Review

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Recent Updates on Modifiable Risk Factors Involved in the Pathogenesis of Autism Spectrum Disorders

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Autism spectrum disorder (ASD) is a composite neurodevelopmental disorder characterized by a broad range of symptoms and varying severity. Although the exact cause of ASD remains unknown, new research has identified modifiable risk factors that may contribute to the pathophysiology of the disease. A combination of environmental and genetic variables influences the risk of ASD. Recent studies suggest that prenatal exposure to environmental pollutants, such as air pollution and certain pesticides, may increase the incidence of ASD. Additionally, maternal health during pregnancy, including obesity and gestational diabetes, has been identified as a modifiable risk factor. Furthermore, ongoing investigations explore probiotics and dietary modifications as potential means to alter these risk factors. There is also increasing attention given to the role of immunological dysregulation and inflammation in ASD. Studies have linked pregnant women with autoimmune diseases or infections to a higher incidence of ASD. Potential preventative interventions being explored include immunomodulatory methods. In summary, recent studies have identified modifiable risk factors contributing to the pathophysiology of ASD, offering the potential for early intervention and prevention. Reducing the prevalence of ASD may require a multimodal strategy addressing immune system regulation, maternal health, and environmental exposures. Further investigation is needed to translate these discoveries into practical therapeutic approaches.

Keywords: autism spectrum disorder; epigenetic factors; genetic factors; environmental factors; pathogenesis; risk factors

Introduction

Autism spectrum disorder (ASD) encompasses a group of neurodevelopment disorders characterized by persistent impairments in social communication, inadequate interaction, restricted and repetitive pattern of behavior, and loss of interest in activities. These conditions arise due to complex interactions of various modifiable risk factors during pre- and post-natal brain development [1–3]. Currently, there is no effective treatment for the primary symptoms of ASD. However, early behavioral intervention and the treatment of associated psychiatric illnesses, supported by some pharmaceutical therapies, can help alleviate ASD symptoms [4]. In the USA, there is a noticeable increase in the prevalence rate of ASD because 1 in 59 children is estimated to have autism [3]. Research data from children aged 2 to 17 showed a prevalence estimate of 3.5% between 2001 and 2011. Presently, ASD prevalence rates are approximately 1% in the United Kingdom, 1.5% in the United States, and 0.2% in India [5].

The precise cause behind the increased incidence of ASD remains unclear despite improved case identification. Brain development from pregnancy to the early postnatal years suggests that significant improvements are likely connected to these network experiences [6]. According to a recent meta-analysis of studies, ASD accounted for 7.7 million disability-adjusted life years (DALYs) in 2021, making it the most common mental health condition causing disability in children under five years of age [7,8]. Our understanding of ASD pathogenesis has evolved over time. A diagnosis can now be made as early as 18 to 24 months of age with specific symptoms distinguishing affected children from normal children [9]. Since 1950, autism has erroneously been attributed to “refrigerator mothers” due to perceived maternal lack of affection. Initially, researchers sought a single explanation, such as a gene or specific brain region, responsible for most cases of autism. However, instead of identifying a universal cause or providing definitive tests or treatments, the pursuit of underlying causes has led to the discovery of various risk factors. Research has identified diverse genetic and non-genetic elements that interact over time to produce distinct traits among individuals [9].

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Epidemiology has recently provided valuable insights into the neurodevelopmental origins of diseases, including autism [10]. By establishing a connection between modifiable environmental risk factors and their impact on both patients with long-term, well-defined conditions, and the general population, neurodevelopmental disorders can be identified more effectively. Comprehensive systemic studies have taken precedence in this field to accommodate new primary data. The epidemiology of autism has improved, increasing the overlap of risk factors in neurodevelopmental diseases. Linguistic impairments appear closely tied to age, especially when considering phenotypic behavior and disparities. Individuals with autism exhibit a range of linguistic abnormalities, including language-related challenges, from early stages that persist into atypical presentations after puberty within neuropsychiatric disorders [10].

Psychiatric episodes occurring during pregnancy affect the long-term health of the fetus [10]. These episodes may stem from various factors, including maternal activation, nutritional deficiencies, toxicity, stress, environmental changes, and various physiological and pathological conditions [11,12]. Their significance becomes particularly pronounced concerning the onset of autism when it arises during pregnancy [11]. Bipolar and spatial autistic disorders are central nervous system illnesses, indicating their origin from both genetic and environmental factors. According to epigenetic research, there is a clear link between autism spectrum disorders in children and immune system overactivation brought on by infection and other stressors [13].

Most cases of severe neurodevelopmental conditions, such as autism, are identified between the ages of three and fourteen months. Prenatal modifiable risk factors relevant to the development of ASD may emerge in the temporal region, where autism can develop [12]. According to a genetic study, the synapse, a crucial cellular organelle, appears particularly vulnerable to genetic variations and disruptions due to associated risk factors. It has been proposed that the prenatal transmission of genetic variables affects the current circumstances in offspring and is influenced by abnormalities in the immune system. Creating a framework to reveal novel processes behind ASD requires examining the interaction between synaptic strength and genetic risk factors [14].

Moreover, it has been hypothesized that numerous risk factors contribute to the development of ASD. These factors encompass the dynamic relationship between the mother and the newborn and the child's subsequent social and repetitive behaviors. Various prenatal influences impact the brain, potentially leading to challenges for the baby after birth and consequently contributing to the development of ASD. Efforts can be made to mitigate factors that may cause distress, mental stress, and physical harm during pregnancy, thereby fostering a healthier and safer environment for the child's development.

Pathogenesis of ASD: Role of Risk Factors

Neuropathological and imaging studies of autistic patients reveal brain overgrowth in 20% of cases. However, these studies failed to detect the significant and recognized structural abnormalities in the brains of autistic patients [15,16]. Similarly, although only a few postmortem studies have been conducted, there is currently no recognized specific microscopic neuropathology associated with autism. Genetic studies revealed that the synaptic CAM pathway as an etiological factor in ASD. Nonetheless, much remains unknown regarding the specific nature of the underlying pathogenic mechanisms and how they contribute to social and communicative difficulties in individuals with ASD. Further research is necessary to fully understand how abnormal expression of ASD risk factor genes may affect critical synaptic functioning [16,17]. Despite various factors being linked to the etiology of autistic disorders, the specific pathogenesis of ASD remains uncertain. For many comorbid disorders like tuberous sclerosis and fragile X syndrome, a robust evidence-based etiology has long been linked to the genetic basis. Siblings of autistic children have a higher prevalence of autism than the general population, and twin studies have also indicated a substantial genetic role [14].

