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Advances in Genetic Discovery and Implications for Counseling of Patients and Families with Autism Spectrum Disorders

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Abstract

The prevalence of autism spectrum disorders (ASD) continues to increase. Genetic factors play an important role in the etiology of ASD, although specific genetic causes are identified in only a minority of cases. Recent advances have accelerated the discovery of genes implicated in ASD through convergent genomic analysis of genome-wide association studies, chromosomal microarray, exome sequencing, genome sequencing, and gene networks. Hundreds of candidate genes for ASD have been reported, yet only a handful have proven causative. Symptoms are complex and highly variable, and most cases are likely due to cumulative genetic factors, the interactions among them, as well as environmental factors. Here we summarize recent findings in genomic research regarding discovery of candidate genes, describe the major molecular processes in neural development that may be disrupted in ASD, and discuss the implication of research findings in clinical genetic diagnostic testing and counseling. Continued advances in genetic research will eventually translate into innovative approaches to prevention and treatment of ASD.

Keywords

Autism Spectrum Disorders (ASD); copy number variation (CNV); *de novo* mutation; incidental findings (IF); next-generation sequencing (NGS); variants of uncertain significance (VUS)

Introduction

The term Autism Spectrum Disorders (ASD) describes a heterogeneous group of neurodevelopmental symptoms including difficulties with social interactions, deficits in verbal and nonverbal communication, and repetitive or stereotypical behaviors. The Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-V) subsumes

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Conflict of interest

J Shen and S Lincoln both declare no conflict of interest. DT Miller is a Clinical Consultant and Medical Director for Claritas Genomics (no equity), a majority owned subsidiary of Boston Children's Hospital.

Human and Animal Rights and Informed Consent

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several earlier diagnostic subgroups into the category of ASD, including autistic disorder, childhood disintegrative disorder, pervasive developmental disorder-not otherwise specified (PDD-NOS), and Asperger syndrome [1•]. Prevalence continues to increase, from ~1/88 children in 2008 to 1/68 in 2010, according to the Autism and Developmental Disabilities Monitoring Network of the Centers for Disease Control and Prevention [2, 3]. Potential reasons for the increase have been summarized elsewhere and are somewhat debatable [4•].

Multiple lines of evidence support that genetic factors predispose to ASD. First, twin studies in different populations consistently show a much higher concordance rate in monozygotic twins than in dizygotic twins [5–7]. Second, family history of ASD predicts an increased risk for ASD in subsequent children born to the same parents. The 2013 American College of Medical Genetics and Genomics (ACMG) revised practice guideline relies on established data to suggest a recurrence risk of 7% if the proband is female and 4% if the proband is male [8••]. If two or more children are affected, recurrence risk increases to about 33% or more. Finally, increased availability of genetic testing in both research and clinical settings has uncovered numerous genetic variants implicated in ASD.

In providing clinical guidance to individuals or families affected by ASD, the goal is to refine the specific recurrence risk for that family, which can range from very low, such as 1% or less in the case of *de novo* genetic variants, to much higher than the population average, such as 25% in the case of an autosomal recessive disorder. Moreover, the cause in many patients cannot be attributed to a single gene or genetic variant, and is more likely to be multifactorial or complex in nature, as reviewed elsewhere [4•]. Clinicians and researchers interested in the genetic underpinnings of ASD have hoped that improved technologies, such as high throughput next-generation sequencing (NGS) and advanced data modeling, would allow collection and analysis of exponentially more genetic data, and subsequently help to elucidate the causes of these more complex forms of ASD. This review will explore the current state of gene discovery and molecular pathway modeling for ASD, with further discussion on the current state of clinical testing and how adding more comprehensive genomic testing, such as exome sequencing, is impacting genetic diagnosis and counseling of patients with ASD and their families.

Approaches to ASD gene discovery

Human disease genes were traditionally discovered through positional cloning, where the genetic locus was first mapped to a chromosomal region followed by extensive sequencing within the candidate region. Loci for ASD have been discovered through linkage analysis of informative pedigrees, genome-wide association studies (GWAS) in large case and control cohorts, and chromosomal analysis of structural abnormalities that disrupt certain loci. Unlike highly penetrant single gene disorders, inheritance of ASD follows a complex pattern, and the penetrance and expressivity of disease phenotypes may vary even within a family. Therefore, conventional family based linkage mapping has been limited to discovery of genes for specific single gene syndromes where ASD may present as a clinical feature or co-morbidity [9]. These genes then became candidate genes to screen for mutations in patients. Alternatively, a significant number of genetic loci were discovered through sib-pair studies in large ASD cohorts as well as through GWAS [10–15]. However, due to the

complexity and extreme heterogeneity of symptoms, few GWAS studies have yielded reproducible results.

