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The Opioid System in Alcohol and Drug Dependence: Family-Based Association Study

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Opioid receptors and their endogenous peptide ligands play important roles in neurotransmission and neuromodulation in response to addictive drugs such as heroin, cocaine, and alcohol. In an earlier study, we reported that variation in the genes encoding the κ-opioid receptor (*OPRK1*) and its peptide ligand (*PDYN*) were associated with the risk for alcoholism. We continued our investigation of the role of the opioid system in alcohol dependence by analyzing the genes encoding the μ- and δ-opioid receptors and their peptide ligands. We analyzed 18 *OPRM1* SNPs, 18 *OPRD1* SNPs, 7 *PENK* SNPs, and 7 *POMC* SNPs in a sample of 1923 European Americans from 219 multiplex alcohol dependent families. Employing a family-based test of association, we found no evidence that these four genes were significantly associated with alcohol dependence. We also did not find association between these genes and illicit drug dependence. Secondary analyses employing the narrower phenotype of opioid dependence (83 affected individuals) demonstrated association with SNPs in *PENK* and *POMC*, but not in *OPRM1* or *OPRD1*. Haplotype analyses provided further support for the association of *PENK* and *POMC* with opioid dependence. Therefore, our data provide no support for the idea that variations in *OPRM1*, *OPRD1*, *PENK* and *POMC* are associated with alcohol dependence or general illicit drug dependence, but variations in *PENK* and *POMC* appear to be associated with the narrower phenotype of opioid dependence in these families.

KEY WORDS: Alcoholism; μ- and δ-opioid receptors; endorphins; enkephalins; genetics

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INTRODUCTION

The endogenous opioid system plays a major role in addiction to opiates, cocaine and alcohol [Kreek, 1996, 2002]. There are three major groups of endogenous opioid peptides: endorphins, enkephalins, and dynorphins and neoendorphins. Each group shows selectivity for one or two of the three classes of opioid receptors, μ, δ and κ (MOR, DOR and KOR, respectively) [Reisine and Bell, 1993]. The ligands and receptors are expressed widely throughout the brain [Nakanishi et al., 1979; Comb et al., 1982; Kakidani et al., 1982; Khachaturian et al., 1985]. Both MOR and DOR have been implicated in the reinforcing effect of alcohol [Di Chiara et al., 1996; Koob et al., 2003] and appear to mediate alcohol-enhanced dopamine release in the ventral tegmental area [Herz, 1997]. Maintenance of basal release of dopamine in the nucleus accumbens relies on a balance between the stimulatory μ- and δ-opioid systems and the inhibitory κ-opioid system [Spanagel et al., 1992; Herz and Spanagel, 1995].

Several studies in mice have examined the effect of ablating different opioid receptors on alcohol preference. Compared to alcohol-preferring C57BL/6 mice, MOR-deficient mice have decreased ethanol self-administration [Roberts et al., 2000; Hall et al., 2001], while DOR-deficient mice show increased alcohol self-administration [Roberts et al., 2001]. β-endorphin deficient B6 mice, heterozygous for ablation of *POMC*, drink more ethanol than wild-type B6 mice; however, the mice with the homozygous deletion drink more than wild-type mice at an ethanol concentration of 7% but not at 10% [Grisel et al., 1999]. MOR and DOR expression is elevated in several brain regions of *PENK*-deficient mice [Clarke, et al., 2003], yet they show no difference in ethanol consumption or preference [Koenig and Olive, 2002; Hayward et al., 2004].

A coding variation of *OPRM1*, A118G (rs1799971; Asn40Asp), has been extensively studied because it alters amino acid 40 from asparagine to aspartate; the Asp40 form of MOR has been reported to bind the endogenous β-endorphin with three-fold greater affinity than the receptor containing Asn40 [Bond et al., 1998], although later studies did not confirm these results [Befort et al., 2001; Beyer et al., 2004]. Naltrexone, a non-selective opioid receptor antagonist, decreases ethanol consumption in human alcoholics [O'Malley et al., 1992; Volpicelli et al., 1992]. Alcohol-dependent individuals with one or two copies of the Asp40 allele who are treated with naltrexone have significantly lower rates of relapse and a longer time interval until they return to heavy drinking than do individuals lacking this allele [Oslin et al., 2003]. Testing for an association of this variant with alcoholism and other drug dependence has been inconclusive: both positive [Town et al., 1999; Szeto et al., 2001; Schinka et al., 2002; Shi et al., 2002; Luo et al., 2003; Tan et al., 2003; Bart et al., 2004; Kim et al., 2004; Bart et al., 2005; Drakenberg et al., 2006; Nishizawa et al., 2006; Zhang et al., 2006] and negative [Bergen et al., 1997; Sander et al., 1998; Gelernter

