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Pharmacogenomics and addiction to opiates

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Abstract

The risk of initiating and maintaining the use of opiates up to the point of abuse and dependence is to a large degree genetically transmitted and is separate from genetic risk factors for addiction to other drugs of abuse. Pharmacogenetic studies have so far focused on obvious candidate genes that are expected to be involved either in the pharmacokinetics or in the pharmacodynamics of opioids in the mesolimbic reward system of the brain. The few findings of a positive allelic association rarely withstand replication in independent case-control or less stratification-prone family-based association samples. A pharmacogenomic approach in the best sense of the word, however, involves an unbiased, genome-wide, parallel search for risk genes and gene expression patterns. So far, only quantitative trait loci mapping studies of inbred rodent strains and differential expression studies using high-density DNA microarrays fulfill these requirements. The present state of pharmacogenomic and pharmacogenetic studies in animals and humans with respect to opiate addiction is reviewed in this paper.

Keywords: Opiate; Heroin; Addiction; Genetic; Pharmacogenomics; Candidate gene

1. Introduction

Recent large-scale twin studies have estimated the relative contribution of additive genetic factors and of a common (or familial) or specific (or non-shared) environment to the liability of consuming different illegal drugs as individuals progress from their initial exposure to the drug to regular drug use and finally to abuse and dependence (Kendler et al., 1999a,b, 2000; Tsuang et al., 1996, 1999). Heroin emerged as the drug whose use, in low to moderate levels, is influenced the most by genetic factors (heritability 0.54), and as the drug that shares the least genetic vulnerability with other substances of abuse (Tsuang et al., 2000). This might seem to make the identification of individual risk genes an easy task, but like all other psychiatric disorders, drug addiction is a genetically complex disease that does not derive from a single major gene following a simple Mendelian transmission pattern. Rather, a large number of vulnerability genes, no single one of them necessary or sufficient to cause disease, and none of them fully penetrant, can be expected, and many different combinations of these may result in a clinically identical outcome (genetic heterogeneity). More than in any other psychiatric disorder, the contribution of environment is obvious in that genetic vulnerability can lead to disease only once the substance of abuse is readily available. The role of the drug is not limited to triggering the onset of the addictive process, but also involves maintaining it once structural adaptations have been achieved. The genetics of opiate addiction as a disease process are thus by definition pharmacogenetics. The view of addiction as a multi-stage process encompassing initial exposure to the drug, repeated intermittent use with increased sensitivity to every following use (sensitization), regular abuse resulting in reduced sensitivity (tolerance), dependence up to compulsive drug-seeking, and repeated cycles of withdrawal and relapse implies that, for any of these stages, pathophysiological processes and underlying genes may differ, which further complicates the task of gene identification. This is the reason why the genetics of opiate addiction that have so far been studied on a pharmacogenetic, that is gene-by-gene, basis, may be more amenable to a pharmacogenomic approach in which the entire genome and its expression are evaluated in an unbiased way and in a highly parallel fashion (Lockhart and Winzeler, 2000).

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2. Review of the recent literature

2.1. The reward system of the brain and opiate addiction

Since the 1950s it has been known from electric self-stimulation experiments in rats that a specific brain circuit exists for the evaluation of rewarding stimuli. Dopaminergic neuronal projections have been identified as the central component of this brain reward system. They extend from the ventral tegmental area of the midbrain to parts of the limbic system, especially to the nucleus accumbens shell and the frontal cortex. Both natural stimuli (such as food, drink, or sex) and several, but not all, substances of abuse (most prominently cocaine, amphetamine, and opiates, but not benzodiazepines, barbiturates or inhalants) are able to increase the release of dopamine in the nucleus accumbens. While it was formerly believed that dopamine directly mediated the subjective effects of well-being upon rewarding actions, or that it maintained the reinforcement of operant (trainable) behaviour needed to receive a reward, recent studies (as reviewed in an article by Wickelgren, 1997, and in detail by Spanagel and Weiss, 1999) have indicated that the function of dopamine is rather to signal novelty in situations where drug consumption is acquired and to facilitate learning and later recognition of these environmental stimuli associated with consumption (which might explain clinical phenomena like cue-elicited drug craving and relapse that complicate the treatment of opiate dependence).

The specific interactions of opiates with the reward system have been reviewed by Simonato (1996) and by Spanagel and Weiss (1999). The firing of dopaminergic neurons in the ventral tegmental area is normally under the control of GABAergic (γ-amino-butyric acid) interneurons. Binding of opiates to μ-opioid receptors of these interneurons causes inhibition, thereby releasing the ventral tegmental area neurons from inhibitory control, which results in an increased release of dopamine in the nucleus accumbens. This mechanism is responsible for the secondary reinforcing effects of opiates as such effects can be measured by conditioned place preference in mice. In contrast, primary reinforcing effects, such as the self-administration of opiates, seem not to depend on functional dopaminergic ventral tegmental area neurons, but rather on the direct action of opiates at postsynaptic opioid receptors in the nucleus accumbens.

Studies on the reinforcing properties of cocaine have shown that the dopaminergic reward system is also under control of serotonin-releasing neurons projecting from the midbrain dorsal raphé nucleus onto the inhibitory GABAergic interneurons mentioned above (Rocha et al., 1998; White, 1998). Several other transmitter systems interact with opiates (Simonato, 1996), among them the cholinergic, glutamatergic, and adrenergic systems. The latter two are of relevance in the emergence of withdrawal symptoms upon opiate abstinence after chronic exposure. These symptoms are to a large extent mediated by the locus coeruleus, the major noradrenergic nucleus of the brain.

Nestler and Aghajanian (1997) have reviewed the cellular events involved in the long-term adaptations leading to maintenance of addiction beyond the rewarding, reinforcing effects of acute opiate exposure. Tolerance, its counterpart sensitization, and dependence evolve as a consequence of receptor adaptations and up-regulation of the cyclic AMP signal transduction pathway, which also contributes to withdrawal states during short-term abstinence. The occurrence of drug craving and stress-induced relapses during long-term abstinence may involve synaptic plasticity, as the basis of learning and memory, as well as alterations in the hypothalamic-pituitary-adrenal axis response to stress (Kreek, 1996).

Altogether, this suggests a large number of candidate genes with some role in opiate addiction, including the opioid receptor genes, the diverse receptor and transporter genes of the above-mentioned neurotransmitter systems, and the genes of the intracellular signal transduction pathways and of the proteins involved in synaptic plasticity and learning.

2.2. Pharmacogenomic studies in animals

2.2.1. Experimental crosses: mapping of quantitative trait loci

Experimental breeding of mouse and rat strains has allowed the study of genetically based differences in sensitivity to acute and chronic drug effects, in the development of tolerance and withdrawal by neuroadaptational changes, and in the rewarding, reinforcing properties of most drugs of abuse (Crabbe et al., 1994). Since under experimental conditions environmental factors are kept constant, any differences can directly be ascribed to genetic effects. Thus, different strains of rodents can be selectively bred for high versus low drug response. While a huge number of mouse and rat strains exist with various responses to alcohol, few have been designed with respect to opiate sensitivity, e.g. mice with high versus low analgesic response (HAR/LAR) to levorphanol (Crabbe et al., 1994). It is, however, doubtful whether the analgesic response to opiates genuinely reflects a vulnerability to the symptoms more characteristic of dependence (Kest et al., 1998).

