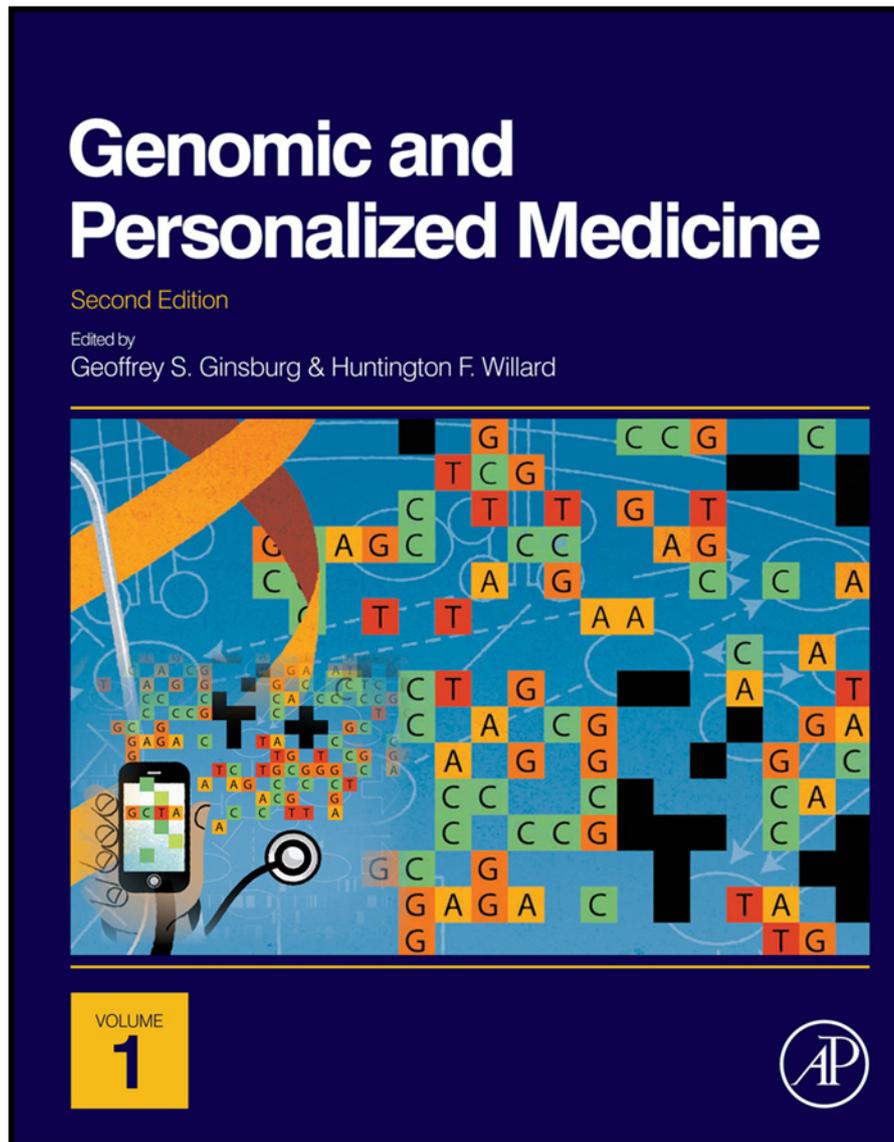


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CHAPTER



Autism Spectrum Disorders

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INTRODUCTION

Views on genetics of autism spectrum disorders (ASD) have changed dramatically in recent years. Although twin studies have supported a strong role for genetics for some time (Bailey et al., 1995; Folstein and Piven, 1991; Hallmayer et al., 2011), the paucity of known specific genetic causes has sustained persistent skepticism about the roles of genes (as opposed to, for example, environmental agents such as vaccines) and controversy as to the sorts of genetic risk factors that might be prevalent in ASD. The last few years have seen an explosion in the identification and study of highly penetrant, quasi-Mendelian ASD genes. The identification of these ASD genes reflects continuous developments in genomic technology, including the ability to determine copy number systematically at all sites in the genome and, more recently, the ability to sequence in a rapid and high-throughput fashion the entire genome or, in other cases, the entire portion of the genome that encodes proteins, known as the exome (Gillis and Rouleau, 2011). Currently, we are in the midst of an explosion of understanding of the genetic architecture of the disease due to the widespread application (as yet only beginning) of these powerful new sequencing methods.