The most significant aspect of a modifiable ASD risk factor is its interaction with other risk factors. The importance of modifiable risk factors in neurodevelopmental disorders has gained prolonged recognition since prenatal behavioral effects lead to neuronal toxicity and alterations in brain growth, impacting accurate neurodevelopment formation. Consequently, the mature brain exhibits extraordinary sensitivity to external stimuli during critical windows of susceptibility. Similar patterns observed in the adult mouse brain are reflected in this process. Human communication occurs during this distinct period, wherein early exposure to risk factors through epigenetics can permanently influence neuronal migration, proliferation, maturation, and differentiation in synaptogenesis, relying on the rehabilitation of synaptic activity [18]. The current scenario aligns with many prevalent autism pathogenesis models. In individuals with autism, genetic variations form complex compounds that significantly heighten vulnerability to the effects of harmful modifiable risk factors contributing to the emergence of ASD. In terms of computation, genetics and epigenetics play a role in the observed phenotypic behavior in adult mice, characterizing ASD [19].

In summary, this discussion has examined the etiological components and their role in autism conclusively. Our focus will shift to investigating recently recognized risk factors, which compelling evidence suggests anticipate a role in ASD.

Treatment of ASD

Pharmacological Treatment

Currently, there are no medications that specifically target the core symptoms of ASD. Instead, pharmaceutical interventions primarily aim to manage comorbid conditions such as anxiety, sleep disturbances, and challenging behaviors resistant to behavioral therapy. Psychotropic drugs are frequently prescribed to a significant proportion of autistic teenagers, especially in cases involving disruptive behaviors and concurrent medical and mental health conditions. The use of psychiatric drugs in ASD cases has markedly increased in recent decades [20–22]. It is estimated that over 70% of individuals with autism also have concurrent mental health disorders, encompassing conditions like attention deficit and hyperactivity disorders, irritability, aggression, mood disorders, anxiety, and various neuropsychiatric disorders [22]. Identifying and managing mental illnesses can be challenging, particularly for individuals with limited language skills, cognitive impairments, and vague symptoms. Collaboration among families, therapists, and teachers is crucial for effective treatment [22]. When pharmaceutical interventions are suggested for children with ASD, the treatment should commence at lower doses and gradually increase. Objective evaluation necessitates quantitative measurement of symptoms before and after medication intervention [18].

The range of therapies available for children and adolescents with ASD is limited. Currently, only two FDA-approved drugs are clinically available for eligible individuals. One of these drugs is Risperidone, specifically approved for children aged five and above [19]. Additionally, Aripiprazole has been sanctioned for use in children aged 6 to 17 [20]. Clinical trials have demonstrated that both medications reduce irritation and repetitive behaviors associated with ASD. Furthermore, these two drugs exhibit an affinity for the brain's 5-HT, dopamine, histaminergic, and alpha-adrenergic receptors [21].

Non-Pharmacological Treatment

Neurofeedback Treatment

Studies involving randomized controlled trials (RCTs) are pivotal as they significantly influence clinical practice [22]. One of the trials revealed that autistic individuals who underwent neurofeedback treatment exhibited more considerable improvements in social interaction and communication skills compared to those who did not. Due to its shorter administration time and long-term effectiveness, neurofeedback holds more advantages over other treatments. There are two primary types of brain stimulation: Transcranial Magnetic Stimulation (TMS) and Deep Brain Stimulation (DBS). TMS uses a simple coil to generate a magnetic field on the head, stimulating specific brain regions. It is often practical and well-tolerated, presenting minimal side effects. On the other hand, DBS involves surgically implanting electrodes in a particular brain region. Studies demonstrating its therapeutic effects have positioned it as an alternative treatment for individuals resistant to pharmaceutical treatment or those with comorbid conditions and low quality of life [23].

Parent-Mediated Treatment

There is growing recognition for the importance of involving parents in the treatment of autistic children. Strauss *et al*. [24] conducted a study to investigate the effects of parental involvement in therapy by monitoring parents' adherence to at-home treatment plans and the level of treatment intensity. The study compared twenty-two children undergoing electrical intervention with twenty children involved in parent-mediated Early Intensive Behavioral Intervention (EIBI). The parental intervention group outperformed the electrical intervention group in measures of autism severity, language, and developmental skills. Furthermore, therapy administered by parents resulted in less complex behavior in the children.

The study also unveiled a link between parental stress and staff fidelity to treatment, which influenced decisions in treatment planning and resulted in positive behavior outcomes. These findings are significant as they shed light on parental factors impacting a child's treatment response [25]. Parents of children with ASD may find this concept helpful, particularly when addressing issues that cannot be immediately resolved. While parents of children with intellectual challenges have explored psychological acceptance, this area has not been extensively studied among parents of children with ASD [26].

Modifiable Therapeutic Targetable Risk Factors and ASD

In this discussion, we delve into modifiable therapeutic targetable risk factors associated with ASD. A primary preventive objective for older children and adolescents involves performing comprehensive screening for any concurrent psychopathology that emerges during these dynamic developmental stages. Typically, there are three categories of mental health prevention. Firstly, preventive measures aim to reduce the incidence of a disease among a large population. Subsequently, secondary steps target specific risk factors such as environmental, genetic, and epigenetic risk factors to halt the progression of the condition. The etiopathology of ASD is believed to be influenced by a combination of these developmental risks, protective factors, and genetic factors. Finally, prevention efforts should focus on altering the developmental pathways that start during the gestation period and continue through the first 2–3 years of life to alleviate or avoid the onset of ASD symptoms [27].

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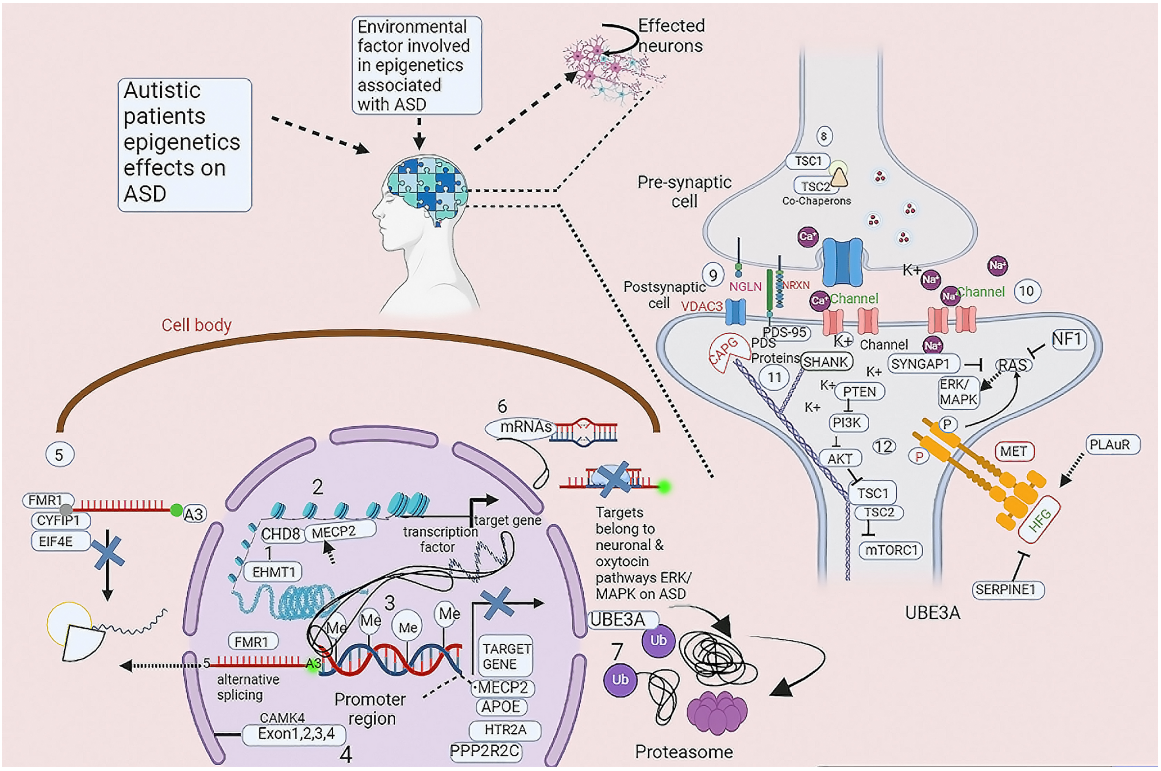

