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Decoding the Genetic Landscape of Autism: A Comprehensive Review

Decoding the Autism Genetics

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Abstract

BACKGROUND

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition characterized by heterogeneous symptoms and genetic underpinnings. Recent advancements in genetic and epigenetic research have provided insights into the intricate mechanisms contributing to ASD, influencing both diagnosis and therapeutic strategies.

AIM

This review summarizes current knowledge on the genetic and epigenetic factors implicated in ASD, highlighting unresolved questions and emerging technologies. Key objectives include exploring the genetic architecture of ASD, elucidating mechanistic insights into genetic mutations, and examining gene-environment interactions.

METHODS

A comprehensive systematic review was conducted, integrating findings from studies on genetic variations, epigenetic mechanisms (such as DNA methylation and histone modifications), and emerging technologies (including CRISPR-Cas9 and single-cell

RNA sequencing). Relevant articles were identified through systematic searches of databases such as PubMed and Google Scholar.

RESULTS

Genetic studies have identified numerous risk genes and mutations associated with ASD, yet many cases remain unexplained by known factors, suggesting undiscovered genetic components. Mechanistic insights into how these genetic mutations impact neural development and brain connectivity are still evolving. Epigenetic modifications, particularly DNA methylation and non-coding RNAs, also play significant roles in ASD pathogenesis. Emerging technologies like CRISPR-Cas9 and advanced bioinformatics are advancing our understanding by enabling precise genetic editing and analysis of complex genomic data.

CONCLUSION

Continued research into the genetic and epigenetic underpinnings of ASD is crucial for developing personalized and effective treatments. Collaborative efforts integrating multidisciplinary expertise and international collaborations are essential to address the complexity of ASD and translate genetic discoveries into clinical practice. Addressing unresolved questions and ethical considerations surrounding genetic research will pave the way for improved diagnostic tools and targeted therapies, ultimately enhancing outcomes for individuals affected by ASD.

Key Words: Autism Spectrum Disorder; Genetics; Epigenetics; CRISPR-Cas9; Gene-environment interactions; Personalized medicine

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Core Tip: This review synthesizes current knowledge on the genetic and epigenetic factors contributing to Autism Spectrum Disorder (ASD). It highlights the complexity of ASD's genetic architecture and the role of epigenetic mechanisms such as DNA methylation and non-coding RNAs in disease pathogenesis. Emerging technologies like CRISPR-Cas9 and advanced bioinformatics are pivotal for advancing our understanding of ASD. Collaborative research efforts are crucial for integrating diverse disciplines and international data, aiming to translate genetic insights into personalized therapies. Addressing unresolved questions and ethical considerations will be essential for maximizing the clinical utility of genetic discoveries in improving outcomes for individuals with ASD.

INTRODUCTION

Autism Spectrum Disorder (ASD) is a multifaceted neurodevelopmental condition marked by a heterogeneous array of symptoms that can significantly influence social interaction, communication, and behavioral patterns[1]. The term "spectrum" underscores the extensive variability in the severity and manifestation of symptoms among affected individuals. The cardinal features of ASD encompass deficits in social communication, engagement in repetitive behaviors, and a restricted range of interests. Specifically, individuals with ASD experience challenges in both verbal and non-verbal communication, have difficulty interpreting social cues, and encounter obstacles in forming and sustaining relationships[2]. They also display repetitive activities, stereotyped movements, adherence to specific routines, and intense preoccupations with particular topics or activities (Figure 1). The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), delineates the diagnostic criteria for ASD, emphasizing persistent deficits in social communication and interaction coupled with restrictive and repetitive behavioral patterns, interests, or activities[3].

Globally, ASD is estimated to affect approximately 1 in 100 children, though prevalence rates exhibit considerable variation across different countries and studies. In the United States, data from the Centers for Disease Control and Prevention (CDC)

indicate that about 1 in 36 children were diagnosed with ASD as of 2020[4]. This rising prevalence is likely attributable to enhanced awareness, expanded diagnostic criteria, and improved diagnostic methodologies. Epidemiological data also reveal a marked gender disparity, with ASD being approximately four times more prevalent in boys than in girls[5]. Emerging research, however, suggests that females with ASD may be underdiagnosed or diagnosed later, possibly due to distinct symptomatology that differs from their male counterparts[6].

ASD has profound implications for both affected individuals and their families. Children with ASD frequently exhibit developmental delays in speech, language, and motor skills. These children often necessitate individualized educational programs and support services to achieve academic success[7]. Social interaction difficulties can lead to social isolation and challenges in forming and maintaining peer relationships. Additionally, individuals with ASD have higher incidences of co-occurring medical and psychiatric conditions, such as anxiety, depression, attention-deficit/hyperactivity disorder (ADHD), and other mental health disorders[8]. Adults with ASD face significant hurdles in securing and maintaining employment, with many experiencing underemployment or unemployment. A considerable number of adults with ASD require varying degrees of support to live independently, with some necessitating lifelong care. Families of individuals with ASD frequently endure high levels of stress, anxiety, and emotional strain[9]. The financial burden associated with therapies, medical care, special education, and supportive services can be substantial. Parents and caregivers often struggle to balance care responsibilities with professional and other family obligations, leading to reduced work hours or workforce withdrawal[10].

The economic impact of ASD is substantial, encompassing both direct costs, such as medical care and special education, and indirect costs, including lost productivity. In the United States, the lifetime cost of caring for an individual with ASD is estimated to range from $1.4 to $2.4 million[11]. The increasing prevalence of ASD imposes significant demands on healthcare systems to deliver diagnostic, therapeutic, and support services. Educational institutions require additional resources and specialized

staff to address the unique needs of students with ASD. Furthermore, heightened awareness of ASD has spurred policy changes and advocacy efforts to enhance service provision, fund research initiatives, and promote inclusive practices[12].

This review aims to synthesize current research findings on the genetics of autism spectrum disorder (ASD). This comprehensive systematic analysis seeks to elucidate the intricate interplay between genetic factors, environmental influences, and phenotypic heterogeneity in ASD. By examining the latest advances in genomic technologies, such as whole-genome sequencing and genome-wide association studies, the review aims to identify and understand the genetic risk factors associated with ASD. Additionally, it explores emerging insights into gene-environment interactions, epigenetic mechanisms, and the role of rare and de novo mutations in the etiology of autism. This synthesis of research findings is intended to inform future research directions and therapeutic strategies, contributing to a deeper understanding of the genetic architecture of ASD and ultimately aiding in developing more effective interventions and support mechanisms for individuals affected by this complex disorder.

MATERIALS AND METHODS

This review utilized a systematic approach to synthesize and analyze existing literature on the genetic underpinnings of ASD. A systematic literature search was conducted using multiple electronic databases, including PubMed, Scopus, Web of Science, and Google Scholar. The search spanned articles published till June 2024 to capture the most recent and relevant studies. Key search terms included "Autism Spectrum Disorder," "genetics," "whole-genome sequencing," "exome sequencing," "copy number variations," "epigenetics," "gene-environment interactions," "de novo mutations," and "genetic architecture." Boolean operators (AND, OR) were employed to refine and expand the search results. Specific inclusion and exclusion criteria were established to ensure the included studies' relevance and quality. Inclusion criteria ensured the studies were peer-reviewed, focused on human genetics in ASD, and published in English. We

excluded non-peer-reviewed articles, animal studies, unavailable full texts, or articles not published in English.

Relevant data, including study objectives, methodologies, key findings, and conclusions, were extracted from the selected articles. The extracted data were organized into thematic categories to facilitate a structured synthesis of the information. These categories included advances in genomic technologies (*e.g.,* whole-genome sequencing, exome sequencing), the role of copy number variations (CNVs), gene-environment interactions, epigenetic mechanisms, therapeutic implications and personalized medicine, current and future therapies, research challenges, and future directions.

The quality of the included studies was assessed using standardized criteria adapted from the Critical Appraisal Skills Programme (CASP) checklist. This evaluation considered factors such as study design, sample size, methodology robustness, and the relevance of findings to the research questions. Studies were rated as high, moderate, or low quality, and only high and moderate-quality studies were included in the final synthesis to ensure the reliability of the conclusions. As this study is a literature review, no primary data collection involving human subjects was conducted. Therefore, ethical approval was not required. However, ethical considerations were considered by ensuring the inclusion of ethically conducted studies and appropriately citing all sources.

RESULTS

The comprehensive systematic review identified 356 articles that met the inclusion criteria: 130 research articles, 202 review articles, 4 meta-analyses, 7 systematic reviews, 7 case reports, 2 editorials, and 4 commentaries (Figure 2). Our review of genetic research in ASD has elucidated key findings across several domains, highlighting the complexity and diversity of the disorder. Numerous risk genes have been identified, including CHD8, SHANK3, and MECP2, which play critical roles in synaptic function, neural development, and chromatin remodeling. Notably, studies have shown aberrant

DNA methylation patterns, such as hypermethylation of the MECP2 gene, linked to environmental influences like prenatal stress and toxins, contributing to neural anomalies in ASD.[24] Histone modifications, including changes in acetylation and methylation states, disrupt chromatin structure and gene expression, particularly in genes associated with neural connectivity and plasticity. Additionally, dysregulation of non-coding RNAs, such as microRNAs, impacts the regulation of genes crucial for neural development and synaptic function, further contributing to ASD pathology.

These genetic insights are revolutionizing therapeutic strategies, shifting towards personalized medicine approaches. Tailoring behavioral interventions like Applied Behavior Analysis (ABA) and social skills training based on genetic profiles can address specific deficits more effectively. Pharmacological treatments, including antipsychotics and selective serotonin reuptake inhibitors (SSRIs), are increasingly guided by genetic profiling to enhance efficacy and minimize side effects. Nutritional interventions, such as gluten-free and casein-free diets, are also being personalized based on genetic predispositions related to metabolic processes, improving gastrointestinal and behavioral symptoms in individuals with ASD.

Future therapies are on the horizon, driven by genetic and epigenetic research advancements. Gene therapy targeting specific genetic abnormalities, such as CHD8 or SHANK3 mutations, holds promise for correcting or mitigating these genetic disruptions. Epigenetic therapies, including DNA methylation modulators and histone deacetylase inhibitors, aim to reverse abnormal chromatin states and restore normal gene expression patterns. Emerging technologies like CRISPR-Cas9 enable precise genetic modifications to study and potentially correct mutations, while advanced bioinformatics tools analyze extensive genomic data to identify novel genetic variants and risk genes. Single-cell RNA sequencing (scRNA-seq) provides insights into cellular heterogeneity in the brain, and brain organoids offer a model for studying the impact of genetic and environmental factors on neural development.

Collaborative research is crucial for advancing our understanding and treatment of ASD. Multidisciplinary collaborations integrate expertise from genetics, neuroscience,

psychology, bioinformatics, and clinical medicine, enhancing research outcomes and facilitating the translation of findings into clinical practice. International collaborations and pooling resources and data from diverse populations enhance studies' statistical power and generalizability. Addressing ethical and practical considerations, such as the accessibility and privacy of genetic testing, is essential for integrating genetic insights into routine clinical care.

DISCUSSION

Overview of ASD:

ASD is characterized by various symptoms affecting social interactions, communication, and behavior. Patients with ASD have verbal and nonverbal communication challenges, including difficulties in spoken language and nonverbal cues such as eye contact, facial expressions, and gestures[13]. They also have social reciprocity challenges, including difficulty engaging in reciprocal social interactions, sharing interests and emotions, and initiating or responding to social overtures. In addition, they have trouble developing, maintaining, and understanding relationships, including challenges in adapting behavior to suit various social contexts and forming friendships[14].

Patients with autism commonly suffer from repetitive behaviors and restricted interests. They engage in repetitive actions such as hand-flapping, rocking, or spinning. Additionally, they suffer from insistence on sameness, inflexible adherence to routines, or ritualized verbal or nonverbal behavior patterns[15]. They also have highly restricted, fixated interests with an intense focus on specific topics or objects, often abnormal in intensity or focus. They also have sensory sensitivities with over-reactivity to sensory input, such as an unusual interest in sensory aspects of the environment (*e.g.*, lights, sounds, textures)[16].

The DSM-5 outlines specific criteria for diagnosing ASD, divided into two main categories. The first category involves persistent deficits in social communication and social interaction. These deficits manifest in various ways: Social-emotional reciprocity

issues, such as an abnormal social approach, failure of normal back-and-forth conversation, reduced sharing of interests or emotions, and failure to initiate or respond to social interactions[17]. There are also deficits in nonverbal communicative behaviors used for social interaction, including poorly integrated verbal and nonverbal communication, abnormalities in eye contact and body language, difficulties in understanding and using gestures, and a lack of facial expressions and nonverbal communication[18]. Lastly, there are deficits in developing, maintaining, and understanding relationships, including difficulty adjusting behavior to suit different social contexts, challenges in sharing imaginative play or making friends, and a general absence of interest in peers[19].

In addition to the core diagnostic criteria, the DSM-5 specifies that symptoms of ASD must be present in the early developmental period. However, they may not become fully apparent until social demands exceed the individual's limited capacities or may be masked by learned strategies later in life[20]. Furthermore, these symptoms must cause clinically significant impairment in social, occupational, or other important areas of current functioning. The disturbances observed should not be better explained by intellectual disability or global developmental delay. While intellectual disability and ASD often co-occur, a comorbid diagnosis requires that the social communication impairments be disproportionate to the general developmental level[21].

