Pharmacogenetics: A Tool for Identifying Genetic Factors in Drug Dependence and Response to Treatment

Pharmacogenetics research looks at variations in the human genome and ways in which genetic factors might influence how individuals respond to drugs. The authors review basic principles of pharmacogenetics and cite findings from several gene-phenotype studies to illustrate possible associations between genetic variants, drug-related behaviors, and risk for drug dependence. Some gene variants affect responses to one drug; others, to various drugs. Pharmacogenetics can inform medication development and personalized treatment strategies; challenges lie along the pathway to its general use in clinical practice.

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Substance dependence is a complex psychiatric disorder that develops in response to a combination of environmental and genetic risk factors and drug-induced effects (Ho et al., 2010). The strong genetic basis of dependence is supported by family, adoption, and twin studies, which demonstrate substantial heritability, estimated to be about 50 percent (Uhl et al., 2008). The evidence suggests that no single variant accounts for a major portion of this risk, but that variations in many genes each contribute a small amount.

Pharmacogenetics is the study of the genetic factors that influence drug response and toxicity. In this review, we briefly state the basic principles of pharmacogenetics and then provide examples of discoveries that demonstrate the impact of genetic variation on drug dependence, drug effects, and drug-induced behaviors. The primary goal of pharmacogenetic research into substance abuse is to better understand the sources of variation in the risk for dependence and the mechanisms involved. Some of the studies we discuss have identified genotypes that confer high risk for drug dependence, information that may be used to develop targeted, effective prevention programs. We also highlight how pharmacogenetics can advance the development of personalized treatments by revealing genetic variations that predict individual responses to therapeutic interventions.

PRINCIPLES OF PHARMACOGENETICS

Pharmacogenetics focuses on variation within the human genome. The human genome consists of some 30,000 genes, each composed of a sequence of hundreds to thousands of nucleotides (units of deoxyribonucleic acid, or DNA) (see Figure 1). Every person inherits two copies of most genes, one from each parent. Although any two individuals' DNA is over 99 percent identical, the number of nucleotides

FIGURE 1. Structure of a Gene
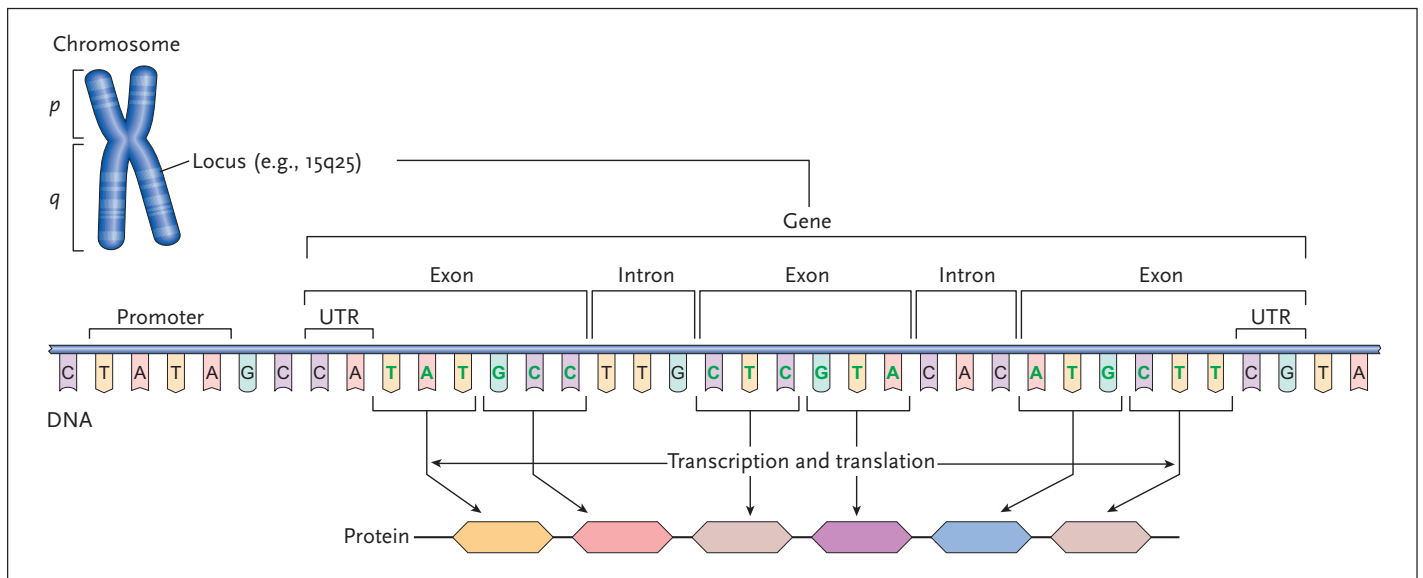
Jim Perkins

Genes are distributed along chromosomes, which are long sequences of DNA. This illustration shows human chromosome 15 and highlights a hypothetical gene at position 25 on the long (q) arm.

A gene is a sequence of DNA units, or nucleotides (adenine [A], cytosine [C], guanine [G], and thymine [T]). For a gene that determines a protein, the order of nucleotides precisely dictates the structure of an RNA intermediate and a subsequent protein product. In contrast to this simplified illustration, actual genes are hundreds to thousands of nucleotides long, untranslated regions (UTRs) are hundreds of nucleotides long, and promoters are typically at least 40 nucleotides long.

Each gene and its related DNA can be divided into segments:

Promoter region: The genetic machinery anchors here to begin building the RNA intermediate; sequence variation in this region may alter the machinery's access to the gene and thereby affect its rate of RNA and protein production.

