

Schizophrenia Candidate Genes: Are We Really Coming Up Blank?

The most comprehensive genetic association study of genes previously reported to contribute to the susceptibility to schizophrenia by Gejman et al. is published in this issue of the *Journal*. The study by Sanders et al. (1) set out to examine whether polymorphic DNA sites, or single nucleotide polymorphisms (SNPs), in 14 candidate genes previously argued by the authors and other researchers to be associated with schizophrenia would continue to do so in a large sample of European ancestry. The authors analyzed 433 SNPs in these genes in the hope of representing, or “tagging,” the known common DNA variation in those genes. They also examined an additional 215 SNPs in these genes used in the earlier published studies or that occur in functionally relevant regions of the genes. Before discussing their results, it may be helpful to set the stage for this article.

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It is often not appreciated that the last decade of genetic studies in schizophrenia has generated findings suggesting that a number of genes are associated with schizophrenia across multiple studies (2). Not all studies of any particular gene are positive, and the contribution toward risk that any individual gene makes appears to be small. Many are also surprised that some of these genes were initially found using positional cloning, the systematic identification and localization of genes using linkage analysis in families segregating schizophrenia. Examples of such genes include dystrobrevin-binding protein 1 (*DTNBP1*), neuregulin 1 (*NRG1*), and D-amino acid oxidase activator (*DAOA*). Similarly, positive studies have emerged for genes pursued after being found at sites of chromosomal abnormalities. Disrupted in schizophrenia 1 (*DISC1*) was identified at the site of a chromosomal translocation segregating with schizophrenia (and other mental disorders) in a single pedigree. Likewise, the catechol-*O*-methyltransferase (*COMT*) gene occurs in a large common deletion of a segment of chromosome 22 that by itself increases the risk of schizophrenia by greater than 20-fold. Sanders et al. (1) chose to examine these genes as well as those connected to animal models (*PP3CC*, also residing in a reported linkage region) or pharmacological hypotheses (*HTR2A*, *DRD2*) in their analyses. This study stands above others because the authors attempt to bring to bear two critical factors on their experiment—a large sample and comprehensive genotyping.

Given that many of the studies suggesting that these 14 genes are associated with schizophrenia were of modest size, the authors' sample of nearly 1,900 cases and 2,000 comparison subjects provides, at least in theory, substantial statistical power for confirming the results of the earlier studies. The authors were able to cobble together 1,952 persons recruited from multiple sources and diagnosed with schizophrenia or schizoaffective disorder, collected over 15 years from 12 sites in 10 U.S. states, plus a single site in Australia, which also contributed the most subjects. About 17% of the individuals were derived from pedigrees with multiple members with schizophrenia. The authors also collected a comparison group composed of adults who completed a short online self-report clinical assessment. Although these screened comparison subjects may be preferable to random uncharacterized individuals, given the high prevalence of psychi-

atric disorders, there may be hidden biases influencing who elected to participate in a comparison group.

With these strengths—reasonable candidate genes, a large, rigorously phenotyped sample, and a dense set of SNPs—what do the authors find? They find that none of the polymorphisms were associated with the schizophrenia phenotype at a reasonable threshold for statistical significance. Removal of schizoaffective disorder cases did not alter the results, nor did careful correction for subtle ancestry differences between cases and comparison subjects. Haplotypes composed of multiple markers were not associated, and neither were imputed genotypes of SNPs not directly examined in the study. Of the 69 SNPs that had shown positive association to these genes in previous studies, only four—in *TAAR6*, *HTR2A*, and *RGS4*—showed even nominal association in Sanders et al. (1). The distribution of test statistics suggests nothing outside of what would be expected by chance.

