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Review

Genetic Vulnerability and Susceptibility to Substance Dependence

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The development of substance dependence requires the initiation of substance use and the conversion from experimental use to established use before development of dependence. Numerous large twin studies have indicated a significant genetic contribution to this process. Genetic studies to date have been most successful at identifying genetic factors that influence the transition from regular use to dependence. The availability of large cohort samples for nicotine and alcohol dependence has resulted in significant progress being made in understanding at least some of the genetic contributions to these addictions. Fewer studies have replicated specific genetic contributions to illicit drug use, though it is clear that there is a strong genetic component involved here as well. Substance dependence can be thought of as a pharmacogenetic illness, and most likely hundreds and more probably thousands of genetic variants will be required to fully explain the genetic input to this disease.

Introduction

Large segments of our population use tobacco, alcohol, and other drugs. Cigarette smoking is common in both industrialized and developing countries. In the United States, over 43 million people use tobacco, and worldwide, over one billion people are tobacco users (CDC, 2010; WHO, 2010). In the U.S., over 400,000 people die every year from tobacco-related illnesses, and smoking remains the greatest contributor to preventable death (Mokdad et al., 2004). With increasing tobacco use in developing countries, it is predicted that the worldwide death toll will rise to eight million people per year by 2030. Alcohol is the most commonly used and abused substance in the population, and 12.5% of adults in the U.S. develop alcohol dependence during their lifetime (Hasin et al., 2007). In 2004, the World Health Organization (WHO) estimated that alcohol use disorders affected 76.3 million people globally (WHO, 2004). In the U.S., almost 80,000 people die per year from the consequences of alcohol consumption, which includes alcohol-related illnesses and accidents (Mokdad et al., 2004). Our society pays a high price for substance use, primarily through increased health care costs and judicial system expenditures. It is estimated that over 11% of federal and state government budgets ($374 billion in 2005) are spent dealing with the consequences of tobacco, alcohol, and other substance use, abuse, and dependence (The National Center on Addiction and Substance Abuse at Columbia University, 2009).

The development of addiction requires the use of a substance and a subsequent chain of behavioral events that leads to addiction. The key steps in the development of addiction include the initiation of substance use and the conversion from experimental use to established use before the actual development of addiction (see Figure 1). Each step is influenced by environmental and genetic factors, some of which are common to all steps, and others that are specific. For example, environmental factors, such as the availability of nicotine, alcohol, and drugs, play a role in each stage in the development of addiction, but accessibility of a substance is relatively more important in the initiation of substance use. Similarly, high cost of a substance through taxation can reduce initiation, use, and addiction; however, taxation has a stronger influence on teenagers who have less money, thus limiting initial use. Family, twin, and adoption studies also convincingly demonstrate a substantial genetic contribution to the development of addiction to nicotine, alcohol, and illicit drugs. Heritability estimates for nicotine, alcohol, and drug addiction are in the range of 50% to 60% (Heath et al., 1997; Tsuang et al., 1998; Kendler et al., 2003; Li, 2006). In general, it appears that environmental factors have a stronger effect on initiation, whereas genetic factors play a larger role in the transition from regular use to the development of addiction (Vink et al., 2005). Given the robust behavioral evidence for the role of genetic influence in addiction, genetic studies are warranted.

Initial inroads into understanding the genetic influences of addiction in humans relied on both genetic linkage mapping and candidate gene association studies, resulting in the identification of hundreds of potential genes contributing to the addiction process. Yet, few of these associations have been replicated in independent studies, potentially reflecting a number of false positives and/or genetic heterogeneity in which multiple genes contribute modest effects. The last decade, however, has seen a revolution in genetic technologies, and now hundreds of thousands of genetic variants (or single nucleotide polymorphisms; SNPs) can be queried in thousands of individuals in a cost-effective manner. This technology facilitates genome-wide association studies (GWAS) that test for an association of genetic variants with an illness in order to discover genetic contributions to complex diseases. Complex diseases are caused by many genetic and environmental factors working together, and GWAS has permitted the discovery of hundreds of genetic variants that alter the risk of developing multiple complex diseases, including type 2 diabetes, Crohn's disease, and Parkinson's disease (Hindorff et al., 2010). More recently, the genetic tools of GWAS have been applied to the study of