Systematic testing of mouse strains that have been developed for genetic research other than for drug abuse showed that they differ in their voluntary acceptance of morphine-tinged drinking solutions in a two-bottle choice paradigm (Belknap et al., 1993). Among 15 inbred mouse strains, the daily dose of ingested morphine varied by more than an order of magnitude, indicating a substantial genetic effect. Comparing high-consuming black C57BL/6J mice with low-consuming DBA/2J mice, Berrettini et al. (1994a) showed that the former maintained a constant intake over various concentrations of drinking solution,

and that their morphine ingestion could be halved by intraperitoneal injection of the opioid receptor antagonist naltrexone. Morphine plasma levels did not differ between the two inbred strains. Thus, strain differences in morphine preference did not simply reflect gustatory or pharmacokinetic strain variation, but differences of opioid receptor-mediated, reward-related phenomena. Likewise, pharmacokinetic differences did not account for the different sensitivity to morphine or for the development of tolerance in two rat strains (Mas et al., 2000).

Typically, drug-related quantifiable traits of interest are normally distributed in the population, indicating the action of a large number of underlying genes, each of which influences the trait only to a minor extent. Mapping these quantitative trait loci by linkage analysis involves determining their locations on a marker map that covers the whole chromosomal genome as well as estimating the strength of their effects. By inbreeding animals from the second generation of intercrossing of two inbred strains for at least 20 generations, a selection of recombinant inbred lines can be created of which each line represents a subsample of the genomes of the two founding inbred strains (Takahashi et al., 1994). Provisional evidence for 10 morphine-related quantitative trait loci was obtained by comparing trait means in the 26 BXD recombinant inbred strains that had been derived from the C57BL/6J and DBA/2J inbred strains mentioned above for their large difference in voluntary morphine intake (Gora-Maslak et al., 1991; Belknap and Crabbe, 1992; Crabbe et al., 1994). Quantitative trait loci that influence locomotor activation by morphine were found on mouse chromosomes 14 [near the 5-hydroxytryptamine (5-HT) receptor gene cluster] and 19. Temperature sensitivity due to morphine treatment depended on quantitative trait loci of subcentromeric chromosome 10 (near the μ-opioid receptor gene) and chromosome 18 (near the glucocorticoid 1 receptor gene). A quantitative trait locus on chromosome 3 (near the acetylcholine receptor β-2 and metabotropic glutamate receptor 2 genes) had an impact on morphine-induced analgesia in the hot-plate test, and a quantitative trait locus on chromosome 4 (between the metabotropic glutamate receptor 7 and the δ-opioid receptor genes) affected the consumption of morphine in saccharine solution. The induction of Straub tail (a contraction of the mouse anal sphincter elicited by high-level intake of morphine, forcing the tail into an upright position) mapped to quantitative trait loci on chromosomes 5 (near the acetylcholine esterase gene), 9 (one quantitative trait locus between the opioid binding protein and the dopamine receptor D2 genes, another quantitative trait locus near the 5-HT receptor 1B gene), and 16 (in the proximity of the dopamine D3 receptor gene).

A substantial number of findings from nuclear genome scans can be expected to be false-positive due to chance; replication in independent samples is therefore needed. Berrettini et al. (1994b) performed a quantitative trait loci mapping study of morphine-drinking preference in the second generation intercross of the above C57BL/6J and DBA/2J inbred strains. Animals of each parental strain are homozygous at almost every genetic locus (by inbreeding); their first generation intercross offspring (F1 generation) are uniformly heterozygous. They tested 606 F2 animals (resulting from brother–sister matings of the F1 generation) for morphine preference and typed the 49 mice with the highest versus 46 mice with the lowest morphine consumption on a genome-wide set of markers less than 10 cM apart. Three quantitative trait loci on chromosomes 1, 6 and 10 were obtained that together accounted for 85–90% of the genetic variance in morphine preference between the two parental strains (that was about 50% of the total variance, genetic and non-genetic). Another study of F2 intercrosses of the C57BL/6J and DBA/2J inbred strains found a quantitative trait locus in the same proximal chromosome 10 region for morphine-induced analgesia in the hot-plate test (Belknap et al., 1995). Thus, the existence of a subcentromeric quantitative trait locus on murine chromosome 10 with an impact on the effects of morphine is strongly supported by its vicinity to the μ-opioid receptor gene (Alexander et al., 1996).

Current strategies to follow-up quantitative trait loci findings include the use of congenic strains, the study of candidate genes within the quantitative trait loci regions by targeted disruption (knockout; see Section 2.4 below), and the study of epistatic interaction between genes, and between genes and the environment (Crabbe et al., 1999).

2.2.2. Expression profiling: differential display of drug-exposed versus non-exposed genomes

Gene expression in drug-exposed animals has been investigated mainly with respect to single genes of interest, in particular the opioid receptors. Therefore, these studies cannot strictly be called pharmacogenomic. Unterwald et al. (1995) found that the well-documented up-regulation of opioid receptors following chronic exposure to antagonists was not caused by increased transcription of the μ-opioid receptor gene or by increased stability of its mRNA. Despite an 83% increase of receptor density in male Sprague–Dawley rats infused for 7 days with naltrexone, mRNA levels did not differ from those of saline-treated control animals in all brain regions tested. Alternative mechanisms may account for this result, such as post-translational modifications, internalization of the receptor into the endosomal compartment (Evans, 2000; Evans et al., 2000), or changed turnover. Likewise, the agonist-induced decrease in receptor number that has been suggested as a mechanism of tolerance was not found to be associated with altered μ- and δ-opioid receptor gene transcription (Buzas et al., 1996; Castelli et al., 1997). However, increased κ-opioid receptor gene mRNA levels could be induced by six intermittent injections of morphine (Wang et al., 1999). Since the opioid system is also involved in the control of the hypothalamic-pituitary-adrenal axis, the transcription of several of its pertinent genes in response to

chronic agonist treatment was studied. Methadone did not change mRNA levels of the genes encoding pro-opiomelanocortin, the corticotropin-releasing factor and its receptor (Zhou et al., 1996).

Recently, the availability of high-density oligonucleotide arrays (so-called DNA chips) has made it possible to obtain an expression profile of thousands of murine genes after experimental drug exposure in a single assay. This is a truly pharmacogenomic approach in that the entire expressed genome can be studied in an unbiased way (Lockhart and Winzeler, 2000). DNA chips should also facilitate the study of epistatic interactions between several genes that are not amenable to simple pharmacogenetic studies on a gene-by-gene basis. In a pharmacogenomic expression study with DNA microarrays in mice (Miles et al., 2000, and manuscript in press in Molecular Pharmacology), three genes were found to be transcriptionally responsive to morphine exposure: *e3B1* (eps8 binding protein), *KRT18* (cytokeratin 18), and *NEF3* (neurofilament 3). It is striking that these three proteins possess some relationship to the cytoskeleton. As there are also several cytoskeletal members among the more than 75 proteins that are part of a huge neuronal membrane complex (the "Hebbosome") that regulates synaptic strength by long-term potentiation and depression (Grant, 2000), it can be speculated that transcriptional regulation of these three genes by morphine exposure contributes to the salient learning processes that make up some of the most relevant phenomena of drug dependence, namely cue-elicited drug craving and "addictive memory".

