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Pharmacogenetics and Human Molecular Genetics of Opiate and Cocaine Addictions and Their Treatments

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***Abstract*——Opiate and cocaine addictions are major social and medical problems that impose a significant burden on society. Despite the size and scope of these problems, there are few effective treatments for these addictions. Methadone maintenance is an effective and most widely used treatment for opiate addiction, allowing normalization of many physiological abnormalities caused by chronic use of short-acting opiates. There are no pharmacological treatments for cocaine addiction. Epidemiological, linkage, and association studies have demonstrated a significant contribution of genetic factors to the addictive diseases. This article reviews the molecular genetics and pharmacogenetics of opiate and cocaine addictions, focusing primarily on genes of the opioid and monoaminergic systems that have been associated with or have evidence for linkage to opiate or cocaine addiction. This evidence has been marshaled either through identification of variant alleles that lead to functional alterations of gene products, altered gene expression, or findings of linkage or association studies. Studies of polymorphisms in the μ opioid receptor gene, which encodes the receptor target of some endogenous opioids, heroin, morphine, and synthetic opioids, have contributed substantially to knowledge of genetic influences on opiate and cocaine addiction. Other genes of the endogenous opioid and monoaminergic systems, particularly genes encoding dopamine β-hydroxylase, and the dopamine, serotonin, and norepinephrine transporters have also been implicated. Variants in genes encoding proteins involved in metabolism or biotransformation of drugs of abuse and also of treatment agents are reviewed.**

I. Introduction

Opiate and cocaine addictions are chronic relapsing diseases with complex etiologies, significant comorbidities (e.g., human immunodeficiency virus, hepatitis B and C infections, depressive and anxiety disorders, and other psychiatric illnesses), and major negative socioeconomic consequences. Opiate addicts frequently suffer from cocaine addiction, and cocaine addicts frequently have comorbid alcohol addiction. There is also significant comorbid nicotine addiction in both groups. In addition, opiate and cocaine addicts often abuse marijuana and benzodiazepines. As with other complex chronic diseases such as hypertension, coronary artery disease, type 2 diabetes mellitus, and unipolar depression, the addictions develop through the interaction of various social-behavioral, physiological, and genetic factors. Because the development of the addictive diseases is predicated on the exposure to a drug of abuse, pharmacological and pharmacogenetic factors must be considered and, for opiate and cocaine addictions, will be the focus of this review.

The addictive diseases are clinically defined through a combination of physiological and behavioral criteria. Currently, the most widely used diagnostic criteria for the addictive diseases are those of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV[1]) (Diagnostic and Statistical Manual of Mental Disorders, 1994). The DSM-IV was written not as a research tool but as a diagnostic tool for identifying patients in need of treatment. Unlike earlier versions (e.g., DSM-III-R), DSM-IV criteria for substance dependence include but do not require physiological phenomena such as tolerance and dependence. Other nondiagnostic tools such as the Addiction Severity Index are available to assess duration and mode of drug use but do little to ascertain the extent of drug exposure (McLellan et al., 1980, 1992). We have recently developed and validated against DSM-IV definitions of opiate, cocaine, and alcohol dependencies a brief instrument, the Kreek-McHugh-Schluger-Kellogg scale, that assesses the amount of drug use at its peak level of intake (Kellogg et al., 2003). The Kreek-McHugh-Schluger-Kellogg scale allows for quantitative analysis of drug use characteristics and may be useful in future genetic and pharmacological studies of the addictive diseases.

According to DSM-IV, a diagnosis of substance dependence is met if three or more of the following occur in the same 12-month period: 1) tolerance, defined by the need

[1]Abbreviations: DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; DSM-III-R, Diagnostic and Statistical Manual of Mental Disorders, Third Edition—Revised; MOR, μ opioid receptor; KOR, κ opioid receptor; SNP, single nucleotide polymorphism; DOR, δ opioid receptor; HPA, hypothalamic-pituitary-adrenal; LAAM, levo-α-acetylmethadol; PET, positron emission tomography; M6G, morphine-6-glucuronide; CREB, cAMP response element-binding protein; CSF, cerebrospinal fluid; RFLP, restriction fragment length polymorphism; VNTR, variable nucleotide tandem repeat; P450, cytochrome P-450; 6-MAM, 6-monoacetylmorphine; hCE, human carboxylesterase; M3G, morphine-3-glucuronide; UGT, UDP glucuronosyltransferase.

for increased amount of substance to achieve the desired effect or diminished effect with continued use of the same amount of the substance; 2) development of a characteristic withdrawal syndrome when the substance is stopped or the use of the substance to prevent the onset of withdrawal; 3) increased or prolonged use; 4) a desire or unsuccessful attempts to cut down or control use; 5) significant time spent in activities related to drug procurement, use, and recovery; 6) important social, occupational, or recreational activities are sacrificed because of substance use; and 7) ongoing use despite knowledge of ongoing physical or psychological harm related to substance use. This is in contrast to the initial, and current, federal criteria for entrance into methadone maintenance treatment for opiate addiction (Rettig and Yarmolinsky, 1995; Kreek and Vocci, 2002). The federal criteria, which require greater than 1 year of multiple daily opiate self-administrations, far exceed the DSM-IV criteria for opioid dependence in that tolerance and withdrawal may develop within several weeks following regular opiate use.

By contrast, the DSM-IV criteria for substance abuse do not include the physiological effects of repeated drug use. Rather, a diagnosis of substance abuse is met if one or more of the following result in clinically significant impairment or distress during a 12-month period: 1) substance use results in failure to fulfill major work, school, or home obligations; 2) recurrent substance use in hazardous situations; 3) recurrent substance-related legal problems; and 4) continued use despite persistent or recurrent social or interpersonal problems related to substance use.

Although the DSM-IV refers to "substance dependence", this causes confusion in nomenclature since frequently encountered, medically expected and accepted dependencies occur with chronically administered therapeutic agents such as all opioid analgesics, adrenocorticosteroids, and several antihypertensives. Throughout this review we will use the term "addiction" rather than "dependence" since it better describes the maladaptive and harmful aspects of persistent compulsive drug use.

II. Addictions

Whereas opiates and cocaine are discussed in this review, all drugs of abuse share a common characteristic which underlies their abuse potential: initial use in the mode and pattern of abuse leads to rapid increase or decrease of receptor and/or transporter function, neurotransmitter/neuropeptide activity, and secondary messenger signaling. Changes in the gene expression of target proteins follow frequent, repeated exposure. Cessation of drug use leads to similarly profound changes. Thus, recurrent "on-off" use of short-acting drugs produces long-term, perhaps permanent, alterations in these affected neuronal systems and underlies the development of tolerance, dependence, withdrawal, and relapse characteristic of the addictive diseases.

A. Opiates and Other Opioids

Opiates are the drugs derived from opium and include morphine, codeine, their congeners (e.g., heroin and oxycodone), and other semisynthetic derivatives of thebaine. Opioids, however, consist of all agonist drugs with morphine-like activity whether they are naturally occurring or synthetic. For the purposes of this review, opiates and opioids are discussed together. The initial effects of opiates are mediated through the endogenous opioid system. Although there are three classes of opioid receptors (μ, δ, and κ), abused opiates primarily interact with μ opioid receptors (MOR). This seven transmembrane G protein-coupled receptor modulates diverse physiological systems including response to pain and reward, stress responsivity, gastrointestinal motility, and immune function. The endogenous ligands for MOR are the 31-amino acid protein β-endorphin and the smaller enkephalin molecules. By inhibiting γ-aminobutyric acid (GABAergic) neurons, stimulation of MOR also results in disinhibition of mesolimbic-mesocortical dopamine pathways central to the reinforcing properties of opiates and other drugs of abuse. Repeated administration of and withdrawal from opiates disrupts these pathways and results in the physiological and behavioral effects of opiate addiction.

1. Heroin. The 2002 National Survey on Drug Use and Health estimated that in 2002, 19.5 million Americans age 12 or older were illicit drug users (Substance Abuse and Mental Health Services Administration, 2003b). Over 3.5 million Americans have used heroin and there are over 1 million heroin addicts in the United States. From 1995 to 2002, the prevalence of lifetime heroin use has increased among youths aged 12 to 25 with greater than 100,000 heroin initiates annually during this period.

2. Codeine. Codeine is an orally administered prescription opiate rarely administered as a sole agent. Codeine is frequently prepared in combination with decongestants and expectorants as a cough remedy and nonopiate analgesics such as acetaminophen for the treatment of pain. Although codeine itself is modestly potent, it is, in part, metabolized into the potent and reinforcing opiate morphine. Recent trends indicate that there has been a decline in the illicit use of codeine (Substance Abuse and Mental Health Services Administration, 2003b). Rather than a sign of decreased demand for prescription opiates, this decrease seems to be offset by increased illicit use of more potent prescription opioids.

3. Noncodeine Prescription Opioids. The prevalence of illicitly used codeine- and noncodeine-containing prescription opioids has been difficult to determine. An estimated 1.9 million Americans used oxycodone illicitly in 2002 (Substance Abuse and Mental Health Services

Administration, 2003b). According to the Drug Abuse Warning Network, from 1995 to 2002 emergency room mentions of illicitly used prescription opiates increased 116% (2775 mentions in 2002) for morphine-containing analgesics, 560% (22,397 mentions in 2002) for oxycodone-containing analgesics, to over 6000% (1506 mentions in 2002) for fentanyl-containing analgesics (Substance Abuse and Mental Health Services Administration, 2003a).

B. Cocaine

Cocaine primarily acts through inhibition of presynaptic dopamine transporters as well as the serotonin and norepinephrine transporters. Increased levels of synaptic dopamine and, thereby, dopamine receptor binding following cocaine administration is a key mechanism through which cocaine is reinforcing. Cocaine also modulates the endogenous opioid system, especially MOR, κ opioid receptors (KOR), and preprodynorphin. Whereas stimulation of dopaminergic pathways may be sufficient to cause the reinforcing effects of cocaine, dopamine transporter gene deletion studies have shown that this pathway is not essential to the development of cocaine self-administration (see below). Selective gene disruption of the MOR will, however, prevent the development of cocaine self-administration (see below).

Close to 34 million Americans have used cocaine and there are over 1.5 million cocaine addicts in the United States (Substance Abuse and Mental Health Services Administration, 2003b). Discussion of other stimulant drugs such as methamphetamine and 3,4-methylenedioxy-methamphetamine (or ecstasy) as well as illicitly used prescription stimulants is beyond the scope of this review.

III. Molecular Genetics of Opioid and Cocaine Addictions

The human genome contains approximately 25–40,000 genes encoded in 3.2 billion nucleotides of DNA (Lander et al., 2001; Venter et al., 2001). It has been predicted that any two genomes, when compared, are nearly 99.9% identical (Kruglyak and Nickerson, 2001). A substantial portion of the 0.1% genetic variability between individuals is due to the 11 million single nucleotide polymorphisms (SNPs) estimated to occur in the human genome with allelic frequencies greater than 1% (Kruglyak and Nickerson, 2001). Variability is also introduced by such processes as alternative splicing of mRNA transcripts and imprinting.

Polymorphisms (or variants) in genes, which code for proteins that are in the pathways where heroin or cocaine act, especially when their expression results in altered protein amounts or when they code for aberrant forms of proteins, may be responsible for some of the observed differences between individuals in their physiological, biochemical, and behavioral responses to those drugs. The acquisition and persistence of, relapse to, and treatment of the addictions may also be influenced by genetic variations.

Although some diseases, such as sickle cell anemia and cystic fibrosis, are single gene disorders, vulnerability to addiction undoubtedly has a more complex genetic basis. Complex diseases may be polygenic (being caused by many genes), but are generally considered to be oligogenic (when only a few genes play a significant role). Classical pharmacogenomics has concentrated on the genetics of an individual and how it relates to their response to therapeutic agents. This has focused on genetic variation related to the absorption, toxicity, and biotransformation of therapeutic agents. However, it has become apparent that physiogenetics, the genetic variability in physiological processes (e.g., endocrine regulation, intercellular signal transduction pathways, and neurochemistry), are of importance. Hence, pharmacogenetics will expand its scope to include physiogenetics.

Many genes have been significantly associated or have displayed evidence of linkage with opiate or cocaine addiction. However, only a small subset of these genes has a polymorphism for which an alteration in function has been verified. Polymorphisms in genes may modify transcriptional regulation or rate, mRNA splicing or stability, protein translation, function (such as enzymatic activity or binding), or stability. There are many factors that may confound results obtained in association or linkage studies, such as population admixture or environmental variables. These factors may obscure the influence of various genetic polymorphisms in the specific cohorts studied, not allowing the studies to reach significance.

In this review, we will discuss several genes for which convincing evidence has been published to indicate that they may be involved in the predisposition to opiate or cocaine addiction. These genes have been chosen due to either a functional alteration in a variant allele affecting expression of this gene, because genetic linkage and/or association have been replicated in a number of different studies and populations, or due to their known role in the manifestation of drug effects. Hence, the genes chosen here for review are hypothesized to be involved in the vulnerability to develop opioid or cocaine addiction based on laboratory studies and, moreover, have been shown to be involved in vulnerability in one or more studies (see Table 1).

A. Epidemiology

Three main factors contribute to the development of addiction. These are: 1) environmental factors, 2) drug-induced physiological effects (such as those effects on neurochemistry, neural networks, mRNA, peptide and protein levels), and 3) genetic factors. Genetic factors may be quite complex in nature. Genetic variants of one gene may interact with other genes, or the effects of one

TABLE 1
Selected genes associated with heroin and cocaine addiction

Gene	Name	Function	System	Chromosomal Location*
OPRM1	μ-Opioid receptor	Receptor	Opioidergic	6q24-q25
OPRK1	κ-Opioid receptor	Receptor	Opioidergic	8q11.2
OPRD1	δ-Opioid receptor	Receptor	Opioidergic	1p34.3-p36.1
PDYN	Preprodynorphin	Ligand	Opioidergic	20p12.2-pter
PENK	Preproenkephalin	Ligand	Opioidergic	8q23-q24
DβH	Dopamine β-hydroxylase	Metabolism	Dopaminergic	9q34
DRD2	Dopamine receptor D2	Receptor	Dopaminergic	11q23
SLC6A3	Dopamine transporter	Transporter	Dopaminergic	5p15.3
SLC6A4	Serotonin transporter	Transporter	Serotonergic	17q11.1-q12
SLC6A2	Norepinephrine transporter	Transporter	Noradrenergic	16q12.2
CYP2D6	Cytochrome P450 2D6	Metabolism	(e.g. opioid metabolism)	22q13.1

* Gene Map Locus: Online Mendelian Inheritance in Man, OMIM. Johns Hopkins University, Baltimore, MD.

gene may mask the effect of another gene (epistasis). Genes have been shown to interact with environmental factors. For example, Caspi and colleagues have found that in males with a variant of the monoamine oxidase A gene encoding a monoamine oxidase A enzyme with low activity (a possible risk factor for violence) and also who had experienced high levels of maltreatment as children had the highest risk of developing antisocial behavior (Caspi et al., 2002). Drug addictions are complex disorders that are likely to involve multiple genes having multiple polymorphisms that work in various combinations with each other in different people and interact with the environment. In addition, many of these alleles may contribute to either protection from, or vulnerability to, the development of a drug addiction and will have frequencies which vary by ethnic/cultural group. The genetic, developmental, and environmental context in which particular variants exist may affect their influence on the ultimate phenotype.

Early studies to determine whether addiction is a heritable disease were family studies on alcoholism (e.g., Kaij, 1960; Partanen et al., 1966; Cloninger et al., 1981). More recently, other addictions have been studied and both similarities and differences between the addictions have been observed. Some of the physiology, neurobiology, and treatments have been found to overlap in various aspects between the addictions, but often they are quite distinct. In addition, genetic variants may be both nonspecific (shared) and specific for various drugs of abuse. However, rigorous studies by leading geneticists into the specificity of genetic factors have found divergent results. For example, the genetic influences determining drug abuse vulnerability were studied by Tsuang and colleagues (Tsuang et al., 1998). Using a study population of 3372 predominately Caucasian (90% non-Hispanic white) male twin pairs, it was found that stimulant abuse/addiction (e.g., cocaine and amphetamines) had a total genetic variance of 0.3, whereas heroin use/addiction had a total genetic variance of 0.5. In this study, stimulants had a low specific genetic variance of 0.09, whereas heroin had a high specific genetic variance of 0.4. A similar study, but with very different findings, was performed by Kendler and colleagues using 1196 U.S. Caucasian male twin pairs (Kendler et al., 2003). Common additive genetic factors for use of cocaine were estimated to have a variance of 0.5, whereas for opiates the variance was estimated at 0.4. However, in contrast with the study of Tsuang and colleagues, the substance-specific additive genetic variance was only 0.07 for cocaine and 0.00 for opiates.

Family studies have also documented familiar transmission of cocaine abuse. Siblings of cocaine-dependent probands had a relative risk of 1.7 to develop cocaine dependence themselves (Bierut et al., 1998). A comparison of other addictions occurring in the probands suggested that the addictions had independent causative factors. Another family study found that the adjusted odds ratio for having the same drug disorder in adult first-degree relatives was over 7 for cocaine and over 10 for opioids, again indicating an involvement of genetic factors (Merikangas et al., 1998).

B. Molecular Genetic Studies

1. Family and Linkage Studies. There are two main types of studies, linkage and association studies, conducted to establish whether genes and their variants may be involved in vulnerability to drug addiction. Linkage studies use families to provide evidence of how close a genetic marker is to an allele causing the phenotype in question, whereas association studies may be performed with unrelated individuals or with parent-offspring trios.

Linkage studies have been used to study subjects with addictive diseases and comorbid conditions. One early and continuing study is the Collaborative Study on the Genetics of Alcoholism (COGA), a multicenter effort to identify genes involved in alcoholism (description available at <www.niaaa.nih.gov/extramural/projcoga.htm>) (Edenberg, 2002; also, see Begleiter et al., 1999). This group, as well as studies by others, have identified many chromosomal regions (including regions on chromosomes 1, 2, 3, 4, 7, and 11) using linkage scans that appear to be involved in the vulnerability to develop alcoholism as well as other addictions (Long et al., 1998; Reich et al., 1998; Foroud et al., 2000; Bergen et al., 2003; Ma et al., 2003; Stallings et al., 2003; Wyszynski et

al., 2003). These studies have identified regions that can be more finely mapped, and the genes within these regions may be studied further.

2. Case Control-Association Studies. Another method to identify variants involved in addictions is to select genes that are likely to be involved in the physiological effect of the specific drug under consideration in a neurotransmitter system related to drug taking behaviors or genes for which there is previous evidence to be of interest. Genetic variants are identified in these candidate genes. Cases and control groups are genotyped for the variant(s), and statistical approaches are then employed to measure the probability that a given variant allele is associated with the drug addiction.

In association studies, the cultural identity and the ethnicity of the subjects must be carefully evaluated because some genes' allelic frequencies vary widely among ethnic/cultural groups. Several studies have found that although approximately 90% of the total genetic variation is between individuals, approximately 10% occurs between ethnic/cultural groups (reviewed in Brown and Armelagos, 2001). Hence, it appears that this 10% difference may be enough to introduce population stratification into association analyses when ethnicity is not matched between subjects and controls. Failure to do so may cause both false-positive and false-negative errors. Such population admixture, or stratification, repeatedly has been shown to confound results of association studies (reviewed in Thomas and Witte, 2002).

The techniques for conducting association studies have been recently expanded to include thousands of variants using gene microarray technology. To locate and identify genes and chromosomal regions that are associated with specific addictions, genome-wide scans can be performed on affected and control subjects. To map genes involved in vulnerability to drug abuse, Uhl and colleagues have used microarray technology (using an Affymetrix microarray that can genotype 1494 SNPs in a single hybridization) (Uhl et al., 2001). Multiple pools, each containing DNA from 20 individuals who were either polysubstance abusers or controls and who were of the same ethnic/cultural group, were genotyped. Using this association genome scanning technique, they identified 42 chromosomal regions that potentially are involved in vulnerability to drug abuse in both European Americans and African Americans. Since these studies were conducted using subjects with alcohol, nicotine, or polysubstance addictions, the chromosomal regions identified may contain loci for vulnerability to addiction to multiple substances. When the variants associated with polysubstance abuse in the microarray study were compared with those regions identified in linkage studies of alcohol (Long et al., 1998; Reich et al., 1998; Foroud et al., 2000) and nicotine addiction (Straub et al., 1999), 15 candidate regions had positive results in at least two of the studies (Uhl et al., 2002b). Again, since these studies were based on polysubstance abuse, alcohol, or nicotine addiction, the regions identified as candidates are most likely associated with vulnerability to drug abuse in general.

