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Search for Genetic Markers and Functional Variants Involved in the Development of Opiate and Cocaine Addiction, and Treatment

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Abstract

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 genome-wide and multi-gene association studies, we have found association of a number of new genes and new variants of known genes with heroin addiction. Finally, we have described 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: (i) environmental factors, including cues, conditioning, external stressors and the stress they cause; (ii) drug-induced factors, that lead to a variety of molecular neurobiological changes resulting in altered behaviors; (iii) 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

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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 one year of daily multiple uses of heroin or other short-acting narcotics). Most of the subjects are currently in methadone maintenance treatment. The subjects for the cocaine association studies were selected based on the DSM-IV criteria for cocaine dependence or combined cocaine/alcohol co-dependence 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 (c) 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

The 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 non-synonymous variant rs1799971 (118A>G, Asn40Asp, exon 1) that removes *N*-glycosylation site in OPRM1 extra cellular domain is the most studied *OPRM1* polymorphism. The Asp40 variant receptor (118G) that was originally shown to be more potent in beta- 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 beta-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 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 while 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 cortisol¹⁸ and greater cortisol responses to opioid receptor blockade with naloxone,^{19, 20} in what may be a population-specific effect since the effect was limited to European Americans (EA) and was not seen in Asians.²¹

The A118A>G variant was also associated with the pharmacogenetics variability morphine response (for review see),²² as was shown by inter-individual 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 10K association study from our laboratory²⁶ identified association of a 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 a single *OPRM1* gene was cloned, one explanation for these observations was the existence of alternative splice variants. A number of 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 non-genetic 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 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, while 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 ^{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 one hour after exposure to fear conditioning.⁶² Aberrant DNA methylation occurs in cancer through the down-regulation of tumor-suppressing genes.^{63–67} Azacitidine, 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

The 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 play 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 counter-modulatory mechanisms of the brain following 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 differential effects of acute and chronic activation of the dynorphin/KORr 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, JDTC, 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 has 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'-UTR region of 3096 nucleotides in length, similar to rodent *Oprk1* genes.⁸¹ In this study, we genotyped twelve 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 Caucasian population.⁸² Another study tested association of seven *OPRK1* gene variants with substance dependence risk in a large cohort of Caucasians.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 *OPRK1* gene in a large group of Caucasians from alcohol-dependent families and found a number 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 Caucasians.

The 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' untranslated region (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-base pair 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 1–4 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 compared with 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 de-repression 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 very 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 (eQTL) between DNA sequence variations and phenotypes and use eQTL 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} A number of 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 using independent allele-specific gene expression assays, such as the SNaPshot Multiplex Kit (Applied Biosystems). 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 to identify *cis*-acting SNPs in the *PDYN* gene.¹¹¹ Six common *PDYN* variants were genotyped in Caucasian 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 Caucasians, 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 Caucasian 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 individual mRNA sample. Our results demonstrate the presence of significant allelic differences in mRNA expression of *PDYN* in seven out 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 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 compared with 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 a multitude of *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 Caucasians. 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 which 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 counter-modulatory 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

The melanocortin receptor type 2 gene—The melanocortin receptor type 2 (*MC2R* or adrenocorticotrophic 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. Hypothalamic-pituitary-adrenal (HPA) axis dysregulation has been found in association with a number of physical and psychological conditions: posttraumatic stress disorder,^(Griep *et al.* 1998¹²¹) fibromyalgia¹²², Alzheimer's disease¹²³, major depression and specific stressors.^{124, 125} Our group has found that specific addictive diseases are also associated with dysregulation of HPA axis: hyperresponsivity to removal of glucocorticoid negative feedback was found in

cocaine addicts;¹²⁶ HPA hypo-activity was found in medication-free illicit drug-free former heroin addicts.¹²⁷

Being derived from anterior pituitary peptide proopiomelanocortin (POMC), 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, a number of 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 (DHEA) response than homozygous GG individuals, while baseline DHEA concentrations did not differ between groups.¹³³ A number of 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 (Caucasians, 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, comprising 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

The 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 animal 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 see¹⁴¹).

A substitution of 472G>A (Val158Met) results in a four-fold decrease of activity of COMT.^{142–144} A study of human lymphoblast cell lines and brains showed that allele 158Met was over-expressed compared to 158Val.¹⁴⁵ As shown in a functional magnetic resonance imaging (fMRI) study, amphetamine administration enhances the prefrontal cortex functioning in individuals homozygous for the 158Val allele during a working memory task, while for individuals homozygous for the Met allele, no enhancement of cortical efficiency was found.¹⁴⁶ The number of 158Met alleles (one vs. 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 Caucasians,¹⁴⁸ heroin addiction in Caucasian¹⁴⁹ and Chinese subjects¹⁵⁰ and abuse of methamphetamine in Han Chinese.¹⁵¹ The 158Met allele was found to be associated with novelty seeking in Caucasian amphetamine abusers.¹⁵² Different specific haplotypes of COMT were associated with cocaine dependence in African Americans.¹⁵³ In studies of human postmortem brain¹⁵⁴ in heroin abusers, levels of proenkephalin (PENK) within the nucleus accumbens correlated to the COMT Val158Met genotype. Control Met/Met subjects expressed lower PENK mRNA

than Val carriers with the opposite pattern in heroin users. Study of dopamine (DA) transporter-COMT gene-gene interaction¹⁵⁵ showed that subjects homozygous for 158Met/Met with lower COMT activity and higher DA availability have larger responses in prefrontal and ventral striatum activities in anticipation of reward, compared to 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 consequences.¹⁵⁶

The effect of association might be gender-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 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 17-beta-estradiol were shown to down-regulate *COMT* gene transcription and protein expression.^{160, 161} This may account in part for gender differences observed.

In recent studies performed by our group¹⁶² we sequenced exon 4 of the *COMT* gene in a search for novel polymorphisms and then genotyped four out of five SNPs identified by direct sequencing, using 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 (Caucasians, Hispanics, and African Americans). Using genotype tests, we found a trend to point-wise 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 gender identified a point-wise significant association of G/A and A/A genotypes with opiate addiction in women, but not men. Linkage disequilibrium patterns were similar for the three ethnic/cultural groups.

The tryptophan hydroxylase genes—Serotonin has long been recognized as a major moderator of impulse control. Levels of cerebral spinal 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 and impulsivity¹⁶⁴ and aggression.^{165, 166} Serotonin is known to be involved in a number of aspects of mood and impulsivity.^{167, 168}

Serotonin biosynthesis is regulated by its rate-limiting biosynthetic enzyme, tryptophan hydroxylase (TPH).¹⁶⁹ Since 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 tryptophan hydroxylase, rs1799913,^{170, 171} and found that this variant was associated with CSF 5- HIAA levels in Finnish violent offenders,¹⁷² with suicidality, and was linked to alcoholism.¹⁷³ This association with alcoholism has been replicated in Taiwanese¹⁷⁴ The variant rs179913, and the nearby variant rs1800532, have been found to be associated with several addiction-related behaviors.

Before 2003, it was believed that only a single gene encoded *TPH*. A number of 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 tryptophan hydroxylase gene was named *TPH2* and is expressed mainly in the raphe nuclei of the brain.^{175–178} *TPH1*, the previously identified tryptophan hydroxylase

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 since *TPH1* had been associated with addiction, polymorphisms in *TPH2* may also be associated with addiction. We resequenced the tryptophan hydroxylase 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 a single *TPH1* variant, we genotyped individuals with addictive diseases and normal volunteers. Since the allele frequencies of five of the variants varied significantly among the ethnicities studied, we conducted associations stratified by ethnicity. In the subjects who either met heroin addiction or control criteria, and who were of African American, Caucasian, or Hispanic ethnicity, significant differences in genotype patterns were observed between the cases and controls. 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 the 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 inter-individual vulnerability to develop heroin addiction. This interaction may differ among ethnic groups.

It has been shown that both pineal and retinal TPHs are the rate-limiting enzymes in melatonin production. Since melatonin controls circadian rhythm, and *TPH2* and *TPH1* have diurnal variations of expression in the pineal¹⁸¹ 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 tryptophan hydroxylase 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 found to be associated with specific addictions. The serotonin receptors 5-HT_{2A}^{187–189} as well as 5-HT_{1B} (see below) 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}

The 5-hydroxytryptamine (serotonin)-1B receptor gene—The 5-hydroxytryptamine (serotonin)-1B receptor (*HTR1B*) is known to be involved in many neuropsychiatric and physiological functions such as thermoregulation, locomotion and feeding. (e.g., Barnes & Sharp. 1999²⁰²) Serotonin receptor knockout mice showed increased special memory performance,²⁰³ increased impulsive aggression,^{204–206} increased locomotor's response to cocaine administration,²⁰⁷ increased cocaine self-administration²⁰⁷, increased alcohol consumption,²⁰⁸ increased exploratory activity²⁰³ 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 level 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 a number of behavioral candidate genes directed by micro-RNA revealed an interaction of microRNA miR-96, which is expressed in brain, with a common SNP rs13212041 in the 3' untranslated region of this *HTR1B* gene²¹² in an allele-specific manner. The presence of allele A in rs13212041 strongly repressed the expression of the gene. This effect was completely eliminated by substitution of A with G. In human studies, individuals homozygous for the A allele of this SNP reported more conduct-disorder behaviors compared to 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 group²¹⁷ no association of polymorphisms of the *HTR1B* gene including -261T>G, 129C>T (rs6298), 861G>C with cocaine abuse and dependence and alcohol abuse and dependence was found.