Three major concepts have emerged recently about the genetics of ASD. First and foremost, ASD are extremely heterogeneous in every way (Abrahams and Geschwind, 2008;

Geschwind, 2008). The behavioral phenotype of autism itself is heterogeneous, in the sense that the term encompasses children with a very wide range of clinical presentations. Moreover, some children diagnosed on the broad autism spectrum can move into or out of the diagnosis of autism over the years, so the diagnosis is not always static. However, beyond even that, even if one focuses on children who look very similar clinically or phenotypically, children with indistinguishable conditions can reflect mutations in a very wide range of different genes, to the point that we presently do not have clinical tools capable of predicting a specific genetic etiology.

A second important concept is the effect of evolutionary selection on ASD-associated mutations. Since ASD patients, like those with intellectual disability or other severely disabling childhood illnesses, show severely reduced fertility, they have a greatly reduced likelihood of passing their disease-associated mutations to progeny, by comparison, for example, to typically developing children and/or those with adult-onset diseases. Therefore, mutations that confer a large relative risk in the heterozygous state tend to be very rarely transmitted. Hence, the presence of these highly penetrant heterozygous mutations in the population typically represents *de novo* mutations, i.e., those present in the affected child but not found in either parent (Abrahams and Geschwind, 2008; Gauthier et al., 2009; Kumar et al., 2008; Moessner et al., 2007; Sebat et al., 2007; Weiss et al., 2008).

Such *de novo* mutations are a much more common cause of severe childhood illness than is generally recognized, though ASD do not so far appear to show a higher *de novo* mutation rate than other diseases with similarly severely reduced rates of transmission.

A third important concept is that no gene identified to date causes only ASD and nothing else; rather, the genes found to be mutated in ASD can be mutated in other developmental brain disorders that do not have ASD as a clinical feature. This observation was known from the earliest stages of analysis for Mendelian disorders such as tuberous sclerosis and fragile X, for which a certain proportion of patients present with typical ASD, whereas other affected patients can show intellectual disability or other features without prominent social defects. However, more recently, the spectrum of behavioral manifestations of virtually any genetic mutation that greatly increases the risk of ASD has been broadened, so that, for example, two well-documented copy number variants (CNVs), deletions at 22q11.2 or 16p11.2, can be associated with ASD, intellectual disabilities, or other neuropsychiatric disorders such as schizophrenia (Guilmatre et al., 2009; Lionel et al., 2011; Stone et al., 2008). No well-documented genetic cause of ASD so far causes “just autism,” and this is true of so many genetic mutations that it has become highly likely to be a general rule. Hence, notwithstanding the clinical and diagnostic challenges, it should not be surprising that children with a variety of phenotypes can have similar underlying genetic pathology.

DEFINING AUTISM IN THE CLINIC AND IN THE LABORATORY

Autism is defined behaviorally by a core triad of social defects: poor language development, poor social behavior, and repetitive or stereotyped behaviors. Although these symptoms may be immediately evident upon clinical assessment, quantitative and standardized behavioral measures are often used to confirm the diagnosis and to quantify specific features in some cases. In the present *Diagnostic and Statistical Manual* version IV (DSM-IV-TR), ASD are synonymous with pervasive developmental disorders (PDD) and comprise five categories: autistic disorders, PDD not otherwise specified (PDD-NOS), Asperger syndrome, Rett syndrome, and childhood disintegrative disorders, though these categories are certain to be reorganized with the impending publication of DSM-V.

