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RESEARCH HIGHLIGHT

Modifier genetics in neuropsychiatric disease: challenges and opportunities

Elizabeth K Ruzzo[1], Andrea L Pappas[2] and David B Goldstein[1]*

Abstract

A new study focuses attention on multigenic interactions influencing the risk of autism spectrum disorders.

In recent years, efforts to uncover the etiology of neuropsychiatric disorders have revealed a number of rare copy number variants (CNVs) that have variable expressivity within and across clinically distinct disorders. For example, CNVs in 1q21.1 are associated with a number of phenotypes, including intellectual disability (ID), microcephaly, schizophrenia and autism spectrum disorder (ASD) [1]. Such observations have brought the potential role of modifier mutations to the forefront of neuropsychiatric disease genetics. A recent study by Leblond *et al.* [2] published in *PLoS Genetics* has investigated the possible impact of genetic variation at other loci in patients carrying *de novo* deletions in an established autism gene.

Given the complexity of neuropsychiatric disease, the increasing support for major risk factors interacting strongly with one another and with the genetic background should come as no surprise. Work in model organisms has revealed rampant genetic interactions. For example, a single mutation in any of the approximately 1,000 essential genes of yeast induces lethality, but it is estimated that there are 200 times as many digenic combinations resulting in synthetic lethality [3]. In contrast, we have only a handful of examples in human diseases where multiple hits are required for manifestation of a disease. Unfortunately, identification of genetic interactions in humans has proved difficult. Even in Mendelian diseases where the genetic architecture is simplified by low locus heterogeneity, progress has been slow, with only a few robust examples, including sickle cell anemia and cystic fibrosis [4]. In both cases, the phenotypic expression is modified by variants outside of the disease-causing gene. In cystic fibrosis, as in many other diseases, different mutations within the disease-causing gene *CFTR* can also result in differences in disease severity [4], making it harder to identify interactions.

A new study by Albers *et al.* [5] published in *Nature Genetics* investigates the genetic interactions responsible for thrombocytopenia with absent radii syndrome. By focusing on patients harboring a previously associated microdeletion in 1q21.1, they identified two different low-frequency variants in the regulatory region of *RBM8A*. The combination of either variant with the original microdeletion is sufficient to cause this disorder. Subsequently, patients lacking the microdeletion were found to carry novel null mutations in *RBM8A*, thus resolving the responsible gene within the 1q21.1 region. This compound inheritance mechanism explained 53 of 55 cases ($P < 5 \times 10^{-228}$) and provides a simplified model that can be applied in studies of neuropsychiatric disorders. However, given the low frequency of risk alleles and the extreme genetic heterogeneity of neuropsychiatric disorders, identifying well-powered cohorts of genetically homogeneous samples will be no small task.

Common complex diseases represent a particular challenge for studying modifier genetics. Perhaps the most fundamental constraint is that the high locus heterogeneity complicates identification of patients with similar 'primary' mutations in order to ask how these interact with modifiers. So far, such efforts have been modest. In the case of heterozygous microdeletions, one possibility is that variable expressivity is due to newly hemizygous deleterious mutations in distinct genes on the remaining chromosome. This possibility has been tested in only a few studies to date [1,6], with no clear evidence of genetic modifiers on the intact chromosome. Admittedly, the small sample sizes in these studies mean that it is difficult to rule out this possibility even for the deletion regions that have been tested. Other studies have looked elsewhere in the genome for evidence of genetic interactions. Girirajan *et al.* [7] performed a genome-wide scan for CNVs in ID patients carrying the 16p12.1 microdeletion and identified a non-specific enrichment of large CNVs that correlated with a more severe clinical phenotype. Perhaps the presence of

*Correspondence: d.goldstein@dm.duke.edu
[1]Center for Human Genome Variation, Duke University School of Medicine, Durham, North Carolina, 27708, USA

one causal CNV more readily allows the presence of others compared with controls. It remains unclear whether these observations are reflective of a primary driver with secondary modifiers or if some combination of multiple hits is necessary for manifestation of the phenotype.

ASD may present one of the clearest cases for strong genetic interactions. The heritability of ASD is well established, and some twin studies indicate that concordance rates for monozygotic twins (70% to 90%) are much higher than for dizygotic twins (0% to 10%) [8]. One explanation for this would be that several interacting risk factors are necessary to confer a higher risk for ASD. Preliminary work supports the idea that multiple risk factors are present in patients with ASD [9]; specifically, that rare CNVs are enriched (approximately 1.2-fold) in ASD cases compared with controls and particularly enriched (approximately 1.7-fold) at known ASD/ID loci. The challenge imposed by locus heterogeneity is exemplified by the fact that the genetic cause of ASD is now thought to be known in 10% to 20% of cases, and yet the variants explaining the highest proportion of cases still individually explain no more than 1% to 2% [8]. This is further complicated by the phenotypic spectrum seen in patients with ASD who carry mutations in the same gene, which is also consistent with the analysis of *Shank3* mutant mice. Unexpectedly, *Shank3* mutant strains with four unique deletions (for example, deleting exons 4 to 9 removes the ANK domain [10]) show phenotypic variability, which may be reflective of the genotype-phenotype variability seen in patients with *SHANK3* mutations.

