Association of Genes Involved in Calcium and Potassium Pathways with Opioid Dependence

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Opioids such as morphine, oxycodone, and fentanyl are widely used as effective analgesics for treating acute and chronic pain. Although appropriate use of opioid analgesics is an essential part of effective pain management, abuse and dependence on opioids pose a threat to health and have a devastating social and economic impact on families, communities, and nations. In a systematic analysis of the epidemiology of drug dependence with "global burden of disease," Degenhardt *et al.* (1) identified opioid dependsence as the largest contributor to the direct burden of disease among all illicit drugs.

In addition to environmental influences, many studies have demonstrated the familial transmission of substance abuse, with heritability estimates ranging from 30%–80% (2). More recently, genome-wide association studies have been used to identify genetic risk factors for complex diseases including substance dependence. The most successful and replicable GWAS associations for substance dependence have been with variants in the gene cluster encoding the α5, α3, and β4 nicotinic receptor subunits on chromosome 15 that are associated with risk of nicotine dependence and other smoking-related traits (3–5). The GWAS on alcohol dependence and related traits have not yet provided conclusive evidence for the role of specific genetic factors, most likely because these studies have been underpowered compared with the nicotine dependence studies, the largest of which have used cigarettes per day as a proxy for nicotine dependence enabling sample sizes in the tens of thousands. Genome-wide significant results have been observed for alcohol dependence with low frequency variants within the alcohol dehydrogenase genes, but these variants are not well tagged by variants on GWAS chips (5,6). There are even fewer GWAS on illicit drug dependence, and similar to the alcohol dependence studies, the sample sizes for these studies are smaller than most of the GWAS for other psychiatric traits that have resulted in multiple genome-wide significant loci (http://www.med.unc.edu/pgc/results#Results2Date). Gelernter *et al.* (7) used imputed minor allele dosage as the dependent variable and DSM-IV cocaine dependence symptom count in an association analysis and identified an intronic variant within the *FAM53B* gene that exhibited genome-wide significant association. In their article in this issue of *Biological Psychiatry*, Gelernter *et al.* (8) use the same analytic approach to identify genome-wide significant associations with opioid dependence.

It is well recognized that the genetic etiology of complex psychiatric disorders such as substance dependence is most likely influenced by a mixture of many common variants with small effect and by rare variants with large effect. A major challenge in detecting these genetic risk factors is the statistical power needed to detect these variants of small effect. The discovery sample cohort used by Gelernter *et al.* consists of 2379 European Americans and 3318 African Americans. Additionally, the authors used publicly available data from the Study of Addiction: Genetics and Environment data set as a replication sample cohort. Combining the two cohorts improved statistical power and led to the detection of genome-wide significant signals associated with opioid symptom count after meta-analysis. A major strength of this study is the use of samples from multiple populations and the relatively large size of the African American sample in particular. The genetic architecture of most traits differs across populations, and it is important to investigate disease risks in populations of various ancestries to determine whether an observed genetic risk factor is population-specific or a common risk factor across populations. The four distinct loci with genome-wide significant support for association with opioid dependence reported in this study were identified in the African American cohorts using imputed genotypes. The minor allele frequency (MAF) of each of these four variants was relatively low and ranged from 3%–8% in African Americans. Three of the four variants are not polymorphic in populations of European descent providing an explanation for why the associations were observed only in the African American subsets of these data sets. Although imputation programs are much improved and can generate high-quality genotypes on millions of single nucleotide polymorphisms (SNPs), it is essential to validate promising findings, such as those reported in the article by Gelernter *et al.*, with actual genotyped data, especially for low-frequency and rare variants.