The term “autism” itself is a difficult one to apply robustly in a genetic research setting, since the diagnosis does not correlate with pathogenesis, level of function, genetic diagnosis, or prognosis. This becomes problematic in a research context since patients ascertained in different settings (e.g., school versus hospital clinic) can have very different levels of function associated with their ASD and hence probably have different sorts of underlying causation. This can be compounded by the fact that some clinicians tend to exclude from ASD those children with clinical features of ASD, but who have a known genetic condition (e.g., fragile X or tuberous sclerosis) even though the

results of the revised autism diagnostic interview (ADI-R) or autism diagnostic observation schedule (ADOS) would classify them as autistic. As more and more specific genetic causes of ASD are identified, this creates a challenge for systematic classification. Fortunately, ASD are increasingly regarded as falling on a continuum with other developmental brain disorders, showing considerable overlap with many of them at a genetic, pathogenetic, syndromic, and behavioral level.

“SYNDROMIC” ASSOCIATIONS WITH ASD

Many of the earliest known genes associated with ASD were identified based on the fact that they also caused broader, multi-organ syndromes, and several such syndromes have ASD as a frequent manifestation. Tuberous sclerosis syndrome (TSC) is an autosomal dominant disorder, associated with a high spontaneous mutation rate, that manifests with cardiac tumors, skin lesions, and hamartomas in many organs, including the brain. Up to 50% of affected children show autistic symptoms, most in the setting of intellectual disability (Wiznitzer, 2004). So far, there is no clear understanding of the determinants of ASD symptoms in patients with TSC. Recently, animal models have shown that there are widespread abnormalities in myelination (Meikle et al., 2007), axonal connectivity, and other developmental events in mice heterozygous for TSC mutations, suggesting that humans with TSC mutations may also have widespread neuronal abnormalities beyond the focal lesions that are only evident with newer, sophisticated magnetic resonance imaging (MRI) methods (Choi et al., 2008).

Fragile X syndrome, associated often with mild dysmorphic features of the ears and head, is associated with ASD in about 50% of children and can rarely present with ASD in the absence of severe intellectual disability. Fragile X is probably the most common single-gene disorder causing ASD (Belmonte and Bourgeron, 2006; Bolton, 2009; McLennan et al., 2011).

The progressive neurodevelopmental disorder Rett syndrome (RTT; Mendelian Inheritance in Man (MIM) 312750) is caused by mutations in the X-linked gene *MECP2* (Amir et al., 1999). RTT is characterized by normal development in the first 6–18 months of life, followed by loss of any acquired speech and the replacement of purposeful hand use with stereotypic movements. *MECP2* mutations, as well as increased gene dosage, can result in a range of neurobehavioral abnormalities, including autism, mild learning disabilities, X-linked mental retardation, and infantile encephalopathy (Chahrour and Zoghbi, 2007). RTT phenotypes overlap with nonsyndromic autism, and RTT is included in DSM-IV. *MECP2* mutations have been reported in ~1% of children diagnosed with autism (Moretti and Zoghbi, 2006), and males with *MECP2* duplications often present with autism (Ramocki and Zoghbi, 2008; Ramocki et al., 2009). The MeCP2 protein regulates the expression of its target genes, and a better understanding of its role in maintaining neuronal function will have implications for autism.

A plethora of other Mendelian syndromes are associated with autistic symptoms as well, but less commonly or universally. As many as 300 different genetic syndromes have been reported at some level to be associated with ASD (Betancur, 2011), although the proportion of patients with the genetic syndrome that manifest ASD is highly variable, so that the strength of these associations is variable. It is important to note that, given that up to 1% of all children suffer from ASD, ASD will of course at some point be reported in children with any possible condition as a coincidence, and larger series will eventually be needed to prioritize which genetic syndromes are truly most commonly associated with ASD.

CHROMOSOMAL DISORDERS AND COPY NUMBER VARIANTS

A growing number of chromosomal rearrangements and duplications or deletions have been associated with ASD. Two of the earliest identified recurrent chromosomal disorders are deletions of 22q11.2 and duplications of 15q11-14. Deletions of 22q11.2, which are associated with the velo-cardio-facial syndrome, or duplications of the same interval, have also been associated with intellectual disability, schizophrenia, and ASD (Eliez, 2007; Fine et al., 2005; Lo-Castro et al., 2009; Ramelli et al., 2008). Inverted duplications of 15q11-14 were found in about 1% of children with ASD and were found to be exclusively maternally inherited, suggesting that an imprinted locus, likely on proximal 15q, is responsible for the ASD symptoms (Cook et al., 1997). These recurrent genomic syndromes served as a model for genetic causation of ASD, but determining the prevalence of such genomic rearrangements, or the potential role of smaller deletions and duplications in ASD, required new technology to scan genomes at high resolution.

