INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by very early onset of dysfunction in social communication and interaction, repetitive behavior, and limited interest. It is now believed that ASD is a result of complex gene–environment interactions, with strong and clear genetic influences. Studies of twin pairs, high-risk infant siblings, families, and populations have estimated concordance rates and segregation of the disorder within families. The concordance rate was reported as 60–70% in monozygous twins and as 5–30% in siblings; this is in agreement with a recent large prospective study revealing a recurrence rate of 18% in infant siblings and of 33% in multiplex families [1, 2]. However, it is currently believed that over 50% of the risk of developing ASD is attributed to genetic variation [3, 4].

Advances in genetic technologies, large cohort studies, and widespread database sharing have contributed to the discovery and validation of causative genes in ASD [5]. Knowledge from genetic studies of ASD also provides insight into other neurodevelopmental disorders, as ASD shares both behavioral characteristics and endophenotypes. However, ASD is one of the most heterogeneous neurodevelopmental disorders, with great variation observed in behavioral manifestations and cognitive profiles, which makes determination of the single most important genetic risk factor extremely difficult.

Identifying biomarkers has been one of the primary goals of biological research of ASD, and current research efforts are directed predominantly toward the identification of markers for risk and early diagnosis [6]. There have been intense research efforts to identify the genetic basis of ASD, with an assumption that the genetic markers can be utilized as essential biomarkers in the diagnosis of, and the development of pharmacological treatments for, ASD. The objective of this paper is to review the current knowledge of genetic variations in ASD, and its role in the identification of genetic biomarkers of ASD for diagnostic and therapeutic purposes.

Genetics of Autism Spectrum Disorder: Current Status and Possible Clinical Applications

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Autism spectrum disorder (ASD) is one of the most complex behavioral disorders with a strong genetic influence. The objectives of this article are to review the current status of genetic research in ASD, and to provide information regarding the potential candidate genes, mutations, and genetic loci possibly related to pathogenesis in ASD. Investigations on monogenic causes of ASD, candidate genes among common variants, rare de novo mutations, and copy number variations are reviewed. The current possible clinical applications of the genetic knowledge and their future possibilities are highlighted.

Key words: Autism spectrum disorder, Syndromic autism, de novo mutations, Genetic diagnosis
GENETIC VARIANTS

Common variants

The genetic architecture of ASD is diverse in frequency (common vs. rare variation), mode of inheritance (inherited vs. de novo variation), type of variation (single nucleotide, indel, or copy number variation [CNV]) and mode of action (dominant, recessive, or additive) [3, 7]. Common variation refers to genetic variation from the reference genome, which is present in >1% of the population. Common variants with small effects are thought to act additively in the development of complex traits in ASD [8]. One recent investigation reported that the liability of ASD is mostly attributed to common variation in the genetic architecture, and that rare de novo mutations contribute to individual liability (49% of common inherited variants, 3% of de novo, 3% of rare inherited variants, and 41% of unaccounted) [7].

Confirmation of the most specific, consistently replicated, and highly effective common variants involved in the pathogenesis of ASD is another issue. The first molecular genetic studies of autism were candidate gene association studies that aimed to discover common genetic variants in the form of single-nucleotide polymorphisms (SNPs). However, a large disadvantage of this is that it requires existing physiological, biochemical, or functional knowledge, which is either finite or unavailable [9]. These investigations have been hindered by inadequate sample sizes and sparse genotyping, resulting in a lack of reproducible markers except for a few plausible genes [9, 10].

The most consistently reported genes among the common variants include the gamma-aminobutyric acid (GABA) A receptor, beta 3 (GABRB3); oxytocin receptor (OXTR); reelin (RELN); serotonin transporter (SLC6A4); N-methyl-D-aspartate receptor (NMDA; GRIN2B); arginine vasopressin receptor 1A (AVPRA); engrailed homeobox 2 (EN2); integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61; ITGB3); met proto-oncogene (hepatocyte growth factor receptor; MET); and contactin-associated protein-like 2 (CNTCAP2) genes [11-22]. GABRB3, which is localized to chromosome 15q11–q13 and is involved in genome instability, gene expression, imprinting, and recombination, was investigated in the first era of ASD genetic research [13, 23]. This region became a major subject of attention because deletion of this locus is related to monogenic causes of ASD, Prader–Willi syndrome, and Angelman’s syndrome, and because GABA may be a pharmacological therapeutic target [13, 24, 25]. Oxytocin acts as a neuromodulator in the central nervous system, and induces social/affective bonding in animal models. The OXTR is a promising biomarker candidate, due to its genetic variants, functions on behavior, and positive results in human clinical trials [17, 26, 27].