Loci for ASD have also been identified through cases of aneuploidy and other chromosomal abnormalities [16, 17]. These were traditionally diagnosed through G-banded karyotyping and fluorescence *in situ* hybridization (FISH), especially in subtelomeric regions [18]. Genes that were deleted or disrupted at the breakpoints became new ASD candidate genes. However, it was difficult to prove causality because multiple disease alleles were rarely found. Although causative genes in many ASD loci remain elusive, according to SFARI Gene (gene.sfari.org/), hundreds of candidate genes with thousands of variants implicated in the etiology of ASD have been reported, especially with recent technological advances in genome-wide chromosomal and sequencing analyses [19].

The majority of genes for ASD have been discovered through a candidate gene approach. As researchers identified the causes of ASD-associated single gene disorders, such as fragile X syndrome, Rett syndrome, macrocephaly/autism syndrome, tuberous sclerosis complex (TSC), Angelman syndrome, and Timothy syndrome, these genes were screened in ASD patients and rare variants were discovered in patients who might or might not show typical features of the single gene disorder [20–23]. Since intellectual disability, epilepsy, and psychiatric disorders show considerable co-morbidity with ASD, many genes associated with these conditions have also been included as candidate genes to screen for mutations in cohorts of patients with ASD [24–28]. However, pathogenic variants in these genes only account for a small subset of patients, and the clinical significance of many rare variants in the candidate genes remain uncertain. Nevertheless, these genes are the basis of current gene panels to test for ASD.

High-throughput genome-wide chromosomal copy number analysis by chromosomal microarray (CMA) has been a major breakthrough in gene discovery and clinical diagnostics. CMA has revealed a great number of copy number variants (CNV), including both gains and losses of chromosomal material, over-represented in patients with ASD. Marshall *et al.* reported that 44% of families have CNVs not found in >1,600 controls and *de novo* CNVs occurred in ~7% of families with one affected child [29]. CMA technology has revealed new microdeletion/microduplication syndromes associated with ASD based on recurrent observation of pathogenic CNVs in affected individuals, such as deletions and duplications at chromosome 1q21.1, 15q13.3, and 16p11.2 [11, 29–34]. Recurrent CNV hotspots are due to non-allelic homologous recombination mediated by segmental duplication genomic architecture and CNV regions associated with ASD are typically large (>400 kb), supporting a multigenic etiology [35•]. Furthermore, the widespread adoption of CMA revealed rare or *de novo* CNVs associated with autistic traits in up to 10% of sporadic ASD cases [36–39]. Because of the relative high detection rate of abnormal findings with this approach, CMA has been recommended as the first tier genetic test for non-specific ASD [8••].

More recently, high-throughput high-resolution sequencing analysis has accelerated gene discovery. Exome sequencing is a large-scale NGS based approach targeting protein coding regions and splicing sites, which comprises ~1.5% of the genome. Because every individual

has millions of variants in the genome including on average ~100 loss-of-function variants [40], presence of a disrupted gene in an individual with ASD alone does not establish a pathogenic role of the gene [41•]. Exome sequencing identified genes contributing to ASD based on several additional lines of evidences. First, variants occur in candidate genes known to cause neural developmental disorders related to ASD, such as intellectual disability, seizures, and schizophrenia, thus strengthening their association with ASD. Second, variants in genes fall within mapped ASD loci, implying a high *a priori* likelihood of being pathogenic [42–44, 45•]. Third, distinct rare *de novo* changes in the same gene are observed in multiple unrelated patient with ASD, and statistically significantly lower in control populations or exhibit transmission disequilibrium in parent-child trios [46, 47•, 48–54]. Finally, variants may be in genes involved in neural development based on animal models and biological functional studies (Table 1).