Exons: The genetic machinery transcribes these segments into an RNA intermediate, then translates the RNA intermediate (except for the RNA that came from the UTRs) into the sequence of amino acids that constitutes the protein product; sequence variation within exons can alter that product (see Figure 2).

Introns: The genetic machinery transcribes these segments into RNA but deletes them before translation into amino acids.

UTRs: These regions serve regulatory functions and contribute to the stability of the RNA intermediate; however, they are not translated into protein.

A genetic variant may alter responses to one drug or to multiple drugs.

is so large—approximately 3 billion—that millions of variant sequences still occur across the human population (Kruglyak and Nickerson, 2001). Variants that are found in more than 1 percent of the population are called polymorphisms. The most abundant type of variant is the single nucleotide polymorphism (SNP, pronounced "snip"); other common types are deletions, insertions, and tandem repeats (see Figure 2).

Each gene's nucleotide sequence encodes (provides a template for) a molecular product, usually a protein. Sequence variation may result in alterations in the gene's product, which in turn may have an effect on phenotypes (traits or characteristics, such as a disease or response to a drug) that the product influences.

Genetic researchers use several types of studies to establish and explore gene-phenotype relationships. Heritability studies can indicate the relative contributions

of genetic and nongenetic (e.g., environmental) influences to a particular phenotype. Linkage studies analyze pedigrees of related individuals and genetic markers to hone in on regions in the genome that may harbor genes associated with phenotypes of interest. Candidate gene association studies can be used to investigate gene-phenotype relationships suggested by linkage studies, as well as to focus on genes selected for their physiological or pharmacologic relevance to a phenotype. Genome-wide association (GWA) studies look for gene-phenotype relationships by simultaneously comparing hundreds of thousands of gene variants in DNA samples taken from large numbers of individuals.

Traditionally, pharmacogenetics has focused on the role of genetic variation in pharmacokinetics (e.g., the absorption, distribution, metabolism, and excretion of drugs) and pharmacodynamics (e.g., drug-response pro-

FIGURE 2. Gene Polymorphisms

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"Wild Type" Allele
The most common variant; note that the sequence of each DNA triplet determines one amino acid of the protein product.
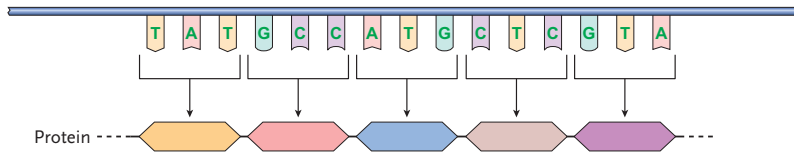
Single Nucleotide Polymorphism (SNP)
Substitution of one DNA unit (a nucleotide) for another at a particular site (here, a C replaces an A found in wild-type triplet 1); the changed sequence of the triplet results in an altered amino acid in the protein product.
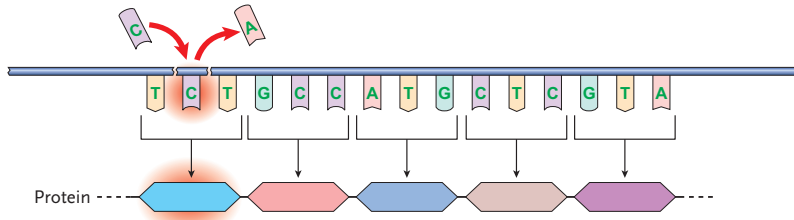
Insertion
Intrusion of an additional DNA unit or units into the gene sequence (here, a C between the T and A in wild-type triplet 1); this may change not only the sequence of the triplet in which it occurs, but of all subsequent triplets, producing alterations in many amino acids of the protein product.
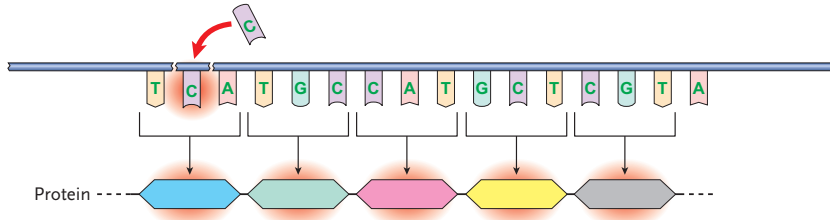
Deletion
Excision of a DNA unit or units (here, an A from wild-type triplet 1); this may change all subsequent triplets and the corresponding amino acids of the protein product.
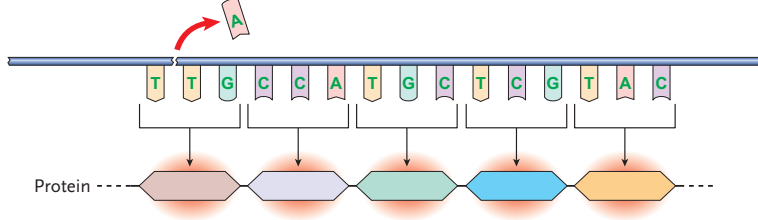
Tandem Repeats
Addition of multiple copies of a DNA triplet (here, TAT), end-to-end; each new triplet can add one more copy of the amino acid it encodes to the protein product; the resulting elongated protein product may function differently than that produced by the gene's wild-type variant.
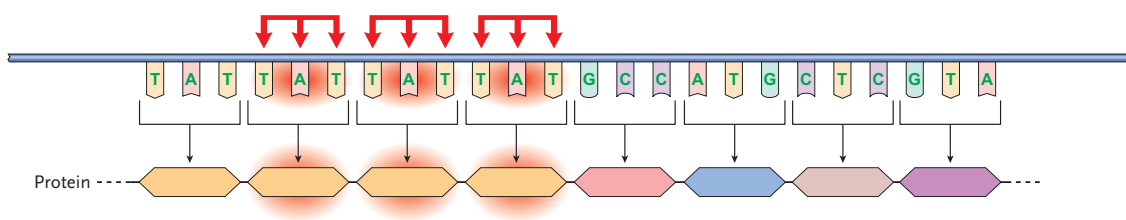
Polymorphism, or genetic variation in a gene's DNA sequence, may result in alterations in the expression, regulation, and/or function of its protein product. The panels show five possible variants (alleles) of a hypothetical gene.

