



Genetic research in autism spectrum disorders

Elise B. Robinson^{a,b}, Benjamin M. Neale^{a,b}, and Steven E. Hyman^{b,c}

Purpose of review

The recent explosion of genetic findings in autism spectrum disorder (ASD) research has improved knowledge of the disorder's underlying biology and etiologic architecture. This review introduces concepts and results from recent genetic studies and discusses the manner in which those findings can influence the trajectory of ASD research.

Recent findings

Large consortium studies have associated ASDs with many types of genetic risk factors, including common polygenic risk, *de novo* single nucleotide variants, copy number variants, and rare inherited variants. In aggregate, these results confirm the heterogeneity and complexity of ASDs. The rare variant findings in particular point to genes and pathways that begin to bridge the gap between behavior and biology.

Summary

Genetic studies have the potential to identify the biological underpinnings of ASDs and other neuropsychiatric disorders. The data they generate are already being used to examine disease pathways and pathogenesis. The results also speak to ASD heterogeneity and, in the future, may be used to stratify research studies and treatment trials.

Keywords

autism, exome sequencing, genetics, GWAS

INTRODUCTION

Autism spectrum disorders (ASDs) are serious neuropsychiatric conditions characterized by impairments in social and communication behavior, as well as restricted and repetitive interests. Approximately, 1% of children in the United States meet the current diagnostic criteria for ASDs, with onset of symptoms in early childhood and persistence through the life course. Although the degree of impairment associated with ASDs varies substantially, affected individuals and their families are confronted by social, educational, and occupational challenges, for which treatment options are limited. The current treatments available for ASDs are predominantly behavioral, and have a limited impact on core behavioral symptomology. No pharmacologic treatments have been empirically associated with a reduction in autism symptoms or their frequent cognitive comorbidities. Unmet treatment need typifies neuropsychiatric disorders and reflects limited knowledge about the biological processes that drive pathogenesis.

Twin studies – notwithstanding some quantitative disagreements – have demonstrated significant heritability of ASDs, thus motivating large-scale genetic studies [1,2^{***}]. Such studies aim to identify genetic variants that contribute to the risk of ASDs; genetic variants (alleles) represent DNA sequences

that differ among individuals at specific genomic locations (loci). Depending on their genomic region, DNA sequence variants may influence the risk of an ASD by altering the amino acid sequence of a functional RNA or protein or by producing a change in the timing, circumstances, or level at which these molecules are expressed. A central goal of genetic research in ASDs and other neuropsychiatric disorders is to nominate molecular pathways for further interrogation and thus to link biological mechanisms with behavior. On the basis of the experience of other fields of medicine, the resulting knowledge should enable the discovery of new

^aAnalytic and Translational Genetics Unit, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston,

^bStanley Center for Psychiatric Research and Program in Medical and Population Genetics, Broad Institute of Harvard and MIT and

^cDepartment of Stem Cell and Regenerative Biology, Harvard University, Cambridge, Massachusetts, USA

Correspondence to Elise B. Robinson, Stanley Center for Psychiatric Research, Broad Institute, 75 Ames Street, Cambridge, MA 02142, USA. Tel: +1 617 714 7543; fax: +1 617 800 1764; e-mail: erob@broadinstitute.org

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KEY POINTS

- A series of recent consortium studies have associated ASDs with many types of genetic risk factors. These projects have greatly improved the understanding of the etiologic architecture of ASDs.
- ASDs are heterogeneous and complex, and genetic data can be used to better understand differences among affected individuals.
- Genetic findings are pointing to genes and pathways that begin to bridge the gap between behavior and biology.

treatments. Recent large consortial studies have identified the first genes that are convincingly associated with ASDs and have produced some early biological insights [3¹¹,4¹²]. These initial successes have also underscored the need for far larger sample sizes if we are to successfully elucidate the genetic architecture of ASDs. This review will highlight the goals and primary outcomes of recent ASD genetic studies, and discuss ways in which those data are being used to guide autism research.

GENETIC INFLUENCES ON AUTISM SPECTRUM DISORDER RISK

Genetic variants differ in nature and in the frequency at which they occur in the human population (Fig. 1). Inherited genetic variants, those that are passed from parents to children, can occur at all frequencies from common to very rare, whereas *de novo* variants, those that are newly arising in offspring and are not seen in a carrier's parents, are typically rare. Genetic studies have strongly suggested that the variant classes contributing to ASD risk are of all structural types, all frequencies, and can be inherited or *de novo*.