3. Haplotype Analysis. Polymorphic variants are known to associate in clusters. Analyses of variants in pedigrees have shown that some alleles that are in close proximity with each other are inherited as a block, whereas others have lower linkage disequilibrium. Linkage disequilibrium patterns of variants have shown that there are characteristic patterns of linkage disequilibrium across the human genome. A map of these patterns is a haplotype map and is a description of variants in a region and the linkage disequilibrium pattern involved. The haplotype map is intended to describe the common variation patterns and to provide a minimal set of variants that capture the full set of diversity in the population.

A haplotype is a defined region of one chromosome from each pair of chromosomes. An individual has two sets of haplotypes, one derived from each parent. Hence, haplotype blocks in a population represent chromosomal regions that were inherited with little past recombination. Haplotypes may be determined directly through the use of various techniques or may be inferred using one of several algorithms. These algorithms predict haplotypes that are most likely to occur. However, haplotypes of equal likelihood are not predicted by these methods, although they may be the real haplotypes.

Haplotypes vary among populations. It has been demonstrated that in European and Asian populations the mean haplotype block is about 22,000 nucleotides with about four common haplotypes per block (Gabriel et al., 2002). In African American and Yoruban (Nigerian) populations, the mean haplotype block is only 11,000 nucleotides with five common haplotypes per block. This is similar to the finding of Reich and colleagues who found that, in populations of European descent, linkage disequilibrium extends to approximately 60,000 nucleotides from common alleles, although only extending less than 5000 nucleotides in a Nigerian population (Reich et al., 2001).

When a new variant arises in a population, it occurs on a specific haplotype. In subsequent generations, the variant and its ancestral haplotype are altered only by recombination or the acquisition of new variants. Hence, it should be possible to identify a rare variant associated with a disease by identifying the ancestral region on which it was first introduced.

Two examples relevant to this review which will be detailed below are the μ opioid receptor (*OPRM1*) and dopamine β-hydroxylase (*DβH*) genes. A haplotype analysis of *OPRM1* was conducted with European American and African American subjects (Luo et al., 2003). Eight variants were genotyped and analyzed using an expectation-maximization (E-M) algorithm (Long et al., 1995). Six haplotypes were predicted in the European Americans and seven in the African Americans. In the Euro-

pean Americans, a significant difference in haplotype frequency was found between the groups who were both alcohol- and opioid-dependent combined versus controls.

In the *DβH* gene, Cubells and colleagues genotyped two variants, one 3000 nucleotides upstream of the transcription start site (DBH*5′-ins/del) and the other at nucleotide 444 (DBH*444g/a) (Cubells et al., 2000). These variants, separated by approximately 8400 nucleotides, were in linkage disequilibrium. One of the haplotypes, "Del-a", was associated with low plasma DβH and with cocaine-induced paranoia in European Americans.

C. Selected Identified Genes

1. Opioid-Related Genes. For millennia, compounds derived from the opium poppy have been used for their medicinal properties as analgesics, antitussives, soporifics, and antidiarrheals. Also, opium and derivatives such as morphine and heroin have long been recognized as drugs of addiction. Endogenous receptors for opiate drugs were first postulated to exist in 1954 (Beckett and Casey, 1954). During the ensuing two decades, efforts to identify these receptors were performed using stereospecific ligand-binding assays (Ingoglia and Dole, 1970; Goldstein et al., 1971), and in 1973, opioid receptors were discovered independently by three groups (Pert and Snyder, 1973; Simon et al., 1973; Terenius, 1973). The availability of increasingly selective opioid ligands subsequently allowed the identification of three receptor types, which were named the μ, κ, and δ opioid receptors (abbreviated in this review as MOR, KOR, and DOR, respectively). The first endogenous ligands for opioid receptors were identified in 1975 by Hughes and colleagues, who isolated Leu- and Met-enkephalins, which are five amino acid peptides with "opiate-like" or "opioid" activity (Hughes et al., 1975). Subsequently, two other endogenous opioid peptides were discovered, β-endorphin and dynorphin A (Bradbury et al., 1976; Cox et al., 1976; Li and Chung, 1976; Goldstein et al., 1979).

The endogenous opioid peptides are derived through proteolytic processing of three propeptide precursors. Met- and Leu-enkephalin, as well as Met-enkephalin-Arg-Phe and Met-enkephalin-Arg-Gly-Leu are derived from proenkephalin. β-Endorphin is derived from pro-opiomelanocortin (which also yields adrenocorticotropic hormone and melanocyte stimulating hormone in equimolar amounts), and processing of prodynorphin produces dynorphins A and B and α-neoendorphin. Each of these classic endogenous opioid peptides contains the N-terminal sequence Tyr-Gly-Gly-Phe, which confers their opiate-like properties.

The cDNA and gene sequences of the endogenous opioid peptide precursors were determined a few years after their discovery (enkephalin: Comb et al., 1982; Noda et al., 1982a,b; dynorphin: Kakidani et al., 1982; Horikawa et al., 1983; pro-opiomelanocortin: Nakanishi et al., 1979, 1981; Takahashi et al., 1981, 1983). Opioid receptors, on the other hand, were first cloned and sequenced almost 20 years following their discovery. The groups of Evans and Kieffer, working independently, isolated, defined, and sequenced cDNA clones of the mouse δ opioid receptor in 1992 (Evans et al., 1992; Kieffer et al., 1992). Following publication of the sequence of the DOR, many groups used homology screening to identify and sequence clones of the MOR and KOR from several species (μ: Chen et al., 1993; Fukuda et al., 1993; Thompson et al., 1993; Wang et al., 1993; κ: Li et al., 1993; Meng et al., 1993; Minami et al., 1993; Yasuda et al., 1993; Mansson et al., 1994; Simonin et al., 1995, Zhu et al., 1995; δ: Knapp et al., 1994; Simonin et al., 1994).

Binding assays with selective ligands allowed classification of the opioid receptors into three general classes: the MOR, which has as endogenous ligands the enkephalins and β-endorphin, the DOR, which selectively binds enkephalins, and the KOR, for which dynorphins are the endogenous ligands. Binding assays using cells expressing cloned receptors indicate that there exists considerable cross-selectivity for some of the peptide ligands.

The endogenous opioid system is centrally important in the responses to addictive opiate drugs such as morphine, codeine, and heroin, as well as to synthetic opioid narcotics such as fentanyl. The receptors mediate both the analgesic and rewarding properties of opioid compounds and opioid effects on the hypothalamic-pituitary-adrenal (HPA) stress-responsive axis, respiratory and pulmonary function, gastrointestinal motility, immune responses, and other functions. Additionally, this system is important in modulating the responses to cocaine and other psychostimulants, as well as to alcohol and other drugs (e.g., see Kreek et al., 2002). Components of the endogenous opioid system and the genes encoding them have therefore been the focus of research into specific addictions since their discovery.

a. μ Opioid Receptor Gene (OPRM1). *OPRM1* has been selected as a candidate for human genetic studies of the addictions for many reasons. The MOR is the molecular target of the active biotransformation products of heroin (6-monoacetylmorphine and morphine), as well as most opiate and opioid analgesic medications such as oxycodone, hydromorphone, and fentanyl, each of which has significant potential for addiction. Abuse of and addiction to these MOR-directed agents is increasingly recognized to constitute a major addiction problem. From our early work that led to the development of methadone maintenance treatment for heroin addiction in the 1960s, we know that μ-selective agonists with long-acting pharmacokinetics such as methadone and levo-α-acetylmethadol (LAAM) or partial agonists (buprenorphine) are the most effective treatments for this disorder (e.g., Dole et al., 1966; Kreek et al., 2002). Clinical studies also point to a relative "endorphin deficiency" in active and former heroin addicts and also in

active or recently abstinent cocaine addicts, although whether this is drug-induced or pre-exists and contributes to the development of addiction is still an unanswered question (e.g., Kreek et al., 1984; Schluger et al., 2001).

Studies of quantitative trait loci in mice identified a chromosomal region containing the μ opioid receptor gene as contributing to a substantial amount of the variance in analgesic and reward responses to morphine (Belknap and Crabbe, 1992; Berrettini et al., 1994; Kozak et al., 1994; Belknap et al., 1995; Crabbe et al., 1999). Also, studies of mice with targeted deletion of the μ opioid receptor gene definitively established this receptor as essential for morphine analgesia, physical dependence, and reward as measured by antinociception, withdrawal, conditioned place preference, and self-administration studies (Matthes et al., 1996; Sora et al., 1997; Kitanaka et al., 1998; Loh et al., 1998; Becker et al., 2000). Chronic morphine administration or heroin self-administration alters naloxone efficacy measured using 5′-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding in several brain regions by increasing MOR binding and decreasing MOR-activated G proteins (Sim et al., 1996; Sim-Selley et al., 2000; Kruzich et al., 2003).

Although the initial molecular target of cocaine is monoamine transporters, expression and function of the MOR is also affected by cocaine, particularly in a chronic experimental administration paradigm and also in long-term human cocaine abusers. For example, in rats administered cocaine for 14 days in a "binge" paradigm, MOR binding density increased in several regions containing terminals of the mesocorticolimbic dopaminergic system, including areas of the cingulate cortex, the nucleus accumbens, caudate-putamen, and basolateral amygdala (Unterwald et al., 1992, 1994). A direct molecular effect of acute or 3-day cocaine administration on MOR mRNA levels has also been observed with increases in dopaminergically innervated brain regions reported (Azaryan et al., 1996; Yuferov et al., 1999). A positron emission tomography (PET) study in cocaine-dependent men also showed increases in MOR binding that were associated with cocaine craving (Zubieta et al., 1996). Additionally, MOR knockout mice show reduced reward responses to both cocaine and alcohol (Roberts et al., 2000; Hall et al., 2001; Becker et al., 2002).

Many variants, particularly SNPs, in the coding, untranslated, flanking, and intronic regions of this gene have been identified and tested for association with opiate, cocaine, and other addictions. A few nonsynonymous variants in the coding region have been studied using in vitro assays to assess possible functional alterations in the encoded proteins.

The most common coding region SNP, the A118G variant, which alters amino acid sequence, was one of the first discovered in the *OPRM1* gene (Bergen et al., 1997; Bond et al., 1998) and has been evaluated in a number of genetic studies of opiate, alcohol, and mixed drug addiction. This variant has also been shown to be of physiogenetic (genetically based difference in physiology) and pharmacogenetic (genetically based difference in response to a pharmacotherapeutic agent) importance, discussed below. The frequency of this polymorphism varies widely across populations, occurring at less than 2% in some populations up to almost 50% in others (Table 2). This SNP is in the first exon of the *OPRM1* gene, and the 118G allele encodes a variant receptor with an aspartic acid at amino acid position 40 instead of asparagine, which is at this position in the prototype receptor. This substitution leads to a charge difference and also to the loss of a putative N-linked glycosylation site in the N-terminal domain of the receptor (Bergen et al., 1997; Bond et al., 1998). Glycosylation of G protein-coupled receptors has been reported to be important in mediating appropriate protein conformation that allows receptor trafficking to the cell membrane (George et al., 1986; Hughes et al., 1997; Petäjä-Repo et al., 2000).

In an extensive in vitro study of function of the variant receptors encoded by the 118A and 118G alleles, we used

TABLE 2
Allelic frequencies of the variant (118G) allele of the A118G single nucleotide polymorphism of the OPRM1 gene in diverse populations

Ethnicity or Population	Bergen et al. (1997)	Bond et al. (1998)	Gelernter et al. (1999)	Szeto et al. (2001)	Tan et al. (2003)	Bart et al. (2004a)	Bart et al. (2004b)
Caucasian							
European American	0.105 (100)	0.115 (52)	0.141 (543)				
Finnish	0.122 (324)						
Swedish						0.107 (187)	0.109 (559)
Indian					0.442 (137)		
Asian							
Japanese			0.485 (34)				
Han Chinese				0.362 (297)			
Chinese					0.351 (208)		
Thai					0.438 (56)		
Malay					0.446 (156)		
Hispanic		0.142 (67)	0.117 (47)				
African American		0.016 (31)	0.028 (144)				
Southwest Native American	0.163 (367)						
Other							
Ethiopian			0.170 (49)				
Bedouin			0.080 (43)				
Ashkenazi			0.210 (93)				

stably transfected cell lines expressing either the variant or prototype receptors and performed binding assays using several ligands, including the five amino acid residue endogenous peptide ligands Met- and Leu-enkephalin, the four amino acid residue peptides endomorphin-1 and -2, the synthetic μ-selective peptide D-Ala^2,*N*-Me-Phe^4,Gly-ol^5-enkephalin, the κ-selective endogenous peptide dynorphin A(1–17), the μ-preferring opioid agonists morphine, methadone, fentanyl, and the opioid antagonist naloxone. Each of these compounds had similar binding affinities to the prototype and 118G variant receptors (Bond et al., 1998).

We also tested the μ- and δ-selective 31-residue endogenous peptide β-endorphin, which is the longest of the endogenous opioid peptides, has the longest half-life, and is found both centrally and peripherally. In our in vitro studies, β-endorphin bound the 118G variant receptors with approximately three times greater affinity than those containing the prototype sequence (Bond et al., 1998).

We also studied the 118A and 118G variant receptors with respect to an important cellular activity of the MOR, that of agonist-mediated regulation of G protein-activated inwardly rectifying K^+ channels. The prototype or 118G receptors were coexpressed in *Xenopus* oocytes with G protein-activated inwardly rectifying K^+ channels; several peptide agonists, including β-endorphin and endomorphin-1 and -2, were tested for their ability to activate the K^+ channels. The short peptide agonists tested in this assay all showed similar EC_{50} values for the prototype and variant receptor; however, β-endorphin caused an approximately 3-fold greater potency in activation of K^+ current in the 118G variant receptor compared with the prototype (Bond et al., 1998).

The differences in binding of β-endorphin and activation of the 118G variant receptors following β-endorphin binding in these in vitro cellular studies led us to predict that persons carrying the gene expressing the variant receptor might show altered function of physiological systems under the control of the MOR, including, for example, pain perception, reproductive function, and responses to stress mediated by the HPA stress-responsive axis, which is under tonic inhibitory control of this receptor (Bond et al., 1998; Kreek 2000; LaForge et al., 2000b). Two studies have identified such a difference in the HPA stress-responsive axis of persons with the 118A/118G or 118G/118G genotype (Wand et al., 2002; Hernandez-Avila et al., 2003). In these studies, healthy control individuals were administered naloxone; subjects who carried one or more 118G alleles showed a greater activation of the axis, as measured by plasma cortisol, demonstrating a physiogenetic role for the A118G variant. Additional studies also suggest that this polymorphism may result in altered pharmacogenetic responses. Differences in physiological and analgesic responses to morphine, its active metabolite morphine-6-glucuronide (M6G), and to alfentanil have been reported in individuals carrying a 118G allele (Lötsch et al., 2002; Skarke et al., 2003a). Also, 118A/118G or 118G/118G genotypes have been reported to be associated with an improved response to treatment of alcoholism with naltrexone (Oslin et al., 2003) as well as to treatment of smoking with transdermal nicotine replacement therapy (Lerman et al., 2004).

The A118G variant has been studied in a number of genetic studies, primarily case-control studies of opiate and other addictions. In some studies, evidence for an association of specific alleles in specific populations has been found; other studies have not obtained such evidence. In the first report of an association of this variant with opiate addiction, we observed a higher proportion of the 118G allele in Hispanic control subjects, but not in American Caucasian or African American subjects (Bond et al., 1998). Tan and colleagues found a higher proportion of the 118A allele in opioid-dependent cases of Indian ancestry who were recruited and studied in Singapore; this finding, however was not replicated in other ethnic groups in that study, including Chinese and Malay subjects (Tan et al., 2003). In both of these studies, the numbers of individuals in each of the groups with a positive association was small (58 cases and 9 controls in the study of Bond and colleagues, 20 cases and 117 controls in the study of Tan and colleagues); also, the likelihood of population admixture in these groups would be expected to be high, which may have contributed to the findings of a positive association with this allele.

Conversely, in two other studies conducted in selected populations, the 118G allele was found to be strongly associated with opiate addiction. These studies had a more robust sample size and, to reduce the possibility of admixture confounding results, were performed with subjects who had parents of the same ethnic/cultural group as the probands. Szeto and colleagues studied 200 cases and 97 controls. Subjects were male Han Chinese individuals with all first-degree relatives from that ethnic group. An association of both the 118G allele and genotypes containing that allele with opiate-dependent subjects was found (Szeto et al., 2001).

In a recent study of heroin addiction in subjects from a geographic region known to have minimal admixture (central Sweden) we studied primarily Swedish individuals (both male and female) from the Stockholm area (139 cases and 170 controls). Like the study of Szeto and colleagues, we observed an association of 118G alleles and genotypes containing at least one 118G allele with opiate addiction (Bart et al., 2004a). Moreover, in that study, we found a substantial attributable risk for heroin addiction contributed by the 118G allele. In this study, the findings were significant if all subjects were included (attributable risk 18%), as well as if analyses were limited to Swedish subjects with both parents Swedish (attributable risk 21%) (Bart et al., 2004a).

Other genetic studies in which the A118G SNP has been evaluated have not found evidence that either allele of the A118G variant is associated with the development of opiate addiction. These include two studies of Han Chinese populations in Sichuan and Nanjing Provinces, respectively, one of German Caucasians and one in a sample of American Caucasians and African Americans (Li et al., 2000; Franke et al., 2001; Shi et al., 2002; Crowley et al., 2003). Li and colleagues studied 282 heroin-abusing subjects (DSM-IV criteria, determined by clinical interview) and 258 control subjects without neurological or psychiatric disorders (determined by questionnaire) recruited from college staff, medical students, and acute medical inpatients from a general hospital. All subjects were Han Chinese recruited in the Sichuan Province. No differences in genotype or allele frequencies between cases and controls were observed (Li et al., 2000). Franke and colleagues used case control and allele transmission analyses to test for an association of the A118G SNP with opiate addiction in German Caucasians from the Bonn area. In the case control analysis, 287 individuals meeting DSM-III-R criteria for opiate dependence and 365 control subjects (with 133 screened using a semistructured instrument and 232 blood donors who were briefly screened with respect to drug abuse history) were genotyped for the A118G variant. No differences in genotype or allele distributions were found between cases and controls (Franke et al., 2001). Additionally, in the family-controlled allele transmission test with 111 opiate-dependent probands, no preferential transmission of alleles of the A118G SNP were observed (Franke et al., 2001). Shi and colleagues studied 148 former heroin addicts in methadone maintenance treatment at the Nanjing Drug Abuse Control Bureau and 48 control individuals (with no drug or alcohol abuse as confirmed by questionnaire). All subjects were Han Chinese. The authors did not find a difference in 118A/118A versus 118A/118G + 118G/118G genotype frequencies in cases compared with controls. However, among former heroin-abusing individuals, those carrying the 118G allele along with a specific allele of an intron 2 SNP of the *OPRM1* gene (the IVS2 + 31A allele) reported higher heroin intake dosages than other addicts (Shi et al., 2002). Finally, Crowley and colleagues studied 225 opioid-dependent (determined by review of medical records) and 200 screened controls matched for ethnicity (European American and African American) recruited from the Philadelphia area. No differences between cases and controls in genotype or allele frequencies for the A118G SNP were found within either ethnic group (Crowley et al., 2003).

The second most common coding region variant of the μ opioid receptor gene is the C17T SNP, which encodes a variant receptor with a valine at amino acid position 6 instead of alanine in the N-terminal domain of the receptor (Bergen et al., 1997; Berrettini et al., 1997; Bond et al., 1998). Allele frequencies of this substitution also vary widely across population groups (Table 3). The potential functional significance of this alteration in protein structure was studied with no differences between prototype and variant receptors in agonist binding to several peptide and nonpeptidic ligands, in agonist-induced 5′-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS)-binding assays (a measure of receptor activation), or in agonist-mediated receptor down-regulation (Befort et al., 2001). It should be noted, however, that in this study no differences in β-endorphin binding between the 118A and 118G receptors were also found and that an undefined biological reason (such as systematically decreased presentation of functional receptors on the plasma membrane) or, alternatively, technical matters may underlie the difference in findings with our earlier study in which binding differences were observed (Bond et al., 1998).