2.3. Pharmacogenomic studies in humans are lacking

2.3.1. Genome-wide linkage studies

Linkage studies in families with several affected members offer the possibility to explore in an unbiased way the entire nuclear genome for loci influencing disease risk (a genome scan). Although the classical domain of monogenic diseases, linkage studies have been successful in mapping risk genes in other complex genetic disorders such as breast cancer and Alzheimer's disease. Linkage studies look for co-transmission of the disease trait within families with any one of a given set of polymorphic DNA markers that are evenly spaced over the genome, mostly simple sequence variants such as CA repeats (so-called microsatellites). They are most powerful when a particular mode of transmission (dominant or recessive), penetrance and population frequency of the assumed risk gene can be specified; however, it is close to impossible to estimate these parameters accurately for complex genetic diseases. Therefore, non-parametric (or model-free) linkage analyses have become popular, in which affected sibling pairs (or pairs of other affected relatives) are studied. Affected siblings will share, by a greater than 50% chance frequency, any marker linked by proximity to an unknown risk gene.

Samples of hundreds of families with affected siblings are needed to provide sufficient power to detect loci of low to moderate effect as they can be expected in complex diseases. However, to our knowledge no sample of this kind has so far been collected with respect to opiate dependence. Mapping the loci that influence the risk for alcoholism and a number of associated, presumably pathophysiological, traits (so-called endophenotypes) was successful in a large multi-center study (Reich et al., 1998). As there is substantial comorbidity in addictive disorders (for instance, there are few alcohol-dependent individuals who do not smoke), it has been possible to identify in the same sample of alcohol-dependent probands risk loci for cocaine dependence, cannabis dependence, and habitual smoking. Some of the linked chromosomal regions were virtually identical in several kinds of addiction, whereas others were unique for one particular type of substance (Bierut et al., 2000). Unfortunately, opiate abuse and dependence were too rare in this particular sample to allow for mapping of linked loci.

2.3.2. Genome-wide association studies

It has been proposed that the high density of base substitutions, so-called single nucleotide polymorphisms that occur at about every 1000 base pairs in the genome, might be helpful in the study of disease associations even in genetically highly heterogenous, mixed populations on a genome-wide basis (Risch and Merikangas, 1996). Linkage disequilibrium, the non-random allelic association of closely neighboring markers of a gene variant that increases disease susceptibility, reflects one or several genetic backgrounds (haplotypes) in a population in which any such disease variant has arisen by mutation. This approach has been applied successfully to the mapping of monogenic disease loci, and it has been useful in the mapping of the risk loci of some genetically complex diseases in isolated, genetically homogeneous populations in whose members linkage disequilibrium extends over large chromosomal distances. Extending this approach from the study of single, pre-selected candidate genes (as they are reviewed in Section 2.5 below) to an unbiased screening of the entire genome for disease association or drug response would indeed constitute a truly pharmacogenomic endeavour. However, this would have to await the availability of denser maps of single nucleotide polymorphisms and of cheaper genotyping techniques. Also, issues concerning sample size and the power of resolution, given the moderate effect sizes of susceptibility alleles, have not been sufficiently resolved (McCarthy and Hilfiker, 2000).

2.4. Pharmacogenetic studies in animals: gene targeting

Gene targeting by homologous recombination makes it possible to disrupt a gene of interest in mouse embryonic stem cells so that expression of that gene is prevented.

Transfected cells can then be introduced into recipient blastocysts, and chimeric animals can be raised in which the gene is nonfunctional in some germ cells. Cross-breeding these chimera will lead to the generation of knockout animals that are totally and ubiquitously deficient for the gene product (as long as disruption of the gene is compatible with survival).

2.4.1. Knockout of opioid receptor genes

Primary interest has focused on the knockout of the μ-, δ-, and κ-opioid receptor genes, to discriminate their relative contribution to the various actions of agonists and antagonists (Kieffer, 1999). In mice lacking the μ-opioid receptor gene (Matthes et al., 1996; Becker et al., 2000) morphine had no analgesic, locomotor hyperactivity-inducing, or rewarding effects (the latter as evidenced by the inability to induce conditioned place preference and to acquire self-administration). Signs of withdrawal after chronic morphine administration was stopped were completely absent. All of these morphine-induced actions normally seen in wild-type control animals are therefore largely dependent on the μ-opioid receptor, except for morphine withdrawal, which was also diminished in κ-opioid receptor knockout mice (Simonin et al., 1998). Heroin or its major metabolite morphine 6-β-glucuronide, like morphine, failed to elicit analgesia in μ-opioid receptor knockout mice (Kitanaka et al., 1998). This showed that no heroin-specific opioid receptor subtype exists. Autoradiography of all three receptor types demonstrated that disruption either of the μ- or of the κ-opioid receptor gene did not provoke a major compensatory up-regulation of the remaining two receptor types (Kitchen et al., 1997; Slowe et al., 1999). Coupling of the δ-, but not of the κ-opioid, receptor to GTP-binding proteins may be altered in μ-opioid receptor knockout mice (Park et al., 2000). Reduced tolerance to the analgesic action of δ-opioid receptor ligands has been shown in mice lacking this receptor type (Zhu et al., 1999).

2.4.2. Knockout of endogenous opioid receptor ligand genes

The effects of a total elimination of the physiological μ- and δ-opioid receptor ligands, β-endorphin and enkephalin, have been studied by targeted disruption of the pro-opiomelanocortin and pro-enkephalin genes (Kieffer, 1999). In mice without β-endorphin, exposure to swim stress did not lead to analgesia, which is usually mediated by this endogenous ligand, while exogenous morphine retained its analgesic effect on the available μ-opioid receptors (Rubinstein et al., 1996). Lack of enkephalin resulted in a compensatory up-regulation of μ- and δ-opioid receptors in frontal brain areas, which was suggested as an explanation for the increased aggressiveness observed in these animals (Brady et al., 1999). However, none of these studies tested directly for putative changes in the addictive properties of abused opiates.

2.4.3. Knockout of other genes involved in the reward system

Disrupting the cannabinoid receptor 1 gene not only led to a lack of response to cannabinoids, but also to a substantial reduction in the rewarding effects of chronic morphine administration and to reduced opiate withdrawal (Ledent et al., 1999). Microdialysis studies of the nucleus accumbens of these mice showed that mesolimbic dopamine release was blunted in knockouts whereas it was normally stimulated by morphine in wild-type controls (Mascia et al., 1999). It may be concluded that the close functional link between the cannabinoid and the opioid systems of the brain does not exclusively depend on a common μ-opioid receptor mechanism (Tanda et al., 1997), but also on the integrity of the cannabinoid receptor gene.