Common polygenic variation

Autism spectrum disorders are highly familial. In the United States, the siblings of children with ASDs are estimated to be at more than a 10-fold increase in risk for an ASD diagnosis themselves [5–8]. Moreover, the family members of children with ASDs are also more likely to have a history of psychiatric diseases defined broadly, including schizophrenia, bipolar disorder, and depression [9]. Such familial aggregation of psychiatric illness is consistent with complex, inherited genetic risk. Genome-wide association studies (GWAS) make it possible to identify common variants contributing to such polygenic risk by the use of DNA microarrays to

detect common single nucleotide polymorphism (SNPs).

Methods for estimating heritability from SNP data – for example Genome-wide Complex Trait Analysis and LD (linkage disequilibrium) score regression [10,11¹³] – have permitted existing ASD GWAS collections to report strong, cumulative contributions of common inherited variants to autism risk. Across several recent studies, common genotyped SNPs are estimated to account for between 20% and 50% of variation in liability to ASDs [2¹⁴,11¹⁵,12]. These estimates are consistent with those for other highly heritable, polygenic neuropsychiatric disorders, and predict that GWAS studies in ASDs will become an increasingly productive source of biological insight as sample size grows. ASD GWAS studies have yet to reliably nominate any specific loci but, as highlighted by the recent extraordinary successes in schizophrenia genetics [13¹⁶], psychiatric disease GWAS require very large case and control collections. As ASD sample collection efforts progress and GWAS studies become adequately powered, SNPs with significant association to ASDs will be identified, laying the foundation for follow-up biological interrogation.

Polygenic scoring and SNP heritability methods, both derived from GWAS, have also been used to identify genetic correlations between ASDs and other neuropsychiatric disorders, such as schizophrenia [12,14]. The genetic correlation between autism and schizophrenia is estimated to be approximately 20% using multiple genome-wide approaches. Overlapping genetic risk is a theme common to neuropsychiatric phenotypes, strongly suggesting that the biology underlying psychiatric disorders is unlikely to adhere to the diagnostic boundaries set out in such traditional classifications as the DSM-5 [15–18,19¹⁷,20].

De novo variation

The contribution of *de novo* (i.e., newly arising) variation to ASD risk has been identified through whole exome sequencing – the deep characterization of protein coding regions, which in aggregate comprise 1–2% of the genome [21–23]. The largest trio sequencing studies of ASDs published to date represent a clear advance in neurodevelopmental genomics, identifying a significant excess of functional mutations in more than 40 genes [3¹⁸,4¹⁹].

Although most people carry at least one *de novo* mutation somewhere in their exome [24], a *de novo* mutation at any given position is rare. The rarity of these events in specific genes means that it is necessary to build models to assess the significance of gene–disease relationships, even in the context of