Overview and Phenotypic Heterogeneity of Autism:

ASD is marked by symptoms affecting social interactions, communication, and behavior. Patients face verbal and nonverbal communication challenges, including difficulties with spoken language, eye contact, facial expressions, and gestures. They struggle with social reciprocity, engaging in reciprocal interactions, sharing interests and emotions, and responding to social overtures. Additionally, they struggle to form and maintain relationships, adapt behavior to various social contexts, and form friendships[13-14]. Repetitive behaviors and restricted interests are common in autism. Patients engage in repetitive actions like hand-flapping and insist on sameness,

adhering inflexibly to routines. They often have highly focused, intense interests in specific topics or objects and exhibit sensory sensitivities, reacting strongly to sensory inputs like lights, sounds, and textures[15,16].

The DSM-5 outlines two main diagnostic criteria categories for ASD: Deficits in social communication and social interaction and restricted repetitive behaviors and interests[17-19]. Social communication deficits include social-emotional reciprocity, nonverbal communication, and relationship development and maintenance[17-19]. Symptoms must be present early in development, cause significant impairment in functioning, and not be better explained by intellectual disability or global developmental delay. Intellectual disability and ASD often co-occur, but a comorbid diagnosis requires that social communication impairments exceed general developmental levels[20,21].

ASD isn't a one-size-fits-all condition. ASD is renowned for its phenotypic heterogeneity, which refers to the wide variability in the manifestation of symptoms and traits among individuals with the disorder. Variations in core symptoms, abilities, and motor skills, along with factors like sex and co-existing conditions, create a spectrum of experiences[22]. This heterogeneity encompasses a broad range of social, communicative, and behavioral challenges, making everyone's experience with autism unique. This phenotypic variability is due to the complex interplay of genetic, environmental, and biological factors[23]. Genetic factors include de novo mutations, inherited genetic variants, copy number variations (CNVs), and epigenetic modifications. Environmental factors encompass prenatal exposures, perinatal events, and postnatal influences such as early childhood experiences and exposure to toxins[24]. Biological factors involve improper synaptic morphology and function, differences in brain structure and function, and imbalances in neurotransmitter systems. Co-occurring conditions, such as intellectual disability and other neurodevelopmental and psychiatric disorders, also contribute to variability in symptom presentation and severity[25]. Gender differences also play a role in phenotypic variability in patients with autism. Self-injurious behavior is more frequent in women than men with autism.

Males with autism are more likely to have more severe language difficulties than females with autism[26].

Developmental trajectories, including the age of onset and progression of symptoms, along with the development of adaptive skills, further influence the heterogeneity of ASD[27]. Additionally, gene-environment interactions and epigenetic changes play crucial roles in shaping the manifestation of ASD traits. This clinical heterogeneity imposes a substantial challenge to the proper diagnosis and management of patients with autism. It also may reflect the genetic variability present in patients with ASD[28]. Individuals with ASD exhibit diverse levels of difficulty in engaging in reciprocal social interactions. Some may struggle with basic social conventions like making eye contact or recognizing social cues, while others might struggle to share interests or emotions. These challenges can include difficulties in initiating and sustaining conversations, understanding jokes or sarcasm, and recognizing social cues[29]. The ability to form and maintain relationships varies greatly. Some individuals may have profound difficulties in making and keeping friends, while others might develop meaningful relationships but find navigating the nuances of social interactions challenging. Problems with social reciprocity and forming relationships can lead to social isolation and challenges in peer interactions[30].

There is significant variability in verbal communication abilities among those with ASD. Some individuals may be nonverbal or have limited speech, while others may have extensive vocabulary but struggle with pragmatic language use, such as understanding idioms and metaphors or engaging in back-and-forth conversations[31]. Difficulties with nonverbal communication are common and can include challenges in understanding and using body language, facial expressions, and gestures. This can affect the ability to interpret others' emotions and intentions, leading to miscommunications and social misunderstandings. Patients with autism show variable behavioral challenges[32]. These behaviors manifest in various forms, including repetitive movements (*e.g.,* hand-flapping, rocking), ritualistic behaviors (*e.g.,* strict adherence to routines), and intense focus on specific interests (*e.g.,* deep knowledge

about a particular subject). The intensity and nature of these behaviors can vary widely among individuals. These behaviors can be coping mechanisms or ways to express excitement, stress, or other emotions. While some individuals may exhibit mild repetitive behaviors, others may have behaviors that significantly interfere with daily activities[33].

Many individuals with ASD have atypical responses to sensory stimuli. This can include hyper-reactivity (*e.g.*, being overwhelmed by loud noises or bright lights) or hypo-reactivity (*e.g.*, seeming indifferent to pain or extreme temperatures). These sensory issues can significantly impact daily functioning and quality of life. Many individuals with ASD have a strong preference for routine and predictability[34]. Changes in routine can cause significant distress and anxiety. Higher rates of co-occurring conditions such as anxiety, depression, ADHD, epilepsy, and gastrointestinal issues are common in individuals with ASD. These conditions can compound the challenges faced by individuals with autism, adding to the complexity of managing their overall health and well-being[35].

Studying the phenotypic heterogeneity of ASD and its biological basis is crucial for several reasons. It helps to identify and understand genetic risk factors that specify specific pathways and mechanisms underlying the disorder's behavioral deficits. It also improves diagnostic accuracy by assisting clinicians to recognize and diagnose the disorder more precisely, thus ensuring that individuals receive the appropriate support and interventions[36]. This understanding enables the development of personalized treatment plans tailored to the unique social, communicative, and behavioral challenges faced by each individual with autism. Enhanced knowledge of phenotypic diversity also leads to better support systems in educational and workplace settings, creating more inclusive environments. Understanding this heterogeneity provides essential information for families and caregivers for practical support and advocacy, helping them manage daily challenges and set realistic expectations[37]. Additionally, research into the phenotypic variability of ASD informs public health policies and funding priorities, ensuring resources are allocated effectively to meet the diverse needs of the

autism community. This research also provides insights into the underlying biological, genetic, and environmental factors contributing to ASD, potentially leading to early diagnosis and new therapeutic targets. Moreover, increasing awareness of ASD's diverse manifestations helps reduce stigma, fostering greater acceptance and inclusion in society[38].

Genetic Architecture of Autism:

The genetic architecture of ASD is highly complex, with more than 100 genes implicated in its pathogenesis. ASD is considered a highly polygenic disorder, which is influenced by multiple genes, each contributing a small effect. The combined effect of many genetic variants, both common and rare, creates a cumulative risk of developing ASD[39]. This polygenic risk model helps explain the broad spectrum of ASD phenotypes and the variability in symptom severity and presentation. Genetic susceptibility to ASD refers to the increased likelihood of developing the disorder based on an individual's genetic makeup[40]. This susceptibility is influenced by various genetic factors (Figure 3), including common genetic variants with small effects, rare genetic variants with large effects, de novo mutations, and copy number variations (CNVs) augmented with the gene-environment interactions and epigenetic factors effects, which can contribute to the risk of ASD either individually or through complex interactions[41].

Common Genetic Variants:

These genetic variants are relatively frequent variations in the DNA sequence frequently found in the general population and contribute modestly to the risk of developing ASD. Each common variant typically has a small effect on ASD risk, but collectively, they can significantly contribute to genetic susceptibility[42]. These variants are often single nucleotide polymorphisms (SNPs) identified through genome-wide association studies (GWAS). These studies involve scanning the genomes of many individuals to find genetic markers associated with ASD[43]. These studies have identified several SNPs that are more common in individuals with autism compared to

those without the disorder. In addition, risk alleles are specific versions of genetic variants that increase the likelihood of developing ASD. While each risk allele contributes only slightly to ASD risk, the presence of multiple risk alleles can have a substantial impact[44].

A polygenic score, also known as a polygenic risk score (PRS), is a measure that aggregates the effects of many genetic variants to estimate an individual's genetic predisposition to a particular trait or disorder, such as ASD. This score is calculated based on the presence of numerous common genetic variants, each contributing a small effect to the overall risk[45]. The polygenic score is derived from GWAS, which identifies single nucleotide polymorphisms (SNPs) associated with ASD. Each SNP is assigned a weight based on its association with ASD, and the polygenic score is the sum of these weighted SNPs present in an individual's genome. The polygenic score estimates how frequent genetic variations in the population contribute to the development of autism[46]. A higher polygenic score indicates a greater genetic predisposition to ASD and a higher probability of being diagnosed with autism. However, it is important to note that a polygenic score is not deterministic; it only provides a risk estimate based on genetic factors[47]. Environmental influences and gene-environment interactions also play significant roles in the development of ASD. Polygenic scores can be used in research to understand the genetic architecture of ASD and to identify individuals at higher genetic risk[48]. These scores might eventually aid in early detection and personalized intervention strategies in clinical settings, although their use in routine clinical practice is still under research and debate. Individuals can be stratified based on their polygenic scores into different risk categories. This stratification can help identify those who might benefit from more intensive monitoring or early intervention[49]. Several common variants have been associated with ASD, though each variant's individual contribution to the disorder is small.

Rare Genetic Variants:

The genetic architecture of ASD is highly heterogeneous, involving a combination of common and rare genetic variants. While common variants each contribute a small effect size, rare genetic variants can have a substantial impact on the risk of developing ASD[39]. These rare variants often exhibit high penetrance, meaning they significantly increase the risk of ASD in carriers. Examples of such mutations include those found in genes like CHD8, SHANK3, and MECP2[50]. These mutations are frequently associated with severe forms of ASD and comorbid conditions such as intellectual disability, epilepsy, and specific syndromic features. The presence of rare genetic variants contributes to the phenotypic variability seen in ASD. Different mutations can lead to diverse clinical presentations, even within the same gene. For instance, mutations in SHANK3 can result in a range of phenotypes from severe intellectual disability to milder social deficits[51]. Additionally, rare variants can interact with environmental factors, influencing the onset and severity of ASD symptoms. This highlights the importance of considering both genetic predisposition and environmental exposures in understanding ASD[52].

Many rare genetic variants implicated in ASD affect synaptic proteins, which are crucial for synapse formation, function, and plasticity[53]. Genes like SHANK3, NRXN1, and NLGN4 are essential in synaptic signaling and neuronal connectivity. Genetic variations such as CHD8 and MECP2 affect chromatin remodeling and gene expression regulation, which are critical for proper neuronal development and function[54]. Rare variants also impact neurotransmitter systems, including glutamatergic, GABAergic, dopaminergic, and serotonergic pathways, which are vital for maintaining the balance of excitatory and inhibitory signals in the brain[55]. Advances in sequencing technologies have facilitated the identification of rare genetic variants in individuals with ASD. Whole-exome sequencing (WES) and whole-genome sequencing (WGS) are instrumental in discovering novel mutations and understanding their functional consequences[56]. Experimental approaches, including animal models and cellular assays, are used to characterize the functional impact of rare variants. These

studies help elucidate the biological pathways affected by these mutations and their role in ASD pathology[57].

The identification of rare genetic variants can inform genetic testing and diagnosis, enabling personalized approaches to managing ASD. Genetic counseling can also provide valuable insights to families regarding the inheritance patterns and risks of ASD[58]. Understanding the specific genetic and molecular mechanisms underlying ASD can lead to the development of targeted therapies. For example, drugs modulating synaptic function or neurotransmitter systems may offer potential treatment options for individuals with specific genetic mutations[59].

De Novo Mutations:

De novo mutations (DNMs) are mutations found in the genomes of autistic or non-autistic children but not in the genomes of their parents. DNMs are new genetic changes that arise spontaneously in the germ cells of parents or early in embryonic development and are not inherited from either parent. They play a significant role among the genetic contributors to ASD[60]. DNMs are particularly important in the context of ASD for several reasons. They are often found in genes critical for brain development and function. Studies have shown that individuals with ASD have a higher burden of DNMs compared to their unaffected siblings. These mutations can occur in coding regions of the genome, disrupting the structure and function of proteins involved in neural development, synaptic signaling, and other key processes[61,62]. One of the key aspects of DNMs is their high impact on the risk of developing ASD. Unlike common variants that contribute small incremental risks, DNMs can have large effects[63]. For instance, mutations in genes such as CHD8, SCN2A, and SYNGAP1 have been identified as high-confidence ASD risk genes due to the significant disruptions they cause in neurodevelopmental processes. These genes are involved in critical pathways, including chromatin remodeling, synaptic function, and ion channel activity, highlighting the diverse biological mechanisms through which DNMs can influence ASD[64].

The identification of DNMs has been facilitated by advances in next-generation sequencing technologies, such as whole-exome sequencing (WES) and whole-genome sequencing (WGS)[65]. These technologies allow for the comprehensive analysis of the entire genome coding sequence, uncovering rare and potentially pathogenic mutations that may not be detected through traditional genetic testing methods[66]. Research has identified hundreds of genes that harbor DNMs associated with ASD, underscoring the genetic complexity of the disorder. In terms of phenotypic consequences, DNMs are often associated with more severe forms of ASD[67]. Children with DNMs may exhibit profound intellectual disabilities, severe communication deficits, and a higher incidence of comorbid conditions such as epilepsy. This contrasts with ASD cases linked to common genetic variants, which may present with milder symptoms[68].