The fourth association was observed with rs62103177, which is located within an intron of the *KCNG2* gene (encodes potassium voltage-gated channel subfamily G member 2). The minor allele of this variant is more common in European Americans than in populations of African descent (e.g., 1000-genome MAF = .21 in CEU [population with Northern and Western European ancestry], MAF = .05 in ASW [Americans of African ancestry in Southwest United States], MAF = .01 in YRI [Yoruba in Ibadan, Nigeria]). However, this variant did not show an association with opioid dependence symptom counts in the European American cohort. The absence of association with this SNP in the European American cohort suggests that the SNP is not itself functional, but that it tags another functional variant in the African American population but not the European American population. Given the substantial difference in frequency for the minor allele of this SNP in these two populations, this suggestion is certainly possible. If genotyping of this SNP in African American samples confirms this association, resequencing of *KCNG2* in people carrying this rare allele should lead to the identification of the functional allele driving the association, providing further insight into the role of *KCNG2* in risk of opioid dependence.

In a case-control model in both African American and European American populations, rs6419156, a variant 196 kb upstream of the *PPP3CA* gene (encodes protein phosphatase 3, catalytic subunit, alpha isozyme) showed evidence of association with opioid dependence. However, the direction of effect for this association differs within the two populations. The authors suggest that haplotype analysis with rs6419156 could be used to determine whether there are population-specific causal

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variants underlying these associations or whether a single causal variant exists that occurs on distinct haplotypes in African American and European American populations. An alternative interpretation of this observation is that it represents a false-positive association. To determine which of these alternatives provides the correct explanation of the observed results, it would be necessary to examine this variant in other opioid dependence data sets.

In addition to single SNP analyses, the authors performed pathway analysis. A strength of this approach is that it provides a means of testing whether variants within genes in a common biological pathway are observed among SNPs with low *p* values more often than would be expected by chance. This pathway analysis identified genes involved in calcium signaling and synaptic long-term potentiation as significantly associated with opioid dependence in the case-control model. Several genes are shared in common across these two pathways, lending support to the role of these genes in risk for opioid dependence. This observation provides a link to the existing animal literature on the neurobiology of addiction. First, *in vitro* and *in vivo* studies have demonstrated that acute treatments with morphine and other opiates can inhibit adenylate cyclase activity resulting in a decrease in cyclic adenosine monophosphate levels, inhibiting calcium channels and stimulating voltage-gated potassium channels (9). In contrast, chronic administration of opioids upregulates the cyclic adenosine monophosphate pathway and cyclic adenosine monophosphate response element binding protein indicating that continued opioid exposure decreases an individual's sensitivity to the rewarding effects and possibly induces tolerance. Second, individuals with chronic exposure to amphetamines and opiates often display pronounced neuropsychological impairment of executive function and memory. Synaptic long-term potentiation is one of the major cellular mechanisms influencing learning and memory. The association of opioid dependence with genes involved in long-term potentiation provides further support for the importance of this pathway in the biology of addiction.

A collaborative mega-analysis combining GWAS for several different psychiatric disorders had demonstrated evidence for genome-wide pleiotropy for major depressive disorder, bipolar disorder, schizophrenia, autism spectrum disorders, and attention-deficit/hyperactivity disorder (10). In particular, variation in the calcium channel genes *CACNA1C* and *CACNB2* was significantly associated with multiple disorders. Results from the pathway analysis suggested that other genes involved in calcium channel activity also play a role in risk for all five psychiatric disorders. The observation in the study by Gelernter *et al.* that variants in calcium channel genes also contribute to risk for opioid dependence suggests that there may be an underlying shared genetic liability to opioid dependence and these other psychiatric disorders.

Unraveling the genetic basis of susceptibility to illicit drug dependence and its possible link with the genetics of other mental disorders would require very large sample sizes. Toward this goal, a meta-analysis of substance dependence data sets is currently underway within the Psychiatric Genomics Consortium. These studies would help not only to strengthen the evidence for the role of specific genes in substance dependence risk, including genes identified in the study by Gelernter *et al.*, but also would enable identification of additional genes underlying the shared vulnerability with other psychiatric disorders.

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