Fig. 1. Figure depicts synapses affected by various genetic, epigenetic, and environmental factors in the emergence of autism spectrum disorder (ASD). ASD-related nuclear and cytoplasmic processes are exiting cell bodies of specific neurons on the bottom. Numerous methods for regulating gene expression are used in the nucleus, which is demonstrated step-by-step. (1) Chromatin modernization involves chromatin packing and factors, (2) transcription factors govern gene transcription, (3) DNA methylation in the gene's promoter areas is akin to preventing the particular focus that forms transcriptionally transforming, (4) prospective braid and miRNA transport to the cytoplasm, (5) via the cytoplasmic Eukaryotic Translation Initiation Factor 4 Fraxile-X mental retardation protein (CYFIP1-EIF4E-FMR1) network, which controls post-transcriptional (6) mRNA regulation, and (7) Protein ubiquitination and proteasome degradation. (8) tuberous sclerosis (TSC) protein effects on exemplary synaptic area architecture and function of mechanisms similar to ASD in the presynaptic cells. (9) The neurexin/ neuroligin trans-synaptic network is depicted (10) in postsynaptic cells, and the voltage-gated ion channel is expressed. (11) Proteins that serve as capping, scaffolds, and actin filaments. (12) The postsynaptic cells contain several Phasphositide-3-Kinase-Protein Kinase-B (PI3K/AKT) pathway members as well as members of the RAS signal transduction network and MET receptor tyrosine kinase pathway. Indicated in beige are chromatin remodelers, pink is a transcription factor, light blue is a protein included in RNA binding and transport, purple is a protein involved in protein ubiquitination, and red is a scaffold protein. Green are proteins involved in cell growth and proliferation. Grey are members of their linked pathways. Created with BioRender.com.

Role of Modifiable Risk Factors in the Development of ASD

Numerous studies have suggested various modifiable risk factors linked to ASD, as summarized in Table 1 ([28–37]). Access to more extensive and more accurate patient and general population samples is necessary to examine the relationship between potentially modifiable environmental risk factors, genetics, epigenetics, and the network of neurodevelopmental disorders (Fig. 1) [10]. According to one study, pregnant female mice exposed to an infection at conception had a higher probability of giving birth to offspring requiring treatment for a neurodevelopmental disorder [38]. Maternal immune activity mediates the impacts of maternal infection on placental and fetal brain growth in preclinical Maternal Immune Activation (MIA) animal models [39]. Nonhuman primates offer a unique system to analyze MIA, as most MIA development increases the risk of neurodevelopment issues in adult offspring. We propose the first long-term study designed using MIA animal models in nonhuman primates. We can employ carefully chosen natural substances and agents to potentially exert a positive effect on the autistic brain, as the MIA model induces neurodevelopment abnormalities, increasing the modifiable risk factors of ASD [40,41].

Epigenetic Risk Factor

The study of differential gene expression, in which various genes may be expressed or repressed, is known as epigenetics. Epigenetics has several phenotypic effects that carry physiological and pathological repercussions. For in-

Table 1. Modifiable risk factors of ASD.

Category of risk factors	Risk factors	Type of study(ies)	Studies/models/tests	Study Parameters (Behavior & Molecular)	Remark	Ref.
Epigenetic	DNA methylation	*In vivo*	Poly (I: C)-induced mouse model	Hypo-methylation on offspring's DNA during gestation	The changes in DNA methylation observed during gestation revealed numerous genes such as *ABCA1*, *IGF2* & *IL10* in children, suggesting substantial epigenetic effects of maternal starvation. Probiotic supplementation during pregnancy reduced MIA cytokine levels & resultant autism spectrum disorder (ASD) symptoms in the offspring, such as sorrow, anxiety, and social deficits.	[28]
Epigenetic	DNA methylation	*In vivo*	Poly (I: C)- induced mouse model	Infection-mediated epigenetic & transgenerational mechanisms	It demonstrates neurodevelopmental impairment in the methyl-CpG-binding protein 2 (*Mecp2*) gene in mutated mice, which plays a critical role in DNA methylation during early brain development.	[35]
Epigenetic	*Mecp2* methylation	*In vivo*	Maternal immune activation (MIA)-induced mouse model	Prenatal inflammation altering DNA methylation & *Mecp2* gene-regulated LINE1 transcription	LINE1 hypomethylation linked to complex neurodevelopmental disorders, including Schizophrenia and ASD.	[29]
Epigenetic	DNA methylation	*In vivo*	Poly (I: C) administered the MIA mouse model	Interaction between MIA and Genetic Risk Factors	MIA-associated alterations have contributed to variations in phenotypes, including autism, schizophrenia, and other neurological disorders affecting behavior. The link between maternal infection and changes in phenotype is not entirely understood. Brain changes and associated behavior could be linked to MIA-induced prolonged immune changes.	[36]
Genetic factor	*Mecp2* & *Mfr1* gene	*In vivo*	LPS-induced mouse model	ASD mice/rat models and genetic risk factors	The study examines multiple Shank3 mice models to explore the *in vivo* function of this high-confidence ASD gene, which is the key to understanding and analyzing the diverse phenotypes observed in ASD single-gene models.	[32]

Table 1. Continued.