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et al., 1999; Gscheidel et al., 2000; Li et al., 2000; Franke et al., 2001; Crowley et al., 2003; Ide et al., 2004; Loh et al., 2004] results have been reported. A meta-analysis by Arias et al. [2006] came to a negative conclusion. Similar inconclusive association results have also been found with other variants of *OPRM1*, including C17T (rs1799972), a rare coding variation which changes amino acid 6 from alanine to valine [Bergen et al., 1997; Gelernter et al., 1999; Gscheidel et al., 2000; Li et al., 2000; Szeto et al., 2001; Shi et al., 2002; Crowley et al., 2003; Luo et al., 2003; Tan et al., 2003; Ide et al., 2004; Loh et al., 2004; Zhang et al., 2006].

A few studies have examined whether two coding variants in *OPRD1*, G80T (rs1042114) and T921C (rs2234918), are associated with alcohol and/or drug dependence. There was no evidence of association with the two SNPs in samples of heroin-dependent Chinese [Xu et al., 2002], alcohol-dependent Taiwanese Hans [Loh et al., 2004], or heroin- and alcohol-dependent Germans [Franke et al., 1999].

A CA repeat in the 5′ flanking region of *PENK* was not associated with alcohol dependence in populations of Asian, African-American, and Caucasian origin [Chan, et al., 1994]. However, an association was reported in a sample of non-Hispanic Caucasians with opiate dependence [Comings et al., 1999]. There has been no report of the association of *POMC* with alcohol or other drug dependence.

In a sample of multiplex alcoholic families, we previously reported the associations of *OPRK1* (encoding KOR) and *PDYN* (encoding its ligand, dynorphin) with alcohol dependence [Xuei et al., 2006]. Here, we extend our investigation in this same sample to the remaining opioid receptor genes, *OPRM1* and *OPRD1*, and the genes encoding their endogenous ligands, *POMC* and *PENK*. To reduce the likelihood of a false positive result due to population stratification, we employed a family-based test of association. We analyzed SNPs across each of the candidate genes to ensure adequate power to detect association.

MATERIALS AND METHODS

Sample and Phenotype

The Collaborative Study on the Genetics of Alcoholism (COGA) is a multi-site study recruiting families at six centers across the United States: Indiana University, State University of New York Downstate Medical Center, University of Connecticut, University of Iowa, University of California/San Diego, and Washington University, St. Louis. The institutional review boards of all participating institutions approved the study. The ascertainment and assessment of this sample has previously been described [Reich et al., 1998; Foroud et al., 2000]. Briefly, probands were identified through alcohol treatment programs; families with at least three first degree relatives with alcohol dependence participated in the genetic part of this study. A sample of 1923 European American individuals from 219 families was used in this study.

Phenotypic diagnoses were based on interview data from the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) [Bucholz et al., 1994; Hesselbrock et al., 1999]. Two primary phenotypes, alcohol dependence and illicit drug dependence, were examined for their association with the genotyped SNPs. To test for association with alcoholism, individuals were classified as affected if they met criteria for both DSM-IIIR alcohol dependence [American Psychiatric Association, 1987] and Feighner definite alcoholism [Feighner et al., 1972] (n = 870). Because a component of the genetic risk might be common to other drugs, individuals who did not meet criteria for alcoholism but did meet DSM-IIIR criteria for dependence on an illicit drug (marijuana, cocaine, stimulant, sedative or opioid) were included in the unknown group rather than the unaffected group (n = 221). To test for association with illicit drug dependence, individuals were classified as affected (n = 508) if they met DSM-IIIR criteria for dependence on any illicit drug (marijuana, cocaine, stimulant, sedative or opioid). Again, because of the potential shared genetic risk for alcoholism and illicit drug dependence, individuals who did not meet criteria for illicit drug dependence but did meet criteria for alcoholism were considered unknown. For both the alcoholism and illicit drug dependence phenotypes, individuals were considered unaffected only if they did not meet criteria for either alcoholism or illicit drug dependence (n = 832).