Several studies have also evaluated this variant with respect to specific addictions in human genetic studies. Berrettini and colleagues reported that the 17T allele was associated with opiate- and/or cocaine-dependent subjects at a borderline level of significance ($p = 0.05$,

TABLE 3
Allelic frequencies of the variant (17T) allele of the C17T single nucleotide polymorphism of the OPRM1 gene in diverse populations

Ethnicity or Population	Bond et al. (1998)	Gelernter et al. (1999)	Szeto et al. (2001)	Tan et al. (2003)	Bart et al. (2004a)	Bart et al. (2004b)
Asian						
Japanese		None observed (35)				
Han Chinese						
Chinese				0.005 (209)		
Thai				None observed (56)		
Malay				0.005 (156)		
Indian				None observed (137)		
Southwest Native American						
Caucasian						
European American	0.019 (52)	0.008 (470)				
Finnish						
Swedish					None observed (187)	None observed (559)
Hispanic	0.037 (66)	0.011 (46)				
African American	0.210 (31)	0.140 (143)				
Other (populations in Israel)						
Ethiopian		0.080 (49)				
Bedouin		0.050 (43)				
Ashkenazi		0.016 (93)				

ethnic groups combined), a finding similar to our study of opioid-dependent individuals in which individuals were stratified by ethnic/cultural group (Berrettini et al., 1997; Bond et al., 1998). Two studies in other populations did not find an association of this variant with alcohol or mixed (opiate and/or cocaine) dependence (Gelernter et al., 1999). Because the 17T allele is at low frequency or absent in a number of the populations in which this variant has been characterized, its usefulness as a genetic marker may be limited to those populations in which it has a higher prevalence (see Table 3, and Bond et al., 1998; Gelernter et al., 1999; Tan et al., 2003; Bart et al., 2004a,b).

Several other variants, primarily SNPs, of the μ opioid receptor gene have been evaluated in the context of opiate, cocaine, and other addictions. For example, an allelic association was reported for the IVS2 C1031G variant in male Han Chinese with both parents also from that ethnic group (Szeto et al., 2001); however, this finding was not observed in a separate study in several populations including Han Chinese, Indian, Thai, and Malay individuals recruited in Singapore (Tan et al., 2003).

Three groups have also reported haplotype approaches to test for associations of this gene with addiction to opiates with or without addiction to other substances. One study used a clustering approach of 43 variants in coding, flanking, and intronic regions in a study group of European Americans and African Americans with opiate and/or cocaine dependencies compared with ethnically matched controls; they reported that specific haplotypes were associated with addiction to these substances (Hoehe et al., 2000). This type of analysis, however, raises issues concerning corrections for multiple testing, an issue that has come under increasing scrutiny in the field of statistical genetics (Levenstien et al., 2003). A second study of six biallelic variants (C-2044A, T-1793A, -1699i nsT, T-1469C, A-1320G, and C-111T) in the 5′-flanking region along with the A118G and C17T SNPs, reported specific differences in haplotype frequencies comparing controls to alcohol- and opioid-dependent patients combined or to alcohol-, opioid-, or cocaine-dependent patients combined (DSM-III-R criteria), although this finding was limited to European American subjects and was not found in African Americans (Luo et al., 2003). An additional study conducted in European American and African American subjects evaluated three 5′-flanking region variants (T-1793A, -1699insT, and A-1320G) along with the two common exon 1 SNPs and did not find evidence for an association of haplotypes with severe opiate dependence (cases had a family history of substance dependence and the age of onset of opiate dependence was 20 or below) (Crowley et al., 2003). In each of the haplotype studies, significant differences in haplotype frequencies were found between the two ethnic/cultural groups. Also, a high degree of linkage disequilibrium was reported across the 5′-flanking and exon 1 variants (Crowley et al., 2003; Luo et al., 2003).

b. κ Opioid Receptor Gene (OPRK1). The κ opioid receptor is also involved in the responses to addictive drugs, particularly cocaine, but also to opiates. κ agonists, including dynorphin, the primary endogenous peptide ligand of this receptor, lower levels of dopamine in the nucleus accumbens and act in a counter-modulatory manner to attenuate increases in synaptic dopamine levels caused by cocaine's blockade of dopamine reuptake into presynaptic terminals (see *Section III.C.1.e.* and *III.C.2.*). Increases in synaptic dopamine in the nucleus accumbens is reinforcing and is thought to be an important, although not necessarily, essential component of addiction to cocaine, opiates, alcohol, and other substances.

Chronic binge administration of cocaine lowers basal dopamine levels in both the caudate-putamen and nucleus accumbens (Maisonneuve and Kreek, 1994; Maisonneuve et al., 1995). Chronic binge cocaine administration also leads to increases in κ opioid receptor-binding density in several brain regions that play a counter-modulatory role in reward or locomotor effects of psychostimulants, including the rostral cingulate cortex, caudal olfactory tubercle, rostral caudate-putamen, and the ventral tegmental area (Unterwald et al., 1994). These two phenomena may be related since cocaine induces dynorphin release from the striatum, and either acute or chronic cocaine administration by a number of paradigms persistently increases preprodynorphin mRNA transcription in the caudate-putamen (e.g., Sivam, 1989; Hurd et al., 1992; Hurd and Herkenham, 1992; Daunais et al., 1993, 1995; Spangler et al., 1993a,b, 1996).

Dynorphin, presumably acting through the κ opioid receptor, also modulates μ opioid receptor responses that may be relevant for opiate addiction. For example, dynorphin A(1–13), a dynorphin peptide that has been approved for study in humans and is a shortened form of the naturally occurring peptide dynorphin A(1–17), attenuates opiate withdrawal symptoms and augments μ opioid receptor-mediated analgesia in chronic pain patients (Specker et al., 1998; Portenoy et al., 1999). Also of interest is the finding that in methadone-maintained patients administered dynorphin A(1–13) the attenuated increase of serum prolactin (which is regulated by the tuberoinfundibular dopaminergic system) is lower than in control subjects, suggesting that alterations in dopaminergic tone may exist in long-term opioid-dependent individuals (Bart et al., 2003).

The initial cloning of the human κ opioid receptor gene identified three exons that contained the complete coding sequence (Simonin et al., 1995; Zhu et al., 1995). However, the rat and mouse genes have been shown to contain an exon upstream of the translation start site that encodes most of the 5′-untranslated region of the mRNA (Liu et al., 1995; Yakovlev et al., 1995).

Recently, Yuferov and colleagues defined an additional 5′ exon in the human κ opioid receptor gene and identified several transcription initiation sites, as well as determining the poly(A) addition site of the mRNA (Yuferov et al., 2004). This has allowed a renumbering of the exons of the gene, and the previously numbered exons 1, 2, and 3 will be referred to as 2, 3, and 4 in this review.

Seven SNPs in the human κ opioid receptor gene have been reported (Höllt, 2000; LaForge et al., 2000a; Mayer and Höllt, 2001). These include the substitutions G36T in exon 2, C459T in exon 3, and A843G, C846T, C852T, C948T, and C1008T in exon 4. The first to be reported were identified in a population of German Caucasians; these were G36T (14%), C459T (2%), C843C (8%), and C846T (2%) (Höllt, 2000) (with allele frequencies in parentheses reported in Mayer and Höllt, 2001). These variants were also detected in our further studies conducted in New York with a study sample of primarily Caucasian, African American, and Hispanic subjects with the identification of additional SNPs that have overall allele frequencies ranging from <1% to 3% (Yuferov et al., 2004). Additionally, preliminary evidence from that study suggests that the G36T SNP may be associated with opiate addiction (Yuferov et al., 2004).

c. δ Opioid Receptor Gene (OPRD1). The DOR functions in nociceptive responses, but has also been shown to be involved in modulating the effects of MOR-directed compounds. For example, mice with targeted deletion of the *OPRD1* gene do not develop tolerance to the analgesic effects of morphine, although still becoming physically dependent on the drug (Zhu et al., 1999; Nitsche et al., 2002). The DOR also may play a role in responses to cocaine. Chronic binge cocaine administration attenuated the ability of the selective δ agonist [D-penicillamine^2, D-penicillamine^5] enkephalin to inhibit adenylyl cyclase in the caudate-putamen and nucleus accumbens (Unterwald et al., 1993).

Three studies have evaluated variants of the *OPRD1* gene for possible association with opiate addiction. Cloning of the *OPRD1* identified three exons containing the complete coding region; however, given that the rat and mouse genes have four exons, the possibility of yet-undiscovered exons remain (Simonin et al., 1994). Two coding region SNPs in the *OPRD1* gene have been defined and studied. These are both in the first exon: a T80G substitution, which results in the replacement of the amino acid phenylalanine with cysteine at position 27, and the synonymous substitution T921C (Mayer et al., 1997; Gelernter and Kranzler, 2000).

Mayer and colleagues studied 103 heroin addicts from the Rhine-Ruhr, Berlin, and Southern Bavaria areas of Germany and 115 unaffected individuals from the Rhine-Ruhr and Southern Bavaria regions, with the T921C variant ascertained in cases and controls (Mayer et al., 1997). This SNP is very common in this population, with 39% frequency of the 921C allele in control subjects. This study identified an association of the 921C allele with opiate addiction. No differences in genotype or allele frequencies were observed between samples collected from different regions within Germany within the case or control groups, respectively.

In a second study of 233 German heroin addicts meeting DSM-III-R criteria for heroin dependence and 173 ethnically and geographically matched controls (all subjects were from the Bonn area), no differences were found in genotype or allele frequencies for the T921C SNP between cases and controls (Franke et al., 1999). Also, an allele transmission test was performed on 90 additional heroin addicts and their parents. No difference in allele transmission to affected offspring was detected (Franke et al., 1999). A third study analyzed the *OPRD1* T921C SNP in 405 patients hospitalized for heroin detoxification (all meeting DSM-IV criteria for opiate abuse or dependence) from two cities in Southwestern China and population-based controls from the same geographic region (Xu et al., 2002). In this study, no differences were observed in genotype or allele frequencies between cases and controls. Genotyping of two unrelated (genomic control) variants (*ADH2* 47 His>Arg and *ALDH2* 487 Glu>Lys) suggested that cases and controls were appropriately matched in this sample. These studies bring into question the findings of Mayer and colleagues, and additional studies in diverse populations will be necessary to determine the importance of variants of the *OPRD1* gene in the development of opiate addiction.

d. Preproenkephalin Gene (PENK). Enkephalin peptides, which are derived from the processing of proenkephalin, function in the modulation of pain perception and are also suggested to play a role in reward and the addictions. For example, "knockout" mice lacking the preproenkephalin gene, develop physical dependence following morphine administration but do not develop tolerance to the antinociceptive effects of the drug (Nitsche et al., 2002). Additionally, mice lacking the enkephalin gene show reduced responding to food reinforcers, which suggests a general reduction in hedonic response (Hayward et al., 2002).

One study of 31 opioid-dependent non-Hispanic Caucasians and several matched control/contrast groups found an association of genotypes and alleles of a $(CA)_n$ repeat polymorphism in the 3′-flanking sequence and opiate dependence, as measured by DSM-III-R criteria (Comings et al., 1999). Preliminary findings of studies conducted by our group also suggest a difference in genotype frequencies between opioid-dependent cases and controls in Caucasian subjects, but not in other ethnic/cultural groups studied (K. S. LaForge, unpublished findings).

e. Preprodynorphin Gene (PDYN). As discussed above, naturally occurring peptides derived from prodynorphin (including dynorphin A, dynorphin B, and

α-neoendorphin) are the primary endogenous ligands of the κ opioid receptor. Microdialysis studies in rats demonstrated that dynorphin A(1–17) lowers basal dopamine tone in the nucleus accumbens, and studies in mice demonstrate that this peptide attenuates the increases in dopamine release in the nucleus accumbens caused by cocaine administration (Claye et al., 1997; Zhang et al., 2004). It is therefore hypothesized that dynorphins acting through the κ opioid receptor may counter-modulate the responses of the dopaminergic system to psychostimulants and, possibly, other drugs of abuse that also directly or indirectly increase synaptic dopamine release in brain reward circuitry. The well documented increases in striatal dynorphin peptide release and increases in mRNA levels caused by psychostimulant administration provide additional evidence that dynorphin is relevant for addictions (e.g., Sivam, 1989; Hurd and Herkenham, 1992; Hurd et al., 1992; Daunais et al., 1993; Spangler et al., 1993a,b).

Variants of the preprodynorphin gene have been studied in addiction to opiates, cocaine, and alcohol, as well as in in vitro functional studies. The most interesting variant is a 68-base repeat polymorphism located in the promoter region. This repeat was identified in the initial sequencing report of the gene (Horikawa et al., 1983) and is located approximately 1200 nucleotides upstream from the primary transcription initiation site identified by Geijer and colleagues (Geijer et al., 1995), thus placing it in the putative promoter region of the gene. Zimprich and colleagues studied this region in heroin addicts and controls and showed that the repeat is polymorphic, with alleles of one, two, three, and four copies identified (Zimprich et al., 2000). In that study, the alleles had overall frequencies of 2.7%, 32.0%, 63.5%, and 1.8%, for the one, two, three, and four repeat alleles, respectively, in control subjects of German Caucasian origin. Interestingly, the repeat contains a near-canonical activator protein 1 (AP-1) binding site that specifically binds the AP-1 protein complex. Using reporter gene constructs containing one to four copies of the repeat, Zimprich and colleagues also found that phorbol ester-induced transcription was greater in cells transfected with plasmids containing three or four copies of the repeat compared with one or two copies, and therefore this 68-base repeat polymorphism represents a potentially functional gene variant (Zimprich et al., 2000).

In their study of this variant and opiate addiction, Zimprich and colleagues found no differences in allele frequencies between heroin-addicted and control subjects (Zimprich et al., 2000). However, given the interactions between cocaine, dopamine, and dynorphin described above, we suggested this variant as an interesting candidate for cocaine addiction (LaForge et al., 2000b; Chen et al., 2002). In our study of cocaine abusing and dependent subjects and controls recruited in New York, we found that alleles containing three or four copies of the repeat were more common in control subjects compared with cases. These findings suggest that longer alleles (i.e., those with three or four copies of the repeat), which may result in greater transcriptional activation of the dynorphin gene and, therefore, greater levels of dynorphin peptides, may be protective against cocaine addiction. Given these provocative findings and the concordance of evidence from human genetic, animal, and in vitro molecular biological studies, further genetic studies into the role of allelic variants of the dynorphin gene in cocaine and other addictions are warranted.

2. Monoaminergic-Related Genes. The monoaminergic neurotransmitter systems include the catecholaminergic and the serotonergic systems. Dopamine, a catecholamine, functions as a neurotransmitter in the central nervous system, whereas norepinephrine, another catecholamine, is also a neurotransmitter in the brain and is the main postganglionic, sympathetic neurotransmitter (Molinoff and Axelrod, 1971). Dopamine is synthesized from the amino acid tyrosine by the subsequent actions of the tyrosine hydroxylase and 3-hydroxytyrosine decarboxylase enzymes. Dopamine can then be biotransformed to norepinephrine by the action of the enzyme DβH. Tryptophan is biotransformed by tryptophan hydroxylase and aromatic amino acid decarboxylase into serotonin. Upon the appropriate signal, e.g., membrane depolarization, the monoaminergic neurotransmitters are released from the presynaptic neurons into the synapse where they bind to both pre- and postsynaptic receptors. The dopamine, serotonin, and norepinephrine transporters then transport dopamine, serotonin, and norepinephrine, respectively, back into the presynaptic neuron for reutilization or degradation. There is a large body of evidence indicating interactions between the dopaminergic, serotonergic, and opioidergic systems in reward and drug dependence and withdrawal (Kreek et al., 2002). The dopaminergic pathways of the mesolimbic and nigrostriatal systems have been shown to be involved in the reward pathway. Disinhibition of dopaminergic neurons in the ventral tegmental area with opioid agonists such as the μ opioid receptor-directed peptides, e.g., β-endorphin, or pharmacological agents activate the reward pathway (Di Chiara and Imperato, 1988a; Herz, 1988).

Cocaine has been demonstrated to activate the expression of many genes in the nucleus accumbens through the downstream changes in adenylyl cyclase which produce an increase in cAMP levels. In response to increased cAMP, CREB (cAMP response element-binding protein) becomes activated via phosphorylation (Nestler, 2001). Phospho-CREB dimerizes with CREB-binding protein to activate transcription through binding to the CRE binding sites of several genes, thereby activating their transcription. Cocaine has been shown to activate a number of target genes in this manner in the nucleus accumbens, including preprodynorphin, preproenkepha-

lin, and several transcription factors (reviewed in McClung and Nestler, 2003).

Serotonin is the neurotransmitter of the serotonergic system. Serotonergic neurons originate in the raphe nuclei of the brain stem and project throughout the brain. Serotonergic function is believed to be involved in impulse control and behavioral suppression (Soubrie, 1986). Medications effective in the treatment of depression, including the prevention of suicidality such as selective serotonin reuptake inhibitors, have the serotonergic system as their sites of action.

Norepinephrine-containing neurons in the central nervous system originate from the locus ceruleus and the lateral tegmental nucleus, both located in the brain stem, and project to the cortex, spinal cord, and cerebellum. The noradrenergic system is involved in the perception of positive motivation. Medications used in the treatment of depression, such as selective serotonin reuptake inhibitors and reboxetine (a nontricyclic antidepressant norepinephrine uptake inhibitor not approved for use in the United States), have been shown in animals to decrease the firing of neurons in the locus ceruleus (Szabo et al., 1999; Szabo and Blier, 2001).

Cocaine's pharmacological actions are produced, in part, by its high-affinity binding to the dopamine transporter and lower-affinity binding to the serotonin transporter and the norepinephrine transporter by inhibiting reuptake of dopamine, serotonin, and norepinephrine, respectively (Uhl et al., 2002a; Rothman and Baumann, 2003). Mice with targeted disruption of the gene encoding the dopamine transporter will self-administer cocaine and establish cocaine-conditioned place preference (Rocha et al., 1998; Sora et al., 1998). In addition, mice with targeted disruption of the gene encoding the serotonin transporter or the norepinephrine transporter still retain conditioned place preference (Sora et al., 2001; Hall et al., 2002). However, mice with disruption of both the genes coding for the dopamine and serotonin transporters no longer display conditioned place preference indicating that both of these transporters are involved in cocaine's action (Sora et al., 2001).

Below we will discuss the relevance of five genes of the monoaminergic system, *DβH*, dopamine receptor D2 (*DRD2*), dopamine transporter (*SLC6A3*), serotonin transporter (*SLC6A4*), and norepinephrine transporter (*SLC6A2*). The structure of the three transporter genes and their relevant variants are shown in Fig. 1.

a. Dopamine β-Hydroxylase Gene (DβH). The enzyme DβH metabolizes dopamine into norepinephrine (Kaufman and Friedman, 1965; reviewed in Weinshilboum, 1978). Many centrally mediated cognitive, behavioral, and physiological functions are modulated by norepinephrine (Grace et al., 1998; Arnsten, 2000a,b). DβH is found within synaptic vesicles that store catecholamines. Most of the DβH is membrane-bound, whereas some is free within the vesicle (Stewart and Klinman, 1988). DβH is coreleased with catecholamines during synaptic transmitter release from neurons and from the neurosecretory cells of the adrenal medulla into the circulation. DβH is quite stable in plasma, with a 4.2-day half-life in rats (Grzanna and Coyle, 1977). Levels of DβH are highly correlated between the cerebrospinal fluid (CSF) and plasma, but vary widely between unrelated individuals (Weinshilboum et al., 1973). The interindividual variation of CSF DβH has been found in twin and family studies to be highly heritable in both

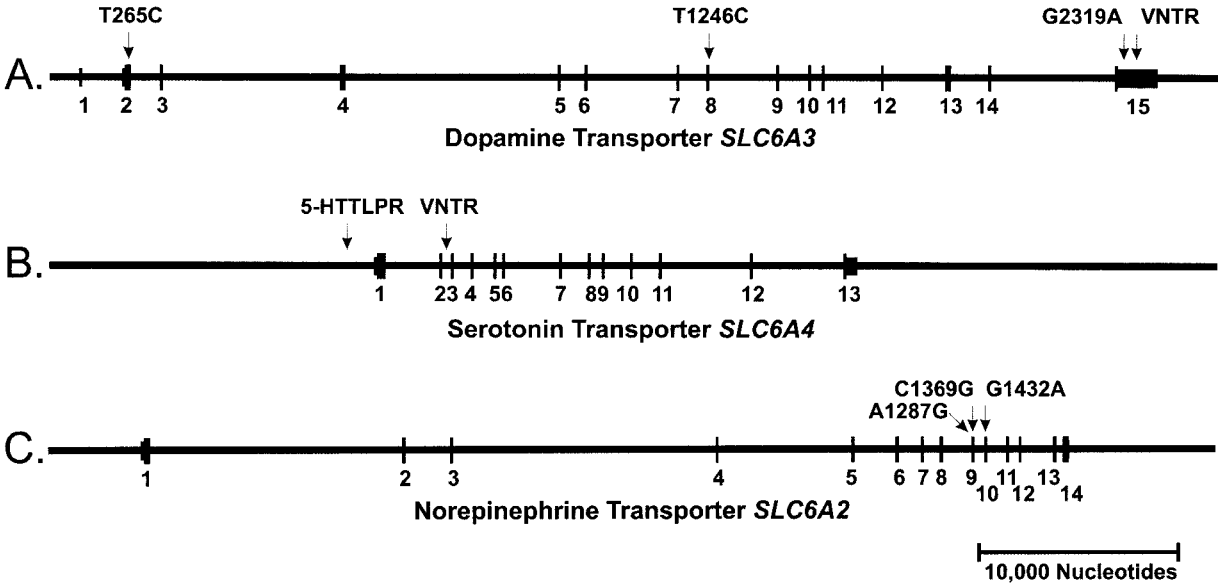

FIG. 1. Structures of the human dopamine transporter gene *SLC6A3*, the serotonin transporter gene *SLC6A4*, and the norepinephrine transporter gene *SLC6A2* and their relevant variants. A, the human dopamine transporter gene *SLC6A*. The exons are represented by black boxes and are numbered. The tall black boxes represent exonic coding regions and the short black boxes represent untranslated exonic regions. B, the serotonin transporter gene *SLC6A4*. C, the norepinephrine transporter gene *SLC6A2*. All three gene structures were derived from the genomic sequence as presented in the UCSC Genome Browser (http://genome.ucsc.edu/) (July 2003 build) and the GENATLAS (http://www.dsi.univ-paris5.fr/genatlas/).

