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Search for genetic markers and functional variants involved in the development of opiate and cocaine addiction and treatment

Vadim Yuferov,[1] Orna Levran,[1] Dmitri Proudnikov,[1] David A. Nielsen,[1,2] and Mary Jeanne Kreek[1]

[1]Laboratory of the Biology of Addictive Diseases, Rockefeller University, New York, New York, USA
[2]Present address: Menninger Department of Psychiatry and Behavioral Sciences, Baylor College of Medicine, Houston, Texas, USA

Address for correspondence: Mary Jeanne Kreek, M.D., Laboratory of the Biology of Addictive Diseases, Rockefeller University, The Rockefeller University Hospital, 1230 York Ave., Box 171, New York, NY 10065. Voice: 212-327-8490; fax: 212-327-8247. kreek@rockefeller.edu

Addiction to opiates and illicit use of psychostimulants is a chronic, relapsing brain disease that, if left untreated, can cause major medical, social, and economic problems. This article reviews recent progress in studies of association of gene variants with vulnerability to develop opiate and cocaine addictions, focusing primarily on genes of the opioid and monoaminergic systems. In addition, we provide the first evidence of a *cis*-acting polymorphism and a functional haplotype in the *PDYN* gene, of significantly higher DNA methylation rate of the *OPRM1* gene in the lymphocytes of heroin addicts, and significant differences in genotype frequencies of three single-nucleotide polymorphisms of the P-glycoprotein gene (*ABCB1*) between "higher" and "lower" methadone doses in methadone-maintained patients. In genomewide and multigene association studies, we found association of several new genes and new variants of known genes with heroin addiction. Finally, we describe the development and application of a novel technique: molecular haplotyping for studies in genetics of drug addiction.

Keywords: opiate and cocaine addiction; genetics of drug addiction; epigenetics; pharmacogenetics; allele-specific gene expression; molecular haplotyping

Introduction

Addiction to opiates and illicit use of psychostimulants is a chronic, relapsing brain disease that, if left untreated, can cause major medical, social, and economic problems. There are at least three different categories of factors that contribute to the vulnerability of developing a specific addiction, once self-exposed: (1) environmental factors, including cues, conditioning, external stressors, and the stress they cause; (2) drug-induced factors, which lead to a variety of molecular neurobiological changes resulting in altered behaviors; and (3) genetic factors, which represent approximately 40–60% of the risk of developing an addiction.[1] Addiction to opioids may arise from illicit use of heroin, or from illicit prescription opioids, or correct or incorrect opioid treatment of acute or chronic pain.[2]

In this review, we present several experimental approaches performed in the Laboratory of the Biology of Addictive Diseases to characterize the relationship of gene variations and epigenetics with heroin and cocaine addictions, and pharmacogenetics. The subjects recruited for our heroin opiate addiction association and pharmacogenetics studies were all unrelated former, or active severe heroin addicts that met the criteria for entry into a methadone maintenance program (i.e., a history of at least 1 year of daily multiple uses of heroin or other short-acting narcotics). Most subjects are currently in methadone maintenance treatment. The subjects for the cocaine association studies were selected based on the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV), criteria for cocaine dependence or combined cocaine/alcohol codependence and the Addiction Severity Index.

The control subjects were recruited based on the following exclusion criteria: (1) at least one instance of drinking to intoxication or any illicit drug use in the previous 30 days; (2) a history of alcohol drinking to intoxication or illicit drug use, more than twice a week, for more than 6 consecutive months; and (3) cannabis use for more than 12 days in the previous 30 days and/or past use for more than twice a week for more than 4 years.

Gene variants involved in heroin and/or cocaine addiction

Opioid system genes

Mu opioid receptor gene

The G protein-coupled mu opioid receptor (encoded by *OPRM1*) is the main target of morphine, heroin, and methadone, and it plays an important role in opioid tolerance and dependence. Individual differences in response to opiate drugs may be attributed in part to genetic variations in the *OPRM1* gene. In this review we will update our previous reviews.[1,3,4]

The nonsynonymous variant rs1799971 (118A→G, Asn40Asp, exon 1) that removes the *N*-glycosylation site in OPRM1 extracellular domain is the most studied *OPRM1* polymorphism. The Asp40 variant receptor (118G) that was originally shown to be more potent in β-endorphin binding and receptor activity,[5] was recently shown to reduce agonist-induced receptor signaling efficacy, but not binding, in human postmortem brain.[6] *In vitro* expression studies of the variant mu opioid receptor in two cell lines (HEK293 and AV-12) reported differences between transient and stable OPRM1 expression.[7] In the stable expression, lower receptor binding site availability and lower forskolin-induced cAMP accumulation were found. In addition, there was a difference in the mediation of cAMP signaling by morphine and methadone, but not β-endorphin.[7] Zhang *et al.*[8] reported an allelic expression imbalance, where the 118G allele has less abundant expression than the 118A allele in autopsy brain samples, indicating loss of OPRM1 function. An impaired opioid neuropeptide transcription system was associated with 118G in heroin abusers in a postmortem brain study.[9] Several studies reported positive association between single-nucleotide polymorphism (SNP) 118A→G and opioid dependence, as well as other substance dependencies, in diverse populations[10,11] (for additional references see our recent review[1]), whereas other studies did not find association with this SNP.[12–16]

The mu opioid receptor also modulates the stress-responsive hypothalamic–pituitary–adrenal (HPA) axis, which is altered in patients with addictive diseases.[3,17] Healthy subjects with the 118G allele showed an increased basal level of cortisol[18] and greater cortisol responses to opioid receptor blockade with naloxone,[19,20] in what may be a population-specific effect because the effect was limited to European Americans and was not seen in Asians.[21]

The 118A→G variant was also associated with the pharmacogenetics variability morphine response (for review see Ref. 22), as was shown by interindividual differences in pain scores and self-administered intravenous morphine,[23] as well as chronic pain that requires higher doses of analgesic.[24] These studies suggested a reduction in morphine effectiveness by this variant.