Category of risk factors	Risk factors	Type of study(ies)	Studies/models/tests	Study Parameters (Behavior & Molecular)	Remark	Ref.
Genetic factor	*Shank3* gene	*In vivo*	Poly (I: C) induced to BTBR mouse model	Complex phenotypic behavior in the rat autism model	ASD genes, syndromic genes, recurrent copy number variants (CNVs), and non-genetic factors contribute to mitigating symptoms relevant to ASD. There is increasing evidence supporting non-genetic factors, such as MIA, linked to ASD etiology.	[32]
Genetic factor	*Shank3* gene	*In vivo*	Poly (I: C)-induced MIA mouse model	Maternal inflammation alteration during pregnancy In adult offspring at 20 mg/kg Poly (I: C)	Developed MIA causes viral and bacterial illness in pregnant mothers. It has been connected to the fetus and escalates the risk of ASD.	[30]
Genetic factor	*Mecp2* gene	*In vivo*	Poly (I: C) induced MIA mouse model	Epigenomic signature of ASD	Postmortem brain analysis revealed hypo-methylation of gene areas related to immune function during brain development in ASD but hyper-methylation in regions containing developmentally regulated neural synaptic genes.	[31]
Environmental factor	Air pollution	*In vivo*	MIA-Induced mouse model	ASD-like behavior assessed in offspring after exposure to air pollution in MIA	Studies have linked MIA to ASD-like outcomes in offspring across various species and have encompassed environmental factors, aside from infection, associated with ASD in humans, including toxins, stress, and maternal obesity.	[33]
Environmental factor	Air pollution	*In vivo*	MIA induced C57BL/6J mouse model	Inflammatory cytokine, Air pollution exposure, and MIA	Diesel Exhaust (DE) exposure during development (from E0 to PND21) has been linked to behavioral alterations in all three hallmark domains of ASD, as well as an increase in repetitive behavior, disruptions in verbal and olfactory communication, and deficiencies in social behavior.	[37]
Environmental factor	Organic compound pollution	*In vivo*	Valproic acid (VPA)-induced mouse model	Behavior alteration VPA exposure	Mitochondrial dysfunction due to environmental exposure, such as air pollution and dietary metals, may lead to ASD.	[34]

stance, mounting evidence demonstrates that ASDs are significantly influenced by abnormal epigenetic pathways. Histone modification and DNA methylation/demethylation are two primary methods involved in the epigenetic control of genes. During histone modification, DNA wraps around histone proteins, allowing for selective acetylation or phosphorylation of these proteins to open the DNA for transcription [42]. Chromatin undergoes compaction and decompaction during this transcriptional process to permit the transcriptional apparatus access to the DNA strands. Conversely, deacetylation or dephosphorylation causes DNA to bind tightly to nucleosomes, compacting chromatin and limiting transcription. DNA methylation is another mechanism used by cells to silence genes. Methylation of a DNA strand's cytosine chain inhibits transcription, reducing gene expression [43,44].

Mechanistic Role of Various Modifiable Epigenetic Risk Factors in ASD

Standard epigenetic marks, susceptible to genetic and environmental modifications, can lead to epigenetic changes that disrupt the control of gene expression and have a detrimental effect on biological pathways crucial for brain development. Research outlines significant evidence supporting the role of epigenetics in the underlying cellular processes of ASD. Genetic research, environmental exposure, and recent studies have drawn evidence identifying unique epigenetic patterns associated with ASD, particularly DNA methylation, DNA histone modification, and microRNA (miRNA) deacetylation. Recent studies have implications for potential epigenetic therapeutic targets and indicate new research directions in various epigenetic changes, which include (1) DNA methylation, (2) Histone deacetylation, and (3) miRNA dysregulation (Fig. 2) [45].

DNA Methylation

Many studies have conducted methylation analyses in peripheral and brain tissues of both control and autistic mice [41]. These studies were designed to identify cell lines and collect complete blood DNA from twins discordant in monozygosity for ASD and compare them with control samples. The objective was to locate various methylated areas between autistic patients and control samples [46]. Subsequently, 400 differentially methylated regions (DMRs) in placentas from ASD-affected children, compared to those from typically developing controls, were found to be enriched at the promoters of genes involved in neural development. Two DMR methylation levels mapped on CYP2E1 and IRS2 showed similarities regarding genotype within the DMR and prenatal vitamin consumption [44].

The studies did not examine DNA methylation patterns that could distinguish controls from heterogeneous cases of ASD. However, specific DNA methylation signatures have been identified in individuals with ASD who have deletions or pathogenic mutations of CHD8. When comparing matched controls to the subgroup of ASD cases based on the results of the Autism Diagnostic Interview, changes in Alu methylation patterns were observed. Recently, genome-wide methylation research was conducted on postmortem tissue samples from various brain regions removed from individuals with ASD and controls. Many significant methylation changes were found between idiopathic ASD cases and their respective controls, as well as in people with the 15q11-13 duplication. These abnormalities were more prominent in cortical areas than in the cerebellum [45,47].

A considerable increase in the total methylation of CCI at CGI-2 and CGI-4 was observed in the brain tissue of individuals with ASD. Additionally, a potent histone deacetylase inhibitor rescued the behavioral phenotype in the SHANK3 deletion mouse model, supporting the importance of epigenetics in ASD. These studies provided evidence of underlying mechanisms behind the development or manifestation of ASD symptoms involving abnormalities in DNA methylation and histone modification of genes associated with ASD [28,48].

Histone Deacetylation

Histone acetyltransferases (HATs) and histone deacetylases (HDACs) catalyze the acetylation and deacetylation of histones, respectively. HDACs facilitate the deacetylation of histone and non-histone substrates. HDAC inhibitors enhance transcription factor accessibility and promote gene expression by suppressing HDAC activity. This alteration affects the balance of histone acetylation/deacetylation, leading to hyperacetylation due to enzymatic activity. HDAC inhibitors restore decreased gene expression caused by the Ash11 deletion during brain development. Histone deacetylation by HDACs at transcriptional regulatory regions induces chromatin compaction and activates transcription [49]. The dynamic alteration of the chromatin network, involving the addition of acetyl groups to essential histone proteins by HATs and their removal by HDACs, provides a mechanism through which genetic and environmental influences may impact phenotypic modifications over time [29,50].