CSF and plasma (Ross et al., 1973; Weinshilboum et al., 1973; Oxenstierna et al., 1986).

A number of polymorphisms at and near the *DβH* gene have been linked to variation in DβH levels (Wei et al., 1997a,b; Cubells et al., 1998, 2000; Zabetian et al., 2001). Initially, Wei and colleagues found an association between a short tandem $(GT)_n$ dinucleotide repeat located approximately 3000 nucleotides upstream of the *DβH* locus and plasma DβH in British subjects (Wei et al., 1997a). This association was confirmed in a group of European Americans (Cubells et al., 1998). A polymorphism located approximately 4700 nucleotides upstream from the *DβH* transcription initiation site (DBH*5′–ins/del, a biallelic 19 nucleotide insertion/deletion) and a synonymous SNP, G444A (DBH*444g/a), which encodes a guanine to adenosine substitution at nucleotide position 444, are in strong linkage disequilibrium and display similar associations to plasma DβH levels (Cubells et al., 1998, 2000). A haplotype analysis of the DBH*5′–ins/del and the G444A (DBH*444g/a) polymorphisms identified an association of the Del-a haplotype with low DβH and also with cocaine-induced paranoia in a group of American Caucasians who abuse cocaine (Cubells et al., 2000). A possibly functional variant, C-1021T (-1021C→T), was associated with plasma DβH activity in European Americans, African Americans, and Japanese (Zabetian et al., 2001). The C-1021T allele was associated with decreased plasma DβH levels in all the populations studied. When DβH activity was compared with this and 11 SNPs across the *DβH* locus, the C-1021T SNP was associated at the highest significance with DβH activity (Zabetian et al., 2003). Eight nonsynonymous (amino acid-substituting) SNPs have been identified in various populations in the *DβH* gene: three located in exon 3, G499C (499G→C or Ala197Thr), G589A (589G→A or Ala97Thr), and G675C (675G→T or Lys225Asn); two in exon 4, G706C (706G→C or Glu236Gln) and G826A (826G→A or Asp276Asn); two in exon 5, T908C (908T→C or Leu303Pro) and G910T (901G→T or Ala304Ser); and one in exon 11, C1603T (1603C→T or Arg535Cys) (Halushka et al., 1999; Zabetian et al., 2001). The Ala304Ser variant has been reported to produce altered activity in transient transfection assays (Ishii et al., 1991); however, others have not been able to replicate this finding (Li et al., 1996).

b. Dopamine Receptor D2 Gene (DRD2). Dopamine receptors appear to play a large role in the rewarding effects of drugs of abuse. Somato-dendritic DRD2 receptors on dopaminergic neurons in the ventral tegmental area are activated by dopamine to inhibit the mesolimbic dopaminergic pathway (Di Chiara and Imperato, 1988b; Koob, 1992). In addition, targeted ablation of the *DRD2* gene has been shown to remove the rewarding effects of opiates (Maldonado et al., 1997).

A polymorphism of the *DRD2* gene, *Taq*I A, is a nonfunctional restriction fragment length polymorphism (RFLP) located approximately 10,000 nucleotides downstream of the *DRD2* gene and has been repeatedly reported to be associated with, and not associated with, alcoholism (*meta*-analysis in Noble, 1998) (Hallikainen et al., 2003). The *Taq*I A RFLP has been associated with heroin use and methadone treatment outcome (Lawford et al., 2000), cocaine dependence (Noble et al., 1993), psychostimulant abuse (Persico et al., 1996), polysubstance abuse (O'Hara et al., 1993), and with early age of onset of multiple substance abuse (Comings et al., 1994). A *DRD2* promoter region variant (-141ΔC) was associated with heroin use in Chinese subjects (Li et al., 2002). When this group was subdivided by route of heroin administration, the association of the promoter variant was of greater significance in the inhaler group, whereas significance was not achieved for the injector group. Another RFLP of *DRD2*, located in intron 2 (*Taq*I B), also was reported to be significantly associated with polysubstance abuse in Caucasians, but not in African Americans (O'Hara et al., 1993) and in another study with cocaine dependence (Noble et al., 1993).

In a recent analysis of DRD2 binding in caudate nuclei isolated postmortem from Caucasians, the minor alleles at the *Taq*I A, *Taq*I B, and an intron 6 variant were significantly associated with lowered B_{max} (40% lower) of the DRD2 receptor (Ritchie and Noble, 2003). In a recent study of six synonymous variants in the *DRD2* gene, one of the variants, 957T, was found to cause a decrease in mRNA stability ($t_{1/2}$ 8 h → 4 h) and a 50% decrease in the translational efficiency of the *DRD2* mRNA (Duan et al., 2003). This C957T variant altered the predicted *DRD2* mRNA folding. Duan and colleagues also found that another naturally occurring variant in *DRD2*, 1101A, annulled the decrease in mRNA stability conferred by the 957T allele. In addition, it was demonstrated that dopamine stabilized the 957C *DRD2* mRNA, whereas there was little stabilization of the 957T *DRD2* mRNA. It has been postulated that subjects with reduced DRD2 receptor content may compensate this deficiency through the use of drugs that stimulate the dopaminergic system (Noble, 2000).

c. Dopamine Transporter Gene (SLC6A3). A key regulator of dopaminergic tone is the dopamine transporter, encoded by the gene *SLC6A3* (see Fig. 1), which regulates the reuptake of dopamine back into the presynaptic neuron, thereby terminating its action. The dopamine transporter is also the major site of action for cocaine's pharmacological actions accounting for cocaine's rewarding properties.

A SNP, G2319A, and a variable number of tandem repeats (VNTR), consisting of a repeat unit of 40 nucleotides, are found in the 3′-untranslated region in exon 15 of the *SLC6A3* gene. Subjects with the 9-/10-repeat genotype had a 22% reduction in dopamine transporter availability in the putamen than in subjects homozygous for the 10-repeat allele, as measured by single photon emission computed tomography and plasma radioligand levels, indicating that the VNTR affects expression of

the dopamine transporter (Heinz et al., 2000). However, using the same techniques and radioligand, Jacobson and colleagues found a 13% increase in striatal binding in the 9-/10-repeat heterozygotes compared with the 10-repeat homozygotes (Jacobsen et al., 2000b). In an in vitro reporter assay, the 10-repeat allele was found to have a greater transcriptional activity than that for the 7- or 9-repeat alleles (Fuke et al., 2001). However, the dopamine transporter VNTR alleles were not associated with the CSF neurometabolite homovanillic acid, the degradation product of dopamine, in a sample of mostly Swedish Europeans (Jönsson et al., 1998).

In a study by Gelernter and colleagues using a Caucasian sample, no association of this dopamine transporter variant with cocaine dependence was observed (Gelernter et al., 1994). However, they did find an association of the VNTR alleles with cocaine-induced paranoia, indicating a possible alteration in the efficiency of the dopamine transporter to remove dopamine from the synapse. A similar finding was reported by Ujike and colleagues, who showed that the 9- or fewer repeat alleles were associated with methamphetamine psychosis which lasted 1 month or more after discontinuing methamphetamine (Ujike et al., 2003). In addition, in a study of Chinese men, no association of the dopamine transporter VNTR alleles was observed in a study of methamphetamine abuse (Hong et al., 2003).

Two less common allelic variants which alter the coding sequence of the dopamine transporter are the T265C (Val55Ala) in exon 2 that substitutes a valine for an alanine at amino acid 55 in the intracellular N terminus and T1246C (Val382Ala) in exon 8, which substitutes a valine for an alanine at amino acid 382 in the fourth extracellular loop (Vandenbergh et al., 2000). Dopamine uptake velocity and cocaine analog binding were reduced by half with the 1246C (382Ala) allele in transient expression assays, whereas the 265C (55Ala) allele had 1.7-fold lower K_m for dopamine uptake (Lin and Uhl, 2003).

d. Serotonin Transporter Gene (SLC6A4). The serotonin transporter, encoded by the *SLC6A4* gene (see Fig. 1), is expressed on the presynaptic terminals of serotonergic neurons. The serotonin transporter directs the reuptake of serotonin from the synapse into the presynaptic neuron. As was noted above, targeted deletion of the serotonin transporter in mice has shown the involvement of this transporter, along with the dopamine transporter, in cocaine's mechanism of action.

In single photon emission computed tomography studies with a serotonin transporter ligand, cocaine-dependent subjects had significantly higher serotonin transporter binding sites in the brainstem and diencephalon (Jacobsen et al., 2000a). However, Little and colleagues found, in postmortem brain studies using quantitative autographic and in situ hybridization assays, lowered serotonin transporter binding in cocaine addicts compared with controls in the dorsal raphe, median raphe, and substantia nigra (Little et al., 1998).

The promoter of the serotonin transporter gene contains a GC-rich 44-nucleotide insertion/deletion (5-HTTLPR). This polymorphic site occurs in a repetitive element located approximately 1400 nucleotides upstream from the transcription start site (Lesch et al., 1996). The short variant (*s*) has been shown in transfection experiments in vivo to have half the transcriptional rate of the long variant (*l*) (Lesch et al., 1996), and the *s* variant has an attenuated gene expression response to the synthetic glucocorticoid dexamethasone compared with the *l* variant (Glatz et al., 2003). Serotonin transporter binding sites and mRNA levels in the dorsal raphe, median raphe, and the substantia nigra of postmortem brain also varied by genotype with the highest level of binding and mRNA in brain regions from subjects with the *l/l* genotype, with lower binding and mRNA levels in the *l/s* and *s/s* genotypes (Little et al., 1998).

In a group of Caucasian Italians the serotonin transporter low activity *s/s* genotype was found to be associated with heroin dependence (Gerra et al., 2004). In addition, the *s/s* genotype was also associated with aggression within the heroin-dependent group. This serotonin transporter promoter polymorphism was found not to be associated with cocaine dependence in African Americans (Patkar et al., 2001), nor with heroin addiction in the Chinese subjects (Li et al., 2002).

A VNTR is located in the second intron of the serotonin transporter with three alleles: a 9-, 10-, and 12-repeat of a 16–17 nucleotide element. This polymorphism has been shown, in differentiating embryonic stem cells, to possess allele-dependent differential enhancer activity (MacKenzie and Quinn, 1999). In a case-control study of Chinese heroin addicts and controls, an association of the 10-repeat allele with heroin addiction was found (Tan et al., 1999). However, no such association of this serotonin transporter VNTR was observed in a study of cocaine dependence in African Americans (Patkar et al., 2002) or in methamphetamine abuse in Chinese subjects (Hong et al., 2003).

e. Norepinephrine Transporter Gene (SLC6A2). Similar to the other monoamine transporters, the norepinephrine transporter clears its cognate monoamine neurotransmitter, norepinephrine, from synapses in the brain and peripheral nervous system by reuptake into presynaptic neurons. The norepinephrine transporter is the site of action of many of the tricyclic antidepressants (e.g., desipramine). The norepinephrine transporter can transport both norepinephrine and dopamine (Horn, 1973; Raiteri et al., 1977) and has been found to have a higher binding affinity for dopamine than does the dopamine transporter (Giros et al., 1994; Gu et al., 1994; Eshleman et al., 1999). Targeted disruption of *SLC6A2* in mice produces an increase in extracellular norepinephrine and a decrease in intracellular norepinephrine in the brain (Xu et al., 2000). Further studies in norepi-

nephrine transporter knockout mice have demonstrated that dopamine uptake in the frontal cortex probably occurs through the action of the norepinephrine transporter (Moron et al., 2002). In these knockout mice, morphine treatment produced greater analgesia than in wild-type mice, indicating that the effect of tricyclic antidepressants, which block reuptake of norepinephrine by binding to the norepinephrine transporter, may function by enhancing the effects of endogenous opioids (Bohn et al., 2000). These knockout mice were hyperresponsive to locomotor stimulation by cocaine or amphetamine (Xu et al., 2000).

Screening of the norepinephrine transporter (*SLC6A2*) gene has identified one promoter variant (T-182C), five nonsynonymous (amino acid altering), and eight synonymous or intronic variants (Stober et al., 1996; 1999). The nonsynonymous coding region variants G205A (Val69Ile), C296T (Thr99Ile), G733A (Val245Ile), G1345A (Val449Ile), and G1432A (Gly478Ser) were all located in transmembrane domains of the norepinephrine transporter in exons 1, 2, 4, 9, and 10, respectively, and in transmembrane domains 1, 2, 4, 9, and 10, respectively. When these five nonsynonymous variants were assayed in cellular constructs, the only difference between these variants and the wild-type norepinephrine transporter was with the G1432A (Gly478Ser) variant (Runkel et al., 2000). This G1432A variant coded for a protein that displayed a 4-fold higher K_m for norepinephrine with no effect on V_{max}, indicative of an altered substrate recognition domain. A synonymous variant, A1287G, has been shown to be associated with CSF 3-methoxy-4-hydroxyphenylglycol, a norepinephrine neurometabolite, in Caucasians (Jönsson et al., 1998). Another nonsynonymous variant, C1369G, results in the substitution of an alanine to proline at amino acid 457 in transmembrane domain 9 of the norepinephrine transporter (Shannon et al., 2000; Paczkowski et al., 2002). The 1369C allele, which codes for the 457Pro substitution, in cellular constructs in transient transfection assays exhibited a 5-fold higher binding affinity for cocaine, a 50-fold higher K_m (lower apparent affinity), a 2-fold higher turnover (V_{max}/B_{max}) for norepinephrine, and a 2-fold lower binding affinity for nisoxetine, a norepinephrine transporter inhibitor, compared with the 457Ala prototype transporter (Paczkowski et al., 2002).

IV. Treatment of Addictions

A. Pharmacotherapies

1. Opiate and Opioid Addictions. Since the original studies of the treatment of heroin addiction with methadone performed at The Rockefeller University by Dole, Nyswander, and Kreek in 1964, significant progress has been made in the understanding of the addictive diseases (Dole et al., 1966). Methadone maintenance treatment has become the standard of treatment for heroin addiction and remains the most efficacious treatment agent in the armamentarium for addictive diseases. Since these initial studies, two other opioid medications have been approved for use in the treatment of heroin addiction, LAAM and buprenorphine (preferably combined with naloxone). Unlike short-acting injected opiates, each of these medications is long-acting and orally (or, in the case of buprenorphine, sublingually) available thereby reducing their pharmacologically and behaviorally reinforcing properties.

a. Methadone. Methadone is a synthetic opioid with high (>90%) oral bioavailability. Following oral administration, peak plasma levels are achieved by 2 to 4 h and maintained for approximately 24 h (Inturrisi and Verebely, 1972a,b; Kreek, 1973). There is significant cross-tolerance between methadone and other opiates which contribute to its ability to ameliorate withdrawal, reduce craving, and block the euphoric effects of coadministered illicit opiates (Dole et al., 1966). Despite cross-tolerance to other opiates, once steady-state dosing is achieved, little further tolerance to methadone develops (possibly through the modest *N*-methyl-D-aspartate antagonism provided by both enantiomers of methadone) allowing for stable dosing, typically in the range of 80 to 120 mg per day (Gorman et al., 1997; Davis and Inturrisi, 1999; Callahan et al., 2004).

Following administration, methadone is more than 90% plasma protein bound (primarily by albumin but also by globulins) and slowly released in unmetabolized form from the liver which contributes to its extended period of activity when administered chronically (Kreek et al., 1978). Methadone is *N*-demethylated in the liver by the cytochrome P450 enzyme family (mostly CYP3A4, but also CYP2D6 and CYP1A2) to inactive metabolites which undergo oxidative metabolism and are excreted in both the urine and the feces (Bowen et al., 1978). Methadone is a full and highly selective MOR agonist. Once steady state is achieved, PET with the μ-preferring opioid antagonist [^{18}F]cyclofoxy in stable dose methadone-maintained former heroin addicts has revealed that approximately 67 to 81% of MOR in different brain regions are unoccupied by methadone and remain available for their usual physiological roles (Kling et al., 2000).

b. Levo-α-acetylmethadol. LAAM is a methadone congener, which, like methadone, also undergoes *N*-demethylation through a CYP3A4-mediated microsomal pathway. Unlike the demethylated metabolites of methadone, the metabolites of LAAM, nor-LAAM, and dinor-LAAM are pharmacologically active and protein bound, contributing to LAAM's extended period of activity (up to 96 h) (Sullivan et al., 1973). Like methadone, LAAM also achieves steady-state occupation of MOR, although the extent of this in vivo in humans has not been fully determined. Ten cases of potential adverse cardiac effects attributed to LAAM caused the European Agency for the Evaluation of Medicinal Products to recommend that LAAM be removed from the market throughout the European Union. In February 2004, citing potential

problems related to prolongation of the cardiac QT interval, Roxane, the U.S. marketer of LAAM (brand name Orlaam), ceased LAAM production, although it remains approved by the Food and Drug Administration for the treatment of heroin addiction.

c. Buprenorphine. Buprenorphine is a synthetic opioid with primarily MOR partial agonism and modest κ opioid receptor activity. It is available for heroin detoxification as a single agent but marketed primarily in the United States in a combination preparation with naloxone (to reduce intravenous abuse potential) for maintenance therapy. Buprenorphine has a much shorter terminal half-life (3 to 5 h) than either methadone (24 to 36 h) or LAAM (greater than 48 h) but dissociates slowly from MOR over 24 to 48 h, allowing for daily or even once every 3-day dosing (Nath et al., 1999). Because it is a partial agonist with strong MOR affinity, buprenorphine can induce withdrawal symptoms in moderately to highly opiate tolerant individuals, and it is recommended that buprenorphine not be administered until tolerance is reduced or withdrawal symptoms are apparent. As a partial MOR agonist, buprenorphine reaches a ceiling effect following doses greater than 24 to 32 mg sublingually (Walsh et al., 1994). A PET study using the MOR agonist [^{11}C]carfentanil performed in heroin addicts maintained on varying doses of buprenorphine for 21 to 68 days found that whole brain MOR occupancy is directly related to buprenorphine dose with 41% occupancy following 2 mg and 84% occupancy following 32 mg (Greenwald et al., 2003). Whether this increase in MOR occupancy compared with that seen in methadone maintenance prevents a return to normal physiological function remains uncertain.

Because of extensive first-pass metabolism buprenorphine is administered sublingually. Buprenorphine is dealkylated by CYP3A4 to the metabolite norbuprenorphine, which is also active at MOR (Kobayashi et al., 1998). Intravenously administered buprenorphine can be euphorogenic and cause life-threatening respiratory depression in nonopiate tolerant individuals and is a significant drug of abuse in several countries (Kintz, 2001). By combining buprenorphine with the MOR-preferring antagonist naloxone, the immediate reinforcing euphorigenic effects of intravenously administered buprenorphine can be significantly reduced (Stoller et al., 2001). Naloxone, however, has low oral bioavailability (<3%) and, following sublingual administration of the combination product, does not lead to increased withdrawal symptoms compared with buprenorphine alone (Stoller et al., 2001). When administered as part of a well structured clinical or private medical office setting, opioid agonist therapy with methadone, LAAM, or buprenorphine can have 1-year treatment retention rates greater than 80% (Johnson et al., 2000; Kakko et al., 2003).

d. Naltrexone. In areas where regulatory constraints prevent agonist therapy from being used (e.g., in opioid-addicted physicians in some, but not all, states), opioid antagonist therapy with naltrexone is attempted. Drug-free retention rates are often less than 20%, and this form of management should not be considered a first-line treatment (San et al., 1991; Kakko et al., 2003).

e. Clonidine. Other medications used in the treatment of opiate addiction are the α-2-adrenergic receptor agonists clonidine and lofexidine (the latter not approved for use in the United States). These medications are hypothesized to reduce the action of excess norepinephrine in the locus ceruleus during acute opiate withdrawal; they have been shown to ameliorate some signs and symptoms, such as the increased lacrimation, rhinorrhea, and gastrointestinal distress experienced during acute opiate withdrawal but have little effect on others.