A major breakthrough in autism genetics was the discovery of spontaneous (i.e., *de novo*) CNVs as a fairly common cause

of ASD (Table 90.1) (Christian et al., 2008; Gilman et al., 2011; Guilmatre et al., 2009; Kusenda and Sebat, 2008; Lee and Lupski, 2006; Marshall et al., 2008; Morrow, 2010; Pinto et al., 2010; Sanders et al., 2011; Schaaf and Zoghbi, 2011; Sebat et al., 2007; Sykes et al., 2009). The availability of higher resolution microarrays has allowed systematic analysis of even small regions of the genome and led to the recognition of spontaneous CNVs in as many as 10% of ASD patients. The most recent and most rigorous studies, using the most modern genome arrays, suggest a consistent excess of CNVs in ~5% of ASD patients, but probably not much higher (Gilman et al., 2011; Levy et al., 2011; Pinto et al., 2010; Sanders et al., 2011; Shen et al., 2010).

Overall, CNVs illustrate several critical aspects of ASD genetics: the high degree of genetic heterogeneity, the important role for *de novo* mutation due to the severe negative evolutionary selection against the condition, and the seemingly indirect mapping of behavior onto genetics, with similar mutations causing a range of phenotypes.

Diagnostic Testing with Chromosomal Microarrays

The identification of spontaneous CNVs as a cause of ASD emphasized that the genetic landscape of ASD is likely to involve a major role of rare variation, including spontaneous mutation, and has hastened the pursuit of other rare variants (e.g., *de novo* point mutations and rare recessive mutations) as well. These chromosome microarray (CMA) studies have led to the recognition of many new regions of the genome that are recurrently affected in unrelated patients, including in 16p11.2, 7q11.23, and 15q13-14. Nonetheless, more than half of the regions that are deleted or duplicated in ASD-related CNVs have very low recurrence and are highly heterogeneous, sometimes almost unique to a given patient. *De novo* CNVs also occur in unaffected individuals (Sebat et al., 2004), making it surprisingly difficult to ascertain exactly which CNVs are likely to be causative and which are benign. Nonetheless, the

TABLE 90.1 Summary of recurrent *de novo* CNVs in ASD

Genomic region	Frequency in ASD	Deletion/duplication	Evidence level	Other disease associations
16p11.2	0.5%	Deletion	++++	ID, ADHD, schizophrenia, obesity
		Duplication	++	ID, epilepsy, schizophrenia, microcephaly
15q11.2-13.1	0.2%	Duplication	+++	ID, epilepsy, schizophrenia
<i>NRXN1</i> locus	0.13%	Deletion	+	Schizophrenia
22q11.2	0.13%	Deletion	++	VCFS, ID, schizophrenia
		Duplication	+	ID, ADHD
7q11.23	0.1%	Duplication	+	

The table summarizes some of the most common CNVs associated with ASD, and indicates the approximate level of evidence supporting their association. The right column also indicates additional neuropsychiatric or other disorders that have been associated with these same CNVs, to illustrate the variety of phenotypes associated with a given mutation. ID=intellectual disability, ADHD=attention deficit hyperactivity disorder, VCFS=velo-cardio-facial syndrome.

Adapted from Sanders et al., 2011.

widespread use of diagnostic CMA has revolutionized much of the culture of ASD clinical testing, since CMA is the single diagnostic test with the highest yield, leading to a consensus that CMA should be performed in all patients who are diagnosed clinically as being on the autism spectrum (Miller et al., 2011).