In another recent study²¹⁸, we tested for association with heroin addiction a number of 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 3 SNPs, and statistically-inferred haplotypes consisting of these 5 SNPs with heroin addiction was done separately for each of the three ethnicities: African American, Caucasian and Hispanic. Significant association of statistically-inferred haplotype TACGG indicating protective effect from heroin addiction was found in Caucasians. Also, an experiment-wise significant association of the minor allele 1180G with protective effect from heroin addiction in Caucasians 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 see¹). 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 ^{220–223}). The involvement of additional CYP enzymes including CYP2C19,²²⁴ CYP3A5²²⁵ and CYP2C8²²⁶ has been suggested. The CYP enzymes are characterized by inter-individual, as well as ethnic and gender 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 inter-individual 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 is 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 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 see²³³). In respect to *CYP2D6* genotypes, the general population is comprised of: extensive (the majority), intermediate, poor, and ultra-rapid metabolizers. Under-representation of poor metabolizers in Caucasians with opiate addiction was reported.²³⁷ Ultra-rapid metabolizers were found to have unsuccessful methadone treatment therapy,²³⁸ but have been reported to do well on buprenorphine that is not significantly metabolized by CYP2D6. Several genetic tests of cytochrome P450 genotypes, including *CYP2D6* and *CYP2C19* are now FDA approved and are available commercially, but there is ongoing debate about their interpretation and their benefits for specific drugs. Part of the inter-individual 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}

The 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 trans-membrane protein that is composed of two homologous sequences, each containing six trans-membrane 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 impact on net methadone intestinal absorption due to the fact that 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), that showed lower *in vivo* duodenal P-gp expression²⁵² 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 Caucasians.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 3-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 above and lower methadone doses.

Genome-wide and multi-gene association studies

Since the development of high-density microarray technology, it has become possible to interrogate large numbers of single nucleotide genetic variants in a single individual. Statistical analyses comparing groups of individuals analyzed using these high-density microarrays have allowed researchers to conduct genome-wide 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 genome-wide association study using the Affymetrix 10K GeneChip, 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 Caucasian, 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 found to be 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 10K 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 multi-gene 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 controls were healthy volunteers that were selected by detailed personal interview and stringent criteria. For this study we used a 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 non-coding 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 (STR) 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 STR markers, a linkage peak was found at D17S785 on chromosome 17 that associated with heroin addiction.²⁶⁰

Other studies have used single nucleotide polymorphisms in linkage studies on opioid dependence. Lachman *et al.* found a region on chromosome 14q that was “suggestive” of genome-wide evidence for linkage.²⁶³ The Gelernter study identified eight variants with point-wise 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 1,494 variant chip, Uhl *et al.* identified several variants associated with vulnerability to develop drug abuse.²⁶⁶ Using the 10K GeneChip and with pools of African- and European-American cohorts, they identified 38 “nominally reproducibly positive” variants associated with non-specific 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 500K 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 genome wide arrays. Confirmation of these findings may lead to the identification of new targets for the treatment and prevention of substance dependence.

The developments 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 small number of *OPRM1* gene SNPs using on-site-made microarrays based on polyacrylamide gel pad technology.²⁷² This technology utilizes polyacrylamide gel pads as base elements of microarrays. Having 3D structure, such microarrays provide higher hybridization signal intensity compared to 2D arrays. Such arrays are simple in preparation and might be manufactured either using a robotic station or manually.²⁷³ Oligonucleotides, proteins² or DNA PCR products²⁷⁴ 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 based on 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 employed. 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 to 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 a single DNA molecule or separation of DNA strands using allele-specific amplification, cloning, hybridization or other means. The products of amplification may be then analyzed by capillary electrophoresis, (e.g., Cañadas *et al.* 2007; Konfortov *et al.* 2007^{281, 282}) mass-spectrometry, (e.g., Tost *et al.* 2002²⁸³) melting curve analysis, (e.g., Millson *et al.* 2005; Pont-Kingdon & Lyon, 2005^{284, 285}) hybridization, microarrays, (e.g., Guo *et al.* 2006²⁸⁶) pyrosequencing and also 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 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 10000 nucleotides and longer. Haplotyping of overlapping amplicons ("tiling" approach) was used for reconstruction of haplotypes consisting of 105 single-nucleotide polymorphisms.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" amplification²⁹¹ 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., 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 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 also 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 compared to other methods of molecular haplotyping. Haplotypes containing polymorphisms separated from each other by 390, 289, 99 or even 2 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 i) data produced by our haplotyping method, ii), genotype analysis using the TaqMan assay and also, iii) 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, Caucasian, Hispanic), a likelihood ratio test that incorporates double-sample information was applied to the molecular haplotype data and statistically inferred haplotype-pair data. Double-sample suggests that two entirely different approaches (direct molecular haplotyping and haplotyping statistically inferred from genotype data) were applied for determining each individual's pair of haplotypes. Every individual's statistically-inferred haplotype pair agreed with the individual's haplotype pair based on molecular haplotype.

Summary

In this article we have reviewed the studies of a number of gene variants that may contribute to the vulnerability to develop cocaine and/or heroin addiction, and to the efficacy of methadone treatment. We have also described multi-gene 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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References

1. Kreek MJ, et al. Pharmacogenetics and human molecular genetics of opiate and cocaine addictions and their treatments. *Pharmacol. Rev.* 2005; 57:1–26. [PubMed: 15734726]
2. Savage SR, et al. Challenges in using opioids to treat pain in persons with substance use disorders. *Addict. Sci. Clin. Pract.* 2008; 4:4–25. [PubMed: 18497713]
3. Kreek MJ, LaForge KS. Stress responsivity, addiction, and a functional variant of the human mu-opioid receptor gene. *Mol. Interv.* 2007; 7:74–78. [PubMed: 17468387]
4. Kreek MJ, et al. Bidirectional translational research: Progress in understanding addictive diseases. *Neuropharmacology.* 2009; 56(Suppl 1):32–43. [PubMed: 18725235]
5. Bond C, et al. Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proc. Natl. Acad. Sci. U. S. A.* 1998; 95:9608–9613. [PubMed: 9689128]
6. Oertel BG, et al. A common human mu-opioid receptor genetic variant diminishes the receptor signalling efficacy in brain regions processing the sensory information of pain. *J. Biol. Chem.* 2009 in press.

7. Krosiak T, et al. The single nucleotide polymorphism A118G alters functional properties of the human mu opioid receptor. *J. Neurochem.* 2007; 103:77–87. [PubMed: 17877633]
8. Zhang Y, et al. Allelic expression imbalance of human mu opioid receptor (OPRM1) caused by variant A118G. *J. Biol. Chem.* 2005; 280:32618–32624. [PubMed: 16046395]
9. Drakenberg K, et al. Mu opioid receptor A118G polymorphism in association with striatal opioid neuropeptide gene expression in heroin abusers. *Proc. Natl. Acad. Sci. U. S. A.* 2006; 103:7883–7888. [PubMed: 16682632]
10. Bart G, et al. Substantial attributable risk related to a functional mu-opioid receptor gene polymorphism in association with heroin addiction in central Sweden. *Mol. Psychiatry.* 2004; 9:547–549. [PubMed: 15037869]
11. Kapur S, et al. A118g polymorphism in mu opioid receptor gene (oprm1): association with opiate addiction in subjects of Indian origin. *J. Integr. Neurosci.* 2007; 6:511–522. [PubMed: 18181266]
12. Levran O, et al. Genetic susceptibility to heroin addiction: a candidate-gene association study. *Genes Brain Behav.* 2008; 7:720–729. [PubMed: 18518925]
13. Zhang H, et al. Association between two mu-opioid receptor gene (OPRM1) haplotype blocks and drug or alcohol dependence. *Hum Mol Genet.* 2006; 15:807–819. [PubMed: 16476706]
14. Glatt SJ, et al. Evaluation of OPRM1 variants in heroin dependence by family-based association testing and meta-analysis. *Drug Alcohol Depend.* 2007; 90:159–165. [PubMed: 17416470]
15. Arias A, et al. Association of an Asn40Asp (A118G) polymorphism in the mu-opioid receptor gene with substance dependence: a meta-analysis. *Drug Alcohol Depend.* 2006; 83:262–268. [PubMed: 16387451]
16. Xuei X, et al. The opioid system in alcohol and drug dependence: family-based association study. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2007; 144:877–884. [PubMed: 17503481]
17. Kreek MJ, Koob GF. Drug dependence: stress and dysregulation of brain reward pathways. *Drug Alcohol Depend.* 1998; 51:23–47. [PubMed: 9716928]
18. Bart G, et al. Altered levels of basal cortisol in healthy subjects with a 118G allele in exon 1 of the mu opioid receptor gene. *Neuropsychopharmacology.* 2006; 31:2313–2317. [PubMed: 16794569]
19. Wand GS, et al. The mu-opioid receptor gene polymorphism (A118G) alters HPA axis activation induced by opioid receptor blockade. *Neuropsychopharmacology.* 2002; 26:106–114. [PubMed: 11751037]
20. Hernandez-Avila CA, et al. Association between the cortisol response to opioid blockade and the Asn40Asp polymorphism at the mu-opioid receptor locus (OPRM1). *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2003; 118:60–65. [PubMed: 12627468]
21. Hernandez-Avila CA, et al. Population-specific effects of the Asn40Asp polymorphism at the mu-opioid receptor gene (OPRM1) on HPA-axis activation. *Pharmacogenet. Genomics.* 2007; 17:1031–1038. [PubMed: 18004207]
22. Lotsch J, Geisslinger G. Are mu-opioid receptor polymorphisms important for clinical opioid therapy? *Trends Mol. Med.* 2005; 11:82–89. [PubMed: 15694871]
23. Sia AT, et al. A118G single nucleotide polymorphism of human mu-opioid receptor gene influences pain perception and patient-controlled intravenous morphine consumption after intrathecal morphine for postcesarean analgesia. *Anesthesiology.* 2008; 109:520–526. [PubMed: 18719451]
24. Janicki PK, et al. A genetic association study of the functional A118G polymorphism of the human mu-opioid receptor gene in patients with acute and chronic pain. *Anesth. Analg.* 2006; 103:1011–1017. [PubMed: 17000822]
25. Zhang D, et al. Effect of mu-opioid receptor gene polymorphisms on heroin-induced subjective responses in a Chinese population. *Biol. Psychiatry.* 2007; 61:1244–1251. [PubMed: 17157823]
26. Nielsen DA, et al. Genotype patterns that contribute to increased risk for or protection from developing heroin addiction. *Mol. Psychiatry.* 2008; 13:417–428. [PubMed: 18195715]
27. Bayerer B, et al. Genomic variations and transcriptional regulation of the human mu-opioid receptor gene. *Eur. J. Pain.* 2007; 11:421–427. [PubMed: 16843022]
28. Pasternak GW. Molecular biology of opioid analgesia. *J. Pain Symptom Manage.* 2005; 29:S2–S9. [PubMed: 15907642]

