
# Genomic Architecture of ASD

# 2

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## 2.1 Introduction

Autism Spectrum Disorders (ASDs) are neurodevelopmental disabilities with a large heritable component. Concordance rate in monozygotic twins is 30–99% depending on the study, whereas concordance rates in dizygotic twins and siblings are 0–65% and 3–30%, with an estimated overall heritability of 0.7–0.8 [1]. ASD is clinically heterogeneous with respect to behavior, intellectual function, anthropometric traits (e.g., head size; BMI), and comorbid conditions [2]. The extreme clinical variability parallels the genetic heterogeneity, which is far to be completely identified. Indeed, even if epidemiological evidence from family and twin studies has convincingly demonstrated a strong genetic component to ASD, identifying the responsible genetic variants has been impaired by the lack of appropriate technical genomic tools. Only in recent years, we have rapidly developed novel and sensitive methods such as microarray analyses and next generation sequencing (NGS), which have allowed identifying several novel ASD-associated genetic and genomic lesions.

Several Mendelian diseases have been linked to ASD and genetic evidence suggests that up to 1500 genes are involved in ASD susceptibility [3]. Copy Number Variants (CNVs) explain 5–15% of ASD cases and pathogenic variants in single Mendelian genes likely account for a further 15–20%. Finally, oligogenic or polygenic inheritance may account for a still undetermined, but surely relevant group of patients.

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On the other hand, it is now clear that the same genetic determinant associated with ASD can also cause other neurodevelopmental anomalies, including isolated intellectual disability, or psychiatric disorders. The reason of this variable clinical expressivity is unknown and may be attributed to the genetic background, epigenetic or environmental factors.

The breakthrough of new genetic technologies has evidenced a determinant contribution of *de novo* genomic and genetic variants in ASD, which account for the rarity of familial cases of ASD. This means that these mutations arise in the parental germ cells or in somatic cells of the developing embryo. As for intellectual disability, the strong impact on phenotype associated with a reduced reproductive fitness of the severe ASD forms indicated *a priori* that *de novo* variants play an important role in ASD [4].

## 2.2 CNVs Associated with Increased Risk for ASD

More than a decade ago, karyotyping and fluorescence in situ hybridization (FISH) have shown the role of rare genomic alterations in ASD [5], including the 7q11.23, 15q11–13, and 22q11.2 regions, already associated with micro-deletion and micro-duplication syndromes, characterized by autistic symptoms as a component [6, 7]. A breakthrough in the discovery of ASD genetic elements was determined by the development of microarray analyses, such as comparative genomic hybridization (CGH), which allowed a higher resolution—as low as 100 kb—compared with karyotype in the detection of CNVs [8]. The first analyses showed that individuals with ASD had 10–20 times the number of CNVs compared to healthy controls [9, 10]. Since then, a number of studies have consistently confirmed that individuals with ASD have more CNVs than non-related controls. In particular, the study of trios (parents and child) has revealed that part of ASD cases are caused by highly penetrant *de novo* CNVs [11, 12] (Table 2.1). The importance of CNVs in ASD is underlined by the fact that microarray methods to search for genomic deletions and duplications are now recommended as first-line genetic tests in ASD [13–16].

Part of the pathogenic CNVs are recurrent, i.e., involve the same genetic region in different affected subjects with *de novo* CNV. These are mediated by unequal crossing-over events due to a peculiar structure of the genomic region involved. On the other hand, many non-recurrent CNVs have been described, and are generated by different and more complex molecular mechanisms [17].

In both cases, a pathogenic CNV involves one or more dose-sensitive gene(s). This term indicates genes whose product amount is critical for the cell function. Its unbalance is thus associated with a genetic disease, both if decreased, such as in deletions, and increased, such as in duplications [18].

Some of the pathogenic CNVs can result in nearly opposite or mirror phenotypes depending on whether they are duplicated or deleted. This reciprocal impact of deletions/duplications is well-known for the 16p11.2 copy number variant. Severe obesity (deletion) and leanness (duplication) have mirror