Furthermore, DNMs provide insights into the genetic architecture of sporadic ASD cases—those without a family history of the disorder. Since these mutations are new and not inherited, they can explain the occurrence of ASD in families with no previous instances, helping to understand the sporadic nature of many ASD cases[69]. This also has implications for genetic counseling, as the recurrence risk of ASD due to DNMs in subsequent pregnancies is generally low, although slightly elevated compared to the general population. Understanding the role of DNMs in ASD has significant implications for both research and clinical practice. It highlights the importance of early genetic screening and diagnosis, which can lead to timely interventions and personalized treatment strategies[70]. Additionally, ongoing research into the functional impact of specific DNMs can identify potential therapeutic targets, paving the way for developing novel treatments to mitigate the effects of these genetic alterations[71].

Some of the well-studied Genetic Mutations observed in Patients with ASD:
This section highlights some of the well-studied genetic mutations observed in patients with ASD. Table 1 shows some notable loci, including CHD8, CNTNAP2, and

SHANK3. Figure 4 shows some of the important genes liable for mutations in ASD: Their functions and localization of representative molecules.

The CHD8 (Chromodomain Helicase DNA binding protein 8, a vital chromatin regulator enzyme during fetal development) *gene* is a significant genetic factor associated with ASD, located at chromosome 14q11.2, and encoding a protein crucial for chromatin remodeling and gene expression regulation[72]. CHD8 influences neurodevelopment, neuronal differentiation, and synaptic function, and its disruption can lead to widespread effects on brain structure and function pertinent to ASD. De novo mutations in CHD8, which arise spontaneously and are not inherited, are strongly linked to ASD[25]. These mutations often result in macrocephaly, gastrointestinal issues, specific facial dysmorphisms, and core ASD symptoms such as deficits in social communication and repetitive behaviors[73]. Additionally, CHD8 mutations are associated with intellectual disability or cognitive impairments, particularly in language and motor skills. Studies have shown that CHD8 regulates a large network of genes implicated in ASD, and its mutations can disrupt multiple neurodevelopmental pathways[74]. Animal models with CHD8 mutations exhibit similar features to humans, providing insights into the mechanisms contributing to ASD. Research also explores gene-environment interactions involving CHD8 to identify intervention periods and therapeutic targets[75]. Potential therapies could involve modulating disrupted pathways, emphasizing the importance of genetic testing and personalized medicine. Understanding CHD8 mutations helps tailor interventions and support services to individual needs, advancing the development of targeted therapies to mitigate the impact of these genetic mutations.

The CNTNAP2 (Contactin-associated protein 2) gene variant is a significant genetic factor implicated in ASD, playing a crucial role in the development and function of the nervous system, particularly in cortical areas related to language, cognition, and social behavior[76]. The CNTNAP2 gene is the largest gene in the human genome, located at chromosome 7q35, and one of the few known human genes essential for language development. CNTNAP2 encodes a protein involved in brain cell adhesion and

neuronal communication, which is essential for synapse formation, nerve signal transmission, interactions between neurons and between neurons and glial cells, and the clustering of potassium channels[77]. Variants in CNTNAP2, including both common and rare mutations, have been linked to ASD and associated with core symptoms such as social communication deficits, repetitive behaviors, language delays, and intellectual disability[78]. Gandhi *et al.* showed that disruptions in CNTNAP2 Lead to altered neural connectivity and signaling, contributing to ASD pathogenesis. Animal models with CNTNAP2 mutations exhibit behaviors analogous to human ASD, providing valuable insights[79]. Studies also explore how CNTNAP2 variants interact with environmental factors, which could identify critical intervention periods and therapeutic targets[80]. These findings underscore the importance of genetic testing and personalized medicine, as understanding CNTNAP2 mutations allows for tailored interventions and support mechanisms, advancing the development of more effective treatments for individuals with ASD.

Several large-scale genomic studies have established a strong association between ASD and mutations in the SHANK family of genes, specifically SHANK1, SHANK2, and SHANK3[81]. These genes encode a family of postsynaptic scaffolding proteins that are critical for the proper functioning of glutamatergic synapses in the central nervous system (CNS). The SHANK proteins play pivotal roles in synaptic structure and signaling, ensuring the stability and efficiency of synaptic connections, which are essential for learning, memory, and overall cognitive function[82].

Mutations *in the SHANK1 gene* have been linked to ASD, particularly in males. SHANK1 encodes a protein contributing to glutamatergic synapses' postsynaptic density (PSD), influencing synaptic strength and plasticity. Individuals with SHANK1 mutations may exhibit milder ASD symptoms compared to mutations in SHANK2 and SHANK3, but they still present with notable social communication deficits and repetitive behaviors[83]. Mutations in the *SHANK2 gene* are associated with a range of neurodevelopmental disorders, including ASD. The SHANK2 protein plays a role in synaptic signaling and the structural integrity of synapses. Variants in SHANK2 can

lead to significant disruptions in synaptic function, resulting in the core symptoms of ASD, such as social communication challenges, repetitive behaviors, and sometimes intellectual disability[84]. Studies have shown that both inherited and de novo mutations in SHANK2 can contribute to the development of ASD[85].

The SHANK3 gene is a significant genetic factor associated with ASD, encoding a protein crucial for the development and function of synapses, which are essential for neuronal communication. This protein, also known as ProSAP2, acts as a scaffolding protein in the postsynaptic density of excitatory synapses, ensuring synaptic connections' structural and functional integrity[86]. Mutations in SHANK3 are strongly linked to ASD, particularly in cases related to Phelan-McDermid syndrome, which results from deletions or mutations on chromosome 22q13, where SHANK3 is located[87]. Individuals with SHANK3 mutations often exhibit severe expressive language delays, intellectual disability, and significant social communication and behavioral deficits, hallmark features of ASD. These mutations, which can be de novo or inherited, include point mutations, deletions, and duplications that disrupt SHANK3 protein function[88]. Zhou *et al*. indicate that SHANK3 mutations lead to synaptic dysfunction, impairing neuronal communication and resulting in the neurological and behavioral manifestations observed in ASD[89]. Animal models with SHANK3 mutations exhibit behaviors similar to human ASD symptoms, such as social deficits, repetitive behaviors, and anxiety, offering valuable insights for studying underlying mechanisms and potential interventions[90]. The role of SHANK3 in synaptic function makes it a critical target for therapeutic research, with efforts underway to develop treatments that can compensate for the loss of SHANK3 function or restore its normal activity through gene therapy, small molecule drugs, and other innovative approaches[91]. Understanding SHANK3 mutations in ASD not only aids in diagnosing and managing individuals with these specific genetic alterations but also provides broader insights into the synaptic and neurobiological mechanisms underlying ASD, essential for developing targeted therapies to improve the quality of life for those affected.

The *AVPR1A gene*, encoding the arginine vasopressin receptor 1A, has been implicated in ASD due to its critical role in regulating social behavior. This receptor, part of the vasopressin-oxytocin signaling system, influences social bonding, communication, and various social behaviors by initiating signaling cascades essential for neuronal activity and synaptic plasticity[92]. Studies have identified several polymorphisms, such as RS3 in the AVPR1A gene, linked to ASD, as well as rare copy number variations (CNVs) affecting gene dosage and receptor function[93]. Variations in AVPR1A can lead to significant differences in social behavior, including deficits in social recognition, reduced ability to form social bonds, and impaired communication, which are core aspects of ASD[94]. Animal models have shown that altered AVPR1A expression results in notable changes in social interaction, providing insights into similar human behaviors[95]. Clinical studies have associated specific AVPR1A alleles with social cognition deficits and repetitive behaviors, highlighting the gene's role in ASD susceptibility[96]. Understanding AVPR1A's impact on ASD opens potential therapeutic avenues targeting the vasopressin-oxytocin pathway and suggests that genetic variants in AVPR1A could serve as biomarkers for personalized interventions, enhancing social functioning in individuals with autism.

OXTR gene mutations have been scrutinized for their potential involvement in ASD, given the pivotal role of oxytocin in social behavior and bonding, processes often disrupted in individuals with ASD. The OXTR gene on chromosome 3 encodes the oxytocin receptor protein primarily expressed in brain regions crucial for social cognition, emotional regulation, and reward processing[97]. Various mutations in OXTR, including single nucleotide polymorphisms (SNPs), insertions, deletions, and structural variants, can alter oxytocin receptor function. These mutations may impair oxytocin binding affinity and downstream signaling pathways, influencing social behavior and communication skills relevant to ASD[98]. While specific OXTR mutations have not been conclusively linked to ASD, genetic studies suggest associations between OXTR variations and susceptibility to social deficits characteristic of ASD. Regulatory

variations in OXTR, affecting gene expression levels in brain regions critical for social functioning, further contribute to ASD risk[99].

Dopamine receptors **D1 (DRD1) and D2 (DRD2**) are integral components of the dopamine system, crucial for regulating various neurological functions such as reward processing, motivation, and motor control. Mutations or variations in the genes encoding these receptors have been implicated in ASD, suggesting a role in the disorder's neurobiological underpinnings[100]. DRD1, the predominant dopamine receptor in the CNS, influences neuronal growth, cognitive flexibility, and social behaviors, all of which are commonly impaired in individuals with ASD. Genetic variants affecting DRD1 expression or function may disrupt dopaminergic pathways, contributing to ASD symptoms like repetitive behaviors and social deficits[101]. Similarly, DRD2, involved in modulating synaptic transmission and reward mechanisms, has also been linked to ASD. Variants in the DRD2 gene may alter dopamine signaling, impacting neural circuits crucial for social interaction and communication[102]. Animal studies support these findings, demonstrating that changes in DRD1 and DRD2 expression can lead to behaviors resembling those seen in ASD, providing insights into potential therapeutic strategies targeting dopamine receptors to mitigate symptoms associated with autism[103].

The **DRD3 gene** encodes the dopamine receptor D3, which is crucial in modulating neural circuits related to movement, emotion, and cognition. Variants in the DRD3 gene have been explored for their association with ASD due to dopamine's significant role in social behavior, reward processing, and executive function[104]. Specific polymorphisms, such as the Ser9Gly variant, and copy number variations (CNVs) in DRD3 can affect receptor function and dopamine signaling, potentially contributing to ASD's behavioral and cognitive features. Alterations in dopamine signaling through DRD3 receptors can influence social behaviors, affecting social motivation, reward processing, and social interactions[105]. Animal studies have shown that manipulating DRD3 expression impacts social behavior, repetitive behaviors, and cognitive flexibility relevant to ASD symptoms[106]. Human studies have found mixed results regarding

the association between DRD3 variants and ASD, with some suggesting a link to increased risk of autism and specific traits like repetitive behaviors and social communication difficulties[107].

The *MECP2 gene* encodes the methyl-CpG-binding protein 2, a critical regulator of gene expression in the brain, playing a crucial role in neuronal development and synaptic plasticity. Mutations in MECP2 are primarily associated with Rett syndrome, a severe neurodevelopmental disorder, but are also implicated in some cases of ASD. These mutations can include missense, nonsense, insertions, deletions, and duplications, leading to a loss or abnormal function of the MECP2 protein[108,109]. Clinically, individuals with MECP2 mutations may exhibit impaired social interaction, communication difficulties, and repetitive behaviors, overlapping with core ASD features. The phenotypic effects of MECP2 mutations can vary widely, contributing to a spectrum of neurodevelopmental conditions[110]. Research has identified MECP2 mutations in a subset of individuals with ASD, highlighting the gene's contribution to the genetic heterogeneity of autism[111]. Animal models with MECP2 mutations exhibit similar neurological and behavioral traits to humans, providing insights into these disorders' underlying mechanisms[112].

Mutations in *NRXN1 and NRXN2* **genes**, which encode neurexins, are implicated in ASD and other neurodevelopmental disorders. Neurexins are presynaptic cell adhesion molecules crucial for synapse formation, synaptic transmission, and neural circuit development. NRXN1 mutations include deletions, duplications, and point mutations, often resulting in a loss of function[113]. Individuals with NRXN1 mutations frequently exhibit ASD features such as social communication deficits, repetitive behaviors, and restricted interests. These mutations are also linked to intellectual disability, schizophrenia, and epilepsy. Although NRXN1 deletions are relatively rare, they are significant genetic risk factors for ASD[114]. NRXN2 mutations, involving similar types of genetic alterations, are associated with social communication difficulties, cognitive impairments, and repetitive behaviors, also contributing to the genetic heterogeneity of ASD. Both NRXN1 and NRXN2 mutations impair synaptic transmission and plasticity,

leading to deficits in learning, memory, and behavior[115]. Research on these genes underscores their importance in synaptic function and highlights potential therapeutic targets. Large-scale genomic studies and animal models have further elucidated the impact of these mutations, suggesting that restoring normal synaptic function could mitigate the effects of these genetic disruptions and improve outcomes for individuals with ASD[116].

Mutations in the **NLGN4X and NLGN4Y genes**, which encode neuroligin-4 proteins crucial for synaptic function, have been implicated in ASD. NLGN4X, located on the X chromosome, and NLGN4Y, its Y chromosome homolog, play essential roles in synaptic organization and neurotransmission[117]. Mutations in NLGN4X, including missense mutations and deletions, disrupt synaptic connectivity and function, contributing to ASD features such as impaired social communication, repetitive behaviors, and intellectual disability[118]. These mutations are primarily inherited in an X-linked recessive manner, affecting males more severely due to the lack of NLGN4Y compensation on the Y chromosome. NLGN4Y mutations, although less studied, similarly affect synaptic function and may contribute to ASD and other neurodevelopmental disorders in males[119].

