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Autism: The Ups and Downs of Neuroligin

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The etiology of autism spectrum disorders (ASD), like many other neurodevelopmental disorders, has a significant contribution from genetic sources. Among the first genetic causes of ASD identified were a number of rare chromosomal disorders (1). However, these conditions were not really considered causes of idiopathic, “mainstream” autism by many because most of these nonidiopathic disorders had dysmorphic features or other signs that were distinguishable from idiopathic autism. Also, like most other common medical conditions, ASD was presumed to be caused primarily by common genetic variation, under a model whereby a combination of multiple common polymorphisms interacted to cause the condition.

Several recent developments reopened the consideration of the role of rare genetic variation as playing a significant role in ASD and other common neuropsychiatric conditions. First, the large genome-wide association studies assessing common variation clearly indicate that the locus-specific effects from common genetic variation are small (2). Second, the major sources of common variation under several linkage peaks cannot account for the linkage signal (3,4). Third, high-resolution studies of structural chromosomal variation, largely fueled by new methods such as microarrays, have identified unique and recurrent copy number variations (CNVs) as significant contributors to ASD (5–7). Copy number variations are among the most common causes of ASD so far, with loci at 15q11-13, 22q, and 16p, each accounting for between .5% and 1% of ASD (6,8–10). Together, already identified unique and recurrent CNVs may account for as much as 10% of ASD (7,9).

However, many of the individual CNVs uncovered by microarray analysis are very rare, and although the overall burden is increased in ASD patients versus control subjects, proving the pathogenicity of individual rare variants is difficult (7). Given current sample sizes, statistical support for each rare variant, whether CNV or point mutation, is not possible; much larger sample sizes will be needed to provide statistical evidence for any form of putative rare mutation. In other words, simply because a given rare genetic variant observed among a cohort of ASD cases leads to a premature stop codon or changes a highly conserved amino acid, it does not prove that it is pathogenic. It may be a rare, but benign, variant. For example, pathogenicity in the original NLGN3 and NLGN4 mutation discovery (11) was presumed due to the nature of the mutations (stop codon, conservative amino acid change, etc.) and their rarity, a level of evidence that probably would not be sufficient now. Statistical support for segregation with disease was provided by study of a large family segregating intellectual disability (mental retardation [MR]) and ASD along with a dominant NLGN4 mutation (12).

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It is in this context that the article by Daoud *et al.* (13) in this issue of *Biological Psychiatry* is worth highlighting. Here, the investigators follow up on previous identification of rare mutations in NLGN genes in MR and ASD by sequencing of the NLGN4X gene in a relatively small cohort of patients with autism. They find one potential mutation, a de novo (not in parents), 1 base pair (bp) substitution in the gene's promoter in one patient and no other mutations, consistent with the rarity of NLGN4X mutations in ASD. They screen 450 normal X chromosomes, not finding this particular variant among control subjects. One could raise the issue that given the rarity of such variants even among subjects with ASD, it may be necessary to fully resequence the gene or assay this variant in a much larger number of control subjects, as others have done (14). However, this mutation is de novo and here the authors take a further step to assess pathogenicity. They hypothesize that promoter variation should modulate expression level, if indeed it is functionally relevant, and show that both in patient lymphoblasts (relative to control subjects) and by luciferase assay in vitro in a nonneural cell line that this variant significantly increases NLGN4X levels. This rare polymorphism also exhibits a change in a gel shift assay, consistent with differential transcription factor binding to the mutant promoter.

As the authors suggest, given the X chromosome dosage differences between males and females, the results may also indicate that increased NLGN4X expression in men at or beyond the levels observed in women is pathogenic. However, since these observations, although consistent, are in nonneural tissues, we do not know how this variant would act to affect NLGN4X expression in the brain. These data likely do reflect a deficit in the ability to regulate neuroligin levels but, in which direction, remains to be determined. These results are consistent with CNV studies in humans and the potential haploinsufficiency mechanism suggested by the dominantly inherited NLGN4X mutation segregating with MR and ASD in the large family mentioned above (12), which indicates that neuroligin dosage is material to normal human neural development and cognition. More mechanistic studies in vivo in neuronal tissue will be necessary to delineate how this specific variant and in general how changes in NLGN4X levels affect neural circuitry leading to ASD or MR. Nevertheless, this work emphasizes the relevance and impact of functional study of rare variants to assess their potential for pathogenicity in humans.

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