Recently, the impact of the dopamine transporter gene on the mesolimbic reward system has been demonstrated (Spielewoy et al., 2000). Despite the tonic overactivity of the dopamine system in these knockout mice, morphine was still able to elicit higher dopamine release and transcription of the immediate-early gene *c-fos* than in wild-type animals. Reward-related behaviour was increased, withdrawal symptoms to naloxone were decreased, analgesia was similar, and induction of locomotion was absent in knockout mice.

Knockout of the dopamine D2 receptor gene resulted in a decreased striatal expression of enkephalin, in reduced locomotion, and in the loss of rewarding effects of morphine administration in a place-preference test. Withdrawal behaviour was not influenced (Maldonado et al., 1997; Drago et al., 1998).

As the receptor of the neuromodulatory tachykinin peptide “Substance P” is also expressed in the nucleus accumbens, disruption of its gene might reveal a possible role in drug reward. Indeed, morphine lost its rewarding properties in knockout mice, and physical signs of withdrawal were diminished (Murtra et al., 2000).

While acute morphine administration inhibits the cyclic AMP (cAMP) signal transduction pathway, chronic exposure leads to its compensatory up-regulation, which is regarded as the basis of the development of drug tolerance, sensitization, dependence, and the onset of withdrawal (Nestler and Aghajanian, 1997). One of the distal effectors of this pathway is the transcription factor cAMP response element binding protein (CREB), which initiates transcription of immediate-early genes in the cell nucleus. Complete disruption of the genes of all three CREB isoforms is lethal shortly after birth, but targeted disruption of two of the genes and consequent upregulation of a minor isoform is compatible with survival and may be regarded as an incomplete knockout. Morphine withdrawal in these animals was strongly reduced (Maldonado et al., 1996), confirming the above hypothesis.

2.5. Pharmacogenetic studies in humans: candidate genes

In the absence of a truly pharmacogenomic approach in humans (see Section 2.3 above), association studies have had to single out candidate genes according to their likely role in opiate metabolism or in the reward system of the brain (Table 1). Most studies have been case-control comparisons, and it should be stressed that these are notoriously prone to sample stratification biases. Other than indicating that the associated marker itself is the susceptibility-increasing gene variant or that it is in linkage disequilibrium with any such variant nearby, any positive finding may be due to unaccounted systematic differences (the most obvious being ethnicity) between cases and controls apart from the presence versus absence of the disease under study. Means to remedy this weakness have been suggested (Spielman and Ewens, 1996), but have rarely been applied to samples of probands with opiate addiction. When parents are available, the transmission of a putatively disease-associated allele from heterozygous parents to affected probands can be studied; departure from random transmission indicates association in an unbiased way.

2.5.1. Pharmacokinetic candidate genes: cytochrome P450 2D6

Tyndale et al. (1997) hypothesized that due to an inability to metabolize oral opiates into more active degradation products, individuals homozygous for deletions of the cytochrome P450 2D6 gene (*CYP2D6*) should be protected from opiate dependence (a negative association with the disease). Indeed, they found no homozygous deletions in their sample of opiate addicts, but did in 4% of never-dependent controls and in 6.5% of multi-drug dependent probands. Though the odds ratio of the effect was impressive, its 95% confidence interval covered unity (Table 1). Other criticism focused on possible sample stratification (as mentioned in Section 2.5 above) and a low correspondence between the poor metabolizer phenotype and genotype when only the two most frequent deletions were typed out of a greater number that are known to occur (Mikus et al., 1998; Tyndale et al., 1998).

2.5.2. Pharmacodynamic candidate genes

2.5.2.1. Dopamine receptor and transporter genes. Evidence that the mesolimbic–mesocortical dopaminergic projections represent the reward system of the brain implicates the different genes of dopamine receptors and the dopamine transporter as candidate genes. In 1990, a restriction fragment length polymorphism of the dopamine D2 receptor gene was the first genetic variant found to be associated with alcoholism (Blum et al., 1990, Smith et al., 1992). Given several successful and numerous negative replication studies in independent samples, a strong debate has waged ever since on whether this finding might have been a false-positive result because of the likelihood that sample stratification occurred, as discussed in Section 2.5. Association with the A1 allele was also found in a comparison between a large sample of polysubstance-abusing individuals and a substantially lower number of drug-free controls (Smith et al., 1992), but it is unclear how much of the effect was due to opiate addiction since substance abuse was not broken down into specific substance abuse patterns. A study on an obviously overlapping sample showed that the finding did not hold for a subsample of 40 opiate-preferring poly-drug users, but rather for individuals with a heavy daily preferential use of psychostimulants (Persico et al., 1996). The same study ruled out an association between opiate preference and a polymorphism of the dopamine transporter gene, but with less than 40 individuals in each group, power was low. In a recent conference abstract, a highly significant association was reported between opiate addiction and an insertion/deletion polymorphism in the promoter of the dopamine D2 receptor gene whose alleles lead to a different extent of reporter gene expression in transfection assays. However, this finding was restricted to a subgroup of Chinese heroin addicts who consumed the drug by inhalation (Li et al., 2000).

As several studies have related the dopamine D3 receptor gene with the actions of cocaine (see Duaux et al., 1998), a single nucleotide polymorphism leading to the presence of either serine or cysteine in the ninth amino terminal position has also been studied in a moderately sized sample of opiate addicts (Duaux et al., 1998). The authors found no difference in allele and genotype frequencies between cases and controls, but homozygosity (i.e. genotype serine/serine, or cysteine/cysteine) was more frequent in those addicts who scored high in sensation seeking, a temperament characteristic known to predispose to substance abuse (similar to the slightly differently conceptualized measure of novelty seeking, see below). Two non-replications in sizable samples from Israel (Kotler et al., 1999) and China (Li et al., 2000) underscore that findings derived from small subgroups of moderately sized samples may be a critical issue.

Another association was based on the observation that novelty seeking, a personality trait increasing the risk for substance dependence, is to a slight extent increased in individuals with longer alleles of several expressed repeat units in the third exon of the dopamine D4 receptor gene. The same polymorphism might thus be associated directly with opiate dependence, as was found in the Israelian and Chinese samples mentioned above (Kotler et al., 1997; Li et al., 1997). However, a study in the US found no association with this and three more polymorphisms of the same gene (Gelernter et al., 1997). Another sizable case-control study in Germany also had a negative result (Franke et al., 2000), and in a family-controlled study, as recommended above (Section 2.5), preferential transmission of the supposedly associated allele 7 from heterozygous parents to heroin-dependent offspring was not observed

Table 1
Association studies of 11 candidate genes in probands with opiate dependence