Because *OPRM1*, *OPRD1*, *PENK* and *POMC* are part of the opioid system, secondary analyses were performed to specifically test for an association between the genotyped SNPs and opioid dependence. In this model, individuals were considered affected if they met DSM-IIIR criteria for opioid dependence (n = 83). As in the other models, individuals were only considered unaffected if they did not meet criteria for either alcoholism or any other illicit drug dependence (n = 832).

SNP Selection and Genotyping

The most extensively studied isoform of *OPRM1* (L25119.1) contains four exons and spans 80 kb on chromosome 6q24 (Fig. 1A). *OPRD1* contains three exons and spans 51.5 kb on chromosome 1p36 (Fig. 2A). *PENK* contains two exons and spans 5 kb on chromosome 8q23 (Fig. 3A). *POMC* contains three exons and spans 8 kb on chromosome 2p23 (Fig. 4A). SNPs distributed throughout the genes were selected from public databases, primarily dbSNP (http://www.ncbi.nlm.nih.gov/SNP/). At the time SNPs were selected, allele frequencies were not usually available. To determine allele frequencies, SNPs were genotyped in two sets of samples, each consisting of 40 unrelated individuals from the Coriell European- and African-American diversity samples. SNPs with greater than 10% heterozygosity were preferentially genotyped; all genotyped SNPs were in Hardy–Weinberg equilibrium in both test populations. Most SNPs were located in non-coding regions of the genes. Key coding SNPs were also genotyped (Table I). Locations of the SNPs were determined from the annotations in the NCBI human genome assembly Build 35.1.

Genotyping was done using a modified single nucleotide extension reaction with allele detection by mass spectrometry (Sequenom MassArray system; Sequenom, San Diego, CA). The success rate of all genotypes was 97% or higher. All SNP genotypes were checked for Mendelian inheritance using the program PEDCHECK [O'Connell and Weeks, 1998]. Marker allele frequencies and heterozygosities were computed in the COGA sample using the program USERM13, part of the MENDEL linkage computer programs [Boehnke, 1991].

Statistical Analyses

To ensure that the SNP density was sufficient to evaluate the evidence of association between each of the genes and substance dependence, the program HAPLOVIEW [Barrett et al., 2005] was employed to examine the extent of linkage disequilibrium (LD) between pairs of SNPs as well as to determine the haplotype block structure within each gene. Blocks were defined using the criteria proposed by Gabriel [Gabriel et al., 2002]. Tagger [de Bakker et al., 2005] (http://www.broad.mit.edu/mpg/tagger/) was used to estimate how well the selected SNPs represented the genetic information contained in non-genotyped SNPs. We analyzed the extent to which the genotyped SNPs correlated with all SNPs of MAF $\geq$0.05 in the region. Because some of the SNPs we genotyped were not in the HapMap database and therefore were not evaluated by Tagger, this method underestimates the extent to which our genotyped SNPs also carry information on the non-genotyped variation in the gene.