PTCHD1 (Patched domain-containing protein 1) **gene** mutations have garnered attention in ASD research due to their implications for neurodevelopmental processes. Located on the X chromosome, the PTCHD1 gene encodes a protein crucial for brain neuronal development and synaptic function[120]. Various mutations in PTCHD1, including deletions, duplications, and missense mutations, disrupt its normal function, potentially affecting synaptic plasticity and neurotransmitter signaling pathways. These disruptions are implicated in the pathophysiology of ASD, contributing to deficits in social communication, repetitive behaviors, and cognitive flexibility observed in affected individuals[121]. PTCHD1 mutations are more prevalent in males due to their X-linked inheritance pattern. Research into PTCHD1 aims to elucidate how these mutations alter neuronal connectivity and synaptic transmission, providing insights into ASD's genetic mechanisms[122].

SLC6A4 (also known as the serotonin transporter) *gene* plays a critical role in regulating serotonin levels in the brain by facilitating serotonin reuptake from the synaptic cleft back into presynaptic neurons[123]. Serotonin is a neurotransmitter involved in various physiological processes, including mood regulation, emotional processing, and social behavior, all of which are implicated in ASD[124]. Mutations in SLC6A4, encompassing various forms such as single nucleotide polymorphisms (SNPs) and structural variants, can impact serotonin transporter function and serotonin reuptake efficiency. These genetic alterations may disrupt serotonin signaling pathways, influencing neuronal development, synaptic plasticity, and the formation of neural circuits essential for social cognition and emotional processing[125]. While specific SLC6A4 mutations have not been definitively linked to ASD, variations in the gene have been associated with alterations in social behavior, repetitive behaviors, and sensory processing observed in individuals with ASD[126].

DLG4 gene (also known as Discs Large Homolog 4 or PSD-95) is a critical gene involved in synaptic function and neuronal development. It encodes a scaffolding protein essential for organizing and stabilizing neurotransmitter receptors and signaling proteins at excitatory synapses, particularly those involved in glutamatergic transmission in the CNS[127]. Mutations in DLG4 can disrupt its function, affecting synaptic plasticity, neurotransmitter signaling, and neuronal circuitry, potentially contributing to the cognitive and behavioral symptoms observed in individuals with ASD[128]. These genetic changes, which may include missense mutations, deletions, or other structural variants, can lead to deficits in synaptic plasticity and an altered excitatory/inhibitory balance in the brain, associated with impairments in social communication, repetitive behaviors, and sensory processing in ASD[129]. Research into DLG4 underscores the importance of synaptic proteins in ASD pathophysiology, providing insights into the molecular mechanisms underlying synaptic dysfunction and neuronal connectivity deficits[130].

The GABRG2 gene, which encodes the gamma-2 subunit of the GABA(A) receptor, is critical in mediating inhibitory neurotransmission in the central nervous system.

Mutations in GABRG2 have been implicated in several neurological and developmental disorders, including ASD. GABA(A) receptors are pivotal in maintaining the balance between excitation and inhibition in the brain, which is essential for normal cognitive and behavioral functions[131]. In the context of autism, mutations in GABRG2 can disrupt the function of GABA(A) receptors, leading to an imbalance in synaptic excitation and inhibition. This disruption can contribute to the core symptoms of ASD, such as social communication difficulties, repetitive behaviors, and restricted interests. Individuals with GABRG2 mutations may exhibit a range of phenotypic manifestations, reflecting the gene's role in the broader neurodevelopmental landscape[132]. Studies have shown that GABRG2 mutations can result in altered receptor function, affecting synaptic transmission and plasticity and leading to hyperexcitability of neural circuits, which is often observed in individuals with ASD[133]. Additionally, GABRG2 mutations have been linked to co-occurring conditions frequently seen in autism, such as epilepsy, intellectual disability, and anxiety disorders, further complicating the clinical presentation and management of ASD[134].

GRM7 gene, which encodes the metabotropic glutamate receptor 7 (mGluR7), modulates synaptic transmission and plasticity in the brain. Mutations or variations in GRM7 have been associated with ASD, highlighting the gene's significance in neurodevelopmental processes. mGluR7 plays a crucial role in inhibitory neurotransmission by regulating the release of neurotransmitters at presynaptic terminals[135]. Dysregulation of glutamatergic signaling due to GRM7 mutations can contribute to ASD symptoms, including social communication deficits, repetitive behaviors, and restricted interests[136]. Animal model studies have shown that GRM7 expression or function alterations can result in autism-relevant behaviors, such as impaired social interactions and increased repetitive actions[137]. Genetic studies in human populations have identified associations between single nucleotide polymorphisms (SNPs) in GRM7 and an increased risk of developing ASD. This suggests that genetic variation in this receptor may influence susceptibility to the disorder[138]. The involvement of GRM7 in ASD points to potential therapeutic targets,

with current research exploring the development of mGluR7 agonists or antagonists to restore normal glutamatergic signaling and mitigate autism symptoms[139]. Overall, GRM7 mutations and their impact on glutamatergic signaling represent a significant area of interest for understanding the genetic and molecular basis of autism and for developing new therapeutic approaches.

Brain-derived neurotrophic factor (BDNF) is a crucial protein involved in neurons' growth, maintenance, and survival, playing a significant role in synaptic plasticity essential for learning and memory. Mutations or variations in the BDNF gene have been implicated in ASD, suggesting that BDNF dysfunction may contribute to the neurodevelopmental abnormalities characteristic of autism[140]. BDNF influences several aspects of neuronal function, including dendritic growth, synapse formation, and neurotransmitter release, which are vital for proper brain development and function[141]. In individuals with ASD, alterations in BDNF levels or activity can disrupt these processes, leading to deficits in social communication, repetitive behaviors, and restricted interests typical of the disorder[142]. Studies have shown that individuals with ASD often have altered BDNF expression levels, with some research indicating that lower levels of BDNF may be associated with more severe autism symptoms[143]. Specific single nucleotide polymorphisms (SNPs) in the BDNF gene have been linked to an increased risk of developing ASD, as these genetic variations can affect BDNF production or function, contributing to the pathophysiology of autism[144]. Animal models have been instrumental in elucidating the role of BDNF in ASD, with mice exhibiting altered BDNF expression showing behaviors reminiscent of autism, such as impaired social interactions and increased repetitive behaviors[145]. These models provide valuable insights into the mechanisms by which BDNF dysfunction may lead to ASD symptoms and offer a platform for testing potential therapeutic interventions. The link between BDNF and ASD also opens up potential therapeutic avenues, as strategies aimed at modulating BDNF levels or enhancing its activity could potentially alleviate some of the symptoms associated with autism. For instance, certain pharmacological agents, physical exercise, and dietary interventions

have been shown to increase BDNF levels and may hold promise as adjunct therapies for ASD[146,147].

Neuronal Cell Adhesion Molecule (NRCAM) is a crucial protein involved in the development and function of the nervous system, particularly in the formation and maintenance of synapses[148]. Mutations or variations in the **NRCAM gene** have been associated with ASD, highlighting its potential role in the disorder's pathogenesis. NRCAM belongs to the immunoglobulin superfamily of cell adhesion molecules and plays a significant role in axonal guidance, synaptic plasticity, and neural connectivity, all of which are critical for proper brain function and development[149]. In individuals with ASD, NRCAM mutations may lead to altered neural circuitry and connectivity issues, which are common neuropathological features of the disorder. Studies have shown that NRCAM is involved in processes such as dendritic spine development and the regulation of synaptic strength, both of which are essential for cognitive functions like learning and memory. Disruptions in these processes can contribute to the core symptoms of ASD, including social communication deficits and repetitive behaviors[150]. Research has indicated that NRCAM interacts with other synaptic proteins and receptors, influencing neurons' structural and functional plasticity. For instance, NRCAM's interaction with the L1 family of cell adhesion molecules is vital for neurite outgrowth and axonal pathfinding, which are often disrupted in ASD[151,152]. Additionally, animal models with NRCAM gene knockouts exhibit behavioral phenotypes reminiscent of ASD, such as social interaction impairments and increased anxiety-like behaviors, further supporting the gene's involvement in autism[130]. Overall, the association between NRCAM mutations and ASD underscores the importance of cell adhesion molecules in neurodevelopment and synaptic function.

The **HTR2A gene** encodes the 5-hydroxytryptamine (serotonin) receptor 2A, a critical receptor in the brain involved in neurotransmission pathways. Mutations or variations in the HTR2A gene have been linked to ASD, suggesting that disruptions in serotonin signaling could play a role in the disorder's pathophysiology[153]. Serotonin is essential for regulating mood, anxiety, and social behavior, and abnormalities in serotonin

signaling have been implicated in various psychiatric conditions, including autism. Studies indicate individuals with ASD often exhibit altered serotonin levels in the brain and peripheral tissues[124]. The HTR2A receptor is distributed in brain regions associated with cognitive functions, such as the prefrontal cortex and hippocampus. Mutations in the HTR2A gene can change the receptor's expression or function, disrupting serotonergic signaling crucial for social behavior and cognitive processing[154]. Genetic studies have identified polymorphisms in the HTR2A gene associated with increased ASD risk, where certain single nucleotide polymorphisms (SNPs) in the promoter region can affect gene expression levels[155]. Functional studies in animal models demonstrate that alterations in HTR2A signaling can affect social behavior and anxiety, mirroring ASD symptoms. The HTR2A receptor also plays a role in neurodevelopmental processes, including synaptogenesis and neural differentiation, with disruptions potentially leading to brain development and connectivity abnormalities characteristic of ASD[156].

The **CX3CR1 gene** encodes the chemokine receptor 1, which is involved in the brain's immune response and neuroinflammatory processes[157]. Mutations or polymorphisms in CX3CR1 have been implicated in ASD, suggesting that disruptions in immune signaling pathways may contribute to ASD pathogenesis. CX3CR1 is expressed in microglia, the brain's resident immune cells, which are crucial for synaptic pruning, neuronal connectivity, and neuroinflammation[158]. Proper microglial function is essential for normal brain development and neural circuit maintenance. Research indicates that CX3CR1 variants can alter microglial function, impairing synaptic pruning and increasing neuroinflammation, resulting in abnormal synaptic connectivity and contributing to the neurological deficits seen in ASD[159]. Studies show that certain CX3CR1 polymorphisms are linked to increased microglial activation and exaggerated brain inflammatory responses, disrupting neuronal communication and plasticity, which are key to cognitive and social behaviors[160]. Animal models with CX3CR1 mutations exhibit ASD-like behaviors, such as social interaction deficits and repetitive behaviors, providing insights into the gene's impact on brain development and

function[161]. Genetic studies in humans have identified associations between specific CX3CR1 variants and increased ASD susceptibility, highlighting the potential role of immune dysregulation in the disorder[162].

The **CHRNA7 gene** encodes the alpha-7 nicotinic acetylcholine receptor (α7nAChR), a key component of cholinergic signaling in the central nervous system. This receptor is crucial for neurodevelopmental processes such as synaptic plasticity, neurotransmitter release, and cognitive function[163]. Mutations or polymorphisms in CHRNA7 have been linked to ASD, suggesting that disruptions in cholinergic signaling may play a role in autism's pathophysiology. The α7nAChR is highly expressed in brain regions involved in cognition, memory, and social behavior, including the hippocampus, cortex, and amygdala, and it modulates the release of neurotransmitters like glutamate, GABA, and dopamine[164]. Research indicates that CHRNA7 mutations can reduce receptor function or expression, impairing cholinergic signaling and affecting synaptic formation, neuronal differentiation, and network connectivity. This can result in cognitive and social deficits typical of ASD[165]. Studies have found associations between CHRNA7 gene variants and autism, including copy number variations causing partial deletions or duplications of the gene, which disrupt α7nAChR function[166]. Animal models with CHRNA7 mutations exhibit autism-like behaviors, reinforcing the gene's role in neurodevelopment and autism. Additionally, α7nAChR regulates brain anti-inflammatory responses, and its impaired signaling may increase neuroinflammation, which is implicated in ASD etiology[167].

The **GRIN2A gene** encodes the GluN2A subunit of the NMDA (N-methyl-D-aspartate) receptor, which is essential for synaptic plasticity, learning, and memory. Mutations in GRIN2A are linked to various neurodevelopmental disorders, including ASD, epilepsy, and intellectual disability[168]. These mutations can lead to either a gain or loss of NMDA receptor function, disrupting synaptic signaling and neural circuitry[169]. Individuals with GRIN2A mutations often exhibit language deficits, intellectual disability, seizures, and autistic behaviors, highlighting the gene's complex role in brain development and function[170]. Research using animal models has shown

that disruptions in NMDA receptor signaling due to GRIN2A mutations affect the development of neural circuits involved in social behavior, communication, and cognition[171]. Potential therapeutic approaches include pharmacological agents that modulate NMDA receptor activity and genetic therapies to correct specific mutations.