Protein	Gene symbol	Cytogenetic location	Publication	Number of cases	Number of control subjects	Results
Cytochrome *P*450, subfamily II D	*CYP2D6*	22q13.1	Tyndale et al., 1997	83 (oral opiate), 93 (multi-drug)	276	Protective effect of homozygote deletion (no poor metabolizers in 83 opiate abusers) [$P = 0.05$, OR 7.2 (95% CI, 0.4–124.1)]
Dopamine receptor D2	*DRD2*	11q23	Smith et al., 1992	232 (poly-substance)	56	Alleles B1 and A1 ↑ in cases ($P < 0.01$, $P < 0.05$)
			Persico et al., 1996	40 (opiate-preferring)	119	No association
			Li et al., 2000	465 (heroin addicts)	298	−141C Ins/Del (insertion/deletion polymorphism) associated in nasal inhalers ($P = 0.006$)
Dopamine receptor D3	*DRD3*	3q13.3	Duaux et al., 1998	54 (opiate addicts)	70	Ser9Gly homoz. ↑ in sensation seekers ($P = 0.034$)
			Kotler et al., 1999	193 (heroin addicts)	134	No association with Serin9Glycin polymorphism
			Li et al., 2000	121 (heroin addicts)	180	No association with Serin9Glycin polymorphism
Dopamine receptor D4	*DRD4*	11p15.5	Kotler et al., 1997	141 (heroin addicts)	110	Allele 7 ↑ [$P = 0.001$, RR 2.5 (95%CI, 1.4–4.4)]
			Li et al., 1997	121 (heroin addicts)	154	Long alleles ↑ [$P = 0.02$, OR 2.3 (95%CI, 1.1–4.9)]
			Gelernter et al., 1997	55 (opiate dependent)	144	No association with 4 coding polymorphisms
			Franke et al., 2000	285 (heroin addicts)	197	No association (allele 7, $P = 0.19$)
			Franke et al., 2000	111 parent–offspring trios		Transmission/disequilibrium test (TDT), $P = 0.74$
Solute carrier family 6, member 3; dopamine transporter	*SLC6A3*; *DATI*	5p14.3	Persico et al., 1996	32 (opiate-preferring)	38	No association with 40 basepair variation in the number of tandem repeats polymorphism in 3' untranslated region
Solute carrier family 6, member 4; 5-hydroxy-tryptamine transporter; serotonin transporter	*SLC6A4*; *5-HTT*; *SERT*	17q11.1–q12	Tan et al., 1999	63 (heroin addicts)	72	Allele 10 ↑ [$P = 0.005$, OR 3.5 (95%CI, 1.4–8.6)]
			Kotler et al., 1999	186 (heroin addicts)	217	No association with promoter variant ($P > 0.1$)
			Li et al., 2000	121 (heroin addicts)	180	No association with promoter variant and intronic variable number of tandem repeat
5-hydroxytryptamine receptor 2A	*HTR2A*	13q14–q21	Li et al., 2000	121 (heroin addicts)	180	No association with A-1438G and T102C single nucleotide polymorphisms
Gamma-aminobutyric acid receptor γ-2	*GABRG2*	5q31.1–q33.1	Li et al., 2000	121 (heroin addicts)	180	No association with G3145A single nucleotide polymorphism
Cannabinoid receptor 1	*CNR1*	6q14–q15	Comings et al., 1997b	29 (opiate users)	114	No association with AAT triplet repeat
Opioid receptor μ-1	*OPRM1*	6q24–q25	Bond et al., 1998	113 (heroin addicts)	39	No association with C17T ($P = 0.054$) or A118G ($P = 0.16$; except in Hispanics, $P = 0.004$)
Opioid receptor δ-1	*OPRD1*	1p36.1–p34.3	Mayer et al., 1997	103 (heroin addicts)	115	T921C: CC homoz. ↑ [RR 4.4 (95%CI, 1.8–10.8)]
			Franke et al., 1999	233 (heroin addicts)	173	T921C: no association ($P = 0.30$)
			Franke et al., 1999	90 parent–offspring trios		Transmission/disequilibrium test (TDT), $P = 0.68$

(Franke et al., 2000), which strongly suggests the possibility of stratification biases in the first two studies.

Studies of the association of polymorphisms in the dopamine D1 and D5 receptor genes with substance addiction have also been reported, but these covered only nicotine (Comings et al., 1997a) or unspecified substance abuse (Vanyukov et al., 1998) and therefore do not appear in Table 1.

2.5.2.2. 5-HT receptor and transporter genes. Two polymorphisms are known in the serotonin transporter gene. Allele 10 of an intronic tandem repeat was found to be associated with opiate addiction in a sample of Chinese descent (Tan et al., 1999); however, this could not be confirmed in another Chinese sample of twice the size (Li et al., 2000). Variable number of tandem repeat polymorphisms, though occurring in non-coding introns, sometimes have an impact on gene transcription, for instance in the insulin gene. Functionally more important is a deletion variant in the promoter of the serotonin transporter gene, the serotonin transporter (SERT) linked polymorphic region. In transfection studies, the presence of a short allele results in less transcription of the gene than the presence of two long alleles. No association was found with this variant in an Israelian and a Chinese sample (Kotler et al., 1999; Li et al., 2000). Unpublished studies of the family-controlled sample of Franke et al. (1999, 2000) also indicated that neither the intronic variable number of tandem repeat nor the promoter variant seem to contribute to heroin dependence (the latter variant is, however, strongly associated with alcohol dependence; Lichtermann et al., 2000).

No association with opiate addiction was seen in a single study of two base exchange polymorphisms in the 5-HT receptor 2A gene (Li et al., 2000).

2.5.2.3. GABA receptor genes. The GABA receptor γ2 subunit gene contains a single nucleotide polymorphism that is not associated with opiate dependence (Li et al., 2000).

2.5.2.4. Cannabinoid and opioid receptor genes. The knockout studies reviewed above (Sections 2.4.1 and 2.4.3, and Uhl et al., 1999) support a prime role of these receptor genes. Comings et al. (1997b) found an association between a triplet repeat polymorphism in the cannabinoid receptor gene and intravenous abuse of various drugs, but not with opiate dependence as such. Opiate users were rare in their sample, so the power of the study may have been limited.

Five single nucleotide polymorphisms have been discovered in the μ-opioid receptor gene (Bond et al., 1998), the alleles of two of which lead to amino acid substitutions and are sufficiently frequent in the population to allow for an association analysis. The presence of the rarer G allele of the A118G single nucleotide polymorphism (encoding aspartic acid instead of asparagine in the amino-terminal position 40) was shown not to influence exogenous ligand binding, but the endogenous ligand β-endorphin was bound three times stronger and activated potassium channels coupled to GTP-binding protein three times more efficiently than the endogenous ligand did in the more frequent variant. However, no association was observed with opiate dependence, except in a Hispanic subsample, where the presence of the G allele reduced the risk of dependence by half. No significant association emerged with the C17T polymorphism, either. Unpublished results on the family-controlled sample by Franke et al. (1999, 2000) were also negative.

An increased risk of opiate dependence in C-allele homozygotes of the T921C single nucleotide polymorphism in the δ-opioid receptor gene was reported from a case-control study in Germany (Mayer et al., 1997). However, no replication was seen in another German sample of double the size and in an additional family-controlled sample (Franke et al., 1999).