Positive responses to heroin after first use were found to be associated with three *OPRM1* intron 1 tag SNPs in Chinese.[25] Analysis of 12 intronic SNPs spanning the gene locus in European Americans revealed association of intron 1 SNPs with drug dependence (cocaine and opioid),[13] but this result was not supported by a similar study.[16] In a hypothesis-driven case–control association study, using a Golden Gate Illumina custom array we analyzed 1350 variants in 130 candidate genes in subjects with European ancestry. Two variants from intron 1 (rs510769 and rs3778151) showed association with heroin addiction.[12] A 10 K association study from our laboratory[26] identified association of an SNP located 11.6 kb upstream of the *OPRM1* gene with heroin addiction. Transcription regulation of OPRM1 was shown to be modified by two promoter variations (positions −554 and −1320).[27]

The existence of subpopulations of mu opioid receptors has been suggested based on binding assays, pharmacological studies, and clinical observations.[28] Because only one *OPRM1* gene was cloned, one explanation for these observations was the existence of alternative splice variants. Several splice variants have been reported in humans and rodents, but their biological function is yet to be elucidated.[29–31] One variant that retains a portion of intron 1 was shown to form a heterodimer with OPRM1, suggesting a possible role as a

modulator.[32] Two SNPs in intron 3, located at a novel exon of an alternative splice variant, were not associated with opioid dependence.[33]

Epigenetic studies of the mu opioid receptor gene
Epidemiological studies indicate that nongenetic factors contribute 40–60% of the risk of developing drug addiction.[34–44] Some of these are environmental and drug-induced factors, but other factors, such as "epigenetic modifications" (i.e., DNA methylation and chromatin remodeling), may also play an important role. The transmission of information not encoded in the DNA sequence is termed epigenetic inheritance. DNA methylation and covalent histone modifications are the primary sources of epigenetic inheritance. DNA methylation of cytosine residues in genomic DNA is a common epigenetic mechanism controlling gene expression and occurs through the addition of a methyl group to cytosine residues in cytosine:guanine (CpG) dinucleotides by DNA methylation enzymes. CpG dinucleotides are often clustered in "CpG islands."[45] CpG islands are at least 200 base pairs (bp) with a CpG percentage that is greater than 50% and a CpG content of at least 60% of that which would be expected (∼4–6%).[46] In humans, there are about 45,000 CpG islands, many of which are found in the promoter regions of genes. These CpG islands are generally located upstream of the transcription start site to within the first exon.[46] Roughly 70% of the CpG dinucleotides in the genome are methylated, whereas most of the CpG islands in the promoters of housekeeping genes (i.e., genes constitutively transcribed in most cells and representing 60% of the genome) are unmethylated.[47] Genes without CpG islands, in general, are repressed by the methylation of CpG dinucleotides in their promoter regions (reviewed in Refs. 48–50). This occurs through the methylation-mediated disruption of the binding of transcription factors that include CpG sites in their cognate transcription binding sites.[51–53.]

We recently reported a study on methylation of CpG sites in the mu opioid receptor gene promoter region in former heroin addicts stabilized in methadone treatment and in control subjects. We hypothesized that there would be differences in methylation at specific CpG sites in the promoter of the *OPRM1* gene between these groups of subjects. We found that in DNA obtained from peripheral lymphocytes, two of 16 CpG sites in a region of

the *OPRM1* gene promoter had significantly higher methylation in former heroin addicts than in controls.[54] The two CpG sites that were hypermethylated in the former heroin addicts are located in binding sites for the potential Sp1 transcription factor. It is possible that the hypermethylation at these sites reduces expression of the *OPRM1* gene in former heroin addicts. Future studies may determine whether the hypermethylation of these CpG sites was due to methadone maintenance pharmacotherapy, heroin, imprinting, or major life events prior to heroin use.

Other studies have shown that drugs of abuse can also alter DNA methylation. In genomic DNA from lymphocytes, overall DNA methylation was higher in alcoholics than controls.[55,56] It was also reported that the alcoholics had a decrease in expression of the DNA methyltransferases DNMT-3a and DNMT-3b.[57] In alcoholics, there was an increase in DNA methylation of the promoter region of the alpha synuclein gene *SNCA*[58] and of the homocysteine-induced endoplasmic reticulum protein gene *HERP*.[55] Another group reported that in lymphoblast cell lines from women, but not men, overall DNA methylation was significantly associated with alcohol dependence and nicotine dependence.[59] Maternal cocaine exposure in mice decreased global methylation at day 3 postnatum (P3) and increased by approximately 35% in global DNA methylation at P30 in hippocampal pyramidal neurons.[60]

Methylation marks (CpG methylation) in the DNA may persist for decades or change rapidly. The imprinted *IGF2* gene promoter was reported to be hypomethylated in subjects who were exposed prenatally to famine during the Dutch Hunger Winter of 1944–1945.[61] Alternatively, methylation levels may change rapidly. In rats, methylation of the *reelin* gene was decreased and methylation of the protein phosphatase 1 gene was increased 1 h after exposure to fear conditioning.[62] Aberrant DNA methylation occurs in cancer through the downregulation of tumor-suppressing genes.[63–67] Azacytidine, a DNA methylation inhibitor whose mechanism of action is to reactivate silenced genes through the hypomethylation of DNA, has been approved for treatment of myelodysplastic syndromes.[68] Recent studies showed that a single cocaine injection induced chromatin remodeling at the *cFos* promoter in rat striatum, and at the *cdk5*, and *bdnf* promoters

after chronic cocaine administration.[69] This remodeling by chronic cocaine administration has been suggested to occur through a decrease in the histone deacetylase HDAC5 function.[70] In other studies, cocaine administration in rats produced decreased histone methylation in the prefrontal cortex.[71]

Kappa opioid receptor gene

Dynorphin and the kappa opioid receptor (KOPr) are localized in several areas of the dopaminergic nigrostriatal and mesolimbic–mesocortical systems, and they play an important role in a modulation of opioid, cocaine, and other rewarding stimuli, presumably through modulation of basal and drug-induced dopaminergic tone.[72] In contrast to mu opioid receptor ligands, dynorphin peptides decrease basal and drug-induced dopamine levels in several areas of the dopaminergic nigrostriatal and mesolimbic–mesocortical system. The KOPr–dynorphin system may therefore be considered to be a part of the countermodulatory mechanisms of the brain after direct or indirect drug-induced dopaminergic stimulation.[1] Earlier studies showed that pretreatment with KOPr agonists decreases the psychostimulant and conditioned rewarding effects of cocaine in rats and decreases the rate of intravenous cocaine self-administration.[73–75] However, recent studies demonstrated different effects of acute and chronic activation of the dynorphin/KOPr system in various models of cocaine-seeking behavior in rodents. Repeated infusion of the KOPr selective agonist U50,488 first suppressed and then potentiated cocaine-induced place preference in rats[76] and produced an increase in the relative reinforcing effects of cocaine in comparison with food in rhesus monkeys.[77] The novel KOPr receptor antagonist, JDTic, significantly reduced footshock-induced reinstatement of cocaine self-administration but did not affect cocaine-primed induced reinstatement.[78] Stress and chronic drug abuse increase dynorphin expression, raising the possibility that dynorphin modulates the depressive-like effects of both stimuli that can be blocked by a KOPr antagonist.[79,80] The mechanisms of this KOPr agonist–induced cocaine reinforcing potentiation have yet to be established.