teins, such as receptors, channels, and transporters) (see Figure 3). However, candidate gene studies and, more recently, GWA studies have identified additional genetic influences on drug abuse and dependence. Because GWA studies cast a wide net and are without a hypothesis about which genes are involved, they are theoretically excellent tools for discovering novel common genetic variants and new genetic biomarkers that associate with particular phenotypes. For instance, GWA studies have shown that genes involved in cell adhesion, enzymatic activities, transcriptional regulation, and many other processes and functions may be associated with dependence phenotypes (e.g., Ishiguro et al., 2008; Uhl et al., 2008).

The following sampling of pharmacogenetic findings is not exhaustive but is intended to demonstrate the associations and predictive validity of some genetic variants conferring susceptibility for drug dependence or treatment response. (For further reading, see: Ho et al., 2010; Ho and Tyndale, 2007; Rutter, 2006; Uhl et al., 2008.)

PHARMACOGENETICS OF SUBSTANCE ABUSE VULNERABILITY, ACQUISITION, AND PERSISTENCE

Some genetic variants alter the risk for dependence on one drug; others affect responses to various drugs. A variant that alters an enzyme that metabolizes a specific drug or a receptor activated by a specific drug is likely to play a role in vulnerability to dependence upon just that drug. In contrast, vulnerability to a variety of drugs could result from a variant that affects the brain reward pathways or neuroplasticity (the brain's formation of new neural connections in response to experience or drug exposures) (Uhl et al., 2008). Genetic variation contributing to vulnerability to, and dependence on, different drug classes has been shown for the drug-metabolizing cytochrome P450 (CYP) enzymes, receptors such as the dopamine D2 receptor (DRD2) and mu opioid receptor (OPRM1), transporters such as the serotonin transporter (5-HTT) and dopamine transporter (DAT1), and enzymes such as dopamine β-hydroxylase (DβH) and monoamine oxidase (MAO).

CYPs and Smoking

Variation in *CYP2A6,* the gene for the nicotine-metabolizing enzyme CYP2A6, influences aspects of smoking dependence by altering nicotine pharmacokinetics. CYP2A6 is primarily responsible for converting nicotine to cotinine, rendering it inactive (Benowitz

and Jacob, 1994; Messina et al., 1997), and the enzyme further metabolizes cotinine to trans-3'-hydroxycotinine (Nakajima et al., 1996). Individuals with different *CYP2A6* variants can be grouped according to the resulting CYP2A6 enzyme activity as normal, intermediate (approximately 75 percent of normal), or slow (less than 50 percent of normal) metabolizers (Schoedel et al., 2004).
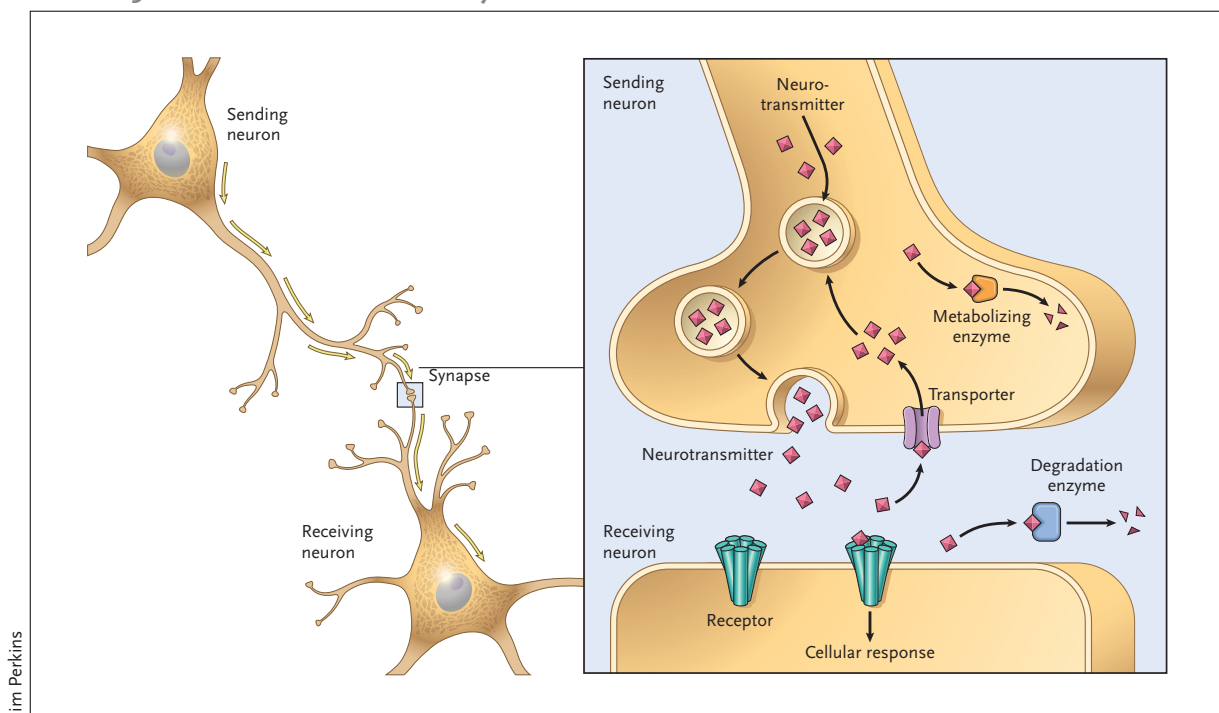
The *CYP2A6* genotype (the pair of specific variants, or alleles, in a gene that a person inherits, one from each parent) has been associated with the risk for being a smoker and with numerous smoking behaviors. For instance, studies in novice adolescent smokers have found that slow and normal metabolizers differ in their risk for conversion to dependence, as defined in the *International Classification of Diseases, 10th Revision* (ICD-10) (O'Loughlin et al., 2004) and in the rate at which they progress to increasingly severe dependence (Audrain-McGovern et al., 2007). Among adult smokers, slow metabolizers are less prevalent than intermediate or normal metabolizers; they smoke fewer cigarettes per day, exhibit reduced cigarette puffing, have decreased dependence, wait longer to smoke the first cigarette of the day, and have fewer nicotine withdrawal symptoms; and they make up a smaller portion of smokers as the duration of smoking increases, suggesting that they quit smoking sooner (Kubota et al., 2006; Malaiyandi et al., 2006; Schoedel et al., 2004; Strasser et al., 2007).