However, these candidate gene studies also revealed that common variation has a weak effect when individual SNPs are investigated. A genome-wide association study (GWAS) avoids the need for a priori hypotheses for the primary cause of illness, and is a more appropriate approach for genetic studies of complex disorders such as autism [10]. GWASs have been applied to psychiatric disorders with complex phenotypes, and several variants have been reported [28]. Several GWASs have been conducted for ASD, and a few well-designed studies reported that common genetic variants on 5p14.1 and 5p15 were highlighted and replicated in two independent samples, each carrying a small increased risk (OR 1.2) or protective effect (OR 0.6) [29-31]. A significant association was observed with the CDH9 and CDH10 genes, but replications of this association was inconsistent [29-33]. Several studies identified significant SNP markers that were replicated in two or more independent samples and were associated with specific phenotypes of ASD, but the effect sizes were relatively small [29-35]. However, significant genome-wide results were not consistently reproduced across studies and ethnicities [33, 35].

Those inconsistencies can be attributed to the phenotypic heterogeneity of ASD and to relatively small sample sizes. Researchers attempted to decrease phenotypic heterogeneity by subphenotyping or using quantitative phenotypes, but this was unsuccessful in enhancing the substantial power of GWAS, resulting in the necessity of very large sample sizes, such as 50,000 individuals [5, 33, 34].

Rare variants and monogenic autism

Rare variation is genetic variation that is present in the population at a frequency of ≤1%. ASD can be expressed as the behavioral manifestation of known genetic syndromes, called syndromic autism, as opposed to idiopathic autism, which does not have known genetic causes. Syndromic autism often has dysmorphic features characterized by the genetic syndrome it belongs to and equal male:female ratios, unlike idiopathic autism, which occurs 4–5 times more frequently in males than in females [36]. Single-gene disorders, including fragile X (mutations in FMR1), tuberous sclerosis complex (mutations in TSC1 and TSC2), Dup15q syndrome, deletions in the 16p11.2 region, Rett syndrome (mutation in MeCP2), and neurofibromatosis (mutations in NF1), are detected in 3–5% of subjects with ASD, and are well-known as having an ASD phenotype as well as comorbid intellectual disabilities and epilepsy [37].

Recent development of whole-exome sequencing (WES) techniques has revealed that more than 25% of individuals with
ASD have identifiable, causative, and protein-disrupting rare genetic mutations [5]. However, single mutations account for no more than 1% of cases, mainly due to phenotypic heterogeneity and variable penetrance. Though the prevalence is not strikingly high, syndromic autism helps to understand core deficits of ASD as one of the phenotypes that specific genetic mutations carry, and acts as a gateway to explore the genetic etiology of ASD. The representative examples of monogenic autism and their clinical implications are summarized in Table 1.