29. Pan YX, et al. Identification and characterization of two new human mu opioid receptor splice variants, hMOR-1O and hMOR-1X. *Biochem. Biophys. Res. Commun.* 2003; 301:1057–1061. [PubMed: 12589820]
30. Pan L, et al. Identification and characterization of six new alternatively spliced variants of the human mu opioid receptor gene, Oprm. *Neuroscience.* 2005; 133:209–220. [PubMed: 15893644]
31. Shabalina SA, et al. Expansion of the human mu-opioid receptor gene architecture: novel functional variants. *Hum. Mol. Genet.* 2009 in press.
32. Choi HS, et al. The opioid ligand binding of human mu-opioid receptor is modulated by novel splice variants of the receptor. *Biochem. Biophys. Res. Commun.* 2006; 343:1132–1140. [PubMed: 16580639]
33. Smith RJ, et al. Novel exonic mu-opioid receptor gene (OPRM1) polymorphisms not associated with opioid dependence. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2005; 133B:105–109. [PubMed: 15558714]
34. Pedersen NL, Floderus-Myrhed B. Twin analysis as a potential tool for examining psychosocial factors associated with and preceding smoking behaviors. *Acta Genet. Med. Gemellol. (Roma).* 1984; 33:413–424. [PubMed: 6543276]
35. Grove WM, et al. Heritability of substance abuse and antisocial behavior: a study of monozygotic twins reared apart. *Biol. Psychiatry.* 1990; 27:1293–1304. [PubMed: 2364118]
36. Rounsaville BJ, et al. Psychiatric disorders in relatives of probands with opiate addiction. *Arch. Gen. Psychiatry.* 1991; 48:33–42. [PubMed: 1984760]
37. Tsuang MT, et al. Genetic influences on DSM-III-R drug abuse and dependence: a study of 3,372 twin pairs. *Am. J. Med. Genet.* 1996; 67:473–477. [PubMed: 8886164]
38. Kendler KS, et al. Temperance board registration for alcohol abuse in a national sample of Swedish male twins, born 1902 to 1949. *Arch. Gen. Psychiatry.* 1997; 54:178–184. [PubMed: 9040286]
39. Tsuang MT, et al. Co-occurrence of abuse of different drugs in men: the role of drug-specific and shared vulnerabilities. *Arch. Gen. Psychiatry.* 1998; 55:967–972. [PubMed: 9819064]
40. Bierut LJ, et al. 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.* 1998; 55:982–988. [PubMed: 9819066]
41. Kendler KS, et al. Illicit psychoactive substance use, heavy use, abuse, and dependence in a US population-based sample of male twins. *Arch. Gen. Psychiatry.* 2000; 57:261–269. [PubMed: 10711912]
42. Fu Q, et al. Shared genetic risk of major depression, alcohol dependence, and marijuana dependence: contribution of antisocial personality disorder in men. *Arch. Gen. Psychiatry.* 2002; 59:1125–1132. [PubMed: 12470129]
43. Kendler KS, et al. The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. *Arch. Gen. Psychiatry.* 2003; 60:929–937. [PubMed: 12963675]
44. Rhee SH, et al. Genetic and environmental influences on substance initiation, use, and problem use in adolescents. *Arch. Gen. Psychiatry.* 2003; 60:1256–1264. [PubMed: 14662558]
45. Antequera F, Bird A. Number of CpG islands and genes in human and mouse. *Proc. Natl. Acad. Sci. U. S. A.* 1993; 90:11995–11999. [PubMed: 7505451]
46. Gardiner-Garden M, Frommer M. CpG islands in vertebrate genomes. *J. Mol. Biol.* 1987; 196:261–282. [PubMed: 3656447]
47. Cooper DN, Krawczak M. Cytosine methylation and the fate of CpG dinucleotides in vertebrate genomes. *Hum. Genet.* 1989; 83:181–188. [PubMed: 2777259]
48. Lande-Diner L, et al. Gene repression paradigms in animal cells. *Cold Spring Harb. Symp. Quant. Biol.* 2004; 69:131–138. [PubMed: 16117642]
49. Robertson KD, Wolffe AP. DNA methylation in health and disease. *Nat. Rev. Genet.* 2000; 1:11–19. [PubMed: 11262868]
50. Bird A, Macleod D. Reading the DNA methylation signal. *Cold Spring Harb. Symp. Quant. Biol.* 2004; 69:113–118. [PubMed: 16117639]

51. Alikhani-Koopaei R, et al. Epigenetic regulation of 11 beta-hydroxysteroid dehydrogenase type 2 expression. *J. Clin. Invest.* 2004; 114:1146–1157. [PubMed: 15489962]
52. Douet V, et al. DNA methylation and Sp1 binding determine the tissue-specific transcriptional activity of the mouse Abcc6 promoter. *Biochem. Biophys. Res. Commun.* 2007; 354:66–71. [PubMed: 17214963]
53. Michelotti GA, et al. Epigenetic regulation of human alpha1d-adrenergic receptor gene expression: a role for DNA methylation in Sp1-dependent regulation. *FASEB J.* 2007; 21:1979–1993. [PubMed: 17384146]
54. Nielsen DA, et al. Increased OPRM1 DNA methylation in lymphocytes of methadone-maintained former heroin addicts. *Neuropsychopharmacology.* 2009; 34:867–873. [PubMed: 18650805]
55. Bleich S, et al. Epigenetic DNA hypermethylation of the HERP gene promoter induces down-regulation of its mRNA expression in patients with alcohol dependence. *Alcohol. Clin. Exp. Res.* 2006; 30:587–591. [PubMed: 16573575]
56. Bonsch D, et al. Homocysteine associated genomic DNA hypermethylation in patients with chronic alcoholism. *J. Neural Transm.* 2004; 111:1611–1616. [PubMed: 15565495]
57. Bonsch D, et al. Lowered DNA methyltransferase (DNMT-3b) mRNA expression is associated with genomic DNA hypermethylation in patients with chronic alcoholism. *J. Neural Transm.* 2006; 113:1299–1304. [PubMed: 16463117]
58. Bonsch D, et al. DNA hypermethylation of the alpha synuclein promoter in patients with alcoholism. *Neuroreport.* 2005; 16:167–170. [PubMed: 15671870]
59. Philibert RA, et al. MAOA methylation is associated with nicotine and alcohol dependence in women. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2008; 147B:565–570. [PubMed: 18454435]
60. Novikova SI, et al. Maternal cocaine administration in mice alters DNA methylation and gene expression in hippocampal neurons of neonatal and prepubertal offspring. *PLoS ONE.* 2008; 3:e1919. [PubMed: 18382688]
61. Heijmans BT, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc. Natl. Acad. Sci. U. S. A.* 2008; 105:17046–17049. [PubMed: 18955703]
62. Miller CA, Sweatt JD. Covalent modification of DNA regulates memory formation. *Neuron.* 2007; 53:857–869. [PubMed: 17359920]
63. Izumi H, et al. Frequent silencing of DBC1 is by genetic or epigenetic mechanisms in non-small cell lung cancers. *Hum. Mol. Genet.* 2005; 14:997–1007. [PubMed: 15746151]
64. van Doorn R, et al. Epigenetic profiling of cutaneous T-cell lymphoma: promoter hypermethylation of multiple tumor suppressor genes including BCL7a, PTPRG, and p73. *J. Clin. Oncol.* 2005; 23:3886–3896. [PubMed: 15897551]
65. Leu YW, et al. Loss of estrogen receptor signaling triggers epigenetic silencing of downstream targets in breast cancer. *Cancer Res.* 2004; 64:8184–8192. [PubMed: 15548683]
66. Sonoda I, et al. Frequent silencing of low density lipoprotein receptor-related protein 1B (LRP1B) expression by genetic and epigenetic mechanisms in esophageal squamous cell carcinoma. *Cancer Res.* 2004; 64:3741–3747. [PubMed: 15172977]
67. Farrell WE. Epigenetic mechanisms of tumorigenesis. *Horm. Metab. Res.* 2005; 37:361–368. [PubMed: 16001328]
68. Issa JP, Kantarjian H. Azacitidine. *Nat. Rev. Drug. Discov.* 2005; (Suppl):S6–S7. [PubMed: 15962522]
69. Kumar A, et al. Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron.* 2005; 48:303–314. [PubMed: 16242410]
70. Renthal W, et al. Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. *Neuron.* 2007; 56:517–529. [PubMed: 17988634]
71. Black YD, et al. Altered attention and prefrontal cortex gene expression in rats after binge-like exposure to cocaine during adolescence. *J. Neurosci.* 2006; 26:9656–9665. [PubMed: 16988036]
72. Kreek MJ, et al. Pharmacotherapy of addictions. *Nat. Rev. Drug Discov.* 2002; 1:710–726. [PubMed: 12209151]
73. Glick SD, et al. Kappa opioid inhibition of morphine and cocaine self administration in rats. *Brain Res.* 1995; 681:147–152. [PubMed: 7552272]

