Zeroing in on a Schizophrenia Gene: A New Tool to Assess the Probability

The article "Identification of a Schizophrenia-Associated Functional Noncoding Variant in *NOS1AP*" in this issue, by Wratten et al. (1), is exciting not only because of the finding of a DNA variant that is closely associated with schizophrenia, but also because it makes one of the first uses in mental illness research of a new statistical method that made this finding possible.

The genetics of schizophrenia has many hurdles to overcome. The illness is complex, with the likely involvement of multiple genes, each giving rise to subtle, often unknown alterations in brain function, and each interacting with multiple environmental factors. Results are often inconsistent across population samples because the statistical power to detect any one genetic factor among all these interactions is low. However, no hurdle has been more frustrating than the inability to detect the actual DNA changes that cause malfunction of a specific gene, even when strong evidence already exists for that gene's role in the transmission of schizophrenia. For classic genetic illnesses, such as sickle cell anemia, the alteration in the DNA is an obvious change in a codon for an amino acid—in the case of sickle cell anemia, the alteration renders the hemoglobin molecule dysfunctional. But for schizophrenia, most changes appear to occur in single nucleotide polymorphisms (SNPs) outside the amino acid coding region, in areas that affect gene expression, not protein structure. There are millions of SNPs throughout the human genome, and the biological significance of most of them is unknown. Thus, it has been nearly impossible to determine whether any specific SNP causes the genetic malfunction responsible for transmission of the illness.

"NOS1AP represents a good example of a gene with a subtle but important neuronal function that could be involved in altering brain activity in schizophrenia."

In 2000, Brzustowicz and colleagues (2) first reported strong evidence for genetic linkage between schizophrenia and a narrow region on chromosome 1 in a sample of Canadian families. Subsequently, using linkage disequilibrium (LD) mapping techniques on that linked region, Brzustowicz et al. (3) determined that all the markers exhibiting significant LD with schizophrenia fell within the gene for nitric oxide synthase 1 adaptor protein, *NOS1AP* (previously called *CAPON*). In 2005, they found increased expression of this same gene in postmortem brain from people with schizophrenia (4), thus providing additional evidence for *NOS1AP*'s role in schizophrenia susceptibility. Two other recent studies also support the involvement of *NOS1AP* in schizophrenia—one in a South American population isolate (5), the other in a Han Chinese sample (6).

How might *NOS1AP* be involved in schizophrenia? *NOS1AP* produces a protein that links the enzyme nitric oxide synthetase to specific targets in the cell. This enzyme is found in high concentration in forebrain inhibitory interneurons. There it is activated by calcium ions that are introduced into the neurons by the action of neurotransmitters such as glutamate and acetylcholine. The nitric oxide produced by the activated enzyme then acts as an intracellular messenger. Thus, *NOS1AP* represents a good example of a gene with a subtle but important neuronal function that could be involved in altering brain activity in schizophrenia. Interestingly, it is not likely that anyone would have targeted this as a candidate gene ahead of time.

This article is featured in this month's AJP **Audio**.

The problem that this research group now faced was how to determine which of the many SNPs in the DNA of this gene might be responsible for the alteration of its function in schizophrenia. In the study reported in this issue, Wratten et al. genotyped 60 SNPs within the gene in a sample of 24 Canadian families known to be transmitting schizophrenia at the chromosome 1 site. They then analyzed the SNPs for LD with schizophrenia. In this context, LD means that a specific allele of the SNP appears in individuals with schizophrenia more frequently than in the general population, thus implying a potential causal connection between that allele and the illness. Three SNPs showed strong evidence for LD. The authors performed functional assessments on them, testing for regulatory activity in postmortem samples from dorsolateral prefrontal cortex. For one of these SNPs, rs12742393, one allele (the *A* allele) produced significantly higher expression than the other allele (the *C*), suggesting that this allele may play a causal role in schizophrenia.

Wratten et al. wanted to incorporate the strong linkage evidence from the previous work into the current study. To do so in a statistically rigorous manner, they employed a relatively new technique, the posterior probability of linkage disequilibrium (PPLD) (7, 8). This is one of a class of methods, all based on the PPL (posterior probability of linkage) approach pioneered by Veronica Vieland, one of the coauthors of this article. These methods follow in the tradition of evaluating the evidence contained in the data, as opposed to focusing on hypothesis testing and p values (9). This posterior probability approach has already proved successful with autoimmune thyroid disease (10). To use these methods, one specifies a "prior probability" of the phenomenon of interest. One then evaluates the evidence in the data in favor of or against that phenomenon and uses that evidence to modify the prior probability up or down, yielding a new probability, the "posterior probability."