### Table 1. Examples of monogenic “syndromic” autism and related phenotypes

| Mutations                  | Phenotypes                                                                 |
|----------------------------|----------------------------------------------------------------------------|
| Fragile X syndrome         | FMR1  Large, protruding ears, long face, hyperextensible joint, macroglossism, hypotonia, learning problem, intellectual disability, language impairment, developmental delay, attention problem, ASD [120] |
| Retts syndrome             | MECP2 Developmental regression, microcephaly, cognitive and motor impairment, epilepsy, stereotyped hand movement, severe repetitive behavior, severe ASD [36] |
| Tuberous sclerosis         | TSC1, TSC2 Brain tumors, multi-organ involvement (kidneys, lungs, heart, eyes and skin), learning difficulties, intellectual disability, self-injurious behavior, obsessive compulsive disorder, attention deficit hyperactivity disorder, aggression, epilepsy, ASD [121] |
| Neurofibromatosis 1        | NF1 Café au lait spots, neurofibromas, scoliosis, iris tumor, cognitive dysfunction, epilepsy, autism [122] |
| Cornelia de Lange syndrome | SMC1A/SMC3 Low birth weight, facial abnormalities, hearing and vision abnormalities, limb differences, heart defect, cleft palate, self-stimulation, self-injurious behavior, aggression, ASD [123] |
| Cohen syndrome             | COH1 Ocular abnormalities, obesity, thin arms and legs, micrognathia, deafness, intellectual disability, epilepsy, ASD [124] |
| Timothy syndrome           | CACNA1C Congenital heart disease, cardiac arrhythmias (long QT syndrome), webbing of fingers and toes (or syndactyly), immune deficiency, cognitive abnormalities, ASD [125] |
| Smith-Lemli-Opitz syndrome | DHCR7 Facial abnormalities (bitemporal narrowing, ptosis, short and upturned nose), micrognathia, finger and feet abnormalities, microcephaly, developmental delay, learning disability, behavioral abnormalities, hand mannerism, ASD [126] |
| Williams-Beuren syndrome   | 7q11.23 del Cardiac and gastrointestinal problems, hyperacusia, phonophobia, strabismus, esotropia, problems with visual processing, cerebellar signs, hypotonia, motor delay, intellectual disability, strong interest in people, lack of social inhibition, ASD [127] |
| Dup15q syndromes           | Del 15q11–q13, GABBR3 Hypotonia, facial dysmorphism (flat nasal bridge, epicanthal folds, deep set ear, high arched palate), small stature, gross and fine motor delays, cognitive delays, speech/language delays, behavior problems, sensory processing problem, epilepsy, ASD [128] |
| Prader-Willi syndrome       | Del 15q11–q13 (paternal allele) Specific face, hypogonadism, small hands and feet, hypopigmentation, hyperphagia, severe obesity, obsessive compulsive disorder, mood and behavior problem, ASD [37] |
| Angelman syndrome          | Del 15q11–q13 (maternal allele, UBE3A) Strabismus, unique facial dysmorphism, prominent mandible, wide mouth, sleep disturbance, severe developmental delay, speech impairment, ataxia, attention problem, frequent laughter, easily excitable personality, epilepsy, ASD [37] |
| 16p11.2 deletion syndrome  | 16p11 del Minor unusual facial and physical features, hypotonia, overweight, language delay, learning difficulty, epilepsy, ASD [49] |
| Smith-Magenis syndrome     | 17p11.2 del Facial dysmorphism (broad, square shaped face, deep set eyes, prominent lower jaw), short stature, scoliosis, eye abnormalities, reduced sensitivity to pain and temperature, sleep disturbances, behavioral problem, self-injurious behavior, stereotyped behavior (finger licking, flipping books), ASD [129] |
| 22q11 duplication syndrome | 22q11.2 dup Growth retardation, hypotonia, delayed psychomotor development, learning difficulty, intellectual disability, ASD [130] |
| DeGeorge syndrome          | 22q11.2 del Multi-organ involvement (heart, kidney, gastrointestinal system, skeletal abnormality), cleft palate, facial dysmorphism, immune system abnormality, low calcium level, hearing loss, developmental delay, learning difficulty, mental illnesses (schizophrenia, anxiety, mood disorders), attention deficit hyperactivity disorder, ASD [131] |
| Phelan-McDermid syndrome   | 23q13.3 del Dolichocephaly, hand and facial dysmorphism, ptosis, kidney problems, neonatal hypotonia, global developmental delay, intellectual disability, reduced sensitivity to pain, absent or severely delayed speech, ASD [39] |

ASD, autism spectrum disorder.
**Copy number variation**

Copy number variants (CNVs) are variations (duplication or deletion) in chromosomal structure of greater than 1,000 nucleotides, usually a section of DNA with a length from 1 kb to several Mb. CNVs can be either common or rare, transmitted or de novo, and are widely distributed in human genome, accounting for a substantial proportion of genetic variation [5, 38]. Studies have revealed an increased frequency of CNVs in individuals with ASD compared to normal controls and several de novo CNVs in children with autism, suggesting excessive genomic instability. The frequency of de novo CNVs in ASD has been reported as 3–19% in ASD from simplex and multiplex families, compared to approximately 1% in healthy controls [39-41].