The human *OPRK1* gene is located on chromosome 8q11.2. Previously, we have identified a full exon–intron structure of the human *OPRK1* gene and demonstrated that the human *OPRK1* gene has at least four major exons and three introns, and the

3′ untranslated region (UTR) of 3096 nucleotides, similar to rodent *Oprk1* genes.[81] In this study, we genotyped 12 SNPs, located in coding and intron 1 regions of the gene. Using logistic regression with opioid dependence as the dependent variable, the 36G→T SNP (rs1051660) exhibited a point-wise significant association with disease status. A haplotype of eight SNPs was identified in Hispanics with significant difference in frequencies between cases and controls. This finding was replicated in an independent study of association of the SNP rs1051660 with opiate addiction in a European American population.[82] Another study tested association of seven *OPRK1* gene variants with substance dependence risk in a large cohort of European Americans.[83] Although no significant differences in allele and genotype frequencies were found between cases and controls, logistic regression analysis showed that two SNPs, including 36G→T, may be associated with cocaine dependence. In addition, a specific *OPRK1* haplotype of seven SNPs was significantly associated with alcohol dependence. A study by Xuei *et al.*[84] examined 13 SNPs throughout the *OPRK1* gene in a large group of European American people from alcohol-dependent families and found several of the gene variants to be associated with increased risk for alcohol dependence. A high frequency 830-bp insertion/deletion (indel) was found 1389 bp upstream of the transcription start site of *OPRK1*.[85] A reporter gene expression assay showed an inhibitory effect of the insert on the *OPRK1* promoter transcription activity. This study showed that the presence of an 830-bp insert, rather than its deletion, is associated with alcohol dependence in European Americans.

Prodynorphin gene

The human prodynorphin gene (*PDYN*) is located at chromosome 20pter-p12.2 and spans 15.3 kb. The gene consists of four exons. Exon 1 and exon 2 contain the 5′ UTR, exon 3 encodes a signal peptide, and exon 4 encodes dynorphin peptides, including a-neoendorphin, b-neoendorphin, dynorphin A, and dynorphin B. Dynorphin peptides and prodynorphin mRNA are particularly abundant in the nucleus accumbens, caudate, amygdala, hippocampus, and hypothalamus.[86–88] Currently, two transcription factor binding sites within the *PDYN* promoter have been shown to play a role in regulation of gene expression. A 68-bp nucleotide tandem repeat polymorphism (rs35286281) is located 1250 bp

upstream of exon 1.[89] This polymorphism, which contains a putative AP-1 transcription complex (c-Fos/c-Jun) binding site, is found in one to four copies. An *in vitro* study, using a minimal *PDYN* promoter in a reporter gene expression assay in mouse neuroblastoma cells (NG108–15), showed that constructs containing three or four copies of the repeat produced approximately 1.5 greater levels of forskolin-induced (but not basal) transcriptional activity than did constructs with one or two copies of the repeat.[90] However, ongoing studies in our laboratory suggest that the opposite may pertain.

Expression of the human *PDYN* gene is also regulated by the calcium-binding protein downstream regulatory element (DRE) antagonist modulator (DREAM).[91] In basal conditions; DREAM is bound to the DRE and represses the expression of target genes. Acute cocaine administration significantly increases the intracellular calcium concentration in cell culture[92] and in rodent brain.[93] The cocaine-induced increased calcium levels lead to release of DREAM from the DRE site and to derepression of *PDYN* transcription.

Several studies have examined an association of this polymorphism with drug dependence, with conflicting results. One study showed that Hispanic individuals with three or four copies of the repeat have a lower risk for development of cocaine dependence.[94] Two subsequent studies using more stringent diagnostic criteria showed increased risk for cocaine/alcohol codependence in African Americans with three or four repeats.[95,96]

Recently, it has been recognized that the classical approach for association of DNA variants with phenotype or disease, being important for identification of the potentially causative gene polymorphisms, provided limited functional information.[97,98] Therefore, a new strategy, called genetical genomics, has been developed, which integrates DNA variation, gene expression, and disease phenotype data.[99,100] Genetical genomics approaches treat gene expression levels as intermediate expression quantitative trait loci between DNA sequence variations and phenotypes and use expression quantitative trait locus mapping to identify genetic loci controlling gene expression. It has been suggested that inherited variations affecting gene expression may play an important role in susceptibility to complex disorders, including drug addiction and alcoholism.[101–103] Several studies have been performed

to elucidate the patterns of genetic variations affecting gene expression in relation to phenotypic variation and disease.[98,104,105]

Although robust and high-throughput methods are available[106,107] for direct measurement of differences in allelic expression, data for candidate genes require validation with independent allele-specific gene expression assays, such as the SNaPshot Multiplex Kit (Applied Biosystems, Foster City, CA). This method is based on a primer extension reaction by comparing the relative level of each variant of mRNA transcript in a tissue from individuals who are heterozygous for an expressed polymorphism.[108–110]

We have applied the SNaPshot assay for allele-specific gene expression analysis and for identification of *cis*-acting SNPs in the *PDYN* gene.[111] Six common *PDYN* variants were genotyped in European and African American individuals. In genotype and allelic tests, we found significant association of three SNPs (rs910080, rs910079, and rs2235749) in the 3′ UTR with both cocaine dependence and cocaine/alcohol codependence in European but not in African Americans. This study extends our earlier work on association of *PDYN* polymorphisms with cocaine dependence[96] and supports a previous study finding an association of these SNPs with alcohol dependence.[84] In our study,[111] analysis of haplotypes revealed only one block of these three SNPs in both ethnic groups. There were only two major complementary haplotypes, TTC and CCT. Haplotype TTC was more frequent in the European American control subjects, whereas the haplotype CCT was associated with a risk for development of cocaine dependence or cocaine/alcohol codependence.