2. Cocaine Addiction.

a. Antidepressants. There are no effective pharmacotherapeutics for cocaine addiction (Lima et al., 2002). Tricyclic antidepressants and serotonin reuptake inhibitors have been widely used in the treatment of cocaine addicts but have not been widely efficacious. In some individuals with underlying comorbid psychiatric conditions, these medications may have a slight benefit.

b. Disulfiram. Disulfiram, traditionally used with minimal success in the treatment of alcoholism, has received attention for the treatment of cocaine addiction because of its ability to block the conversion of dopamine to norepinephrine through inhibition of the enzyme DβH (Carroll et al., 1993, 2004; Higgins et al., 1993). This compound is now under study to determine whether it has any general usefulness in the treatment of cocaine addiction.

c. Dopaminergic Agents. Therapeutics such as methylphenidate and other medications (e.g., bromocriptine, pergolide, and amantadine), which act as dopamine agonists, also show limited efficacy in the treatment of cocaine addiction (Khantzian et al., 1984; Grabowski et al., 1997; Handelsman et al., 1997; Malcolm et al., 2000; Shoptaw et al., 2002). The efficacy of methylphenidate, however, may be apparent only in cocaine addicts with comorbid attention deficit/hyperactivity disorder rather than cocaine addicts in general (Gawin et al., 1985; Levin et al., 1998; Schubiner et al., 2002).

d. $GABA_A$-$GABA_B$ Directed Drugs Indirectly modulating dopamine through agonism of GABAergic neurons has also been studied as a means of treating cocaine addiction. Trials using the $GABA_B$ agonist baclofen have shown reductions in cocaine craving, but long-term outcome studies looking at craving and continued cocaine use are needed (Ling et al., 1998; Shoptaw et al., 2003). The GABA analog gabapentin has also been evaluated and has shown modest benefit, although larger placebo-controlled efficacy trials are needed (Myrick et al., 2001).

Although none of the medications for cocaine addiction mentioned above have achieved the success of phar-

macotherapeutics used for heroin addiction, or even pharmacotherapeutics for alcohol addiction, the recent advances in human molecular genetics related to their proposed mechanisms of action make them worthy of discussion and further pharmacogenetic and pharmacotherapeutic investigation.

V. Pharmacogenetics Related to the Treatment of Addictions

Variants of genes encoding proteins involved in the metabolism or biotransformation of drugs of abuse may affect vulnerability to develop an addiction. A frequently cited example is the SNP encoded dysfunction of aldehyde dehydrogenase (produced by the *ALDH2*2* allele variant), an enzyme responsible for the biotransformation of the toxic alcohol metabolite acetaldehyde (Yoshida et al., 1984, 1991). This SNP is present in approximately 40% of Asians and results in a flushing response following alcohol consumption that most people find unpleasant (Harada et al., 1980; Higuchi et al., 1992). Subjects homozygous for the *ALDH2*2* allele were less likely to become alcohol dependent than heterozygous subjects who were less likely to be alcohol dependent than subjects with the prototypic gene (Harada et al., 1982; Higuchi et al., 1992, 1996). Variants of genes encoding proteins involved in the metabolism of opiates and cocaine have been identified and may be associated with the vulnerability to develop or affect the treatment of the addictive diseases. Most of the enzymes involved in opiate metabolism are part of the P450 family of microsomal enzymes; however, heroin and morphine also undergo non-P450-mediated biotransformation.

A. Metabolism/Biotransformation of Opiates and Other Opioids

1. Morphine and Heroin. Morphine, a phenanthrene alkaloid, still is derived today (due to the difficulty of synthetic production) from the milky extract of the unripe seed pods of the poppy plant, *Papaver somniferum*. Morphine comprises approximately 10% of the opium extract from the plant. Diacetylmorphine (heroin) was first synthesized in 1874 and then marketed as heroin in 1898 by Bayer. Heroin is a lipid soluble prodrug that exerts its effect only after metabolism to 6-monoacetylmorphine (6-MAM) and morphine. Heroin has little oral bioavailability and undergoes complete first-pass metabolism with blood clearance greater than the upper limit of hepatic blood flow, indicating additional extrahepatic metabolic factors.

Heroin is metabolized to 6-MAM and then morphine by hydrolysis of ester linkages catalyzed by three esterases: pseudocholinesterase, human carboxylesterase-1 (hCE-1), and human carboxylesterase-2 (hCE-2). In humans, heroin is metabolized by hydrolysis of the 3-acetyl group to 6-MAM in the liver by hCE-1 and hCE-2, in the serum by pseudocholinesterase, and also nonenzymatically in the serum. Whereas all three enzymes catalyze the rapid hydrolysis of heroin to 6-MAM, only hCE-2 catalyzes hydrolysis of 6-MAM to morphine with high efficiency (Kamendulis et al., 1996).

Morphine undergoes glucuronidation by uridine diphosphate glucuronosyltransferases (UDP glucuronosyltransferases) to the inactive metabolite morphine-3-glucuronide (M3G) and, to a lesser extent, the MOR agonist M6G. A recent study of five subjects with Gilbert syndrome, characterized by impaired glucuronidation due to a polymorphism in the gene encoding UDP glucuronosyltransferase (UGT) 1A1, did not show altered morphine clearance or difference in the plasma concentration versus time curves for M6G or M3G compared with controls (Skarke et al., 2003b). A promoter region SNP (C-161T) in the gene encoding UGT 2B7 (*UGT2B7*) has been identified in individuals with low rates of glucuronidation, and subjects with this SNP show reduced M6G/morphine ratios; since this SNP was in complete linkage disequilibrium with the nonsynonymous C802T SNP (His268Tyr) in exon 2, it is unclear which of these two SNPs is the functional variant (Sawyer et al., 2003). Other studies have also identified promoter or coding region polymorphisms of *UGT2B7*, however, none significantly alter morphine clearance or M6G and M3G formation and clearance (Holthe et al., 2002, 2003; Duguay et al., 2004). Further studies to identify functional polymorphisms and their effect on morphine metabolism are needed to determine whether there are physio- or pharmacogenetic factors contributing to morphine addiction and/or analgesia.

2. Codeine. Most opiates (other than heroin and morphine) are metabolized by P450 enzymes. While a portion of codeine undergoes glucuronidation, it is also *O*-demethylated (as are its congeners oxycodone and hydrocodone) to the active and more potent metabolite morphine (oxymorphone and hydromorphone for oxycodone and hydrocodone, respectively) by CYP2D6. Over 60 variants (including gene duplications, gene deletions, alternative splicing, insertions and deletions with changes in the reading frame, and SNPs imparting amino acid substitutions) of the *CYP2D6* gene have been identified (for a review, see Howard et al., 2002). Some of these variants increase metabolism of these drugs into their more potent metabolites, whereas others decrease metabolism. The analgesic potency and abuse liability of opioid medications may, therefore, be influenced by variants in this gene (Sindrup et al., 1991, 1993; Kathiramalainathan et al., 2000). Tyndale et al. (1997) reported that no subjects meeting DSM-IV criteria for oral opioid dependence had either of the defective mutant alleles *CYP2D6*3* or *CYP2D6*4* which reduce metabolism compared with control and multidrug-dependent individuals whose frequency for these alleles did not differ from that of previously reported Caucasian control populations. Pharmacological inhibition of CYP2D6 with fluoxetine or quinidine, which significantly reduces the formation

of morphine following codeine administration (Kathiramalainathan et al., 2000; Romach et al., 2000), however, failed to reduce daily codeine intake in a small cohort of codeine addicts (Fernandes et al., 2002). The effect of *CYP2D6* alleles resulting in low versus ultrarapid metabolism has been investigated in methadone-maintained subjects. Although the metabolism of methadone is primarily mediated by CYP3A4, the investigators did find a significant decrease in dose-to-weight corrected methadone concentrations in the ultrarapid metabolizers, but this did not appear to influence treatment outcome compared with the low metabolizer group (Eap et al., 2001).

3. Methadone, Levo-α-acetylmethadol, and Buprenorphine. The standard medications used in the treatment of opiate addiction, methadone, LAAM, and buprenorphine, are all primarily metabolized by CYP3A4. Concomitant use of medications that induce (e.g., rifampin, phenytoin) or inhibit (e.g., fluoxetine, cimetidine, saquinavir) CYP3A4 may result in withdrawal symptoms or sedation, respectively. Polymorphisms that affect CYP3A4 function may similarly influence the efficacy of these treatment agents. Over 20 variants of *CYP3A4* have been identified (http://www.imm.ki.se/CYPalleles/cyp3a4.htm), and two studies using cellular constructs have identified variants that increase or decrease CYP3A4 function and alter testosterone (a CYP3A4 substrate) metabolism (Dai et al., 2001; Eiselt et al., 2001). The functional effects of other *CYP3A4* variants have not been determined and there are no reports on whether *CYP3A4* variants alter the metabolism of medications used in the treatment of the addictive diseases.

B. Metabolism/Biotransformation of Cocaine

Cocaine is a tropane ester alkaloid extracted from the leaves of the coca bush, *Erythroxylon coca*, which grows in the Andean region of South America. Similar to heroin, cocaine metabolism is catalyzed by pseudocholinesterase, hCE-1, and hCE-2. Hydrolysis of cocaine to ecgonine methyl ester is catalyzed by pseudocholinesterase and hCE-2. Ecgonine methyl ester is then nonenzymatically hydrolyzed. hCE-1 catalyzes transesterification of cocaine to cocaethylene, a toxic metabolite, in the presence of ethanol and also hydrolysis to benzoylecgonine, the primary metabolite excreted in the urine. Cocaethylene can be further hydrolyzed by hCE-1 or hCE-2, producing benzoylecgonine or ecgonine ethyl ester, respectively (Dean et al., 1991; Brzezinski et al., 1994; Laizure et al., 2003).

Phenotypic variation in pseudocholinesterase is associated with prolonged apnea in patients receiving the muscle relaxant drug succinylcholine during surgery. The dibucaine number, a method for measuring activity of pseudocholinesterase, has for many years been used to identify atypical phenotypes of this enzyme that display decreased or even complete absence of activity (Kalow and Genest, 1957; Kalow and Staron, 1957). Several genetic variants have been identified that are responsible for some of these phenotypic abnormalities (e.g., McGuire et al., 1989; Nogueira et al., 1990a,b; Maekawa et al., 1997). An earlier investigation of serum from a person identified as having an inactive phenotype of this enzyme revealed that it did not hydrolyze heroin, and serum from a patient with an atypical, partially active, phenotype hydrolyzed heroin but with less efficiency than the typical enzyme (Lockridge et al., 1980). More recently, the activity of several human cholinesterase variants with cocaine has been examined (Xie et al., 1999). One atypical cholinesterase (Asp70Gly) had 10-fold lower binding efficiency for cocaine and 10-fold lower catalytic efficiency (k_{cat}/K_m). Although this evidence suggests the possibility that individual responses to heroin and cocaine may be mediated in part by greater or lesser metabolic capacity, genetically determined variations in cholinesterase activity, or in activity of the carboxylesterase (referred to above), have not been investigated in persons with addictive diseases.

VI. Summary

Herein we have reviewed a number of genes that appear to be involved in the vulnerability to and the treatment of heroin and/or cocaine addiction. The evidence for the roles of a few of these genes and the influence of their variants in these diseases remain tenuous and will require replication, whereas the roles of others appear to be fairly well established. It must be remembered that association or linkage results may not directly identify a functional polymorphism, but may only indicate that a functional polymorphism is in linkage disequilibrium. Functional variants may alter the primary structure of the resultant protein, modify the transcriptional profile of the gene, alter splicing or stability of the mRNA, or alter translational efficiency. Furthermore, some alleles may only exert an effect in the context of other alleles within the same gene or of other genes.

To further elucidate the genetic variability that may contribute to the vulnerability, acquisition, and treatment of cocaine and/or heroin addiction, studies will be required to identify both new alleles as well as to confirm the role of previously identified alleles. If the diseases of addiction are to be understood, treated, and prevented, these studies will be necessary and invaluable. However, genetics are only one aspect that contributes to the development of heroin or cocaine addiction. Environmental factors are also of importance. To further our knowledge into the physiology of these addictions, the interplay of genes with environmental factors will have to be evaluated to a greater extent. Exposure to heroin or cocaine are necessary factors. Investigations will have to take into account how specific genes and their specific alleles interact with each other and with

the environment. New analysis techniques such as haplotype analyses should aid in attaining these goals. To develop novel pharmacotherapies as well as behavioral therapies and to create new prevention and treatment programs, the roles of genes, their variants, and the environment in which they are expressed will have to be elucidated.

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References

Arnsten AF (2000a) Through the looking glass: differential noradrenergic modulation of prefrontal cortical function. *Neural Plast* **7:**133–146.

Arnsten AF (2000b) Stress impairs prefrontal cortical function in rats and monkeys: role of dopamine D1 and norepinephrine α-1 receptor mechanisms. *Prog Brain Res* **126:**183–192.

Azaryan AV, Coughlin LJ, Buzas B, Clock BJ, and Cox BM (1996) Effect of chronic cocaine treatment on μ- and δ-opioid receptor mRNA levels in dopaminergically innervated brain regions. *J Neurochem* **66:**443–448.

Bart G, Borg L, Schluger JH, Green M, Ho A, and Kreek MJ (2003) Suppressed prolactin response to dynorphin A(1–13) in methadone maintained versus control subjects. *J Pharmacol Exp Ther* **306:**581–587.

Bart G, Heilig M, LaForge KS, Pollak L, Leal SM, Ott J, and Kreek MJ (2004a) Substantial attributable risk related to functional μ-opioid receptor gene polymorphism in association with heroin addiction in central Sweden. *Mol Psychiatry* **9:**547–549.

Bart G, Heilig M, Proudnikov D, LaForge KS, Pollak L, Ott J, and Kreek MJ (2004b) Increased attributable risk related to a functional μ-opioid receptor gene polymorphism in association with alcohol dependence in central Sweden (Abstract). *Neuropsychopharmacology*, in press.

Becker A, Grecksch G, Brodemann R, Kraus J, Peters B, Schroeder H, Thiemann W, Loh HH, and Höllt V (2000) Morphine self-administration in μ-opioid receptor-deficient mice. *Naunyn-Schmiedeberg's Arch Pharmacol* **361:**584–589.

Becker A, Grecksch G, Kraus J, Loh HH, Schroeder H, and Höllt V (2002) Rewarding effects of ethanol and cocaine in mu opioid receptor-deficient mice. *Naunyn-Schmiedeberg's Arch Pharmacol* **365:**296–302.

Beckett AH and Casey AF (1954) Stereochemistry of certain analgesics. *Nature (Lond)* **173:**1231–1232.

Befort K, Filliol D, Decaillot FM, Gaveriaux-Ruff C, Hoehe MR, and Kieffer BL (2001) A single nucleotide polymorphic mutation in the human μ-opioid receptor severely impairs receptor signaling. *J Biol Chem* **276:**3130–3137.

Begleiter H, Reich T, Nurnberger J Jr, Li TK, Conneally PM, Edenberg H, Crowe R, Kuperman S, Schuckit M, Bloom F, et al. (1999) Description of the Genetic Analysis Workshop 11 Collaborative Study on the genetics of alcoholism. *Genet Epidemiol* **17:**S25–S30.

Belknap JK and Crabbe JC (1992) Chromosome mapping of gene loci affecting morphine and amphetamine responses in BXB recombinant inbred mice. *Ann NY Acad Sci* **654:**311–323.

Belknap JK, Mogil JS, Helms ML, Richards SP, O'Toole LA, Bergeson SE, and Buck KJ (1995) Localization to chromosome 10 of a locus influencing morphine analgesia in crosses derived from C57BL/6 and DBA/2 strains. *Life Sci* **57:**PL117–PL124.

Bergen AW, Kokoszka J, Peterson R, Long JC, Virkkunen M, Linnoila M, and Goldman D (1997) μ opioid receptor gene variants: lack of association with alcohol dependence. *Mol Psychiatry* **2:**490–494.

Bergen AW, Yang XR, Bai Y, Beerman MB, Goldstein AM, and Goldin LR (2003) Genomic regions linked to alcohol consumption in the Framingham Heart Study. *BMC Genet* **4:**S101.

Berrettini WH, Ferraro TN, Alexander RC, Buchberg AM, and Vogel WH (1994) Quantitative trait loci mapping of three loci controlling morphine preference using inbred mouse strains. *Nat Genet* **7:**54–58.

Berrettini WH, Hoehe MR, Ferrada TN, and Gottheil E (1997) Human mu opioid receptor gene polymorphisms and vulnerability to substance abuse. *Addict Biol* **2:**303–308.

Bierut LJ, Dinwiddie SH, Begleiter H, Crowe RR, Hesselbrock V, Nurnberger JI Jr, Porjesz B, Schuckit MA, and Reich T (1998) Familial transmission of substance dependence: alcohol, marijuana, cocaine, and habitual smoking: a report from the Collaborative Study on the Genetics of Alcoholism. *Arch Gen Psychiatry* **55:**982–988.

Bohn LM, Xu F, Gainetdinov RR, and Caron MG (2000) Potentiated opioid analgesia in norepinephrine transporter knock-out mice. *J Neurosci* **20:**9040–9045.

Bond C, LaForge KS, Tian M, Melia D, Zhang S, Borg L, Gong J, Schluger J, Strong JA, Leal SM, et al. (1998) Single nucleotide polymorphism in the human mu opioid receptor gene alters β-endorphin binding and activity: possible implications for opiate addiction. *Proc Natl Acad Sci USA* **95:**9608–9613.

Bowen DV, Smit ALC, and Kreek MJ (1978) Fecal excretion of methadone and its metabolites in man: application of GC-MS, in *Advances in Mass Spectrometry* (Daly NR ed) pp 1634–1639, Heyden and Sons, Philadelphia, PA.

Bradbury AF, Smyth DG, Snell CR, Birdsall NJM, and Hulme EC (1976) C fragment of lipotropin has a high affinity for brain opiate receptors. *Nature (Lond)* **260:**793–795.

Brown RA and Armelagos GJ (2001) Apportionment of racial diversity: a review. *Evol Anthropol* **10:**34–40.

Brzezinski MR, Abraham TL, Stone CL, Dean RA, and Bosron WF (1994) Purification and characterization of a human liver cocaine carboxylesterase that catalyzes the production of benzoylecgonine and the formation of cocaethylene from alcohol and cocaine. *Biochem Pharmacol* **48:**1747–1755.

Callahan RJ, Au JD, Paul M, Liu C, and Yost CS (2004) Functional inhibition by methadone of *N*-methyl-D-aspartate receptors expressed in *Xenopus* oocytes: stereospecific and subunit effects. *Anesth Analg* **98:**653–659.

Carroll KM, Fenton LR, Ball SA, Nich C, Frankforter TL, Shi J, and Rounsaville BJ (2004) Efficacy of disulfiram and cognitive behavior therapy in cocaine-dependent outpatients. *Arch Gen Psychiatry* **61:**264–272.

Carroll KM, Ziedonis D, O'Malley SS, McCance-Katz E, Gordon LT, and Rounsaville BJ (1993) Pharmacological interventions for abusers of alcohol and cocaine: a pilot study of disulfiram versus naltrexone. *Am J Addict* **2:**77–79.

Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A, and Poulton R (2002) Role of genotype in the cycle of violence in maltreated children. *Science (Wash DC)* **297:**851–854.

Chen ACH, LaForge KS, Ho A, McHugh PF, Bell K, Schluger RP, Leal SM, and Kreek MJ (2002) A potentially functional polymorphism in the promoter region of prodynorphin gene may be associated with protection against cocaine dependence or abuse. *Am J Med Genet* **114:**429–435.

Chen Y, Mestek A, Lui J, Hurley JA, and Yu L (1993) Molecular cloning and functional expression of a μ-opioid receptor from rat brain. *Mol Pharmacol* **44:**8–12.

Claye LH, Maisonneuve IM, Yu J, Ho A, and Kreek MJ (1997) Local perfusion of dynorphin A_{1-17} reduces extracellular dopamine levels in the nucleus accumbens, in *Problems of Drug Dependence, 1996; Proceedings of the 58th Annual Scientific Meeting of the College on Problems of Drug Dependence. National Institute of Drug Abuse Research Monograph Series* (Harris LS ed) pp 113, U.S. Department of Health and Human Services, National Institutes of Health. NIH Publication No (ADM)97-4236, Washington DC.

Cloninger R, Bohman M, and Sigvardsson S (1981) Inheritance of alcohol abuse: cross-fostering analysis of adopted men. *Arch Gen Psychiatry* **38:**861–868.

Comb M, Seeburg PH, Adelman J, Eiden L, and Herbert E (1982) Primary structure of the human Met- and Leu-enkephalin precursor and its mRNA. *Nature (Lond)* **295:**663–666.