**Table 2.1** CNVs frequently associated with ASD, and their counterparts

| Alternative titles/related syndromes | Locus | CNV | Clinical features | OMIM number |
|---|---|---|---|---|
| Chromosome 1q21.1 deletion syndrome | 1q21.1 | Loss | Developmental delay, bone and cardiac anomalies | 612474 |
| Chromosome 1q21.1 duplication syndrome | 1q21.1 | Gain | ASD, schizophrenia | 612475 |
| Chromosome 2q37 deletion syndrome | 2q37.2-q37.3 | Loss | ASD | 600430 |
| | 2q24.2 | Loss | ASD | 605556 |
| Chromosome 2p16.3 deletion syndrome | 2p16.3 | Loss | ASD, MR, schizophrenia | 614332 |
| Chromosome 3q29 deletion syndrome | 3q29 | Loss | MR, ASD, schizophrenia, BD | 609425 |
| | 3p14.2 | Loss | ASD | |
| | 3p14.2 | Gain | ASD | |
| | 6p23 | Loss | ASD | 600511 |
| Williams–Beuren region duplication syndrome | 7q11.23 | Gain | ASD, speech delay, craniofacial anomalies | 609757 |
| | 7q36.3 | Gain | ID, schizophrenia | 613959 |
| | 7q31.1 | Loss | | 602081 |
| Saethre–Chrotzen syndrome | 7p21.1 | Loss | ASD | 101400 |
| | 10q11.23-q21.2 | Gain | ASD | 610987 |
| | 11q13.3-q13.4 | Loss | ASD, ID | 603290 |
| | 13q14.12-q14.13 | Loss | ASD | 615609 |
| Chromosome 15q13.3 micro-deletion syndrome | 15q13.3 | Loss | ID, seizures, schizophrenia, ASD, BPD | 612001 |
| Duplication 15q11-q13 syndrome | 15q11-q13.33 | Gain | ASD | 608636 |
| Chromosome 15q11.2 deletion syndrome | 15q11.2 | Loss | ID, ADHD, schizophrenia, ASD | 615656 |
| Chromosome 16p13.3 deletion syndrome (Rubinstein–Taybi) | 16p13.3 | Loss | ASD | 610543 |
| Chromosome 16p13.3 duplication syndrome | 16p13.3 | Gain | ID, speech problems, mild skeletal anomalies | 613458 |
| | 16p13.1 | Gain | ASD, ID, schizophrenia | |
| Chromosome 16p11.2 deletion syndrome, 220 kb | 16p11.2 | Loss | Obesity, obesity with developmental delay | 613444 |
| Chromosome 16p11.2 deletion syndrome, 593 kb | 16p11.2 | Loss | ASD | 611913 |
| Chromosome 16p11.2 duplication syndrome | 16p11.2 | Gain | ASD, schizophrenia, ADHD, microcephaly | 614671 |
| Chromosome 16p12.1 deletion syndrome, 520-kb | 16p12.1 | Loss | Developmental delay and learning disability | 136570 |

(continued)

**Table 2.1** (continued)

| Alternative titles/related syndromes | Locus | CNV | Clinical features | OMIM number |
|---|---|---|---|---|
| Smith–Magenis syndrome | 17p11.2 | Loss | ASD | 182290 |
| Potocki–Lupski syndrome | 17p11.2 | Gain | ASD | 610883 |
| Chromosome 17q12 deletion syndrome | 17q12 | Loss | ID, ASD, schizophrenia | 614527 |
| Chromosome 17q12 duplication syndrome | 17q12 | Gain | ID, behavioral abnormalities, psychomotor delay | 614526 |
| | 22q13.33 | Loss | ASD | 606230 |
| Chromosome 22q13 duplication syndrome | 22q13.33 | Gain | ASD, ID | 615538 |
| | 20p13 | Loss | ASD | |
| Chromosome 22q11.2 deletion syndrome | 22q11.2 | Loss | ID, schizophrenia, ADHD, other psychiatric disturbances | 188400 |
| Chromosome 22q11.2 duplication syndrome | 22q11.2 | Gain | ASD | 608363 |
| | Xp22.11 | Loss | ASD, ADHD | 300830 |
| | Xq13.1 | Loss | ASD, ID | 300336 |

*ADHD* attention deficit hyperactivity disorder, *ID* intellectual disability

etiologies, possibly through contrasting effects on metabolism regulating energy balance [19]. A similar mirror phenotype is associated with the 7q11.23 deletion/duplication, both leading to multisystem neurodevelopmental disorders. The former is associated with the Williams–Beuren syndrome characterized by extreme friendliness and sociable traits lying at opposite ends of the same behavioral spectrum in duplication 7q11.23 which has language impairment, and autistic like features [20].

