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Recent Advances in Understanding the Genetic Architecture of Autism

Caroline M. Dias[1,2,3] and Christopher A. Walsh[2,3,4,5,6]

[1]Division of Developmental Medicine, Boston Children's Hospital, Boston, Massachusetts 02115, USA; email: caroline.dias@childrens.harvard.edu

[2]Division of Genetics and Genomics, Boston Children's Hospital, Boston, Massachusetts 02115, USA; email: christopher.walsh@childrens.harvard.edu

[3]Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115, USA

[4]Department of Neurology, Harvard Medical School, Boston, Massachusetts 02115, USA

[5]Howard Hughes Medical Institute, Boston Children's Hospital, Boston, Massachusetts 02115, USA

[6]Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02142, USA

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Abstract

Recent advances in understanding the genetic architecture of autism spectrum disorder have allowed for unprecedented insight into its biological underpinnings. New studies have elucidated the contributions of a variety of forms of genetic variation to autism susceptibility. While the roles of de novo copy number variants and single-nucleotide variants—causing loss-of-function or missense changes—have been increasingly recognized and refined, mosaic single-nucleotide variants have been implicated more recently in some cases. Moreover, inherited variants (including common variants) and, more recently, rare recessive inherited variants have come into greater focus. Finally, noncoding variants—both inherited and de novo—have been firmly implicated in the last few years. This work has revealed a convergence of diverse genetic drivers on common biological pathways and has highlighted the ongoing importance of increasing sample size and experimental innovation. Continuing to synthesize these genetic findings with functional and phenotypic evidence and translating these discoveries to clinical care remain considerable challenges for the field.

INTRODUCTION

Autism spectrum disorder (here referred to simply as autism) is a heterogenous disorder defined by deficits in social communication in conjunction with highly restricted interests and/or repetitive behaviors, first manifesting early in development and leading to significant functional impairment. Serious comorbidities frequently occur and include challenges related to intellectual disability, epilepsy, feeding, and sleep. The fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (2) groups all individuals who meet certain criteria under the umbrella term autism spectrum disorder and eliminates previous designations, such as Asperger's disorder. It is important to understand that although autism is a neurobiological condition, it is currently defined by a set of observed behaviors, and objective biomarkers have not been implemented in routine clinical use. Thus, there is no a priori guarantee that autism, as currently defined, represents a unitary genetic or biological mechanism. To the contrary, the clinical presentation of autism spectrum disorder, as the name implies, is highly heterogeneous and encompasses a wide range of cognitive and adaptive abilities. This suggests that distinct genetic pathways contribute regardless of the terminology used. In fact, identifying distinct genetic and biological subtypes of autism is critical to better understand this complex diagnosis (29).

Autism can no longer be considered a rare disorder; the Centers for Disease Control and Prevention estimated the prevalence of autism in the United States to be around 1 in 59 as of 2014, with a significantly higher proportion of males affected as compared with females (5). Despite the high prevalence and progress that has been made in the past decade, autism remains enigmatic. Estimates of its heritability have varied substantially over the years depending on the population examined and methodology employed (27, 61, 72), but there is now robust evidence that the heritability is high, with increasing relatedness to an affected individual increasing risk (60). When considering autism liability, it is important to distinguish between individual and population risk and to account for the caveats of population-level ascertainment. For example, a rare de novo mutation in a highly constrained, highly penetrant gene may confer high risk for an individual who carries this variant but contribute relatively little risk to the population. On the other hand, although common variation is thought to contribute to a significant proportion of the variance in autism liability on the population level, for the most part we are not yet able to identify the specific factors in common variation that contribute to risk in any given individual. With respect to ascertainment, the relative proportions of distinct genetic components vary depending on the sample of individuals studied. For example, simplex families with a single affected individual tend to be enriched for de novo variation, while studies of families with shared common ancestry often show enrichment for inherited factors, especially recessive mutations in the case of consanguineous pedigrees.

