histocompatibility complex I and synapsin protein expression. Front Psychiatr. 2022;13:836217.
94. Gomes AKS, Dantas RM, Yokota BY, Silva A, Griesi-Oliveira K, Passos-Bueno MR, et al. Interleukin-17a Induces Neuronal Differentiation of Induced-Pluripotent Stem Cell-Derived Neural Progenitors From Autistic and Control Subjects. Front Neurosci. 2022;16:828646.
95. Lin M, Zhao D, Hrabovsky A, Pedrosa E, Zheng D, Lachman HM. Heat shock alters the expression of schizophrenia and autism candidate genes in an induced pluripotent stem cell model of the human telencephalon. PLoS ONE. 2014;9(4) (no pagination).
96. Pinto L, Goetz M. Radial glial cell heterogeneity-The source of diverse progeny in the CNS. Prog Neurobiol. 2007;83(1):2-23.
97. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol. 2004;75(2):163-89.
98. Salomoni P, Betts-Henderson J. The role of PML in the nervous system. Mol Neurobiol. 2011;43(2):114-23.
99. Onishi RM, Gaffen SL. Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. Immunology. 2010;129(3):311-21.
100. Anitha A, Thanseem I, Nakamura K, Vasu MM, Yamada K, Ueki T, et al. Zinc finger protein 804A (ZNF804A) and verbal deficits in individuals with autism. J Psychiatry Neurosci. 2014;39(5):294-303.
101. Zaslavsky K, Zhang W-B, McCready FP, Rodrigues DC, Deneault E, Loo C, et al. SHANK2 mutations associated with autism spectrum disorder cause hyperconnectivity of human neurons. Nature Neuroscience. 2019;22(4):556-64.
102. Quartier A, Courraud J, Thi Ha T, McGillivray G, Isidor B, Rose K, et al. Novel mutations in NLGN3 causing autism spectrum disorder and cognitive impairment. Hum Mutat. 2019;40(11):2021-32.
103. Berkel S, Marshall CR, Weiss B, Howe J, Roeth R, Moog U, et al. Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. Nat Genet. 2010;42(6):489-91.
104. Jamain S, Quach H, Betancur C, Råstam M, Colineaux C, Gillberg IC, et al. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nat Genet. 2003;34(1):27-9.
105. Di Napoli A, Warrier V, Baron-Cohen S, Chakrabarti B. Genetic variant rs17225178 in the ARNT2 gene is associated with Asperger Syndrome. Molecular Autism. 2015;6(1):9.
106. Wong G, Goldshmit Y, Turnley AM. Interferon-gamma but not TNF alpha promotes neuronal differentiation and neurite outgrowth of murine adult neural stem cells. Exp Neurol. 2004;187(1):171-7.
107. Song JH, Wang CX, Song DK, Wang P, Shuaib A, Hao C. Interferon gamma induces neurite outgrowth by up-regulation of p35 neuron-specific cyclin-dependent kinase 5 activator via activation of ERK1/2 pathway. J Biol Chem. 2005;280(13):12896-901.
108. Prem S, Millonig JH, DiCicco-Bloom E. Dysregulation of Neurite Outgrowth and Cell Migration in Autism and Other Neurodevelopmental Disorders. Adv Neurobiol. 2020;25:109-53.
109. Jan Y-N, Jan LY. Branching out: mechanisms of dendritic arborization. Nature Reviews Neuroscience. 2010;11(5):316-28.

110. Hu C, Yang J, Qi Z, Wu H, Wang B, Zou F, et al. Heat shock proteins: Biological functions, pathological roles, and therapeutic opportunities. MedComm (2020). 2022;3(3):e161.
111. Miller DJ, Fort PE. Heat Shock Proteins Regulatory Role in Neurodevelopment. Front Neurosci. 2018;12:821.
112. Gevezova M, Minchev D, Pacheva I, Todorova T, Yordanova R, Timova E, et al. Association of NGF and Mitochondrial Respiration with Autism Spectrum Disorder. Int J Mol Sci. 2022;23(19).
113. Sun YY, Chen WJ, Huang ZP, Yang G, Wu ML, Xu DE, et al. TRIM32 Deficiency Impairs the Generation of Pyramidal Neurons in Developing Cerebral Cortex. Cells. 2022;11(3):28.
114. Singh R, Kisku A, Kungumaraj H, Nagaraj V, Pal A, Kumar S, et al. Autism Spectrum Disorders: A Recent Update on Targeting Inflammatory Pathways with Natural Anti-Inflammatory Agents. Biomedicines. 2023;11(1).
115. Hafizi S, Tabatabaei D, Lai M-C. Review of Clinical Studies Targeting Inflammatory Pathways for Individuals With Autism. Frontiers in Psychiatry. 2019;10.