These findings suggest that nicotine levels remain elevated longer in smokers with slow-metabolizing *CYP2A6* variants than those with fast-metabolizing variants, resulting in a decreased need to light up or puff as often to avoid withdrawal. This reduced frequency of exposure to nicotine over time may decrease nicotine-induced changes in the brain, resulting in less severe dependence and perhaps more successful quitting attempts. Although not all studies agree on these associations, variation in CYP2A6's metabolic inactivation of nicotine appears, on balance, to be associated with variation in smoking behavior and may alter cessation rates (Munafo et al., 2004).

CYPs and Opioid Dependence

Several oral opioids, such as codeine, oxycodone, and hydrocodone, are metabolized by another CYP enzyme, CYP2D6, to more psychoactive metabolites, such as morphine, oxymorphone, and hydromorphone (Otton et al., 1993). The *CYP2D6* gene is highly polymorphic, with some variants leading to a completely inactive enzyme.

A dopamine transporter gene variant may play a role in drug-induced paranoia and psychosis.

FIGURE 3. Generic Neurotransmitter System

Sending neuron

Receiving neuron

Synapse

Sending neuron

Neurotransmitter

Metabolizing enzyme

Transporter

Neurotransmitter

Degradation enzyme

Receiving neuron

Receptor

Cellular response

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Neurotransmitters, such as dopamine and acetylcholine, are chemicals that carry signals from neuron to neuron across gaps called synapses. A sending neuron synthesizes neurotransmitter molecules and bundles them into packages; when the neuron becomes electrically excited, it releases the neurotransmitter molecules into the synapse. Once in the synapse, each molecule may:

- **dock on a receptor on the receiving neuron, initiating a cellular response;**

- **re-enter the sending neuron via a molecular conduit called the transporter;**

- **encounter a metabolizing or degrading enzyme that destroys it.**

Drugs of abuse produce psychoactive effects by disrupting the normal balance of neurotransmitter release, signaling, recovery, and metabolism. Genetic variation in receptors, transporters, or enzymes can limit or exacerbate these effects and thereby affect susceptibility to drug abuse and dependence.

GWA studies have made nicotinic acetylcholine subunit genes a focus of pharmacogenetic research on smoking.

Individuals who inherit such defective *CYP2D6* alleles from both parents are referred to as poor metabolizers (Alvan et al., 1990). Poor *CYP2D6* metabolizers are underrepresented among people dependent on oral opioids, suggesting that the *CYP2D6* defective genotype is a pharmacogenetic protection factor against oral opioid dependence (Tyndale et al., 1997). Of note, *CYP2D6* variation should not play a role in dependence on intravenously administered opioids (e.g., morphine) as these drugs are already psychoactive and do not depend on CYP2D6 activity for metabolic activation.

Dopamine Receptors

Drugs of abuse activate the mesolimbic dopaminergic pathway, which plays an essential role in drug reward and reinforcement (Di Chiara and Bassareo, 2007). A key mechanism in this pathway is dopamine activation

of DRD2 receptors on dopaminergic neurons in the ventral tegmental area (Cohen et al., 2007; Di Chiara and Imperato, 1988; Koob, 2006). Accordingly, studies have examined the impact of genetic variation in *DRD2* on responses to several drugs of abuse, and several polymorphisms have been implicated in susceptibility and dependence.