MicroRNA Dysregulation

Small, non-coding RNA molecules, known as microRNAs (miRNAs), regulate gene expression at the post-transcriptional level [4,30]. Residing in the brain, they govern half of all human genes. Unregulated miRNAs were identified through small RNA sequencing analysis conducted on genome-wide DNA methylation data. High expression of miRNAs in the brains of ASD patients has been linked to synaptic function, contributing to neurodevelopmental issues due to miRNA release dysregulation. miRNA therapy presents a promising treatment for ASD as it can penetrate cells without being integrated into the host DNA. Furthermore, therapies utilizing miRNA antagonists and

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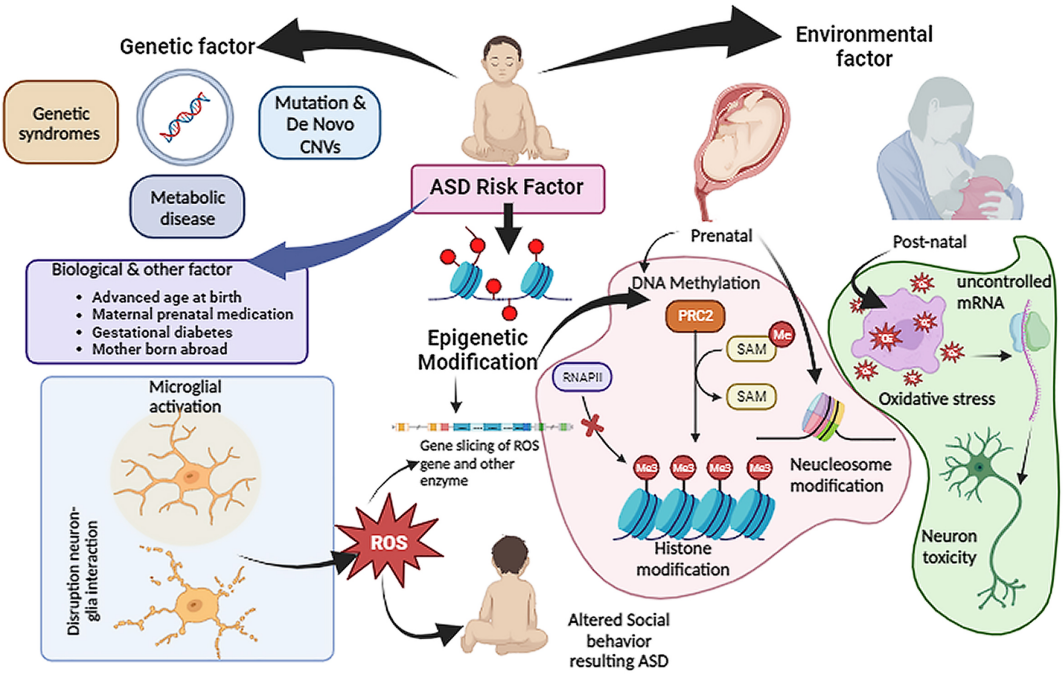

Fig. 2. Illustrations depict complex interactions among genetic, epigenetic, environmental, and biological factors in autism spectrum disorder (ASD) pathogenesis. Various modifiable risk factors, including genetic, epigenetic, environmental, and biological, have complex interactions and play significant roles in the pathogenesis of ASD. Firstly, genetic factors, including *de novo* copy number variants (CNVs), genetic-related syndromes, and metabolic disorders, as well as biological factors, including late pregnancy, prenatal medication use, gestational diabetes, and the mother's foreign birthplace together activate the microglia cells of the brain causing disruption of neuron-glia interactions which leads to the generation of reactive oxygen species (ROS) that results in altered social behavior leading to ASD. ROS also cause epigenetic modification through gene slicing of ROS genes and other enzymes, further causing oxidative stress. Further, prenatal environmental factors lead to epigenetic changes, including DNA methylation, nucleosome, and histone modification, whereas postnatal environmental factors result in uncontrolled mRNA generation, oxidative stress, and neuronal toxicity in the brain; both prenatal and postnatal changes ultimately lead to alteration in social behavior that results in ASD. ASD neurobiology is complex in which each factor alters the others through different mechanisms. Created with BioRender.com.

inhibitors can downregulate overexpressed miRNA, while low expression of overexpressed miRNA in ASD patients can be compensated for by miRNA substitution therapy using miRNA mimics [31]. The specific location, such as the open-chromatin structure, determines the relationship between miRNAs and gene expression [32,33].

Epigenetic alterations are also believed to contribute to ASD significantly. Many candidate genes for ASD discovered through genetic screening involve epigenetic pathways [47]. Dysregulated miRNAs alter the expression levels of genes and biological pathways associated with autism biology. The oxytocin receptor (*OXTR*) gene, responsible for regulating social, cognitive, and emotional behavior, is linked to subgroups of individuals with ASD, including those with Asperger's syndrome [30,34]. The epigenetic modulator miR-142 negatively regulates monoamine oxidase transcription, affecting translation and neurotransmitter metabolism. Upregulated miR-142-5p might lead to HDAC2 mRNA degradation, impacting cell proliferation and differentiation [31,34]. Several miRNAs, such as miRNA-125b, miRNA-132, miRNA-146a, miRNA-146b, miRNA-137, miRNA-134, miRNA-134-5p, miRNA-138-5p, miRNA-34a, miRNA-181c, and miRNA-30d, have demonstrated a clear correlation with ASD. These miRNAs directly control the expression of numerous genes involved in neurodevelopment. These findings suggest that miRNAs are crucial for discovering novel treatments for the improvement of outcomes for patients with ASD [31,51].

Genetic Risk Factors and ASD

Research was conducted to understand the pathogenesis of ASD after its establishment as a disorder. Earlier, it was known that environmental factors were the only cause of autism but later on genetic causes of ASD in humans garnered much attention [51]. A study on twins was conducted after discovering that the incidence of siblings was 50% higher than average. Monozygotic twins showed a 60% concordance rate compared to non-concordant dizygotic pairs, indicating a genetic component more likely shared by monozygotic twins than by dizygotic twins [52,53]. Additionally, the likelihood of developing ASD correlated with the percentage of shared genomes with a parent or sibling

having the condition [54]. Research has sought to identify specific genes responsible for each ASD symptom and their connection to the disorder's pathogenesis. Genes associated with ASD are strongly linked to other neurodevelopment conditions like Fragile X syndrome and Rett syndrome due to the wide range of ASD symptoms [55,56].

As ASD is associated with multiple genes, it is now recognized as a polygenic disorder, where a combination of genes increases its occurrence. Many of these genes are involved in neurotransmitter modulation, synapse function, and early brain development. Furthermore, a significant proportion of individuals with ASD exhibit *de novo* mutations, novel genetic alterations not present in their parents [57]. Fraternal twin concordance rates range from 0–10%, while identical twin concordance rates range from 70–90% in families with an incidence of ASD [58]. Familial clustering has been observed in families with existing ASD cases. The prevalence of ASD is higher among younger siblings or those already diagnosed with autism, especially among younger male siblings [59]. Genetic causes, in the form of *de novo* mutations, frequent and atypical genetic changes, and ASD-associated common polymorphisms, can be detected in approximately 20–25% of children or adults with ASD. The *SFARI* gene database, a collection of potential genes for autism, links approximately 1000 genes to ASD [60]. Understanding these genetic constituents is imperative in augmenting our cognizance of ASD, enhancing prompt diagnosis, and potentially formulating more targeted interventions and treatments.