The 16p11.2 Deletion/Duplication Syndrome

The 16p11.2 deletion/duplication syndrome, noted previously (Kumar et al., 2008; Sebat et al., 2007) but most clearly delineated by Weiss and colleagues (2008), is now arguably one of the best-characterized ASD genetic syndromes. The 16p11.2 deletion syndrome illustrates themes that recur with other genetic forms of ASD. The 16p11.2 deletions are frequently *de novo*, but can be inherited in perhaps 50% of families, from parents who are either mildly affected or clinically normal, emphasizing the importance of *de novo* variation (Weiss et al., 2008). Deletions of 16p11.2 are not by any means invariably associated with ASD. Overall, about one third of deletion patients meet strict research criteria for autism, being indistinguishable in any syndromic or psychological way from “idiopathic” autism (Hanson et al., 2010; Miller et al., 1993; Sanders et al., 2011), even when phenotyped “blind” to genotype. On the other hand, another third or so of patients fulfill some criteria for ASD but not others; and the last third of patients with 16p11.2 deletion do not have ASD at all. Instead, they may be clinically normal or may have other neurodevelopmental conditions such as intellectual disability, schizophrenia, obesity, ADHD, epilepsy, or multiple disorders (Fernandez et al., 2010; Hanson et al., 2010; Miller et al., 1993; Yu et al., 2011). This heterogeneity even holds when the same deletion is inherited within a family (Shen et al., 2011), and in some families in which a deletion segregates, affected patients may lack a deletion present in other family members, or *vice versa* (Sanders et al., 2011). Duplication of the identical region on 16p11.2 also greatly increases the risk of ASD (Weiss et al., 2008), but 16p11.2 duplications are even more heterogeneous, being associated with brain malformations in some cases (agenesis of the corpus callosum, microcephaly), a high rate of epilepsy (Bedoyan et al., 2010), schizophrenia (McCarthy et al., 2009), and often more severe intellectual disability (Shinawi et al., 2010).

The Simons Simplex Collection

The Simons Simplex Collection (SSC) was initiated by the Simons Foundation in 2007 as a prospective registry and DNA sample collection that combined detailed phenotyping with intensive genetic study (Fischbach and Lord, 2010). It was conceived based on the suggestion that spontaneous mutation in ASD might be more common in “simplex” families, in which only a single individual is affected with ASD (Sebat et al., 2007), but the remarkable structure of the pedigrees has also facilitated studies of other genetic mechanisms in ASD. Recent study of the SSC has highlighted several important points. The study confirms that CNVs are a consistently reproducible

and highly significant cause of ASD, present and likely causative in ~5% of patients in the SSC. The SSC has confirmed the high statistical association of 16p11.2 CNV with ASD and has highlighted other newly described CNV regions (Gilman et al., 2011; Levy et al., 2011; Sanders et al., 2011). The SSC studies also show that patients with CNVs as a cause of ASD are not typically distinguishable from patients with “idiopathic” autism by any observable feature (e.g., IQ, social scales, etc.).

One region discovered to be associated with ASD in the SSC studies is the Williams syndrome region on 7q11.23. The Williams syndrome region is intriguing, because here a deletion is associated with a condition characterized by abnormal positive sociability, whereas the duplication is associated with poor socialization, suggesting a “dose-response” relationship to behavior. Hints of similar “dose effects” are seen for 16p11.2 deletion/duplication in head size (large and small, respectively), and body mass (obese and thin, respectively) (Hanson et al., 2010; Tannour-Louet et al., 2010; Yu et al., 2011), and have also been claimed for duplication/deletion of the 22q11.2 regions (Eliez, 2007; Lo-Castro et al., 2009; Niklasson et al., 2009). The physiological bases of these apparent dose effects are not clear.

“NONSYNDROMIC” GENES FOR ASD

A major step forward in our understanding of autism genetics came with identification of *NLGN3* and *NLGN4X* as the first clear, “nonsyndromic” causes of ASD, meaning that patients with these mutations can be indistinguishable from ASD patients without a clear genetic diagnosis (unlike, for example, TSC or fragile X syndrome, in which patients have additional somatic signs that can be diagnostic) (Jamain et al., 2003). *NLGN4X* was first identified as an X-linked candidate gene because it was deleted in a patient with ASD (Jamain et al., 2003); subsequent sequence analysis revealed additional point mutations in other males with ASD and identified a large family with an inherited mutation in *NLGN4X* present in 19 affected males, four of whom showed ASD, with the remaining affected males showing intellectual disability, emphasizing the overlap of ASD and intellectual disabilities (Laumonnier et al., 2004).