3. Conclusion

Although studies with mice bearing disrupted genes for opioid, cannabinoid or dopamine receptors clearly confirm that the rewarding properties of opiates depend largely on the functionality of these structures, surprisingly little evidence has been found in association studies of opiate-addicted humans for a contribution of these genes to the vulnerability for opiate dependence, which may be marginal. This may indicate that the selective approach of pharmacogenetic studies on a gene-by-gene basis might miss important genetic interactions as well as single gene variation with a larger effect size, particularly below the level of receptor-ligand interactions. While suitable samples of affected sibling pairs for unbiased, genome-wide linkage mapping of vulnerability genes are missing and the applicability of single nucleotide polymorphism maps for genome-wide linkage disequilibrium mapping has not yet been proven, pharmacogenomic approaches are beginning to be successfully applied to the monitoring of whole-genome expression patterns in animal studies.

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References

Alexander, R.C., Heydt, D., Ferraro, T.N., Vogel, W., Berrettini, W.H., 1996. Further evidence for a quantitative trait locus on murine chromosome 10 controlling morphine preference in inbred mice. Psychiatr. Genet. 6, 29–31.

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

Belknap, J.K., Crabbe, J.C., 1992. Chromosome mapping of gene loci affecting morphine and amphetamine responses in BXD recombinant inbred mice. Ann. N. Y. Acad. Sci. 654, 311–323.

Belknap, J.K., Crabbe, J.C., Riggan, J., O'Toole, L.A., 1993. Voluntary consumption of morphine in 15 inbred mouse strains. Psychopharmacology (Berlin) 112, 352–358.

Belknap, J.K., Mogil, J.S., Helms, M.L., Richards, S.P., O'Toole, L.A., Bergeson, S.E., Buck, K.J., 1995. Localization to chromosome 10 of a locus influencing morphine analgesia in crosses derived from C57BL/6 and DBA/2 strains. Life Sci. 57, 117–124.

Berrettini, W.H., Alexander, R., Ferraro, T.N., Vogel, W.H., 1994a. A study of oral morphine preference in inbred mouse strains. Psychiatr. Genet. 4, 81–86.

Berrettini, W.H., Ferraro, T.N., Alexander, R.C., Buchberg, A.M., Vogel, W.H., 1994b. Quantitative trait loci mapping of three loci controlling morphine preference using inbred mouse strains. Nat. Genet. 7, 54–58.

Bierut, L., Rice, J., Goate, A., Foroud, T., Edenberg, H., Crowe, R., Hesselbrock, V., Li, T.K., Nurnberger, J., Porjesz, B., Schuckit, M., Begleiter, H., Reich, T., 2000. Common and specific factors in the familial transmission of substance dependence. Am. J. Med. Genet. 96, 459.

Blum, K., Noble, E.P., Sheridan, P.J., Montgomery, A., Ritchie, T., Jagadeeswaran, P., Nogami, H., Briggs, A.H., Cohn, J.B., 1990. Allelic association of human dopamine D2 receptor gene in alcoholism. JAMA 263, 2055–2060.

Bond, C., LaForge, K.S., Tian, M., Melia, D., Zhang, S., Borg, L., Gong, J., Schluger, J., Strong, J.A., Leal, S.M., Tischfield, J.A., Kreek, M.J., Yu, L., 1998. Single-nucleotide polymorphism in the human mu opioid receptor gene alters β-endorphin binding and activity: possible implications for opiate addiction. Proc. Natl. Acad. Sci. U. S. A. 95, 9608–9613.

Brady, L.S., Herkenham, M., Rothman, R.B., Partilla, J.S., König, M., Zimmer, A.M., Zimmer, A., 1999. Region-specific up-regulation of opioid receptor binding in enkephalin knockout mice. Brain Res. Mol. Brain Res. 68, 193–197.

Buzas, B., Rosenberger, J., Cox, B.M., 1996. μ- and δ-opioid receptor gene expression after chronic treatment with opioid agonist. Neuroreport 7, 1505–1508.

Castelli, M.P., Melis, M., Mameli, M., Fadda, P., Diaz, G., Gessa, G.L., 1997. Chronic morphine and naltrexone fail to modify μ-opioid receptor mRNA levels in the rat brain. Brain Res. Mol. Brain Res. 45, 149–153.

Comings, D.E., Gade, R., Wu, S., Chiu, C., Dietz, G., Muhleman, D., Saucier, G., Ferry, L., Rosenthal, R.J., Lesieur, H.R., Rugle, L.J., MacMurray, P., 1997a. Studies of the potential role of the dopamine D1 receptor gene in addictive behaviors. Mol. Psychiatry 2, 44–56.

Comings, D.E., Muhleman, D., Gade, R., Johnson, P., Verde, R., Saucier, G., MacMurray, J., 1997b. Cannabinoid receptor gene (CNR1): association with i.v. drug use. Mol. Psychiatry 2, 161–168.

Crabbe, J.C., Belknap, J.K., Buck, K.J., 1994. Genetic animal models of alcohol and drug abuse. Science 264, 1715–1723.

Crabbe, J.C., Phillips, T.J., Buck, K.J., Cunningham, C.L., Belknap, J.K., 1999. Identifying genes for alcohol and drug sensitivity: recent progress and future directions. Trends Neurosci. 22, 173–179.

Drago, J., Padungchaichot, P., Accili, D., Fuchs, S., 1998. Dopamine receptors and dopamine transporter in brain function and addictive behaviors: insights from targeted mouse mutants. Dev. Neurosci. 20, 188–203.

Duaux, E., Gorwood, P., Griffon, N., Bourdel, M.C., Sautel, F., Sokoloff, P., Schwartz, J.C.., Ades, J., Loo, H., Poirier, M.F., 1998. Homozygosity at the dopamine D3 receptor gene is associated with opiate dependence. Mol. Psychiatry 3, 333–336.

Evans, C.J., 2000. Agonist selective μ-opioid receptor trafficking in rat central nervous system. Mol. Psychiatry 5, 121.

Evans, C.J., Monteillet-Agius, G., Saliminejad, N., Zaki, P.A., 2000. Opiate drugs: 'guilt by association'. Mol. Psychiatry 5, 122–123.

Franke, P., Nöthen, M.M., Wang, T., Neidt, H., Knapp, M., Lichtermann, D., Weiffenbach, O., Mayer, P., Höllt, V., Propping, P., Maier, W., 1999. Human δ-opioid receptor gene and susceptibility to heroin and alcohol dependence. Am. J. Med. Genet. 88, 462–464.

Franke, P., Nöthen, M.M., Wang, T., Knapp, M., Lichtermann, D., Neidt, H., Sander, T., Propping, P., Maier, W., 2000. DRD4 exon III VNTR polymorphism- susceptibility factor for heroin dependence? Results of a case-control and a family-based association approach. Mol. Psychiatry 5, 101–104.

Gelernter, J., Kranzler, H., Coccaro, E., Siever, L., New, A., Mulgrew, C.L., 1997. D4 dopamine-receptor (DRD4) alleles and novelty seeking in substance-dependent, personality-disorder, and control subjects. Am. J. Hum. Genet. 61, 1144–1152.

Gora-Maslak, G., McClearn, G.E., Crabbe, J.C., Phillips, T.J., Belknap, J.K., Plomin, R., 1991. Use of recombinant inbred strains to identify quantitative trait loci in psychopharmacology. Psychopharmacology (Berlin) 104, 413–424.