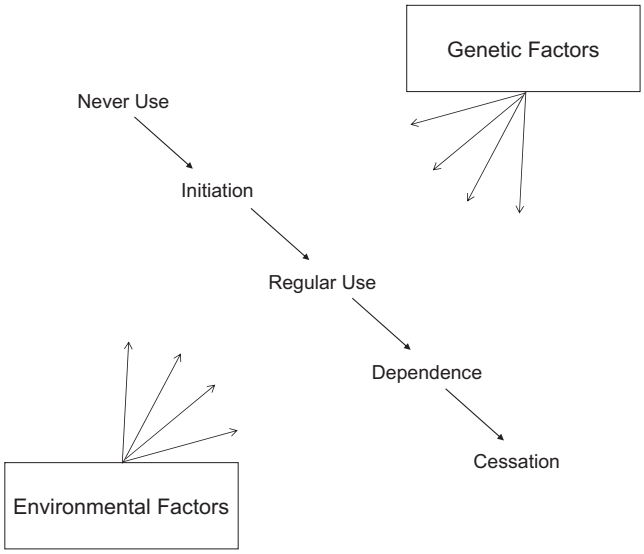

Figure 1. Steps in the Development of Dependence

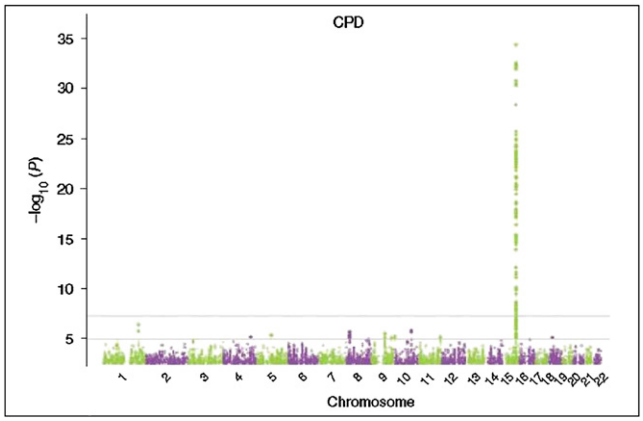

Figure 2. Genome-Wide Association Results for Cigarettes Per Day
Manhattan plot, indicating significance of association of all SNPs in the TAG Consortium meta-analysis for cigarettes per day. Manhattan plot shows SNPs plotted on the x axis according to their position on each chromosome, and plotting on the y axis is shown as negative $\log_{10}$ p value. Chromosome 15 contains the strongest genetic contribution to the risk of developing nicotine dependence. Figure courtesy of TAG Consortium (2010).

addiction to identify genetic variations that contribute to this illness. The success of this approach has been in part due to the creation of genetic research consortia for the study of nicotine and illicit drugs (NIDA Genetics Consortium; http://www.nida.nih.gov/about/organization/genetics/consortium/index.html) and alcohol (e.g., NIAAA's Collaborative Study on the Genetics of Alcoholism [COGA]; http://www.niaaa.nih.gov/ResearchInformation/ExtramuralResearch/SharedResources/projcoga.htm), permitting the collection of the massive numbers of comprehensively assessed subjects and DNA samples required for large-scale studies. These resources are also shared with the scientific community through the database of Genotypes and Phenotypes (dbGaP; http://www.ncbi.nlm.nih.gov/gap) so that scientists around the world can test new hypotheses about the genetic underpinnings of addiction.

This review will give a synopsis of the current understanding of genetic contributions to the vulnerability of substance dependence. There have been extensive discussions about the terminology used to define substance use disorder—"dependence" versus "addiction." Substance dependence is the official diagnostic nomenclature used in the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; APA, 1994) to represent the syndrome of substance misuse that leads to adverse consequences and includes a cluster of symptoms such as tolerance, withdrawal, and inability to stop using (see DSM-IV substance dependence for the complete diagnostic criteria). The creators of the DSM-IV criteria selected the term "dependence" because of the concern of stigmatization associated with "addiction." At this time, revisions to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) are underway for release in 2013. In this revision, issues have again been raised about the term used to define this clinical syndrome. In order to differentiate from the normal physiologic development of tolerance and withdrawal that develops with substance use from the compulsive drug use with loss of control, DSM-V proposes the use of the word "addiction" to define substance use disorder. The words "dependence" and "addiction" are used interchangeably in this review to represent the same underlying concept of substance use disorder.

Genome-Wide Association Studies of Nicotine Dependence

The strongest genetic contribution to nicotine dependence comes from variation in the nicotinic receptor subunits, and the most compelling genetic evidence is provided by several large-scale GWAS meta-analyses of smoking behavior (Liu et al., 2010; Thorgeirsson et al., 2010; TAG Consortium, 2010). Because smoking is a major contributor to many illnesses, cigarettes smoked per day (CPD), a proxy phenotype for nicotine dependence, has been measured in many genetic studies, and this has allowed meta-analyses of over 80,000 individuals of European ancestry. These genetic meta-analyses of CPD confirm that two chromosomal regions containing nicotinic receptor subunit gene clusters influence smoking behavior.

