Objective: The completion of the human genome sequence has spurred investigation of the genetic contribution to substance dependence. In this article some of the recent scientific evidence for genetic determinants of opioid and cocaine dependence is reviewed. Method: An electronic search of the medical literature was conducted to locate published studies relevant to the genetics of opioid and cocaine dependence. The collected information judged to be most pertinent is described and discussed. Results: Genetic epidemiologic studies support a high degree of heritable vulnerability for both opioid and cocaine dependence. Polymorphisms in the genes coding for dopamine receptors and transporter, opioid receptors, endogenous opioid peptides, cannabinoid receptors, and serotonin receptors and transporter all appear to be associated with the phenotypic expression of this vulnerability once opioids or cocaine are consumed. Conclusions: Despite this initial progress, identification of specific genes and quantification of associated risk for the expression of each gene remain to be elucidated. While alteration of an individual's genome to change the phenotype seems remote, future interventions for treatment of opioid and cocaine dependence may include precise medications targeted to block the effects of proteins that have been identified through genetic research. (HARV REV PSYCHIATRY 2005;13:218–232.)

Keywords: genomics, polymorphism, substance-related disorders

As in other areas of medicine, recent years have witnessed rapidly emerging evidence for the importance of genetic explanatory models in the etiology of addictive disorders. Numerous genetic epidemiological studies demonstrate conclusively that heritability plays a major role in the development of these disorders. Genetic studies conducted thus far have begun to suggest the contribution of specific genes to this heritability.

An electronic search of the medical literature was conducted to locate published studies relevant to the genetic determinants of opioid and cocaine dependence. This review covers the genetic epidemiology of opioid and cocaine dependence, summarizes biological aspects of addiction essential to understanding potential mechanisms of genetic contributions, provides a synopsis of current knowledge concerning potential genetic markers for opioid and cocaine dependence, and integrates these current findings in the context of their meaning for the genetics of addiction and the clinical management of patients. The review focuses on the genetics of opioid and cocaine dependence because the genetics of alcohol dependence and nicotine dependence have recently been reviewed elsewhere.1–3
At the outset, a few general points will underscore the complexity of the topic of addiction genetics. First, defining phenotypes in addiction, as in most other psychiatric disorders, poses a challenge. Contrary to the situation with many medical disorders—for which a tissue, blood, or radiological examination confirms the diagnosis and phenotype—no specific, objective test currently exists that definitively confirms the addiction phenotype. For the past decade the seven physiologic and behavioral criteria set forth in the Diagnostic and Statistical Manual for Mental Disorders have served as the most widely accepted approach to defining a diagnosis or phenotype for substance dependence. Generally, the presence of the diagnosis can be established with good reliability by administration of a structured clinical interview. The precise type of structured interview may vary across studies.

Second, addiction represents a disease state that cannot be explained purely on the basis of genetics. Environmental exposure to the addictive substance (and possibly other environmental insults) must occur to create this disease state. Thus, the disease necessarily develops as a consequence of gene-environment interactions.

Third, data accrued thus far indicate that the genetic propensity to addiction, as with other complex disorders, most likely arises from the combined influence of several genes rather than from a single gene. Thus, genetic models of addiction, while evolving, remain far from complete.

Fourth, while separate consideration of the genetics of opioid and cocaine dependence makes organizational sense and may have some theoretic underpinnings, in the real world most individuals with addiction problems suffer from dependence on more than one substance. It will become readily apparent that substantial overlap exists for potential genetic causes of addiction to both of these substances.

Finally, considerable previous work has looked at the genetic aspects of addiction in laboratory animals, but most of the information presented here concerns humans. Animal studies are noted here only to the extent that they inform efforts at grappling with the conundrums posed by human addiction genetics.

GENETIC EPIDEMIOLOGY OF ADDICTION

Broadly defined, genetic epidemiology is a discipline that focuses on the role of genetic factors and their interactions with environment in the occurrence of disease in the human population. It uses several population- and family-based approaches. Commonly, the degree to which a genetic contribution exerts control over the trait/disease of interest is described as heritability. Although heritability is calculated differently with different models (e.g., family studies vs. twin studies; see below), it can best be understood as the proportion of variation directly attributable to genetic differences among individuals, relative to the total variation among the individuals in a population.

Genetic epidemiological methods applied to addiction include family studies, adoption studies, and twin studies—which have largely reached similar conclusions in regard to the heritability of addiction. Family studies recruit probands with the phenotype of a specified substance use disorder. An attempt is then made to determine the phenotypes of blood relatives. The rate of the disorder in the blood relatives of affected probands may be compared either to the rate of the disorder in blood relatives of a specifically recruited control sample whose members do not have the disorder or to the known rate of the disorder in the general population. If blood relatives of the affected probands have an elevated rate of the disorder, it suggests that a genetic factor, a familial-specific environmental factor, or both contribute etiologically to the disorder. For example, one study evaluated 231 probands dependent upon alcohol, opioids, cocaine, or cannabis and 61 control subjects, along with 1,267 adult first-degree relatives of both groups. Whereas the rate of drug dependence among relatives of controls was 3.5%, the rate was 20.5% among relatives of opioid-dependent probands, 14.9% among relatives of cocaine-dependent probands, and 21.3% among relatives of cannabis-dependent probands. Overall, this study indicated an eightfold increased risk of addiction among relatives of probands with addiction and also suggested that the predominant substance of dependence was aggregated within families. While this study demonstrates that addiction runs in families, the obvious limitation is that such a study cannot sort out the degree to which familial transmission springs from genes versus shared environment.