In other CNVs, two pathogenic different models have been proposed: (1) in the dominant model, gene expression changes in one direction only (decrease or increase) may contribute to a specific phenotype, with no effect (on the same trait) for a change in the other direction. An example is the immunodeficiency associated with the 22q11.2 deletion, but not observed in the corresponding duplication. (2) In the U-shaped model, genotype–phenotype correlations in reciprocal CNVs have allowed to demonstrate that a reduced or increased number in the number of copies of causal genes can lead to the same phenotype. Among many examples, the 15q13.3 deletion and duplication syndromes are overlapping (i.e., ID, DD, ASD, schizophrenia, ADHD) except for aggressive/impulsive behavior which are reported in deletion, but not duplication, carriers (up to 35%) [20].

Most recurrent CNVs are large (>400 kb), involving dozens of genes, and are individually rare (<0.1%). There are now several well-characterized rare CNVs, clearly associated with a high risk of ASD (Table 2.1). Very large CNVs seem to

be further enriched in individuals who have comorbidity with intellectual disability.

Recurrent CNVs can be distinguished in "syndromic," where they are associated to a highly reproducible set of congenital anomalies, or "variable expressive CNVs," resulting in a broad spectrum of disease phenotypes [21].

One emerging aspect of CNVs associated with ASD is that most manifest a wide range of clinical phenotypes. As an example, the 15q13.3 deletion and duplication are now clearly associated with ASD [22], ID [23], epilepsy [24], and schizophrenia [25].

Some CNVs are not only associated with a variable expressivity, but also with an incomplete penetrance. This is clear in families where the transmitting parent is apparently "unaffected" suggesting these CNVs are not sufficient to determine the disease. Indeed, recent works provide evidence for an oligogenic CNV model, where in addition to the primary CNV, a second CNV (inherited or *de novo*) is required at a different locus for a child to develop ASD. This phenomenon is exemplified by the 520-kb deletion on chromosome 16p12.1 (MIM# 136570), which is associated with developmental delay and extensive phenotypic variability [26]. Interestingly, in most cases, this deletion was inherited from a parent who also manifested mild neuropsychiatric features, and the severely affected children were more likely to carry another large (>500 kb) rare CNV.

These results suggest a contribution of rare variants in the genetic background toward neurodevelopmental disorders, depending upon the extent to which the primary variant sensitizes an individual toward a specific pathological phenotypic trajectory [26].

## 2.3 Rare Highly Penetrant ASD Genes

CNVs are causative in only 5–15% of individuals with ASD, suggesting that other types of mutations must be operant in ASD as well. Rare Mendelian syndromes have been associated with ASD, showing that at least in part ASD is a monogenic disorder [27, 28]. Among these, fragile X syndrome (*FMR1* gene), Rett syndrome (*MECP2* gene), tuberous sclerosis (*TSC1* and *TSC2* genes), Timothy syndrome (*CACNA1C* gene), all display partial comorbidity with ASD [18].

The recent widespread availability of next generation sequencing (NGS) allowed a further increase in the resolution to detect genetic alteration in ASD. Starting from 2008, NGS has allowed to sequence the coding genes of an entire human genome, the so-called whole exome sequencing (WES) strategy, at affordable prices and without the need of an *a priori* hypothesis on the disease gene. A number of large WES studies have been completed in ASD, now encompassing several thousands of individuals [29–35]. Rare autosomal recessive disorders were identified in consanguineous families, affecting for instance the *AMT, BCKDK, C3ORF58, CNTNAP2, NHE9, PCDH10, PEX7, SYNE1, VPS13B, PAH, POMGNT1,* and *SLC9A9* genes [36–38]. These are associated with highly variable clinical presentation, and ASD

can be isolated in patients lacking the diagnostic criteria and features of the associated Mendelian disorders. A limited number of X-linked genes have also identified to contribute to ASD, among these, the already cited *FMR1* and *MECP2*, and neuroligins *NLGN3* and *NLGN4* [39, 40]. However, recently new important X-linked genes are emerging such as the X-linked dominant *DDX3X* gene, affecting females only [41].

The list of these genes is still limited and will surely expand in the next years thanks to the new sequencing methods.

## 2.4 Novel Highly Penetrant ASD Genes

As for CNVs, also single-nucleotide pathogenic variations so far discovered are mainly *de novo* in highly penetrant ASD. These genes behave as autosomal dominant and are rarely found segregating in families (e.g., *SHANK1, SHANK2,* and *SHANK3*) often because their strong effect on reproductive fitness reduction. WES studies identified a number of high-confidence ASD candidate genes that likely may represent up to 20% of cases [42, 43]. Some of them are recurrently hit among families, such as *CHD8, DYRK1A, KATNAL2, GRIN2B, POGZNTNG1*, and *SCN2A* [44]. However, the general notion is that many genes associated with ASD phenotype are likely to be very rare or even "private," unlikely to be found in many individuals. This suggests that rare variants have a larger than originally expected impact on ASD risk, although large cohorts of patients are needed to deepen the knowledge on this issue.