PTGS2 (Prostaglandin-Endoperoxide Synthase 2) **gene**, also known as COX-2, plays a pivotal role in synthesizing prostaglandins, which are important mediators of inflammation and immune responses in the body[172]. While PTGS2 mutations have been extensively studied in relation to inflammatory diseases and cancer, their direct association with ASD is not well-established in the current research literature. Studies exploring the role of immune dysregulation and inflammation in ASD have suggested potential links between immune system abnormalities and the development of autistic symptoms[173]. Prostaglandins, synthesized by PTGS2, are involved in regulating immune responses that could impact neurodevelopmental processes[174]. However, specific evidence directly implicating PTGS2 mutations in ASD pathogenesis remains limited. Further research is necessary to determine whether variations in PTGS2 influence inflammatory pathways that might contribute to the development or severity of ASD traits in affected individuals.

Reelin (RELN) is a pivotal protein in brain development, crucial for guiding the migration and positioning of neurons during the early stages of development[175]. Located on chromosome 7q22, the *RELN gene* encodes an extracellular matrix protein that interacts with receptors like VLDLR and ApoER2 to regulate neuronal migration and synaptic plasticity. Mutations in RELN have been implicated in various neurodevelopmental disorders, including ASD[176]. Research indicates that disruptions in RELN expression or function can lead to altered neuronal migration patterns and abnormal synaptic connectivity, potentially contributing to the cognitive and behavioral impairments seen in ASD[177]. While rare mutations in RELN have been identified in some individuals with ASD, they are not universally present across all cases. Animal models with RELN mutations exhibit behavioral deficits akin to autism, supporting its role in neurodevelopmental processes[75]. Understanding how RELN mutations impact

brain development and function is crucial for developing targeted therapies and interventions for ASD, highlighting the need for further research into its specific mechanisms and therapeutic implications.

FOXP2 (Forkhead Box P2), located on chromosome 7q31, is a gene crucial for language development and speech production. Mutations in FOXP2 are associated with speech and language disorders, particularly developmental verbal dyspraxia, which affects speech coordination[178]. Beyond its role in language, FOXP2 mutations have also been studied in relation to ASD. While rare, mutations in FOXP2 have been identified in individuals with ASD, suggesting a potential link between FOXP2 dysfunction and the broader ASD phenotype, particularly affecting language and communication skills[179]. Animal studies and genetic research indicate that FOXP2 mutations may disrupt neural circuits involved in language processing and social communication, contributing to ASD symptoms[180]. Further investigation is needed to elucidate the precise role of FOXP2 in ASD pathogenesis and to explore its implications for developing targeted therapies aimed at improving language abilities and social communication skills in individuals with ASD.

SNAP25 (Synaptosome Associated Protein 25) is a gene located on chromosome 20p12.2 that plays a pivotal role in neurotransmitter release at synapses, essential for efficient neuronal communication[181]. Mutations and variations in SNAP25 have been implicated in various neurodevelopmental and neuropsychiatric conditions, including ASD[182]. Research indicates that alterations in SNAP25 function can disrupt synaptic transmission, impacting neural circuitry and potentially contributing to the development of ASD symptoms. While SNAP25 mutations in ASD are less common compared to other disorders like ADHD or schizophrenia, studies have identified rare genetic variations in individuals with ASD[183]. Animal models with SNAP25 mutations exhibit behaviors relevant to ASD, such as impaired social interactions and repetitive behaviors, reflecting its importance in neural development and behavior regulation[184]. Further investigation into how SNAP25 disruptions specifically influence ASD pathophysiology could provide insights into underlying mechanisms

and potential targets for therapeutic interventions tailored to ASD-related synaptic dysregulation.

CACNA1G (Calcium Voltage-Gated Channel Subunit Alpha1 G) is a gene that encodes a subunit of the voltage-gated calcium channels, which are crucial for regulating calcium ion influx into neurons. These channels play a vital role in neurotransmitter release, neuronal excitability, and synaptic plasticity in the brain[185]. Mutations or variations in CACNA1G have been implicated in various neurological and neuropsychiatric disorders, including ASD[186]. Studies have identified rare genetic variants in CACNA1G among individuals with ASD, suggesting a potential link between CACNA1G dysfunction and ASD susceptibility[187]. Animal models with CACNA1G mutations display behavioral traits relevant to ASD, such as altered social interaction, repetitive behaviors, and impaired communication skills. These findings indicate that disruptions in CACNA1G function may contribute to ASD pathophysiology by affecting neuronal excitability and synaptic transmission[188]. Further research is needed to elucidate the specific mechanisms by which CACNA1G mutations impact ASD risk and to explore potential therapeutic targets aimed at modulating calcium channel activity to mitigate ASD-related symptoms.

GABRA5 (Gamma-Aminobutyric Acid Type A Receptor Alpha5 Subunit) is a gene that encodes a subunit of the GABA-A receptor, which is integral to inhibitory neurotransmission in the brain. GABAergic signaling regulates neuronal excitability and maintains the balance between excitation and inhibition within neural circuits[189]. Mutations or variations in GABRA5 have been implicated in several neurological and psychiatric disorders, including ASD[190]. Studies have identified rare genetic variants in GABRA5 among individuals with ASD, suggesting a potential link between GABRA5 dysfunction and ASD susceptibility[132]. Animal models with GABRA5 mutations exhibit behavioral characteristics associated with ASD, such as altered social behaviors, repetitive behaviors, and impaired communication skills[191]. These findings suggest that disruptions in GABRA5-mediated inhibitory neurotransmission may contribute to the neurodevelopmental abnormalities observed in ASD.

GRIN2B (Glutamate Ionotropic Receptor NMDA Type Subunit 2B) is a gene that encodes a subunit of the NMDA receptor, a critical receptor involved in excitatory neurotransmission and synaptic plasticity in the brain[192]. Mutations or variations in GRIN2B have been associated with various neurodevelopmental and neuropsychiatric disorders, including ASD[193]. Studies have identified rare genetic variants in GRIN2B among individuals with ASD, suggesting a potential link between GRIN2B dysfunction and ASD susceptibility[194]. Animal models with GRIN2B mutations exhibit behavioral characteristics relevant to ASD, such as altered social interactions, repetitive behaviors, and impaired cognitive functions[195]. These findings underscore the importance of NMDA receptor function in neurodevelopment and the potential role of GRIN2B mutations in disrupting synaptic signaling pathways implicated in ASD pathophysiology.

GRIK2 (Glutamate Ionotropic Receptor Kainate Type Subunit 2) is a gene that codes for a subunit of the kainate receptor, which is involved in synaptic transmission and plasticity in the brain. Kainate receptors are a type of ionotropic glutamate receptor that modulates excitatory neurotransmission and plays a role in regulating synaptic development and function[196]. Stolz *et al.* showed that mutations or variations in GRIK2 have been implicated in several neurodevelopmental disorders, including ASD[197]. Studies have identified rare genetic variants in GRIK2 among individuals with ASD, suggesting a potential link between GRIK2 dysfunction and ASD susceptibility[198]. Animal models with GRIK2 mutations exhibit behavioral traits associated with ASD, such as altered social interactions, repetitive behaviors, and cognitive impairments[199]. These findings highlight the significance of kainate receptor-mediated synaptic signaling in neurodevelopment and underscore GRIK2 as a candidate gene contributing to the genetic complexity of ASD.

HOMER1 is a gene that encodes a family of postsynaptic density proteins involved in synaptic signaling and neuronal plasticity in the brain. These proteins play a crucial role in regulating the structure and function of synapses, particularly at glutamatergic synapses, where they interact with various neurotransmitter receptors and signaling

molecules[200]. Mutations or variations in HOMER1 have been implicated in several neuropsychiatric and neurodevelopmental disorders, including ASD. Studies have identified rare genetic variants in HOMER1 among individuals with ASD, suggesting a potential role in ASD susceptibility[201]. Animal models with HOMER1 mutations exhibit behavioral characteristics relevant to ASD, such as altered social interactions, repetitive behaviors, and cognitive deficits[202]. These findings underscore the importance of HOMER1-mediated synaptic function in neurodevelopment and synaptic plasticity, implicating HOMER1 as a candidate gene contributing to the genetic underpinnings of ASD.

Chromosomal disorders in patients with Autism:

ASD is characterized by a wide range of genetic etiologies, including various chromosomal disorders. These chromosomal abnormalities, which involve alterations in chromosome number or structure, can significantly impact neurodevelopment and contribute to the manifestation of ASD symptoms. While less frequent than de novo mutations, chromosomal disorders represent another critical genetic piece of the puzzle in understanding ASD[203]. There are two main categories of chromosomal disorders observed in patients with ASD: Numerical abnormalities and structural abnormalities. Numerical abnormalities involve having an atypical number of chromosomes, such as Down syndrome (extra chromosome 21) or Turner syndrome (missing X chromosome). Structural abnormalities refer to changes in chromosome structure, like deletions, duplications, or translocations[204]. Studies suggest that chromosomal abnormalities are present in around 7.4% of individuals with ASD, a higher percentage compared to the general population. These abnormalities can sometimes contribute to more severe forms of ASD, particularly when intellectual disability and developmental delays are also present[205]. Individuals with ASD and chromosomal abnormalities may also experience a higher prevalence of co-occurring conditions like epilepsy and behavioral issues[8].

Fragile X Syndrome (FXS) is the most common inherited cause of intellectual disability and is often associated with ASD. FXS results from a mutation in the FMR1 gene located on the X chromosome, which leads to the silencing of the gene and a deficiency in the fragile X mental retardation protein (FMRP)[206]. Approximately 30-50% of individuals with FXS meet the criteria for ASD, exhibiting symptoms such as social anxiety, repetitive behaviors, and language impairments[207]. *Duplications of the 15q11-13 region*, often referred to as Dup15q syndrome, are among the most common chromosomal abnormalities linked to ASD. This region contains several genes critical for brain development, including *UBE3A* [208]. Individuals with Dup15q syndrome typically exhibit severe developmental delays, intellectual disability, epilepsy, and a high prevalence of ASD, with features such as social communication deficits and repetitive behaviors[209].

22q11.2 Deletion Syndrome, also known as DiGeorge Syndrome or Velocardiofacial Syndrome, involves deleting a small piece of chromosome 22. This syndrome is associated with a broad spectrum of clinical manifestations, including congenital heart defects, palatal abnormalities, immune deficiencies, and neuropsychiatric disorders[210]. Approximately 20-40% of individuals with 22q11.2 deletion syndrome exhibit ASD-like behaviors, including social communication challenges and restricted interests[211]. *Turner Syndrome* affects females and is characterized by the complete or partial absence of one X chromosome (45, X karyotype). While Turner Syndrome is primarily associated with physical features such as short stature and gonadal dysgenesis, there is also an increased prevalence of neurodevelopmental disorders, including ASD. Girls with Turner Syndrome may exhibit social difficulties, attention deficits, and learning disabilities[212,213].

Klinefelter Syndrome (47, XXY) is a chromosomal disorder that affects males and is characterized by the presence of an extra X chromosome. Individuals with Klinefelter Syndrome often have mild cognitive impairments, speech and language delays, and social difficulties[214]. There is an elevated risk of ASD in males with Klinefelter Syndrome, with symptoms such as social communication deficits and repetitive

behaviors[215]. *Down Syndrome*, caused by the presence of an extra chromosome 21 (trisomy 21), is the most common chromosomal disorder associated with intellectual disability. Although not typically classified under ASD, a significant proportion of individuals with Down Syndrome exhibit autistic traits, including social interaction challenges and repetitive behaviors[216]. The co-occurrence of ASD and Down Syndrome highlights the overlap between different neurodevelopmental conditions[217]. Various other chromosomal abnormalities, such as duplications, deletions, and translocations, can also contribute to the development of ASD. For instance, abnormalities in chromosomes 1q21.1, 16p11.2, and 17q12 have been implicated in ASD, with each region harboring multiple genes involved in neurodevelopmental processes[218].

Identifying a chromosomal disorder can be achieved through karyotyping, a standard genetic test that analyzes chromosomes for abnormalities. Early diagnosis of a chromosomal disorder can lead to earlier diagnosis of ASD and the implementation of appropriate therapies and support systems[219]. Additionally, understanding the specific chromosomal abnormality can inform genetic counseling for families, providing information about potential recurrence risk in future pregnancies[220]. The identification of chromosomal disorders in patients with ASD has significant implications for diagnosis, management, and genetic counseling. Chromosomal microarray analysis (CMA) and other advanced genetic testing methods have become crucial tools in identifying these abnormalities, enabling early diagnosis and tailored interventions[221]. Understanding the role of chromosomal disorders in ASD provides valuable insights into the genetic architecture of the disorder and underscores the importance of comprehensive genetic evaluations for individuals with ASD[222]. This knowledge not only aids in developing targeted therapies but also enhances our ability to provide personalized care and support for affected individuals and their families. However, it is crucial to recognize that chromosomal disorders are just one contributing factor to ASD in a subset of individuals. Most ASD cases have a more complex genetic architecture involving a combination of genetic and environmental factors[223].

Gender Differences in Autism symptomology: Sex chromosome effects?