74. Schenk S, et al. U69593, a kappa-opioid agonist decreases cocaine self administration and decreases cocaine-produced drug-seeking. *Psychopharmacology (Berl.)*. 1999; 144:339–346. [PubMed: 10435406]
75. Zhang Y, et al. Effect of the endogenous kappa opioid agonist dynorphin A(1–17) on cocaine-evoked increases in striatal dopamine levels and cocaine-induced place preference in C57BL/6J mice. *Psychopharmacology (Berl.)*. 2004; 172:422–429. [PubMed: 14712335]
76. McLaughlin JP, et al. Prior activation of kappa opioid receptors by U50,488 mimics repeated forced swim stress to potentiate cocaine place preference conditioning. *Neuropsychopharmacology*. 2006; 31:787–794. [PubMed: 16123754]
77. Negus SS. Effects of the kappa opioid agonist U50,488 and the kappa opioid antagonist nor-binaltorphimine on choice between cocaine and food in rhesus monkeys. *Psychopharmacology (Berl.)*. 2004; 176:204–213. [PubMed: 15112031]
78. Beardsley PM, et al. Differential effects of the novel kappa opioid receptor antagonist, JD1c, on reinstatement of cocaine-seeking induced by footshock stressors vs cocaine primes and its antidepressant-like effects in rats. *Psychopharmacology (Berl.)*. 2005; 183:118–126. [PubMed: 16184376]
79. Carlezon WA, et al. Depressive-like effects of the kappa-opioid receptor agonist salvinorin A on behavior and neurochemistry in rats. *J. Pharmacol. Exp. Ther.* 2006; 316:440–447. [PubMed: 16223871]
80. Land BB, et al. The dysphoric component of stress is encoded by activation of the dynorphin kappa-opioid system. *J. Neurosci.* 2008; 28:407–414. [PubMed: 18184783]
81. Yuferov V, et al. Redefinition of the human kappa opioid receptor gene (OPRK1) structure and association of haplotypes with opiate addiction. *Pharmacogenetics*. 2004; 14:793–804. [PubMed: 15608558]
82. Gerra G, et al. Human kappa opioid receptor gene (OPRK1) polymorphism is associated with opiate addiction. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2007; 144B:771–775. [PubMed: 17373729]
83. Zhang H, et al. The OPRD1 and OPRK1 loci in alcohol or drug dependence: OPRD1 variation modulates substance dependence risk. *Mol. Psychiatry*. 2008; 13:531–543. [PubMed: 17622222]
84. Xuei X, et al. Association of the kappa-opioid system with alcohol dependence. *Mol. Psychiatry*. 2006; 11:1016–1024. [PubMed: 16924269]
85. Edenberg HJ, et al. A regulatory variation in OPRK1, the gene encoding the kappa-opioid receptor, is associated with alcohol dependence. *Hum. Mol. Genet.* 2008; 17:1783–1789. [PubMed: 18319328]
86. Mansour A, et al. Kappa 1 receptor mRNA distribution in the rat CNS: comparison to kappa receptor binding and prodynorphin mRNA. *Mol. Cell. Neurosci.* 1994; 5:124–144. [PubMed: 8032682]
87. Hurd YL. Differential messenger RNA expression of prodynorphin and proenkephalin in the human brain. *Neuroscience*. 1996; 72:767–783. [PubMed: 9157322]
88. Akil H, et al. Endogenous opioids: overview and current issues. *Drug Alcohol Depend.* 1998; 51:127–140. [PubMed: 9716935]
89. Horikawa A, et al. Isolation and structural organization of the human preproenkephalin B gene. *Nature*. 1983; 306:611–614. [PubMed: 6316163]
90. Zimprich A, et al. An allelic variation in the human prodynorphin gene promoter alters stimulus induced expression. *J. Neurochem.* 2000; 74:472–477. [PubMed: 10646497]
91. Carrion A, et al. DREAM is a Ca²⁺-regulated transcriptional repressor. *Nature*. 1999; 398:80–84. [PubMed: 10078534]
92. Zhang A, et al. Acute cocaine results in rapid rises in intracellular free calcium concentration in canine cerebral vascular smooth muscle cells: possible relation to etiology of stroke. *Neurosci. Lett.* 1996; 215:57–59. [PubMed: 8880753]
93. Du C, et al. Cocaine increases the intracellular calcium concentration in brain independently of its cerebrovascular effects. *J. Neurosci.* 2006; 26:11522–11531. [PubMed: 17093073]

94. Chen AC, et al. Potentially functional polymorphism in the promoter region of prodynorphin gene may be associated with protection against cocaine dependence or abuse. *Am. J. Med. Genet.* 2002; 114:429–435. [PubMed: 11992566]
95. Dahl JP, et al. Confirmation of the association between a polymorphism in the promoter region of the prodynorphin gene and cocaine dependence. *Am. J. Med. Genet. B Neuropsychiatr. Genetics.* 2005; 139:106–108. [PubMed: 16184603]
96. Williams TJ, et al. Prodynorphin gene promoter repeat associated with cocaine/alcohol codependence. *Addict. Biol.* 2007; 12:496–502. [PubMed: 17559549]
97. Sieberts SK, Schadt EE. Moving toward a system genetics view of disease. *Mamm. Genome.* 2007; 18:389–401. [PubMed: 17653589]
98. Chen Y, et al. Variations in DNA elucidate molecular networks that cause disease. *Nature.* 2008; 452:429–435. [PubMed: 18344982]
99. Jansen RC, Nap JP. Genetical genomics: the added value from segregation. *Trends Genet.* 2001; 17:388–391. [PubMed: 11418218]
100. Li J, Burmeister M. Genetical genomics: combining genetics with gene expression analysis. *Hum. Mol. Genet.* 2005; 14::R163–R169. [PubMed: 16244315]
101. Knight JC. Regulatory polymorphisms underlying complex disease traits. *J. Mol. Med.* 2005; 83:97–109. [PubMed: 15592805]
102. Sadée W, Dai Z. Pharmacogenetics/genomics and personalized medicine. *Hum. Mol. Genet.* 2005; 14:R207–R214. [PubMed: 16244319]
103. Le-Niculescu H, et al. Convergent functional genomics of bipolar disorder: from animal model pharmacogenomics to human genetics and biomarkers. *Neurosci. Biobehav. Rev.* 2007; 31:897–903. [PubMed: 17614132]
104. Morley M, et al. Genetic analysis of genome-wide variation in human gene expression. *Nature.* 2004; 430:743–747. [PubMed: 15269782]
105. Pastinen T, et al. Mapping common regulatory variants to human haplotypes. *Hum. Mol. Genet.* 2005; 14:3963–3971. [PubMed: 16301213]
106. Bibikova M, et al. High-throughput DNA methylation profiling using universal bead arrays. *Genome Res.* 2006; 16:383–393. [PubMed: 16449502]
107. Serre D, et al. Differential allelic expression in the human genome: a robust approach to identify genetic and epigenetic cis-acting mechanisms regulating gene expression. *PLoS Genet.* 2008; 4(2):e1000006. [PubMed: 18454203]
108. Yan H, et al. Allelic variation in human gene expression. *Science.* 2002; 297:1143. [PubMed: 12183620]
109. Bray NJ, et al. Cis-acting variation in the expression of a high proportion of genes in human brain. *Hum. Genet.* 2003; 113:149–153. [PubMed: 12728311]
110. Pastinen T, et al. Influence of human genome polymorphism on gene expression. *Hum. Mol. Genet.* 2006; 15:R9–R16. [PubMed: 16651375]
111. Yuferov V, et al. A functional haplotype implicated in vulnerability to develop cocaine dependence is associated with reduced *PDYN* expression in human brain. *Neuropsychopharmacology.* 2009 in press.
112. Koob G, Kreek MJ. Stress, dysregulation of drug reward pathways, and the transition to drug dependence. *Am. J. Psychiatry.* 2007; 164:1149–1159. [PubMed: 17671276]
113. Mignone F, et al. Untranslated regions of mRNAs. *Genome Biol.* 2002; 3 Review S0004, Epub 2002 Feb 28.
114. Xie X, et al. Systematic discovery of regulatory motifs in human promoters and 3' UTRs by comparison of several mammals. *Nature.* 2005; 434:338–345. [PubMed: 15735639]
115. Chen JM, et al. A systematic analysis of disease-associated variants in the 3' regulatory regions of human protein-coding genes II: the importance of mRNA secondary structure in assessing the functionality of 3' UTR variants. *Hum. Genet.* 2006; 120:301–333. [PubMed: 16807757]
116. Tao H, et al. Allele-specific KRT1 expression is a complex trait. *PLoS Genet.* 2006; 2:e93. [PubMed: 16789827]