Comings DE, Blake H, Dietz G, Gade-Andavolu R, Legro RS, Saucier G, Johnson P, Verde R, and MacMurray JP (1999) The proenkephalin gene (*PENK*) and opioid dependence. *Neuroreport* **10:**1133–1135.

Comings DE, Muhleman D, Ahn C, Gysin R, and Flanagan SD (1994) The dopamine D2 receptor gene: a genetic risk factor in substance abuse. *Drug Alcohol Depend* **34:**175–180.

Cox BM, Goldstein A, and Li CH (1976) Opioid activity of a peptide, β-lipotropin-(61-91), derived from β-lipotropin. *Proc Natl Acad Sci USA* **73:**1821–1823.

Crabbe JC, Phillips TJ, Buck KJ, Cunningham CL, and Belknap JK (1999) Identifying genes for alcohol and drug sensitivity: recent progress and future directions. *Trends Neurosci* **22:**173–179.

Crowley JJ, Oslin DW, Patkar AA, Gottheil E, DeMaria PA Jr, O'Brien CP, Berrettini WH, and Grice DE (2003) A genetic association study of the mu opioid receptor and severe opioid dependence. *Psychiatr Genet* **13:**169–173.

Cubells JF, Kranzler HR, McCance-Katz E, Anderson GM, Malison RT, Price LH, and Gelernter J (2000) A haplotype at the DBH locus, associated with low plasma dopamine β-hydroxylase activity, also associates with cocaine-induced paranoia. *Mol Psychiatry* **5:**56–63.

Cubells JF, van Kammen DP, Kelley ME, Anderson GM, O'Connor DT, Price LH, Malison R, Rao PA, Kobayashi K, Nagatsu T, et al. (1998) Dopamine β-hydroxylase: two polymorphisms in linkage disequilibrium at the structural gene DBH associate with biochemical phenotypic variation. *Hum Genet* **102:**533–540.

Dai D, Tang J, Rose R, Hodgson E, Bienstock RJ, Mohrenweiser HW, and Goldstein JA (2001) Identification of variants of CYP3A4 and characterization of their abilities to metabolize testosterone and chlorpyrifos. *J Pharmacol Exp Ther* **299:**825–831.

Daunais JB, Roberts DCS, and McGinty JF (1993) Cocaine self-administration increases preprodynorphin, but not c-fos, mRNA in rat striatum. *Neuroreport* **4:**543–546.

Daunais JB, Roberts DCS, and McGinty JF (1995) Short-term cocaine self administration alters striatal gene expression. *Brain Res Bull* **37:**523–527.

Davis AM and Inturrisi CE (1999) d-Methadone blocks morphine tolerance and *N*-methyl-D-aspartate (NMDA)-induced hyperalgesia. *J Pharmacol Exp Ther* **289:**1048–1053.

Dean RA, Christian CD, Sample RH, and Bosron WF (1991) Human liver cocaine esterases: ethanol-mediated formation of ethylcocaine. *FASEB J* **5:**2735–2739.

Di Chiara G and Imperato A (1988a) Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. *J Pharmacol Exp Ther* **244:**1067–1080.

Di Chiara G and Imperato A (1988b) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* **85:**5274–5278.

Dole VP, Nyswander ME, and Kreek MJ (1966) Narcotic blockade. *Arch Intern Med* **118:**304–309.

Duan J, Wainwright MS, Comeron JM, Saitou N, Sanders AR, Gelernter J, and Gejman PV (2003) Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. *Hum Mol Genet* **12:**205–216.
Duguay Y, Skorpen F, and Guillemette C (2004) A novel functional polymorphism in the uridine diphosphate-glucuronsyltransferase 2B7 promoter with significant impact on promoter activity. *Clin Pharmacol Ther* **75:**223–233.
Eap CB, Broly F, Mino A, Hämmig R, Déglon JJ, Uehlinger C, Meili D, Chevalley AF, Bertschy G, Zullino D, et al. (2001) Cytochrome P450 2D6 genotype and methadone steady-state concentrations. *J Clin Psychopharmacol* **21:**229–234.
Edenberg HJ (2002) The collaborative study on the genetics of alcoholism: an update. *Alcohol Res Health* **26:**214–218.
Eiselt R, Domanski TL, Zibat A, Mueller R, Presecan-Siedel E, Hustert E, Zanger UM, Brockmoller J, Klenk HP, Meyer UA, et al. (2001) Identification and functional characterization of eight CYP3A4 protein variants. *Pharmacogenetics* **11:** 447–458.
Eshleman AJ, Carmolli M, Cumbay M, Martens CR, Neve KA, and Janowsky A (1999) Characteristics of drug interactions with recombinant biogenic amine transporters expressed in the same cell type. *J Pharmacol Exp Ther* **289:**877–885.
Evans CJ, Keith DE Jr, Morrison H, Magendzo K, and Edwards RH (1992) Cloning of a delta opioid receptor by functional expression. *Science (Wash DC)* **258:**1952–1955.
Fernandes LC, Kilicarlsan T, Kaplan HL, Tyndale RF, Sellers EM, and Romach MK (2002) Treatment of codeine dependence with inhibitors of cytochrome P450 2D6. *J Clin Psychopharmacol* **22:**326–329.
Foroud T, Edenberg HJ, Goate A, Rice J, Flury L, Koller DL, Bierut LJ, Conneally PM, Nurnberger JI, Bucholz KK, et al. (2000) Alcoholism susceptibility loci: confirmation studies in a replicate sample and further mapping. *Alcoholism Clin Exp Res* **24:**933–945.
Franke P, Nöthen MM, Wang T, Neidt H, Knapp M, Lichtermann D, Weiffenbach O, Mayer P, Höllt V, Propping P, et al. (1999) Human δ-opioid receptor gene and susceptibility to heroin and alcohol dependence. *Am J Med Genet* **88:**462–464.
Franke P, Wang T, Nöthen MK, Knapp M, Neidt H, Albrecht S, Jahnes E, Propping P, and Maier W (2001) Nonreplication of association between μ-opioid-receptor gene (OPRM1) A118G polymorphism and substance dependence. *Am J Med Genet* **105:**114–119.
Fuke S, Suo S, Takahashi N, Koike H, Sasagawa N, and Ishiura S (2001) The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression. *Pharmacogenomics J* **1:**152–156.
Fukuda K, Kato S, Mori K, Nishi M, and Takeshima H (1993) Primary structures and expression from cDNAs of rat opioid receptor δ- and μ-subtypes. *FEBS Lett* **327:**311–314.
Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, et al. (2002) The structure of haplotype blocks in the human genome. *Science (Wash DC)* **296:**2225–2229.
Gawin F, Riordan C, and Kleber H (1985) Methylphenidate treatment of cocaine abusers without attention deficit disorder: a negative report. *Am J Drug Alcohol Abuse* **11:**193–197.
Geijer T, Telkov M, and Terenius L (1995) Characterization of human prodynorphin gene transcripts. *Biochem Biophys Res Commun* **215:**881–888.
Gelernter J, Kranzler H, and Cubells J (1999) Genetics of two mu opioid receptor gene (OPRM1) exon I polymorphisms: population studies, and allele frequencies in alcohol- and drug-dependent subjects. *Mol Psychiatry* **4:**476–483.
Gelernter J and Kranzler HR (2000) Variant detection at the delta opioid receptor (OPRD1) locus and population genetics of a novel variant affecting protein sequence. *Hum Genet* **107:**86–88.
Gelernter J, Kranzler HR, Satel SL, and Rao PA (1994) Genetic association between dopamine transporter protein alleles and cocaine-induced paranoia. *Neuropsychopharmacology* **11:**195–200.
George ST, Ruoho AE, and Malbon CC (1986) N-Glycosylation in expression and function of β-adrenergic receptors. *J Biol Chem* **261:**16559–16564.
Gerra G, Garofano L, Santoro G, Bosari S, Pellegrini C, Zaimovic A, Moi G, Bussandri M, Moi A, Brambilla F, et al. (2004) Association between low-activity serotonin transporter genotype and heroin dependence: behavioral and personality correlates. *Am J Med Genet B Neuropsychiatr Genet* **126:**37–42.
Giros B, Wang YM, Suter S, McLeskey SB, Pifl C, and Caron MG (1994) Delineation of discrete domains for substrate, cocaine, and tricyclic antidepressant interactions using chimeric dopamine-norepinephrine transporters. *J Biol Chem* **269:** 15985–15988.
Glatz K, Mossner R, Heils A, and Lesch KP (2003) Glucocorticoid-regulated human serotonin transporter (5-HTT) expression is modulated by the 5-HTT gene-promoter-linked polymorphic region. *J Neurochem* **86:**1072–1078.
Goldstein A, Lowney LI, and Pal BK (1971) Stereospecific and nonspecific interactions of the morphine congener levorphanol in subcellular fractions of mouse brain. *Proc Natl Acad Sci USA* **68:**1742–1747.
Goldstein A, Tachibana S, Lowney LI, Hunkapiller M, and Hood L (1979) Dynorphin-(1–13), an extraordinarily potent opioid peptide. *Proc Natl Acad Sci USA* **76:**6666–6670.
Gorman AL, Elliott KJ, and Inturrisi CE (1997) The *D*- and *L*-isomers of methadone bind to the noncompetitive site on the *N*-methyl-D-aspartate (NMDA) receptor in rat forebrain and spinal cord. *Neurosci Lett* **223:**5–8.
Grabowski J, Roache JD, Schmitz JM, Rhoades H, Creson D, and Korszun A (1997) Replacement medication for cocaine dependence: methylphenidate. *J Clin Psychopharmacol* **17:**485–488.
Grace AA, Gerfen CR, and Aston-Jones G (1998) Catecholamines in the central nervous system. Overview. *Adv Pharmacol* **42:**655–670.
Greenwald MK, Johanson CE, Moody DE, Woods JH, Kilbourn MR, Koeppe RA, Schuster CR, and Zubieta JK (2003) Effects of buprenorphine maintenance dose on μ-opioid receptor availability, plasma concentrations, and antagonist blockade in heroin-dependent volunteers. *Neuropsychopharmacology* **28:**2000–2009.
Grzanna R and Coyle JT (1977) Immunochemical studies on the turnover of rat serum dopamine β-hydroxylase. *Mol Pharmacol* **13:**956–964.
Gu H, Wall SC, and Rudnick G (1994) Stable expression of biogenic amine transporters reveals differences in inhibitor sensitivity, kinetics, and ion dependence. *J Biol Chem* **269:**7124–7130.
Hall FS, Li XF, Sora I, Xu F, Caron M, Lesch KP, Murphy DL, and Uhl GR (2002) Cocaine mechanisms: enhanced cocaine, fluoxetine and nisoxetine place preferences following monoamine transporter deletions. *Neuroscience* **115:**153–161.
Hall FS, Sora I, and Uhl GR (2001) Ethanol consumption and reward are decreased in μ-opiate receptor knockout mice. *Psychopharmacology* **154:**43–49.
Hallikainen T, Hietala J, Kauhanen J, Pohjalainen T, Syvälahti E, Salonen JT, and Tiihonen J (2003) Ethanol consumption and DRD2 gene TaqI A polymorphism among socially drinking males. *Am J Med Genet A* **119:**152–155.
Halushka MK, Fan JB, Bentley K, Hsie L, Shen N, Weder A, Cooper R, Lipshutz R, and Chakravarti A (1999) Patterns of single-nucleotide polymorphisms in candidate genes for blood pressure homeostasis. *Nat Genet* **22:**239–247.
Handelsman L, Rosenblum A, Palij M, Magura S, Foote J, Lovejoy M, and Stimmel B (1997) Bromocriptine for cocaine dependence. A controlled clinical trial. *Am J Addict* **6:**54–64.
Harada S, Agarwal DP, Goedde HW, Tagaki S, and Ishikawa B (1982) Possible protective role against alcoholism for aldehyde dehydrogenase isozyme deficiency in Japan. *Lancet* **2:**827.
Harada S, Misawa S, Agarwal DP, and Goedde HW (1980) Liver alcohol dehydrogenase and aldehyde dehydrogenase in the Japanese: isozyme variation and its possible role in alcohol intoxication. *Am J Hum Genet* **32:**8–15.
Hayward MD, Pintar JE, and Low MJ (2002) Selective reward deficit in mice lacking β-endorphin and enkephalin. *J Neurosci* **22:**8251–8258.
Heinz A, Goldman D, Jones DW, Palmour R, Hommer D, Gorey JG, Lee KS, Linnoila M, and Weinberger DR (2000) Genotype influences in vivo dopamine transporter availability in human striatum. *Neuropsychopharmacology* **22:**133–139.
Hernandez-Avila CA, Wand G, Luo X, Gelernter J, and Kranzler HR (2003) Association between the cortisol response to opioid blockade and the Asn40Asp polymorphism at the μ-opioid receptor locus (OPRM1). *Am J Med Genet B Neuropsychiatr Genet* **118:**60–65.
Herz A (1988) Bidirectional effects of opioids in motivational processes and the involvement of D1 dopamine receptors. *NIDA Res Monogr* **90:**17–26.
Higgins ST, Budney AJ, Bickel WK, Hughes JR, and Foerg F (1993) Disulfiram therapy in patients abusing cocaine and alcohol. *Am J Psychiatry* **150:**675–676.
Higuchi S, Matsushita S, Muramatsu T, Murayama M, and Hayashida M (1996) Alcohol and aldehyde dehydrogenase genotypes and drinking behavior in Japanese. *Alcoholism Clin Exp Res* **20:**493–497.
Higuchi S, Muramatsu T, Shigemori K, Saito M, Kono H, Dufour MC, and Harford TC (1992) The relationship between low Km aldehyde dehydrogenase phenotype and drinking behavior in Japanese. *J Stud Alcohol* **53:**170–175.
Hoehe MR, Kopke K, Wendel B, Rohde K, Flachmeier C, Kidd KK, Berrettini WH, and Church GM (2000) Sequence variability and candidate gene analysis in complex disease: association of mu opioid receptor gene variation with substance dependence. *Hum Mol Genet* **9:**2895–2908.
Höllt V (2000) Allelic variation of delta and kappa opioid receptors and its implication for receptor function, in *Problems of Drug Dependence, 1999; Proceedings of the 61st Annual Scientific Meeting of the College on Problems of Drug Dependence. National Institute of Drug Abuse Research Monograph Series* (Harris LS ed) pp 50, U.S. Department of Health and Human Services, National Institutes of Health. NIH Publication No (ADM)00-4737, Bethesda, MD.
Holthe M, Klepstad P, Idle JR, Kaasa S, Krokan HE, and Skorpen F (2003) Sequence variations in the UDP-glucuronsyltransferase 2B7 (*UGT2B7*) gene: identification of 10 novel single nucleotide polymorphisms (SNPs) and analysis of their relevance to morphine glucuronidation in cancer patients. *Pharmacogenomics J* **3:**17–26.
Holthe M, Klepstad P, Zahlsen K, Borchgrevink PC, Hagen L, Dale O, Kaasa S, Krokan HE, and Skorpen F (2002) Morphine glucuronide-to-morphine plasma ratios are unaffected by the *UGT2B7* H268Y and *UGT1A1*28* polymorphisms in cancer patients on chronic morphine therapy. *Eur J Clin Pharmacol* **58:**353–356.
Hong CJ, Cheng CY, Shu LR, Yang CY, and Tsai SJ (2003) Association study of the dopamine and serotonin transporter genetic polymorphisms and methamphetamine abuse in Chinese males. *J Neural Transm* **110:**345–351.
Horikawa S, Takai T, Toyosato M, Takahashi H, Noda M, Kakidani H, Kubo T, Hirose T, Inayama S, Hayashida H, et al. (1983) Isolation and structural organization of the human preproenkephalin B gene. *Nature (Lond)* **306:**611–614.
Horn AS (1973) Structure-activity relations for the inhibition of catecholamine uptake into synaptosomes from noradrenaline and dopaminergic neurones in rat brain homogenates. *Br J Pharmacol* **47:**332–338.
Howard LA, Sellers EM, and Tyndale RF (2002) The role of pharmacogenetically-variable cytochrome P450 enzymes in drug abuse and dependence. *Pharmacogenomics* **3:**185–199.
Hughes J, Smith TW, Kosterlitz HW, Fothergill LA, Morgan BA, and Morris HR (1975) Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature (Lond)* **258:**577–579.
Hughes RJ, Pasillas M, Faiz J, Jasper J, and Insel PA (1997) Decreased transcript expression coincident with impaired glycosylation in the beta2-adrenergic receptor gene does not result from differences in the primary sequence. *Biochim Biophys Acta* **1356:**281–291.
Hurd Y and Herkenham M (1992) Influence of a single injection of cocaine, amphetamine or GBR 12909 on mRNA expression of striatal neuropeptides. *Mol Brain Res* **16:**97–104.
Hurd YL, Brown EE, Finlay JM, Fibiger HC, and Gerfen CR (1992) Cocaine self-administration differentially alters mRNA expression of striatal peptides. *Mol Brain Res* **13:**165–170.
Ingoglia NA and Dole VP (1970) Localization of *d*- and *l*-methadone after intraventricular injection into rat brains. *J Pharmacol Exp Ther* **175:**84–87.
Inturrisi CE and Verebely K (1972a) Disposition of methadone in man after a single oral dose. *Clin Pharmacol Ther* **13:**923–930.