The list of candidate genes involved in ASD is continuously increasing as the complexity of data supporting their pathogenicity. Several groups have tried to develop criteria to rank and assess the strength of evidence associated with candidate genes. Among these one of the most complete databases is SFARI gene (https://gene.sfari.org/), built on information extracted from peer-reviewed scientific and clinical studies on the molecular genetics and biology of ASD [45]. SFARI gene integrates genetic, neurobiological, and clinical information about genes associated with ASD, reporting a total of 956 genes (version 3.0). The annotation criteria used allow dividing genes into seven categories: syndromic genes predisposing to autism in the context of a syndromic disorder (e.g., fragile X syndrome); categories 1 and 2 (high and strong confidence) contain genes with a genome-wide statistical significance, with independent replication; categories 3 and 4 (suggestive and minimal evidence) list genes reported in relatively small studies, whose evidence is still incomplete. Finally, in category 5 (hypothesized but untested) are reported genes that have been implicated solely by evidence in model organisms or other evidence of a marginal nature, and category 6 (evidence does not support a role) is for those genes that have been tested in a human cohort, but the weight of the evidence argues against a role in ASD (Table 2.2).

**Table 2.2** ASD genes reported in SFARI database (November 2018)

| Category | Definition | N. of genes |
|---|---|---|
| Syndromic | Genes with a substantial degree of increased risk for ASD, and consistently linked to additional characteristics not required for an ASD diagnosis | 79 |
| Category 1, high confidence | Genes with evidence of recurrent and convincing mutations (functional or large pedigree segregation) accompanied by a rigorous statistical comparison with the mutation frequency in controls. This category also includes single genes that reached genome-wide significance in association studies independently replicated, or which reached genome-wide significance via meta-analysis of all current association studies | 25 |
| Category 2, strong candidate | Rare mutations that are recurrent and convincing (see above), accompanied by a rigorous statistical comparison with the mutation frequency in controls. Rare *de novo* variants, likely to be disruptive, in three or more unrelated cases. Results from association studies must reach genome-wide significance, uniquely implicating a single gene, but with no independent replication. Alternatively, consistently replicated association of the same allele, falling short of genome-wide significance, that must be accompanied by evidence that the risk variant has a relevant functional effect in humans | 61 |
| Category 3, suggestive evidence | Genes with consistently replicated association of the same allele, without functional support. Rare *de novo* variants, likely to be disruptive, in two or more unrelated cases. Genes within a CNV, or near a GWAS peak close to significance, with additional accessory evidence | 184 |
| Category 4, minimal evidence | Genes in an ASD-associated multi-genic CNV, proximal to genome-wide significant intergenic variants for which there is no other independent evidence. Any significant, convincing, but unreplicated association study data, along with any instances of multiple but inconsistent reports of association that are not overall significant by meta-analysis<br>Genes with a series of two or more putative mutations identified (e.g., non-synonymous substitutions, single-gene deletion, duplication, disruption by translocation) for which there is not rigorous statistical comparison with controls<br>Single rare de novo variants, likely to be disruptive | 437 |
| Category 5, hypothesized | Genes for which the only evidence comes from studies of model organisms. Genes in a region of linkage with no unique evidence for that gene versus others. Genes shown to functionally interact with category ASD strong candidates. Genes with a single rare variant observed in a single ASD case/family are placed here | 170 |

This table reports a summary whose full text is available at https://gene.sfari.org/about-gene-scoring/

## 2.5 Common Variants Risk to ASD

High-throughput genotyping of single-nucleotide polymorphisms (SNP) allowed a large number of genome-wide association studies (GWAS) to identify common variants to ASD risk [46]. The potential number of genes likely able to confer moderately-sized risk for ASD is large. In fact, statistical modeling based on published results of both rare and common variation has predicted that up to 1000–1500 genes may ultimately be found to be associated with ASD [35, 47]. The comprehension of how such a large and varied number of genes can all be associated with one common clinical phenotype will be the major challenge to the field. The challenge of understanding how combinations of susceptibility genes interact during human brain development to cause disease (epistasis) has only begun to be explored.