Additionally, even today there remain significant disparities in diagnosis in the United States along socioeconomic, racial, and ethnic lines (21), which presumably have important ramifications for research studies that may reflect these diagnostic biases. Thus, any precise estimate of specific contributions to risk should be taken with a grain of salt. Despite these important caveats, significant advances have been made in the past two decades. In the year 2000, identified causes of autism were limited to grossly visible abnormalities on karyotype and identification of monogenic disorders with characteristic comorbidities and presentations, such as tuberous sclerosis complex or fragile X syndrome. We now understand that other underlying genetic drivers, such as de novo variation, represent important components of the genetic architecture of autism. Nonetheless, even today a majority of individuals with autism do not have a single identifying cause (**Figure 1**). This review highlights recent and ongoing work that is seeking to bridge this gap and elucidate how genetic risk affects cellular functioning and clinical phenotypes. We hope that an improved

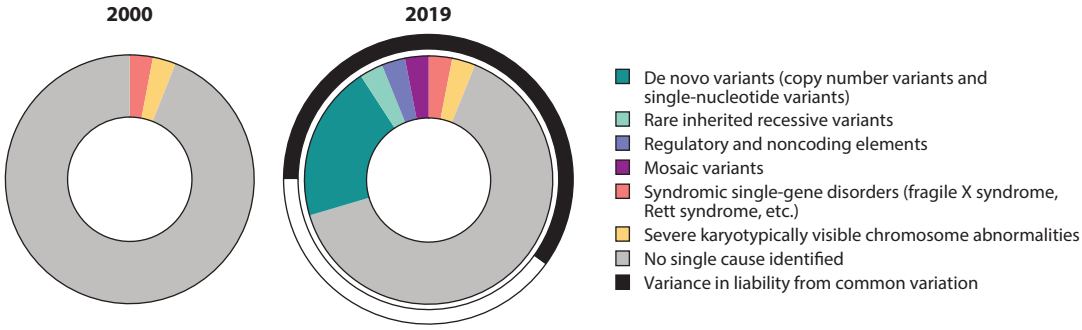

Figure 1

Advances in our understanding of the genetic architecture of autism from 2000 to 2019. The inner circle depicts the approximate estimated percentage of cases attributed to specific genetic causes; the outer ring highlights the role of common variation that is now understood to contribute to risk variance in the population. Common variation, which includes a large number of variants with very small effect size, affects the genetic background of all individuals, influencing the expression of other sources of variation. Although we have made significant strides in the past two decades, a significant portion of risk remains undefined.

understanding of the genetic architecture of autism will ultimately lead to improvements in clinical care (**Figure 2**). We should note that, although we discuss these recent advances categorically for the convenience of the reader, many of these genetic mechanisms are not mutually exclusive, and understanding the interplay is itself an important topic.

DE NOVO COPY NUMBER VARIANTS IN AUTISM

A rich body of literature has examined the role of de novo variation in the etiology of autism (3, 8, 30, 31, 34, 35, 47, 58, 59, 64, 73). In 2007, comparative genomic hybridization was used in groundbreaking work to establish a significant association between de novo submicroscopic structural variation and autism (64). This finding is now one of the most robustly replicated features of the genetic architecture of autism (44, 57, 58). Collectively, this source of variation represents one of the largest contributors to known autism risk, and as such, it represents one of the few genetic mechanisms that is routinely included in the current clinical assessment of patients. Specific loci have emerged as being recurrently linked to autism risk, including but not limited to 7q11.23, 15q11.2–11.3, 16p11.2, and 22q11.2 (20, 38, 44, 57, 78).

Given that copy number variants (CNVs) can represent deletions or duplications of dozens of genes, precisely localizing the source and mechanism of pathogenicity is challenging. The association of phenotypes with gene dosage presents clues, but the relationship is hardly straightforward. For example, microdeletions at 7q11.23 cause Williams syndrome, which has been classically associated with heightened sociability, while duplications at this same locus have been preferentially linked to autism and communication deficits (32, 57, 69), which might suggest that copy number gain and loss represent opposite effects on gene function and phenotype. However, closer inspection of the social functioning of individuals with microdeletions at this locus reveals coexisting deficits in core social skills, making the matter more complex (32). Given the complexity of social functioning and behavior, it is perhaps not surprising that the gene–phenotype relationship is more nuanced.