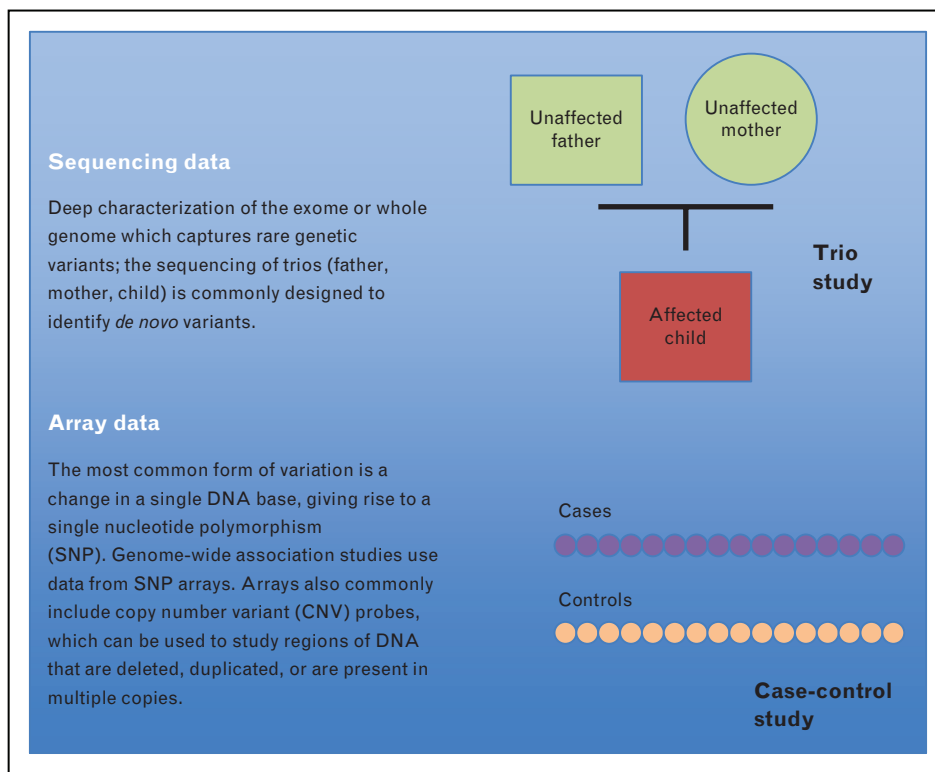


FIGURE 1. Study designs in autism spectrum disorders genetics.

genes in which multiple *de novo* mutations have been found in ASD cases. One such modeling approach estimates the mutation rate of variants of a given functional class (e.g., synonymous, missense) at a gene level, which can then be used to calculate the probability of observing a given number of mutations in the sample size in question [19[¶]].

Statistical models can also be used to assess whether there is enrichment of certain functional classes of variants genome wide. For example, *de novo* loss of function (LoF) variants – *de novo* variants that result in loss of gene function – are seen in approximately 9% of people in the general population, and 16–18% of people with an ASD diagnosis [3^{¶¶},4^{¶¶}]. This suggests that *de novo* LoFs, on average, approximately double an individual's ASD risk, increasing the probability of being diagnosed with an ASD from approximately 1% to approximately 2%. Individuals with ASDs also manifest a significant excess of *de novo* missense mutations, but the risk conferred by them is, on average, smaller. This level of enrichment suggests that additional genes will be significantly associated with ASDs as sequencing activities grow [3^{¶¶},4^{¶¶}].

On the basis of these results, recent studies have estimated that approximately 3 to 10% of ASD risk is attributable to *de novo* single-nucleotide variation in

the exome [2^{¶¶},3^{¶¶},25^{¶¶}]. The current meta-analytic picture reflects a significant but limited contribution that, as with GWAS associations, can be used to link behavior and biology. GWAS associations, however, identify a locus rather than a causal variant; moreover, approximately 90% of GWAS associations across diseases are located within non-coding regions of the genome. Clearly identifiable mutations in protein-coding regions represent a more tractable situation for biological experimentation, given the broader toolkit currently available for interrogating protein function rather than gene regulation. The genes that have been strongly linked with ASDs in trio sequencing studies are associated with diverse biological functions [4^{¶¶}], including both neuronal function and development processes. As a class, genes associated with ASDs show clear evidence of evolutionary constraint, as they are deficient in functional mutations in the population [19[¶]].

Similar efforts at the association of *de novo* mutations with intellectual disability (ID) have identified more than 50 genes as significant contributors to risk [26[¶]]. The great majority of those genes have also been implicated in ASD [3^{¶¶},4^{¶¶}], suggesting limited phenotypic specificity among neurodevelopmentally sensitive genes.

Copy number variants and inherited rare variation

Copy number variants (CNVs) comprise the most common form of structural variation in the genome, and can be inherited or *de novo*. As with *de novo* SNVs, significant enrichment in the rate of *de novo* CNVs is observed in individuals with ASDs. Furthermore, CNVs in specific regions of the genome are associated with increased risk of ASDs [27]. Region-specific CNV analyses were some of the first to consistently tie locations in the genome to ASD risk [17]. As discussed below, several specific CNVs that have been associated with ASDs are now being targeted for biological and phenotypic study.

An inherited rare variation, which can be investigated through both trio sequencing and the sequencing of cases and controls, is among the most difficult type of event to interrogate given the combination of low frequency and, on average, small effect size [28]. ASDs have been associated with inherited, two-hit (recessive) loss of function mutations and, more recently, a global excess of rare inherited protein-truncating variants in genes that are intolerant of mutation [25²²,29]. It is likely that additional rare inherited variant types will be associated with ASDs as sequencing studies increase in sample size and new techniques are developed to filter and highlight classes of variation most likely to be deleterious.

Risk factors for ASDs can be found across the full spectrum of genetic variation. Such a diverse landscape of genetic risk clearly demonstrates that ASDs are a polygenic trait, with a myriad of different risk factors in the population. These genetic clues are the first step in gaining insight into the biological underpinnings of ASDs.