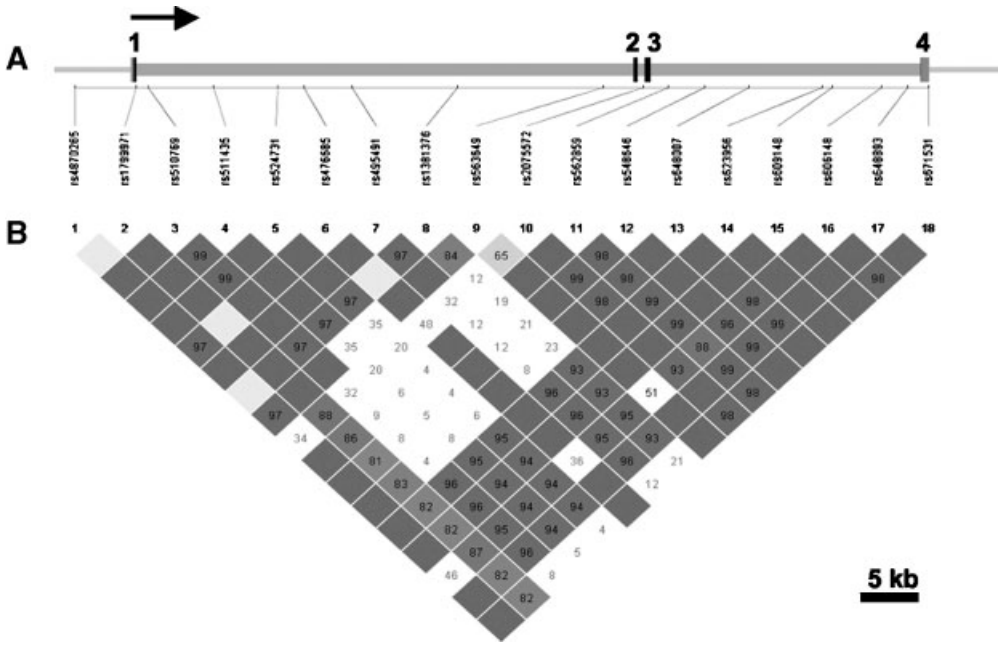

Fig. 1. (**A**) Gene structure of *OPRM1*. The gene structure is shown across the top of the figure, with SNPs positioned below. The exons are numbered, the untranslated regions are gray and the coding regions are black. The direction of transcription is represented by an arrow. The size of the gene is indicated at the lower right side. **(B)**. Pairwise linkage disequilibrium between the genotyped SNPs. Within each box is the pair-wise estimate of D′.

The Pedigree Disequilibrium Test (PDT) [Martin et al., 2000], as implemented in the program UNPHASED [Dudbridge, 2003], was used to test for association in the extended, multiplex COGA pedigrees. The PDT utilizes data from all available trios in a family, as well as from discordant sibships. Evidence for association is assessed based on the overtransmission of a particular allele to affected individuals, and the greater frequency of the allele in affected individuals as compared to their unaffected siblings. We report results from the "avg-PDT" statistic, which weighs each family equally in computing the overall test statistic [Martin et al., 2001].

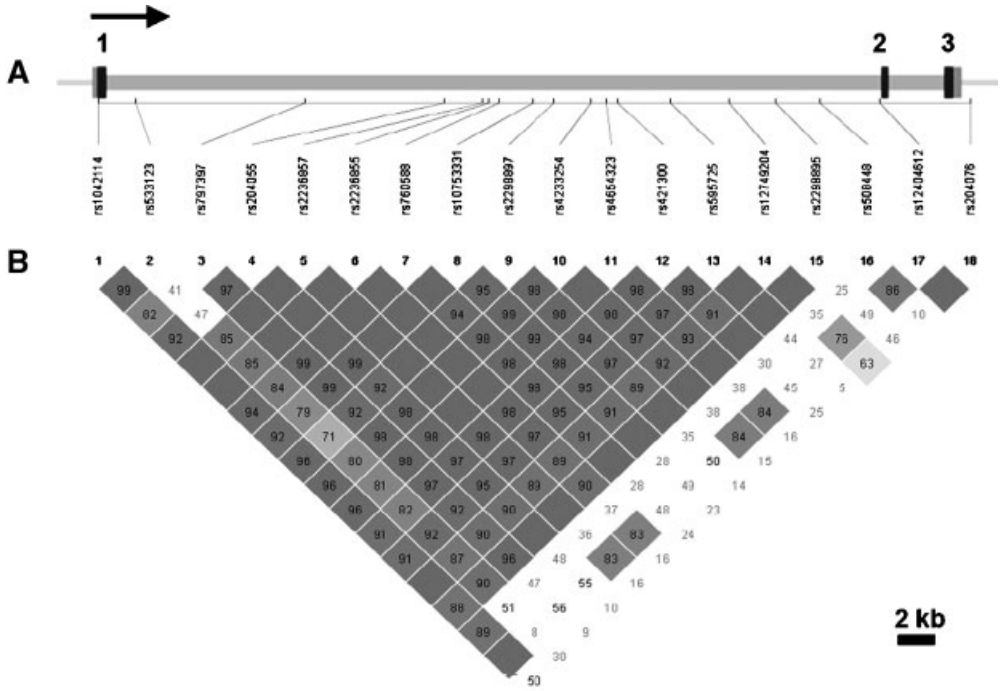

Fig. 2. **(A)** Gene structure of *OPRD1*. The gene structure is shown across the top of the figure, with SNPs positioned below; symbols as in Figure 1. **(B)** Pairwise linkage disequilibrium between the genotyped SNPs. Within each box is the pair-wise estimate of D′.