Another example of the complexity in interrogating the functional effects of structural variation on development and behavior is the 16p11.2 locus (28). Deletion and duplication at 16p11.2 are associated with autism at similar rates (11), but deletion is associated with macrocephaly and

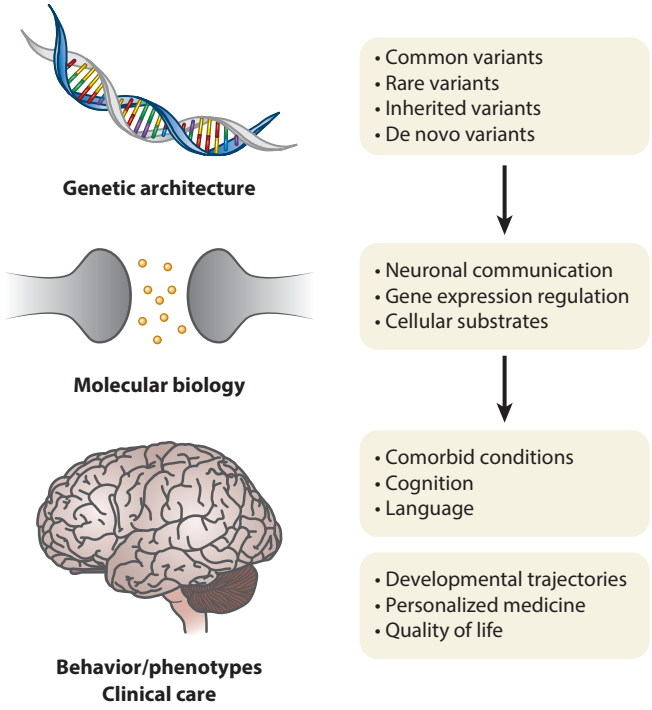

Figure 2

Illustration of how a better understanding of the underlying genetic architecture of autism advances clinical care. Understanding of the genetic architecture of autism has shed light on the underlying biological processes that are particularly important in its etiology, including gene expression regulation and synaptic communication. However, important questions remain, including how variants affect different cell types and lead to different phenotypes in different individuals. Ultimately, a better understanding of the genetic underpinnings is needed to improve clinical care in a variety of domains.

obesity, while duplication is associated with poor growth and microcephaly (11, 79). In an attempt to characterize the mechanism of this paradoxical phenotypic relationship, Deshpande et al. (14) utilized human induced pluripotent stem cells to study the cellular basis of these genetic differences in differentiated neurons from deletion and duplication carriers and discovered elegant cellular correlates of the clinical phenotypes. Such work represents the power afforded by systematically integrating human genetics with molecular neurobiology and model systems.

DE NOVO SINGLE-NUCLEOTIDE VARIANTS IN AUTISM AND IDENTIFICATION OF UNDERLYING NEUROBIOLOGY

Just as the technical ability to resolve submicroscopic CNVs allowed us to better understand the role of structural variation, the advent of exome sequencing subsequently revealed the important contribution of de novo single-nucleotide variants (SNVs) to autism risk (30, 31, 47, 49, 59). De novo SNVs associated with autism have been particularly important in advancing our understanding of the underlying neurobiology, given that these variants usually affect a single gene, thus allowing for greater genetic precision as compared with CNVs. Loss-of-function variants, in specific genes that can increasingly be identified as intolerant of mutation based on their limited variation in unaffected individuals, have provided particularly powerful evidence about the

biological networks altered in autism (12, 35). Exploration of missense variation represents a natural extension of this work, albeit through presumably more complex mechanisms, including gain of function in some cases.

The largest exome sequencing study to date harnessed bioinformatic innovation and increased sample size to shed light on these more complex genetic mechanisms in autism. Satterstrom et al. (62) studied 11,986 samples from individuals with autism from both family-based and case–control studies totaling 35,584 exomes. They applied enhanced bioinformatic analyses that integrated evolutionary constraint to identify more than 100 autism-associated genes with a false discovery rate less than or equal to 0.1. In addition to utilizing probability of loss of function (pLI) to identify variants affecting genes with greater functional severity, they also applied the missense badness, PolyPhen-2 constraint (MPC) score to similarly categorize missense variants by predicted impact (39, 56). Analysis not only confirmed enrichment of de novo mutations affecting highly constrained genes, as indicated by a high pLI score, but also identified a significant burden of more severe missense mutations, which parallels the finding described below in the context of rare inherited recessive variation (18). In fact, as the authors noted, the top tier of missense variants was more enriched in cases as compared with the second tier of protein-truncating variants, suggesting that missense variation represents a significant subset of variation with critical clinical implications. Furthermore, they were able to identify genes that were associated with autism more often through missense variation than through protein-truncating variants (PTVs). By systematically analyzing both the functional impact of the residues and the clinical phenotype, they argued that these mutations were likely contributory and, in some cases, acting by gain of function. Of course, bona fide experimental interrogation is particularly critical in these cases to better understand the mechanism of pathogenicity.