Targeted sequencing methods, including exome sequencing, only focus on a very small part of the genome, and are limited in the ability to detect structural variation such as deletion or duplication of chromosomal regions. Sequencing may detect balanced chromosomal abnormalities (BCA) if the breakpoints happen to occur within the captured region, but such information is not currently available from clinical exome sequencing. While CMA can detect CNVs, it cannot detect BCAs and does not reveal precise breakpoints at nucleotide resolution. In theory, whole genome sequencing (WGS) can detect structural and sequence variants if sequenced at sufficient read-depth. However, at the present time, deep WGS is still cost prohibitive and computationally demanding. Jiang *et al.* reported only 12% specificity and 75% sensitivity in detecting CNVs of >10 kb with WGS at >30× average read-depth coverage [55•]. An intermediate approach that is currently feasible in the clinical setting is to map BCAs by NGS of whole-genome large-insert libraries with tremendously improved resolution comparing to the traditional karyotyping method, and several new ASD candidates were identified through this approach [56, 57••].

Furthermore, convergent genomic analysis combining GWAS, CNV analysis, exome sequencing, and/or transcriptome profiling has been proven to achieve substantially increased statistical power [58, 59•]. Comparing the transcriptome profiles of autistic vs. normal brains, Voineagu *et al.* identified modules of gene networks based on gene co-expression patterns exhibiting distinct regional patterns in normal brains, and found the distinctions were lost in brains of those with ASD. Furthermore, they found susceptibility genes were enriched in a neuronal module and under-expressed in cases [60••]. Integrative approaches have begun to shed light on the convergent molecular pathways involved in the pathophysiology of ASD [61]. In addition, it has been suggested that epigenetic factors regulating DNA methylation and chromatin modification, as well as non-coding regulatory elements and microRNAs, also play a role in the pathogenesis of ASD due to dysregulation of gene expression in the central nervous system [62•, 63•].

Molecular Processes Involved in ASD

Recent advances in genetic analysis technologies have led to the discovery of many new candidate genes for ASD. Convergent evidence for involvement of candidate genes in neural development and plasticity has emerged from co-expression, co-regulation, and protein

interaction network analyses of these genes [64]. Studies have shown that genes involved in important neural developmental processes including cortical organization, synaptic formation, and regulation of gene expression in the central nervous system are implicated in ASD [60••, 65–68]. Functional imaging studies in patients with ASD support a model of atypical neural connectivity as the common underpinning of the impaired social cognition [69]. Many candidate genes facilitate normal cortical architecture, and alterations in these genes contribute to abnormal neural connectivity. Genes associated with brain malformation are known to cause intellectual disability and epilepsy, which are common co-morbidities of ASD. Neuroimaging studies of patients and RNA *in situ* hybridization studies of postmortem brains from children with ASD showed abnormal laminar organization [70]. Developmental co-expression analysis revealed that genes with rare *de novo* variants in affected probands exhibit enriched expression in superficial layers of the cortex [67]. Mouse models of ASD associated genes showed structural defects in the brain, such as macrocephaly / neuronal hypertrophy (Pten and Tsc1/Tsc2) and lissencephaly / abnormal neuronal migration (Dcx, Reln, and Cntnap2), prior to the onset of behavioral abnormalities, suggesting symptoms arise from abnormal brain development [71]. MRI of patients with ASD and brains of mouse models of 16p11.2 CNV also showed changes in brain architecture [69, 72].

Alterations in genes related to synaptic function also contribute to ASD susceptibility through impairment in synapse development, neurotransmission, and activity-dependent synaptic plasticity that lead to improper neuronal connectivity. Recent *in vitro* studies and animal models have continued to demonstrate that the most convincing susceptibility genes are all involved in this process (Table 1). Neuronal activity induces both local changes at the synapse and transcriptional regulation in the nucleus. The fragile X mental retardation protein (FMRP), an RNA binding protein, as well as hamartin (TSC1) and tuberin (TSC2) complex that inhibits the mammalian target of rapamycin (mTOR), regulate local protein synthesis at synapses. The mTOR pathway is regulated by PTEN. Presynaptic neurexins (NRXN) interact with postsynaptic neuroligins (NRLGN) and SHANK proteins at synaptic junctions to help regulate synapse formation. NRXN1 undergo activity-dependent splicing, which regulates neurotransmitter release, and binds to neuroligins to modulate specific types of neurotransmitter receptors to maintain the balance of excitatory and inhibitory synapses [73–76]. The SHANK proteins have multidomain scaffolds at the postsynaptic densities, which organize neurotransmitter receptors, ion channels and cytoskeleton. Disruption of any components of the synaptic function may lead to impaired activity-dependent neural circuitry formation.