As a simple example of this principle, imagine someone gives you a game token and asks you to evaluate whether it is "fair," that is, whether it has equal probabilities of landing with its red side up or its green side up when tossed. The token comes from a batch of tokens in which, due to a manufacturing error, only 60% were fair and the rest were biased toward green. To apply the approach being discussed here, you would set the prior probability that the token is fair at 60%. You could then decide to perform the experiment of tossing the token 10 times, in order to see what the evidence from your tosses tells you about the fairness of the token. Based on how many tosses come up red, one can calculate the new probability that the token is fair. For example, if the token comes up red in five of those 10 tosses, you would expect this outcome to support the fairness hypothesis, and indeed, in that case, the probability of fairness increases from 60% to 80.2%. In contrast, if the token comes up red in only two of the tosses, the probability should decrease, which it does, to 7.5%. In either case, this new probability represents the "posterior" probability of fairness.

Wratten et al. already knew there was linkage between schizophrenia and the *NOS1AP* locus. So to evaluate the evidence for LD, they first assumed linkage, then specified a prior probability of LD *conditioned* on the presence of linkage. They set this conditional prior probability to a low value, 2%. Of the 60 SNPs that they tested, 56 yielded posterior probabilities either lower than 2% (representing evidence against LD) or only slightly higher than 2% (representing negligible evidence in favor of LD). (One SNP yielded a posterior probability of 15%, which is also relatively low.) The remaining three SNPs had posterior probabilities of over 40%. These were the three that the authors further investigated; as described above, one of them, rs12742393, did yield positive results.

The evidence Wratten et al. present in this article for involvement of *NOS1AP* in schizophrenia is among the strongest, best-replicated findings so far in genetic studies of this disorder. Moreover, this evidence supports biological plausibility, not just a statistically significant result. We do not know yet just how major a role *NOS1AP* will prove to play outside these Canadian families, but the replicated associations and the post-

mortem work suggest that the role may be more major than appreciated until now. Thus, the work reported by these authors may bring us closer to understanding a causal genetic variant for schizophrenia.

References

1. Wratten NS, Memoli H, Huang Y, Dulencin AM, Matteson PG, Cornacchia MA, Azaro MA, Messenger J, Hayter JE, Bassett AS, Buyske S, Millonig JH, Vieland VJ, Brzustowicz LM: Identification of a schizophrenia-associated functional noncoding variant in *NOS1AP*. Am J Psychiatry 2009; 166:434–441
2. Brzustowicz LM, Hodgkinson KA, Chow EW, Honer WG, Bassett AS: Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21-q22. Science 2000; 288:678–682
3. Brzustowicz LM, Simone J, Mohseni P, Hayter JE, Hodgkinson KA, Chow EW, Bassett AS: Linkage disequilibrium mapping of schizophrenia susceptibility to the CAPON region of chromosome 1q22. Am J Hum Genet 2004; 74:1057–1063
4. Xu B, Wratten N, Charych EI, Buyske S, Firestein BL, Brzustowicz LM: Increased expression in dorsolateral prefrontal cortex of CAPON in schizophrenia and bipolar disorder. PLoS Med 2005; 2:e263
5. Kremeyer B, García J, Kymäläinen H, Wratten N, Restrepo G, Palacio C, Miranda A, López C, Restrepo M, Bedoya G, Brzustowicz L, Ospina-Duque J, Arbeláez M, Ruiz-Linares A: Evidence for a role of the *NOS1AP* (*CAPON*) gene in schizophrenia and its clinical dimensions: an association study in a South American population isolate. Hum Hered 2008; 67:163–173
6. Zheng Y, Li H, Qin W, Chen W, Duan Y, Xiao Y, Li C, Zhang J, Li X, Feng G, He L: Association of the carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase gene with schizophrenia in the Chinese Han population. Biochem Biophys Res Commun 2005; 328:809–815
7. Yang X, Huang J, Logue MW, Vieland VJ: The posterior probability of linkage allowing for linkage disequilibrium and a new estimate of disequilibrium between a trait and a marker. Hum Hered 2005; 59:210–219
8. Vieland VJ: Thermometers: something for statistical geneticists to think about. Hum Hered 2006; 61:144–156
9. Royall R: Statistical Evidence: A Likelihood Paradigm. London, Chapman & Hall, 1997
10. Vieland VJ, Huang Y, Bartlett C, Davies TF, Tomer Y: A multilocus model of the genetic architecture of autoimmune thyroid disorder, with clinical implications. Am J Hum Genet 2008; 82:1349–1356

SUSAN E. HODGE, D.Sc.
ROBERT FREEDMAN, M.D.

Address correspondence and reprint requests to Dr. Hodge, Columbia University, New York State Psychiatric Institute, Unit 24, 1051 Riverside Dr., New York, NY 10032; seh2@columbia.edu (e-mail).

Dr. Hodge reports no competing interests. Disclosures of The American Journal of Psychiatry *editors are published in each January issue.*