Solving the Autism Puzzle a Few Pieces at a Time

Christian P. Schaaf[1,*] and Huda Y. Zoghbi[1,2,3,4,*]
[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA
[2]Howard Hughes Medical Institute, USA
[3]Departments of Pediatrics and Neuroscience, Program in Developmental Biology, Baylor College of Medicine, Houston, TX 77030, USA
[4]Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, Houston, Texas 77030, USA
*Correspondence: schaaf@bcm.edu (C.P.S.), hzoghbi@bcm.edu (H.Y.Z.)
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In this issue, a pair of studies (Levy et al. and Sanders et al.) identify several de novo copy-number variants that together account for 5%–8% of cases of simplex autism spectrum disorders. These studies suggest that several hundreds of loci are likely to contribute to the complex genetic heterogeneity of this group of disorders. An accompanying study in this issue (Gilman et al.), presents network analysis implicating these CNVs in neural processes related to synapse development, axon targeting, and neuron motility.

Autism spectrum disorders (ASDs) are among the most common neuropsychiatric disorders, with an estimated worldwide prevalence of 1%–2.6% (Kogan et al., 2009; Kim et al., 2011). Almost 70 years after the description of autism by Leo Kanner and Hans Asperger, tremendous progress has been made in the recognition and diagnosis of children with ASDs. It is well established that ASDs represent a heterogeneous group of disorders that are highly heritable, with heritability indices estimated at 85%–92%. Advances in identifying the genetic causes of ASDs first came from the study of syndromic autism (ASDs in conjunction with congenital malformations and/or dysmorphic features), which pinpointed the causes of disorders, such as fragile X syndrome, Rett syndrome, *PTEN* macrocephaly syndrome, Timothy syndrome, and Joubert syndrome, to name a few (Miles, 2011). The challenge, however, was identifying the genetic cause of nonsyndromic or idiopathic autism given the lack of defining features besides the neurobehavioral phenotypes and the fact that the majority of cases were simplex (one affected in a family). This issue of *Neuron* highlights three studies of simplex, mostly nonsyndromic, relatively high-functioning ASDs (Levy et al., 2011; Sanders et al., 2011; Gilman et al., 2011), that establish de novo copy-number variants (CNVs) as the cause of 5%–8% of cases of simplex autism.

Copy-Number Variants and ASD

Using different array platforms on practically the same cohort of patients, both Sanders et al. (2011) and Levy et al. (2011) confirmed the role of de novo CNVs in the etiology of idiopathic autism. The analysis of a large number of families from the Simons Simplex Collection (SSC)—887 families in the Levy paper and 1174 families in the Sanders paper—allows them to confirm multiple known ASD loci but also to identify novel loci, such as 16p13.2 and the *CDH13* locus. The sheer number of different de novo CNVs identified in the probands, but not their unaffected siblings, supports the conclusion that autism is mostly caused by rare mutations (at least for CNVs that is), with most de novo events being unique to each proband.

As previously established, and now confirmed in larger data sets, deletions and duplications of 16p11.2 are the single most common cause of ASDs identifiable by DNA array analysis. This is the only locus known to date that accounts for > 1% of ASD cases, i.e., 1.1%–1.2%, with deletions being slightly more common than duplications. Given the relatively large number of individuals with CNVs identified in these studies, it would have been interesting to learn of any genotype-phenotype correlations, but none were reported except for body mass index, which negatively correlates with copy number at the 16p11.2 locus.

It is quite interesting that both studies revealed the importance of 7q11.23 as an ASD locus. While deletions of this region cause Williams syndrome, a multiple congenital anomaly syndrome with hypersociable behaviors, duplications of the same region cause ASDs. The opposing social phenotypes of 7p11.23 deletions and duplications provide a fascinating basis for studies in animal models to pinpoint the genes and neurons mediating these phenotypes. Within the genomic region, *CLIP2*, *LIMK1*, *GTF2i*, and *STX1A* have been proposed as potential culprit genes, but the exact underlying pathomechanisms are far from being understood.