HETEROGENEITY IN AUTISM SPECTRUM DISORDERS – GENOTYPE TO PHENOTYPE

The recent explosion of genetic associations in ASD research has highlighted the diversity of the disorder's etiologic influences. The phenotypic heterogeneity of ASDs has been recognized far longer, and several avenues of research now aim to associate genetic risk factors for ASDs with specific phenotypic differences.

The prevalence of individuals diagnosed with ASDs has increased rapidly over the past 20 years. Despite concerns that higher prevalence might suggest new pathogenic environmental factors, the majority of the increase appears to reflect diagnostic expansion – both diagnostic substitution between ID and ASDs and, to an even greater degree, diagnostic extension into the high functioning range [30–32]. The most recent US population surveys suggest that

the ID rate in ASD has dropped by 50% in only 25 years, indicating a striking shift in diagnostic practice and the average phenotypic profile of those diagnosed [33]. Lower intelligence quotient (IQ) in ASDs is correlated with increased behavioral symptom severity and an increased rate of comorbid epilepsy and other neurological symptoms. Thus, an increase in mean IQ predicts higher functioning across a variety of phenotypic dimensions [34¹].

Several 'genotype to phenotype' relationships in ASDs have recently been examined [3²²,34¹,35,36], with the association between *de novo* mutations and proband IQ emerging as particularly significant. Specifically, the rate of *de novo* LoFs in low IQ ASDs substantially exceeds that seen in high IQ ASDs. The association between IQ and *de novo* LoFs in the Simons Simplex Collection is shown in Fig. 2. The LoF rate in ASDs crosses the rate seen in other disorders at several points along the IQ distribution. At very low IQ, the LoF rate is similar to that seen in samples ascertained for severe ID [19¹]. In high IQ groups, it intersects with the average rates seen in people with schizophrenia [37,38] and the rates seen in healthy control populations.

Some of this trend is likely to reflect a general relationship between cognition and rare, damaging mutations in the population as a whole, in both those with and without diagnosable neuropsychiatric disorders. To the extent that differences in genetic architecture in ASDs, however, predict differences in underlying biology, clinical features, or treatment response, such genotype–phenotype relationships warrant further exploration, whether starting with phenotypes or genotypes. For example, recent studies have been conditioned on specific individual genes and CNVs, such that the spectrum of biological and clinical findings associated with those genomic events can be better characterized [39²²,40,41¹]. The Simons Variation in Individuals Project is one such experiment. The investigators have collected extensive behavioral, cognitive, and biological data from several hundred carriers of an ASD-associated CNV, typically a duplication or deletion at a particular chromosomal locus, 16p11.2, as well as the carriers' family members. The size and diversity of the data set permits consideration of several questions that cannot be addressed in a study of individuals ascertained only for ASDs. For example, Moreno-De-Luca *et al.* [39²²] recently reported an association between parental cognitive and behavioral profiles and phenotypic variation in carriers of *de novo* 16p11.2 deletions, strongly suggesting an additive relationship between inherited polygenic and *de novo* risk on neuropsychiatric phenotypes. Another recent study carefully examined the phenotype associated with mutations in the *CHD8* gene, which has been