For instance, one *DRD2* variant, called *TaqI A,* results from a SNP (32806C>T) that occurs in the *DRD2*-neighboring *ankyrin repeat and kinase domain containing 1(ANKK1)* gene. Although this variant does not lie within the DNA region that encodes the DRD2 protein, it is nonetheless associated with a lower density of DRD2 receptors and consequently decreased dopaminergic activity (Noble et al., 1993). The *TaqI A* polymorphism may contribute to vulnerability to substance abuse and has been associated with polysubstance abuse (O'Hara

et al.,1993), heroin use (Lawford et al., 2000), cocaine dependence (Noble et al., 1993), and psychostimulant polysubstance abuse (Persico et al., 1996). Some studies have suggested that *TaqI A* is a risk factor for smoking behaviors (Comings et al., 1996; Erblich et al., 2005; Huang et al., 2009), while other studies have not found these associations (Berlin et al., 2005; Johnstone et al., 2004; Singleton et al., 1998).

Another variant associated with the *DRD2* gene, called *TaqI B,* located in exon 2, also results in lower density of DRD2 receptors in the striatum. Individuals with this variant are more likely than those without it to have smoked and to have started smoking at an earlier age (Spitz et al., 1998; Wu et al., 2000) to be cocaine-dependent (Noble et al., 1993), and to abuse psychostimulants (Persico et al., 1996). Additionally, a deletion variant (-141C Del) in the promoter region was associated with higher density of DRD2 receptors in the striatum (Jonsson et al., 1999) and with a higher likelihood of heroin abuse by inhalation, but not by injection (Li et al., 2002). These data suggest that, consistent with the key role of the dopamine receptor in the reward pathway, variation in *DRD2* may alter multiple aspects of dependence for many drugs of abuse.

An opioid receptor gene variant may predict responses to naltrexone therapy for alcoholism.

Other Dopamine Pathway Components

Genetic variation in other components of the dopamine transmission system has also been implicated in substance abuse. For instance, the gene *SLC6A3* encodes the dopamine transporter (DAT1), which regulates dopamine activity by drawing the neurotransmitter back into pre-synaptic neurons and terminating its action. Cocaine inhibits the dopamine transporter, which contributes to the drug's reinforcing effects.

Genetic variants consisting of variable numbers of tandem repeats (VNTR) of a 40-nucleotide unit can occur in exon 15 of *SLC6A3* (Vandenbergh et al., 1992). Studies have associated these variants with cocaine-induced paranoia but not cocaine dependence (Gelernter et al., 1994) and with methamphetamine-induced psychosis (Ujike et al., 2003) but not with methamphetamine abuse or subjective responses to acute methamphetamine (Hong et al., 2003; Lott et al., 2005). These findings suggest a distinct role for this *SLC6A3* genetic variation in drug-induced paranoia and psychosis, which appears to be unrelated to drug abuse and dependence. However, a different VNTR variant of *SLC6A3*—consisting of repeats of a unit of 30 nucleotides in intron 8—has been associated with cocaine abuse in a Brazilian population (Guindalini et al., 2006).

Variation in genes for dopamine-metabolizing enzymes has also been implicated in drug effects. The *DβH* gene encodes dopamine beta hydroxylase (DβH), an enzyme that metabolizes dopamine to norepinephrine (Stewart and Klinman, 1988). Two polymorphisms in *DβH*—an insertion-deletion variant (DβH5'-Ins/Del) and a SNP (444G>A)—are often inherited together and have been associated with cocaine-induced paranoia (Yamamoto et al., 2003). The enzyme MAO-A metabolizes a broad array of drugs and other molecules, including the neurotransmitters serotonin, dopamine, and norepinephrine (Shih et al., 1993). A VNTR polymorphism in the promoter region of the *MAO-A* gene has been associated with risk for substance use disorders (Vanyukov et al., 2004; Vanyukov et al., 2007). A variant with three repeats of a 30-nucleotide segment results in decreased expression of the *MAO-A* gene compared with variants having 3.5 or 4 repeats (Deckert et al., 1999; Denney et al., 1999; Sabol et al., 1998). Some studies have associated the low-activity variant with increased susceptibility to alcoholism (Contini et al., 2006; Huang et al., 2007; Saito et al., 2002) and antisocial alcoholism (Samochowiec et al., 1999; Schmidt et al., 2000), although other studies have found no such associations (Lu et al., 2002; Mokrovic et al., 2008).

The Opioid System

Genetic variation in the opioid system has been implicated in altered risk for drug dependence. For instance, the *OPRM1* gene encodes the receptor for beta-endorphin (an opioid produced naturally by the body) as well as for opiate and opioid drugs and the psychoactive metabolites of heroin (morphine and 6-monoacetylmorphine) (Ho et al., 2010). The most common *OPRM1* SNP (A118G) occurs in exon 1 and alters an amino acid (Asn40Asp) in the receptor (Bond et al., 1998; Zhang et al., 2005).

Studies of the effect of this variant on receptor function have yielded contradictory results. Some suggest that receptors encoded by this *OPRM1* variant have an increased affinity for beta-endorphin and greater receptor activation upon binding (Bond. et al., 1998), while others have found no change in receptor function, signaling, or binding affinities for various opioids (Befort et al., 2001; Beyer et al., 2004). Although variation in *OPRM1* has been found to contribute to the risk for heroin addiction in some populations (Bart et al., 2004; Szeto et al., 2001), not all studies agree (Bond et al., 1998; Tan et

al., 2003). The A118G variant was also associated with increased risk of developing alcoholism (Bart et al., 2005).

The Serotonin Transporter

The serotonin transporter (5-HTT) is encoded by the *SLC6A4* gene and directs the reuptake of serotonin from the synapse into the presynaptic neuron. This gene's promoter region *(5-HTTLPR)* occurs in short and long variants, depending on whether a 44-nucleotide sequence is deleted or not. The short variant reduces the transcriptional efficiency of the gene promoter, leading to decreased production of 5-HTT and hence a dysfunctional serotonin reuptake mechanism (Heils et al., 1996; Lesch et al., 1996).