The most robust genetic finding that alters the risk of developing heavy smoking is in the chromosome 15q25 region, which contains the α5, α3, and β4 nicotinic receptor subunit gene cluster (*CHRNA5*, *CHRNA3*, and *CHRNB4*). The SNP rs16969968 is unequivocally associated with smoking behavior ($p = 4.48 \times 10^{-33}$ and $p = 5.57 \times 10^{-72}$ in combined analyses) (Figure 2; TAG Consortium, 2010). Further examination of the chromosome 15 region demonstrates that there are at least two distinct genetic risk variants that contribute to heavy smoking behavior (Saccone et al., 2010a; TAG Consortium, 2010).

Variation in an independent group of nicotinic receptors is also associated with the development of heavy smoking and nicotine dependence. The nicotinic receptor gene cluster on chromosome 8 that includes the α6 and β3 nicotinic receptor subunit gene cluster (*CHRNA6*, *CHRNB3*) is correlated with smoking behavior. This region generated genome-wide significant association with nicotine dependence, though the strength of this

association is much less (rs6474412 $p = 1.4 \times 10^{-8}$) (Thorgeirsson et al., 2010).

In addition to genetic variants in the nicotinic receptors contributing to the development of nicotine dependence, genetic variation in nicotine metabolism plays an important role in cigarette consumption (Schoedel et al., 2004; Minematsu et al., 2006) and nicotine dependence (Audrain-McGovern et al., 2007). Conversion of nicotine to cotinine accounts for 70% of initial nicotine metabolism and is performed by the *CYP2a6* enzyme (Yamazaki et al., 1999; Su et al., 2000; Malaiyandi et al., 2006). Important functional polymorphisms of *CYP2a6* include large deletions and gene recombinations that involve neighboring genes (Oscarson et al., 1999, 2002). The importance of nicotine metabolism and variation in the *CYP2a6* region on chromosome 19 was recently reinforced by the GWAS meta-analysis studies in which variants in this region were associated with number of CPD (Thorgeirsson et al., 2010; TAG Consortium, 2010). The most significant SNP reported in this region, the intergenic variant rs41405144, lies within two large deletions (defined as CYP2a6*4 and CYP2a6*12). This variant, rs41405144, is correlated with rs1801272, a nonsynonymous SNP that defines the CYP2a6*2 loss-of-function allele. These findings confirm that variation in nicotine metabolism contributes to the number of cigarettes smoked daily and the development of nicotine dependence.

Genetics of Alcohol Dependence

Alcohol dependence was one of the first behavioral disorders shown to have validated genetic contributions. Polymorphisms in the alcohol metabolizing enzymes are the most strongly associated genetic variants that influence alcohol consumption and alcohol dependence. In 1972, individuals of Asian descent were reported to have facial flushing and decreased tolerance when exposed to alcohol (Wolff, 1972). The flushing reaction after ingesting alcohol is secondary to a deficiency of aldehyde dehydrogenase (specifically ALDH2), an enzyme involved in the metabolism of ethanol (Goedde et al., 1980). The ALDH2 deficiency was found to be present in a large part of the general Japanese population, but uncommon in alcohol-dependent individuals, implying a protective role for the deficiency of ALDH2 in alcohol dependence (Harada et al., 1982).

Since these initial discoveries, much has been learned about alcohol metabolism. Ethanol metabolism occurs predominantly in the liver in two steps: the oxidation of ethanol to acetaldehyde catalyzed by alcohol dehydrogenases (ADHs), and the oxidation of acetaldehyde to acetate by acetaldehyde dehydrogenases (ALDHs). Several known genetic variants cause amino acid changes in these proteins and alter enzymatic activity. For instance, ADH1B*2, or rs1229984, diminishes ADH1b enzymatic activity several-fold, and ALDH2*2, or rs671, results in a nearly inactive enzyme (Edenberg, 2007). These genetic variants reduce the probability of heavy alcohol consumption and the development of alcohol dependence (Edenberg, 2007; Macgregor et al., 2009; Sherva et al., 2009). The mechanism by which variants of these enzymes influence the risk of developing alcohol dependence is hypothesized to be through an elevation of acetaldehyde levels after drinking, leading to facial flushing, nausea, and other adverse reactions.

In terms of GWAS assessments, in contrast to the GWAS of smoking behaviors to date, GWAS of alcohol dependence have been less consistent in identifying genetic variants associated with alcoholism (Treutlein et al., 2009; Bierut et al., 2010; Edenberg et al., 2010). One main reason for the differences in results is that these initial studies of alcohol dependence are of modest size by GWAS standards, with only a few thousand subjects compared with the tens of thousands of subjects in the GWAS of smoking behaviors. Each study identified novel regions that have suggestive evidence of association with alcohol dependence, including *PECR* (Treutlein et al., 2009), an enzyme involved in fatty acid metabolism; *PKNOX2* (Bierut et al., 2010), which plays a role in cell proliferation, differentiation, and death; and *SLC22A18* (Edenberg et al., 2010), a solute carrier. However, there is not consistent replication across studies. In addition, the alcohol metabolizing genes previously found to be associated with alcohol dependence are not well queried on the genetic platforms used by these studies, so they remain to be validated by GWAS. For instance, rs1229984 and rs671 are not genotyped on many of the initial GWAS chips (www.broadinstitute.org/mpg/snap; Johnson et al., 2008). Overall, this variation in results suggests that individual genetic contributions to alcohol dependence will be of modest effect. Larger-scale meta-analyses are underway, and hopefully these studies will discover unique genetic associations with alcoholism.