This study (62) also attempted to assess whether the autism-associated genes overlapped with the recently identified common variation found to contribute to autism risk (26). Although the authors identified one gene that emerged from both data sets, *KMT2E*, overall they did not find enrichment of a genome-wide association study (GWAS) signal in their list of autism-associated genes, despite identifying significant enrichment in schizophrenia and educational attainment data sets. They hypothesized that autism GWAS data sets may remain underpowered at this point in time.

Autism and intellectual disability are frequently comorbid. To identify genes that may preferentially affect one condition or another, the researchers examined the relative frequency of the disruptive de novo variants in a severe neurodevelopmental disorder ascertained group or an autism ascertained group and identified a paucity of inherited PTVs in the severe neurodevelopmental group (62), consistent with prior work (13). Importantly, they also noted that individuals with autism who carry disruptive severe neurodevelopmental variants walk significantly later and have a significantly lower IQ (by more than 11 IQ points) than individuals with autism who carry variants in autism ascertained genes, suggesting further genetic and phenotypic distinctions.

Functional annotation of autism-associated genes confirmed previous identification (12) of two important gene categories: gene expression regulation and neuronal communication (62). A new category of genes important in cytoskeletal organization was also identified. By overlaying the genes with developmental and cell type–specific experimental data from the Genotype-Tissue Expression (GTEx) data set, BrainSpan bulk RNA sequencing (RNA-seq) data sets, and single-cell RNA-seq data from the fetal human brain (1, 40, 48), the researchers were able to identify several intriguing patterns (62). The most dramatic increase in expression of implicated genes occurred during mid-fetal development. Assessment of the developmental trajectories of different categories of gene function showed that genes that fell within the gene expression regulation category were expressed at higher levels in prenatal development, while the expression of neuronal

communication genes demonstrated a trend toward a postnatal bias in expression. This intriguing finding presents the provocative possibility that there are distinct developmental periods of autism susceptibility. Although speculative, such findings could be critically important in understanding the variable responses to behavioral interventions in autism. For example, one might posit that individuals who carry variants in genes that continue to have important roles in postnatal development may show a more dramatic response to treatment if these neural substrates are targeted directly or indirectly by such behavioral therapies. Another important finding was that the genes implicated in expression regulation did not seem to target the neuronal communication gene category or significantly interact with it, suggesting potential unique biological drivers.

This study also took advantage of a single-cell RNA-seq data set spanning the developing human fetal forebrain (48) and tracked expression of the identified autism-related genes within different cell types and across developmental time points (62). Expression of genes of interest was enriched in both developing and mature excitatory and inhibitory neurons, with early excitatory neurons and striatal interneurons demonstrating the greatest enrichment. Interestingly, enrichment was not seen for microglia, despite studies in postmortem brain from individuals with autism that implicate this cell type in disease pathogenesis (23, 77). Although oligodendrocyte precursors and astrocytes were also significantly enriched for autism-associated genes, more than 95% of these genes were expressed in radial glia as well, illustrating the importance of better understanding cell type–specific expression repertoires. Transcriptomic analyses from several mouse models of Pitt–Hopkins syndrome, as well as postmortem human brain, also provided important evidence that oligodendrocytes and myelination dysregulation may represent an overlooked source of cellular pathology in autism (50). Other recent work has specifically investigated single-cell expression patterns from postmortem brain from individuals with autism and identified upper-layer excitatory neurons and microglia as differentially regulated in autism (77). Although further research is needed to untangle these somewhat conflicting results, studying alterations in gene function within distinct cellular subtypes offers a unique opportunity to address one the most significant unanswered questions facing the autism research community today: Are there specific cells and circuits responsible for the manifestation of symptoms in autism? Together, these studies emphasize the importance of better understanding the cell type specificity of genetic variation in the developing human brain.

Another recent study also evaluated whether inherited and de novo risk genes share biological pathways as well as how the genetic architecture of autism fits into the developing neurobiology of the fetal brain (55). The researchers utilized whole-genome sequencing from 2,308 individuals with autism from multiplex families. Although they found no significant difference in rates of rare inherited variants in affected individuals, they attributed this result to the fact that unaffected siblings may also have increased variation in risk, requiring a larger sample size than they analyzed. Nonetheless, they successfully identified 98 highly constrained genes that segregated with all affected individuals. When examining the protein–protein interaction network of high-risk inherited variation, they uncovered enrichment for both unique biological pathways (e.g., the cell cycle) and members of chromatin remodelers previously implicated in de novo autism risk, suggesting that rare inherited and de novo variants affect risk through both overlapping and distinct neurobiological mechanisms.