Genomic imbalances associated with ASD are classified as recurrent and nonrecurrent events. Recurrent events are non-allelic homologous recombination, with reciprocal dosage imbalances (deletion and duplication) in different individuals [42]. Notable examples of recurrent CNVs are microdeletions and duplications in chromosome 1q21, 15q13, and 16p11.2, and microdeletion syndrome in chromosomes 2p15-2p16.1, 17p11.2, and 17q12 [43-49]. CNVs are associated with a wide range of phenotypic heterogeneity, including dysmorphic features, intellectual disabilities, language impairments, attention problems, hyperactivity, aggression and other behavioral problems, mood disorders, and schizophrenia, which implicates those variants might not be a specific cause of social disability in ASD [18].

**Synaptic genes**

Of the genetic variations studied regarding ASD, the most consistently reported genetic abnormalities are mutations in synaptic genes, including neuroligins (NLGN), SH3 and multiple ankyrin repeat domains (SHANK), neurexin (NRXN) families, and contactin associated protein-like 2 (CNTNAP2) [50-62]. Mutations in synaptic genes are not specific to ASD, and are also found in other neuropsychiatric disorders, such as schizophrenia and Alzheimer’s disease [63, 64]. However, as these neuropsychiatric conditions share common features with ASD, such as cognitive dysfunction, limited emotional expression, and lack of social reciprocity, synaptic dysfunction is still considered a common pathway of these major, chronic neuropsychiatric illnesses [5, 18].

NLGNs are known to act as splice site-specific ligands for beta-neurexins and be involved in the formation and remodeling of central nervous system synapses (http://www.ncbi.nlm.nih.gov/gene/54413). The identification of a de novo, loss of function mutation in neuroligin 4, X-linked (NLG4X) in an affected mother that was transmitted to two affected boys first suggested the possibility of synaptic dysfunction involvement in ASD [65]. This was followed by the identification of a single-base missense mutation of NLGN3 in another family [53]. These findings have been replicated in other studies, and NLGN3, NLGN4, and NLGN4Y were found to be possibly associated with ASD. However, mutation of those genes in ASD is relatively low (0.6–3.3%), and the clinical phenotypes and neurobiological characteristics of these mutations are also quite diverse, including ASD, intellectual disabilities, and Tourette syndrome, and inconsistent across ethnicities [51, 54, 55, 61, 62].

A second family of genes possibly associated with ASD is the SHANK genes (SHANK1, SHANK2, and SHANK3), encoding synaptic proteins that may function as molecular scaffolds in the postsynaptic density of excitatory synapses (http://www.ncbi.nlm.nih.gov/gene/22941). SHANK3 is the most widely studied, but SHANK1 and SHANK2 are also implicated by de novo deletions observed in subjects with ASD [50, 57-59]. Durant et al. (2007) reported eight non-synonymous mutations in ASD patients that were not present in healthy controls; rare de novo mutations in SHANK3 located in chromosome 22q13.3 were identified in probands and families with ASD in many studies [50]. Rare mutations and genomic deletions have been reported in different SHANK3 loci, with a frequency of 0.2–0.8% of probands in ASD [50, 57, 66-69]. Mutations in SHANK3 are gaining attention, as they are related to Phelan-McDermid syndrome (PMS) and 22q13 deletion syndrome, and are one of the known genetic causes of ASD. PMS is characterized by autism or autistic-like behavior in more than 50% of subjects, and is accompanied by neurological deficits, including global developmental delay, moderate to severe intellectual impairment, absent or severely delayed speech, and neonatal hypotonia [59].

The transmission pattern of SHANK3 mutations is variable; inheritance from healthy parents and existence in unaffected siblings were reported [50, 57, 67]. Recently, Nemirovsky et al. (2015) reported germline mosaicism for a heterozygous cytosine deletion in exon 21 of SHANK3 by whole-genome sequencing in three male siblings from a segregated family exhibiting phenotypes of severe intellectual disability, absence of language, autism spectrum symptoms, and epilepsy [58]. As with other potential candidates, the associated phenotypes of SHANK3 mutations are not specific for ASD, but SHANK3 is regarded as one of the potential causative genes and therapeutic targets of ASD, based on animal and cellular model studies.