Aside from the 68-bp tandem repeat variants in the *PDYN* promoter, the functionality of other *PDYN* SNPs has not been previously described. To test the hypothesis that the haplotypes TTC and CCT were associated with alterations in *PDYN* mRNA levels, we measured allelic expression of the gene in human postmortem brain tissues from eight subjects heterozygous for rs910079, using the SNaPshot assay. In this method, each allele serves as an internal control against which expression of the other allele can be measured within each mRNA sample. Our results demonstrate the presence of significant allelic differences in mRNA expression of *PDYN* in seven of eight samples analyzed in both the caudate and nucleus accumbens regions, with greater

expression of the common rs910079 T allele and lower expression of the C allele. Because only two major complementary haplotypes (TTC and CCT) were found, the high linkage disequilibrium (LD) of rs910079 with two other 3′ UTR SNPs (rs910080 and rs2235749) suggests that the CCT haplotype is associated with lower *PDYN* expression in the striatum.[111] However, without further experimental data it is not clear which of these SNPs is functional.

Our study provided the first evidence that the SNP rs910079 in the gene was a *cis*-acting polymorphism, related to differential *PDYN* gene expression in an allele-specific manner. Importantly, the allelic-gene expression assay was performed in the caudate and nucleus accumbens, which are principal brain regions in the rewarding effects of drugs of abuse.[112] Moreover, the measurements of the total *PDYN* mRNA levels in the caudate from 43 postmortem brains demonstrated a strong effect of the TTC and CCT haplotypes. The subjects with homozygous diplotypes consisting of the "protective" TTC haplotypes had significantly higher levels of *PDYN* mRNA than the mRNA levels in the subjects with homozygous diplotypes of "risk" CCT haplotypes. It is of interest that the significant relationship observed between the genotypes and total *PDYN* mRNA levels in our rather small sample was not dependent on ethnicity or other variables of postmortem tissues.

The discovery of allelic *PDYN* expression differences raises the question of whether the 3′ UTR SNP rs910079 is functional or linked to other functional variants. The 3′ UTR of genes are rich in regulatory elements essential for mRNA stability and degradation, nuclear transport, and translation.[113] These diverse regulatory roles are executed via *cis*-acting elements that interact with many *trans*-acting factors in a given cellular environment, including targeting by microRNAs.[114,115] Further studies of the promoter and the 3′ UTR regulatory elements in *PDYN* mRNA are required to elucidate their functional roles.

Among the two variants in the *PDYN* promoter region analyzed in this study, only the −301A→G SNP (rs1997794) was in linkage with the *cis*-acting 3′ UTR SNP rs910079 in European American people. The minor G allele of rs1997794 eliminates a putative binding site TGTGTCA for the AP-1 transcription factor. Because the G allele of this SNP is more frequently associated with the risk haplotype CCT,

it may contribute to lower expression of the *PDYN* gene in cocaine-dependent and cocaine/alcohol-codependent subjects. Therefore, rs1997794 is a good candidate to be a *cis*-regulatory SNP. Additional *cis*-acting elements in the *PDYN* gene may exist, particularly *cis*-regulatory elements and epigenetic factors that may be involved in differential gene expression. A recent study of keratin 1 gene (*KRT1*) expression in white blood cells suggests that allelic expression differences result from the cumulative contribution of multiple *cis*-regulatory sequences, interacting with both transcriptional activators and transcriptional repressors.[116]

We have measured *PDYN* mRNA levels in the caudate first because rodent studies in our laboratory and others showed a robust response in this region to acute and chronic cocaine administration.[117–119] Our laboratory has long hypothesized that the dorsal striatum (caudate and putamen) is centrally involved in drug addiction. Neuroimaging of cocaine-dependent subjects showed the largest dopamine changes in the dorsal striatum, and the magnitude of these changes was correlated with self-reports of craving.[120] It has been suggested that the dynorphin–KOPr system might be part of the countermodulatory mechanisms of the brain after drug-induced dopaminergic stimulation, and dysregulation of this system may contribute to the development of cocaine dependence and cocaine/alcohol codependence.[4,112]

Hypothalamic–pituitary–adrenal axis genes

Melanocortin receptor type 2 gene
The melanocortin receptor type 2 (*MC2R* or adrenocorticotropic hormone, ACTH receptor) gene is part of the superfamily of G protein-coupled membrane receptors and is involved in regulation of adrenal cortisol secretion, important in the physiological response to stressors. HPA axis dysregulation has been found in association with several physical and psychological conditions: posttraumatic stress disorder,[121] fibromyalgia,[122] Alzheimer's disease,[123] major depression, and specific stressors.[124,125] Our group has found that specific addictive diseases are also associated with dysregulation of the HPA axis: hyperresponsivity to removal of glucocorticoid negative feedback was found in cocaine addicts[126]; HPA hypoactivity was found in medication-free illicit drug–free former heroin addicts.[127]

Being derived from anterior pituitary peptide proopiomelanocortin, hormone ACTH regulates adrenal glucocorticoid and androgen synthesis in the zonae fasciculata and reticularis in the adrenal cortex. ACTH binds to its specific receptor, MC2R or ACTH receptor.[128]

In genetics studies, several SNPs in the *MC2R* gene have been linked to familial glucocorticoid deficiency.[129–131] Studies of healthy volunteers led to the discovery of the possible involvement of MC2R in stress regulation mechanisms. Substitution of A to G in the −179A→G (also called −2T→C) SNP results in lower promoter activity *in vitro* and is found in association with impaired cortisol response to ACTH stimulation *in vivo*.[132] A clinical study with ACTH stimulation tests showed that homozygous AA individuals have a significantly higher dehydroepiandrosterone response than homozygous GG individuals, whereas baseline dehydroepiandrosterone concentrations did not differ between groups.[133] Several putative transcription factor binding sites, including AP1, CRE and Sp1, have been identified in the promoter region of the *MC2R* gene.[134]

In recent studies performed by our group,[135] we sequenced the coding region of the *MC2R* gene in a search for novel polymorphisms in three different ethnicities (European Americans, African Americans, and Hispanics) and tested a series of individual SNPs and statistically inferred haplotypes of the *MC2R* gene in association with vulnerability to develop a heroin addiction. In Hispanics, we found an experiment-wise significant association of the minor allele A of the −184G→A (rs2186944) and the haplotype AACT, consisting of −184G→A, −179A→G, 833A→C (resulting in F278C; rs28926182), and 1005C→T (rs4797824), with a protective effect from the development of heroin addiction.

Dopamine and serotonin pathway genes

Catechol-*O*-methyltransferase gene

Catechol-*O*-methyltransferase (COMT) is important in metabolism of catechol neurotransmitters, including dopamine. Alterations in the dopaminergic system might be caused by administration of drugs of abuse. Reduction of levels of striatal dopamine and dopamine D2 receptors has been found after chronic administration of cocaine in an-

imal models.[136–139] In human studies, brain imaging shows reductions in striatal dopamine D2 receptors in subjects addicted to drugs of abuse.[140] COMT has been found in both peripheral and central tissues (for review see Ref. 141).