The genotype in which both copies of the gene are short (*s/s*) has been associated with heroin dependence, particularly in violent heroin-dependent users, among Caucasian Italians. This finding is consistent with a hypothesis linking the *5-HTTLPR s/s* genotype to a general behavioral disorder characterized by aggressiveness, impulsiveness, and vulnerability to addiction (Gerra et al., 2004). However, a study in Chinese subjects did not find an association between *5-HTTLPR* variation and heroin dependence (Li et al., 2002).

Studies have suggested a possible role for serotonin transmission in susceptibility to nicotine dependence with the *5-HTTLPR s/s* genotype being associated with personality traits (e.g., neuroticism) typical of smoking behavior (Hu et al., 2000; 1996; Lerman et al., 2000; Lesch et al., 1996). However, not all studies agree. In adolescents, the *s/s* genotype frequency was significantly higher among smokers compared with nonsmokers, and among heavy smokers who started smoking early compared with moderate smokers who started smoking later (Gerra et al., 2005). Other studies found that individuals with the *s/s* genotype were less inclined to smoke (Ishikawa et al., 1999) or found no association between *5-HTTLPR* and smoking (Lerman et al., 1998; Sieminska et al., 2008; Trummer et al., 2006).

Genome-Wide Association Studies

Whole-genome association techniques have provided some novel insights into genetic influences on drug dependence (Liu et al., 2006). For instance, GWA studies have revealed previously unrecognized influences on the development of nicotine dependence (Bierut et al., 2007). As a result of those studies, variation in nicotinic receptor genes has become a focus of pharmacogenetic research (see Figure 4).

This research has drawn attention to region 15q25 (region 25 of the long arm of chromosome 15), which includes a cluster of genes that encode subunits of the nicotinic acetylcholine receptor: *CHRNA3* (encodes the α3 subunit), *CHRNA5* (encodes the α5 subunit), and *CHRNB4* (encodes the β4 subunit). Some GWA studies have found a direct association between 15q25 variation and lung cancer risk that may be independent of smoking behavior or nicotine addiction (Amos et al., 2008; Hung et al., 2008), while others have associated 15q25 variation with smoking quantity, nicotine dependence, and lung cancer risk (Thorgeirsson et al., 2008). This gene cluster has also been associated with risk for chronic obstructive pulmonary disease (Pillai et al., 2009).

The GWA findings are in accord with candidate gene studies that have linked variation in *CHRNA3* and *CHRNA5* with the number of cigarettes smoked per day (Berrettini et al., 2008) and increased absorption of nicotine and tobacco toxins (e.g., nitrosamines) per cigarette (Le Marchand et al., 2008). Candidate gene studies have also associated variation in *CHRNA5* with risk of developing nicotine dependence once a person begins to smoke cigarettes (Saccone et al., 2007) and with experiencing a pleasurable rush or buzz during the initial phases of smoking (Sherva et al., 2008). These findings suggest that variation in nicotinic receptor subunit genes may be implicated in smoking behaviors, nicotine dependence, and subsequent tobacco-related illnesses, such as lung cancer and chronic obstructive pulmonary disease.

The cost-effectiveness of GWA studies has been questioned because some of the gene-phenotype relationships they reveal do not appear to be very strong, while others would be logical targets for exploration using less expensive candidate gene studies. For example, nicotinic acetylcholine receptors are the primary drug targets for nicotine in the brain, and thus their biological relevance makes them promising subjects for candidate gene studies independent of the GWA results.

However, other GWA studies have identified genetic variants that were not obvious targets for candidate gene studies and may contribute to addiction vulnerability through previously unsuspected mechanisms, including cell adhesion; protein translation, trafficking, and degradation; transcriptional regulation; transport processes; and cell structures. Once discovered by GWA, these genes become high-priority subjects for candidate studies and biochemical pathway analyses.

To be accepted, the use of pharmacogenetic testing to guide treatment must demonstrate improved clinical outcomes.