Mechanistic Role of Various Modifiable Genetic Risk Factors in ASD

Genetic factors, specifically gene mutations, seem to contribute to ASD based on available information. Although single-gene abnormalities like FXS account for a small fraction of ASD cases, the majority are influenced by the subtle effects of potentially hundreds of genes. Approximately 10% of all ASD cases are the result of spontaneous genetic changes. Additionally, various known genes associated with ASD directly or indirectly influence synapse development and function. Over 100 genes show a strong association with susceptibility and pathogenesis. Mutations in genes like *SERBP1*, *BOLA2*, *STXBP1*, *CDLK56*, *NLGN*, *NRXN*, *PTEN*, *MECP2*, *UBE3a*, and *SHANK* have been linked to synaptogenesis and epileptic encephalopathy. Understanding the genes and genetic pathways of ASD is crucial for comprehending the related pathophysiology and investigating any potential therapeutics. There is significant research focused on how specific signaling genes contribute to the onset of ASD and its comorbid conditions. This research includes incorporation of proteins involved in protein synthesis and degradation, such as mTOR and DIA-1, while disregarding proteasomal degradation players like UBE3A and the protease RELN [52,56,58,61–65].

NLGN3 Gene and ASD

NLGN3 and *NLGN4* were the first ASD-associated genes identified in this pathway [62]. A significant breakthrough in this line of research occurred when two brothers, one with Asperger syndrome and the other with autism, were found to have a transformative mutation in the esterase domain of the *NLGN4* gene. This discovery sparked genetic and molecular research on neuroligins in ASD. Approximately ten other studies have identified non-synonymous mutations or deletions of NLGN4 in ASD. Only two male ASD siblings carrying the potential causal missense mutation R451C in NLGN3 have been reported [63]. NLGN4 has been found to possess rare beneficial mutations associated with ASD more frequently in the coding zone or promoter domain than NLGN3 [64]. Even though NLGN4 mutations occur in only a small percentage of ASD cases, their impact on synaptic functionality provides a crucial perspective on the pathophysiology of the disorder. In addition to the coding regions, the research investigated NLGN4's regulatory domain and copy number variations in ASD, as these factors may influence the extent of NLGN4 expression [52].

SHANK2 Gene and ASD

The *SHANK* gene (*SHANK1*, *SHANK2/ProSAP1*, and *SHANKProSAP2*) plays a vital role in development and processes by acting as a postsynaptic anchoring platform for receptor complexes and signaling molecules. Genetic variations in the three *SHANK* gene loci have been linked to ASD. Anxiety-like behavior, hyperactivity, and abnormal social behavior have been observed in *SHANK2* deficit mice. Furthermore, dysregulation of synaptic agents, including receptors—particularly the N-methyl-D-aspartate (NMDA), cell adhesion proteins, and members of many signaling cascades—has been observed in the affected brain area. Thus, maintaining an excitatory-inhibitory balance is a crucial process in ASD [56,57].

The *SHANK* gene is also associated with other mental disorders, such as intellectual impairments and Phelan-McDermid Syndrome. This gene encodes excitatory synapse postsynaptic scaffold proteins (SHANK1, 2, and 3) and numerous other ankyrin repeat domain proteins. Over 80 ASD-related mutations are associated with the *SHANK3* gene, while the *SHANK2* gene has over 30 such mutations. *Shank2* knockout in inadequate exons 6 and 7 demonstrated NMDA hypo-function and extensive deficits, while the new *Shank2* inadequate exon 7 showed NMDA hyperfunction and elevation in the adult hippocampus. The authors showed that differences in gene expression, experimental conditions, genetic makeup, and mouse developmental phases may partially explain the opposing cellular abnormalities observed in the two *Shank2* mutant mouse models [59].

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NRXN1 Gene and ASD

The *NRXN1* gene, responsible for encoding presynaptic proteins involved in neurotransmitter delivery, harbors some of the most prevalent single-gene variants linked to ASD. *NRXN1* is one of the longest human genes, spanning more than 1 Mb with 24 exons on chromosome 2. Non-recurrent copy number variants (CNVs) are particularly frequent at this extensive locus. These CNVs, which vary in magnitude and location across the *NRXN1* gene, are associated with various psychiatric and neurodevelopmental abnormalities, including intellectual disability and autism. It took two decades to create the first *NRXN1* animal model, which involved deleting the promoter and exon1 to eliminate *NRXN1* transcription. Behavioral phenotype studies conducted on mice with the *NRXN1* promoter/exon1 revealed diverse traits relevant to autism, including alterations to aggression, locomotion, self-protection, motor learning, decision-making, and changes in social affiliative behavior [11,65].

Over the past 30 years, several genetically altered mouse models have been generated for human genetic illnesses and neuropsychiatric disorders. Mice share a 90% gene congruency and 85% similarity in the protein-coding region with humans, making them valuable models for studying the cellular and molecular alterations underlying behavior and their contributions to neurological illnesses [66]. The identification of genetic risk factors contributing to autism dates back to the discovery of identical twins sharing similar traits in the past. Subsequent research indicated an inherited rate of over 80% in identical twins, while fraternal twins showed a concordance rate of about 40% [53]. Many communication-related traits observed in autism are also evident in well-known syndromes that frequently co-occur with the disorder. These syndromic ASDs, which account for about 10% of all autism cases, are typically characterized by dysmorphic features and abnormalities. Studies have reported a notable increase in autism prevalence among patients with these syndromes [67]. Research has demonstrated the widespread development of neuropsychiatric conditions in young children with autism, often attributed to the investigation of genetic risk factors across various domains. Approximately 2–8% of children display autistic traits, with related impairments potentially elevating this percentage to 12–20%, underscoring the impact of impairments on autism diagnosis. Infection and prenatal nutrition have been suggested modifiable genetic risk factors supported by numerous studies and theories [68,69].

Environmental Risk Factors of ASD

An elevated risk of poor neurodevelopment and specific conditions, such as ASD, has been associated with environmental chemicals and toxins. As a modifiable component, a prenatal diet may alter the relationships with such environmental influences. Studies have reviewed research on prenatal dietary factors as moderators influencing the association between environmental exposure and ASD or other related neurodevelopmental outcomes. Twelve studies were identified, five of which examined the diagnosis of ASD or its associated qualities as the outcome, while the other studies focused on the correlation with neurodevelopmental scores. The primary research focused on omega-3 fatty acids, prenatal vitamins, and folic acid as potential effect modifiers. Environmental risk variables included pesticides, heavy metals, endocrine-disrupting substances, and air pollution. Results demonstrated that prenatal significantly attenuated the ASD induced by environmental exposure, establishing the strong link between environmental exposure and ASD [59,60].