Other important synaptic genes are NRXN1, NRXN2 and NRXN3, encoding neuroligins. This trans-synaptic complex is required for efficient neurotransmission, and they are involved in the formation of synaptic contacts by interaction with
neurexins [60]. Neuriligin aggregation is synaptogenic, but exhibits specificity: NLGN1, NLGN3 and NLGN4 link only to glutamategic postsynaptic proteins, but NLGN2 links to both glutamategic and GABAergic postsynaptic proteins [52]. In the earlier era of ASD genetic studies, CNVs were found to disrupt the locus containing NRXN1, but this was inconsistent, with a high unaffected carrier frequency of deletions [70-73]. More recently, there have been relatively large cohort studies that describe a higher rate of deletions in the NRXN1 region located in the probes of chromosome 2p16.3 associated with ASD, compared to healthy controls, with an overrepresentation of small-sized inverted repeats [72, 74]. Shared psychopathologies related to the deletions were developmental delays, speech delays, abnormal behaviors, including ASD, and some degree of dysmorphism [72]. CNTNAP2 is another candidate gene suggested to be associated with ASD by human and animal model studies. Family-based association studies identified a common variant (rs7794745) that was associated with increased risk of autism, and another study revealed an amino acid substitution in the CNTNAP2 protein in children with autism [75-77]. Variation of the CNTNAP2 gene and age at first word, a language development phenotype, are both related to autism rather than to the diagnosis itself, and raises the implication that genetic variants of a quantitative phenotype of ASD interact with FOXP2 [75]. The functional relevance of CNTNAP2 genetic variants has been validated in animal models; CNTNAP2 (-/-) mice exhibited deficits in all three core behavioral deficits of ASD, as well as hyperactivity and epileptic seizures, and improved repetitive behavior of mutant mice [78]. A large-sized GWAS study failed to demonstrate a significant association of the marker noted in the previous studies, and no associations between rare heterogeneous mutations of CNTNAP2 and ASD were observed [29, 79]. However, a recent investigation revealed the possible involvement of novel functional variants of the 5' -promotor region, mediated by alterations in transcription-binding sites in subjects with ASD. CNTNAP2 is still regarded as one of the potential causative genes of ASD that warrants further research [80].

These findings indicate the possibility that ASD might be caused by abnormalities in synaptic plasticity, as indicated by proteins that play essential roles in synaptic development and modification. There is evidence that NRXN (presynaptic) and NLGN (postsynaptic) interact as synaptic adhesion molecules, providing mechanistic support in synaptic formation and maintenance [81]. Alternate splicing of NLGN controls selective binding with α- and β-NRXN, provides synaptic diversity, and regulates function. Alternate splicing also controls the variable interaction of NRXN with other postsynaptic ligands, such as in glutamategic, GABA-ergic, and cholinergic synapses [81, 82]. The SHANK family includes postsynaptic scaffolding proteins, which link multiple receptor signaling complexes and the actin cytoskeleton that are essential for maintaining synaptic function [83]. SHANK3 directly and indirectly binds to CNTN and NLGNs and interacts with presynaptic glutamategic receptors [83, 84]. The molecular function of CNTNAP2 is relatively unclear, but it is known to encode a neuronal transmembrane protein member of the NRXN superfamily that is involved in neural–glia interactions and clustering of potassium channels in myelinated axons (http://www.omim.org/entry/604569).

Overall, despite of low frequency of de novo mutations in affected individuals, inconsistencies in genetic analysis results, and phenotypic heterogeneity, synapse-related genes are crucial candidates of ASD, and provide baseline evidence for testing compounds that can enhance synaptogenesis for the treatment of the core symptoms of ASD [25].