FIGURE 4. Nicotinic Acetylcholine Receptor
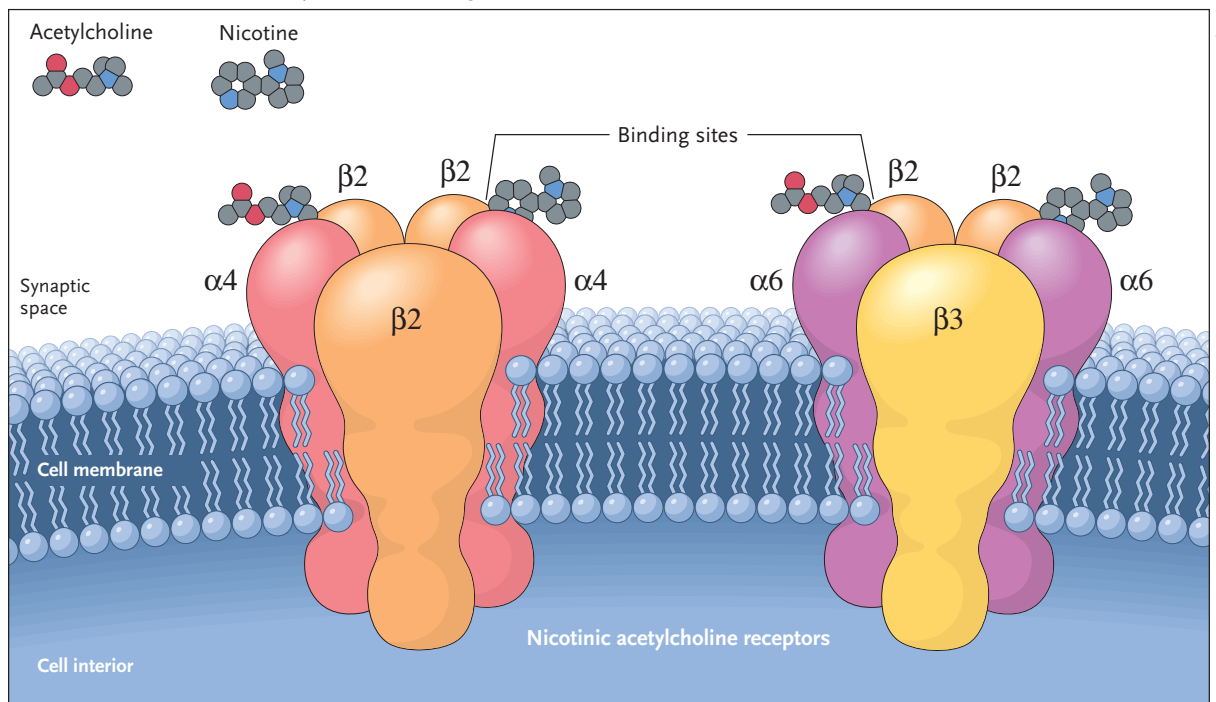
Nicotine initiates its effects by activating nicotinic acetylcholine receptors (nAChRs). The normal function of these receptors is to respond to the neurotransmitter acetylcholine, but the nicotine molecule binds to the same sites that acetylcholine does and also stimulates the receptors.

When nicotine attaches to an nAChR, its impact depends on the combination of subunits making up the receptor. Each nAChR consists of five subunits, drawn from a set of twelve types, designated alpha 2 to 10 and beta 2 to 4. Some types are more responsive to nicotine than others. Genetic variation in the subunits also can affect their sensitivity to nicotine and thereby alter vulnerability to smoking and many aspects of the smoking experience and behavior.

PHARMACOGENETICS OF SUBSTANCE ABUSE TREATMENT

The enormous personal and societal costs of substance use and abuse (Rehm et al., 2006) dictate a need for effective interventions. Two strategies for achieving improved treatment outcomes are to optimize pharmacotherapies and to personalize treatment options (Rutter, 2006). Here we review a selection of studies that have linked genetic variation to treatment response and hence may advance progress toward these two goals.

As mentioned above, *CYP2A6* genetic variation has been associated with smoking dependence and behavior, suggesting that it might also affect response to nicotine replacement therapy. Researchers investigated this hypothesis in a study with Caucasian smokers who were given standard, 8-week courses of the nicotine patch or spray (Malaiyandi et al., 2006). The results confirmed that *CYP2A6* genotype influences smoking behavior, but the impact on quitting could not be determined due to the small sample size. However, slow CYP2A6 activity, as measured by blood levels of nicotine metabolites,

was associated with higher plasma nicotine levels and substantially greater quitting success with the nicotine patch in multiple studies (Lerman et al., 2006a; Schnoll et al., 2009). In contrast, slow metabolizers had equal quit rates relative to normal metabolizers in the group that used the nicotine spray. Nicotine spray, like cigarette smoking, allows titration for differences in nicotine need and rates of metabolism. Recently we have also shown, using either the *CYP2A6* genotype or the nicotine metabolite phenotype measure, that slow metabolizers respond better to extending the duration of nicotine patch treatment (Lerman et al., 2010).

In a study comparing placebo with bupropion (Zyban), slow CYP2A6 metabolizers achieved superior quit rates during treatment with placebo compared with fast metabolizers (Patterson et al., 2008). This finding is consistent with a role for CYP2A6 in smoking behaviors—such as amount smoked and smoking duration—that can alter smoking cessation outcomes. In addition, when bupropion was compared with placebo, only fast CYP2A6 metabolizers received any additional

benefit (Patterson et al., 2008). Together, these data suggest that CYP2A6 slow metabolizers have superior quit rates even in the absence of active drug, and this effect is enhanced by the nicotine patch. In contrast, CYP2A6 fast metabolizers do poorly in the absence of pharmacotherapy and respond relatively well to bupropion.

The cytochrome P450 enzyme CYP2B6 is responsible for metabolizing bupropion to hydroxybupropion (Faucette et al., 2000). The *CYP2B6* gene sequence is variable, and some variants result in altered CYP2B6 activity (Hesse et al., 2004; Kirchheiner et al., 2003). For example, the *CYP2B6*6* variant (G516T and A785G), which is found in 45 percent of Caucasians, 50 percent of African-Americans, and 25 percent of Asians, results in decreased bupropion metabolism (Hesse et al., 2004). In a clinical trial of bupropion versus placebo (Lee et al., 2007), smokers with one or two *CYP2B6*6* alleles achieved significantly higher abstinence rates with bupropion than with placebo. In contrast, smokers with two copies of the more common *CYP2B6*1* allele showed no difference in abstinence between bupropion and placebo treatment. This study, if replicated, would suggest that smokers with the *CYP2B6*6* variant should be treated with bupropion, but smokers with the *CYP2B6*1/*1* genotype are unlikely to benefit from this medication (Lee et al., 2007).