Dysregulation of global gene expression in the central nervous system is another molecular hallmark of ASD revealed through genetic research. This may occur at the epigenetic, transcriptional, splicing, translational, or post-translational level. Epigenetic mechanisms including genomic imprinting, DNA methylation, and histone modification have been linked to ASD. For example, activity-dependent phosphorylation at critical sites of MeCP2 leads to genome-wide change of transcriptions [77]. The role of MeCP2 in chromatin remodeling has long been established [78, 79]. New evidence suggests that it may also regulate gene expression through suppressing microRNA processing [80]. Topoisomerase regulates the transcription of long transcripts including non-coding ones, many of which are implicated in

ASD [81]. New evidence suggests that alternatively spliced isoforms from brain contributes to 30% of unknown protein-protein interactions [68]. Single-cell long-read mRNA sequencing confirmed extensive alternative splicing in generating the diversity of neurexins [82, 83]. DNA methylation can also modulate splicing [84]. Modulation of protein homeostasis by ubiquitin-protein ligase UBE3A adds additional dynamic control of synaptic proteins [85]. All these findings are consistent with a multigenic complex model for ASD.

Evaluating a Patient to Select an Appropriate Genetic Test for ASD

Choosing the most appropriate genetic testing for patients with ASD may seem overwhelming due to the wide variety of tests available and the wide variety of genetic variants contributing to the susceptibility for ASD. Guidelines exist for the clinical evaluation of ASD and include taking a 3-generation pedigree and dysmorphology examination. Consultation with a clinical geneticist should be considered for patients with dysmorphism or other syndromic features. If a specific syndrome is suspected, targeted testing should be performed first; but if the evaluation is non-specific, testing via CMA and fragile X syndrome (for males) is indicated. Second tier testing recommends *MECP2* analysis for all females with ASD and *PTEN* analysis only if the head circumference is >2.5 SD above the mean [8]. Recently, multi-gene panels for ASD have become clinically available. These panels target genetic syndromes that include autism or autistic features as part of the clinical profile and genes that have been associated with non-syndromic ASD, including many of those listed in Table 1. At this time, guidelines have not been established as to when these panels should be ordered, and studies have not been performed to assess the clinical utility of these panels. Our clinical experience has been that these panels are most helpful in individuals with ASD and dysmorphic features, congenital anomalies, seizures, or other medical issues [4•].

Counseling Challenges Related to Genome-Wide Genetic Testing

Selecting appropriate tests for a given patient is only one challenge. Genome-wide approaches to testing, such as CMA and WES, create many challenges for result interpretation and counseling. Many of these issues are not unique to testing for ASD, but are properties of the testing methodology. First, testing multiple genes or genomic regions either by CMA, gene panels, or WES/WGS, increases the likelihood of identifying variants of uncertain significance (VUS). VUS are relatively common findings, but there is little empiric data about the impact of receiving VUS results. Studies suggest that VUSs can cause concern for families if not expected or explained correctly [86–88]. Reiff et al. (2012) studied how families understand CMA results using semi-structured interviews with 31 parents of 25 pediatric outpatients who received either pathogenic (n=11) or VUS (n=14) results and found that incomplete comprehension (defined as an individual's self-reported ability to grasp the meaning of the result) of test results and a need for more information to improve understanding of results were prominent issues for parents [86]. A survey of 40 physicians found that their comfort levels of explaining CMA results to families were lowest for VUS (score of 3.46 on a 6-point Likert scale with 6 being the highest comfort level) compared to a normal or abnormal result [87]. Physicians also felt that parents did not have a good understanding of CMA results (score of 2.49 on a 6-point Likert scale), despite

families reporting a good understanding of CMA results in a prior study by the same group [86–88].

Second, genomic testing by CMA, WES, or WGS may identify variants that have clear clinical significance but are unrelated to the reason for testing, so-called incidental findings (IF). For example, CMA may identify CNVs conferring an increased risk of adult-onset cancer in approximately 0.1–0.2% of individuals tested [89–91]. A review of CMA testing on 18,437 patients identified 34 patients with copy-number gains or losses that included genes or gene regions associated with recognized cancer syndromes, and 24 of these patients were referred for CMA for suspicion of syndromes not related to cancer [89]91]. Twenty-nine of 4,805 patients (0.6%) referred for developmental delay, behavioral abnormalities, and birth defects had CNVs involving cancer predisposition genes, and 23 had no symptoms or family history for a cancer predisposition syndrome [90]. In another study, 5,548 CNVs were identified among 9,005 patients, fetuses, and their parents referred for clinical suspicion of a genetic/genomic disorder, and 85 CNVs affected 41 unique genes associated with adult-onset disorders, including *PMS2*, *DMD*, and *SPAST*. None of the cases had clinical symptoms highly suggestive of a phenotype related to the affected gene [91]. Data on the frequency of IFs in WES/WGS is limited, but is estimated as 3.4% and 1.6% for individuals of European and African descent, respectively, for high-penetrance actionable pathogenic or likely pathogenic variants in adults [92]. Both the ACMG and National Society of Genetic Counselors (NSGC) have published policies for reporting of IFs [93].