The above limitation can be addressed, in part, by adoption studies, which involve longitudinal follow-up of adoptees whose biological parents either had or did not have a substance use disorder. This methodology attempts to eliminate the confound of shared environmental effects on a child growing up in a household with an addicted parent. If the adoptees with a biological parent positive for the disorder develop the disorder at a higher rate than adoptees with a biological parent negative for the disorder, one can reasonably conclude that heritable traits contribute to the development of the disorder. For example, one study that examined drug abuse in adoptees found that those with a biologic first-degree relative with an alcohol problem had four times the rates of drug abuse compared to adoptees without an alcoholic first-degree relative.

Twin studies provide further support for the heritability of substance use disorders. In twin studies, a comparison occurs between monozygotic twins who possess identical genotypes, and dizygotic twins who, on average, share half of their genes, as do any other siblings. Thus, the extent
to which the concordance rate for a disorder in monozygotic twins exceeds the rate in dizygotic twins affords an estimate of heritability.\textsuperscript{15,16} For example, in Kendler and colleagues’ study\textsuperscript{17} of 1,198 male twin pairs, the concordance rate for cocaine dependence among monozygotic twins was 0.41, whereas the concordance rate for dizygotic twins was only 0.13. These results suggest that 79% of the variance for liability to cocaine dependence is attributable to additive genetic factors. Shared familial environment did not contribute to the variance, and unique, individual-specific environmental factors and measurement error accounted for 21% of the variance. The data from this sample, where six different classes of illicit substances were examined, were further analyzed to determine whether risk factors for substance use disorders are substance-specific or nonspecific.\textsuperscript{18} The results indicated that the vulnerability to substance use disorders is largely or entirely nonspecific—meaning that the same genetic and shared environmental factors account for dependence on any of these substances—though a twin study by Tsuang and colleagues\textsuperscript{16} has suggested that the heritability of heroin dependence (0.38) is more specific than the heritability of some other substances. Overall, the number of opioid-dependent individuals in published twin studies is small, but at least one study suggested that the concordance rate for this disorder, as in the case of cocaine dependence, is higher in monozygotic than in dizygotic twins.\textsuperscript{19}

**BIOLOGIC ASPECTS OF ADDICTION**

Since epidemiologic studies demonstrate that a substantial proportion of the risk for substance use disorders is heritable, it follows that genes must be affecting some biologic characteristics of the organism to produce this risk. These characteristics would likely involve either the way that the body handles or metabolizes a specific substance (pharmacokinetics) or the way that the body responds to the substance (pharmacodynamics). These events could occur in the central nervous system or in the periphery. Metabolic events commonly occur in both places. The important response events that lead to addiction occur mainly in the brain.

The development of substance dependence necessarily requires frequent self-administration of a foreign substance. Many individuals self-administer substances that can cause dependence, yet never progress to excessive use of, or dependence on, that substance. Clearly, the body’s capacity to metabolize the substance in question would have an influence on the likelihood of becoming dependent. If the body metabolizes the substance quickly, the individual might either have no interest in the substance because she or he feels little effect, or conversely, use large quantities of the substance in an effort to build upon the small effect experienced. If the body metabolizes the substance slowly, small quantities administered will exert large effects. In this case the individual might either experience a preponderance of toxic effects and dislike the substance, or appreciate the positive effects he or she receives and continue to use the substance in order to repeat that experience.

The cytochrome P450 system plays a key role in the metabolism of opioids such as buprenorphine, codeine, methadone, and oxycodone. The metabolism of substrates that are processed by this system shows high interindividual variability that is at least in part related to genetic polymorphisms within the cytochrome P450 system.\textsuperscript{20} The opioids heroin and morphine are metabolized via enzyme systems distinct from the P450 system—some of which also display potentially significant polymorphisms.\textsuperscript{24} The relevance of the genetics of each of these enzyme systems to the phenotype of substance dependence will be discussed in more detail below, in the sections relevant to the genetics of opioid and cocaine dependence.

More than half of all human genes are expressed in the central nervous system, but scientific inquiry about genes and the brain has thus far been directed at less than 1% of the genome.\textsuperscript{4} In regard to substance dependence, most genetic investigation has targeted brain processes known to contribute to dependence, which may vary somewhat by substance, although there is substantial overlap.\textsuperscript{23}

The primary neurobiologic system that appears to mediate substance dependence is the brain reward pathway.\textsuperscript{26,27} This system relies primarily on the neurotransmitter dopamine.\textsuperscript{26,27} The cell bodies that contain the dopamine reside in an area in the midbrain called the ventral tegmental area, and project anteriorly to the nucleus accumbens and also to the prefrontal cortex.\textsuperscript{28} Dopamine release in these areas results in reward or reinforcement, and current theory suggests that all substances of dependence directly or indirectly cause dopamine release in this system.\textsuperscript{26,29} In normal physiology, brain reward pathways serve an important evolutionary function. These pathways are stimulated when the organism performs an activity conducive to survival—including eating when hungry; ingesting fluids when thirsty; sex; nest building; care of young; and so on. When substances of dependence are used repeatedly and stimulate this pathway by directly or indirectly increasing the effects of dopamine, the organism mistakenly behaves as if use of the substance will enhance survival, and thus continues to use it.\textsuperscript{30} There are at least five different subtypes of dopamine receptors to which dopamine can bind, enabling its signal to be transmitted to the receiving neuron after it has been released into the synaptic.\textsuperscript{31} Each of these receptors is a distinct protein (although the amino acid sequences are similar) coded by a distinct gene.\textsuperscript{32} Neurons that release dopamine into the synapse also have a dopamine
transporter—another distinct protein coded by a distinct gene—that can take dopamine back out of the synapse and terminate the transmission. Since the dopamine system exhibits such critical involvement in substance dependence, considerable interest has developed in the possible relation of dependence to variations in the genes coding for the dopamine receptors and transporter. Indeed, experimental evidence suggests that variability in the length of the untranslated portion of the dopamine transporter gene may affect its expression in brain.