SHANK3 was also identified as a candidate gene based on the discovery of small deletions (Durand et al., 2007), and subsequent resequencing of *SHANK3* in patients with ASD has shown point mutations in up to 0.75% of ASD patients (Gauthier et al., 2009; Moessner et al., 2007). A chromosome rearrangement, as well as a rare recessive disorder associated with autistic symptoms (Strauss et al., 2006), also led to the identification of *CNTNAP2* as an ASD gene (Alarcon et al., 2008; Arking et al., 2008; Bakkaloglu et al., 2008; Stephan, 2008).

Although deletions were instrumental in implicating *SHANK3* and *NLGN4X* as ASD candidate regions, resequencing of genes contained in other recurrent CNV intervals has not always been successful in implicating a single gene as

causative for the ASD. For example, in the 22q11.2 region, the 16p11.2 region (Konyukh et al., 2011), and the 7q11.23 region, the specific ASD gene is not yet known, suggesting the possibility that ASD in these cases reflects the combinatorial action of more than one gene in the interval. Suggestive evidence has also recently been presented for “oligogenic heterozygosity,” implicating the coexistence of heterozygous mutations at more than one candidate gene as a potential causative mechanism of ASD (Schaaf et al., 2011).

RECESSIVE MUTATIONS IN ASD

The observation that all known ASD mutations appear to act by a loss-of-function mechanism suggests that other ASD mutations may also be loss-of-function; wherever this is the case, recessively acting or inherited mutations are likely to be important. For example, for human developmental brain malformations, one or two of the most common genetic mutations are dominant or X-linked, but the vast bulk of the heterogeneity of the condition arises from many rare autosomal recessive syndromes (Manzini and Walsh, 2011). Similarly, intellectual disability (whether associated with autistic features or not) is known to be caused by >70 X-linked recessive genes (Ropers, 2006), but there are already estimates of hundreds of autosomal recessive causes (Ropers, 2006, 2008), of which only dozens have been identified so far (Basel-Vanagaite et al., 2006; Mochida et al., 2009; Najmabadi et al., 2011).

Identification of recessive mutations for rare diseases has been aided recently by the advent of whole-exome sequencing (Bilguvar et al., 2010; Choi et al., 2009; Ng et al., 2010; Shendure and Ji, 2008). Typically, this still requires the ascertainment of large families, often with parental consanguinity (Mochida et al., 2009; Najmabadi et al., 2011; Yu et al., 2010), because the tremendous diversity/heterogeneity of the condition makes it virtually impossible to confidently identify recurrent mutations in unrelated families. Thus, while recessive mutations have been barely explored as a cause of ASD, one would expect that, by analogy to other brain disorders, recessive mutations may contribute greatly to the genetic heterogeneity of ASD. In support of this model, many syndromes associated with ASD also act in a recessive fashion (Betancur, 2011).

In the sole study of consanguineous families with ASD reported to date, several lines of evidence support a contribution of recessive genes to ASD. First, multiplex families (i.e., with more than one affected family) in which the parents are also related, show a lower male/female ratio of affected children (<3M/1F) than offspring of unrelated parents (>4M/1F); a more equal male/female ratio would be consistent with a higher contribution of autosomal mutations. Moreover, multiplex offspring of consanguineous parents show a lower frequency of *de novo* CNVs that segregate with disease, again suggesting other mechanisms at work. Finally, CNV analysis in a cohort of 78 probands identified five probands with homozygous deletions that appear to be tolerated in the heterozygous carrier state, but are causative

in the homozygous state. At least one of these deletions implicated a gene (*SLC9A9/NHE9*) that showed a significant excess of mutations in unrelated cases of ASD as well (Morrow et al., 2008). Some analysis of non-consanguineous families also suggests potential roles of recessive mutations (Casey et al., 2011; Chahrour et al., 2012). These data suggest that appropriate study designs to identify recessive mutations in ASD (e.g., further study of consanguineous families) may be very valuable.

WHAT ARE THE ROLES OF COMMON VARIANTS IN ASD?