Common variation throughout the genome exerts substantial additive genetic effects on ASD liability, with simplex/multiplex family status having an impact on the identified composition of that risk. As a fraction of the total variation in liability, the estimated narrow-sense heritability exceeds 60% for ASD individuals from multiplex families and is approximately 40% for simplex families. Genome-wide association studies demonstrate that a myriad of common variants of very small effect impacts ASD liability. The identification of such variants needs huge cohorts of patients to be analyzed and represents the challenge of ASD genetics for the next decades [48].

## 2.6 Biological Insights into ASD

The genetic architecture of ASD has been proved to be complex and the large majority of cases still have no identifiable genetic cause [3, 49]. Despite these limitations, ASD-causing genes have started providing clues on functional pathways involved in the pathogenesis. WES studies have demonstrated grouping of protein–protein interaction networks, enriched for involvement in beta-catenin, p53 signaling, chromatin remodeling, ubiquitination, and neuronal function [29, 34, 43, 47, 50]. The analyses of convergent pathways integrated with experimental findings based on transcriptomic, and cellular and mouse models are now pointing toward three major cellular pathways interconnected through neuronal activity [44, 51].

1. Synapse development and function. The development and/or maintenance of synaptic function seem a critical factor in development of ASD [52]. Among the important genes are those encoding the presynaptic cell-adhesion molecules (CAMs) neurexins (*NRXN1*) and their postsynaptic partners, neuroligins (*NLGN3* and *NLGN4*). Other molecules involved in pre-post synaptic anchoring are the SHANK family (*SHANK3*) and other molecules connected with the actin cytoskeleton (*CNTNAP2*). The most common electrophysiological and neuroanatomical findings evidenced by mouse models of these genes are altered glutamatergic synaptic transmission, loss of inhibitory GABA interneurons, and impairment in synaptic plasticity attributable to dysfunction of NMDA and

AMPA receptors. Similar findings have been reported in the duplication 15q syndrome mouse models (*UBE3A* gene) which recapitulates the three core ASD features [53]. Glutamatergic transmission might represent a targetable pathway in ASD. Indeed, *Fmr1* knockout mice show a hyperactive mGluR5 signaling, leading to excessive protein synthesis at the synapse and increased trafficking of AMPA receptors [54, 55].

2. Growth, transcription regulation, and protein synthesis. Many ASD risk genes (e.g., *TSC1, TSC2,* and *PTEN*) lie downstream the signaling pathway containing mTOR, a key regulator of cell growth, proliferation, and survival. These genes are predicted to alter protein synthesis within synaptic spines, which is necessary for neuronal plasticity and thus proper cognitive function. Among the recently introduced genes in this list, *CDKL5* (Rett-like syndrome) has recently been shown to affect the mTOR pathway [56]. WNT pathway signaling is also considered to have a key role in the etiology of ASD [57, 58]. Defective synaptogenesis (or synaptic function), altered WNT signaling during brain development, and altered transcription and/or translation in neurons can influence neuronal circuit formation and activity [51].
3. Serotonin signaling and neuropeptides. Alterations in the serotoninergic system were among the earliest evidence of abnormal brain function in ASD [59]. Serotonin mediates neurogenesis, cell migration and survival, synaptogenesis and plasticity [60]. Several variants in the serotonin system have been linked to ASD (*SERT/SLC6A4, MAOA*) [61].

A complementary approach to identify biological relationships among identified genes is to analyze the specific expression time window or molecular process. Two independent works showed that ASD genes are likely expressed in the mid-fetal brain (10–12 weeks of gestation), spatially corresponding to superficial glutamatergic neurons [62, 63]. Interestingly, ASD genes encode messenger RNAs interacting with FMRP, encoded by the *FMR1* gene, suggesting that convergence at common pathways of synaptic plasticity associated with gene regulation is mediated by this protein [47, 64].

Overall, a key role for fetal glutamatergic neuron development has been established for ASD, with a growing evidence for converging pathways in ASD-causing genes, with spatiotemporal specific expression pathways [44].

## 2.7 Conclusion

In recent years, major progress in understanding the genetic architecture of ASD has been made. We now know that both rare and common variants contribute to ASD, with a number of genes and loci implicated. Much remains unknown: the penetrance and expressivity of many ASD genes is still to be determined, as well as the contribution of low penetrance genes in oligogenic forms. Several large-scale projects have just begun to understand both the genetic architecture and the pathophysiological mechanism of these heterogeneous disorders.

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