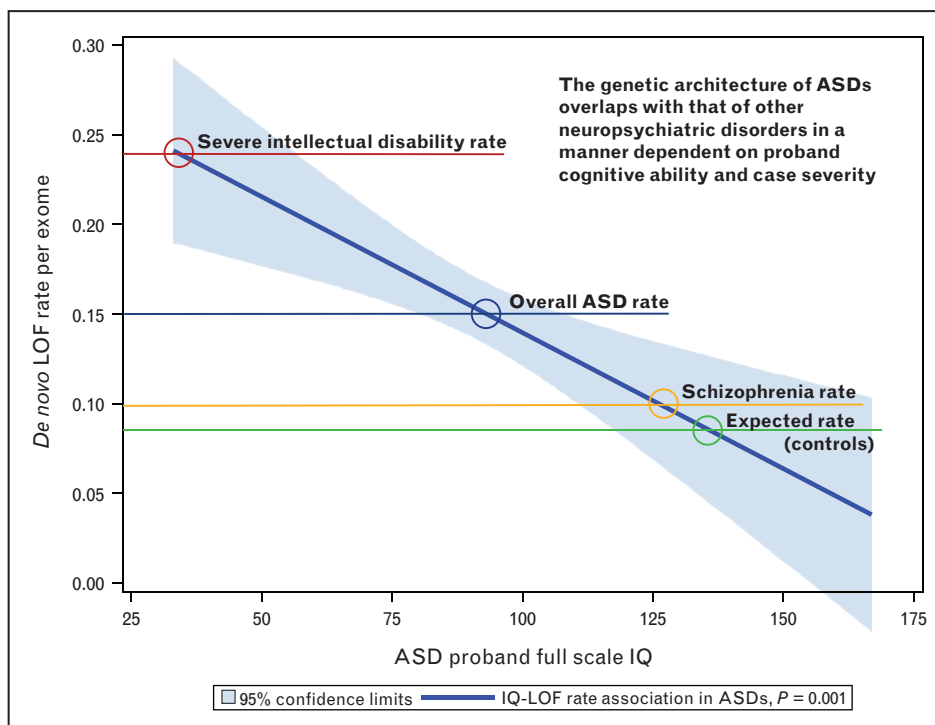


FIGURE 2. Loss of function (LoF) regression generated using data from the Simons Simplex Collection as described by Robinson *et al.* [34[■]]; LoF rate for severe intellectual disability and controls from Samocha *et al.* [19[■]]; LoF rate for schizophrenia from Fromer *et al.* [38]. ASD, autism spectrum disorder; IQ, intelligence quotient.

associated with ASDs in multiple exome sequencing studies [3[■],41[■],42]. Compared with other individuals with ASDs, *CHD8* carriers were more likely to have macrocephaly and gastrointestinal symptoms, along with other distinguishing features.

A limit to the ‘genotype first’ line of inquiry may come from the limited number of genes, or the limited number of recurrent CNVs, that exert a strong enough influence on ASD risk and are seen in more than a handful of cases. As described above, the average odds ratio for ASDs associated with *de novo* LoF mutations is approximately 2. There is likely to be substantial variability surrounding that average [4[■]], but one can use it to estimate limits on the fraction of variants within the class that confer very strong ASD risk. For example, using an odds ratio of 2, fewer than 5% of *de novo* LoF variants could have an ASD odds ratio of 20 before having to postulate that the remaining 95% of *de novo* LoFs variants are collectively protective against autism. *CHD8* is likely to be an uncommonly affected gene with a large odds ratio and as such it is very well chosen to begin the search for genetically defined ASD subtypes. Other syndromic forms of ASDs (e.g., Fragile X, 22q11.2 deletion syndrome) are also more likely to have common phenotypic presentations, but as the landscape of contributing variation continues to be uncovered, it is likely that most ASD cases will be complex (i.e., cannot be

strongly associated with any given genetic or environmental risk factor).

The complexity of most cases will similarly place limits on the diagnostic yield of clinical sequencing and genotyping panels. Families and clinicians have become increasingly interested in associating individual ASD cases with genetic risk factors. Although a small percentage of ASD cases will carry a clearly established, large ASD risk factor, the majority of returns from such efforts are unlikely to be easily interpreted. For the limited subset of families whose affected child carries an established risk factor – for example a *de novo* LoF mutation in *CHD8* – the return from clinical sequencing could be emotionally valuable and could meaningfully inform the parents’ concept of familial ASD risk. However, the great majority of *de novo* or inherited events detected through clinical panels will have made a very small contribution, if any contribution at all, to the development of ASD in a given case. The interpretation of the information returned must then be cautious. In most ASD patients in whom a *de novo* LoF mutation or other event type is found, the majority of the genetic contribution to that patient is still likely to be inherited. In other words, if the average *de novo* LoF mutation doubles ASD risk (from approximately 1% to 2%), about 98% of individual liability is left to be explained, and familial factors are likely to be the largest remaining

contributors [2^{***}]. The clear communication of clinical sequencing results to families will substantially affect the value of those activities.

CONCLUSION

As with other studies of neuropsychiatric traits, genetic studies of ASDs have enormous potential to point to the biology underlying behavior. In addition to aiding in the discovery and development of therapeutics by nominating molecular targets and suggesting new hypotheses for biomarkers, a more complete picture of genetic architecture could lead to the productive stratification of epidemiologic studies and clinical trials. Particularly with continued aggregation of samples in consortium studies, genomics will greatly improve the understanding of ASD biology in the coming years, and may contribute significantly to much needed new treatments.

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Conflicts of interest

S.E.H. is a principal investigator on a collaboration with Novartis Institutes for Biomedical Research, and has advised Novartis, AstraZeneca, and Sunovion on early drug discovery during the past three years.

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