While alcoholism GWAS await further validation, some of the candidates coming out of these earlier human genetic approaches have support from work in animal model systems, and therefore seem like potentially stronger alcoholism risk candidate genes. Animal models support the human genetic studies implicating the γ-aminobutyric acid (GABA) system as fundamentally involved in alcohol intoxication and withdrawal and other behavioral aspects of alcoholism (Krystal et al., 2006). Ethanol enhances $GABA_A$ receptor function (Bowen and Grant, 1998) and electrophysiologic studies implicate $GABA_A$ receptors as targets for the effect of ethanol in the central nervous system (Suzdak et al., 1986). Multiple candidate gene reports show an association between variants in *GABRA2* and alcohol dependence (Enoch, 2008). In a hypothesis-driven approach to test this association as part of a GWAS, we find a modest association with *GABRA2* (Odds Ratio = 1.1 and $p \approx 0.01$), further supporting the role of this candidate gene in the development of alcoholism.

Genetic Influences for Other Drug Addictions

Though less common in the general population, illicit addictions such as cocaine and opiate dependence can be more devastating socially, cause more physical illnesses, and represent an extreme of addiction. Because illicit drug addiction is less common, large-scale GWAS have not been undertaken as yet. Instead, the approach to studying the genetics of drug addiction has been through candidate genes. Hundreds of candidate gene association studies for drug addiction as well as for nicotine and alcohol dependence had been performed in the pre-GWAS era. Thousands more candidate gene studies have been undertaken for other medical illnesses. However, a disconnect between these reported candidate gene associations and the findings from GWAS exists in both the addiction field and across all of

medicine. If these candidate gene studies are valid, then the GWAS should identify thousands of genetic variants that play a role in disease. Though hundreds of genetic variants have been conclusively confirmed by GWAS as contributors to complex diseases, the number of confirmed genetic variants is more modest than what is expected from the candidate gene studies. Overall, only a modest percentage of the numerous genetic associations proposed in the candidate gene era have been subsequently replicated in GWAS (Siontis et al., 2010), which suggests that many of the candidate gene studies contained false positive reports. Interestingly, those that conclusively replicate have strong genetic effects.

This lack of replication across methods reflects two distinct issues in these different study designs: the low threshold for significance in candidate gene studies results in a high false positive rate, and the high threshold for significance in the GWAS design leads to a low sensitivity to true genetic contributions to disease. While candidate gene studies of addiction should therefore be interpreted with caution, they should not be dismissed, because they may have captured unique phenotypes of genetic variation that will not be seen in the large-scale heterogeneous GWAS. Regardless, validation of human genetic mutations linked to illicit drug use awaits further study.

In the interim, animal models of addiction continue to provide insights into potential candidate genes that would benefit from more directed study in humans. A number of these studies have targeted the known neurobiological systems regulating the dopamine reward system and the endogenous opioid system. Dopamine plays a key role in reward behavior, yet the association with alcoholism and other drugs remains controversial. There are equally prominent association studies of *DRD2* with alcoholism and other drug addictions and failures to replicate (Gelernter et al., 1991; Parsian et al., 1991, Le Foll et al., 2009). Similarly, the endogenous opioid system clearly plays a role in addiction, and an amino acid change in the μ opioid receptor (*OPRM1*) displays functional changes with up to 3-fold variation in the affinity of the receptor to bind beta-endorphin, the endogenous opioid (Bond et al., 1998). However, a large-scale meta-analysis does not demonstrate that this variant alters the risk of developing addiction (Arias et al., 2006).

We have the tools in hand now to directly test many of these candidate genes in large-scale studies using uniform criteria for diagnosis and outcomes along with tools to genotype the specific variant needed, and so the contributions and controversy of these potential associations will be resolved in the coming years.