A substitution of 472G→A (Val158Met) results in a fourfold decrease of activity of COMT.[142–144] A study of human lymphoblast cell lines and brains showed that allele 158Met was overexpressed compared to 158Val.[145] As shown in a functional magnetic resonance imaging study, amphetamine administration enhances the prefrontal cortex functioning in individuals homozygous for the 158Val allele during a working memory task, whereas for individuals homozygous for the Met allele, no enhancement of cortical efficiency was found.[146] The number of 158Met alleles (one versus two) was correlated with the ability to experience reward in daily life of the subject.[147]

The 158Val allele was associated with polysubstance abuse in European American people,[148] heroin addiction in European American[149] and Chinese[150] subjects, and abuse of methamphetamine in Han Chinese.[151] The 158Met allele was found to be associated with novelty seeking in European American amphetamine abusers.[152] Different specific haplotypes of COMT were associated with cocaine dependence in African Americans.[153] In studies of human postmortem brain[154] in heroin abusers, levels of proenkephalin within the nucleus accumbens correlated to the COMT Val158Met genotype. Control Met/Met subjects expressed lower proenkephalin mRNA than Val carriers, with the opposite pattern in heroin users. Study of dopamine transporter–COMT gene–gene interaction[155] showed that subjects homozygous for 158Met/Met with lower COMT activity and higher dopamine availability have larger responses in prefrontal and ventral striatum activities in anticipation of reward than that in 158Val/Val homozygous subjects. Recent global scanning of 63 SNPs, in DNA samples collected from 45 populations, in the 172-kb region surrounding the *COMT* gene revealed haplotypes that may harbor functional concequences.[156]

The effect of association might be sex specific: in one study,[157] the 158Met allele was associated with obsessive–compulsive disorder in males but not in females; allele 158Val was associated with alcoholism in American Indian females but not

in males.[158] *COMT* homozygous knockout female mice develop increased anxiety in a light–dark model compared to *COMT* knockout males; also in male mice only an increased aggressive behavior in *COMT* heterozygous knockouts compared to other genotypes was found.[159] In *in vitro* cellular studies, physiological concentrations of 17β-estradiol were shown to downregulate *COMT* gene transcription and protein expression.[160,161] This may account in part for the differences observed between the sexes.

In recent studies performed by our group[162] we sequenced exon 4 of the *COMT* gene in a search for novel polymorphisms and then genotyped four of five SNPs identified by direct sequencing, using the TaqMan assay. Genotype frequencies of the 472G→A SNP, previously identified as one that changes enzymatic activity of COMT, varied significantly among the three main ethnic/cultural groups (European Americans, Hispanics, and African Americans). Using genotype tests, we found a trend to pointwise significant association of the 472G→A SNP in Hispanic subjects with opiate addiction. Further analysis of 472G→A genotypes in Hispanic subjects with data stratified by sex identified a pointwise significant association of G/A and A/A genotypes with opiate addiction in women but not in men. Linkage disequilibrium patterns were similar for the three ethnic/cultural groups.

Tryptophan hydroxylase genes
Serotonin has long been recognized as a major moderator of impulse control. Levels of cerebrospinal fluid 5-hydroxyindolacetic acid (CSF 5-HIAA), a metabolite of serotonin, was first associated with addiction in depressed patients with a family history of alcoholism.[163] Follow-up studies showed that CSF 5-HIAA concentrations were low in subjects with behaviors characterized by a deficit in impulse control, such as impulsivity[164] and aggression.[165,166] Serotonin is involved in several aspects of mood and impulsivity.[167,168]

Serotonin biosynthesis is regulated by its rate-limiting biosynthetic enzyme, tryptophan hydroxylase (TPH).[169] Because TPH controls serotonin biosynthesis, we hypothesized that variants in this gene will be associated with CSF 5-HIAA concentrations as well as with behaviors influenced by serotonin levels.

Early on, we identified a variant in TPH, rs1799913,[170,171] and found that this variant was associated with CSF 5-HIAA levels in Finnish violent offenders,[172] was associated with suicidality, and was linked to alcoholism.[173] This association with alcoholism has been replicated in Taiwanese.[174] The variant rs179913, and the nearby variant rs1800532, have been found associated with several addiction-related behaviors.

Before 2003, it was believed that only one gene encoded *TPH*. Several inconsistencies were observed in studies on the expression of TPH. These inconsistencies were resolved in 2003 when a gene coding for an isozyme of TPH was identified.[175] This newly discovered TPH gene was named *TPH2* and is expressed mainly in the raphe nuclei of the brain.[175–178] *TPH1*, the previously identified *TPH* gene, was found to be expressed in the raphe nuclei only during the late developmental stage and to a high level in the enterochromaffin cells of the gut.[177,179] In the adult brain, *TPH1* and *TPH2* are expressed in the amygdala, cerebellum, cortex, hippocampus, hypothalamus, and thalamus.[178]

We hypothesized that because *TPH1* had been associated with addiction, polymorphisms in *TPH2* may also be associated with addiction. We resequenced the *TPH2* gene (5′ upstream, coding, and 3′ downstream regions, including all 11 exons) in 185 subjects and identified 23 novel and 14 known variants.[180] Using six of the *TPH2* variants and one *TPH1* variant, we genotyped individuals with addictive diseases and healthy volunteers. Because the allele frequencies of five of the variants varied significantly among the ethnicities studied, we conducted associations stratified by ethnicity. In the subjects who met either heroin addiction or control criteria, and who were of African American, European American, or Hispanic ethnicity, significant differences in genotype patterns were observed between the cases and control subjects. In Hispanics, the *TPH1* rs1799913 variant was found to significantly interact with the *TPH2* rs4290270 variant and heroin addiction, and with the *TPH2* variant rs7963720 and heroin addiction. In African Americans, a *TPH2* haplotype was found to be in association with heroin addiction. It is possible that the two TPHs coordinately interact to regulate serotonergic metabolism and influence interindividual vulnerability to develop heroin addiction. This interaction may differ among ethnic groups.

Both pineal and retinal TPHs are the rate-limiting enzymes in melatonin production. Because melatonin controls circadian rhythm, and *TPH2* and *TPH1* have diurnal variations of expression in the pineal gland[181] and in the retina,[182] it is possible that disruption of circadian rhythm is involved in vulnerability to develop an addiction. Rodent studies have shown that serotonin and melatonin may influence the dopaminergic reward pathway.[183–186] Perhaps variants in the *TPH* genes could alter serotonin and melatonin production, thereby altering dopaminergic tone and addiction vulnerability.