This study also explored distinctions in the underlying genetic architecture of simplex versus multiplex families (55). In particular, the frequency of rare de novo PTVs in affected children from multiplex families was half of that seen in simplex families. The authors also found that the high-confidence autism-risk genes (from both de novo and inherited signals) identified novel gene candidates and uncovered a subset of genes where enrichment was driven by rare inherited PTVs as opposed to de novo variation, suggesting that inherited variation remains a source for

novel biological insight that has not yet been fully tapped. They also demonstrated the utility of a novel bioinformatics tool to remove de novo artifacts from cell-line data, expanding the potential repertoire of samples available for more robust analysis.

MOSAICISM IN AUTISM

Mosaic mutations, another source of de novo variants that involve only some cells of the body due to their occurrence after fertilization, represent a known mechanism of disease in cancer and various neurological disorders (16, 25, 51, 54, 66). Mutations that confer grossly visible anatomical or histological changes allow elegant tracing from genetic sequence to the cellular phenotype. In autism, by contrast, identifying the functional consequences of somatic mutations is more challenging. Access to the tissue of greatest interest, the brain, is limited largely to postmortem tissue, an invaluable but scarce resource. Nonetheless, study of mosaic mutations in autism provides a powerful approach to better understand cells and circuits critical to the underlying neurobiology, as well as to potentially explain a fraction of cases that do not yet have a single genetic cause identified.

Although it is not yet clear what fraction of autism cases are affected by somatic variants, several recent studies suggest a modest but consistent contribution of somatic variants. For example, recent studies of somatic mutation have suggested that up to 5–7.5% of de novo mutations in autism may in fact be postzygotic mosaic mutations (22, 42). Additionally, the contribution of somatic mutation to autism liability may be underexplored because the genes and pathways affected in a mosaic state might be distinct from those implicated in germline studies. Variants that cause autism in a mosaic state could plausibly cause a different or more severe phenotype in the germline state and therefore might have not been considered as possible contributors to autism risk. Possible interactions between somatic mutations and germline mutations in regard to compounding and modulating risk also remain to be studied.

Studies of mosaic mutations in postmortem brain tissue are severely limited by the small sample sizes of postmortem tissue available as well as by the absence of parental samples, but they nonetheless suggest the presence of damaging mosaic mutations in some autism brains. Targeted sequencing in postmortem autism brain has previously identified potential contributory mosaic mutations (15), raising the tantalizing possibility that, given sufficient sample size, a more precise understanding of the contribution of mosaic mutation to autism risk will be achieved. In fact, a group of researchers have conducted a study on the largest set of autism-brain-derived whole-genome sequencing data to date, including ultradeep coverage from the prefrontal cortex of 59 autism cases and 15 controls (53). They identified potentially risk-modifying somatic mutations present in brain DNA, including a predicted damaging mutation in *CACNA1A* in one case. They also discovered a modest excess of somatic mutations in brain-active enhancers in autism cases compared with controls, suggesting that somatic mutations may contribute to risk through disruptions in the regulation of gene expression in addition to coding variation.

This work underlines the importance of future studies on noncoding mutations to further elucidate their role in autism pathogenesis in both germline and somatic states. Future studies of somatic mutation would benefit from including brain-derived DNA sources, as mutations at low cell fractions in the brain might not be detectable in peripheral DNA. There is also utility in combining assessments for somatic mutation specifically in noncoding and regulatory sequences in the much larger samples of peripheral DNA that are available for study, including those described below, with the caveat that peripherally detected somatic mutations may be present in different cell fractions and distributions within the brain.

THE CONTRIBUTION OF COMMON GENETIC VARIATION

The relative contributions of rare and common genetic variation to autism risk have been a long-standing point of debate in the field. Although the concept that common and rare variants interact in mediating the risk for autism is certainly not new (9, 10, 33, 76, 80), recent work has allowed for a more comprehensive understanding regarding this topic. In 2014, Gaugler et al. (24) demonstrated that although de novo variants contribute significantly to individual liability, they contribute only nominally to the variance in liability on a population level. They concluded that the majority of variance in population-level genetic risk arises from common heritable variation. In 2017, Weiner et al. (80) established that polygenic variation contributes additive risk with rare variation regardless of IQ. Their paper also highlighted the ongoing conundrum that polygenic risk for autism correlates positively with IQ, a finding that has yet to be satisfactorily resolved.