The Diverse Genetic Causes of ASDs

While the high heritability of autism is well established, the exact underlying causes and mutations are identifiable only in a minority of patients. Using current clinical DNA arrays, relevant de novo genomic imbalances can be identified in 7%–20% of individuals with autism of unknown cause. As expected, the yield is higher in those individuals with "syndromic" autism. Known single-gene disorders account for another 5%–7% of cases, with fragile X syndrome being the most common (1%–3% of cases), followed by *PTEN* macrocephaly syndrome, tuberous sclerosis complex, and Rett syndrome (each accounting for approximately 1% of children diagnosed with autism) (Miles, 2011). Timothy syndrome, Joubert syndrome, *SHANK3* mutations, *NRXN1* mutations, and a handful of other genes account for rare cases. There remain large cohorts of patients that have to be screened for the incidence of respective point mutations and their contribution to the overall number of autism cases. Lastly, several metabolic conditions have been associated with ASDs, including mitochondrial disorders,

phenylketonuria, adenylosuccinate lyase deficiency, creatine deficiency, and some disorders of sterol biosynthesis. In total, known metabolic disorders may account for approximately 5% of cases of ASDs. This leaves us with at least 70% of cases, for which the genetic cause of ASDs cannot yet be identified (Figure 1). The percentage is even higher for the nonsyndromic cases of ASDs.

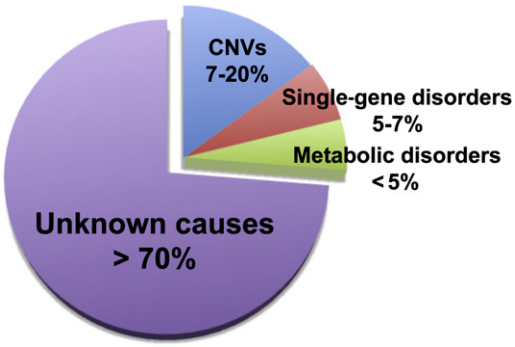

Figure 1. Genetic Causes of Autism Spectrum Disorders

One would have hoped that the type of detailed analysis of large ASD cohorts using very high-resolution arrays as those used in the Sanders and Levy studies would have yielded a high number of identifiable mutations, yet the results are humbling. There is no remarkable increase in pickup rate of CNVs, despite much increased density when compared to previous studies, pointing to the limitations of array analysis and the contributions of de novo and rare inherited CNVs to the etiology of ASDs overall. There is hope that whole-exome and whole-genome sequencing will fill the gap and identify coding variants causing autism, some of which may be in genes involved in CNVs already associated with ASDs and others of which will be in novel ASD susceptibility genes. The total number of ASD genes and target loci is estimated at 250–400 by Levy et al. (2011) and around 130 by Sanders et al. (2011). However, both of these are calculations based only on existing CNV data. The actual number of autism susceptibility genes may be very different, depending on what the large-scale sequencing studies reveal. The number of genes, mutations of which account for the majority of ASD cases may be as small as a dozen or two, but may also be in the thousands.

Mutational Mechanisms Contributing to ASDs

Different mutational mechanisms have been shown to contribute to ASDs, including de novo and inherited CNVs, as well as de novo and inherited point mutations. As shown for 16p11.2 deletions and duplications, specific mutations manifest variable expressivity and incomplete penetrance, even within the same family. These phenomena are applicable to neuropsychiatric disorders in general (Sebat et al., 2009). What is unique about ASDs is the male predominance of the phenotype, with an overall 4:1 male-to-female sex ratio. Why this is the case remains unknown. Sanders et al. (2011) state that based on their data there is no evidence for a causal role of rare X-chromosomal CNVs accounting for this sex ratio. Levy et al. (2011) found that females with ASDs have a higher frequency of de novo CNVs when compared to males; furthermore, they found more genes to be present in events from female probands than in those from male probands. They speculate that females have greater resistance to autism from genetic causes. This idea is supported by the companion paper by Gilman et al. (2011), who describe a large biological network of genes affected by rare de novo CNVs and show convincingly that stronger functional perturbations are required to trigger the autistic phenotype in females compared to males. Given these findings, what accounts for the female resistance to autism? Earlier this year, it was proposed that sex hormonal expression patterns may account for at least part of that, as androgens and estrogens differentially and reciprocally regulate RORA, a novel candidate gene for autism (Sarachana et al., 2011). Genetic modifiers may also account for a sex bias. Several autism-causing genes are located on the X chromosome (*FMR1*, *NLGN4X*, *MECP2*, etc.). Hypomorphic variants of such genes, which do not manifest a phenotype per se, might still alter the individual's overall penetrance of autistic traits. Their presence in hemizygosity in males would lead to a stronger effect than in females.