Inturrisi CE and Verebely K (1972b) The levels of methadone in the plasma in methadone maintenance. *Clin Pharmacol Ther* **13:**633–637.
Ishii A, Kobayashi K, Kiuchi K, and Nagatsu T (1991) Expression of two forms of human dopamine-β-hydroxylase in COS cells. *Neurosci Lett* **125:**25–28.
Jacobsen LK, Staley JK, Malison RT, Zoghbi SS, Seibyl JP, Kosten TR, and Innis RB (2000a) Elevated central serotonin transporter binding availability in acutely abstinent cocaine-dependent patients. *Am J Psychiatry* **157:**1134–1140.
Jacobsen LK, Staley JK, Zoghbi SS, Seibyl JP, Kosten TR, Innis RB, and Gelernter J (2000b) Prediction of dopamine transporter binding availability by genotype: a preliminary report. *Am J Psychiatry* **157:**1700–1703.
Johnson RE, Chutuape MA, Strain EC, Walsh SL, Stitzer ML, and Bigelow GE (2000) A comparison of levomethadyl acetate, buprenorphine, and methadone for opioid dependence. *N Engl J Med* **343:**1290–1297.
Jönsson EG, Nothen MM, Gustavsson JP, Neidt H, Bunzel R, Propping P, and Sedvall GC (1998) Polymorphisms in the dopamine, serotonin, and norepinephrine transporter genes and their relationships to monoamine metabolite concentrations in CSF of healthy volunteers. *Psychiatry Res* **79:**1–9.
Kaij L (1960) *Alcoholism in Twins: Studies on the Etiology and Sequels of Abuse and Alcohol*, Almqvist and Wiksell, Stockholm.
Kakidani H, Furutani Y, Takahashi H, Noda M, Morimoto Y, Hirose T, Asai M, Inayama S, Nakanishi S, and Numa S (1982) Cloning and sequence analysis of cDNA for porcine β-neo-endorphin/dynorphin precursor. *Nature (Lond)* **298:**245–249.
Kakko J, Svanborg KD, Kreek MJ, and Heilig M (2003) High 1-year retention and improved social function in a buprenorphine-assisted relapse prevention treatment for heroin dependence: a randomized, placebo-controlled Swedish trial. *Lancet* **361:**662–668.
Kalow W and Genest K (1957) A method for the detection of atypical forms of human serum cholinesterase; determination of dibucaine numbers. *Can J Med Sci* **35:** 339–346.
Kalow W and Staron N (1957) On distribution and inheritance of atypical forms of human serum cholinesterase, as indicated by dibucaine numbers. *Can J Biochem Physiol* **35:**1305–1320.
Kamendulis LM, Brzezinski MR, Pindel EV, Bosron WF, and Dean RA (1996) Metabolism of cocaine and heroin is catalyzed by the same human liver carboxylesterases. *J Pharmacol Exp Ther* **279:**713–717.
Kathiramalainathan K, Kaplan HL, Romach MK, Busto UE, Li NY, Tyndale RF, and Sellers EM (2000) Inhibition of cytochrome P450 2D6 modifies codeine abuse liability. *J Clin Psychopharmacol* **20:**435–444.
Kaufman S and Friedman S (1965) Dopamine-β-hydroxylase. *Pharmacol Rev* **17:**71–100.
Kellogg SH, McHugh PF, Bell K, Schluger JH, Schluger RP, LaForge KS, Ho A, and Kreek MJ (2003) The Kreek-McHugh-Schluger-Kellogg Scale: a new, rapid method for quantifying substance abuse and its possible applications. *Drug Alcohol Depend* **69:**137–150.
Kendler KS, Jacobson KC, Prescott CA, and Neale MC (2003) Specificity of genetic and environmental risk factors for use and abuse/dependence of cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiates in male twins. *Am J Psychiatry* **160:**687–695.
Khantzian EJ, Gawin F, Kleber HD, and Riordan CE (1984) Methylphenidate (Ritalin) treatment of cocaine dependence—a preliminary report. *J Subst Abuse Treat* **1:**107–112.
Kieffer BL, Befort K, Gaveriaux-Ruff C, and Hirth CG (1992) The δ-opioid receptor: isolation of a cDNA by expression cloning and pharmacological characterization. *Proc Natl Acad Sci USA* **89**:12048–12052.
Kintz P (2001) Deaths involving buprenorphine: a compendium of French cases. *Forensic Sci Int* **121:**65–69.
Kitanaka N, Sora I, Kinsey S, Zeng Z, and Uhl GR (1998) No heroin or morphine 6 beta-glucuronide analgesia in μ-opioid receptor knockout mice. *Eur J Pharmacol* **355:**R1–R3.
Kling MA, Carson RE, Borg L, Zametkin A, Matochik JA, Schluger J, Herscovitch P, Rice KC, Ho A, Eckelman WC, et al. (2000) Opioid receptor imaging with PET and [^{18}F]cyclofoxy in long-term methadone-treated former heroin addicts. *J Pharmacol Exp Ther* **295:**1070–1076.
Knapp RJ, Malatynska E, Fang L, Li X, Babin E, Nguye M, Santoro G, Varga EV, Hruby VJ, Roeske WR, et al. (1994) Identification of a human delta opioid receptor: cloning and expression. *Life Sci* **54:**PL467–PL469.
Kobayashi K, Yamamoto T, Chiba K, Tani M, Shimada N, Ishizaki T, and Kuroiwa Y (1998) Human buprenorphine N-dealkylation is catalyzed by cytochrome P450 3A4. *Drug Metab Dispos* **26:**818–821.
Koob GF (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol Sci* **13:**177–184.
Kozak CA, Fillie J, Adamson MC, Chen Y, and Yu L (1994) Murine chromosomal location of the mu and kappa opioid receptor genes. *Genomics* **21:**659–661.
Kreek MJ (1973) Plasma and urine levels of methadone. *NY State J Med* **73:**2773–2777.
Kreek MJ (2000) Opiates, opioids, SNP's and the addictions: Nathan B. Eddy Memorial Award for lifetime excellence in drug abuse research lecture, in *Problems of Drug Dependence, 1999; Proceedings of the 61st Annual Scientific Meeting of the College on Problems of Drug Dependence. National Institute of Drug Abuse Research Monograph Series* (Harris LS ed) pp 3–22, U.S. Department of Health and Human Services, National Institutes of Health. NIH Publication No (ADM)00-4737, Bethesda, MD.
Kreek MJ, LaForge KS, and Butelman E (2002) Pharmacotherapy of addictions. *Nat Rev Drug Discov* **1:**710–726.
Kreek MJ, Oratz M, and Rothschild MA (1978) Hepatic extraction of long- and short-acting narcotics in the isolated perfused rabbit liver. *Gastroenterology* **75:** 88–94.
Kreek MJ, Ragunath J, Plevy S, Hamer D, Schneider B, and Hartman N (1984) ACTH, cortisol and β-endorphin response to metyrapone testing during chronic methadone maintenance treatment in humans. *Neuropeptides* **5:**277–278.
Kreek MJ and Vocci FJ (2002) History and current status of opioid maintenance treatments: blending conference session. *J Subst Abuse Treat* **23:**93–105.
Kruglyak L and Nickerson DA (2001) Variation is the spice of life. *Nat Genet* **27:**234–236.
Kruzich P, Chen ACH, Unterwald EM, and Kreek MJ (2003) Subject-regulated dosing alters morphine self-administration behavior and morphine-stimulated [^{35}S]GTPγS binding. *Synapse* **47:**243–249.
LaForge KS, Kreek MJ, Uhl GR, Sora I, Yu L, Befort K, Filliol D, Favier V, Hoehe M, Kieffer BL, et al. (2000a) Symposium XIII: allelic polymorphism of human opioid receptors: functional studies: genetic contributions to protection from, or vulnerability to, addictive diseases, in *Problems of Drug Dependence, 1999; Proceedings of the 61st Annual Scientific Meeting of the College on Problems of Drug Dependence. National Institute of Drug Abuse Research Monograph Series* (Harris LS ed) pp 47–50, U.S. Department of Health and Human Services, National Institutes of Health. NIH Publication No (ADM)00-4737, Bethesda, MD.
LaForge KS, Yuferov V, and Kreek MJ (2000b) Opioid receptor and peptide gene polymorphisms: potential implications for addictions. *Eur J Pharmacol* **410:**249–268.
Laizure SC, Mandrell T, Gades NM, and Parker RB (2003) Cocaethylene metabolism and interaction with cocaine and ethanol: role of carboxylesterases. *Drug Metab Dispos* **31:**16–20.
Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, et al. (2001) Initial sequencing and analysis of the human genome. *Nature (Lond)* **409:**860–921.
Lawford BR, Young RM, Noble EP, Sargent J, Rowell J, Shadforth S, Zhang X, and Ritchie T (2000) The D(2) dopamine receptor A(1) allele and opioid dependence: association with heroin use and response to methadone treatment. *Am J Med Genet* **96:**592–598.
Lerman C, Wileyto EP, Patterson F, Rukstalis M, Audrain-McGovern J, Restine S, Shields PG, Kaufmann V, Redden D, Benowitz N, et al. (2004) The functional mu opioid receptor (OPRM1) Asn40Asp variant predicts short-term response to nicotine replacement therapy in a clinical trial. *Pharmacogenomics J* **4:**184–192.
Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, and Murphy DL (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science (Wash DC)* **274:**1527–1531.
Levenstien MA, Yang Y, and Ott J (2003) Statistical significance for hierarchical clustering in genetic association and microarray expression studies. *BMC Bioinformatics* **4:**62.
Levin FR, Evans SM, McDowell DM, and Kleber HD (1998) Methylphenidate treatment for cocaine abusers with adult attention-deficit/hyperactivity disorder: a pilot study. *J Clin Psychiatry* **59:**300–305.
Li B, Tsing S, Kosaka AH, Nguyen B, Osen EG, Bach C, Chan H, and Barnett J (1996) Expression of human dopamine β-hydroxylase in Drosophila Schneider 2 cells. *Biochem J* **313:**57–64.
Li CH and Chung D (1976) Isolation and structure of an untriakontapeptide with opiate activity from camel pituitary glands. *Proc Natl Acad Sci USA* **73:**1145–1148.
Li S, Zhu J, Chen C, Chen Y-W, Deriel JK, Ashby B, and Liu-Chen L-Y (1993) Molecular cloning and expression of a rat κ opioid receptor. *Biochem J* **295:**629–633.
Li T, Liu X, Zhao J, Hu X, Ball DM, Loh el-W, Sham PC, and Collier DA (2002) Allelic association analysis of the dopamine D2, D3, 5-HT2A, and GABA(A)gamma2 receptors and serotonin transporter genes with heroin abuse in Chinese subjects. *Am J Med Genet* **114:**329–335.
Li T, Liu X, Zhu ZH, Zhao J, Hu X, Sham PC, and Collier DA (2000) Association analysis of polymorphisms in the μ opioid gene and heroin abuse in Chinese subjects. *Addict Biol* **5:**181–186.
Lima MS, Soares BG, Reisser AA, and Farrell M (2002) Pharmacological treatment of cocaine dependence: a systematic review. *Addiction* **97:**931–949.
Lin Z and Uhl GR (2003) Human dopamine transporter gene variation: effects of protein coding variants V55A and V382A on expression and uptake activities. *Pharmacogenomics J* **3:**159–168.
Ling W, Shoptaw S, and Majewska D (1998) Baclofen as a cocaine anti-craving medication: a preliminary clinical study. *Neuropsychopharmacology* **18:**403–404.
Little KY, McLaughlin DP, Zhang L, Livermore CS, Dalack GW, McFinton PR, DelProposto ZS, Hill E, Cassin BJ, Watson SJ, et al. (1998) Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. *Am J Psychiatry* **155:**207–213.
Liu HC, Lu S, Augustin LB, Felsheim RF, Chen HC, Loh HH, and Wei LN (1995) Cloning and promoter mapping of mouse kappa opioid receptor gene. *Biochem Biophys Res Commun* **209:**639–647.
Lockridge O, Mottershaw-Jackson N, Eckerson HW, and La Du BN (1980) Hydrolysis of diacetylmorphine (heroin) by human serum cholinesterase. *J Pharmacol Exp Ther* **215:**1–8.
Loh HH, Liu H-C, Cavalli A, Yang W, Chen Y-F, and Wei L-N (1998) μ opioid receptor knockout in mice: effects on ligand-induced analgesia and morphine lethality. *Mol Brain Res* **54:**321–326.
Long JC, Knowler WC, Hanson RL, Robin RW, Urbanek M, Moore E, Bennett PH, and Goldman D (1998) Evidence for genetic linkage to alcohol dependence on chromosomes 4 and 11 from an autosome-wide scan in an American Indian population. *Am J Med Genet* **81:**216–221.
Long JC, Williams RC, and Urbanek M (1995) An E-M algorithm and testing strategy for multiple-locus haplotypes. *Am J Hum Genet* **56:**799–810.
Lötsch J, Skarke C, Grösch S, Darimont J, Schmidt H, and Geisslinger G (2002) The polymorphism A118G of the human μ-opioid receptor gene decreases the pupil constrictory effect of morphine-6-glucuronide but not that of morphine. *Pharmacogenetics* **12:**3–9.
Luo X, Kranzler HR, Zhao H, and Gelernter J (2003) Haplotypes at the OPRM1 locus are associated with susceptibility to substance dependence in European-Americans. *Am J Med Genet B Neuropsychiatr Genet* **120:**97–108.

Ma JZ, Zhang D, Dupont RT, Dockter M, Elston RC, and Li MD (2003) Mapping susceptibility loci for alcohol consumption using number of grams of alcohol consumed per day as a phenotype measure. *BMC Genet* **4 (Suppl 1):**S104.
MacKenzie A and Quinn J (1999) A serotonin transporter gene intron 2 polymorphic region, correlated with affective disorders, has allele-dependent differential enhancer-like properties in the mouse embryo. *Proc Natl Acad Sci USA* **96:**15251–15255.
Maekawa M, Sudo K, Dey DC, Ishikawa J, Isumi M, Kotani K, and Kanno T (1997) Genetic mutations of butyrylcholine esterase identified from phenotypic abnormalities in Japan. *Clin Chem* **43:**924–929.
Maisonneuve IM, Ho A, and Kreek MJ (1995) Chronic administration of a cocaine "binge" alters basal extracellular levels in male rats: an in vivo microdialysis study. *J Pharmacol Exp Ther* **272:**652–657.
Maisonneuve IM and Kreek MJ (1994) Acute tolerance to the dopamine response induced by a binge pattern of cocaine administration in male rats. *J Pharmacol Exp Ther* **268:**916–921.
Malcolm R, Kajdasz DK, Herron J, Anton RF, and Brady KT (2000) A double-blind, placebo-controlled outpatient trial of pergolide for cocaine dependence. *Drug Alcohol Depend* **60:**161–168.
Maldonado R, Saiardi A, Valverde O, Samad TA, Roques BP, and Borrelli E (1997) Absence of opiate rewarding effects in mice lacking dopamine D2 receptors. *Nature* (*Lond*) **388:**586–589.
Mansson E, Bare L, and Yang D (1994) Isolation of a human κ opioid receptor cDNA from placenta. *Biochem Biophys Res Commun* **202:**1431–1437.
Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, Befort K, Dierich A, Le Meur M, Dolle P, et al. (1996) Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the μ-opioid-receptor gene. *Nature (Lond)* **383:**819–823.
Mayer P and Höllt V (2001) Allelic and somatic variations in the endogenous opioid system of humans. *Pharmacol Ther* **91:**167–177.
Mayer P, Rochlitz H, Rauch E, Rommelspacher H, Hasse HE, Schmidt S, and Höllt V (1997) Association between a delta opioid receptor gene polymorphism and heroin dependence in man. *Neuroreport* **8:**2547–2550.
McClung CA and Nestler EJ (2003) Regulation of gene expression and cocaine reward by CREB and DeltaFosB. *Nat Neurosci* **6:**1208–1215.
McGuire MC, Nogueira CP, Bartels CF, Lightstone H, Hajra A, Van der Spek AF, Lockridge O, and La Du BN (1989) Identification of the structural mutation responsible for the dibucaine-resistant (atypical) variant form of human serum cholinesterase. *Proc Natl Acad Sci USA* **86:**953–957.
McLellan AT, Kushner H, Metzger D, Peters R, Smith I, Grissom G, Pettinati H, and Argeriou M (1992) The Fifth Edition of the Addiction Severity Index. *J Subst Abuse Treat* **9:**199–213.
McLellan AT, Luborsky L, Woody GE, and O'Brien CP (1980) An improved diagnostic evaluation instrument for substance abuse patients. The Addiction Severity Index. *J Nerv Ment Dis* **168:**26–33.
Meng R, Xie G-X, Thompson RC, Mansour A, Goldstein A, Watson SJ, and Akil H (1993) Cloning and pharmacological characterization of a rat κ opioid receptor. *Proc Natl Acad Sci USA* **90:**9954–9958.
Merikangas KR, Stolar M, Stevens DE, Goulet J, Preisig MA, Fenton B, Zhang H, O'Malley SS, and Rounsaville BJ (1998) Familial transmission of substance use disorders. *Arch Gen Psychiatry* **55:**973–979.
Minami M, Toya T, Katao Y, Maekawa K, Nakamura S, Onogi T, Kaneko S, and Satoh M (1993) Cloning and expression of a cDNA for the rat κ-opioid receptor. *FEBS Lett* **329:**291–295.
Molinoff PB and Axelrod J (1971) Biochemistry of catecholamines. *Annu Rev Biochem* **40:**465–500.
Moron JA, Brockington A, Wise RA, Rocha BA, and Hope BT (2002) Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. *J Neurosci* **22:**389–395.
Myrick H, Henderson S, Brady KT, and Malcolm R (2001) Gabapentin in the treatment of cocaine dependence: a case series. *J Clin Psychiatry* **62:**19–23.
Nakanishi S, Inoue A, Kita T, Nakamura M, Chang ACY, Cohen SN, and Numa S (1979) Nucleotide sequence of cloned cDNA for bovine corticotropin-β-lipotropin precursor. *Nature (Lond)* **278:**423–427.
Nakanishi S, Teranishi Y, Watanabe Y, Notake M, Noda M, Kakidani H, Jingami H, and Numa S (1981) Isolation and characterization of the bovine corticotropin/β-lipotropin precursor gene. *Eur J Biochem* **115:**429–438.
Nath RP, Upton RA, and Everhart ET (1999) Buprenorphine pharmacokinetics: relative bioavailability of sublingual tablet and liquid formulations. *J Clin Pharmacol* **39:**619–623.
Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. *Nat Rev Neurosci* **2:**119–128.
Nitsche JF, Schuller AG, Kink MA, Sengh M, Pasternak GW, and Pintar JE (2002) Genetic dissociation of opiate tolerance and physical dependence in δ-opioid receptor-1 and preproenkephalin knock-out mice. *J Neurosci* **22:**10906–10913.
Noble EP (1998) The D2 dopamine receptor gene: a review of association studies in alcoholism and phenotypes. *Alcohol* **16:**33–45.
Noble EP (2000) Addiction and its reward process through polymorphisms of the D2 dopamine receptor gene: a review. *Eur Psychiatry* **15:**79–89.
Noble EP, Blum K, Khalsa ME, Ritchie T, Montgomery A, Wood RC, Fitch RJ, Ozkaragoz T, Sheridan PJ, Anglin MD, et al. (1993) Allelic association of the D2 dopamine receptor gene with cocaine dependence. *Drug Alcohol Depend* **33:**271–285.
Noda M, Furutani Y, Takahashi H, Toyasato M, Hirose T, Inayama S, Nakanishi S, and Numa S (1982a) Cloning and sequence analysis of cDNA for bovine adrenal preproenkephalin. *Nature (Lond)* **295:**202–206.
Noda M, Teranishi Y, Takahashi H, Toyosato M, Notake M, Nakanishi S, and Numa S (1982b) Isolation and structural organization of the human preproenkephalin gene. *Nature (Lond)* **297:**431–434.
Nogueira CP, Bartels CF, McGuire MC, Adkins S, Lubrano T, Rubinstein HM, Lightstone H, Van der Spek AF, Lockridge O, and La Du BN (1990a) Identification of two different point mutations associated with the fluoride-resistant phenotype for human butyrylcholinesterase. *Am J Hum Genet* **51:**821–828.
Nogueira CP, McGuire MC, Graeser C, Bartels CF, Arpagaus M, Van der Spek AF, Lightstone H, Lockridge O, and La Du BN (1990b) Identification of a frameshift mutation responsible for the silent phenotype of human serum cholinesterase, Gly 117 (GGT—GGAG). *Am J Hum Genet* **46:**934–942.
O'Hara BF, Smith SS, Bird G, Persico AM, Suarez BK, Cutting GR, and Uhl GR (1993) Dopamine D2 receptor RFLPs, haplotypes and their association with substance use in black and Caucasian research volunteers. *Hum Hered* **43:**209–218.
Oslin DW, Berrettini W, Kranzler HR, Pettinati H, Gelernter J, Volpicelli JR, and O'Brien CP (2003) A functional polymorphism of the μ-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. *Neuropsychopharmacology* **28:**1546–1552.
Oxenstierna G, Edman G, Iselius L, Oreland L, Ross SB, and Sedvall G (1986) Concentrations of monoamine metabolites in the cerebrospinal fluid of twins and unrelated individuals –a genetic study. *J Psychiatr Res* **20:**19–29.
Paczkowski FA, Bonisch H, and Bryan-Lluka LJ (2002) Pharmacological properties of the naturally occurring Ala(457)Pro variant of the human norepinephrine transporter. *Pharmacogenetics* **12:**165–173.
Partanen J, Bruun K, and Markkanen T (1966) *Inheritance of Drinking Behavior: A Study on Intelligence, Personality, and Use of Alcohol of Adult Twins.* The Finnish Foundation for Alcohol Studies, Helsinki.
Patkar AA, Berrettini WH, Hoehe M, Hill KP, Gottheil E, Thornton CC, and Weinstein SP (2002) No association between polymorphisms in the serotonin transporter gene and susceptibility to cocaine dependence among African-American individuals. *Psychiatr Genet* **12:**161–164.
Patkar AA, Berrettini WH, Hoehe M, Hill KP, Sterling RC, Gottheil E, and Weinstein SP (2001) Serotonin transporter (5-HTT) gene polymorphisms and susceptibility to cocaine dependence among African-American individuals. *Addict Biol* **6:**337–345.
Persico AM, Bird G, Gabbay FH, and Uhl GR (1996) D2 dopamine receptor gene TaqI A1 and B1 restriction fragment length polymorphisms: enhanced frequencies in psychostimulant-preferring polysubstance abusers. *Biol Psychiatry* **40:**776–784.
Pert CB and Snyder SH (1973) Opiate receptor: demonstration in nervous tissue. *Science (Wash DC)* **179:**1011–1014.
Petäjä-Repo U, Morello JP, Laperrière A, Walker P, and Bouvier M (2000) Export from the endoplasmic reticulum represents the limiting step in the maturation and cell surface expression of the human δ-opioid receptor. *J Biol Chem* **275:**13727–13736.
Portenoy RK, Caraceni A, Cherny NI, Goldblum R, Ingham J, Inturrisi CE, Johnson JH, Lapin J, Tiseo PJ, and Kreek MJ (1999) Dynorphin A(1–13) analgesia in opioid-treated patients with chronic pain: a controlled pilot study. *Clin Drug Investig* **17:**33–42.
Raiteri M, Del Carmine R, Bertollini A, and Levi G (1977) Effect of sympathomimetic amines on the synaptosomal transport of noradrenaline, dopamine and 5-hydroxytryptamine. *Eur J Pharmacol* **41:**133–143.
Reich DE, Cargill M, Bolk S, Ireland J, Sabeti PC, Richter DJ, Lavery T, Kouyoumjian R, Farhadian SF, Ward R, et al. (2001) Linkage disequilibrium in the human genome. *Nature (Lond)* **411:**199–204.
Reich T, Edenberg HJ, Goate A, Williams JR, Rice JP, van Eerdewegh P, Foroud T, Hesselbrock V, Schuckit MA, Bucholz K, et al. (1998) Genome-wide search for genes affecting the risk for alcohol dependence. *Am J Med Genet* **81:**207–215.
Rettig RA and Yarmolinsky A (1995) *Federal Regulation of Methadone Treatment*. Institute of Medicine: National Academy Press, Washington, DC.
Ritchie T and Noble EP (2003) Association of seven polymorphisms of the D2 dopamine receptor gene with brain receptor-binding characteristics. *Neurochem Res* **28:**73–82.
Roberts AJ, McDonald JS, Heyser CJ, Kieffer BL, Matthes HW, Koob GF, and Gold LH (2000) μ-Opioid receptor knockout mice do not self-administer alcohol. *J Pharmacol Exp Ther* **293:**1002–1008.
Rocha BA, Fumagalli F, Gainetdinov RR, Jones SR, Ator R, Giros B, Miller GW, and Caron MG (1998) Cocaine self-administration in dopamine-transporter knockout mice. *Nat Neurosci* **1:**132–137.
Romach MK, Otton SV, Somer G, Tyndale RF, and Sellers EM (2000) Cytochrome P450 2D6 and treatment of codeine dependence. *J Clin Psychopharmacol* **20:**43–45.
Ross SB, Wetterberg L, and Myrhed M (1973) Genetic control of plasma dopamine-β-hydroxylase. *Life Sci* **12:**529–532.
Rothman RB and Baumann MH (2003) Monoamine transporters and psychostimulant drugs. *Eur J Pharmacol* **479:**23–40.
Runkel F, Bruss M, Nothen MM, Stober G, Propping P, and Bonisch H (2000) Pharmacological properties of naturally occurring variants of the human norepinephrine transporter. *Pharmacogenetics* **10:**397–405.
San L, Pomarol G, Peri JM, Olle JM, and Carni J (1991) Follow-up after a six-month maintenance period on naltrexone versus placebo in heroin addicts. *Br J Addict* **86:**983–990.
Sawyer MB, Innocenti F, Das S, Cheng C, Ramírez J, Pantle-Fisher FH, Wright C, Badner J, Pei D, Boyett JM, et al. (2003) A pharmacogenetic study of uridine diphosphate-glucuronosyltransferase 2B7 in patients receiving morphine. *Clin Pharmacol Ther* **73:**566–574.
Schluger JH, Borg L, Ho A, and Kreek MJ (2001) Altered HPA axis responsivity to metyrapone testing in methadone maintained former heroin addicts with ongoing cocaine addiction. *Neuropsychopharmacology* **24:**568–575.
Schubiner H, Saules KK, Arfken CL, Johanson CE, Schuster CR, Lockhart N, Edwards A, Donlin J, and Pihlgren E (2002) Double-blind placebo-controlled trial of methylphenidate in the treatment of adult ADHD patients with comorbid cocaine dependence. *Exp Clin Psychopharmacol* **10:**286–294.
Shannon JR, Flattem NL, Jordan J, Jacob G, Black BK, Biaggioni I, Blakely RD, and Robertson D (2000) Orthostatic intolerance and tachycardia associated with norepinephrine-transporter deficiency. *N Engl J Med* **342:**541–549.