There is no doubt that environmental risk factors can influence the development of autism. Several known prospective causes may provide different perspectives on similar events, such as prenatal stress, prenatal infection, maternal Zn^{2+} deficiency, and the mother's susceptibility to unique hazardous factors [70]. It has been reported that over 200 substances are potential teratogens with neurotoxic effects on the adult brain. The sustained release of neurotoxins and an extended period of heightened sensitivity in the central nervous system can adversely impact neurodevelopmental processes. Preliminary findings suggest that diverse organic compounds, including organophosphates, halogenated hydrocarbons, heavy metals, air pollutants, and other substances, can permeate the placental barrier due to concentration gradient, extent, and thickness. This transference potentially contributes to behavioral teratogenesis, especially concerning ASD [5,71].

Nutritional Factors

Prenatal nutritional deprivation has been investigated as a potential risk factor for ASD [72]. Studies have demonstrated a profound link between nutritional alterations and autism. Both hunger and maternal infection result in fluctuations of micronutrients and homocysteine levels during pregnancy among mothers of specific autistic children. There is evidence suggesting that lower levels of vitamin D during pregnancy and early life might be associated with ASDs, and vitamin D deficiency could potentially elevate the likelihood of autism in future generations [73]. This theory has focused on understanding the relationship between omega-3 fatty acids, vitamin D deficiency, and ASD, areas highlighted for dietary intervention studies [56]. However, only two small, randomized studies have been conducted which met the criteria for a Cochrane review. Researchers in these studies found no evidence supporting the beneficial effect of prenatal omega-3 supplementation in ASD patients, which may indicate an epidemiological trait of autism [74]. They suggested that prenatal nutrition could be the most probable causative factor. Still, there are limitations in solely focusing on the epidemiological theory concerning the heightened risk of autism in offspring due to maternal prenatal nutrition [34,75].

Air Pollutants Factors

The global environmental issue of air pollution significantly impacts the health of individuals worldwide, with exposure often commencing during gestation. As of 2012, according to the US Census Bureau, over 142 million Americans resided in counties where air pollutants exceeded US standards despite a demonstrable improvement in air quality regulations compared to other countries [76]. Air pollution consists of particulate matter (PM), gases, organic compounds, and metals like nickel and manganese, both internally and externally present in the air, constituting a diverse mixture of pollutants [6,77]. Many communities globally consistently experience air contaminant levels that surpass established safety thresholds. There are multiple contributors to air pollution, including wood burning, construction activities, industrial operations (e.g., metal processing plants, refineries), emissions from mobile sources, and domestic fuel usage. Extensive research has demonstrated the adverse effects of air pollution exposure during pregnancy, increasing the risk of low birth weight and premature birth, thereby impacting prenatal development [78].

High levels of carbon or air pollution have been linked to decreased cognition, attention, and memory at advanced ages of human life. There is a profound connection between prenatal air pollution exposure and the increased prevalence of ASD. For instance, a significant cohort study, the CHARGE trial, indicated a 1.86 odds ratio for a higher risk of autism among children residing within 309 meters of a motorway around the time of birth [79,80]. This trial utilized a comprehensive dispersion model encompassing traffic-related air pollutants such as nitrogen (NO_2), ozone (O_3), and PM10 and PM25 air concentrations. The findings revealed a twofold increase in autism risk in children exposed to traffic-related air contaminants during pregnancy and a threefold elevation when the exposure occurred in the first year of life.

Moreover, exposure to PM25, PM10, or NO_2 individually was associated with a twofold rise in autism incidence [80]. In a large-scale, community-based study involving 7603 autistic patients and ten control matches per case, all born to mothers residing in Los Angeles between 1995 and 2006, an analysis of the interquartile range (IQR) of exposure to NO/NO_2, PM25, and O_3 in California and the USA revealed a 3–9%, 5–15%, and 6–12% relative increase in the likelihood of autism, respectively, throughout the entire pregnancy. These results confirm the initial findings but suggest relatively minor effects. These air pollutants primarily stem from traffic, and their association with autism is more pronounced in children born to women with lower socioeconomic status and education [17,19].

Organic Pollutants

Persistent organic pollutants are both discontinued and contemporary carbon-based substances that accumulate in the environment and human tissues. Various organic pollutants, including Polychlorinated Biphenyl (PCBs), 2,3,7,8-tetrachlorodibenzodioxin (TCDD), Perfluorinated compounds (PFCs), and Polybrominated Diphenyl Ethers (PBDEs) are used in industries and households, playing a role on the health of individual's directly or indirectly [81–83]. Phthalates and bisphenol A (BPA) are examples of non-persistent organic pollutants. High-molecular-weight phthalates function as plasticizers in polyvinyl chloride (PVC) plastics, flooring, and medical tubing, while low-molecular-weight phthalates are present in cosmetics, fragrances, and pharmaceuticals [84]. Though not permanent and rapidly eliminated from the body due to water solubility, exposure to these compounds is widespread and continuous, warranting investigation into their chronic impacts. BPA is found in plastics and resins used in food cans, receipts, toys, and medical equipment [84,85].

Humans constantly encounter low doses of common endocrine-disrupting chemicals (EDCs). Studies have revealed detectable levels of BPA in the urine of over 92% of Americans and 4-tetra-octyl phenol (tOP), which is commonly used in rubber tire and printer ink manufacturing, in over 57% of Americans. Medications like ethinyl estradiol, diethylstilbestrol (DES), and valproic acid (VPA) can also act as organic pollutants. Interestingly, VPA is recognized as a risk factor for autism. Its use during pregnancy has been associated with an increased risk of ASD, and DES, a now-discontinued drug used to prevent miscarriages, has been linked to daughters of mothers not receiving human chorionic gonadotropin (hCG), resulting in clear cell adenocarcinoma and other cervical abnormalities. Prenatal and perinatal exposure to administered EDCs may heighten autism risk by causing lasting effects on the nervous system and behavior through improper regulation or alteration of hormonal signaling pathways, given the crucial role of sex hormones in brain development. Early epidemiology studies suggest an elevated ASD risk linked to geographical or chronological exposure, particularly in areas with agricultural pesticide use, quantities of pesticides sprayed, and dispersion of hazardous air pollutants. Recent studies establish a connection between prenatal exposure to evaluated chemicals and autism risk in the offspring, involving biomonitoring of ADCs in bodily fluids from pregnant women, such as urine or serum [86,87].

Several cohort studies have explored the correlation between a mother's exposure to organophosphate pesticides (OPPs) and the development of autistic symptoms in her children, measuring various OPP metabolites during pregnancy. While some concurrent studies found no association between OPP exposure and autistic traits in adolescence, a particular study revealed that prenatal phosphate exposure was linked to increased autistic symptoms in toddlers. Furthermore, another study showed that prenatal exposure to drugs like chlorpyrifos and its metabolic byproduct chlorpyrifos oxon was associated with a higher likelihood of autistic features at age 11. These findings indicated

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that gender did not affect the relationship between prenatal vulnerability to OPP and the emergence of autistic traits in children and adolescents [10,87].