Variation in the genes that encode nicotinic receptors also alters smoking behaviors and smoking cessation rates. In one study, a SNP (rs2072661) in the 3' untranslated region of the *CHRNB2* gene, which encodes the β2 subunit of the nicotinic receptor, affected abstinence rates at the end of smoking cessation treatment; individuals with the less common allele also had substantially decreased odds of being abstinent at the 6-month followup (Conti et al., 2008). Furthermore, this SNP was associated with reduced withdrawal symptoms at the target quit date and increased the time to relapse. Overall, while these results provide strong evidence for *CHRNB2* in the ability to quit smoking, they require replication in an independent sample.

The dopaminergic system has also been implicated in the response to therapeutic interventions for drug dependence. For instance, just as the *TaqI A* variant of the *DRD2* gene has been associated with heroin dependence, it has also been associated with poor methadone treatment outcomes (Lawford et al., 2000). Additionally, smokers with the InsC genotype of the *DRD2* promoter region polymorphism at -141C responded more favorably to smoking cessation treatment with bupropion,

but less favorably to nicotine replacement therapy with the patch or spray (Lerman et al., 2006b). Furthermore, smokers with two copies of a *DRD2* SNP (957C>T) responded better to nicotine replacement therapy than smokers with one or no copies of the variant.

Beta-endorphin is released upon acute and short-term nicotine administration and exhibits rewarding effects. The common *OPRM1* A118G variant was thought to alter the receptor's binding affinity for beta-endorphin, but it may play a larger role in altering messenger RNA (mRNA; see Figure 1) and OPRM1 receptor levels (Bond et al., 1998; Zhang et al., 2005). Smokers with this variant were more likely to be abstinent at the end of 8 weeks of nicotine replacement therapy, with more pronounced effects in those receiving the patch versus the spray, compared with smokers homozygous for the most common *OPRM1* allele (Lerman et al., 2004).

The *OPRM1* A118G variant may also predict naltrexone response for the treatment of alcoholism. In placebo-controlled clinical trials, individuals with this SNP were more responsive to naltrexone treatment (Anton et al., 2008; Oroszi et al., 2009), took a longer time to relapse to drinking, and relapsed at lower rates (Oslin et al., 2003; Kim, et al., 2009) compared with individuals without the variant. However, the association between *OPRM1* A118G and response to treatment was not replicated in other clinical trials with naltrexone (Gelernter et al., 2007; Mitchell et al., 2007) or nalmefene (Arias et al., 2008).

The progression from pharmacogenetic discovery to better substance abuse treatment may be shortened if researchers develop and use phenotype measures (e.g., amount smoked, ability to stop for a short time, motivation to stop, treatment seeking) that are informative both for pharmacogenetic studies and in the screening of human medication development (Perkins et al., 2008). Such an effort should also address the need for uniform phenotype measures that will facilitate comparison and replication of pharmacogenetic findings. Researchers' use of broad or inconsistent phenotype definitions is a major reason why contradictory conclusions about genetic effects on phenotypes—such as many noted above—are common in the pharmacogenetic literature (Szatmari et al., 2007).

PHARMACOGENETICS IN THE CLINIC
The Food and Drug Administration (FDA) recognizes the utility of pharmacogenetics in drug development

and patient care, provides information for understanding the role of such discoveries in regulatory judgments, and has approved the inclusion of pharmacogenetic test information in the labeling of selected drugs (Food and Drug Administration, 2008; Frueh et al., 2008; Shin et al., 2009). However, challenges will need to be met before the full potential of pharmacogenetic discoveries to advance clinical practice can be realized.

Demonstrating the clinical validity and utility of pharmacogenetic testing is among the greatest hurdles facing the widespread application of pharmacogenetics in clinical practice. To be accepted, the use of pharmacogenetic testing to guide treatment must demonstrably improve clinical outcomes (Hunter et al., 2008). However, as this article has highlighted, attempts to replicate studies that have shown benefit to such testing have often failed. The question arises: What degree of clinical benefit is sufficiently robust to warrant clinical implementation?

Once the clinical benefits and risks of pharmacogenetic optimization of a treatment are clearly defined, the question of cost-effectiveness may still remain (Heitjan et al., 2008). Complicated ethical and privacy issues raised by the use of pharmacogenetic tests are the focus of other reviews (Marx-Stolting, 2007; Shields and Lerman, 2008; van Delden et al., 2004).

If pharmacogenetic testing is to become widely accepted as a clinical diagnostic tool, who should be carrying out the tests? Currently, testing is done by using FDA-approved *in vitro* diagnostic devices and kits sold by manufacturers or, more commonly, by clinical laboratories that are not FDA-approved (Shin et al., 2009). With such a variety of testing options, strict monitoring systems are needed to guarantee reliable results (Hunter et al., 2008).

The limited availability and cost of pharmacogenetic testing are additional challenges (Tucker, 2008). Most insurance plans will reimburse the cost of pharmacogenetic testing only if it is required by the FDA, medically necessary, or has proven clinical utility (Shin et al., 2009).

Studies have shown that pharmacogenetic variation can significantly alter susceptibility to, and response to treatment for, drug dependence. It is important that we evaluate current approaches and address concerns appropriately in order to optimize the management of drug dependence through the use of pharmacogenetics.

CONCLUSIONS

We have highlighted several pharmacogenetic findings that contribute to the understanding of substance dependence and to the variation in responses to substance abuse treatment. Clearly, environmental factors also play a role, and future studies of pharmacogenetics will be improved if they are large enough to investigate both gene-gene and gene-environment interactions. We hope that a better understanding of the role of genetic factors will contribute to the optimal use of current therapies and the development of novel and potentially more effective therapeutic strategies.

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