Genetic networks

For some of the identified genes, genetic pathways were identified at the cellular and molecular level based on genetic network analyses and genetic functional studies. Besides synaptic development and function mentioned in the previous section, protein synthesis and metabolism, modulation of transcription process, chromatin remodeling, calcium signaling, and mTOR and oxytocin pathways have also been implicated [85-87]. This suggests that genes involved in ASD might be related each other in several convergent functional units, especially in neuronal development, modulation, and intracellular transcriptional mechanisms. As more and more causative genes of ASD are identified, their interaction within the context of functional significance would be clarified. Molecular pathways possibly involved in pathogenesis of ASD are summarized in Fig. 1 [86].

Genes and brain circuits

Theoretically, ASD is an end-product of gene-environment interaction, mediated by changes in brain physiology, function, and morphology causing cognitive and behavioral dysfunction. Multiple brain areas involving facial recognition, emotion evaluation, empathy, mentalization, social cognition and behavior are known to be associated with ASD as part of the “social brain network” [88]. Alterations in brain connectivity and morphology might be a potential endophenotypes of ASD. However, not many human studies have explored associations between specific genetic variants and brain circuits or morphological phenotypes. One recent study examined phenotypic characteristics of subjects with ASD carrying germline heterogeneous PTEN mutations...
Heejeong Yoo

Heejeong Yoo compared subjects with idiopathic (non-PTEN) ASD and healthy controls, subjects with PTEN-ASD showed prominent cognitive dysfunction and white-matter abnormalities, mediated by reductions in the PTEN protein [89]. Moreover, significant differences in fMRI activation and deactivation patterns to social stimuli as well as functional and structural connectivity in the temporo-parietal region based on the existence of the rs1858830 MET risk allele (C/C) highlighted alterations in gene-brain pathway in ASD [14]. More data are necessary to understand the complex pathways connecting specific genetic mutation/genotype and brain changes, which have a direct impact on specific behavioral phenotypes of ASD.

**Fig. 1.** Molecular pathways implicated in neurodevelopmental disorders. RTKs, receptor tyrosine kinases; iGluRs, metabotropic glutamate receptors; PGC-1α, peroxisome proliferator–activated receptor gamma coactivator 1-alpha; SREBP, sterol regulatory element–binding proteins; HIF1α, hypoxia-inducible factor 1 alpha; ULK1, unc-51-like kinase 1; ARC, activity-regulated cytoskeleton-associated protein; UBE3A, ubiquitin protein ligase E3A; MeCP2, methyl CpG binding protein 2; FMRP, fragile X mental retardation protein; PI3K/mTOR, phosphatidylinositol 3-kinase/mammalian target of rapamycin; PSD-95, postsynaptic density protein 95; CNTNAP2, Contactin-associated protein-like 2; NFI, neurofibromin 1; PLCβ, Abstract Phospholipase C β; SYNGAP1, Synaptic Ras GTPase-activating protein 1; ERK1/2, extracellular signal-regulated kinase; PTEN, Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase; PDK1, Pyruvate dehydrogenase lipoamide kinase isozyme 1; PKC, Paroxysmal kinesogenic choreoathetosis, neurological disorder Protein kinase C; AKT, Protein kinase B; AMPK, AMP-activated protein kinase; TSC2, Tuberous Sclerosis Complex 2; TSC1, tuberous sclerosis 1; RHEB, GTP-binding protein Rheb, DEPDC5, DEP domain-containing 5; mTORC1, mammalian target of rapamycin complex 1; mGluR, metabotropic glutamate receptor; SHANK, Shank protein; ATG13, Autophagy-related protein 13; HDAC, Histone deacetylases; CHD8, Chromodomain-helicase-DNA-binding protein 8; MEF2, myocyte enhancer factor-2; RAS, Ras protein; TBC1D7, TBC1 domain family, Member 7; 4E-BPs, eIF4E-binding proteins; FIP200, a ULK-interacting protein; S6K1/2, Anti-RIBOSOMAL S6 KINASE 1/2; HOMER, homer scaffolding protein; RAGA/B, Ras-related GTP binding A/B ortholog; RAGC/D, Ras-related GTP binding C/D ortholog. From M. Sahin and M. Sur, Genes, circuits, and precision therapies for autism and related neurodevelopmental disorders, Science 350, aab3897 (2015). DOI: 10.1126/science.aab3897. Reprinted with permission from The American Association for the Advancement of Science (AAAS).
In its current status, clinical value is primarily focused on the identification of known genetic causes of ASD. It is recommended that once a clinical diagnosis of ASD is made, genetic testing should be initiated [96]. This includes single-gene tests for monogenic causes of ASD, including fragile X syndrome, tuberous sclerosis complex, Rett syndrome, Angelman syndrome, Prader-Willi syndrome, phosphatase and tensin homolog (PTEN)-associated disorders, Noonan syndrome, cortical dysplasia-focal epilepsy syndrome (associated with CNTNAP2), and Phelan-McDermid syndrome, by detecting point mutations, microdeletions, duplications, and large repeat expansions using sequencing, fluorescence in situ hybridization (FISH), and Southern blotting technologies [95]. An assay for CNVs with array comparative genomic hybridization (aCGH) is also available for known variations of ASD [96]. At the clinical level, practice guidelines of the American College of Medical Genetics (ACMG) recommends a three generation family history with pedigree analyses; an initial evaluation for known syndromes associated with ASD, especially if the subject has dysmorphic features or specific clinical indicators; a chromosomal microarray; and DNA testing for fragile X for all male children suspected of an ASD as the first tier genetic evaluation. ACMG recommends sequencing and duplication testing for the MECP2 gene in female patients, and PTEN testing for those with macrocephaly as second tier genetic testing [97, 98].