117. Spangler R, et al. "Binge" cocaine administration induces a sustained increase of prodynorphin mRNA in rat caudate-putamen. *Mol. Brain Res.* 1993; 19:323–327. [PubMed: 7694032]
118. Daunais JB, McGinty JF. Acute and chronic cocaine administration differentially alters striatal opioid and nuclear transcription factor mRNAs. *Synapse.* 1994; 18:35–45. [PubMed: 7825122]
119. Yuferov V, et al. Elevation of guinea pig brain preprodynorphin mRNA expression and hypothalamic-pituitary-adrenal axis activity by "binge" pattern cocaine administration. *Brain Res. Bull.* 2001; 55:65–70. [PubMed: 11427339]
120. Volkow ND, et al. Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. *J. Neurosci.* 2006; 26:6583–6588. [PubMed: 16775146]
121. Yehuda R. Biology of posttraumatic stress disorder. *J. Clin. Psychiatry.* 2001; 62(Suppl 17):41–46. [PubMed: 11495096]
122. Griep EN, et al. Function of the hypothalamic-pituitary-adrenal axis in patients with fibromyalgia and low back pain. *J. Rheumatol.* 1998; 25:1374–1381. [PubMed: 9676772]
123. Aisen PS, Pasinetti GM. Glucocorticoids in Alzheimer's disease. The story so far. *Drugs Aging.* 1998; 12:1–6. [PubMed: 9467682]
124. Holsboer F, Barden N. Antidepressants and hypothalamic-pituitary-adrenocortical regulation. *Endocr. Rev.* 1996; 17:187–205. [PubMed: 8706631]
125. Gold PW, Chrousos GP. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs. low CRH/NE states. *Mol. Psychiatry.* 2002; 7:254–275. [PubMed: 11920153]
126. Schluger JH, et al. Altered HPA axis responsivity to metyrapone testing in methadone maintained former heroin addicts with ongoing cocaine addiction. *Neuropsychopharmacology.* 2001; 24:568–575. [PubMed: 11282257]
127. Kreek MJ, et al. Circadian rhythms and levels of beta-endorphin, ACTH, and cortisol during chronic methadone maintenance treatment in humans. *Life Sci.* 1983; 33(Suppl. 1):409–411. [PubMed: 6319894]
128. Mountjoy KG, et al. The cloning of a family of genes that encode the melanocortin receptors. *Science.* 1992; 257:1248–1251. [PubMed: 1325670]
129. Clark AJ, et al. Familial glucocorticoid deficiency associated with point mutation in the adrenocorticotropin receptor. *Lancet.* 1993; 341:461–462. [PubMed: 8094489]
130. Tsigos C, et al. Hereditary isolated glucocorticoid deficiency is associated with abnormalities of the adrenocorticotropin receptor gene. *J. Clin. Invest.* 1993; 92:2458–2461. [PubMed: 8227361]
131. Elias LL, et al. Functional characterization of naturally occurring mutations of the human adrenocorticotropin receptor: poor correlation of phenotype and genotype. *J. Clin. Endocrinol. Metab.* 1999; 84:2766–2770. [PubMed: 10443676]
132. Slawik M, et al. Characterization of an adrenocorticotropin (ACTH) receptor promoter polymorphism leading to decreased adrenal responsiveness to ACTH. *J. Clin. Endocrinol. Metab.* 2004; 89:3131–3137. [PubMed: 15240582]
133. Reisch N, et al. Genetic influence of an ACTH receptor promoter polymorphism on adrenal androgen secretion. *Eur. J. Endocrinol.* 2005; 153:711–715. [PubMed: 16260430]
134. Naville D, et al. Genomic structure and promoter characterization of the human ACTH receptor gene. *Biochem. Biophys. Res. Commun.* 1997; 230:7–12. [PubMed: 9020063]
135. Proudnikov D, et al. Association of polymorphisms in the melanocortin receptor type 2 (MC2R, ACTH receptor) gene with heroin addiction. *Neurosci. Lett.* 2008; 435:234–239. [PubMed: 18359160]
136. Maggos CE, et al. Sustained withdrawal allows normalization of in vivo [¹¹C]N-methylspiperone dopamine D2 receptor binding after chronic binge cocaine: a positron emission tomography study in rats. *Neuropsychopharmacology.* 1998; 19:146–153. [PubMed: 9629568]
137. Maisonneuve IM, et al. Chronic administration of a cocaine "binge" alters basal extracellular levels in male rats: an in vivo microdialysis study. *J. Pharmacol. Exp. Ther.* 1995; 272:652–657. [PubMed: 7853178]
138. Tsukada H, et al. Effects of binge pattern cocaine administration on dopamine D1 and D2 receptors in the rat brain: an in vivo study using positron emission tomography. *J. Neurosci.* 1996; 16:7670–7677. [PubMed: 8922423]

139. Zhang Y, et al. Effect of chronic "binge cocaine" on basal levels and cocaine-induced increases of dopamine in the caudate putamen and nucleus accumbens of C57BL/6J and 129/J mice. *Synapse*. 2003; 50:191–199. [PubMed: 14515336]
140. Volkow ND, et al. Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. *Mol. Psychiatry*. 2004; 9:557–569. [PubMed: 15098002]
141. Männistö PT, Kaakkola S. Catechol-*O*-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacol. Rev.* 1999; 51:593–628. [PubMed: 10581325]
142. Lachman HM, et al. Human catechol-*O*-methyltransferase pharmacogenetics: description of functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics*. 1996; 6:243–250. [PubMed: 8807664]
143. Lotta T, et al. Kinetics of human soluble and membrane-bound catechol *O*-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry*. 1995; 34:4202–4210. [PubMed: 7703232]
144. Weinshilboum R, Dunnette J. Thermal stability and the biochemical genetics of erythrocyte catechol-*O*-methyl-transferase and plasma dopamine-beta-hydroxylase. *Clin. Genet.* 1981; 19:426–437. [PubMed: 7296933]
145. Zhu G, et al. Differential expression of human COMT alleles in brain and lymphoblasts detected by RT-coupled 5' nuclease assay. *Psychopharmacology (Berl.)*. 2004; 177:178–184. [PubMed: 15290009]
146. Mattay VS, et al. Catechol *O*-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. *Proc. Natl. Acad. Sci. U. S. A.* 2003; 100:6186–6191. [PubMed: 12716966]
147. Wichers M, et al. The catechol-*O*-methyl transferase Val(158)Met polymorphism and experience of reward in the flow of daily life. *Neuropsychopharmacology*. 2008; 33:3030–3036. [PubMed: 17687265]
148. Vandenbergh DJ, et al. High-activity catechol-*O*-methyltransferase allele is more prevalent in polysubstance abusers. *Am. J. Med. Genet.* 1997; 74:439–442. [PubMed: 9259381]
149. Horowitz R, et al. Confirmation of an excess of the high enzyme activity COMT Val allele in heroin addicts in a family-based haplotype relative risk study. *Am. J. Med. Genet.* 2000; 96:599–603. [PubMed: 11054766]
150. Cao L, et al. Association study of heroin dependence and catechol-*O*-methyltransferase gene. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*. 2003; 20:127–130. (Translated from Chinese). [PubMed: 12673581]
151. Li T, et al. Association analysis of the DRD4 and COMT genes in methamphetamine abuse. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2004; 129:120–124. [PubMed: 15274053]
152. Hosák L, et al. The COMT Val158Met polymorphism is associated with novelty seeking in Czech methamphetamine abusers: preliminary results. *Neuro Endocrinol. Lett.* 2006; 27:799–802. [PubMed: 17187009]
153. Lohoff FW, et al. Association between the catechol-*O*-methyltransferase Val158Met polymorphism and cocaine dependence. *Neuropsychopharmacology*. 2008; 33:3078–3084. [PubMed: 18704099]
154. Nikoshkov A, et al. Opioid neuropeptide genotypes in relation to heroin abuse: dopamine tone contributes to reversed mesolimbic proenkephalin expression. *Proc. Natl. Acad. Sci. U. S. A.* 2008; 105:786–791. [PubMed: 18184800]
155. Yacubian J, et al. Gene-gene interaction associated with neural reward sensitivity. *Proc. Natl. Acad. Sci. U. S. A.* 2007; 104:8125–8130. [PubMed: 17483451]
156. Mukherjee N, et al. The complex global pattern of genetic variation and linkage disequilibrium at catechol-*O*-methyltransferase. *Mol. Psychiatry*. 2009 in press.
157. Karayiorgou M, et al. Family-based association studies support a sexually dimorphic effect of COMT and MAOA on genetic susceptibility to obsessive-compulsive disorder. *Biol. Psychiatry*. 1999; 45:1178–1189. [PubMed: 10331110]