Shi J, Jui L, Xu Y, Wang F, Huang W, and Hu G (2002) Sequence variation in the μ-opioid receptor gene (OPRM1) associated with human addiction to heroin. *Hum Mutat* **19:**459–460.
Shoptaw S, Kintaudi PC, Charuvastra C, and Ling W (2002) A screening trial of amantadine as a medication for cocaine dependence. *Drug Alcohol Depend* **66:**217–224.
Shoptaw S, Yang X, Rotheram-Fuller EJ, Hsieh YC, Kintaudi PC, Charuvastra VC, and Ling W (2003) Randomized placebo-controlled trial of baclofen for cocaine dependence: preliminary effects for individuals with chronic patterns of cocaine use. *J Clin Psychiatry* **64:**1440–1448.
Sim LJ, Selley DE, Dworkin SI, and Childers SR (1996) Effects of chronic morphine administration on mu opioid receptor-stimulated [35S]GTPγS autoradiography in rat brain. *J Neurosci* **16:**2684–2692.
Simon EJ, Hiller JM, and Edelman I (1973) Stereospecific binding of the potent narcotic analgesic [^{3}H]etorphine to rat-brain homogenate. *Proc Natl Acad Sci USA* **70:**1947–1949.
Simonin F, Befort K, Gaveriaux-Ruff C, Matthes H, Nappey V, Lannes B, Micheletti G, and Kieffer B (1994) The human δ-opioid receptor: genomic organization, cDNA cloning, functional expression, and distribution in the human brain. *Mol Pharmacol* **46:**1015–1021.
Simonin F, Gaveriaux-Ruff C, Befort K, Matthes H, Lannes B, Micheletti G, Mattei MG, Charron G, Bloch B, and Kieffer B (1995) κ-Opioid receptor in humans: cDNA and genomic cloning, chromosomal assignment, functional expression, pharmacology, and expression pattern in the central nervous system. *Proc Natl Acad Sci USA* **92:**7006–7010.
Sim-Selley LJ, Selley DE, Vogt LJ, Childers SR, and Martin TJ (2000) Chronic heroin self-administration desensitizes mu opioid receptor-activated G-proteins in specific regions of rat brain. *J Neurosci* **20:**4555–4562.
Sindrup SH, Brøsen K, Bjerring P, Arendt-Nielsen L, Larsen U, Angelo HR, and Gram LF (1991) Codeine increases pain thresholds to copper vapor laser stimuli in extensive but not poor metabolizers of sparteine. *Clin Pharmacol Ther* **49**:686–693.
Sindrup SH, Poulsen L, Brøsen K, Arendt-Nielsen L, and Gram LF (1993) Are poor metabolizers of sparteine/debrisoquine less pain tolerant than extensive metabolizers? *Pain* **53:**335–349.
Sivam SP (1989) Cocaine selectively increases striatonigral dynorphin levels by a dopaminergic mechanism. *J Pharmacol Exp Ther* **250:**818–824.
Skarke C, Darimont J, Schmidt H, Geisslinger G, and Lötsch J (2003a) Analgesic effects of morphine and morphine-6-glucuronide in a transcutaneous electrical pain model in healthy volunteers. *Clin Pharmacol Ther* **73:**107–121.
Skarke C, Schmidt H, Geisslinger G, Darimont J, and Lötsch J (2003b) Pharmacokinetics of morphine are not altered in subjects with Gilbert's Syndrome. *Br J Clin Pharmacol* **56:**228–231.
Sora I, Hall FS, Andrews AM, Itokawa M, Li XF, Wei HB, Wichems C, Lesch KP, Murphy DL, and Uhl GR (2001) Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. *Proc Natl Acad Sci USA* **98:**5300–5305.
Sora I, Takahashi N, Funada M, Ujike H, Revay RS, Donovan DM, Miner LL, and Uhl GR (1997) Opiate receptor knockout mice define mu receptor roles in endogenous nociceptive responses and morphine-induced analgesia. *Proc Natl Acad Sci USA* **94:**1544–1549.
Sora I, Wichems C, Takahashi N, Li XF, Zeng Z, Revay R, Lesch KP, Murphy DL, and Uhl GR (1998) Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. *Proc Natl Acad Sci USA* **95:**7699–7704.
Soubrie P (1986) Reconciling the role of central serotonin neurons in human and animal behavior. *Behav Brain Sci* **9:**319–364.
Spangler R, Ho A, Zhou Y, Maggos C, Yuferov V, and Kreek MJ (1993a) Regulation of kappa opioid receptor mRNA in the rat brain by "binge" pattern cocaine administration and correlation with preprodynorphin mRNA. *Mol Brain Res* **38:**71–76.
Spangler R, Unterwald EM, and Kreek MJ (1993b) "Binge" cocaine administration induces a sustained increase of prodynorphin mRNA in rat caudate-putamen. *Mol Brain Res* **19:**323–327.
Spangler R, Zhou Y, Maggos CE, Zlobin A, Ho A, and Kreek MJ (1996) Dopamine antagonist and "binge" cocaine effects on rat opioid and dopamine transporter mRNAs. *Neuroreport* **7:**2196–2200.
Specker S, Wananukul W, Hatsukami DE, Nolin K, Hooke L, Kreek MJ, and Pentel PR (1998) Effects of dynorphin A(1–13) on opiate withdrawal in humans. *Psychopharmacology* **137:**326–332.
Stallings MC, Corley RP, Hewitt JK, Krauter KS, Lessem JM, Midulich SK, Rhee SH, Smoler A, Young SE, and Crowley TJ (2003) A genome-wide search for quantitative trait loci influencing substance dependence vulnerability in adolescence. *Drug Alcohol Depend* **70:**295–307.
Stewart LC and Klinman JP (1988). Dopamine β-hydroxylase of adrenal chromaffin granules: structure and function. *Annu Rev Biochem* **57:**551–592.
Stober G, Hebebrand J, Cichon S, Bruss M, Bonisch H, Lehmkuhl G, Poustka F, Schmidt M, Remschmidt H, Propping P, et al. (1999) Tourette syndrome and the norepinephrine transporter gene: results of a systematic mutation screening. *Am J Med Genet* **88:**158–163.
Stober G, Nothen MM, Porzgen P, Bruss M, Bonisch H, Knapp M, Beckmann H, and Propping P (1996) Systematic search for variation in the human norepinephrine transporter gene: identification of five naturally occurring missense mutations and study of association with major psychiatric disorders. *Am J Med Genet* **67:**523–532.
Stoller KB, Bigelow GE, Walsh SL, and Strain EC (2001) Effects of buprenorphine/naloxone in opioid-dependent humans. *Psychopharmacology* **154:**230–242.
Straub RE, Sullivan PF, Ma Y, Myakishev MV, Harris-Kerr C, Wormley B, Kadambi B, Sadek H, Silverman MA, Webb BT, et al. (1999) Susceptibility genes for nicotine dependence: a genome scan and followup in an independent sample suggest that regions on chromosomes 2, 4, 10, 16, 17 and 18 merit further study. *Mol Psychiatry* **4:**129–144.
Substance Abuse and Mental Health Services Administration, Office of Applied Studies (2003a) *Emergency Department Trends From the Drug Abuse Warning Network, Final Estimates 1995–2002*, DAWN Series: D-24, DHHS Publication No. (SMA) 03-3780, Rockville, MD.
Substance Abuse and Mental Health Services Administration, Office of Applied Studies (2003b) *Results from the 2002 National Survey on Drug Use and Health: National Findings*. NHSDA Series H-22, DHHS Publication No. SMA 03-3836, Rockville, MD.
Sullivan HR, Due SL, and McMahon RE (1973) Metabolism of alpha-*l*-methadol: *N*-acetylation, a new metabolic pathway. *Res Comm Chem Path Pharmacol* **6:**1072–1078.
Szabo ST and Blier P (2001) Effect of the selective noradrenergic reuptake inhibitor reboxetine on the firing activity of noradrenaline and serotonin neurons. *Eur J Neurosci* **13:**2077–2087.
Szabo ST, de Montigny C, and Blier P (1999) Modulation of noradrenergic neuronal firing by selective serotonin reuptake blockers. *Br J Pharmacol* **126:**568–571.
Szeto CY, Tang NL, Lee DT, and Stadlin A (2001) Association between mu opioid receptor gene polymorphisms and Chinese heroin addicts. *Neuroreport* **12:**1103–1106.
Takahashi H, Hakamata Y, Watanabe Y, Kikuno R, Miyata T, and Numa S (1983) Complete nucleotide sequence of the human corticotropin-β-lipotropin precursor gene. *Nucleic Acids Res* **11:**6847–6858.
Takahashi H, Teranishi Y, Nakanishi S, and Numa S (1981) Isolation and structural organization of the human corticotropin-β-lipotropin precursor gene. *FEBS Lett* **135:**97–102.
Tan EC, Tan CH, Karupathivan U, and Yap EP (2003) Mu opioid receptor gene polymorphisms and heroin dependence in Asian populations. *Neuroreport* **14:**569–572.
Tan EC, Yeo BK, Ho BK, Tay AH, and Tan CH (1999) Evidence for an association between heroin dependence and a VNTR polymorphism at the serotonin transporter locus. *Mol Psychiatry* **4:**215–217.
Terenius L (1973) Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. *Acta Pharmacol Toxicol* **32:**317–320.
Thomas DC and Witte JS (2002) Point: population stratification: a problem for case-control studies of candidate-gene associations? *Cancer Epidemiol Biomark Prev* **11:**505–512.
Thompson RC, Mansour A, Akil H, and Watson SJ (1993) Cloning and pharmacological characterization of a rat μ opioid receptor. *Neuron* **11:**903–913.
Tsuang MT, Lyons MJ, Meyer JM, Doyle T, Eisen SA, Goldberg J, True W, Lin N, Toomey R, and Eaves L (1998) Co-occurrence of abuse of different drugs in men: the role of drug-specific and shared vulnerabilities. *Arch Gen Psychiatry* **55:**967–972.
Tyndale RF, Droll KP, and Sellers EM (1997) Genetically deficient CYP2D6 metabolism provides protection against oral opiate dependence. *Pharmacogenetics* **7:**375–379.
Uhl GR, Hall FS, and Sora I (2002a) Cocaine, reward, movement and monoamine transporters. *Mol Psychiatry* **7:**21–26.
Uhl GR, Liu QR, and Naiman D (2002b) Substance abuse vulnerability loci: converging genome scanning data. *Trends Genet* **18:**420–425.
Uhl GR, Liu QR, Walther D, Hess J, and Naiman D (2001) Polysubstance abuse-vulnerability genes: genome scans for association, using 1,004 subjects and 1,494 single-nucleotide polymorphisms. *Am J Hum Genet* **69:**1290–1300.
Ujike H, Harano M, Inada T, Yamada M, Komiyama T, Sekine Y, Sora I, Iyo M, Katsu T, Nomura A, et al. (2003) Nine- or fewer repeat alleles in VNTR polymorphism of the dopamine transporter gene is a strong risk factor for prolonged methamphetamine psychosis. *Pharmacogenomics J* **3:**242–247.
Unterwald EM, Cox BM, Kreek MJ, Cote TE, and Izenwasser S (1993) Chronic repeated cocaine administration alters basal and opioid-regulated adenylyl cyclase activity. *Synapse* **15:**33–38.
Unterwald EM, Horne-King J, and Kreek MJ (1992) Chronic cocaine alters brain μ receptors. *Brain Res* **584:**314–318.
Unterwald EM, Rubenfeld JM, and Kreek MJ (1994) Repeated cocaine administration upregulates κ and μ, but not δ, opioid receptors. *Neuroreport* **5:**1613–1616.
Vandenbergh DJ, Thompson MD, Cook EH, Bendahhou E, Nguyen T, Krasowski MD, Zarrabian D, Comings D, Sellers EM, Tyndale RF, et al. (2000) Human dopamine transporter gene: coding region conservation among normal, Tourette's disorder, alcohol dependence and attention-deficit hyperactivity disorder populations. *Mol Psychiatry* **5:**283–292.
Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, et al. (2001) The sequence of the human genome. *Science (Wash DC)* **291:**1304–1351.
Walsh SL, Preston KL, Stitzer ML, Cone EJ, and Bigelow GE (1994) Clinical pharmacology of buprenorphine: ceiling effects at high doses. *Clin Pharmacol Ther* **55:**569–580.
Wand GS, McCaul M, Yang X, Reynolds J, Gotjen D, Lee S, and Ali A (2002) The μ-opioid receptor gene polymorphism (A118G) alters HPA axis activation induced by opioid receptor blockade. *Neuropsychopharmacology* **26:**106–114.
Wang JB, Imai Y, Eppler CM, Gregor P, Spivak CE, and Uhl GR (1993) μ opiate receptor: cDNA cloning and expression. *Proc Natl Acad Sci USA* **90:**10230–10234.
Wei J, Ramchand CN, and Hemmings GP (1997a) Possible control of dopamine β-hydroxylase via a codominant mechanism associated with the polymorphic $(GT)_n$ repeat at its gene locus in healthy individuals. *Hum Genet* **99:**52–55.
Wei J, Xu HM, Ramchand CN, and Hemmings GP (1997b) Is the polymorphic microsatellite repeat of the dopamine β-hydroxylase gene associated with biochemical variability of the catecholamine pathway in schizophrenia? *Biol Psychiatry* **41:**762–767.
Weinshilboum RM (1978) Serum dopamine β-hydroxylase. *Pharmacol Rev* **30:**133–166.
Weinshilboum RM, Raymond FA, Elveback LR, and Weidman WH (1973) Serum

dopamine-β-hydroxylase activity: sibling-sibling correlation. *Science (Wash DC)* **181:**943–945.
Wyszynski DF, Panhuysen CI, Ma Q, Yip AG, Wilcox W, Erlich P, and Farrer LA (2003) Genome-wide screen for heavy alcohol consumption. *BMC Genet* **4:**S106.
Xie W, Altamirano CV, Bartels CF, Speirs RJ, Cashman JR, and Lockridge O (1999) An improved cocaine hydrolase: the A328Y mutant of human butyrylcholinesterase is 4-fold more efficient. *Mol Pharmacol* **55:**83–91.
Xu F, Gainetdinov RR, Wetsel WC, Jones SR, Bohn LM, Miller GW, Wang YM, and Caron MG (2000) Mice lacking the norepinephrine transporter are supersensitive to psychostimulants. *Nat Neurosci* **3**:465–471.
Xu K, Liu XH, Nagarajan S, Gu XY, and Goldman D (2002) Relationship of the δ-opioid receptor gene to heroin abuse in a large Chinese case/control sample. *Am J Med Genet* **110:**45–50.
Yakovlev AG, Krueger KE, and Faden AI (1995) Structure and expression of a rat kappa opioid receptor gene. *J Biol Chem* **270:**6421–6424.
Yasuda K, Raynor K, Kong H, Breder CD, Takeda J, Reisine T, and Bell GI (1993) Cloning and functional comparison of κ and δ opioid receptors from mouse brain. *Proc Natl Acad Sci USA* **90:**6736–6740.
Yoshida A, Hsu LC, and Yasunami M (1991) Genetics of human alcohol-metabolizing enzymes. *Prog Nucleic Acid Res Mol Biol* **40:**255–287.
Yoshida A, Huang IY, and Ikawa M (1984) Molecular abnormality of an inactive aldehyde dehydrogenase variant commonly found in Orientals. *Proc Natl Acad Sci USA* **81:**258–261.
Yuferov V, Fussell D, LaForge KS, Nielsen DA, Gordon D, Ho A, Leal SM, Ott J, and Kreek MJ (2004) Redefinition of the human kappa opioid receptor gene (*OPRK1*) structure and association of haplotypes with opiate addiction. *Pharmacogenetics* **14:**793–804.
Yuferov V, Zhou Y, Spangler R, Maggos CE, Ho A, and Kreek MJ (1999) Acute "binge" cocaine increases μ-opioid receptor mRNA levels in areas of rat mesolimbic mesocortical dopamine system. *Brain Res Bull* **48:**109–112.
Zabetian CP, Anderson GM, Buxbaum SG, Elston RC, Ichinose H, Nagatsu T, Kim K-S, Kim C-H, Malison RT, Gelernter J, et al. (2001) A quantitative-trait analysis of human plasma-dopamine β-hydroxylase activity: evidence for a major functional polymorphism at the *DBH* locus. *Am J Hum Genet* **68:**515–522.
Zabetian CP, Buxbaum SG, Elston RC, Kohnke MD, Anderson GM, Gelernter J, and Cubells JF (2003) The structure of linkage disequilibrium at the DBH locus strongly influences the magnitude of association between diallelic markers and plasma dopamine β-hydroxylase activity. *Am J Hum Genet* **72:**1389–1400.
Zhang Y, Butelman E, Schlussman SD, Ho A, and Kreek MJ (2004) Effect of the endogenous κ opioid agonist dynorphin A(1–17) on cocaine-evoked increases in striatal dopamine levels and cocaine-induced place preference in C57BL/6J mice. *Psychopharmacology* **172:**422–429.
Zhu J, Chen C, Xue J-C, Kunapuli S, DeRiel JK, and Lui-Chen L-Y (1995) Cloning of a human κ opioid receptor from the brain. *Life Sci* **56:**PL201–PL207.
Zhu Y, King MA, Schuller AG, Nitsche JF, Reidl M, Elde RP, Unterwald E, Pasternak GW, and Pintar JE (1999) Retention of supraspinal δ-like analgesia and loss of morphine tolerance in δ opioid receptor knockout mice. *Neuron* **24:**243–252.
Zimprich A, Kraus J, Woltje M, Mayer P, Rauch E, and Höllt V (2000) An allelic variation in the human prodynorphin gene promoter alters stimulus-induced expression. *J Neurochem* **74:**472–477.
Zubieta J, Gorelick DA, Stauffer R, Ravert HT, Dannals RF, and Frost JJ (1996) Increased mu opioid receptor binding detected by PET in cocaine-dependent men is associated with cocaine craving. *Nat Med* **2:**1225–1229.