There are ethical considerations of the clinical use of genetic testing and counseling for ASD. First, it is important that biomarker discovery, especially commercialization of biomarker data in autism, does not result in children being given a biological label that fixes and defines their potential and treatment [99, 100]. Second, genetic biomarker results have a huge impact on parental decision-making toward reproduction; therefore, more research and communication is needed for a better understanding of parental needs and attitudes [100].

Pharmacological treatment of ASD is primarily focused on improving comorbid behavioral emotional symptoms, such as irritability, aggression, anxiety, tics, self-injurious behaviors, and epilepsy [101]. One of the ultimate goals of the discovery and validation of biomarkers is developing molecular therapeutics to treat the core symptoms of ASD, based on knowledge about disease modifying mechanisms. Identification of causative genes, especially from high-throughput methods such as NGS, is paving the way for developing pharmacological agents to treat the core symptoms of ASD, including abnormal reciprocal social interaction and communication. Several recent well-designed studies have modeled human genetic variants associated with ASD in mice, using SHANK3, CNTNAP2, MECP2, UBE3A, and
**NGS in ASD Genetics**

High-throughput technologies have facilitated gene discovery in ASD. The most recent development, NGS, which include whole-genome sequencing (WGS), whole-exome sequencing (WES), and targeted sequencing, promotes the precise identification of genetic variants at base-by-base levels. NGS promotes the identification of rare alleles to a degree not possible using genotyping platforms, and allows identification of single gene defects and partial variations of gene function [37, 115]. NGS in ASD is still emerging; it is useful to identify novel de novo variants not observed by conventional methods. Recent studies have confirmed de novo CNV loci in large-sized cohorts of idiopathic ASD families, observed new candidate loci, and confirmed de novo mutations in subjects with ASD and other neuropsychological abnormalities [58, 116-118]. Regarding diagnostic utility, one recent study reported complicated results that the diagnostic yield of WES is comparable to chromosomal microarray, while combined diagnostic yield is higher among children with more complex morphological phenotypes, emphasizing the importance of phenotypic classification [119].

Although NGS is not yet widespread due to its relatively high cost and the demand for techniques involving large-scale data and bioinformatics, it is predicted that large-scale investigations combining NGS technology and accumulated clinical data will facilitate the genetic study of ASD from diagnoses to targeted treatments in the future [5].

**Conclusion**

A wide range of genetic variation is involved in ASD, with interplays of gene–gene and gene–environment interactions. Both genotypic and phenotypic heterogeneity contribute to the difficulty in the thorough exploration and confirmation of causative genetic factors. However, recent technological developments, including NGS, and the accumulation of clinical information are bridging the gap in the application of genetic knowledge towards clinical practice.

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