Several other neurotransmitter systems, each with its own set of specific proteins, also impinge upon and help to regulate the dopamine system. Each of these systems could be involved in the genetics of substance dependence. Important among these are brain inhibitory and excitatory systems based upon the neurotransmitters γ-aminobutyric acid (GABA) and glutamate (or its analogues), respectively. Both of these systems are complex in that their receptors comprise several different protein subunits. Each of these is coded by a separate gene, and each also has a variety of transporters—all distinct proteins—that can remove it from the synapse. When GABA binds to a receptor, it tends to hyperpolarize or inhibit the neuron that contains the receptor, whereas glutamate binds to a receptor, it tends to depolarize or excite the neuron that contains the receptor. Thus, in most instances, GABA binding would inhibit dopamine release, whereas glutamate binding would promote it.

Another important brain system is the endogenous opioid system, which directly mediates the effects of endogenous opioid neuropeptides and of exogenously administered opioids. There are three subtypes of opioid receptors and several different types of endogenous opioid peptides that serve as neurotransmitters and bind to these receptors. A different gene codes for each subtype of opioid receptor and for the specific proteins from which endogenous opioid peptides are cleaved. Abused opioids bind primarily to the μ-opioid receptor. When an exogenously administered opioid or endogenous opioid peptide binds to an opioid receptor, it (like GABA) tends to hyperpolarize or inhibit the neuron that contains the receptor. Since GABA-releasing neurons inhibit dopamine release, when opioid receptors on these GABA neurons are activated by the binding of an opioid, the GABA inhibition of dopamine neurons is reduced. Thus opioids acting via opioid receptors and GABA neurons indirectly induce dopamine release in brain reward pathways. Opioids also suppress pain—an action that may contribute to their potential to cause dependence.

The brain contains both an endogenous cannabinoid system and cannabinoid receptors that serve as the mechanism through which marijuana exerts its effects. There are two types of cannabinoid receptors: CB1, expressed in the central and peripheral nervous systems; and CB2, expressed predominantly in the immune system. Three endogenous cannabinoids—ethanol amide molecules, not proteins—that act as the neurotransmitters for this system have been discovered thus far. A separate gene codes for each type of cannabinoid receptor. Since CB1 resides in many brain areas, the effects of stimulating this receptor are complex—and not fully understood. It is believed, however, that the cannabinoid system modulates the effects of several other neurotransmitters, including GABA, glutamate, opioids, and monoamines such as dopamine and serotonin (see below). Marijuana causes dopamine release in brain reward pathways via an as yet undetermined mechanism. Like opioids, cannabinoids and marijuana can modulate pain. The effects of other substances of dependence may also be mediated, in part, through indirect activation of cannabinoid receptors.

Serotonin (5-hydroxytryptamine), a monoamine neurotransmitter, has effects on mood, sleep, appetite, impulse control, and cognition. Serotonin-containing neurons in the brain exist in a midbrain area called the raphe nuclei and send fiber tracts to numerous other areas in the central nervous system. There are at least fifteen subtypes of serotonin receptors, which are related proteins all coded by different genes. As in other neurotransmitter systems, neurons that release serotonin into the synapse also have a serotonin transporter—again a distinct protein coded by a distinct gene—that can remove serotonin from the synapse and terminate the transmission. These genes have polymorphisms, some of which are thought to be functional. Cocaine also blocks the serotonin transporter and undoubtedly exerts some of its dependence-inducing effects via this mechanism. Stimulation of a specific subtype of serotonin receptor, known as the 5-HT3 receptor, causes rapid release of dopamine in brain reward pathways.

GENETIC DETERMINANTS OF ADDICTION

Before going into detail about genetic studies of cocaine and opiate addiction, some reflections on several aspects of the genetics of complex diseases will help to explain the challenges facing the field. In human genetics a complex trait is a genetic condition for which the mode of inheritance does not follow established Mendelian laws, by which a single gene codes for a single, specific trait. Complex behavioral phenotypes have emerging properties; that is, the properties of the parts (genes, neural systems, and environment) do not fully predict the properties of the whole (the phenotype under study). This situation presents a challenge for a reductionist scientific paradigm based on an understanding of component elements. Finally, for complex traits, just how
to define a phenotype may be somewhat arbitrary; although DSM-IV has defined clinical syndromes with high inter-rater reliability, questions remain regarding the extent to which genes act to produce DSM-IV-defined phenotypes.

In order to address such questions, the concept of endophenotype was initially formulated to denote biological attributes such as enzyme activity, neurotransmitter plasma levels, or other characteristics that, while related to the phenotype of interest, may represent more proximal functions of genes. This concept was subsequently broadened to encompass a wider range of biological and psychological processes that may represent an intermediate state between causative mechanisms, such as genes, and behavioral phenotypes. For example, in the Collaborative Study on the Genetics of Alcoholism, electrophysiological phenotypes, EEGs, and evoked response potentials have been used to conduct genetic-linkage analysis in families with a high density of alcohol dependence.