What might explain these negative findings? The simplest explanation is that for the broadly recruited cases and comparison subjects used in this study, common DNA variation in these genes is just not correlated with the schizophrenia phenotype, as defined by the investigators. This does not necessarily mean we should dismiss the findings of the previous studies, where those results may in fact be valid for specific samples. For example, the authors previously reported positive associations to *DTNBP1* and *TAAR6* in a subset of the same families that contributed to the sample used in the current article (3, 4). The discrepancy between that earlier positive finding and the current negative one is not addressed in Sanders et al. (1) but may highlight the relative merits of family-based studies versus methods using unrelated cases and comparison subjects. Unless we believe that the current results completely negate the previous findings, the difference in results may argue for stronger confidence in family-based analyses. Although the authors have cast a negative interpretation of their findings, a simple thought experiment underscores the problem of accommodating corrections for multiple testing while simultaneously examining several hypotheses backed by prior evidence. If the authors had genotyped only the single SNP in the *STX7* gene that provided their strongest result instead of all 648 SNPs, would the interpretation of the data have fared differently despite arriving at the same statistic? Additionally, the large study of Sanders et al. (1) may not have had the required power to detect genetic effects of small magnitude. Although the authors report that they should be able to detect genotypic relative risks (the risk of being affected for an individual with a single susceptibility allele compared to an individual with no susceptibility alleles) as low as 1.25, even this may be insufficient for reliably observing weak associations. Using recent genome-wide association studies of type 2 diabetes as a way to illustrate this problem, not a single study among three large samples, together comprising 32,000 subjects, was able to conclusively show the *HHEX* gene as a type 2 diabetes risk locus. Combining data from the three independent samples showed the same small odds ratio (1.13) seen in the individual studies but with more persuasive statistical significance ($p=5.70 \times 10^{-10}$) (5), implying that tens of thousands of samples may be required to detect small but real genetic effects. This same problem had been faced years earlier in type 2 diabetes research, with a strong candidate gene called *PPARG*, now well established as a type 2 diabetes susceptibility gene and the target for thiazolidinediones, such as rosiglitazone. In one large study, nominal statistical significance was reached for only one of four independent samples, yet when data from all four groups were combined, a stronger association emerged (6). Another explanation involves the type of DNA variation tested in this study. It is plausible that instead of common SNPs distributed widely across populations, schizophrenia may be influenced by numerous rare but deleterious mutations or by large-scale variation in chromosome structure, models increasingly observed in other disorders, such as autism (7, 8). It may also be that the phenotypic definition of schizophrenia in common use is not entirely reflective of the underlying genetic architecture, perhaps requiring al-

ternative classification schemes taking into account clinical features shared between disorders (9). It is also possible that the majority of the samples in the authors' study had a less heritable form of the disorder; only 17% of the subjects were from pedigrees segregating schizophrenia (i.e., 100% had a positive family history), while less than 5% of the remaining large fraction of the participants reported a family history of disease. Sporadic and familial forms of schizophrenia may share common genetic features, but there is some indication that *de novo* mutation may play a prominent role in sporadic schizophrenia (10), which would render standard linkage disequilibrium gene mapping approaches less useful, if not futile.

The most worrisome explanation for the lackluster results may derive from genetic heterogeneity intrinsic to such studies, despite the great care the authors exerted in the design and execution of this work. This heterogeneity may be due to stochastic factors, local environmental interactions, or ascertainment biases that may apply differentially across sites. For example, it is possible that cases at any one site may be more related to one another than they are to comparison subjects or to cases collected at other sites. Thus, some fraction of a single subsample may be potentially seen as a loose but extended pedigree segregating a limited number of risk alleles not seen in other cases. This situation becomes more complex if one contemplates the role of combinations or interactions between risk variants (11). This could lead to a situation in which a robust finding in one segment of the overall sample is washed out by mixing multiple samples from across the country or, in the case of Sanders et al. (1), from across hemispheres. A direct test of this intra-Caucasian heterogeneity would involve stratification of analysis by site.