Until recently, specific common variants had not been identified. It was predicted that GWASs of autism risk would identify significant risk factors only once a sufficient sample size was obtained, and this prediction came to fruition as 2019 welcomed the first such GWAS in the autism field (26). This study utilized a Danish population from which blood spot samples from birth were available, allowing researchers to increase the sample size from the Psychiatric Genomics Consortium to conduct an analysis on a total of 18,381 individuals with autism and 27,969 controls—the largest study of its kind to date. Several important conclusions arose from this study. Not only were several significant common risk loci identified, but the authors also delineated the genetic heterogeneity of phenotypic subgroups. Specifically, they concluded that the single-nucleotide polymorphism (SNP) heritability was nearly three times higher in cases without comorbid intellectual disability than in cases with intellectual disability, and the heritability of Asperger's syndrome was twice that of childhood autism. The authors therefore argued that common variants play a larger role in high-functioning autism. This work also identified at least a component of a shared polygenic architecture with other psychiatric diagnoses and psychosocial constructs, including educational attainment, consistent with previous studies (19, 68). The authors noted that common variation in autism was enriched in regulatory elements predicted to play a role in the development of the human cortex and utilized Hi-C data from the developing fetal brain to identify potential regulatory targets.

This study provided the first glimpse of how common genetic risk variants contribute mechanistically. Given the relatively small effect size that individual common variants are expected to exert on autism risk, interrogating the effects of these variants on function is not a simple task. Verifying the functional importance of these variants and understanding in greater resolution how they converge on biology will require further investigation and experimental evidence. Additionally, we expect that, with continued increases in GWAS sample size and power, more common risk loci will be identified, which will provide rich genetic data for further exploration.

ADVANCES IN IDENTIFYING NONCODING AND REGULATORY PATHOGENIC VARIATION

Another initial focus in identifying causative factors in autism has more generally, and logically, centered on variants affecting protein-coding sequences, particularly those predicted to have larger functional consequences. However, the majority of the human genome consists of noncoding and regulatory regions. The improved cost and high-throughput analysis of whole-genome sequencing has led to the identification of variants associated with autism in this relatively unexplored domain. However, identifying variants of interest is only the first step. Attributing causality for noncoding and regulatory variants remains challenging for numerous reasons. First,

noncoding variants may on average have relatively smaller effect sizes. Second, even for variants that may have a stronger effect, our understanding of noncoding and regulatory elements lags behind our knowledge of coding sequences, which complicates attempts at predicting pathogenicity. Although recent advances in this field have been thoroughly reviewed (74), we briefly highlight topical work that has been critical in advancing the understanding of noncoding variation in autism risk below.

Both de novo and inherited noncoding variants have been implicated in autism risk (4, 7, 17, 67, 75, 81, 82). Given the lack of a robust functional categorization of the noncoding genome, creative approaches from both bioinformatics and experimental biology are being utilized to drive progress in this area. For example, Doan et al. (17) used comparative genomics to identify regions of the human genome with accelerated divergence from otherwise evolutionarily conserved sequences [human accelerated regions (HARs)], which presumably reflect critical function in the human lineage specifically, and many of which have predicted regulatory function in brain development. An analysis of HARs identified a significant burden of both de novo CNVs and biallelic SNVs in individuals with autism, and in-depth functional validation was conducted with in vitro cellular reporter assays as well as in vivo mouse models, providing strong functional evidence with respect to the impact of the identified variants (7). We should also note that this study harnessed comparative genomics to identify uniquely human sequences that could have particular significance for human cognition. In other studies, evolutionarily conserved loci have been implicated in noncoding sequence variation in autism cases (e.g., see 4). That both of these approaches can independently derive unique insights into autism risk highlights the importance of complementary methodologies in tackling such a complex subject.