In order to identify genetic determinants of a phenotype under study, molecular geneticists have utilized two main approaches: genetic linkage and association studies. Historically, these approaches have roots in experimental biology and epidemiology, respectively. Linkage is a genetic biological concept based on the principle that if the phenotype under study and a random genetic marker (locus) are inherited together in a family, the most obvious biological explanation is that they are physically “close”—that is, they are “linked” on the gene. Demonstrating linkage therefore represents the highest level of statistical proof that the studied phenotype is related to a genetic mechanism. Rather than being dependent on knowing the biology of the disease, linkage studies commonly query the whole genome. They have been successful in identifying the loci, and subsequently specific genes, that are responsible for the diseases that exhibit clear, Mendelian modes of inheritance. Such studies require access to multigenerational families, however, and can be compromised, in part, by incomplete penetrance and high levels of genetic heterogeneity, among other factors. As noted earlier, addiction represents a genetically complex phenotype not ideally suited to linkage analysis, and so, to date, there have been no published linkage studies of opioid or cocaine addiction.

In contrast to linkage, genetic association is a statistical concept and is a property of a specific allele (polymorphism) at a given locus. In essence, association studies compare the allele frequency of cases and controls under the assumption that the only reason for the observed difference in the allele frequency is a consequence or correlate of the phenotype. One advantage of association studies is that the mode of inheritance need not be specified, and another is that, with sufficient sample size, relatively small genetic effects and gene-environment interactions can be identified. Association studies can also assess the effects of candidate genes, and more recently—as a result of technological and statistical advances—whole genome screening has become feasible; for example, Uhl and colleagues have used a whole genome case-control association scan to identify multiple loci contributing to polysubstance abuse vulnerability. In addition to being used in traditional epidemiologic case-control designs, association studies can be adapted to family-based designs. The advantage of family-based studies is that they use unaffected family members as controls and thus account for population stratification such as different ethnic and racial backgrounds. Given these advantages, it is not surprising that association studies have been the design of choice for the genetics of addiction research.

GENETIC DETERMINANTS OF OPIOID DEPENDENCE

As mentioned above, different opioid drugs have different metabolic routes. Heroin (diacetyl morphine) is first transformed to monoacetyl morphine by acetycholinesterase, butyrylcholinesterase, and carboxylesterase. Monoacetyl morphine is metabolized to morphine by human erythrocyte acetycholinesterase. Morphine undergoes transformation to glucuronide metabolites via the enzyme uridine diphosphate glucuronosyltransferase 2B7 and other glucuronosyltransferases, some of which are expressed in the brain. The semisynthetic opioids oxymorphone and hydromorphone are also glucuronidated by these enzyme systems. Although no studies have been done regarding the genetics of these systems in relation to dependence on heroin or morphine, some of them exhibit polymorphisms that appear to have the capacity to alter morphine pharmacokinetics in some individuals. Thus, they would make good candidate genes to evaluate for opioid dependence.

Codeine, oxycodone, and hydrocodone serve as prodrugs and undergo transformation to the active drugs morphine, oxymorphone, and hydromorphone, respectively, via cytochrome P450 2D6. When 2D6 polymorphisms were examined among individuals with oral opioid dependence, none of these individuals had genotypes that result in a “poor metabolizer” phenotype. This finding suggests that having a “poor metabolizer” genotype provides protection against dependence on these specific opioids.

The semisynthetic opioids methadone and buprenorphine are metabolized by cytochrome P450 3A4. The gene that codes cytochrome P450 3A4 also demonstrates numerous polymorphisms, some of which may have functional significance. No studies thus far have examined the relation of these polymorphisms to dependence on methadone or buprenorphine.
Since opioids indirectly facilitate the release of dopamine in brain reward pathways, a few studies have looked at the relation of dopamine receptor genes to opioid dependence. One study assessed, in regard to opioid dependence, the Taq1 A polymorphism in the gene that codes for the dopamine D2 receptor and found not only an association, but also a relation between homozygosity for the A1 allele and the amount of heroin used. In a different study the Taq1 B polymorphism of the D2 receptor gene showed an association with heroin dependence in a Chinese, but not a German, sample. Two studies suggest an association between the dopamine D4 exon III seven-repeat allele and opioid dependence, particularly in combination with the personality trait of sensation seeking. Also, in a study with a Chinese sample, the dopamine D3 receptor Bal 1 polymorphism showed an association with opioid dependence and sensation seeking. Variable nucleotide tandem repeat (VNTR) polymorphisms of both the dopamine and serotonin transporters were examined in a sample of male Russians and Tatars. Homozygosity for specific variants of VNTR polymorphism in both of these genes demonstrated an association with opioid dependence. The genetics of one enzyme involved in dopamine catabolism, catechol-O-methyltransferase (COMT), have also been investigated in relation to opioid dependence. Humans display a variation in enzyme activity level based on a single nucleotide polymorphism that results in the substitution of a valine for a methionine in exon 4 of the gene coding for the enzyme. Individuals with the methionine substitution have a three- to fourfold decrease in COMT activity level. Although the dopamine transporter removes the vast majority of dopamine from the extracellular space, additional differences in the catabolism of dopamine based on COMT activity could conceivably affect dopamine levels and hence dopaminergic function. A family association study using 38 opioid-dependent individuals and their parents showed a significantly higher rate of the val allele in the opioid-dependent probands compared to their nondependent parents. No association was found, however, between the val allele and opioid dependence when a separate group of opioid-dependent individuals was compared to an unrelated, non-opioid-dependent control group.

Opioid receptor genes have, of course, been examined in relation to opioid dependence. The gene coding for the mu-opioid receptor has numerous polymorphisms, the most common and most studied of which is A118G. One study found that the receptors created by this polymorphism (a single amino acid alteration) had much higher binding affinity for beta-endorphin, an endogenous ligand for this receptor. Several studies have looked at the relation of this and other mu-opioid receptor gene polymorphisms to opioid dependence and found either no association or that these polymorphisms represent a general risk factor for substance dependence not necessarily specific to opioid dependence. One study concluded that a single nucleotide polymorphism (-2044C/A, near the gene) represented a risk factor for combined opioid and alcohol dependence. A polymorphism in the delta-opioid receptor gene had an association with opioid dependence in one study of a German sample but not in another.