Levy et al. (2011) conclude that "the hypothesis that autism results from an unfortunate combination of common low-risk variants can be safely rejected." This conclusion seems premature, especially given that it is based solely on CNV data, while large-scale sequencing data on large cohorts of autistic individuals are still being collected. Sequencing studies on individuals with ASDs support a multihit model for disease risk (O'Roak et al., 2011) and a model of oligogenic heterozygosity has been proposed, especially for individuals with high-functioning ASDs (Schaaf et al., 2011). Considering that de novo CNVs are more commonly detected in simplex cases of ASDs when compared to familial cases of ASDs, one could envision that oligogenic and complex patterns of inheritance may play a more important role in families with multiple individuals affected with ASDs.

Several hypomorphic variants may accumulate either in a specific signaling pathway or in a subcellular compartment (such as the synapse) to exceed a threshold and result in phenotypic manifestation. This would be consistent with data from clinical studies whereby children from families in which both parents manifest subthreshold autistic traits are more likely to show more severe impairment in reciprocal and social behavior (Constantino and Todd, 2005).

Functional Considerations

The study presented by Gilman et al. (2011) widens the perspective from sheer identification of CNVs to a more functional interpretation. They identify a large biological network of genes affected by rare de novo CNVs. This can be seen as a proof of principle that networks underlying complex human phenotypes can be identified by a network-based functional analysis of rare genetic variants. Most importantly, the network links molecules to biological functions and cellular compartments, i.e., synaptogenesis, axon guidance, and neuronal motility. Several signaling pathways important in the regulation of dendrite morphogenesis stand out as core elements of the overall network, including the WNT pathway, the reelin pathway, the mTOR pathway, and the Rho/LINK1 pathway. Using an

experimental approach Sakai et al. recently identified a protein interaction network, functionally connecting hundreds of proteins to known and novel ASD proteins. In particular, they exemplified how a protein interaction network based on proteins primarily associated with syndromic autism can be used to identify causative mutations in individuals with nonsyndromic autism (Sakai et al., 2011). This suggests a significant overlap in the genetics of syndromic and nonsyndromic autism.

The identification of key molecular pathways that link many ASD-causing genes is of utmost importance when it comes to potential therapeutic interventions. It is very likely that there will be hundreds of autism genes and proteins; thus designing treatments for ASDs tackling one gene at a time will be a challenge. Identifying functional relationships and interactions between various ASD-associated proteins is likely to identify signaling pathways and subcellular compartments that encompass a whole subgroup of such genes. Having such rich functional pathway information might unearth common targets that are amenable to therapy.

This is a very exciting time for autism research. Large, thoroughly phenotyped cohorts and collections of biospecimens are available. Many ASD loci and genes have been identified and are just beginning to be connected in functional networks. While we are awaiting the results of multiple large-scale sequencing efforts, the field is poised to move on to functional studies that will help understand the molecular underpinnings and neural substrates of this disorder in hopes of developing more effective interventions.

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VEGF Shows Its Attractive Side at the Midline

Travis L. Dickendesher[1,2] and Roman J. Giger[1,2,3,*]
[1]Neuroscience Program
[2]Department of Cell & Developmental Biology
[3]Department of Neurology
University of Michigan School of Medicine, 3065 BSRB, 109 Zina Pitcher Place, Ann Arbor, MI 48109-2200, USA
*Correspondence: rgiger@umich.edu
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Vascular endothelial growth factor (VEGF) family members are best known for their powerful mitotic and angiogenic activities toward endothelial cells. Two independent studies in this issue of *Neuron* now provide compelling evidence that VEGF-A secreted at the CNS midline functions as an attractant for developing axons of spinal commissural neurons and contralaterally projecting retinal ganglion cells.

The assembly of a highly organized network of neuronal connections is a key developmental process and essential for all neural function, ranging from simple movement to complex cognitive processes. Research focused on the cellular strategies and molecular mechanisms that orchestrate neural network assembly led to the discovery of a wide variety of axon guidance molecules and receptors (Kolodkin and Tessier-Lavigne, 2010). Many guidance molecules are evolutionarily conserved and, based on their mode of action, are categorized into short- or long-range guidance cues that influence growth cone steering in a positive (attractive) or negative (repulsive/inhibitory) manner. We now know that the activity of an individual guidance cue is not absolute, but instead interpreted by the neuronal growth cone in a