Brandler et al. (7) sought to quantify the segregation and enrichment of structural variants that encompass *cis*-regulatory regions. Using a discovery cohort of 829 families, they found that paternally inherited *cis*-regulatory structural variants were preferentially transmitted to affected offspring, a result that they then replicated in an independent sample. These findings are intriguing given previous studies that have demonstrated a maternal bias of inherited truncating variants, presumably driven by the female protective effect and the larger genetic load required for susceptibility in females (37). Although the authors presented several arguments as to why there might be a paternal bias in transmission (a two-hit model, epigenetics, or meiotic drive), further study and replication will be needed to specifically address the mechanism that may contribute to this finding. Interestingly, unlike the work above that identified an increased burden of de novo CNVs involving HARs (17), these authors did not identify a role for more general de novo structural variation involving *cis*-regulatory elements in autism risk. Given that regulatory elements may have more moderate effects on risk in general, one way to explain this discrepancy is that assessment for HARs allowed the identification of a subset of variants with higher impact, which in turn might be more likely to enrich for de novo variation. The robust functional validation of the variants identified in the HARs study supports this possibility.

Another recent creative approach to understanding noncoding variation utilized a deep-learning method in conjunction with extensive experimental data to annotate the functional impact of de novo variants in probands in the Simons Simplex Collection (84). Using this approach, researchers demonstrated that regulatory de novo mutations in probands had a significantly higher predicted functional impact than those in unaffected siblings. They also noted that at least some of the variants affected the regulation of previously identified biological pathways, suggesting that noncoding and coding variation may represent risk from two sides of the same coin.

Another group examined the contribution of noncoding mutations to autism risk in 1,902 quad families, including one unaffected sibling (4). These researchers also did not identify increased noncoding de novo rates in cases in their data set. They thus implemented a rigorous and

unbiased genome-wide category-based de novo risk score, which identified de novo mutations at distal conserved promotors as contributing to autism risk. It is also important to note that this study was unable to replicate previous findings that had identified a significant burden of noncoding variation in specific regulatory elements (67, 73, 75). The authors emphasized the need for appropriate application of a conservative correction for multiple-hypothesis testing (81) and for using an unbiased genome-wide approach. More work with larger samples will be needed to determine the nuances of how noncoding and regulatory variation affects risk. Given the promise of recent biological and bioinformatic innovation, we are certain that further insights are on the horizon.

THE CONTRIBUTION OF INHERITED RECESSIVE VARIANTS

Given how informative de novo variants have been, it is somewhat understandable that there has been relatively less exploration of inherited factors (37, 71). Predictions that recessive inheritance contribute to risk in a subset of individuals with autism date back more than 30 years (52), and evidence has since been found that inherited recessive variation plays a role in autism liability (46); however, it has taken several decades to better establish the extent of its contribution. In 2013, Lim et al. (41) estimated that rare complete knockouts may contribute to as many as 5% of cases, with approximately 3% (both homozygous and compound heterozygous) on autosomes and another 2% from rare complete hemizygous knockouts on the X chromosome. A complementary study at that time utilized a cohort recruited by the Homozygosity Mapping Collaborative for Autism, comprising families with a history of consanguinity and/or multiple affected individuals, to study inherited biallelic variation (83). Both of these studies implicated rare inherited variation in autism risk. In addition to identifying likely inherited causative factors, the latter study also provided evidence that hypomorphic variants led to distinct phenotypes from their null mutation counterparts, which were associated with distinctive Mendelian disorders. This finding highlights an important more general feature of the heterogeneity of autism: As opposed to one gene being associated with one phenotype, different sources of variation—even with the same gene—can lead to distinct clinical consequences. Another example is the gene *SCN2A*, where different de novo variants have distinct impacts on ion channel functioning, which in turn lead to distinct clinical phenotypes (6): autism in some individuals, and infantile epilepsy in others. Such detailed interrogations of function will be increasingly required in order to better understand variant pathogenicity of all types.

Work from 2019 has expanded our understanding of the genetic contribution of inherited recessive risk. Researchers utilized data from the Autism Sequencing Consortium to study exome data from 2,343 individuals with autism and 5,852 unaffected individuals (18). This study estimated that approximately 5% of total cases are caused by biallelic loss-of-function or damaging missense mutations. An excess of damaging biallelic missense variation was identified as significantly enriched in cases. Interestingly, analysis of cases with de novo loss-of-function variants found no significant burden of biallelic damaging variants. Increased sample sizes will be needed to clarify whether this truly represents biology or whether the signal of recessive variation was being overshadowed by the larger effect of de novo variation. This study also replicated the female protective effect for rare recessive mutations, with almost twice as many cases attributable to this mechanism when considering females alone.