A number of genetic association studies have been performed on large cohorts of ASD patients and have also suggested potential roles for “common” alleles in ASD, although these studies remain somewhat underpowered, and further work in the upcoming years is likely to be more illuminating (Levitt and Campbell, 2009). One interesting association has been with the c-Met gene (Campbell et al., 2006). A number of genome-wide association studies (GWAS) have recently been published, though the results have not been entirely clear. Two loci on chromosome 5 have been implicated (Glessner et al., 2009; Wang et al., 2009; Weiss et al., 2009), although one of the studies has been criticized on technical grounds (McClellan and King, 2010), and a subsequent meta-analysis did not confirm these two regions (Anney et al., 2010). Given the great heterogeneity in autism and the confirmed role for rare variants, larger studies may be needed, perhaps even studies in which patients with obvious high-risk CNVs or other high-risk alleles are removed, in order to detect a stronger signal of common variation.

EARLY RESULTS OF WHOLE-EXOME AND WHOLE-GENOME SEQUENCING IN ASD

Given that ASD mutations are strongly selected against evolutionarily, several studies have attempted to identify risk mutations directly by increasingly large-scale sequencing of candidate genes to identify mutations not present in parents but present in patients. One large study performed systematic resequencing of genes encoding proteins of the synapse and spine apparatus of neurons, testing for *de novo* mutations, and identified such mutations in *SHANK3* in several patients with ASD (Gauthier et al., 2009). Another study pioneered the use of whole-exome sequencing in ASD and suggested that a significant proportion of ASD patients may have *de novo* mutations (Ng et al., 2009; O’Roak et al., 2011). If further confirmed, these results would greatly expand the proportion of autism that can be explained genetically and would begin to argue strongly that whole-exome sequencing, along with a sensitive analysis of CNVs, would be central to the diagnostic evaluation of children on the autism spectrum in the not-too-distant future.

MECHANISTIC INSIGHTS INTO AUTISM FROM GENETIC STUDIES

Since the ultimate goal of genetic analysis in disease is the development and improvement of treatment, it is appropriate to ask what we have learned thus far from the genetic analysis of ASD. Space does not permit an in-depth analysis of this topic, which has been reviewed extensively elsewhere (Kelleher and Bear, 2008; Ramocki and Zoghbi, 2008; Schaaf and Zoghbi, 2011; Walsh et al., 2008), but a few comments may provide some general perspective.

A great deal of evidence supports the general interpretation that most, if not all, genes associated with ASD encode proteins with roles in the regulation of synapses. This association with synapses includes very direct roles, such as *NRXN1* and *NLGN3/4* genes, which encode structural adhesion molecules that constitute important components of the synaptic specialization itself. Other ASD genes encode intracellular scaffolding proteins that function in synaptic spines, such as *SHANK3* (Schaaf and Zoghbi, 2011). Additional ASD-related genes regulate levels of local protein translation in the dendritic spine, such as the FMRP protein, or the TSC proteins. Proteomic studies have identified up to 1000 proteins in synapses and spines, which would conveniently account for the expected tremendous genetic heterogeneity of ASD. Finally, still other ASD-related proteins function “downstream,” in the nucleus, to regulate mRNA synthesis that is tightly regulated by neuronal activity (Guy et al., 2011). In some sense then,

ASD-related genes are increasingly defining in some depth a pathway that appears to take us from neuronal activity to the plastic changes that underlie learning and memory.

The increasing focus of ASD genes on synaptic plasticity is tremendously exciting therapeutically, because it suggests a potential that drugs affecting many steps in this pathway may nonetheless hold some promise for children with diverse genetic mutations (Abrahams and Geschwind, 2008; Geschwind, 2008; Kelleher and Bear, 2008; Walsh et al., 2008). A number of animal studies have demonstrated the remarkable degree to which several mutant mice strains can improve dramatically by gene replacement (Giacometti et al., 2007; Guy et al., 2007), or pharmacological manipulation (Kelleher and Bear, 2008; Tropea et al., 2009), even after much of early brain development is complete, creating a surge of interest in the pharmacological treatment of ASD. While it is extremely early days in this challenging area, there is reason for some optimism.

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