In a study investigating a polymorphism in the gene coding for proenkephalin—the precursor peptide to the enkephalins, important endogenous opioid peptides—the CA(n) repeat polymorphism demonstrated a positive association with opioid dependence.

A single study that looked specifically at a polymorphism in the cannabinoid receptor (CB1) gene among a Chinese population found no association with heroin dependence.

The genetics of GABA, glutamate, and serotonin receptors await exploration in regard to human opioid dependence.

**GENETIC DETERMINANTS OF COCAINE DEPENDENCE**

Cocaine has one major and two minor metabolic pathways. The major route involves transformation to benzoylecgonine mediated by hepatic microsomal carboxylesterase. It can also be metabolized by the plasma enzyme butyrylcholinesterase to ecgonine methyl ester and to nor cocaine by cytochrome P450 3A. It is certainly of interest that some of the enzymes involved in opioid metabolism are also involved in cocaine metabolism, and that, as described above, these enzymes have apparent functional polymorphisms. Nevertheless, the possible genetic contribution of polymorphisms among these enzyme systems to the development of cocaine dependence has not yet been assessed.

Cocaine directly blocks dopamine and serotonin transporters, thereby preventing the reuptake of these neurotransmitters back into the releasing neuron. An initial study suggested that, as with other forms of substance use, the Taq1 A and B polymorphisms in the gene that codes for the dopamine D2 receptor showed an association with heavy stimulant use. Two subsequent studies looked specifically at these polymorphisms in cocaine-dependent individuals. One confirmed the findings in a purely Caucasian sample, whereas the other, using a racially stratified sample, failed to demonstrate any association. The former study also delineated an additive association with cocaine dependence of a polymorphism in the D3 dopamine receptor gene and the polymorphism in the D2 receptor gene, despite an earlier claim that polymorphisms in the D3 gene did not affect the likelihood of cocaine dependence. A study of a polymorphism in the dopamine-metabolizing enzyme dopamine beta-hydroxylase indicates an association of specific alleles
with both low dopamine β-hydroxylase plasma activity and cocaine-induced paranoia in cocaine users.102 Two studies that examined serotonin-related genes have found no association between cocaine dependence and polymorphisms in either the 5 HT1B gene103 or in the serotonin transporter gene.104

An attempt to find an association between cocaine dependence and polymorphisms in the µ-opioid receptor gene failed to find any association.91 A single study looked at the gene coding for prodynorphin, the precursor to another opioid peptide, and found that an allelic variation that results in enhanced transcription of the gene may protect against cocaine dependence.105

Two studies have investigated the relation of the CB1 receptor gene to cocaine dependence. One found an association with CB1 polymorphisms,106 but the other did not.107 Numerous genes potentially related to the neurobiology of cocaine dependence have yet to be studied.

As elaborated in the above sections, Table 1 summarizes information currently available regarding association studies with genetic polymorphisms that have potential influence on the development of opioid or cocaine dependence.

**DISCUSSION**

The foregoing compilation of findings makes it apparent that the search for specific genes that definitively contribute to the risk for opioid and cocaine dependence remains elusive. Initially positive results seem often to get contradicted rather than confirmed. Given the complex relation between genotype and phenotype, however, this situation is not unexpected. For example, issues of power and statistical significance in association studies are far from being resolved. Freimer and Sabatti108 argue that, for association studies, when proper prior probability of association for any of 30,000 genes in the genome is taken into account, the corresponding p-value to establish significant findings would have to be as low as $< 2.6 \times 10^{-7}$. This contention would suggest that even the most publicized results for any genetic findings related to complex phenotypes are probably false positives. Nevertheless, review of the research performed to date in this field offers intriguing hints. Some evidence supports the notion that the genetics of the dopamine, endogenous opioid, endogenous cannabinoid, and serotonin systems could have a relationship to the development of opioid or cocaine dependence. The potential explanations for contradictions in prior research also point the way toward design of future studies that might obtain more decisive outcomes.

Future investigations must take into account that, in many instances, the contribution of a single gene to the risk for substance dependence is small. In order to overcome this problem, more definitive studies will require relatively large sample sizes or the application of methods that can detect small genetic effects. In addition, findings can vary considerably among different racial and ethnic groups, and between genders. Future research must either confine itself to a single ethnic group and gender, or perform separate analyses for different subsets of subjects. Another approach is to use a “genomic control” method that controls for population structure by comparing random polymorphic loci throughout the genome. The distribution of these loci is then compared for cases and controls, and adjustments can be made for additional stratification effects not taken into account by ethnic and environmental covariates. That approach has been successfully applied in association studies of heroin dependence.77 Since it remains uncertain, however, whether genetic risk for substance dependence is substance specific13,91,109 or generalized,18,85,90 all subjects must be carefully phenotyped for the full range of substance-dependence diagnoses—and, when such diagnoses are discovered, excluded from the control groups. Another problem in selecting subjects derives from the necessity of environmental exposure for the development of opioid and cocaine dependence. Use of case-control designs does not always take into account that not all of the control individuals had the opportunity to use the substances and, as it were, become cases. Finally, the high probability that substance dependence has a polygenic etiology, along with the tantalizing evidence from a few studies that concomitant polymorphisms in two distinct candidate genes may have an additive impact,81,99 supports the strategy of testing subjects for polymorphisms in a multitude of candidate genes. Statistical tools are now being developed that will allow investigators to look explicitly for interactions between loci even in the context of whole-genome studies.110 Including all of these design elements demands considerable time, a huge organizational commitment, and infusion of resources, but the ongoing examples of this kind of integrated undertaking indicate its feasibility.6