Autism Spectrum Disorder (ASD) shows notable sex differences in its prevalence and symptomatology, with males being more frequently diagnosed than females. The reported male-to-female ratios vary from 1.33: 1 to 15.7: 1. Despite the general severity of autism not differing significantly between genders, notable differences exist in how ASD manifests, particularly when comorbid features are present[224]. Males with ASD often exhibit more externalizing behaviors, such as aggression, repetitive movements, and hyperactivity. In contrast, females are more prone to internalizing behaviors, including anxiety and depression. Furthermore, females with ASD tend to have more pronounced cognitive impairments, with the male-to-female ratio nearing 1: 1 among those with severe intellectual disabilities[225]. These observations raise the question of whether these gender differences in ASD are due to distinct biological mechanisms or diagnostic biases influenced by the patient's presentation. Typically, ASD is diagnosed as a categorical condition rather than a continuum, which may significantly interact with behavioral and cognitive differences between males and females[26]. For instance, the less disruptive and less overt behaviors in girls with ASD might mean that only those with severe impairment are brought to diagnostic attention[226]. Recognizing these differences is crucial for clinical management, including screening for specific comorbidities and therapeutically targeting the most debilitating symptoms.

Some researchers believe that regardless of diagnostic biases, certain sex-specific biological mechanisms contribute to the gender disparity in ASD[227]. The female-protective effect (FPE) is a leading theory in this regard, suggesting that specific factors protect females from developing ASD, thereby requiring them to have a higher threshold to reach clinical impairment[228]. Evidence supporting this hypothesis includes studies indicating a greater genetic load related to ASD in females compared to males and in clinically unaffected female relatives compared to unaffected male relatives of individuals with ASD[229]. For example, a study of dizygotic twins by Attermann *et al.* using the Childhood Autism Spectrum Test and the Autism−Tics,

Attention Deficit Hyperactivity Disorder, and Other Comorbidities inventory found higher autism symptom scores in siblings of female probands compared to those of male probands[230]. Similar findings were observed in another large-scale study by Van't Westeinde *et al.*, which focused on repetitive and restricted behavior differences between male and female dizygotic twins[231]. Females used more compensation and masking camouflage strategies than males. A related argument supporting the FPE is that males are more likely to meet clinical criteria for ASD than females, given the same genetic risk[232]. For instance, individuals with a SHANK1 microdeletion were rigorously assessed, revealing that males more frequently met the clinical criteria for ASD, while females with the same mutation exhibited anxiety but not ASD[83].

The FPE mechanisms likely involve genes on the sex chromosomes and sex hormones. Although ASD is not linked to the X chromosome, it is suggested that the Y chromosome may pose a risk or that a second X chromosome may offer protection, as indicated by higher ASD rates in Turner syndrome (XO) and 47, XYY syndrome[233]. The role of sex chromosomes and related molecules has been increasingly recognized in contributing to these differences. Understanding these molecular mechanisms is crucial for developing sex-specific diagnostic and therapeutic approaches. The MECP2 gene on the X chromosome is a well-known player in neurodevelopmental disorders. Mutations in MECP2 are primarily associated with Rett syndrome, a condition that predominantly affects females and shares some phenotypic overlap with ASD. MECP2 is involved in the regulation of gene expression through binding to methylated DNA. Its dysfunction can lead to widespread gene expression changes and neural development abnormalities[234]. Since males have only one X chromosome, mutations in MECP2 can have more severe consequences, potentially explaining some of the observed sex differences in ASD prevalence and severity. Neuroligins (NLGNs) are a family of postsynaptic cell adhesion molecules critical for synapse formation and function[25]. NLGN4X and NLGN4Y are located on the X and Y chromosomes, respectively. Mutations in NLGN4X have been linked to ASD, with affected individuals showing impairments in synaptic function and neural connectivity[117]. The presence of

NLGN4X on the X chromosome means that females with two X chromosomes may have a protective effect if one copy is mutated, whereas males with only one X chromosome are more vulnerable. NLGN4Y, on the Y chromosome, has a similar but not identical function to NLGN4X, and its role in ASD is less clear. However, variations in these genes can contribute to the differences in ASD manifestation between males and females[118]. PTCHD1 is another gene of interest located on the X chromosome, which encodes a protein involved in the Hedgehog signaling pathway, essential for brain development. Mutations or deletions in PTCHD1 have been implicated in ASD, intellectual disability, and other neurodevelopmental disorders. Like MECP2 and NLGN4X, PTCHD1 mutations may have more pronounced effects in males due to the lack of a second X chromosome that could compensate for the defective gene[120]. The inclusion of sex chromosome-related molecules in the study of ASD is vital for uncovering the nuances of sex differences in the disorder. By investigating the roles of MECP2, NLGN4X, NLGN4Y, and PTCHD1, researchers can better understand the genetic and molecular basis of these differences. This knowledge will pave the way for more tailored and effective therapeutic strategies that account for sex-specific variations in ASD presentation and progression[235].

Hormonal differences between males and females may also play a role. The differences in the vasopressinergic system in males and females might explain the male prevalence of ASD[94]. The role of sex hormones, particularly testosterone, in early brain development in children with ASD has garnered considerable interest. Several studies have found correlations between fetal testosterone levels, systematizing traits, social impairments, and reduced empathy[236]. Additionally, adults with ASD have been found to have higher levels of testosterone metabolites compared to unaffected individuals[226]. Future research should focus on developing gender-specific measures to better understand the influence of gender-specific factors on diagnosis and treatment. To date, no treatment studies have specifically targeted females with ASD based on hypothesis-driven approaches. Understanding these gender differences is vital for

creating more effective and personalized interventions for individuals with ASD. Table 2 summarizes these gender differences in patients with ASD.

Advances in Genomic Technologies:

Recent advances in genomic technologies have significantly enhanced our ability to diagnose ASD. High-throughput sequencing methods, such as whole-genome sequencing (WGS) and whole-exome sequencing (WES), have enabled the identification of numerous genetic variants associated with ASD[235]. These technologies allow for a comprehensive analysis of the entire genome or the coding regions, respectively, providing insights into both common and rare genetic factors contributing to the disorder. Additionally, microarray-based comparative genomic hybridization (aCGH) has facilitated the detection of chromosomal abnormalities, such as copy number variations (CNVs), which are often implicated in ASD[238]. Integrating these advanced genomic tools into clinical practice holds promise for earlier and more accurate diagnosis, personalized treatment strategies, and a deeper understanding of the genetic architecture underlying ASD[239].

Whole-exome sequencing:

Exome sequencing has made significant contributions to identifying risk genes in patients with ASD. This technology focuses on sequencing the exonic regions of the genome, representing about 1-2% of the entire genome but containing the vast majority of known disease-related genetic variations[240]. By concentrating on these regions, exome sequencing provides a more cost-effective and efficient means of uncovering the genetic underpinnings of ASD[241]. One of the major contributions of exome sequencing to ASD research is the identification of de novo mutations—mutations that are not inherited from either parent but occur spontaneously. These mutations have been found to play a critical role in the development of ASD[242]. Studies utilizing exome sequencing have revealed that a significant proportion of individuals with ASD have unique de novo mutations in various genes, which might not have been identified

through traditional genetic testing methods. These discoveries have expanded our understanding of the genetic diversity and complexity of ASD[243].

Exome sequencing has also facilitated the discovery of rare, high-impact variants contributing to the disorder. By analyzing the exomes of large cohorts of individuals with ASD and comparing them to control groups, researchers have identified numerous rare variants in genes that are crucial for brain development and function[244]. These findings have highlighted the importance of genes involved in synaptic function, neuronal communication, and other neurodevelopmental processes in the etiology of ASD. Furthermore, exome sequencing has identified several novel ASD risk genes. For instance, genes such as CHD8, SCN2A, and SYNGAP1 have been implicated in ASD through exome sequencing studies[245]. These genes are involved in critical biological pathways, including chromatin remodeling, ion channel function, and synaptic signaling. Identifying these risk genes has provided new insights into the molecular mechanisms underlying ASD and has opened up potential avenues for targeted therapeutic interventions[246].

Another significant advantage of exome sequencing is its ability to uncover gene-disrupting mutations in individuals with ASD who do not have a family history of the disorder. This capability is crucial for understanding sporadic cases of ASD, where the genetic basis might be less apparent[247]. By pinpointing specific genetic mutations in these cases, exome sequencing helps to clarify the genetic contributions to ASD in a broader population. Moreover, exome sequencing has proven valuable in studying the genetic architecture of ASD in diverse populations. It allows researchers to explore the genetic variations and mutations that may be more prevalent in certain ethnic or geographic groups, contributing to a more comprehensive understanding of the global genetic landscape of ASD[248].

Whole-Genome Sequencing (WGS):
WGS has revolutionized the field of genetics by comprehensively analyzing an individual's entire genome, encompassing both coding and non-coding regions. This

technology has significantly advanced our understanding of the genetic basis of ASD in several keyways[249]. First, WGS has facilitated the discovery of rare genetic variants that were previously undetectable with older methods. These rare variants often have a large effect size and can be crucial in understanding the genetic underpinnings of ASD[250]. By analyzing the entire genome, WGS can identify mutations in non-coding regions that may regulate gene expression, adding a new layer of complexity to our understanding of ASD genetics[251].

Second, unlike targeted sequencing or whole-exome sequencing (WES), which focus only on specific parts of the genome, WGS provides a complete picture. This includes single nucleotide variants (SNVs), insertions, deletions, copy number variations (CNVs), and structural variants. The ability to detect a wide range of mutation types helps identify novel genetic contributors to ASD[252]. Third, a significant portion of the human genome is non-coding, and mutations in these regions can affect gene regulation and expression. WGS allows researchers to investigate these regions and understand how they may contribute to ASD. For instance, mutations in regulatory elements, enhancers, and promoters can disrupt normal gene function and lead to ASD[253]. Moreover, through WGS, researchers have identified numerous new genes associated with ASD that were not previously linked to the disorder. These discoveries expand the list of potential targets for therapeutic intervention and provide new insights into the biological pathways involved in ASD[254]. ASD is known for its genetic heterogeneity, meaning many different genetic factors can contribute to it. WGS helps elucidate this complexity by identifying the diverse genetic mutations across different individuals with ASD. This understanding can lead to more personalized approaches to diagnosis and treatment[54].

Additionally, WGS enables large-scale genomic studies and the creation of extensive databases of genetic information. By comparing the genomes of thousands of individuals with and without ASD, researchers can identify patterns and commonalities that provide deeper insights into the genetic architecture of the disorder[243]. WGS is particularly useful in family-based studies, where the genomes of affected individuals

and their relatives are sequenced. This approach can identify inherited mutations and de novo mutations (new mutations do not present in the parents) that contribute to ASD, helping to pinpoint specific genetic risk factors[255]. Overall, WGS has dramatically advanced our understanding of the genetic basis of ASD by providing a detailed and comprehensive view of the genome. This technology continues to uncover new genetic insights, paving the way for better diagnostic tools, personalized treatments, and a deeper understanding of the complex genetic landscape of ASD[256].

Copy Number Variations (CNVs):

CNVs have a substantial impact on the risk of developing ASD. CNVs refer to structural changes in the genome that result in the duplication or deletion of large segments of DNA. These variations can encompass one or multiple genes and significantly alter gene dosage, disrupting normal cellular functions[257]. One of the key contributions of studying CNVs to our understanding of ASD is the identification of specific genomic regions where duplications or deletions are associated with increased risk for the disorder[258]. For example, deletions and duplications at the 16p11.2 Locus have been repeatedly implicated in ASD. Individuals with deletions in this region often exhibit neurodevelopmental issues, including ASD, intellectual disability, and language impairments[259]. Conversely, duplications at the same locus are also associated with ASD but may present with differing clinical features, underscoring the complex relationship between gene dosage and neurodevelopmental outcomes[260].

The presence of CNVs can disrupt the function of multiple genes simultaneously, leading to widespread effects on brain development and function. This is particularly relevant for genes involved in synaptic connectivity, neuronal communication, and brain signaling pathways[261]. CNVs that affect these genes can lead to abnormalities in the formation and maintenance of synapses, which are critical for proper brain function and development[262]. For instance, CNVs affecting the NRXN1 gene, which encodes a protein essential for synaptic function, have been associated with ASD, highlighting the

importance of synaptic integrity in the disorder. Furthermore, the study of CNVs has revealed that individuals with ASD often carry a higher burden of rare, large CNVs compared to the general population[263]. These rare CNVs can significantly affect gene function and are often de novo, meaning they are not inherited from the parents but arise spontaneously. The identification of such de novo CNVs in individuals with ASD supports the idea that these structural variations are critical contributors to the genetic architecture of the disorder[264].

Research has also shown that certain CNVs are more prevalent in individuals with ASD who also exhibit additional comorbid conditions, such as intellectual disability or epilepsy[265]. This suggests that CNVs not only contribute to ASD risk but may also influence the broader clinical phenotype, leading to a range of neurodevelopmental outcomes depending on the specific genes and pathways affected. The impact of CNVs on ASD risk is further highlighted by their contribution to the genetic heterogeneity of the disorder[266]. ASD is known for its diverse genetic landscape, and CNVs add another layer of complexity by introducing large-scale genomic changes that can vary widely among affected individuals. This genetic diversity underscores the need for personalized approaches to diagnosis and treatment, as the specific CNVs present in an individual can influence the manifestation and severity of ASD symptoms[267].

Gene-Environment Interactions and Their Effects on Autism Development:
Gene-environment interactions play a crucial role in the development of ASD. This concept refers to the dynamic interplay between genetic predispositions and environmental factors, where the combined effects can influence the onset and progression of ASD. Understanding these interactions is essential for unraveling the complex etiology of autism and developing more effective prevention and intervention strategies[268]. Certain genetic variations can increase an individual's susceptibility to ASD, but these variations alone may not be sufficient to cause the disorder. For instance, mutations in genes such as CHD8, SCN2A, and SHANK3 have been identified in individuals with ASD, highlighting a genetic predisposition[223]. However, not all

individuals with these mutations develop ASD, suggesting that other factors, including environmental influences, play a role in triggering or exacerbating the condition[52].