158. Enoch MA, et al. Sex differences in the influence of COMT Val158Met on alcoholism and smoking in plains American Indians. *Alcohol. Clin. Exp. Res.* 2006; 30:399–406. [PubMed: 16499480]
159. Gogos JA, et al. Catechol-*O*-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. *Proc. Natl. Acad. Sci. U. S. A.* 1998; 95:9991–9996. [PubMed: 9707588]
160. Xie T, et al. Characterization and implications of estrogenic down-regulation of human catechol-*O*-methyltransferase gene transcription. *Mol. Pharmacol.* 1999; 56:31–38. [PubMed: 10385681]
161. Jiang H, et al. Human catechol-*O*-methyltransferase down-regulation by estradiol. *Neuropharmacology.* 2003; 45:1011–1018. [PubMed: 14573393]
162. Oosterhuis BE, et al. Catechol-*O*-methyltransferase (COMT) gene variants: possible association of the Val158Met variant with opiate addiction in Hispanic women. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2008; 147B:793–798. [PubMed: 18270997]
163. Rosenthal NE, et al. Monoamine metabolites in cerebrospinal fluid of depressive subgroups. *Psychiatry Res.* 1980; 2:113–119. [PubMed: 6158064]
164. Linnoila M, et al. Low cerebrospinal fluid 5-hydroxyindoleacetic acid concentration differentiates impulsive from nonimpulsive violent behavior. *Life Sci.* 1983; 33:2609–2614. [PubMed: 6198573]
165. Brown, GL., et al. Human aggression and its relationship to cerebrospinal fluid 5-hydroxyindoleacetic acid, 3-methoxy-4-hydroxyphenylglycol, and homovanilic acid. In: Sandler, M., editor. *Psychopharmacology of Aggression*. New York, NY: Raven Press; 1979. p. 131-148.
166. Roy A, et al. Acting out hostility in normal volunteers: negative correlation with levels of 5HIAA in cerebrospinal fluid. *Psychiatry Res.* 1988; 24:187–194. [PubMed: 2457227]
167. Lucki I. The spectrum of behaviors influenced by serotonin. *Biol. Psychiatry.* 1998; 44:151–162. [PubMed: 9693387]
168. Soubrie P. Reconciling the role of central serotonin neurons in human and animal behavior. *Behav. Brain Sci.* 1986; 9:319–364.
169. Cooper JR, Melcer I. The enzymatic oxidation of tryptophan to 5-hydroxytryptophan in the biosynthesis of serotonin. *J. Pharmacol. Exp. Ther.* 1961; 132:265–268. [PubMed: 13695323]
170. Nielsen DA, et al. Genetic mapping of the human tryptophan hydroxylase gene on chromosome 11, using an intronic conformational polymorphism. *Am. J. Hum. Genet.* 1992; 51:1366–1371. [PubMed: 1463016]
171. Nielsen DA, et al. Sequence, splice site and population frequency distribution analyses of the polymorphic human tryptophan hydroxylase intron 7. *Brain Res. Mol. Brain Res.* 1997; 45:145–148. [PubMed: 9105682]
172. Nielsen DA, et al. Suicidality and 5-hydroxyindoleacetic acid concentration associated with a tryptophan hydroxylase polymorphism. *Arch. Gen. Psychiatry.* 1994; 51:34–38. [PubMed: 7506517]
173. Nielsen DA, et al. A tryptophan hydroxylase gene marker for suicidality and alcoholism. *Arch. Gen. Psychiatry.* 1998; 55:593–602. [PubMed: 9672049]
174. Hsu YP, et al. Allelic association of tryptophan hydroxylase with alcoholism in five Taiwanese ethnic groups. *Mol. Psychiatry.* 1998; 3:213–214. [PubMed: 9672895]
175. Walther DJ, et al. Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science.* 2003; 299:76. [PubMed: 12511643]
176. Walther DJ, Bader M. A unique central tryptophan hydroxylase isoform. *Biochem. Pharmacol.* 2003; 66:1673–1680. [PubMed: 14563478]
177. Patel PD, et al. Robust and tissue-specific expression of TPH2 versus TPH1 in rat raphe and pineal gland. *Biol. Psychiatry.* 2004; 55:428–433. [PubMed: 14960297]
178. Zill P, et al. Analysis of tryptophan hydroxylase I and II mRNA expression in the human brain: A post-mortem study. *J. Psychiatr. Res.* 2007; 41:168–173. [PubMed: 16023677]
179. Nakamura K, et al. Late developmental stage-specific role of tryptophan hydroxylase 1 in brain serotonin levels. *J. Neurosci.* 2006; 26:530–534. [PubMed: 16407550]

180. Nielsen DA, et al. TPH2 and TPH1: association of variants and interactions with heroin addiction. *Behav. Genet.* 2008; 38:133–150. [PubMed: 18181017]
181. Sugden D. Comparison of circadian expression of tryptophan hydroxylase isoform mRNAs in the rat pineal gland using real-time PCR. *J. Neurochem.* 2003; 86:1308–1311. [PubMed: 12911638]
182. Liang J, et al. Diurnal rhythms of tryptophan hydroxylase 1 and 2 mRNA expression in the rat retina. *Neuroreport.* 2004; 15:1497–1500. [PubMed: 15194882]
183. Williams RG, Dockray GJ. Distribution of enkephalin-related peptides in rat brain: immunohistochemical studies using antisera to met-enkephalin and met-enkephalin Arg6Phe7. *NeuroScience.* 1983; 9:563–586. [PubMed: 6312371]
184. Harlan RE, et al. Localization of preproenkephalin mRNA in the rat brain and spinal cord by in situ hybridization. *J. Comp. Neurol.* 1987; 258:159–184. [PubMed: 3584538]
185. Leander P, et al. Neuronal projections from the mesencephalic raphe nuclear complex to the suprachiasmatic nucleus and the deep pineal gland of the golden hamster (*Mesocricetus auratus*). *J. Comp. Neurol.* 1998; 399:73–93. [PubMed: 9725702]
186. Miguez JM, et al. Effects of single doses and daily melatonin treatments on serotonin metabolism in rat brain regions. *J. Pineal Res.* 1994; 17:170–176. [PubMed: 7536843]
187. Nakamura TS, et al. Association of a polymorphism of the 5HT2A receptor gene promoter region with alcohol dependence. *Mol. Psychiatry.* 1999; 4:85–88. [PubMed: 10089015]
188. Aubert R, et al. 5-HT2A receptor gene polymorphism is associated with food and alcohol intake in obese people. *Int. J. Obes. Relat. Metab. Disord.* 2000; 24:920–924. [PubMed: 10918541]
189. Hwu HG, Chen CH. Association of 5HT2A receptor gene polymorphism and alcohol abuse with behavior problems. *Am. J. Med. Genet.* 2000; 96:797–800. [PubMed: 11121184]
190. Gade R, et al. Correlation of length of VNTR alleles at the X-linked MAOA gene and phenotypic effect in Tourette syndrome and drug abuse. *Mol. Psychiatry.* 1998; 3:50–60. [PubMed: 9491813]
191. Tan EC, et al. Evidence for an association between heroin dependence and a VNTR polymorphism at the serotonin transporter locus. *Mol. Psychiatry.* 1999; 4:215–217. [PubMed: 10395208]
192. Gerra G, et al. Association between low-activity serotonin transporter genotype and heroin dependence: behavioral and personality correlates. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2004; 126B:37–42. [PubMed: 15048645]
193. Hallikainen T, et al. Association between low activity serotonin transporter promoter genotype and early onset alcoholism with habitual impulsive violent behavior. *Mol. Psychiatry.* 1999; 4:385–388. [PubMed: 10483057]
194. Hammoumi S, et al. Does the short variant of the serotonin transporter linked polymorphic region constitute a marker of alcohol dependence? *Alcohol.* 1999; 17:107–112. [PubMed: 10064377]
195. Lichtermann D, et al. Support for allelic association of a polymorphic site in the promoter region of the serotonin transporter gene with risk for alcohol dependence. *Am. J. Psychiatry.* 2000; 157:2045–2047. [PubMed: 11097976]
196. Matsushita S, et al. Association study of serotonin transporter gene regulatory region polymorphism and alcoholism. *Am. J. Med. Genet.* 2001; 105:446–450. [PubMed: 11449397]
197. Turker T, et al. High ethanol tolerance in young adults is associated with the low-activity variant of the promoter of the human serotonin transporter gene. *Neurosci. Lett.* 1998; 248:147–150. [PubMed: 9654330]
198. Herman AI, et al. Serotonin transporter promoter polymorphism and differences in alcohol consumption behaviour in a college student population. *Alcohol Alcohol.* 2003; 38:446–449. [PubMed: 12915525]
199. Ishiguro H, et al. Association between drinking-related antisocial behavior and a polymorphism in the serotonin transporter gene in a Japanese population. *Alcohol. Clin. Exp. Res.* 1999; 23:1281–1284. [PubMed: 10443997]
200. Munafo MR, et al. Association between the serotonin transporter gene and alcohol consumption in social drinkers. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2005; 135B:10–14. [PubMed: 15729746]