The study by Leblond *et al.* [2] investigates the possible impact of genetic variation at other loci by examining the CNVs of three patients carrying *de novo* deletions in an established autism gene, *SHANK2*. They found that all three patients had CNVs within the 15q11-q13 region: two causing a duplication of *CHRNA7* (15q13.3) and the third causing a deletion of *NIPA1*, *NIPA2*, *CYFIP1* and *TUBGCP5* (15q11.2). In their case-control cohort, none of the CNVs showed significant enrichment in patients with ASD. In contrast to neuropsychiatric-disease-associated deletions encompassing this gene, the *CHRNA7* duplication is of unknown clinical significance and was seen in about 1% of patients with ASD and about 0.6% of controls. These results provide a tantalizing suggestion that nicotinic cholinergic receptors may act in a pathway that is shared with *SHANK2* to confer risk of ASD. If confirmed, this observation would provide an important indicator of the precise pathways that underlie risk. But this work also clearly illustrates the challenges involved in ASD modifier studies. It is worth noting that 15q11-q13 is a hotspot for structural rearrangements, meaning that the region often carries CNVs, even in controls, and also that it is difficult to accurately call CNVs in this region. As an illustration, we observed in our own unpublished raw data (not experimentally validated) that 4.56% of our healthy controls (59/1,295) harbor a CNV in this region that is large enough to be called by PennCNV (>2 kb). Even the *CHRNA7* duplication observed in this paper is identified in 1.16% of our control cohort (15/1,295), and the reciprocal deletion of *CHRNA7* is called in 1% of controls (13/1,295).

This study also illustrates the challenge imposed by locus heterogeneity. Analyzing only three patients with *de novo SHANK2* deletions hinders identification of modifiers with statistical certainty. Thus, in an effort to identify additional patients with causal *SHANK2* mutations, Leblond *et al.* investigated the likelihood of pathogenicity for candidate variants seen in patients with ASD by overexpressing them in neuronal cultures. Overexpression of *SHANK2* variants seen in only cases, only controls or both cases and controls revealed an interesting pattern among the 16 tested variants. Four variants significantly reduced synaptic density, and three of these variants were seen only in cases, while the fourth was seen in both cases and controls. Importantly, none of the control-only variants had an impact on synaptic density. This functional work demonstrates the potential utility of *in vitro* or *in vivo* models to gauge pathogenicity among a set of candidate variants, but also shows the difficulty of confident assignment of pathogenicity based on such analyses.

Developing well-powered modifier studies for ASD will be a very significant challenge. Leblond *et al.* [2] took a key first step by jointly analyzing three patients with *de novo SHANK2* deletions, and this raises the captivating possibility that modifiers within 15q11-q13 interact with *SHANK2* mutations to confer ASD. It is unclear whether identifying and considering other presumably causal *SHANK2* mutations would strengthen the initial observation of the role of CNVs within 15q11-q13, or if the inability to securely identify pathogenic variants would dilute this signal. Either way, to obtain the statistical evidence needed to prove such associations, large well-phenotyped and homogeneous datasets must be compiled, highlighting the need for collaborative efforts among researchers.

We are in the earliest days of identifying genes contributing to ASD. However, already it seems likely that synaptic structure and function will play a central role. On a broader level, identification of multiple genetic aberrations in patients, or unaffected individuals, may reveal important combinatorial effects on phenotypic variability and novel underlying biological interactions.

Making effective clinical use of genetic risk factors depends critically on understanding the basis of pathogenicity in individual genomes. Only through such understanding will it be possible to organize all the different rare genetic risk factors, which many expect to underlie common diseases, into a discrete number of alternative disease-associated pathways. Once such mappings are

established between collections of genetic risk factors and the pathways they affect, it will become possible to target those pathways in drug development efforts and tailor treatment for patients having different pathways perturbed. As challenging as they may be, one critical direction for relating genetic risk factors to such pathways is through modifier genetic studies such as the one reported by Leblond *et al*. [2].

Abbreviations
ASD, autism spectrum disorder; CNV, copy number variant; ID, intellectual disability.

Competing interests
The authors declare that they have no competing interests.

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Author details
[1]Center for Human Genome Variation, Duke University School of Medicine, Durham, North Carolina, 27708, USA. [2]Department of Neurobiology, Duke University, Durham, North Carolina, 27708, USA.

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