Grant, S.G.N., 2000. Molecular mechanisms of cognition: genetics of mouse learning and memory as a route to human psychiatry. Am. J. Med. Genet. 96, 455.

Kendler, K.S., Karkowski, L.M., Corey, L.A., Prescott, C.A., Neale, M.C., 1999a. Genetic and environmental risk factors in the aetiology of illicit drug initiation and subsequent misuse in women. Br. J. Psychiatry 175, 351–356.

Kendler, K.S., Karkowski, L., Prescott, C.A., 1999b. Hallucinogen, opiate, sedative and stimulant use and abuse in a population-based sample of female twins. Acta Psychiatr. Scand. 99, 368–376.

Kendler, K.S., Karkowski, L.M., Neale, M.C., Prescott, C.A., 2000. Illicit psychoactive substance use, heavy use, abuse, and dependence in a US population-based sample of male twins. Arch. Gen. Psychiatry 57, 261–269.

Kest, B., McLemore, G.L., Sadowski, B., Mogil, J.S., Belknap, J.K., Inturrisi, C.E., 1998. Acute morphine dependence in mice selectively-bred for high and low analgesia. Neurosci. Lett. 256, 120–122.

Kieffer, B.L., 1999. Opioids: first lessons from knockout mice. Trends Pharmacol. Sci. 20, 19–26.

Kitanaka, N., Sora, I., Kinsey, S., Zeng, Z., Uhl, G.R., 1998. No heroin or morphine 6β-glucuronide analgesia in μ-opioid receptor knockout mice. Eur. J. Pharmacol. 355, R1-3.

Kitchen, I., Slowe, S.J., Matthes, H.W., Kieffer, B., 1997. Quantitative autoradiographic mapping of μ-, δ- and κ-opioid receptors in knockout mice lacking the μ-opioid receptor gene. Brain Res. 778, 73–88.

Kotler, M., Cohen, H., Segman, R., Gritsenko, I., Nemanov, L., Lerer, B., Kramer, I., Zer-Zion, M., Kletz, I., Ebstein, R.P., 1997. Excess dopamine D4 receptor (D4DR) exon III seven repeat allele in opioid-dependent subjects. Mol. Psychiatry 2, 251–254.

Kotler, M., Cohen, H., Kremer, I., Mel, H., Horowitz, R., Ohel, N., Gritsenko, I., Nemanov, L., Katz, M., Ebstein, R.P., 1999. No association between the serotonin transporter promoter region (*5-HTTLPR*) and the dopamine D3 receptor (*BalI D3DR*) polymorphisms and heroin addiction. Mol. Psychiatry 4, 313–314.

Kreek, M.J., 1996. Opiates, opioids and addiction. Mol. Psychiatry 1, 232–254.

Ledent, C., Valverde, O., Cossu, G., Petitet, F., Aubert, J.F., Beslot, F., Böhme, G.A., Imperato, A., Pedrazzini, T., Roques, B.P., Vassart, G., Fratta, W., Parmentier, M., 1999. Unresponsiveness to cannabinoids

and reduced addictive effects of opiates in CB1 receptor knockout mice. Science 283, 401–404.

Li, T., Xu, K., Deng, H., Cai, G., Liu, J., Liu, X., Wang, R., Xiang, X., Zhao, J., Murray, R.M., Sham, P.C., Collier, D.A., 1997. Association analysis of the dopamine D4 gene exon III VNTR and heroin abuse in Chinese subjects. Mol. Psychiatry 2, 413–416.

Li, T., Liu, X., Zhao, J., Hu, X., Sham, P.C., Collier, D.A., 2000. Allelic association analysis of dopamine D2, D3, 5-HT2A and GABAg2 receptors and the serotonin transporter genes with heroin abuse in chinese subjects. Am. J. Med. Genet. 96, 520.

Lichtermann, D., Hranilovic, D., Trixler, M., Franke, P., Jernej, B., Delmo, C.D., Knapp, M., Schwab, S.G., Maier, W., Wildenauer, D.B., 2000. 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 157 (12), 2045–2047.

Lockhart, D.J., Winzeler, E.A., 2000. Genomics, gene expression and DNA arrays. Nature 405, 827–836.

Maldonado, R., Blendy, J.A., Tzavara, E., Gass, P., Roques, B.P., Hanoune, J., Schütz, G., 1996. Reduction of morphine abstinence in mice with a mutation in the gene encoding CREB. Science 273, 657–659.

Maldonado, R., Saiardi, A., Valverde, O., Samad, T.A., Roques, B.P., Borrelli, E., 1997. Absence of opiate rewarding effects in mice lacking dopamine D2 receptors. Nature 388, 586–589.

Mas, M., Sabater, E., Olaso, M.J., Horga, J.F., Faura, C.C., 2000. Genetic variability in morphine sensitivity and tolerance between different strains of rats. Brain Res. 866, 109–115.

Mascia, M.S., Obinu, M.C., Ledent, C., Parmentier, M., Böhme, G.A., Imperato, A., Fratta, W., 1999. Lack of morphine-induced dopamine release in the nucleus accumbens of cannabinoid CB(1) receptor knockout mice. Eur. J. Pharmacol. 383, R1–2.

Matthes, H.W., Maldonado, R., Simonin, F., Valverde, O., Slowe, S., Kitchen, I., Befort, K., Dierich, A., Le Meur, M., Dolle, P., Tzavara, E., Hanoune, J., Roques, B.P., Kieffer, B.L., 1996. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the μ-opioid-receptor gene. Nature 383, 819–823.

Mayer, P., Rochlitz, H., Rauch, E., Rommelspacher, H., Hasse, H.E., Schmidt, S., Höllt, V., 1997. Association between a delta opioid receptor gene polymorphism and heroin dependence in man. Neuroreport 8, 2547–2550.

McCarthy, J.J., Hilfiker, R., 2000. The use of single-nucleotide polymorphism maps in pharmacogenomics. Nat. Biotechnol. 18, 505–508.

Mikus, G., Mörike, K., Griese, E.U., Klotz, U., 1998. Relevance of deficient CYP2D6 in opiate dependence. Pharmacogenetics 8, 565–566.

Miles, M.F., Wang, L., Ravindranathan, A., Thibault, C., Olive, M.F., Lai, C., Lockhart, D.J., Hodge, D.W., 2000. Expression profiling and behavioral responses to drugs of abuse. Am. J. Med. Genet. 96, 456–457.

Murtra, P., Sheasby, A.M., Hunt, S.P., De Felipe, C., 2000. Rewarding effects of opiates are absent in mice lacking the receptor for substance P. Nature 405, 180–183.

Nestler, E.J., Aghajanian, G.K., 1997. Molecular and cellular basis of addiction. Science 278, 58–63.

Park, Y., Ma, T., Tanaka, S., Jang, C., Loh, H.H., Ko, K.H., Ho, I.K., 2000. Comparison of G-protein activation in the brain by μ-, δ-, and κ-opioid receptor agonists in μ-opioid receptor knockout mice. Brain Res. Bull. 52, 297–302.