201. Sander T, et al. Association analysis of a regulatory variation of the serotonin transporter gene with severe alcohol dependence. *Alcohol. Clin. Exp. Res.* 1997; 21:1356–1359. [PubMed: 9394104]
202. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. *Neuropharmacology.* 1999; 38:1083–1152. [PubMed: 10462127]
203. Malleret G, et al. 5-HT1B receptor knockout mice exhibit increased exploratory activity and enhanced spatial memory performance in the Morris water maze. *J. Neurosci.* 1999; 19:6157–6168. [PubMed: 10407051]
204. Brunner D, Hen R. Insights into the neurobiology of impulsive behavior from serotonin receptor knockout mice. *Ann. N.Y. Acad. Sci.* 1997; 836:81–105. [PubMed: 9616795]
205. Saudou F, et al. Enhanced aggressive behavior in mice lacking 5-HT1B receptor. *Science.* 1994; 265:1875–1878. [PubMed: 8091214]
206. Zhuang X, et al. Altered emotional states in knockout mice lacking 5-HT1A or 5-HT1B receptors. *Neuropsychopharmacology.* 1999; 21:52S–60S. [PubMed: 10432489]
207. Rocha BA, et al. Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. *Nature.* 1998; 393:175–178. [PubMed: 9603521]
208. Crabbe JC, et al. Elevated alcohol consumption in null mutant mice lacking 5-HT1B serotonin receptors. *Nat. Genet.* 1996; 14:98–101. [PubMed: 8782828]
209. Parsons LH, et al. Serotonin 1B receptor stimulation enhances cocaine reinforcement. *J. Neurosci.* 1998; 18:10078–10089. [PubMed: 9822762]
210. Sun HFS, et al. Association study of novel human serotonin 5-HT1B polymorphisms with alcohol dependence in Taiwanese Han. *Biol. Psychiatry.* 2002; 51:896–901. [PubMed: 12022963]
211. Duan J, et al. Polymorphisms in the 5'-untranslated region of the human serotonin receptor 1B (HTR1B) gene affect gene expression. *Mol. Psychiatry.* 2003; 8:901–910. [PubMed: 14593427]
212. Jensen KP, et al. A common polymorphism in serotonin receptor 1B mRNA moderates regulation by miR-96 and associates with aggressive human behaviors. *Mol. Psychiatry.* 2009 in press.
213. Huang YY, et al. Substance abuse disorder and major depression are associated with the human 5-HT1B receptor gene (HTR1B) G861C polymorphism. *Neuropsychopharmacology.* 2003; 28:163–169. [PubMed: 12496953]
214. Hasegawa Y, et al. Association of a polymorphism of the serotonin 1B receptor gene and alcohol dependence with inactive aldehyde dehydrogenase-2. *J. Neural Transm.* 2002; 109:513–521. [PubMed: 11956970]
215. Lappalainen J, et al. Linkage of antisocial alcoholism to the serotonin 5-HT1B receptor gene in 2 populations. *Arch. Gen. Psychiatry.* 1998; 55:989–994. [PubMed: 9819067]
216. New AS, et al. Suicide, impulsive aggression, and HTR1B genotype. *Biol. Psychiatry.* 2001; 50:62–65. [PubMed: 11457425]
217. Cigler T, et al. Novel and previously reported single-nucleotide polymorphisms in the human 5-HT(1B) receptor gene: no association with cocaine or alcohol abuse or dependence. *Am. J. Med. Genet.* 2001; 105:489–497. [PubMed: 11496363]
218. Proudnikov D, et al. Association analysis of polymorphisms in serotonin 1B receptor (HTR1B) gene with heroin addiction: a comparison of molecular and statistically estimated haplotypes. *Pharmacogenet. Genomics.* 2006; 16:25–36. [PubMed: 16344719]
219. Kreek, MJ. Introduction to addictive disorders: implications for pharmacotherapies. In: Sibley, DRD., et al., editors. *Handbook of Contemporary Neuropharmacology.* Vol. Vol. 1. Hoboken, NJ: Wiley; 2007. p. 451-463.
220. Haile CN, et al. Pharmacogenetic treatments for drug addiction: alcohol and opiates. *Am. J. Drug Alcohol Abuse.* 2008; 34:355–381. [PubMed: 18584566]
221. Li Y, et al. Interindividual variability of methadone response: impact of genetic polymorphism. *Mol. Diagn. Ther.* 2008; 12:109–124. [PubMed: 18422375]
222. Crettol S, et al. ABCB1 and cytochrome P450 genotypes and phenotypes: influence on methadone plasma levels and response to treatment. *Clin. Pharmacol. Ther.* 2006; 80:668–681. [PubMed: 17178267]

223. Shiran MR, et al. Contribution of the activities of CYP3A, CYP2D6, CYP1A2 and other potential covariates to the disposition of methadone in patients undergoing methadone maintenance treatment. *Br. J. Clin. Pharmacol.* 2009; 67:29–37. [PubMed: 19133059]
224. Gerber JG, et al. Stereoselective metabolism of methadone N-demethylation by cytochrome P4502B6 and 2C19. *Chirality.* 2004; 16:36–44. [PubMed: 14628297]
225. De Fazio S, et al. Role of CYP3A5 in abnormal clearance of methadone. *Ann. Pharmacother.* 2008; 42:893–897. [PubMed: 18460585]
226. Wang JS, DeVane CL. Involvement of CYP3A4, CYP2C8, and CYP2D6 in the metabolism of (R)- and (S)-methadone in vitro. *Drug Metab. Dispos.* 2003; 31:742–747. [PubMed: 12756206]
227. Lamba JK, et al. Genetic contribution to variable human CYP3A-mediated metabolism. *Adv. Drug Deliv. Rev.* 2002; 54:1271–1294. [PubMed: 12406645]
228. Wang H, Tompkins LM. CYP2B6: new insights into a historically overlooked cytochrome P450 isozyme. *Curr. Drug Metab.* 2008; 9:598–610. [PubMed: 18781911]
229. Lamba V, et al. Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. *J. Pharmacol. Exp. Ther.* 2003; 307:906–922. [PubMed: 14551287]
230. Anderson GD. Gender differences in pharmacological response. *Int. Rev. Neurobiol.* 2008; 83:1–10. [PubMed: 18929073]
231. Saito Y, et al. Genetic polymorphisms and haplotypes of major drug metabolizing enzymes in East Asians and their comparison with other ethnic populations. *Current Pharmacogenomics.* 2007; 5:49–78.
232. Perera MA, et al. Prediction of CYP3A4 enzyme activity using haplotype tag SNPs in African Americans. *Pharmacogenomics J.* 2008; 9:49–60. [PubMed: 18825162]
233. Zanger UM, et al. Polymorphic CYP2B6: molecular mechanisms and emerging clinical significance. *Pharmacogenomics.* 2007; 8:743–759. [PubMed: 17638512]
234. Totah RA, et al. Role of CYP2B6 in stereoselective human methadone metabolism. *Anesthesiology.* 2008; 108:363–374. [PubMed: 18292673]
235. Lang T, et al. Multiple novel nonsynonymous CYP2B6 gene polymorphisms in Caucasians: demonstration of phenotypic null alleles. *J. Pharmacol. Exp. Ther.* 2004; 311:34–43. [PubMed: 15190123]
236. Zukunft J, et al. A natural CYP2B6 TATA box polymorphism (–82T→C) leading to enhanced transcription and relocation of the transcriptional start site. *Mol. Pharmacol.* 2005; 67:1772–1782. [PubMed: 15722458]
237. Tyndale RF, et al. Genetically deficient CYP2D6 metabolism provides protection against oral opiate dependence. *Pharmacogenetics.* 1997; 7:375–379. [PubMed: 9352573]
238. Perez de los Cobos J, et al. Association of CYP2D6 ultrarapid metabolizer genotype with deficient patient satisfaction regarding methadone maintenance treatment. *Drug Alcohol Depend.* 2007; 89:190–194. [PubMed: 17234366]
239. Miksys S, Tyndale RF. Brain drug-metabolizing cytochrome P450 enzymes are active in vivo, demonstrated by mechanism-based enzyme inhibition. *Neuropsychopharmacology.* 2009; 34:634–640. [PubMed: 18668033]
240. Miksys S, Tyndale RF. The unique regulation of brain cytochrome P450 2 (CYP2) family enzymes by drugs and genetics. *Drug Metab. Rev.* 2004; 36:313–333. [PubMed: 15237857]
241. Crettol S, et al. In vitro P-glycoprotein-mediated transport of (R)-, (S)-, (R,S)-methadone, LAAM and their main metabolites. *Pharmacology.* 2007; 80:304–311. [PubMed: 17690563]
242. Somogyi AA, et al. Pharmacogenetics of opioids. *Clin. Pharmacol. Ther.* 2007; 81:429–444. [PubMed: 17339873]
243. Dagenais C, et al. Variable modulation of opioid brain uptake by P-glycoprotein in mice. *Biochem Pharmacol.* 2004; 67:269–276. [PubMed: 14698039]
244. Marzolini C, et al. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin. Pharmacol. Ther.* 2004; 75:13–33. [PubMed: 14749689]
245. Fojo AT, et al. Expression of a multidrug-resistance gene in human tumors and tissues. *Proc. Natl. Acad. Sci. U. S. A.* 1987; 84:265–269. [PubMed: 2432605]