Another general issue that arises with finding VUS and IFs is the need for testing parents and possibly other family members to assess *de novo* status in the child, segregation with ASD traits in the family, or bi-parental origin of variants in a recessive gene. Parents may not be available for testing or may not wish to be tested for a VUS or IF. Parental testing also may not be sufficient in interpreting VUS in ASD. Although *de novo* mutation plays an important role in ASD, and hypermutability is a characteristic of genes involved in ASD [94•, 95••], *de novo* status alone does not establish causality[41•]. Therefore, parental testing may confirm a *de novo* variant, but additional information is still needed to determine the clinical significance of the variant. Another reason parental testing may not be sufficient is that some CNV may include an autosomal recessive gene. One study showed that the average genomic carrier burden for severe pediatric recessive mutations was 2.8 and ranged from 0–7 [96, 97]. Should one parent be found to carry the same CNV as in the child, the question of doing full gene analysis for the other parent arises. This may not be feasible as clinical testing may not be available or insurance may not cover the cost of this testing.

Counseling Regarding Risk for ASD in Offspring

If no genetic etiology for ASD is identified, counseling families for recurrence risk is based on epidemiological data. The risk to siblings of individuals with ASD is considered to range from 3–10% [98–100]. However, one study found the rate to be as high as 18.7% in infants with at least one older sibling with ASD, with male gender and having more than one sibling with ASD increasing the risk of developing ASD [101]. It is important to note that these previous studies were based on DSM-IV criteria, and recurrence risk numbers may change with the new DSM-5 criteria for ASD. Based on a cohort of 2,049,973 Swedish children

born between 1982 and 2006, a recent study estimated the heritability of ASD at 0.50 (95% CI, 0.45–0.56), and may provide the most accurate estimates regarding recurrence risk [102••]. The authors calculated a relative recurrence risk (RRR) to measure familial aggregation of disease. Based on a cohort of 14,516 children diagnosed with ASD, the RRR among dizygotic twins and full siblings were similar with RRR of 8.2 (95% CI, 3.7–18.1) and 10.3 (95% CI, 9.4–11.3), respectively. Overall, these recurrence risk numbers are similar to prior estimates endorsed in the ACMG 2013 Guideline [8••]. One limitation of the study is the lack of data regarding gender of the affected sibling, which may influence recurrence risk counseling.

Finally, WES has clarified the role of advanced paternal age (APA) and *de novo* mutations causing ASD [94•, 95••]. In general, with every year older, the risk increases by two mutations per year [95••]. The association of APA and an increase rate of *de novo* autosomal dominant conditions are widely accepted, but studies have shown that APA also appears to be associated with an increased risk for ASD [95••, 103]. Hultman *et al*. evaluated the association of APA and autism using multiple different methodologies in an analytic cohort of 1,035,487 subjects, showing that the risk started to increase at the paternal age of 30, plateaued after age 40, and further increased from the age of 50 years, with odds ratios of 1.22, 1.58, and 2.66 respectively for paternal ages 30–39, 40–49, and 50 and higher. The association of ASD with APA persisted after controlling for maternal age, parental psychiatric history, perinatal conditions, year of birth, and socioeconomic status. Paternal age was also examined within a subset of families of individuals with ASD who also had at least one non-autistic child (n=660 families). Within these families, paternal age when the offspring with autism was born was higher than the paternal age at the time the unaffected offspring was born (mean age 32.7 +/− 6.3 vs. 30.8 +/− 6.4). Hultman *et al*. also did a meta-analysis as part of their study, and pooled results of the meta-analysis were consistent with increasing paternal age and risk of ASD [103]. Further research is needed to determine the relative risk associated with APA, but these recent studies highlight the need for counseling regarding APA and the increased risk for autism.