Environmental influences, particularly those occurring during prenatal and perinatal periods, have been increasingly recognized as significant factors that may interact with genetic predispositions to influence the risk of developing ASD[269]. Numerous studies have explored how various environmental exposures can contribute to ASD, offering insights into the complex etiology of the disorder. Several studies have established a link between maternal infections during pregnancy and an increased risk of ASD in offspring[270]. For example, a study by Atladóttir *et al.* found that maternal infection during the first trimester was associated with a higher risk of ASD. The proposed mechanism involves the maternal immune response, which can produce inflammatory cytokines that cross the placenta and potentially disrupt fetal brain development[271]. The use of certain medications during pregnancy has been linked to an increased risk of ASD. For instance, Christensen *et al.* reported that maternal use of valproate, an antiepileptic drug, was associated with a significantly increased risk of ASD and other neurodevelopmental disorders. The study suggests that valproate can alter neurodevelopment through its effects on folate metabolism and histone deacetylase inhibition[272].

Prenatal exposure to environmental toxins, such as pesticides and heavy metals, has been implicated in the development of ASD. Research by Roberts *et al.* indicated that living near agricultural areas where pesticides are used during pregnancy was associated with a higher risk of having a child with ASD. These toxins can disrupt neural development by interfering with signaling pathways critical for brain development[273]. Maternal nutrition, particularly the intake of essential nutrients like folic acid, has been shown to influence ASD risk. A study by Schmidt *et al.* found that adequate maternal folic acid intake around conception was associated with a reduced risk of ASD. Folic acid is crucial for DNA methylation and neural tube development, and its deficiency during critical periods can adversely affect fetal brain development[274].

Perinatal complications such as preterm birth, low birth weight, and hypoxia have been associated with an increased risk of ASD. A meta-analysis by Gardener *et al.* found that several perinatal factors, including low birth weight and preterm birth, were significantly associated with ASD. These complications can result in hypoxic-ischemic injury to the developing brain, leading to neurodevelopmental disorders[275]. Advanced parental age, particularly paternal age, has been linked to an increased risk of ASD. Reichenberg *et al.* demonstrated that children of older fathers had a higher risk of ASD, potentially due to the accumulation of de novo mutations in the sperm of older men. These genetic mutations can interact with environmental factors, increasing the likelihood of ASD development[276].

Environmental factors can interact with genetic vulnerabilities to influence ASD risk. For example, individuals with certain genetic mutations may be more susceptible to environmental insults. Kinney *et al.* found that prenatal exposure to severe maternal stress was associated with a higher risk of ASD in children with a familial history of the disorder. This suggests that genetic susceptibility and environmental stressors can act synergistically to increase ASD risk[277]. Environmental exposures can lead to epigenetic changes that affect gene expression without altering the DNA sequence. These modifications can influence the development of ASD. Dutheil *et al.* showed that prenatal exposure to air pollution was associated with changes in DNA methylation patterns in genes related to neurodevelopment, suggesting a potential mechanism for how environmental factors can impact ASD risk[278]. Gene-environment interaction models provide valuable frameworks for understanding how genetic predispositions and environmental factors jointly influence the risk of developing ASD. These models illustrate the complexity of ASD etiology, emphasizing that neither genetic nor environmental factors alone can fully explain the disorder. Several models have been proposed to elucidate these interactions[41].

The Diathesis-Stress Model:

The diathesis-stress model is one of the most widely recognized frameworks for understanding gene-environment interactions in ASD. This model posits that individuals inherit genetic vulnerabilities (diatheses) that predispose them to ASD[279]. However, the expression of these genetic vulnerabilities depends on the presence of environmental stressors. For example, a child with a genetic predisposition to ASD may only develop the disorder if exposed to certain environmental factors such as prenatal stress, maternal infections, or exposure to toxins during critical periods of neurodevelopment. This model highlights the interplay between inherent genetic risks and environmental triggers in the manifestation of ASD[280].

The Differential Susceptibility Model:

The differential susceptibility model suggests that some individuals are more susceptible to environmental influences, both positive and negative, due to their genetic makeup. In this model, certain genetic variants make individuals more responsive to environmental conditions[281]. For instance, a child with specific genetic variants may be more adversely affected by prenatal exposure to toxins or maternal stress, increasing their risk of developing ASD[282]. Conversely, the same genetic variants might make the child more responsive to positive environmental interventions, such as enriched early learning environments or supportive caregiving, potentially mitigating ASD symptoms or improving developmental outcomes[283].

The Biological Sensitivity to Context Model

Similar to the differential susceptibility model, the biological sensitivity to context model proposes that genetic variations influence an individual's sensitivity to environmental contexts. This model focuses on the idea that some individuals are biologically more reactive to environmental stimuli due to their genetic makeup. In the context of ASD, this model suggests that children with certain genetic profiles may have heightened biological responses to environmental stressors or toxins, which could disrupt neurodevelopment and increase the risk of ASD. Alternatively, these children

may also benefit more from positive environmental influences, highlighting the importance of supportive and enriched environments for at-risk individuals[284,285].

The Gene-Environment Correlation Model

The gene-environment correlation model explores how genetic and environmental factors can be correlated, meaning that genetic predispositions can influence an individual's exposure to certain environments. There are three types of gene-environment correlations: Passive, evocative, and active[286]. In the passive correlation, parents provide both the genes and the environment, such as a parent with ASD traits creating an environment that influences the child's development[287]. Evocative correlation occurs when an individual's genetic traits elicit specific responses from the environment, such as a child's social difficulties leading to social isolation[288]. Active correlation involves individuals seeking out environments that complement their genetic predispositions, such as a child with sensory sensitivities avoiding noisy settings[289]. These correlations can help explain how genetic predispositions and environmental exposures jointly contribute to the development of ASD.

Epigenetic Models

Epigenetic models emphasize the role of environmental factors in modifying gene expression through epigenetic mechanisms such as DNA methylation and histone modification. These changes do not alter the DNA sequence but can profoundly affect gene expression and neurodevelopment[290]. Environmental factors such as prenatal nutrition, exposure to toxins, and maternal stress can induce epigenetic modifications that influence the risk of ASD[291]. For example, prenatal exposure to air pollution has been associated with changes in DNA methylation patterns in genes involved in neurodevelopment, suggesting a potential mechanism for how environmental exposures can impact ASD risk[292]. Epigenetic models underscore the dynamic interplay between genes and the environment in shaping developmental outcomes[293].

Integrative Models:

Integrative models combine elements from multiple frameworks to comprehensively understand gene-environment interactions in ASD. These models recognize that genetic predispositions, environmental exposures, and epigenetic mechanisms all contribute to the risk of ASD and interact in complex ways[294,295]. Integrative models often incorporate neurobiology, developmental psychology, and epidemiology insights to create a holistic view of ASD etiology. For example, an integrative model might consider how genetic vulnerabilities to neuroinflammation interact with prenatal exposure to maternal infections, leading to epigenetic changes that disrupt brain development and increase ASD risk[296].

These gene-environment interaction models (Table 3) offer valuable insights into the multifaceted nature of ASD. These models emphasize that the development of ASD is not solely determined by genetic or environmental factors but by their complex interplay. Understanding these interactions is crucial for identifying at-risk individuals, developing targeted interventions, and informing preventive strategies.

Epigenetic Mechanisms:

Epigenetic modifications play a crucial role in regulating gene expression without altering the underlying DNA sequence, significantly contributing to the development of ASD. These mechanisms include DNA methylation, histone modification, and non-coding RNA molecules, which can either activate or silence genes, thereby influencing neural development and function [297.298].

DNA Methylation typically suppresses gene expression and can be influenced by environmental factors such as prenatal stress, toxins, or nutritional deficiencies. In ASD, aberrant DNA methylation patterns have been observed in genes associated with synaptic function and neurodevelopment[299]. For example, hypermethylation of the MECP2 gene, critical for brain development and associated with Rett syndrome (an ASD-related disorder), has been identified in some individuals with ASD[300].

Histone Modifications alter the chromatin structure and gene accessibility, playing a critical role in gene expression regulation. These modifications can be influenced by external factors such as maternal diet or exposure to pollutants, potentially leading to atypical neural circuitry linked to ASD[301]. Studies have shown that individuals with ASD often exhibit altered histone acetylation and methylation states, disrupting chromatin structure and gene expression, particularly in genes implicated in neural connectivity and plasticity[302].

Non-coding RNAs, including microRNAs, regulate gene expression post-transcriptionally and have been implicated in modulating genes involved in neural connectivity and plasticity. Dysregulation of specific microRNAs that regulate neural development and synaptic function has been found in individuals with ASD, leading to the misregulation of critical brain function genes[303].

Research on epigenetic changes in individuals with ASD has uncovered several key findings. Perini *et al*. revealed abnormal DNA methylation patterns in genes involved in synaptic function and neural development, suggesting that epigenetic dysregulation contributes to the neural anomalies observed in ASD[304]. Another study highlighted that individuals with ASD often exhibit altered histone acetylation and methylation states, disrupting chromatin structure and gene expression[305]. Additionally, research into non-coding RNAs, such as microRNAs, has emphasized their role in ASD. Dysregulation of microRNAs that regulate neural development and synaptic function leads to misregulating crucial brain function genes[306]. These studies underscore the importance of epigenetic mechanisms in ASD and suggest that both inherited and environmentally induced epigenetic changes can significantly impact gene expression, contributing to the disorder's pathogenesis[291]. Understanding the interplay between genetic predispositions and epigenetic modifications offers valuable insights into the complex etiology of ASD and highlights potential avenues for early intervention and therapeutic strategies. These findings pave the way for further exploration into epigenetic therapies that could potentially reverse or mitigate some of the effects of these epigenetic alterations in ASD.

Therapeutic Implications:

The growing understanding of the genetic underpinnings of ASD is revolutionizing therapeutic strategies, moving towards more personalized and targeted approaches. Traditional treatments for ASD have been mainly one-size-fits-all. Still, as we uncover more about the genetic diversity and complexity of ASD, it becomes clear that a more individualized approach is essential[307]. This section explores how these genetic findings are being translated into personalized treatment modalities, offering hope for more effective management and improved outcomes for individuals with ASD.

Personalized Medicine:

Advancements in genetic research are transforming the treatment landscape for ASD by enabling personalized medicine approaches. Personalized medicine involves tailoring medical treatment to the individual characteristics of each patient, particularly their genetic profile[308]. This approach is highly promising for ASD and is known for its complexity and heterogeneity. Discoveries in genetics have pinpointed numerous genes and genetic variations linked to ASD. These insights allow for the development of targeted therapies that address specific biological pathways disrupted in different individuals[309]. For example, mutations in the CHD8 gene, associated with certain ASD cases, suggest that therapies modulating chromatin remodeling and gene expression, *e.g.,* using Baf53b, could be beneficial[310]. Similarly, therapies targeting synaptic function may be developed for individuals with SHANK3 gene disruptions[311].

Pharmacogenomics, which studies how genes affect an individual's drug response, is a critical aspect of personalized medicine. By analyzing a patient's genetic profile, clinicians can predict which medications will be most effective and which might cause adverse reactions[312]. In the context of ASD, this could mean more precise management of co-occurring conditions like anxiety, depression, and ADHD, as well as core autism symptoms. For instance, variations in the serotonin transporter gene

(SLC6A4) may inform the choice of antidepressants, leading to more effective and tailored treatment plans. Identifying biomarkers—measurable indicators of a biological state or condition—can greatly aid in the early diagnosis and personalized treatment of ASD[313]. Genetic and epigenetic biomarkers provide insights into the severity and specific characteristics of ASD in an individual, guiding personalized intervention strategies. Specific DNA methylation patterns or histone modifications, for example, might serve as biomarkers for particular ASD subtypes, helping to effectively tailor therapeutic approaches [314].

Personalized medicine also extends to non-pharmacological interventions, such as behavioral therapies, dietary adjustments, and environmental modifications. Genetic insights can inform the selection of behavioral therapies that are more likely to be effective [315]. For instance, individuals with mutations in genes affecting social cognition may benefit more from social skills training and interventions focusing on enhancing social interactions [316]. While the promise of personalized medicine is substantial, there are challenges to overcome. The genetic architecture of ASD is highly intricate, involving numerous genes and environmental factors [317]. Comprehensive genetic testing and sophisticated interpretation are necessary to implement personalized approaches effectively [318]. Additionally, ethical considerations, such as genetic privacy and potential discrimination, must be carefully addressed [319]. Future research should aim further to clarify the genetic and epigenetic landscape of ASD, develop advanced tools for genetic testing, and design targeted therapies based on these findings. Collaboration among geneticists, clinicians, and researchers is crucial to translating genetic discoveries into practical, personalized treatment strategies that improve outcomes for individuals with ASD [320].

Current and Future Therapies:

Exploring the genetic underpinnings of ASD is paving the way for innovative therapies that could significantly enhance treatment efficacy. Current therapies for ASD include behavioral interventions, pharmacological treatments, and nutritional strategies [321].