Persico, A.M., Bird, G., Gabbay, F.H., Uhl, G.R., 1996. D2 dopamine receptor gene TaqI A1 and B1 restriction fragment length polymorphisms: enhanced frequencies in psychostimulant-preferring polysubstance abusers. Biol. Psychiatry 40, 776–784.

Reich, T., Edenberg, H.J., Goate, A., Williams, J.T., Rice, J.P., Van Eerdewegh, P., Foroud, T., Hesselbrock, V., Schuckit, M.A., Bucholz, K., Porjesz, B., Li, T.K., Conneally, P.M., Nurnberger Jr., J.I., Tischfield, J.A., Crowe, R.R., Cloninger, C.R., Wu, W., Shears, S., Carr, K., Crose, C., Willig, C., Begleiter, H., 1998. Genome-wide search for genes affecting the risk for alcohol dependence. Am. J. Med. Genet. 81, 207–215.

Risch, N., Merikangas, K., 1996. The future of genetic studies of complex human diseases. Science 273, 1516–1517.

Rocha, B.A., Scearce-Levie, K., Lucas, J.J., Hiroi, N., Castanon, N., Crabbe, J.C., Nestler, E.J., Hen, R., 1998. Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. Nature 393, 175–178.

Rubinstein, M., Mogil, J.S., Japon, M., Chan, E.C., Allen, R.G., Low, M.J., 1996. Absence of opioid stress-induced analgesia in mice lacking β-endorphin by site-directed mutagenesis. Proc. Natl. Acad. Sci. U. S. A. 93, 3995–4000.

Simonato, M., 1996. The neurochemistry of morphine addiction in the neocortex. Trends Pharmacol. Sci. 17, 410–415.

Simonin, F., Valverde, O., Smadja, C., Slowe, S., Kitchen, I., Dierich, A., Le Meur, M., Roques, B.P., Maldonado, R., Kieffer, B.L., 1998. Disruption of the κ-opioid receptor gene in mice enhances sensitivity to chemical visceral pain, impairs pharmacological actions of the selective κ-agonist U-50,488H and attenuates morphine withdrawal. EMBO J. 17, 886–897.

Slowe, S.J., Simonin, F., Kieffer, B., Kitchen, I., 1999. Quantitative autoradiography of μ-, δ- and κ_1 opioid receptors in κ-opioid receptor knockout mice. Brain Res. 818, 335–345.

Smith, S.S., O'Hara, B.F., Persico, A.M., Gorelick, D.A., Newlin, D.B., Vlahov, D., Solomon, L., Pickens, R., Uhl, G.R., 1992. Genetic vulnerability to drug abuse. The D2 dopamine receptor Taq I B1 restriction fragment length polymorphism appears more frequently in polysubstance abusers. Arch. Gen. Psychiatry 49, 723–727.

Spanagel, R., Weiss, F., 1999. The dopamine hypothesis of reward: past and current status. Trends Neurosci. 22, 521–527.

Spielewoy, C., Gonon, F., Roubert, C., Fauchey, V., Jaber, M., Caron, M.G., Roques, B.P., Hamon, M., Betancur, C., Maldonado, R., Giros, B., 2000. Increased rewarding properties of morphine in dopamine-transporter knockout mice. Eur. J. Neurosci. 12, 1827–1837.

Spielman, R.S., Ewens, W.J., 1996. The TDT and other family-based tests for linkage disequilibrium and association. Am. J. Hum. Genet. 59, 983–989.

Tan, E.C., Yeo, B.K., Ho, B.K., Tay, A.H., Tan, C.H., 1999. Evidence for an association between heroin dependence and a VNTR polymorphism at the serotonin transporter locus. Mol. Psychiatry 4, 215–217.

Tanda, G., Pontieri, F.E., Di Chiara, G., 1994. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common μ_1 opioid receptor mechanism. Science 276, 2048–2054.

Takahashi, J.S., Pinto, L.H., Vitaterna, M.H., 1994. Forward and reverse genetic approaches to behavior in the mouse. Science 264, 1724–1733.

Tsuang, M.T., Lyons, M.J., Eisen, S.A., Goldberg, J., True, W., Lin, N., Meyer, J.M., Toomey, R., Faraone, S.V., Eaves, L., 1996. Genetic influences on DSM-III-R drug abuse and dependence: a study of 3,372 twin pairs. Am. J. Med. Genet. 67, 473–477.

Tsuang, M.T., Lyons, M.J., Harley, R.M., Xian, H., Eisen, S., Goldberg, J., True, W.R., Faraone, S.V., 1999. Genetic and environmental influences on transitions in drug use. Behav. Genet. 29, 473–479.

Tsuang, M.T., Lyons, M.J., Faraone, S.V., 2000. Using twin data to define drug abuse phenotypes. Am. J. Med. Genet. 96, 474–475.

Tyndale, R.F., Droll, K.P., Sellers, E.M., 1997. Genetically deficient CYP2D6 metabolism provides protection against oral opiate dependence. Pharmacogenetics 7, 375–379.

Tyndale, R.F., Droll, K.P., Sellers, E.M., 1998. Relevance of deficient CYP2D6 in opiate dependence. Pharmacogenetics 8, 567–568.

Uhl, G.R., Sora, I., Wang, Z., 1999. The μ opiate receptor as a candidate gene for pain: polymorphisms, variations in expression, nociception, and opiate responses. Proc. Natl. Acad. Sci. U. S. A. 96, 7752–7755.

Unterwald, E.M., Rubenfeld, J.M., Imai, Y., Wang, J.B., Uhl, G.R., Kreek, M.J., 1995. Chronic opioid antagonist administration upregulates mu opioid receptor binding without altering mu opioid receptor mRNA levels. Brain Res. Mol. Brain Res. 33, 351–355.

Vanyukov, M.M., Moss, H.B., Gioio, A.E., Hughes, H.B., Kaplan, B.B.,

Tarter, R.E., 1998. An association between a microsatellite polymorphism at the DRD5 gene and the liability to substance abuse: pilot study. Behav. Genet. 28, 75–82.

Wang, X.M., Zhou, Y., Spangler, R., Ho, A., Han, J.S., Kreek, M.J., 1999. Acute intermittent morphine increases preprodynorphin and kappa opioid receptor mRNA levels in the rat brain. Brain Res. Mol. Brain Res. 66, 184–187.

White, F.J., 1998. Cocaine and the serotonin saga. Nature 393, 118–119.

Wickelgren, I., 1997. Getting the brain's attention. Science 278, 35–37.

Zhou, Y., Spangler, R., Maggos, C.E., LaForge, K.S., Ho, A., Kreek, M.J., 1996. Steady-state methadone in rats does not change mRNA levels of corticotropin-releasing factor, its pituitary receptor or proopiomelanocortin. Eur. J. Pharmacol. 315, 31–35.

Zhu, Y., King, M.A., Schuller, A.G., Nitsche, J.F., Reidl, M., Elde, R.P., Unterwald, E., Pasternak, G.W., Pintar, J.E., 1999. Retention of supraspinal delta-like analgesia and loss of morphine tolerance in δ opioid receptor knockout mice. Neuron 24, 243–252.