Conclusion

Recent advances in genetic analysis approaches have led to accelerated discovery of ASD associated genes and begun to elucidate underlying molecular mechanisms. Convergent evidence supports a complex genetic etiology for ASD. Multiple genes involved in large CNVs and single ASD genes regulating the function of many other genes to modulate neural connectivity partially explain the complex nature of ASD. New high-throughput CMA or NGS genetic tests have allowed rapid identification of numerous variants in ASD candidate genes. Identification of causative genes for ASD facilitates a better understanding of molecular pathways, and may lead to the development of innovative and rationally designed treatments [104••]. In particular, rare forms of ASD due to single gene defects resulting in inborn errors of metabolism may be treatable by dietary restriction, supplementation, or enzyme replacement therapy [105–107, 108•].

Tremendous progress has been made in identifying genetic susceptibility loci for ASD, and this has informed clinical genetic testing. Currently, genetic testing may indicate a cause of

ASD in approximately 10% of patients, and improve the accuracy of risk assessment for family members. As genomic testing becomes more widely available, the need for adequate genetic counseling about test results, including VUS and IF, will be more pronounced. Providers should be comfortable with discussing these possibilities, and if they are not, should consider referral to a clinical geneticist and/or genetic counselor.

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Table 1

Genes conferring susceptibility to ASD

Gene	Description	Related syndrome / co-morbidity	Approaches	Molecular Function
CACNA1C	calcium channel, voltage-dependent, L type, alpha 1C subunit	Timothy	syndrome, WGS	Ion channel
CNTN4	contactin 4	3p deletion	CNV, BCA	Synaptic formation and maintenance
CNTNAP2	contactin associated protein-like 2	Pitt-Hopkins like	BCA, GWAS, targeted NGS, animal model	Synaptic adhesion
FMR1	fragile X mental retardation 1	fragile X	syndrome, animal model	Regulation of protein synthesis
GABRB3	gamma-aminobutyric acid (GABA) A receptor, beta 3	Angelman	GWAS, CNV, animal model	Neurotransmitter receptor
GRIN2A	glutamate receptor, ionotropic, N-methyl D-aspartate (NMDA) 2A	Epilepsy and speech disorder	GWAS, CNV	Neurotransmitter receptor
GRIN2B	glutamate receptor, ionotropic, N-methyl D-aspartate (NMDA) 2B	intellectual disability	GWAS, BCA, WES	Neurotransmitter receptor
MBD5	Methyl-CpG binding domain protein 5	intellectual disability	CNV, WGS	Epigenetic regulation
MECP2	Methyl CpG binding protein 2	Rett	syndrome, animal model	Epigenetic regulation
NLGN1	neuroligin 1	intellectual disability	CNV, animal model	Synaptic adhesion
NLGN3	neuroligin 3	developmental delay	CNV, WGS, animal model	Synaptic adhesion
NLGN4X	neuroligin 4, X-linked	developmentaldelay	CNV, WES	Synaptic adhesion
NRXN1	neurexin 1	Pitt-Hopkins like	CNV, BCA, animal model	Synaptic adhesion
PTEN	phosphatase and tensin homolog	Macrocephaly/autism	syndrome, WES, animal model	Regulation of protein synthesis
RELN	Reelin	lissencephaly	GWAS, WES, animal model	Brain architecture
SCN2A	sodium channel, voltage-gated, type II, alpha subunit	epilepsy	WES	Ion channel
SHANK2	SH3 and multiple ankyrin repeat domains 2	intellectual disability	CNV, animal model	Synaptic scaffolding
SHANK3	SH3 and multiple ankyrin repeat domains 3	22q13.3del	CNV, animal model	Synaptic scaffolding
SYNGAP1	synaptic Ras GTPase activating protein 1	intellectual disability	CNV, animal model	Synaptic formation and maintenance
TSC1	tuberous sclerosis 1	Tubular sclerosis	syndrome	Regulation of protein synthesis
TSC2	tuberous sclerosis 2	Tubular sclerosis	syndrome, WES, animal model	Regulation of protein synthesis
UBE3A	ubiquitin protein ligase E3A	Angelman	syndrome, CNV, animal model	Regulation of protein degradation

Genes that have been identified in multiple unrelated ASD patients and with a strong genetic and functional evidence are listed in the alphabetical order. The approaches to which the genes are established as ASD susceptible genes are indicated, where “syndrome” indicates that the gene was first identified as the cause of a syndrome related the ASD and “animal model” indicates supporting functional evidence from animal models of the gene.

Abbreviations: BCA, balanced chromosomal abnormality; CNV, copy number variation, GWAS, genome-wide association studies; NGS, next-generation sequencing; WES, whole exome sequencing; WGS, whole genome sequencing.