TRANSLATING GENETIC ADVANCES TO CLINICAL CARE

Despite the rapid advances in understanding the genetic architecture of autism in recent years, there has been a relatively slower translation of this information to clinical care. For example, the

first-tier genetic assessment of individuals with unexplained autism for many years has been chromosomal microarray and fragile X testing (45, 63, 65). However, as clinical exome sequencing has become more common and has identified SNVs as an important contributor of risk, the question has arisen of whether these recommendations remain appropriate. In fact, a recent meta-analysis by Srivastava et al. (70) estimated a yield of exome sequencing for neurodevelopmental disorders (global developmental delay, intellectual disability, and/or autism) at 36% overall, higher than that previously estimated for chromosomal microarray, which is closer to 15–20% (45). They argued that exome sequencing should be considered the first line in genetic assessment for individuals with neurodevelopmental disorders.

Genetic testing has utility on many fronts. For example, it has the potential to provide answers and allows patients and families to connect with support groups for individuals affected by similar conditions. It also has important implications for medical management and prediction of future risk to other offspring. Furthermore, discovery of genetic mechanisms allows for genotype–phenotype correlation, which enhances our understanding of the genetic underpinnings of the heterogeneity of autism. As we continue to identify causative genetic mechanisms in patients, we may be able to group patients into cohorts with similar biological causes to better understand developmental trajectories and develop more precise personalized therapeutic interventions (29). Based on this reasoning, there is a strong argument for employing the most informative genetic diagnostic strategies as a first-line approach. Given that structural variants can be called from exome sequencing data (36) with appropriate processing pipelines, there should be no significant loss of information in making exome or genome sequencing first tier as opposed to other methods, such as chromosomal microarray—in fact, this has already been demonstrated in a comparison of whole-genome sequencing and microarray in the case of intellectual disability (43).

All of the genetic advances may be for naught if the wealth of information generated from research studies is not applied to improving clinical care for individuals with autism and their families. Even more simply, the importance of communicating our basic understanding of the genetics and biology of autism to the general public cannot be overstated. Given the complexity of the genetic architecture of autism, the challenge of this mandate is not trivial. Unfortunately, a lack of treatment options (aside from behavioral therapy and treatment of comorbidities) has driven many families to seek alternative, unproven therapies that can be expensive and even harmful, such as chelation therapy and hyperbaric oxygen. To combat the pseudoscience and stigma that unfortunately still surround neurodevelopmental disorders, a better understanding of the biological underpinnings of autism must be developed and communicated.

CONCLUSIONS

In the past year alone, there have been tremendous advances in the understanding of the underlying genetic architecture of autism. For the first time, increased sample sizes have permitted identification of specific common genetic risk factors implicated in autism risk. Technological and bioinformatic innovation has facilitated exploration of regulatory and other noncoding regions of the genome. New statistical and biological approaches, as well as increases in sample sizes, will continue to be needed to advance the field. Although many conclusions from prior studies have been replicated with more recent work (including the female protective effect and the importance of networks involved in gene expression regulation and synaptic signaling), others have been more inconsistent (such as differences in the burdens in different categories of regulatory elements and the role of specific cellular subtypes), highlighting the need for continued interrogation of these open questions. One final challenge will be translating the wealth of genetic knowledge that is rapidly emerging in this genomic era to improving clinical care for patients and families.

Improving communication among geneticists, basic neurobiologists, and clinicians as well as with patients and families will be critical in expanding on the technological and experimental innovation that is needed to confront the challenging task at hand.

SUMMARY POINTS

1. Evidence continues to support the idea that diverse genetic mechanisms converge on common biological pathways.
2. Ongoing exploration of mosaic mutations in autism promises to shed light on critical mechanisms, cellular subtypes, and circuits.
3. Genome-wide association studies, powered by increased sample size, have enabled the first identification of specific common loci significantly associated with risk for autism.
4. Noncoding and regulatory regions of the human genome represent an important avenue of investigation, powered in part by bioinformatic and experimental innovation.
5. Rare inherited recessive variation represents a small but not insignificant fraction of cases of autism.
6. Whole-exome sequencing should be considered a first-line genetic test in neurodevelopmental disorders.

FUTURE ISSUES

1. There is an ongoing need to combine recent genetic and genomic discoveries with functional evidence to better understand the impact of genetic variation on risk, whether it be on cellular functioning or clinical phenotype.
2. Future studies should seek to understand the interplay among different genetic sources of risk.
3. Communicating the plethora of information generated in the genomics era to clinicians, patients, and families is critical to truly advancing the field.

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