Common and Specific Factors in Addiction

The above sections have focused on candidate genes for specific addictions (summarized in Table 1), and several of the confirmed genetic findings support that specific genetic variants contribute to specific substance dependence risk. For instance, variants in the alcohol metabolizing genes specifically contribute to differences in alcohol consumption and alcohol dependence, but not to other addictive behaviors. Similarly, the variation in nicotine metabolizing genes contributes to smoking behavior and CPD, but not alcoholism or other drug addiction. Yet data from family and twin analyses also support the idea that there is a strong contribution from common genetic factors to the development of dependence on various classes of drugs (Bierut et al., 1998; Merikangas et al., 1998; Tsuang et al., 1998; Kendler et al., 2003). In fact, twin studies have convincingly shown that most of the genetic variation to addiction is shared across the liability to develop nicotine, alcohol, and illicit drug addiction (Tsuang et al., 1998; Kendler et al., 2003). As a result, once an association is identified, the next step is to test whether this genetic variant influences multiple drug dependencies.

Table 1. Genes and Proteins Associated with Addiction

Dependence	Gene or Protein
Nicotine	*CHRNA5-CHRNA3-CHRNB4*
	CHRNA6-CHRNB3
	Cyp2a6
Alcohol	ADH1B
	ALDH2
Cocaine	*CHRNA5-CHRNA3-CHRNB4*

The chromosome 15 variant in the α5 nicotinic receptor, rs16969968, which influences the development of nicotine dependence, has also been independently shown to contribute to the occurrence of alcohol and cocaine dependence. The minor allele of rs16969968 that is correlated with an increased risk for nicotine dependence is associated with a decreased risk for alcohol and cocaine dependence (Grucza et al., 2008; Chen et al., 2009; Sherva et al., 2010). This bidirectional association is hypothesized to be due to the involvement of nicotinic receptors with both excitatory and inhibitory modulation of dopamine-medicated reward pathways. These data reinforce the importance of variation in the *CHRNA5-CHRNA3-CHRNB3* gene cluster for risk of dependence on multiple substances, although the direction of the effects varies across substances. In addition, variants in this region influence the initial responses to alcohol and nicotine in adolescents (Schlaepfer et al., 2008).

As we identify other genetic variants associated with addiction, it will therefore behoove us to test the potential contribution of each variant across the wide range of abused substances, as it is likely that some variants will be common risk factors of relevance to multiple addictive substances.

Where Is the Unexplained Variance?

Though the GWAS-based approach has been successful for investigating the genetic influences of nicotine dependence and other complex traits, a significant fraction of the genetic variance remains unexplained (Frazer et al., 2009). The heritability of addiction is approximately 50%, yet the confirmed genetic contributions to nicotine dependence (through the nicotinic receptors and nicotine metabolizing genes) and alcohol dependence (through alcohol metabolizing genes) explain only a small fraction of this heritability. There are two main explanations for the missing variance: rare variation not queried on the current GWAS chips, and many genes of small effect. It is likely that both of these contribute to the missing genetic variance.

Clearly, part of this missing variance is related to coverage of the existing GWAS chips. By design, GWAS test for association

with common variants (allele frequencies >5%). The less common (or "rare" variants with allele frequencies <5%) are not adequately represented on the existing arrays. For example, the well-known genetic variants that alter alcohol metabolism, rs1229984 in *ADH1b* and rs671 in *ALDH2*, are not queried on most of the commercial GWAS chips. Although individually rare, these variants are collectively frequent, and their contribution to disease can be greater than those observed for common variants (Bodmer and Bonilla, 2008). Several other rare mechanisms can contribute to the modest explanation of variance to date. Structural variants, which include insertions and deletions, inversions, and translocations, can account for some of the unexplained heritability. Sequencing will be needed to allow us to definitively detect and test this class of variation.

Yet, there is also evidence that multiple common variants can begin to explain more of genetic variation in addiction. For smoking behavior, for example, we know that individual genetic variants contribute only a small effect to the development of nicotine dependence. Yet in combination, these genetic factors play a substantial role in the development of heavy smoking. For example, in our study from the Collaborative Genetic Study of Nicotine Dependence (COGEND), approximately nine variants in the nicotinic receptors explain 5% of the phenotypic variance in the sample (Saccone et al., 2010b). Though this explained variance estimate is likely higher than what will be seen in a general population study of smoking behavior, it demonstrates that collectively common genetic polymorphisms of small effect can begin to explain a larger proportion of genetic variation related to disease. Most likely hundreds, and more probably thousands, of genetic variants will be required to explain the genetic input to disease.

An additional potential drawback to GWAS is that there is heterogeneity of study design that may obscure true genetic contributors to disease, and careful consideration in the design of future addiction GWAS may help to alleviate this issue. An example is seen in the comparison of our study (COGEND), designed to examine genetic influences on smoking behavior and nicotine dependence, and the large-scale GWAS of smoking (Saccone et al., 2009, 2010b; Thorgeirsson et al., 2010). Our COGEND study compared very light smokers and current nicotine-dependent smokers, thus focusing on differences between those who can smoke a little and not become addicted and individuals with addiction. In addition, our sample recruited subjects using a systematic strategy and in a relatively narrow age range (25–44) to avoid the confounding of secular trends in smoking. Conversely, the large-scale GWAS of smoking were based on current and former smokers, and the entire range of smoking amount was included. The age range in these studies encompassed different generations in which we know smoking behavior has changed. In addition, some subjects were recruited for lung cancer, others for heart disease, and others for many other medical illnesses.