The fact that the odds ratios seen in this study are in the range of positive results seen in other complex disorders suggests to some that even larger samples might further pin down these results, spurring great hope that meta-analysis of multiple samples will provide greater clarity. It will be exciting to see how the authors' ongoing genome-wide study (12) using these samples will fare. The great hope for this strategy is the identification of novel risk genes for schizophrenia.

The main lesson of this article is that the 14 genes studied may play little role in schizophrenia in a large and heterogeneous sample of European ancestry. But this does not mean that these genes may not contribute to schizophrenia in specific subgroups, nor does it suggest that these genes are not influencing schizophrenia in non-European samples. Much work, at both the population and molecular levels, remains before dismissing these genes and their connection to schizophrenia.

References

1. Sanders AR, Duan J, Levinson DF, Shi J, He D, Hou C, Burrell GJ, Rice JP, Nertney DA, Olincy A, Rozycki P, Vinogradov S, Buccola NG, Mowry BJ, Freedman R, Amin F, Black DW, Silverman JM, Byerley WF, Crowe RR, Cloninger CR, Martinez M, Gejman PV: No significant association of 14 candidate genes with schizophrenia in a large European ancestry sample: implications for psychiatric genetics. *Am J Psychiatry* 2008; 165:497–506
2. Owen MJ, Craddock N, O'Donovan MC: Schizophrenia: genes at last? *Trends in Genetics* 2005; 21:518–525
3. Duan J, Martinez M, Sanders AR, Hou C, Saitou N, Kitano T, Mowry BJ, Crowe RR, Silverman JM, Levinson DF, Gejman PV: Polymorphisms in the trace amine receptor 4 (TRAR4) gene on chromosome 6q23.2 are associated with susceptibility to schizophrenia. *Am J Hum Genet* 2004; 75:624–638
4. Duan J, Martinez M, Sanders AR, Hou C, Burrell GJ, Krasner AJ, Schwartz DB, Gejman PV: DTNPBL (dystrobrevin binding protein 1) and schizophrenia: association evidence in the 3' end of the gene. *Hum Hered* 2007; 64:97–106
5. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University and Novartis Institutes for BioMedical Research, Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PIW, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Speliotes EK, Taskiran MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjogren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, DeFelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Ricke D, Purcell

- S: Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 2007; 316:1331–1336
6. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES: The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 2000; 26:76–80
 7. Jamain S, Quach H, Betancur C, Rastam M, Colineaux C, Gillberg IC, Soderstrom H, Giros B, Leboyer M, Gillberg C, Bourgeron T: Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nat Genet* 2003; 34:27–29
 8. Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, Saemundsen E, Stefansson H, Ferreira MA, Green T, Platt OS, Ruderfer DM, Walsh CA, Altshuler D, Chakravarti A, Tanzi RE, Stefansson K, Santangelo SL, Gusella JF, Sklar P, Wu BL, Daly MJ, the Autism Consortium: Association between microdeletion and microduplication at 16p11.2 and autism. *N Engl J Med* 2008 (Epub ahead of print)
 9. Owen MJ, Craddock N, Jablensky A: The genetic deconstruction of psychosis. *Schizophr Bull* 2007; 33:905–911
 10. Malaspina D, Corcoran C, Fahim C, Berman A, Harkavy-Friedman J, Yale S, Goetz D, Goetz R, Harlap S, Gorman J: Paternal age and sporadic schizophrenia: evidence for de novo mutations. *Am J Med Genet* 2002; 114:299–303
 11. Straub RE, Lipska BK, Egan MF, Goldberg TE, Callicott JH, Mayhew MB, Vakkalanka RK, Kolachana BS, Kleinman JE, Weinberger DR: Allelic variation in the GAD1 (GAD67) is associated with schizophrenia and influences cortical function and gene expression. *Mol Psychiatry* 2007; 12:854–869
 12. GAIN Collaborative Research Group: New models of collaboration in genome-wide association studies: the Genetic Association Information Network. *Nat Genet* 2007; 39:1045–1051

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The author reports no competing interests.