Heavy Metal

While heavy metal pollution is pervasive, toddlers are typically more susceptible to heavy metal poisoning compared to adults. Determining whether a child's early development involves heavy metal exposure can be challenging. However, there is mounting evidence suggesting that exposure to heavy metals contributes to the autistic phenotype [88]. The metals most frequently linked to autism prevalence and risk are arsenic, cadmium, lead, and mercury, which are sulfhydryl-reactive. Scientists discovered lower levels of sulfhydryl-reactive metals in autistic toddlers compared to control toddlers. This discovery raises the possibility that a particular subclass of autistic patients may be vulnerable to certain metals due to deficient excretory or detoxification systems. The risk may also increase with exposure to other metals. Researchers reported that urine excretion measurements of aluminum, antimony, lead, and mercury body burdens impacted the severity of autistic symptoms. Investigating the relationship between heavy metal exposure and the risk of developing autism is crucial to identifying the metals most frequently linked to this risk and the best biomarkers for risk assessment [75,89].

Infectious Factors

Ecological studies served as the foundation for early research to establish the correlation between prenatal virus exposure and autism incidence. Offspring face a higher risk of autism if their mothers experience a sickness requiring hospitalization during the first or second trimester. However, access to biobanks has enabled the evaluation of these assumptions using extensive analytical techniques. Presently, evidence indicates a heightened prenatal exposure risk for Toxoplasmosis gondii, rubella, and influenza as potential causes of autism. For type 2 of the herpes simplex virus, the evidence remains conflicting. No explicit research has investigated prenatal infection markers regarding autism risk [90].

The connection between illness and the progression of neurodevelopmental problems has spurred animal investigations on prenatal immune activation and brain development. It is plausible that detrimental pregnancy and birth-related issues indirectly affect the mother's brain through a similar maternal immune response rather than from an immediate effect of a specific infectious pathogen. Meta-analyses have examined the relationship between autism risk and pregnancy and birth complications (PBCs). Overall, there is substantial evidence that PBCs increase the likelihood of developing autism later in life. Specific exposures such as bleeding, gestational diabetes, rhesus, insufficient body weight, congenital deformity, reduced head circumference, uterine issues, hypoxia, and emergency cesarean delivery have been linked to a higher autism risk based on prospective population-based studies. The elevated risk of autism in children may emerge at any point during pregnancy, even after childbirth. Comprehensive meta-analysis research on autism risk, PBCs, and related topics have been summarized. Mothers experiencing blood loss during pregnancy were associated with an 81% higher risk of autism in their children, while gestational diabetes was linked to a twofold elevated concern. A 46% higher incidence of autism was consistent with any maternal treatment utilized during pregnancy. There is a positive correlation between the incidence of ASD and the use of mental diagnosis during pregnancy. Animal models are currently being used to better understand how administered drugs may increase the risk of autism. One theory posits that drugs alter the functioning of genes controlling neuronal function [91,92].

Other Factors

Another factor that could influence the development of ASD involves potential in-utero events. An additional discovery from the twin study highlights the importance of the fetus's prenatal environment for its development [93]. Twins exhibit a much greater compliance rate for autism than singletons when both grow in a similar chorion sac during pregnancy. Those who are genetically predisposed to ASD have a considerably higher prevalence or risk of passing it on. Pregnancy illnesses occurring in the second trimester, weight loss, and birthing complications are more prevalent during colder months. Biological variables can have an impact on the occurrence of autism or other mental diseases in individuals who are genetically predisposed to them. Due to genetic makeup, the developing brain may be more vulnerable to viruses during fetal transformation. A transient oxygen shortage or other obstetric delivery issues may be associated with one or more of these severely unfavorable episodes. These incidents may reveal excessively aberrant brain structure, shape, or tissue and could affect the human brain's chemistry and structure. The main categories of pregnancy complications, such as blood loss, diabetes, and rhesus factor incompatibility, atypical fetal transformation and maturation, carriage asphyxia, maternal contamination, among others, can be used to compare the modifications in ASD [92,94].

Conclusion and Future Prospective

In this review, we have examined modifiable risk factors contributing to the emergence of ASD, including genetic, epigenetic, and environmental risk factors. Epigenetic factors such as DNA methylation, histone deacetylation, and miRNA alter gene expression, thereby influencing the biological pathways responsible for brain development. These epigenetic changes also affect genetic, biological, metabolic, and environmental factors which lead to neuronal damage and synaptic disruption through oxida-

tive stress and neuronal toxicity. ASD can also result from mutations of various genes, including *SHANK2*, *CDLK56*, *NLGN3*, *NRXN1*, *PTEN*, and *MECP2*, that play a significant role in synapse development and function. Exposure to environmental factors such as pesticides, heavy metals, pollutants, prenatal nutrition, and infections during the gestational period affects brain development of the fetus. Together, the causative factors have complex interactions that result in changes in brain architecture and disruption of synaptic function.

According to recent research, the most significant factors associated with ASD involve immunization, maternal smoking, thimerosal exposure, and vaccination. Over the past few decades, the prevalence of autism has increased, highlighting the importance of prevention over treatment for all disorders. Researchers have primarily focused on specific elements related to autism, as avoiding these factors could potentially prevent the condition**.** While some pregnancy-related factors, such as maternal obesity, have demonstrated a weak association with ASD risk, birth complications linked to trauma, ischemia, or hypoxia have shown a stronger correlation with ASD.

Factors like maternal medication use during pregnancy might suggest confounding by indication. Although there has been limited attention to the benefits of omega-3 fatty acids in dietary component analyses, a widespread deficiency in vitamin D has been observed in toddlers with autism. While research on malignant features has generally been limited in scope, the evidence supporting the link between specific heavy metals and ASD warrants further investigation. Among modifiable risk factors for ASD, maternal infection stands out as the sole component showing some relationship risk. The biological mechanisms underlying modifiable risk factors for ASD may include non-causal associations, effects related to ASD-associated genes, oxidative stress, inflammation, maternal infection, neurotransmitter changes, and disruption in various signaling pathways. Future research on modifiable risk factors for ASD would benefit from illuminating non-ASD aspects, designing studies from different perspectives, accurately measuring vulnerability, and establishing a clear timeline of interventions concerning key expenditures.

Additionally, using a genetic implication framework should consider the dynamic interaction between genes and the environment. Consequently, numerous risk factors influence the development of ASD. Researchers may also explore the effectiveness of plant-derived bioactive molecules by targeting the various modifiable risk factors discussed above. Various alternative therapies such as mindfulness, behavioral, meditation, music, yoga, and exercise may be investigated to alter risk factors responsible for ASD.

Availability of Data and Materials

Not applicable.

Author Contributions

RS, AK, KS, VP, and SK conceptualized the idea and wrote the first draft of the manuscript. HJK and DP analyzed the data and revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final version of the manuscript. All authors have contributed sufficiently to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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