Recent meta-analyses have suggested that our more focused study design has paid off—our ascertained sample that included a narrow age range and specific smoking behavior requirements increased power to detect genetic variation compared with a more heterogeneous GWAS. Two of the top genetic findings—rs16969968 in *CHRNA5* and rs6474412 in *CHRNB3*—showed significance levels of 5.57×10^{-72} with a sample size of n = 73,853 (TAG Consortium, 2010) and 1.4×10^{-8} with a sample size of n = 84,956 (Thorgeirsson et al., 2010). In our COGEND sample of 2062 subjects of European descent, we have a significance level of 4×10^{-7} for the *CHRNA5* variant and 1.37×10^{-3} for the variant in *CHRNB3* (Saccone et al., 2010a, 2010b), representing a 3-fold and 10-fold increase, respectively, in the power to detect genetic variation compared with a more heterogeneous GWAS. These comparisons demonstrate the amplified power of a study design through the systematic ascertainment, targeted age range, and phenotypic contrast of lifetime light smokers versus current heavy smokers.

From Genetic Association to Function

The above sections have highlighted how human genetic tools have aided in the identification of genetic variants contributing to the addiction cycle. Yet it needs to be understood that a genetic association characterizes only the first stage in understanding the underlying biology that leads to disease. A genetic association represents not only an association with tested genetic variants, but also an association with untested, highly correlated SNPs that can span across many genes on the same chromosome. A challenge once a genetic association is confirmed is to then understand which of these variants contribute to the biological mechanism underlying the correlation with a disease.

In the chromosome 15 region, the most biologically credible variant associated with nicotine dependence is rs16969968, a polymorphism that causes an amino acid change from aspartic acid to asparagine (Asp398Asn) in the α5 nicotine receptor subunit. Several lines of evidence point to this variant as having functional importance. The specific region in the α5 protein that includes this polymorphism is highly conserved across different species, which implies biological importance (aspartic acid is conserved in chimpanzee, Bolivian squirrel monkey, domestic cow, mouse, chicken, and African clawed frog) (Bierut et al., 2008). An in vitro functional study found that $(\alpha4\beta2)_2\alpha5$ receptors that only differed by the asparagine amino acid substitution exhibited altered response to a nicotine agonist compared with receptors containing the aspartic acid amino acid (Bierut et al., 2008). Further studies of the nicotinic receptors show that the α5 Asn 398 protein (high risk variant) in the nicotinic acetylcholine receptor lowers Ca^{2+} permeability and increases short-term desensitization in $(\alpha4\beta2)_2\alpha5$, but does not alter the receptor sensitivity to activation (Kuryatov et al., 2011). The high sensitivity to activation and desensitization of $(\alpha4\beta2)_2\alpha5$ nicotine acetylcholine receptors by nicotine results in a narrow concentration range in which activation and desensitization curves overlap at nicotine concentrations typically sustained in smokers. It is predicted that smokers would desensitize most of these receptors while permitting a smoldering activation of the remainder of the receptors. In addition, the α5 nicotinic receptor subunit is expressed in the brain regions that are important in the pathways relevant to the development of dependence. Finally, this key α5 gene variant is associated with a dorsal anterior cingulate-ventral striatum/extended amygdala circuit, and the "risk allele" decreases the intrinsic resting functional connectivity strength in this circuit (Hong et al.,

2010). Importantly, this effect is observed in nonsmokers and it appears to represent a trait circuitry biomarker.

In the chromosome 15 region, the second independent genetic association with nicotine dependence is marked by rs880395 (Saccone et al., 2010a), and functional studies suggest a distinct biological mechanism: altered α5 nicotinic receptor mRNA expression (Wang et al., 2009; Falvella et al., 2010; Smith et al., 2011). Variants tagged by rs880395, which are more than 10 kb upstream of *CHRNA5*, result in a 2.5- to 4-fold difference in α5 nicotinic receptor mRNA expression in the brain. High expression of *CHRNA5* mRNA is correlated with an increased risk of heavy smoking and nicotine dependence (Wang et al., 2009). This change in expression is not seen in lymphocytes, which demonstrates that genetic variants can have tissue-specific biologic effects (Smith et al., 2011).