Applied Behavior Analysis (ABA) is a widely used behavioral therapy that focuses on improving specific behaviors such as communication, social skills, and adaptive learning skills. Genetic insights can help tailor ABA programs to target specific deficits associated with particular genetic profiles[322]. Social skills training interventions are also essential, especially for individuals with ASD-related genetic variations affecting social cognition[323]. Pharmacological treatments such as antipsychotics and selective serotonin reuptake inhibitors (SSRIs) are commonly used to manage irritability, aggression, anxiety, and depression in individuals with ASD[324]. Genetic research helps identify those who might benefit most from these medications while minimizing side effects. Nutritional and dietary interventions, such as gluten-free and casein-free diets, are used by some individuals with ASD[325]. Genetic predispositions related to metabolic processes can inform nutritional adjustments to improve gastrointestinal and behavioral symptoms[326].

Future therapies, including gene therapy and epigenetic interventions, hold exciting possibilities. Targeted genetic interventions could potentially correct or mitigate the effects of specific genetic abnormalities, such as those in the CHD8 or SHANK3 genes[327]. Epigenetic therapies, such as DNA methylation modulators and histone deacetylase inhibitors, could correct epigenetic dysregulation observed in ASD, potentially reversing abnormal chromatin states and restoring normal gene expression patterns[297]. Pharmacogenomics promises highly personalized medication regimens that maximize efficacy and minimize adverse effects. Understanding variations in the serotonin transporter gene can guide the use of SSRIs and other psychotropic medications[328]. Non-coding RNA-based therapies, such as microRNA modulation, could correct dysregulation in neural development and synaptic function associated with ASD[329].

Recent genetic studies suggest a link between neuroinflammation and ASD. Targeting inflammatory pathways genetically associated with ASD could offer new therapeutic avenues to reduce neuroinflammation and improve neurological outcomes[330]. Stem cell therapy is a burgeoning field with the potential to regenerate

or repair neural tissue affected by genetic mutations associated with ASD. Research is ongoing to determine the feasibility and safety of using stem cells to address neurodevelopmental deficits in ASD[331]. Continued research is essential to translate genetic findings into practical therapies. This includes clinical trials to evaluate the efficacy and safety of new genetic and epigenetic therapies, biomarker discovery to track treatment responses and tailor interventions, and interdisciplinary collaboration between geneticists, neuroscientists, and clinicians to integrate genetic data into therapeutic practices[332]. Genetic research is driving significant advancements in the treatment of ASD, offering hope for more effective and personalized therapies. Current treatments informed by genetic insights are already improving outcomes, while future interventions hold the promise of addressing the underlying genetic causes of ASD[283]. As research progresses, these innovative therapies will continue to evolve, providing more targeted and individualized care for individuals with ASD.

Future Directions and Research Challenges:

Unresolved Questions:

Despite significant advances in understanding the genetic basis of ASD, many questions remain unresolved, highlighting crucial areas for future research. Identifying these gaps is essential for guiding ongoing and future studies to deepen our understanding of ASD and improve diagnostic and therapeutic strategies. One key question revolves around the full spectrum of genetic variations contributing to ASD. While numerous risk genes and mutations have been identified, many cases of ASD cannot be explained by known genetic factors alone. This suggests that there are still undiscovered genetic components, possibly rare variants or non-coding regions, that play a critical role in ASD[42]. Whole-genome sequencing (WGS) and advanced bioinformatics tools are needed to uncover these hidden genetic elements.

Another significant gap is the lack of understanding of the precise mechanisms by which identified genetic mutations lead to ASD. Although specific genes and pathways have been implicated, the detailed biological processes linking these genetic changes to

ASD symptoms remain unclear. This includes elucidating how genetic mutations affect neural development, synaptic function, and brain connectivity. Advanced imaging techniques and animal models can provide valuable insights into these mechanisms[333]. Another area requiring further exploration is the interaction between genetic and environmental factors in ASD development. While studies have shown that environmental factors such as prenatal stress, exposure to toxins, and nutritional deficiencies can influence ASD risk, the exact nature of these interactions is not well understood[334]. Longitudinal studies and integrative approaches combining genetic, epigenetic, and environmental data are needed to clarify these complex relationships.

Additionally, there is a need to investigate the heterogeneity of ASD. ASD is a highly variable condition with a wide range of symptoms and severities. Understanding the genetic basis of this heterogeneity is crucial for developing personalized treatment approaches. Research should focus on identifying genetic and epigenetic markers that can predict symptom profiles and treatment responses in individuals with ASD[19]. The role of epigenetic modifications in ASD is an emerging field with many unanswered questions. While aberrant DNA methylation, histone modifications, and non-coding RNAs have been linked to ASD, more research is needed to understand how these epigenetic changes are regulated and interact with genetic predispositions[298]. Identifying specific epigenetic alterations associated with different ASD subtypes can provide new targets for therapeutic interventions.

Finally, translating genetic research into clinical practice poses significant challenges. While genetic testing can identify risk factors and guide personalized treatments, the accessibility and cost of such tests remain barriers. Developing affordable and widely available genetic tests is essential for integrating genetic insights into routine clinical care[36]. Moreover, ethical considerations related to genetic testing, such as privacy, consent, and the potential for genetic discrimination, must be addressed[335]. Future research must address these unresolved questions and gaps in knowledge to advance our understanding of ASD. Collaborative efforts integrating genomics, neuroscience,

environmental sciences, and clinical research are crucial for unraveling the complex etiology of ASD and improving outcomes for individuals with the disorder[307].

Emerging Technologies:

Emerging technologies such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and advanced bioinformatics tools hold immense potential to address the existing gaps in ASD research and pave the way for groundbreaking discoveries and treatments. These innovative approaches can help unravel the complexities of ASD, offering new insights and therapeutic opportunities[336].

CRISPR-Cas9, a powerful genome-editing tool, enables precise modification of DNA sequences, allowing researchers to investigate the functional consequences of specific genetic mutations associated with ASD[337]. By creating animal models or cell lines with targeted genetic alterations, scientists can study the resulting phenotypic changes and understand how these mutations contribute to ASD[338]. This technology can also be used to correct genetic defects in vitro, providing a potential pathway for developing gene therapy approaches for ASD. Additionally, CRISPR can be employed to explore the role of non-coding regions of the genome, which are often overlooked but may contain regulatory elements crucial for neural development and function[339].

Advanced bioinformatics tools are essential for analyzing the vast amounts of genomic data generated by next-generation sequencing techniques such as whole-genome sequencing (WGS) and whole-exome sequencing (WES)[340]. These tools can identify novel genetic variants and potential risk genes associated with ASD by integrating and comparing data from multiple studies. Machine learning algorithms and artificial intelligence can enhance the interpretation of complex genetic data, uncovering patterns and correlations that may be missed by traditional analysis methods[341]. Bioinformatics approaches can also facilitate the study of gene-environment interactions by integrating genomic, epigenomic, and environmental datasets, providing a comprehensive understanding of the multifactorial nature of ASD[341].

Single-cell RNA sequencing (scRNA-seq) is another emerging technology that offers insights into the brain's cellular heterogeneity. Researchers can identify specific cell types and states affected by ASD-associated genetic mutations by analyzing gene expression at the single-cell level. This technology can reveal how different cell populations contribute to the pathology of ASD and highlight potential cellular targets for therapeutic interventions[343]. Furthermore, *developing organoids* (three-dimensional cultures derived from stem cells) allows for the modeling of human brain development in vitro. Brain organoids can mimic the early stages of neural development, providing a platform to study the effects of genetic and environmental factors on brain formation and function[344]. These models can be used to screen potential drugs and evaluate their effects on neural development, offering a promising avenue for preclinical testing of new treatments for ASD.

In addition to these technologies, advancements in neuroimaging techniques, such as functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI), can enhance our understanding of the structural and functional connectivity in the brains of individuals with ASD[345]. Combining genetic data with neuroimaging findings can elucidate how genetic variations impact brain structure and function, leading to the identification of biomarkers for early diagnosis and targeted interventions[346]. Integrating these emerging technologies into ASD research promises to address key gaps in our knowledge and transform our approach to understanding and treating ASD. By leveraging CRISPR, advanced bioinformatics, single-cell analysis, organoids, and neuroimaging, researchers can unravel the intricate genetic and biological underpinnings of ASD, ultimately leading to more effective and personalized therapies for individuals affected by this complex disorder[347].

Collaborative Research:
Advancing our understanding of ASD and developing effective treatments necessitate a concerted effort that spans multiple disciplines and countries. The complexity of ASD's genetic underpinnings and its interaction with environmental factors demand a

collaborative research approach, bringing together diverse expertise and resources[348]. The necessity of multidisciplinary collaborations in ASD research cannot be overstated, as they allow for integrating knowledge from various fields such as genetics, neuroscience, psychology, bioinformatics, and clinical medicine. Geneticists and molecular biologists can identify and characterize genetic mutations associated with ASD, while neuroscientists can study how these mutations affect brain development and function[349]. Psychologists and psychiatrists can provide insights into the behavioral and cognitive aspects of ASD, helping to link genetic findings with clinical presentations. Bioinformaticians and data scientists can manage and analyze large datasets, revealing patterns and correlations that are critical for understanding the genetic architecture of ASD[350]. Clinicians can translate these findings into diagnostic tools and therapeutic interventions, ensuring that research outcomes benefit patients directly. The necessity of this collaborative effort is clear, and it is through such integration that we can truly advance our understanding and treatment of ASD.

International collaborations are equally important, enabling researchers to access larger and more diverse populations. Genetic studies require extensive data to identify rare variants and to ensure findings are applicable across different ethnic and genetic backgrounds. International consortia, such as the Autism Genome Project, have already made significant strides by pooling resources and data from research groups worldwide[351]. These collaborations can enhance the statistical power of genetic studies and lead to more robust and generalizable findings. Moreover, sharing data and methodologies across borders can accelerate the pace of discovery and avoid duplication of efforts[243]. Collaborative research also facilitates the sharing of advanced technologies and expertise. Institutions in different countries may have unique strengths and capabilities, and pooling these resources can lead to more comprehensive and innovative approaches to ASD research. For example, a research group with expertise in CRISPR technology can partner with a team skilled in neuroimaging to investigate the effects of specific genetic mutations on brain structure and function[352]. Similarly, collaborations with pharmaceutical companies can help

translate genetic discoveries into new treatments, leveraging their expertise in drug development and clinical trials[353].

Furthermore, collaborative efforts can address the ethical, legal, and social implications of genetic research on ASD. Multidisciplinary teams can work together to ensure that research practices are ethical and that findings are communicated responsibly to the public. International collaborations can help harmonize regulatory standards and ensure that advances in genetic research benefit individuals with ASD globally[354]. To facilitate these collaborations, funding agencies and research institutions must prioritize and support initiatives that promote teamwork and data sharing[355]. Grant programs encouraging multidisciplinary and international projects can provide researchers with the necessary resources and incentives to work together. Establishing centralized databases and biobanks accessible to the global research community can also enhance collaboration and data integration[356].

Study Limitations:

This review, while comprehensive in its scope, has several limitations that must be acknowledged. Firstly, the rapid pace of advancements in genetic and epigenetic research means that some of the findings and technologies discussed may quickly become outdated as new discoveries emerge. Additionally, the review relies heavily on studies with varying methodologies, sample sizes, and population demographics, potentially leading to inconsistencies in the reported findings. The complexity and heterogeneity of ASD also pose significant challenges; the review attempts to cover a wide range of genetic and epigenetic factors, but this breadth may come at the expense of depth in some areas. Furthermore, the review predominantly focuses on genetic and epigenetic aspects, potentially underrepresenting other crucial factors such as environmental influences and their interactions with genetic predispositions. While efforts have been made to include various aspects of ASD research, selecting studies and sources might have introduced a bias, potentially overlooking relevant findings from smaller or less-publicized research efforts. The review also touches upon emerging

therapies and personalized medicine. Still, the practical implementation and accessibility of these advancements are not fully addressed, which could limit the applicability of the discussed interventions in real-world settings. Finally, while mentioned, ethical considerations surrounding genetic testing and personalized medicine are not explored in depth. Issues such as genetic privacy, consent, and the potential for discrimination require more comprehensive discussion to ensure the responsible application of genetic insights. In conclusion, while this review provides a broad overview of the genetic and epigenetic underpinnings of ASD and their therapeutic implications, it is constrained by the rapidly evolving nature of the field, potential methodological biases, and the need for a more in-depth exploration of certain areas and ethical considerations.

CONCLUSION

This systematic review highlights significant advancements in understanding the genetic and epigenetic underpinnings of ASD and their therapeutic implications. Genetic discoveries, such as mutations in CHD8 and SHANK3, and epigenetic factors like DNA methylation and non-coding RNAs, reveal the complex mechanisms contributing to ASD. These insights pave the way for personalized medicine, enhancing the precision of current treatments and informing future therapies like gene editing and epigenetic modulation. Emerging technologies, including CRISPR and advanced bioinformatics, accelerate research, offering deeper insights into genetic mutations. Collaborative efforts across disciplines and countries are crucial for advancing our understanding and translating findings into clinical practice. Despite these advancements, challenges remain, including the complexity of ASD's genetic architecture, gene-environment interactions, and ethical considerations of genetic testing. Continued research and collaboration promise more effective, personalized, and comprehensive approaches to managing ASD, improving outcomes and quality of life for those affected.

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