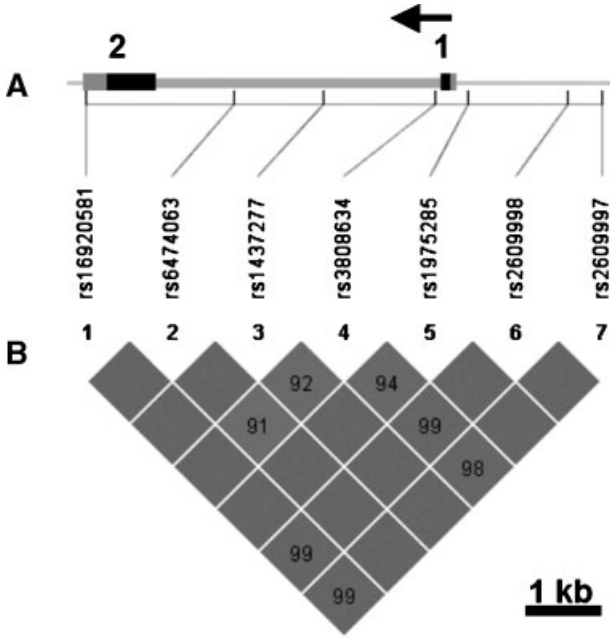

Fig. 3. **(A)** Gene structure of *PENK*. The gene structure is shown across the top of the figure, with SNPs positioned below; symbols as in Figure 1. **(B)** Pairwise linkage disequilibrium between the genotyped SNPs. Within each box is the pair-wise estimate of D′.

For each gene with significant ($P \leq 0.05$) SNP association results, haplotype analyses were performed to further examine the evidence of linkage. Haplotypes were constructed using consecutive sets of three adjacent SNPs [Edenberg et al., 2004]. The EM algorithm, as implemented in UNPHASED, was employed to estimate missing parental genotypes and thereby maximize haplotype estimation. Rare haplotypes (frequency $\leq$ 0.05), were not included in the haplotype-based association analyses.

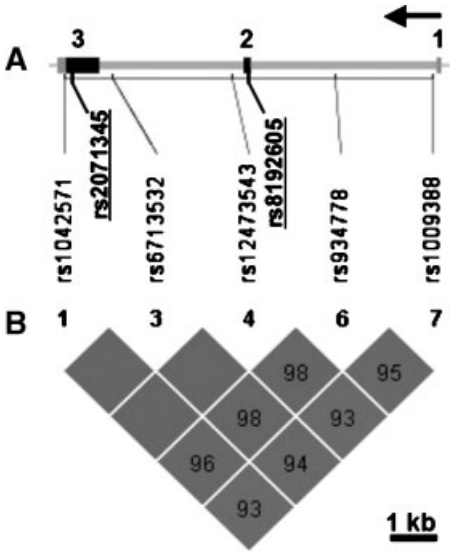

Fig. 4. **(A)** Gene structure of *POMC*. The gene structure is shown across the top of the figure, with SNPs positioned below; symbols as in Figure 1. **(B)** Pairwise linkage disequilibrium between the genotyped SNPs. Within each box is the pair-wise estimate of D′. The underlined SNPs are not included in the pairwise linkage disequilibrium analysis due to their low minor allele frequency (MAF/0.006).