These findings of the α5 nicotinic receptor in humans have motivated further animal studies of this receptor subunit, which show that the habenulo-interpeduncluar pathway is a key neurocircuit controlling nicotine consumption (Fowler et al., 2011). This circuit acts as a negative feedback response, opposite to the mesoaccumbens positive reward pathway. This animal work suggests that individuals with the α5 nicotinic receptor risk alleles for nicotine dependence are relatively insensitive to the inhibitory effects in the reward pathway. This type of work—spanning humans, other animals, individual cells, and then back to humans—represents the power of genetic studies. We can identify associations, target new genes for study, and then test hypotheses in both other animals and humans.

These genetic associations with nicotine and alcohol dependence and these proposed mechanisms of biologic action including neurotransmission and metabolism provide new insights into the underlying biology associated with addiction. Identifying how specific variants and genes associated with addictive behavior affect brain function will be key to understanding the development of dependence; yet, numerous questions remain. For example, will these mechanisms of action associated with genetic variation be expressed in all regions of the brain, or will the genetic effect be region specific? Will these variants have a similar influence throughout the lifespan, or will there be critical periods when these genetic variations alter the risk of developing addiction? Though these biological mechanisms are proposed to lead to the altered risk for the development of addiction, they represent but an initial understanding of the mechanisms of dependence, and it is likely that there will be more complex biologic functions underlying these genetic associations.

Convergence of Genetic Findings of Addiction and Cancer

There is an intriguing convergence of genetic findings for nicotine and alcohol dependence and medical disorders. Smoking is the strongest risk factor for the development of lung cancer and chronic obstructive pulmonary disease (COPD). Large-scale genetic studies demonstrate that the same variants on chromosome 15 that are associated with smoking behavior are also the strongest genetic risk factors for lung cancer and COPD (Amos et al., 2008, 2010; Hung et al., 2008; Liu et al., 2008; Thorgeirsson et al., 2008; Broderick et al., 2009; Pillai et al., 2009; Shiraishi et al., 2009; Lips et al., 2010). The convergence of these genetic findings associated with smoking behavior and smoking-related illnesses raises the question of whether this locus has a direct biologic effect on the risk of developing lung cancer and COPD, or if the increased genetic risk of lung cancer and COPD can be explained solely through the genetic influences on smoking behavior.

The data remain mixed as to whether the genetic risk on chromosome 15 and lung cancer and COPD is related to heavier smoking (an indirect effect) or whether a direct biological mechanism increases lung cancer and COPD risk independent of smoking (a direct effect). Evidence in favor of a direct biological effect is that this genetic risk for lung cancer and COPD association with these variants remains after statistically accounting for duration of smoking history and number of CPD (Lips et al., 2010). The α5 nicotinic receptor subunit is expressed in lung tissue, and a 30-fold upregulation of expression of *CHRNA5* is seen in lung cancer tissue compared with normal lung tissue (Falvella et al., 2010).

On the other hand, this chromosomal region does not increase the risk of lung cancer among nonsmokers (Lips et al., 2010). Furthermore, CPD may not fully account for the exposure to carcinogens in cigarette smoke. An intriguing study demonstrated that smokers with the risk variants in the chromosome 15 region ingested more toxins even after controlling for the number of cigarettes smoked (Le Marchand et al., 2008). This implies that the smokers with the risk variants are inhaling more intensely and increasing their exposure to nicotine and other carcinogens in cigarette smoke. Thus the measurement of CPD is an imprecise measure of the risk of smoking related to lung cancer and COPD.

A parallel finding is seen with genetic variants that influence alcohol consumption, alcohol dependence, and esophageal cancer. Large studies of esophageal cancer, a cancer related to alcohol use, identify two genetic variants in alcohol metabolizing genes that influence alcohol consumption and alcohol dependence (*ADH1b* variant rs1229984, and *ALDH2* variant rs671) and also contribute to the risk of esophageal cancer (Hashibe et al., 2008; Tanaka et al., 2010). Even after controlling for alcohol consumption in the analyses, the protective effects of these variants for esophageal cancer remain strong. This implies that variants in alcohol metabolizing genes not only reduce alcohol consumption and decrease the risk for alcohol dependence, but also lower the susceptibility to esophageal cancer, perhaps by reducing the carcinogenic effects of alcohol, its metabolites, and other toxins.

Both of these examples challenge paradigms about the relationship between addiction and cancer. Epidemiologic data clearly support the association of addiction with cancer: smoking and nicotine dependence are associated with lung cancer; and alcohol consumption and alcohol dependence are associated with esophageal cancer. As a result, exposure to smoking and alcohol has been considered an environmental variable to be controlled in the study of cancer. However, the strongest genetic findings for the development of addiction are also the strongest genetic predictors for the correlated cancers. These findings blur the distinction between genetic and environmental risks with nicotine and alcohol addiction. It also remains

unclear if the mechanism of these associations of cancer with the genetic variants can be completely explained through addictive behaviors, or if biologic mechanisms act in the brain to increase the risk of addiction while also acting in the lung and esophagus to increase the risk of cancer. Only through animal models will we be able to separate the genetic influence of these variants on the development of dependence from the genetic contribution to the development of cancer.