246. Sakaeda T, et al. Pharmacogenetics of drug transporters and its impact on the pharmacotherapy. *Curr. Top. Med. Chem.* 2004; 4:1385–1398. [PubMed: 15379652]
247. Kerb R. Implications of genetic polymorphisms in drug transporters for pharmacotherapy. *Cancer Lett.* 2006; 234:4–33. [PubMed: 16504381]
248. Kim RB, et al. Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin. Pharmacol. Ther.* 2001; 70:189–199. [PubMed: 11503014]
249. Takane H, et al. Haplotype-oriented genetic analysis and functional assessment of promoter variants in the MDR1 (ABCB1) gene. *J. Pharmacol. Exp. Ther.* 2004; 311:1179–1187. [PubMed: 15280437]
250. Tang K, et al. Distinct haplotype profiles and strong linkage disequilibrium at the MDR1 multidrug transporter gene locus in three ethnic Asian populations. *Pharmacogenetics.* 2002; 12:437–450. [PubMed: 12172212]
251. Kroetz DL, et al. Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. *Pharmacogenetics.* 2003; 13:481–494. [PubMed: 12893986]
252. Hoffmeyer S, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 2000; 97:3473–3478. [PubMed: 10716719]
253. Wang D, et al. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability. *Pharmacogenet. Genomics.* 2005; 15:693–704. [PubMed: 16141795]
254. Kimchi-Sarfaty C, et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science.* 2007; 315:525–528. [PubMed: 17185560]
255. Salama NN, et al. MDR1 haplotypes significantly minimize intracellular uptake and transcellular P-gp substrate transport in recombinant LLC-PK1 cells. *J. Pharm. Sci.* 2006; 95:2293–2308. [PubMed: 16883550]
256. Tang K, et al. Genomic evidence for recent positive selection at the human MDR1 gene locus. *Hum. Mol. Genet.* 2004; 13:783–797. [PubMed: 14976162]
257. Levran O, et al. ABCB1 (MDR1) genetic variants are associated with methadone doses required for effective treatment of heroin dependence. *Hum. Mol. Genet.* 2008; 17:2219–2227. [PubMed: 18424454]
258. Collier JK, et al. ABCB1 genetic variability and methadone dosage requirements in opioid-dependent individuals. *Clin. Pharmacol. Ther.* 2006; 80:682–690. [PubMed: 17178268]
259. Hodgkinson CA, et al. Addictions biology: haplotype-based analysis for 130 candidate genes on a single array. *Alcohol Alcohol.* 2008; 43:505–515. [PubMed: 18477577]
260. Gelernter J, et al. Genomewide linkage scan for opioid dependence and related traits. *Am. J. Hum. Genet.* 2006; 78:759–769. [PubMed: 16642432]
261. Glatt SJ, et al. Genome-wide linkage analysis of heroin dependence in Han Chinese: Results from wave one of a multi-stage study. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2006; 141B:648–652. [PubMed: 16856125]
262. Glatt SJ, et al. Genome-wide linkage analysis of heroin dependence in Han Chinese: results from Wave Two of a multi-stage study. *Drug Alcohol Depend.* 2008; 98:30–34. [PubMed: 18538955]
263. Lachman HM, et al. Genomewide suggestive linkage of opioid dependence to chromosome 14q. *Hum. Mol. Genet.* 2007; 16:1327–1334. [PubMed: 17409192]
264. Yu Y, et al. Substance dependence low-density whole genome association study in two distinct American populations. *Hum. Genet.* 2008; 123:495–506. [PubMed: 18438686]
265. Sham P, Bader JS, Craig I, et al. DNA pooling: a tool for large-scale association studies. *Nat. Rev. Genet.* 2002; 3:862–871. [PubMed: 12415316]
266. Uhl GR, et al. Polysubstance abuse-vulnerability genes: genome scans for association, using 1,004 subjects and 1,494 single-nucleotide polymorphisms. *Am. J. Hum. Genet.* 2001; 69:1290–1300. [PubMed: 11704927]
267. Liu QR, et al. Pooled association genome scanning: validation and use to identify addiction vulnerability loci in two samples. *Proc. Natl. Acad. Sci. U.S.A.* 2005; 102:11864–11869. [PubMed: 16091475]

268. Johnson C, et al. Pooled association genome scanning for alcohol dependence using 104,268 SNPs: Validation and use to identify alcoholism vulnerability loci in unrelated individuals from the collaborative study on the genetics of alcoholism. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2006; 141B:844–853. [PubMed: 16894614]
269. Liu QR, et al. Addiction molecular genetics: 639,401 SNP whole genome association identifies many "cell adhesion" genes. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2006; 141B:918–925. [PubMed: 17099884]
270. Uhl GR, et al. Genome-wide association for methamphetamine dependence: convergent results from 2 samples. *Arch. Gen. Psychiatry.* 2008; 65:345–355. [PubMed: 18316681]
271. Johnson C, et al. Convergent genome wide association results for bipolar disorder and substance dependence. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2009; 150B:182–190. [PubMed: 19127564]
272. Khrapko KR, et al. A method for DNA sequencing by hybridization with oligonucleotide matrix. *DNA Seq.* 1991; 1:375–388. [PubMed: 1768861]
273. Guschin D, et al. Manual manufacturing of oligonucleotide, DNA, and protein microchips. *Anal. Biochem.* 1997; 250:203–211. [PubMed: 9245440]
274. Proudnikov D, et al. Immobilization of DNA in polyacrylamide gel for the manufacturing of DNA and DNA-oligonucleotide microchips. *Anal. Biochem.* 1998; 259:34–41. [PubMed: 9606140]
275. Fotin AV, et al. Parallel thermodynamic analysis of duplexes on oligonucleotide microchips. *Nucleic Acids Res.* 1998; 26:1515–1521. [PubMed: 9490800]
276. Guschin DY, et al. Oligonucleotide microchips as genosensors for determinative and environmental studies in microbiology. *Appl. Environment. Microbiol.* 1997; 63:2397–2402.
277. Proudnikov D, et al. Analysis of mutations in oral poliovirus vaccine by hybridization with oligonucleotide microchips. *Biologicals.* 2000; 28:57–66. [PubMed: 10885613]
278. Drobyshev AL, et al. Massive parallel analysis of DNA-Hoechst 33258 binding specificity with a generic oligodeoxyribonucleotide microchip. *Nucleic Acids Res.* 1999; 27:4100–4105. [PubMed: 10497276]
279. Chechetkin VR, et al. Sequencing by hybridization with the generic 6-mer oligo-nucleotide microarray: an advanced scheme for data processing. *J. Biomol. Struct. Dyn.* 2000; 18:83–101. [PubMed: 11021654]
280. LaForge KS, et al. Detection of single nucleotide polymorphisms of the human mu opioid receptor gene by hybridization or single nucleotide extension on custom oligonucleotide gelpad microchips: potential in studies of addiction. *Am. J. Med. Genet.* 2000; 96:604–615. [PubMed: 11054767]
281. Cañadas C, et al. Molecular haplotyping of tandem single nucleotide polymorphisms by allele-specific PCR. *Anal. Biochem.* 2007; 364:153–158. 2. [PubMed: 17399677]
282. Konfortov BA, et al. An efficient method for multi-locus molecular haplotyping. *Nucleic Acids Res.* 2007; 35:e6. [PubMed: 17158153]
283. Tost J, et al. Molecular haplotyping at high throughput. *Nucleic Acids Res.* 2002; 30:e96. [PubMed: 12364613]
284. Millson A, et al. Direct molecular haplotyping of the IVS-8 poly(TG) and polyT repeat tracts in the cystic fibrosis gene by melting curve analysis of hybridization probes. *Clin. Chem.* 2005; 51:1619–1623. [PubMed: 16020494]
285. Pont-Kingdon G, Lyon E. Direct molecular haplotyping by melting curve analysis of hybridization probes: beta 2-adrenergic receptor haplotypes as an example. *Nucleic Acids Res.* 2005; 33:e89. [PubMed: 15937194]
286. Guo Z, et al. Long-range multilocus haplotype phasing of the MHC. *Proc. Natl. Acad. Sci. U. S. A.* 2006; 103:6964–6969. [PubMed: 16632595]
287. Oberacher H, et al. Direct molecular haplotyping of multiple polymorphisms within exon 4 of the human catechol-O-methyltransferase gene by liquid chromatography-electrospray ionization time-of-flight mass spectrometry. *Anal. Bioanal. Chem.* 2006; 386:83–91. [PubMed: 16816940]
288. Hurley JD, et al. A simple, bead-based approach for multi-SNP molecular haplotyping. *Nucleic Acids Res.* 2005; 32:e186. [PubMed: 15637233]

289. Wetmur JG, et al. Molecular haplotyping by linking emulsion PCR: analysis of paraoxonase 1 haplotypes and phenotypes. *Nucleic Acids Res.* 2005; 33:2615–2619. [PubMed: 15886392]
290. Woolley AT, et al. Direct haplotyping of kilobase-size DNA using carbon nanotube probes. *Nat. Biotechnol.* 2000; 18:760–763. [PubMed: 10888845]
291. Mitra RD, et al. Digital genotyping and haplotyping with polymerase colonies. *Proc. Natl. Acad. Sci. U. S. A.* 2003; 100:5926–5931. [PubMed: 12730373]
292. Proudnikov D, et al. High-throughput molecular haplotype analysis (allelic assignment) of single-nucleotide polymorphisms by fluorescent polymerase chain reaction. *Anal. Biochem.* 2004; 335:165–167. [PubMed: 15519584]