The enormous investment required for such endeavors raises the question of what, beyond pure scientific knowledge, might result from them. Although the idea of altering an individual’s genome to change the phenotype currently seems remote, the era of changing phenotypes through targeted pharmacologic manipulation is already upon us, as witnessed by the emergence of the field of pharmacogenomics. The dual concerns of that field are to understand how individual genetic differences cause varied responses to therapeutic regimens, and to develop drug therapies to compensate for individual genetic differences/deficiencies. This concept is not an entirely new one. In fact, the first medication to treat alcohol dependence—disulfiram (Antabuse), which has been available for more than fifty years—does just that. It targets a genetic pathway that is variable in the population to produce a phenotype that is less likely
TABLE 1. Summary of Genetic Association Studies in Opioid and Cocaine Dependence

<table>
<thead>
<tr>
<th>Gene name, symbol, location</th>
<th>Variants studied</th>
<th>Population</th>
<th>Phenotype assessment and group sizes</th>
<th>Results</th>
<th>Study</th>
</tr>
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<tbody>
<tr>
<td>Cytochrome P450 subfamily IID</td>
<td>Wild type vs. poor metabolizer (CYP2D6<em>3 (2549A/del) and CYP2D6</em>4 (1846G/A))</td>
<td>Caucasian</td>
<td>DSM-based interview 83 oral opioid dependent, 93 multi-substance dependent, 276 nondependent controls</td>
<td>Significant difference between poor metabolizer frequency in oral opioid dependent subjects vs. both controls and multi-substance dependent subjects (Fisher's exact ( p = 0.05 ); ( p = 0.02 )); poor metabolizer a protective factor in oral opioid dependent subjects (OR &gt; 7)</td>
<td>Tyndale et al. (1997)</td>
</tr>
<tr>
<td>Dopamine D2 receptor</td>
<td>DRD2 Taq1 A, B, D†</td>
<td>Caucasian</td>
<td>Drug Use Survey Interview 62 stimulant use, 40 opioid use, 47 stimulant + opioid use, 42 polysubstance use</td>
<td>Taq1 A, stimulant users 43.5% vs. controls 27.7% (( \chi^2(1) = 3.92; p = 0.024 ))</td>
<td>Persico et al. (1996)</td>
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<td>11q23</td>
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<td>Dopamine transporter</td>
<td>SLC6A3 (DAT1) 5p15.3</td>
<td>Caucasian</td>
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<tr>
<td>Dopamine D2 receptor</td>
<td>Taq1 A, B, D†</td>
<td>African-American, European-American, Hispanic-American</td>
<td>DSM-based C-DIS-R^5 or SCID^5 Cocaine dependent 77 African-American, 96 European-American, 25 Hispanic-American Controls 45 African-American, 87 European-American</td>
<td>( \chi^2 ) analysis: no association detected</td>
<td>Gelernter et al. (1999)</td>
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<tr>
<td>Dopamine D2 receptor</td>
<td>Taq1 A†</td>
<td>Australian, Caucasian</td>
<td>DSM-based interview and chart review 95 opioid dependent, 50 controls divided into several post hoc control groups</td>
<td>A^+ in 19% of opioid-dependent subjects vs. 4.6% of selected controls (Fisher's exact ( p = 0.009 )) 54 opioid-dependent subjects had successful treatment (A^+, 9%); 19 had poor treatment outcome (A^−, 42%) (Fisher's exact ( p = 0.00002 )) A^+ group heroin consumption 0.55 ± 10 grams/day vs. A^− group, 0.25 ± 5 grams/day (Fisher's exact ( p = 0.003 ))</td>
<td>Lawford et al. (2000)</td>
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<tr>
<td>Dopamine D2 receptor</td>
<td>10 SNPs, including Taq1 A, B, D† and representative haplotypes</td>
<td>Han Chinese and German</td>
<td>DSM-based Chinese 486 heroin dependent, 313 controls German 471 heroin dependent, 192 controls</td>
<td>Taq1 B associated with heroin dependence in Chinese sample (( \chi^2 = 16.94; p = 0.00038 )) Different haplotypes associated with dependence and lower risk for dependence in Chinese and German populations</td>
<td>Xu et al. (2004)</td>
</tr>
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<td>11q23</td>
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<tr>
<td>Dopamine D2 receptor</td>
<td>Msc I/Bal l polymorphism (A/G SNP Ser9Gly)</td>
<td>North American, black and white</td>
<td>62 black and 62 white cocaine dependent Several control groups</td>
<td>No association detected</td>
<td>Franke et al. (1999)</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Gene name, symbol, location</th>
<th>Variants studied</th>
<th>Population</th>
<th>Phenotype assessment and group sizes</th>
<th>Results</th>
<th>Study</th>
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</thead>
<tbody>
<tr>
<td>Dopamine D3 receptor, DRD3 3q13.3</td>
<td>Msc l/Bal l polymorphism (A/G SNP Ser9Gly)</td>
<td>French ancestry</td>
<td>DSM-based DIGS, † † Zuckerman's sensation-seeking scale, Barrat's impulsiveness scale</td>
<td>No association with opioid dependence</td>
<td>Duaux et al. (1998)</td>
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<tr>
<td>Dopamine D3 receptor, DRD3 3q13.3</td>
<td>Msc l/Bal l polymorphism (A/G SNP Ser9Gly)</td>
<td>Caucasian</td>
<td>DIS-III-R, ‡ ‡ ASI § §</td>
<td>Heterozygosity as a risk factor</td>
<td>Comings et al. (1999)</td>
</tr>
<tr>
<td>Dopamine D4 receptor, DRD4 11p15.5</td>
<td>Exon 3 48 BP VNTR</td>
<td>Israeli Arabs</td>
<td>DSM based</td>
<td>7-repeat allele in opioid-dependent subjects vs. controls; 29.1% vs. 11.8% ($\chi^2(1) = 10.9; p = 0.0003; RR = 2.46 (95% CI, 1.38–4.35))</td>
<td>Kotler et al. (1997)</td>
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<tr>
<td>Dopamine D4 receptor, DRD4 11p15.5</td>
<td>Exon 3 48 BP VNTR</td>
<td>Han Chinese</td>
<td>DSM based</td>
<td>Excess of long (5 to 7) repeats in opioid abuse group (Fisher's $p = 0.023; OR = 2.30 (95% CI, 1.07–4.93))</td>
<td>Li et al. (1997)</td>
</tr>
<tr>
<td>Dopamine β-hydroxylase, DBH 9q34</td>
<td>Low DβH activity haplotype consisting of 5′ 19 BP deletion and intronic SNP 444G/A</td>
<td>European-Americans</td>
<td>DSM based and Cocaine Experience Questionnaire</td>
<td>No association with cocaine dependence</td>
<td>Cubells et al. (2000)</td>
</tr>
<tr>
<td>µ-opioid receptor, OPRM 16q24-q25</td>
<td>Exon 1 Asn40Asp polymorphism</td>
<td>German ethnicity</td>
<td>DSM based</td>
<td>No evidence for association with diagnosis</td>
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</table>