Variants in other genes encoding proteins that are involved in serotonin biosynthesis metabolism or neurotransmission have been associated with specific addictions. The serotonin receptors 5-HT$_{2A}$[187–189] as well as 5-HT$_{1B}$ (see following discussion) have been associated with substance abuse or specific addiction. Variants in the metabolizing enzyme monoamine oxidase A have been associated with drug dependence.[190] A repeat polymorphism in the promoter of the serotonin transporter gene has been reported to be associated with heroin dependence[191,192] as well as alcoholism.[193–201]

5-Hydroxytryptamine (serotonin)-1B receptor gene

The 5-hydroxytryptamine (serotonin)-1B receptor (*HTR1B*) is involved in many neuropsychiatric and physiological functions, such as thermoregulation, locomotion, and feeding.[202] Serotonin receptor–knockout mice showed increased spatial memory performance,[203] increased impulsive aggression,[204–206] increased locomotor response to cocaine administration,[207] increased cocaine self-administration,[207] increased alcohol consumption,[208] increased exploratory activity,[203] and decreased anxiety.[206] In rats, administration of serotonin 1B receptor agonists reduced cocaine self-administration.[209]

In gene expression studies, the −161T (rs130058) variant was reported to be expressed consistently in higher levels than −161A.[210] However, the haplotype consisting of −261G (rs11568817) and −161A was reported to enhance transcriptional activity 2.3-fold,[211] compared to the haplotype consisting of −261T and −161A.

A recent study of regulation of silencing of several behavioral candidate genes directed by microRNA revealed an interaction of microRNA miR-96, which is expressed in brain, with a common SNP rs13212041 in the 3′ UTR of this *HTR1B* gene[212] in an allele-specific manner. The presence of allele A in rs13212041 strongly repressed the expression of the gene. This effect was eliminated by substitution of A with G. In human studies, individuals homozygous for the A allele of this SNP reported more conduct-disorder behaviors than individuals having GA or GG genotypes.

A significant association of synonymous polymorphism 861G→C (rs6296) have been reported with a history of substance abuse disorder and with diagnosis of major depressive episode in a population of mixed ethnicities.[213] The same study did not find an association of the same SNP with bipolar disorder, schizophrenia, or alcoholism. For the same SNP, 861G→C, an overrepresentation of allele C was found in alcoholics with inactive aldehyde dehydrogenase-2 in a Japanese cohort.[214] In another study the same SNP was associated with antisocial alcoholism in a Finnish population and also, in a population of Southwestern American Indian tribe.[215] In a group of patients with personality disorder, an association of the polymorphism 861G→C with suicide attempts was found.[216] Another SNP of the *HTR1B* gene, −161A→T, was found in association with alcohol dependence in Taiwanese Han.[218] In study performed by our group[217] no association of polymorphisms of the *HTR1B* gene, including −261T→G, 129C→T (rs6298), and 861G→C, with cocaine abuse and dependence and alcohol abuse and dependence was found.

In another recent study,[218] we tested for association with heroin addiction several SNPs, including −261T→G, −161A→T, 129C→T, 861G→C, and 1180A→G (rs6297), of the *HTR1B* gene, and used a subset of these polymorphisms for molecular haplotype studies. Association analysis of both molecular haplotypes consisting of three SNPs and statistically inferred haplotypes consisting of these five SNPs with heroin addiction was done separately for each of the three ethnicities: African American, European American, and Hispanic. Significant association of statistically inferred haplotype TACGG indicating protective effect from heroin addiction was found in European Americans. Also, an experiment-wise significant association of the minor allele 1180G with protective effect from heroin addiction in European Americans was found.

Pharmacogenetics of methadone treatment

Methadone-metabolizing enzymes

Methadone maintenance is the standard treatment for heroin addiction, and successful treatment relies to a certain extent on individual dose optimization (for review and references see Ref. 1). Methadone is a synthetic opioid that is administered as a racemic mixture of (R) and (S)-enantiomers, yet the (R)-methadone accounts for the opioid effects. Methadone is rapidly absorbed with peak plasma concentrations 2–4 h after oral administration and is metabolized primarily in the liver.[1,219]

The major methadone-metabolizing enzymes are cytochrome P450 CYP3A4, CYP2D6, and CYP2B6 (for recent reviews see Refs. 220–223). The involvement of additional CYP enzymes, including CYP2C19,[224] CYP3A5[225] and CYP2C8,[226] has been suggested. The CYP enzymes are characterized by interindividual, as well as ethnic and sex variation in expression.[227–230] A comprehensive list of *CYP* variants has been compiled (http://www.cypalleles.ki.se) and an ethnic variability in allele frequencies is documented.[231] The large interindividual variation in the pharmacokinetics and response to methadone may be explained in part by some of these genetic variants.

CYP3A4 is the most predominant enzyme of the CYP3A subfamily in the human liver. Conflicting data are available on the functionality and outcome of CYP3A4 variants.[222] An association was recently reported between an upstream variant and CYP3A4 hepatic expression.[232] CYP2B6 has been thought to a have a minor role in methadone metabolism but was recently found to have a larger contribution.[228,233,234] Several *CYP2B6* variants were functionally characterized to be associated with gene expression[229,235,236] (for extensive review of *CYP2B6* variants see Ref. 233). With respect to *CYP2D6* genotypes, the general population consists of extensive (most), intermediate, poor, and ultrarapid metabolizers. Underrepresentation of poor metabolizers in European Americans with opiate addiction was reported.[237] Ultrarapid metabolizers had unsuccessful methadone treatment therapy[238] but have been reported to do well on buprenorphine, which is not significantly metabolized by CYP2D6. Several genetic tests of cytochrome P450 genotypes, including *CYP2D6* and *CYP2C19*, are now U.S. Food and Drug Administration approved and are available commercially, but there is ongoing debate about their interpretation and their benefits for specific drugs. Part of the interindividual variation in response to methadone may be accounted for by CNS CYP enzymes. Brain CYPs may be induced similar to hepatic CYPs, but some inducers may differentially affect liver and brain CYP expression.[239,240]

P-glycoprotein gene (ABCB1/MDR1)

