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Genetic analyses of complex behavioral disorders

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Many behavioral disorders are likely to be "complex," influenced by several genetic and environmental factors. Although a single gene's variants might thus account for only a portion of the vulnerability to the disorder, the high population frequencies of many of these problems makes understanding such gene variants important for public health.

Insights into identifying single gene influences in human complex behavioral disorders have come from family, twin, and adoption studies, mathematical models for complex genetics, identification of candidate genes, elucidation of functional variants in some of the perhaps 40,000 genes expressed in the human brain, and studies in transgenic mice with experimental manipulations of single genes.

Modeling studies now suggest that the magnitude of gene effects on a disease is a primary determinant of our ability to identify a disease-associated gene variant (1). *Heritability* reflects the proportion of the total interindividual variation due to a gene variant, reflecting both the gene variant's frequency in the population and the size of the effects that the gene variant causes. *Sibling relative risk* assesses the increased disease risk to siblings that share one-half of the genes with affected probands. Values for heritability, sibling relative risk, environmental contributions, and the power of genetic approaches can be estimated from studies of disease frequencies and patterns in monozygotic and dizygotic twins and in biologically related and adoptive family members.

Identifying molecular gene variants that contribute to complex behaviors often uses linkage disequilibrium, nonrandom association between a DNA marker, and a nearby gene variant that enhances disease vulnerability. A disease–vulnerability–enhancing gene variant can arise in an individual whose descendants form a large pedigree of individuals whose DNAs bear the variant and nearby gene markers. Studying DNA markers of individuals in the present generation can allow inferences about the chromosomal regions of genetic identity among currently affected individuals who are related to the initial mutant individual. Affected individuals could share gene markers around the disease locus, allowing searches of surrounding DNA for candidate genes and telltale mutations.

One approach to this problem assesses coinheritance of the trait or disease with specific genetic markers using simple pairs of relatives, most often sibling pairs and their parents. A candidate region containing potential vulnerability-enhancing genes is identified if affected siblings display specific gene markers more often than they should by chance inheritance based on parental genotypes. Modelling studies indicate that these methods have good power for detecting genes of substantial effect, but less power in identifying gene variants that account for much less than 5–10% of the variance in the population.

When neurobiological investigations identify a strong candidate gene, association testing can reveal if certain allelic variants of the gene are more common in affected than in unaffected individuals. As the molecular neurobiological underpinnings of complex behavioral disorders are better understood in humans and in animal models, the number of strong candidate genes will grow and increase the power of candidate gene searches. Testing markers at each of the 40,000–60,000 genes expressed in the brain can now be approached, especially comparing marker frequencies in pooled DNA samples from affected and from unaffected control individuals.

Vulnerability to drug abuse, and the results of recent animal and human studies of this common but complex problem, provide a model complex behavioral disorder to illustrate some of these points. Classical human genetic studies indicate significant genetic contributions to drug abuse (2, 3). Genetic influences on several animal models for human drug abuse behaviors can be found in strain comparison, selective breeding, quantitative trait locus, and transgenic mouse studies (4–7).

Many candidate genes for interindividual differences in drug abuse vulnerability have been identified through analyses of genes expressed in dopamine neurons that play prominent roles in drug reward, genes whose expression is altered by abused substances, and human homologs of murine drug response genes identified through quantitative trait locus studies (7, 8).

In transgenic mouse models, altered expression of specific dopaminergic genes can substantially influence models of drug abuse vulnerability. These studies support the importance of the plasma membrane dopamine transporter, the synaptic vesicular monoamine transporter, and D_1 and D_2 class dopamine receptors. Mice overexpressing the dopamine transporter in catecholaminergic neurons show significantly greater cocaine preference than control mice (D. Donovan and G.R.U., unpublished work). Conversely, mice with reduced expression of the VMAT2 gene show no effect on cocaine reward, but reduced amphetamine reward (N. Takahashi and G.R.U., unpublished work). Mice with dopamine D_1 receptor knockouts show slower acquisition of cocaine self-administration, but ultimate expression of cocaine reward similar to that of control mice (4, 9).

Human studies of COMT functional alleles in polysubstance abusers and nonusers provide a provisional association of a functional gene variant with substance abuse vulnerability. The proportion of substance abusers with high activity COMT genotypes was substantially more frequent than in drug-free controls (D. Vandenbergh and G.R.U., unpublished work). These observations fit with initial power calculations concern-

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ing the abilities to identify disease vulnerability genes in drug abuse. One initial estimate based on genetic contributions to the heritability of drug abuse of 0.45, largely nonshared environmental influences, 3- to 4-fold sibling relative risks for developing the disorder (based on human studies summarized in ref. 2), and models of 4–5 loci contributing 3/4 of the genetic influence (based on murine quantitative trait locus estimates for several models of the genetics of drug responsiveness, e.g., ref. 7) suggests an average 1.5-fold locus-specific relative risk for a hypothetical drug-abuse vulnerability model gene. Assessing genotypes at several hundred polymorphic DNA markers spaced at intervals across the human genome in several hundred affected sibling pair pedigrees would provide a reasonable statistical opportunity to detect a locus that produced such an effect size, although false-positive and false-negative results could also be produced (1).

Studies of complex disorders will be likely to reveal genetic heterogeneities in which individuals will have similar phenotypes based on differing genotypes, gene–environment, and gene–gene interactions that will complicate interpretation of results. Identification of gene variants contributing to complex behavioral disorders will also improve understanding of disease nosology, improve prevention strategies, and better target behavioral and pharmacological treatments. These potential benefits should spur the search for genes whose variants can provide insights into these important aspects of the human condition.

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