† † DIGS, ‡ ‡ ASI, § § Cocaine Experience Questionnaire.
<table>
<thead>
<tr>
<th>Receptor</th>
<th>Chromosome</th>
<th>SNP Type</th>
<th>Ethnicity</th>
<th>Methodology</th>
<th>Controls</th>
<th>Results</th>
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<tbody>
<tr>
<td><strong>µ-opioid receptor</strong>&lt;br&gt;Oprm1&lt;br&gt;16q24-q25</td>
<td>8 SNPs, including 2 coding SNPs and representative haplotypes&lt;br&gt;African-American&lt;br&gt;European-American</td>
<td>SCID&lt;sup&gt;5&lt;/sup&gt;</td>
<td>African-American&lt;br&gt;124 alcohol, cocaine, opioid, or multi-substance dependent&lt;br&gt;55 controls&lt;br&gt;European-American&lt;br&gt;318 alcohol, opioid, cocaine, or multi-dependent&lt;br&gt;179 controls</td>
<td>Significant difference in haplotype distribution between “alcohol + opioid”-dependent vs. controls (p = 0.0036)&lt;br&gt;Luo et al. (2003)&lt;sup&gt;94&lt;/sup&gt;</td>
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<td><strong>δ-opioid receptor</strong>&lt;br&gt;Oprd1&lt;br&gt;1p36.1-p34.3</td>
<td>Third exon codon 307&lt;br&gt;T-C noncoding SNP&lt;br&gt;German</td>
<td>Clinical interview, urine toxicology&lt;br&gt;103 heroin dependent&lt;br&gt;115 controls</td>
<td>Association with C allele in heroin abusers&lt;br&gt;χ²(1) = 8.90; p &lt; 0.01&lt;br&gt;Mayer et al. (1997)&lt;sup&gt;92&lt;/sup&gt;</td>
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<td>Third exon codon 307&lt;br&gt;T-C noncoding SNP&lt;br&gt;German</td>
<td>DSM based&lt;br&gt;323 heroin dependent w/ 90 family trios&lt;br&gt;262 alcohol dependent w/ 72 family trios&lt;br&gt;173 controls</td>
<td>Association with C allele in heroin abusers&lt;br&gt;χ²(1) = 8.90; p &lt; 0.01&lt;br&gt;Mayer et al. (1997)&lt;sup&gt;92&lt;/sup&gt;</td>
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<tr>
<td>Proenkephalin&lt;br&gt;Penk&lt;br&gt;8q23-q24</td>
<td>CA repeat polymorphism (two groups: ≤79, ≥81 BP)&lt;br&gt;Caucasian</td>
<td>DIS-III-R&lt;sup&gt;i&lt;/sup&gt;,&lt;sup&gt;‡&lt;/sup&gt;, ASI&lt;sup&gt;ii&lt;/sup&gt;</td>
<td>≥81 BP repeat more common in opioid-dependent subjects&lt;br&gt;χ² = 6.0; p &lt; 0.015&lt;br&gt;Comings et al. (1999)&lt;sup&gt;94&lt;/sup&gt;</td>
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<tr>
<td>Prodynorphin&lt;br&gt;Pdyn&lt;br&gt;20pter-p12.2</td>
<td>68 BP VNTR in promoter region, 1 to 4 copies</td>
<td>African-American&lt;br&gt;European-American&lt;br&gt;Hispanic-American</td>
<td>1–2 copies protective vs. 3–4 copies (RR = 0.59&lt;br&gt;95% CI, 0.37–0.95; χ²(1) = 4.14; p = 0.042&lt;br&gt;Chen et al. (2002)&lt;sup&gt;105&lt;/sup&gt;</td>
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<td>Cannabinoid receptor&lt;br&gt;Cnr1&lt;br&gt;6q14-q15</td>
<td>AAT repeat polymorphism&lt;br&gt;Caucasian</td>
<td>Self-administered questionnaire&lt;br&gt;92 polysubstance use&lt;br&gt;114 controls</td>
<td>Association with ≥5≥5 repeats genotype with no. of IV substances (ANOVA p = 0.005) and cocaine dependence (χ²(2) = 5.36; p = 0.020)&lt;br&gt;Comings et al. (1997)&lt;sup&gt;106&lt;/sup&gt;</td>
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<td>Cannabinoid receptor&lt;br&gt;Cnr1&lt;br&gt;6q14-q15</td>
<td>AAT repeat polymorphism&lt;br&gt;Han Chinese</td>
<td>DSM-based interview&lt;br&gt;375 opioid abuse&lt;br&gt;198 controls</td>
<td>χ² analysis: no association detected&lt;br&gt;Li et al. (2000)&lt;sup&gt;95&lt;/sup&gt;</td>
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<tr>
<td>Cannabinoid receptor&lt;br&gt;Cnr1&lt;br&gt;6q14-q15</td>
<td>AAT repeat polymorphism&lt;br&gt;African-American&lt;br&gt;European-American</td>
<td>DSM-based interview&lt;br&gt;130 polysubstance use&lt;br&gt;49 controls&lt;br&gt;European-American&lt;br&gt;399 polysubstance use&lt;br&gt;113 controls</td>
<td>χ² analysis: no association detected&lt;br&gt;Covault et al. (2001)&lt;sup&gt;107&lt;/sup&gt;</td>
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| Serotonin receptor 5-HT₁B 6q13 | 7 noncoding SNPs | African-American, European-American, Hispanic-American | DSM-based interview 108 alcohol or cocaine, abuse or dependence 92 controls | χ² analysis: no association detected | Cigler et al. (2001) 