Methadone is a substrate of P-glycoprotein 170 (P-gp).[241–243] P-gp is a member of the subfamily B of the ATP-binding cassette (ABC) superfamily. It is a transmembrane protein that is composed of two homologous sequences, each containing six transmembrane domains and an ATP-binding domain.[244] P-gp has a significant role in drug pharmacokinetics and is expressed in tissues with barrier function, including the epithelia of the liver, kidney, intestine, and the endothelial cells lining of brain capillaries.[245] It has a broad range of substrates that are also often substrates of the CYP450 enzymes. It has been suggested that P-gp variants will have little effect on net methadone intestinal absorption because even relatively low doses of methadone (80–150 mg/day), as used in methadone treatment of opiate addiction, would reach a sufficient concentration to saturate the transporters.[246,247]

P-gp is encoded by the highly polymorphic *ABCB1* gene with variation in allele frequencies among different populations.[248] Genetic variability in the *ABCB1* gene may influence methadone distribution by altering P-gp expression and function. The functional significance of various *ABCB1* polymorphisms is not clear.[246,248–251] The most studied SNP is the synonymous 3435C→T (rs1045642), which showed lower *in vivo* duodenal P-gp expression[252] and lower mRNA expression in human liver samples.[253] An altered substrate specificity, as a result of distorted conformation, but similar mRNA and protein levels, was found to be caused by this variant in human and monkey cell lines.[254] Variants 1236T (rs1128503), 2677T (rs2032582), and 3435T were reported to minimize P-gp activity *in vitro* in a substrate-specific manner.[255] A difference in the *ABCB1* haplotype profile was found between different ethnic groups and low haplotype diversity was observed in European Americans.[256]

In a recent study,[257] we showed significant difference in genotype frequencies between the "higher" (>150 mg/day) and "lower" (≤150 mg/day) methadone dose groups for SNP 1236C→T

and the three-locus genotype pattern (rs1045642, rs2032582, and rs1128503) in Israeli methadone-maintained patients. In a similar study from Australia,[258] in which the methadone levels were much lower (<110 mg/day), there was association between a similar variant haplotype that includes the three SNPs mentioned earlier and lower methadone doses.

Genomewide and multigene association studies

Since the development of high-density microarray technology, it has become possible to interrogate many single-nucleotide genetic variants in one individual. Statistical analyses comparing groups of individuals analyzed using these high-density microarrays have allowed researchers to conduct genomewide association studies. These studies have provided confirmatory evidence for the involvement of previously identified genetic variants and the genes containing these variants, as well as evidence for the involvement of genes and genomic regions that have not been previously associated with the addictions.

Recently, we reported on a genomewide association study using the Affymetrix 10 K GeneChip (Santa Clara, CA), which simultaneously genotyped 10,000 variants used by us to identify genetic variants in genes involved in the vulnerability to develop heroin addiction.[26] DNA specimens from former severe heroin addicts who met federal criteria for methadone maintenance treatment and control subjects, all of whom were European American, were analyzed. We performed separate analyses for the autosomal and the X chromosomal variants. When allele frequency was analyzed for association with heroin addiction, the strongest association was with the autosomal variants rs965972, located in a UniGene cluster of unknown function and in a region predicted to have high regulatory potential, and rs1986513, which is found in a region of high conservation in mammals. When genotype frequency was analyzed for association with heroin addiction, the strongest association was found with a variant in the gene coding for the transcription factor myocardin, *MYOCD*. We analyzed the three most significant variants identified by association with genotype frequency with heroin addiction for common genotype patterns that may be associated with heroin addiction. One genotype pattern of these unlinked alleles

was found to be significantly associated with vulnerability to develop heroin addiction. The pattern that had a 27% population-attributable risk for the development of heroin addiction had an odds ratio of 6.25. Another genotype pattern of these same variants was significantly associated with protection from developing this addiction. This genotype pattern had an odds ratio of 0.13 and explained 83% of the population-attributable risk for developing heroin addiction. An assessment of 393 genes, identified by our laboratory as being involved in some aspects of the development of an addiction, that had variants on the 10 K chip identified five genes associated with the development of heroin addiction. The most significant genes identified were those coding for the mu opioid receptor, the mGluR6 and mGluR8 metabotropic receptors, nuclear receptor NR4A2, and cryptochrome 1 (photolyase-like).

In a recent hypothesis-driven multigene study,[12] we scanned 1350 variants in 130 candidate genes in subjects with European ancestry. The "case" subjects were former severe heroin addicts in methadone maintenance treatment and the control subjects were healthy volunteers who were selected by detailed personal interview and stringent criteria. For this study we used an SNP array that was designed by the group of D. Goldman at the National Institute of Alcohol Abuse and Alcoholism.[259] This approach is based on physiological hypotheses and the genes were selected based on their function and related pathways. Nine variants, in six genes, showed nominal significant associations, but none of these associations remained significant after adjustment for multiple testing. These variants were in noncoding regions of the genes encoding the mu (*OPRM1*), kappa (*OPRK1*), and delta opioid receptors (*OPRD1*); the neuropeptide galanin (*GAL*); the serotonin receptor subtype 3B (*HTR3B*); and the casein kinase 1 isoform epsilon (*CSNK1E*).

Several linkage studies have provided evidence for the involvement of different chromosomal regions in the development of heroin addiction.[260–262] The Tsuang group studied Chinese families using short tandem repeat markers and found evidence for linkage for a region on chromosome 4 at D4S1644 with heroin dependence.[263] In a follow-up study that included the original and additional families, they found a linkage peak on chromosome 4 at D4S1644.[262] In another linkage study using short tandem repeat markers, a linkage peak was found

at D17S785 on chromosome 17 that associated with heroin addiction.[260]

Other studies have used SNPs in linkage studies on opioid dependence. Lachman *et al.* found a region on chromosome 14q that was "suggestive" of genomewide evidence for linkage.[263] The Gelernter study identified eight variants with pointwise significance for association with opiate dependence.[264]

To reduce genotyping costs, an alternative method of genotyping has been developed that analyzes pools of multiple DNA samples.[265] This pooling technique allows a comparison of allele frequencies between case and control pools. This technique has been successfully used to find differences in allele frequencies in studies of addiction. In an earlier study using a 1494-variant chip, Uhl *et al.* identified several variants associated with vulnerability to develop drug abuse.[266] Using the 10 K GeneChip and with pools of African and European American cohorts, they identified 38 "nominally reproducibly positive" variants associated with nonspecific substance abuse.[267] Next using the 100 K GeneChip, the Uhl group found in European Americans 51 "clustered positive" regions associated with alcohol dependence.[268] From these studies and an additional study that used the 500 K GeneChips, they found 89 genes that may play a role in the vulnerability to develop an addiction.[269] Using the pooling methodology and samples from Japan and Taiwan, Uhl *et al.* identified 39 genes that were associated with methamphetamine dependence.[270] In a recent study using pools of subjects and the Illumina Human HapMap550 array, the Uhl group identified 23 genes that overlap for the development of both substance dependence and bipolar disorder.[271]

Studies will be needed using additional cohorts of well-defined ethnicity and carefully defined addiction phenotypes to replicate the myriad of findings using genomewide arrays. Confirmation of these findings may lead to the identification of new targets for the treatment and prevention of substance dependence.