Genetic Implications for Different World Populations

Although dependence is common in all populations, to date all of the large-scale GWAS have been performed in populations of European descent. Though the underlying biological mechanisms that lead to the development of substance dependence are most likely the same across populations, varying allele frequencies can alter the relative importance of specific genetic risk factors in different populations. For example, the variant rs16969968, which is relatively frequent in populations of European descent (37% allele frequency), is rare in populations of African or Asian descent (0% to 3% allele frequency) (Bierut et al., 2008). Similarly, the polymorphisms that cause amino acid changes in alcohol metabolizing genes, rs671 and rs1229984, are common in Asian populations, but are rare in populations of European and African descent (Edenberg, 2007). Thus, rs16969968 will play a larger role in the development of heavy smoking and nicotine dependence in populations of European ancestry compared with populations of African and Asian ancestry. Similarly, rs671 and rs1229984 will more strongly influence alcohol consumption and alcohol dependence in Asian populations compared with European and African populations. A new genetic frontier is to leverage these differences in genetic architecture across populations to refine association signals and narrow down associations to the most likely biologically causative variants. This strategy highlights the importance of recruiting, assessing, and studying diverse populations.

Future of Genetic Studies

As we are beginning to understand some of the genetic factors that alter our individual vulnerability to dependence, the future of genetic studies has the potential to personalize our treatments for addiction. We have unequivocal evidence of genetic variation that contributes to the development of this behavioral disorder. Addiction represents a great success in psychiatric genetics. The strongest specific genetic contributors to dependence are related to the pharmacologic responses to nicotine and alcohol and include variation in nicotinic receptor genes, nicotine metabolizing genes, and alcohol metabolizing genes.

Though some might say that GWAS have failed because we cannot account for the genetic variance associated with disease, this represents a very narrow view of the field. We have convincingly identified genetic variants that contribute to addiction. If the progress in other medical disorders can be used as an example, the ''big science'' consortia that include the study of tens of thousands and potentially hundreds of thousands of people will soon discover new variants that contribute to addiction. We now know that the genetic risk is modest (OR 1.3 or less) for variants that are common in the population, but rarer variants may have somewhat stronger effects. The genetic vulnerability to addiction represents the combination of hundreds or thousands of genes of modest effect.

We are at a stage where we can take several productive paths. First, we must integrate the results from candidate gene studies with the findings from GWAS. By synthesizing both approaches into a cohesive model, we will be able to balance the high false positive rate in candidate gene studies with the high false negative rate in GWAS. This will allow us to separate the wheat from the chaff in the candidate gene studies and aid in the discovery of more variants from the GWAS approach. Second, in genetic studies, ascertainment and phenotypes matter and size is not everything. Though we have thrown together GWAS from many different fields, it is time to go back and more carefully select studies for inclusion so that similar ascertainments can be used to reduce heterogeneity. An improvement of phenotypes should also aid in the discovery of genes. For example, CPD is an effective, but imprecise, measurement of nicotine addiction. Though a large sample size can overcome a crude measure, there is a gain of power with more exact assessments. A balance must be reached between the smaller sample sizes in genetic studies with comprehensive assessments and large samples with simpler phenotypes.

Tools are under development to aid scientists, physicians, and the public in the synthesis, interpretation, and dissemination of findings in human genetic variation in health and disease. One mechanism is the Human Genome Epidemiology Network (HuGENet) (http://hugenavigator.net/HuGENavigator/home.do). Since 2001, HuGENet has maintained a searchable database of published, population-based epidemiologic studies of human genes extracted and curated from PubMed. This website allows the user to search by disease and gene with the goal of aiding in the translation and integration of genomics into public health research, policy, and practice.

Finally, we must remember that the major purpose for the study of the genetics of addiction is to ultimately improve our care for individuals with this disorder. Our current treatments for alcohol and nicotine dependence are related to the pharmacologic response of these substances. For example, we exploit the aversion to alcohol by administering disulfiram, a medication that interferes with ALDH, and thus increases acetaldehyde levels when alcohol is ingested. This build up of acetaldehyde causes symptoms of nausea, vomiting, flushing, and headache, and is similar to the biologic response seen in individuals who carry an alcohol metabolizing gene deficiency. We may be able to utilize the variation in nicotinic receptors and nicotine metabolizing genes to improve our treatments for smoking. As we begin to understand more of the genetic diversity that influences an individual's specific risk of dependence, we will highlight new biologic pathways and neural circuitry that may be exploited pharmacologically. By identifying genetic risks that contribute to dependence, we can begin to dissect different contributions of genes and environments that lead to dependence, and in turn we can improve interventions to reduce dependence and improve cessation.

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