| Serotonin transporter SLC6A4 (SERT) 17q11.1-q12 | 17 BP second intron VNTR | African-American SCID, ASI | 156 cocaine dependent 82 controls | χ² analysis: no association detected | Patkar et al. (2002) 

| Catechol-O-methyltransferase | Exon 4 G-A substitution, coding change val-met | Israelis: Arabs, Ashkenazi Jews, Non-Ashkenazi Jews | DSM-IV 101 heroin dependent w/ 38 family trios 126 controls | Haplotype relative risk analysis: excessive val transmission in heroin dependence (likelihood ratio = 4.97; p = 0.03) | Horowitz et al. (2000) |

A, adenine; AAT, adenine/adenine/thymine; BP, base pair; C, cytosine; CA, cytosine/adenine; G, guanine; SNP, single nucleotide polymorphism; T, thymine; VNTR, variable nucleotide tandem repeat.

† DRD2 Taq1 A = T/C SNP 3′ 10kb; DRD Taq1 B = A/G SNP intron1; DRD Taq1 D = T/C SNP intron2.
†† Computerized Diagnostic Interview Schedule for DSM-III-R.
§ Structured Clinical Interview for DSM-III-R.
††† Diagnostic Interview for Gambling Severity.
†††† Diagnostic Interview Schedule, Version III Revised.
§§ Addiction Severity Index.
to consume alcohol. Disulfiram blocks the enzyme acetalddehyde dehydrogenase, rendering the individual taking it similar in phenotype to someone with the form of the enzyme that causes slower metabolism of acetalddehyde. The resulting acetalddehyde buildup from the ingestion of alcohol causes flushing and other toxic reactions, making alcohol aversive.

Disulfiram also blocks dopamine β-hydroxylase—the enzyme catalyzing the metabolism of dopamine into norepinephrine—the low activity of which is associated with cocaine-induced paranoia. Recently, disulfiram was demonstrated to have efficacy in the treatment of cocaine dependence. A theoretical mechanism of action involves its effects on dopamine β-hydroxylase by essentially converting a phenotype with greater enzyme activity into one with less activity. Disulfiram also appears to increase cocaine plasma levels through an unknown mechanism. Thus, another way in which it may affect cocaine use is by transforming a “rapid metabolizer” phenotype into a “poor metabolizer” phenotype.

Using this example of disulfiram, it is easy to imagine the development of other pharmacogenetic treatments specific to an underlying genetic etiology of substance dependence. For example, if we knew that individuals with opioid or cocaine dependence were rapid metabolizers of those substances, we could potentially use medications to block the relevant metabolic enzymes, perhaps causing toxic effects of the abused substances and making them aversive instead of reinforcing.

It is also conceivable that further understanding of the genetic determinants of opioid and cocaine dependence could lead to the use of genetic testing to identify individuals who have not yet developed these disorders but are at high risk of doing so. Such an approach could offer an excellent avenue for the aggressive use of primary prevention programs for such individuals. The use and availability of this kind of information has far-reaching ethical implications, however, as the same type of information that could be effectively used to benefit identified persons through primary prevention programs could also be used to discriminate against those same persons, who might be labeled as susceptible to addictions, denied certain standard benefits (such as health insurance), or deemed unsuitable for specific types of employment (such as health care provider or first responder). Our society obviously has to proceed with extreme caution should we embark on an endeavor so fraught with potential ethical problems.

With the knowledge of addiction genetics that has already accrued, these clinical scenarios seem reasonably close at hand. We know with certainty that genetics contributes a large proportion of the risk for substance dependence. We have already embarked on an effort to study numerous candidate genes. If we invest the resources, we have the technical capacity and scientific acumen ultimately to identify the genetic mechanisms that underlie the development of substance dependence. Armed with that information, we would have an improved capacity to treat, and perhaps even to devise biologically based strategies for preventing, these common and oftentimes lethal disorders.

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59. Solinas M, Panilio LV, Antoniou K, Pappas LA, Goldberg SR. The cannabinoid CB1 antagonist N-piperidinyl-5-(4-
chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (SR-141716A) differentially alters the reinforcing effects of heroin under continuous reinforcement, fixed ratio, and progressive ratio schedules of self-administration in rats. J Pharmacol Exp Ther 2003;306:93–102.