Development of related techniques

Development and application of the custom on-site-made microarrays for genotyping of polymorphisms of opioid genes

We designed and tested several approaches for genotyping of a few *OPRM1* gene SNPs by using on-site-made microarrays based on polyacrylamide gel pad technology.[272] This technology uses polyacrylamide gel pads as base elements of microarrays. Having three-dimensional structure, such microarrays provide higher hybridization signal intensity than two-dimensional arrays. Such arrays are simple in preparation and might be manufactured either using a robotic station or manually.[273] Oligonucleotides, proteins,[2] or DNA PCR products[274] might be immobilized within elements of such arrays as probes. These arrays have been widely used for different purposes, including analysis of thermodynamic parameters of DNA duplexes,[275] detection of pathogenic microorganisms,[276] quantification of viral mutants in vaccines,[277] analysis of DNA–ligand interactions,[278] and *de novo* sequencing of short DNA.[279]

Using gel pad microarray technology, we developed two separate approaches for genotyping of the polymorphisms at positions 17 and 118 of the *OPRM1* gene.[280] The first approach was based on the hybridization of the fluorescently labeled DNA fragment that encompasses the polymorphisms of interest, with complementary oligonucleotide probes immobilized on microarrays. Thirty-six human DNA samples were analyzed by both custom microarrays and by conventional direct sequencing, with concordant identification of both heterozygous and homozygous substitutions. The second approach for microarray SNP analysis was based on the enzymatic extension of the immobilized oligonucleotide with fluorescently labeled nucleotide triphosphate, using DNA as a matrix. These custom gel pad microchips have potential for the rapid and inexpensive detection of specific SNPs for genetic studies.

Development of a novel technique of molecular haplotyping based on the use of fluorescent PCR; practical application of molecular haplotyping for genetics of drug addiction

Recent studies showed that haplotypes might be more relevant in association studies than individual SNPs. The most common and least expensive approach for haplotyping suggests assignment of statistically inferred haplotypes for each sample on the basis of available genotype data. Our recent studies have shown that different statistical

algorithms may provide different results in assignment of statistically inferred haplotypes.[180] As an alternative method to study haplotypes, the genotyping of the polymorphisms in the families of the subjects is used. This approach is difficult to use for the alcoholism or drug addiction studies. Without breaking the ethics of confidentiality, it is extremely difficult to conduct family-based genetic studies of addiction, which makes it difficult or impossible to recruit family members. Molecular means provide an alternative approach for haplotype analysis, which does not require involvement of a patient's family members. At least one DNA sample is required for performing molecular haplotyping, compared with several hundred samples required for a statistical approach. Therefore, the molecular approach allows for analyzing data without any assumptions.

Most common methods for molecular haplotype identification are based on either amplification of one DNA molecule or separation of DNA strands by using allele-specific amplification, cloning, hybridization, or other means. The products of amplification may be then analyzed by capillary electrophoresis,[281,282] mass spectrometry,[283] melting curve analysis,[284,285] hybridization, microarrays,[286] pyrosequencing, and microchip electrophoresis. Among other detection approaches that were used for molecular haplotyping are a combination of liquid chromatography and electrospray ionization time-of-flight mass spectrometry,[287] bead-based approach,[288] linking emulsion PCR,[289] and the combination of atomic force spectrometry and carbon nanotubes.[290] The combination of allele-specific amplification and long-range amplification technologies allows one to haplotype amplicons up to 10,000 nucleotides and longer. Haplotyping of overlapping amplicons ("tiling" approach) was used for reconstruction of haplotypes consisting of 105 SNPs.[282] Although some techniques were demonstrated to be useful for haplotyping genome fragments consisting of tens of thousands of nucleotides including polymerase colony or "polony" amplification[291] or analysis of overlapping amplicons that are results of long-range amplification,[282] only a few of these methods were demonstrated to be useful for high throughput applications. Most of these methods (e.g., the mass spectrometry–based approach) require complicated instrumentation or are difficult to apply for high-throughput studies.

We developed a novel approach for the performance of the molecular haplotyping by using a joint application of allele-specific amplification and a variation of fluorescent PCR, TaqMan.[218,292] Allele-specific primers having a 3′-terminal base complementary to the flanking polymorphisms of the haplotype region are used for the allelic assignment of the flanking polymorphisms in combination with fluorescently labeled TaqMan probes complementary to the internal polymorphisms. The change in the fluorescence of the solution during the PCR amplification is observed when both primers and fluorescently labeled probe are specific for the allele presented in the DNA sample. This approach requires only one enzymatic reaction, which makes this method more reliable, considerably less expensive, and less susceptible to errors due to a lower probability of cross-contamination of samples than other methods of molecular haplotyping. Haplotypes containing polymorphisms separated from each other by 390, 289, 99, or even two bases were successfully identified in our studies. All these features allowed us to apply the method easily for molecular haplotyping of SNPs of *HTR1B* and *OPRK1* genes in high-throughput mode.

Complete concordance was found among (1) data produced by our haplotyping method, (2), genotype analysis using the TaqMan assay, and (3) results of statistical haplotyping performed using the SNPHAP program.[218] To compare the individual haplotyping pairs determined by molecular haplotyping with statistically inferred haplotypes and to test for genetic association with heroin addiction in three different case–control groups (African American, European American, and Hispanic), a likelihood ratio test that incorporates information collected using molecular haplotyping, and also haplotyping statistically inferred from genotype data, was applied. Every individual's statistically inferred haplotype pair agreed with the individual's haplotype pair based on molecular haplotype.

Summary

In this article we reviewed the studies of several gene variants that may contribute to the vulnerability to develop cocaine and/or heroin addiction, as well as to the efficacy of methadone treatment. We also described multigene association studies of heroin addiction and relevant technique developments.

Some of the findings are well supported and some are still tentative. Additional studies are necessary to confirm the role of the identified variants; to identify novel ones; and to characterize their interaction with other variants, other genes, and the environment.

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Conflicts of interest

The authors declare no conflicts of interest.

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