RESULTS

Eighteen SNPs covering the *OPRM1* region were genotyped (Fig. 1A and B); there was substantial pair-wise linkage disequilibrium (LD) between adjacent markers ($0.65 \leq D' \leq 1.0$). Analysis employing the program Tagger [de Bakker et al., 2005] found that the 15 SNPs which could be analyzed in the HapMap database captured 85% of all SNPs in the region (MAF ≥ 0.05) at $r^2 > 0.5$ (mean $r^2 = 0.82$). Eighteen SNPs were genotyped in *OPRD1* (Fig. 2A and B); LD (D′) between adjacent markers (except between markers rs533123 and rs797397) ranged from 0.86 to 1.00. The 16 SNPs that could be evaluated in *OPRD1* captured 89% of the SNPs in the region at $r^2 > 0.8$ (mean $r^2 = 0.91$). Seven SNPs in *PENK* were genotyped (Fig. 3A and B); LD was nearly complete between adjacent markers ($0.92 \leq D' \leq 1.0$). The four SNPs which could be analyzed captured 100% of the SNPs in the region at $r^2 > 0.5$ with mean $r^2 = 1.0$. Seven SNPs in *POMC* were genotyped (Fig. 4A and B); two of the SNPs (rs2071345 and rs8192605, in the coding region but synonymous) had very low MAF (0.006 and 0.005, respectively) and were excluded from HAPLOVIEW analysis. The remaining five SNPs showed nearly complete LD between adjacent markers ($0.95 \leq D' \leq 1.0$); Tagger was unable to reliably estimate coverage in our data for *POMC* because only two SNPs were genotyped by HapMap. Thus, the SNPs analyzed (Table I) captured most of the variation in these four genes as would be required for a comprehensive evaluation of the association of each gene with alcoholism and/or illicit drug dependence. The patterns of LD seen in all the genes evaluated in our European-American sample were similar to those for Europeans in the HapMap database.

Our primary analyses focused on two phenotypes, alcoholism and illicit drug dependence. As shown in Table I, there was no consistent evidence of association in any of the four tested genes using either the phenotype of alcohol dependence or the phenotype of illicit drug dependence. Only one of the 50 markers analyzed reached nominal significance (*POMC*: rs934778; $P = 0.05$).

Because these genes encode the ligands and receptors of the opioid system, a secondary analysis was performed to test whether the narrower phenotype of opioid dependence was associated with any of the genes tested in this study (Table I). There was no evidence of association with either *OPRM1* or *OPRD1*. Three SNPs (rs1437277, rs1975285, and rs2609997) in the 5′ promoter region and intron one of *PENK* provided evidence of association ($P < 0.05$). Two SNPs in intron one of *POMC* (rs934778 and rs1009388) were also associated with opioid dependence ($P \leq 0.05$).

To further examine the evidence of association of *PENK* and *POMC* with opioid dependence, haplotype analyses were performed. *PENK* fell into a single haplotype block (Fig. 3B). There was consistent evidence of association across *PENK* when haplotypes constructed using consecutive sets of three adjacent SNPs were analyzed (four of the five analyses were significant; Table II).

POMC also falls within one haplotype block (Fig. 4B). Association of the 5-SNP haplotypes was significant (global $P = 0.046$), with one haplotype overtransmitted to opioid dependent individuals (G-T-T-G-C; $P = 0.02$). Since the last two SNPs (rs934776 and rs1009388) appeared to determine the significance of the association, a 2-SNP haplotype was analyzed and was significant (global $P = 0.03$) with haplotype G-C overtransmitted to opioid dependent individuals ($P = 0.02$; 1.6-fold overtransmission) and its opposite haplotype A-G

TABLE I. SNPs Analyzed

SNP_ID	Chr position[a]	SNP location	MAF[b]	Alcohol dependence[c]	Drug dependence[c]	Opioid dependence[c]
OPRM1						
rs4870265	154,446,651	upstream	0.03	0.61	0.89	0.90
rs1799971	154,452,911	exon 1, Asp40Asn	0.13	0.36	0.28	0.16
rs510769	154,454,133	intron 1	0.25	0.12	0.53	0.41
rs511435	154,460,661	intron 1	0.21	0.30	0.54	0.13
rs524731	154,467,206	intron 1	0.21	0.20	0.45	0.35
rs476685	154,469,855	intron 1	0.03	0.96	0.94	0.39
rs495491	154,474,656	intron 1	0.25	0.24	0.74	0.36
rs1381376	154,485,372	intron 1	0.18	0.39	0.73	0.20
rs563649	154,500,081	intron 1	0.08	0.44	0.79	0.15
rs2075572	154,504,118	intron 2	0.42	0.77	0.98	0.54
rs562859	154,506,687	intron 3	0.34	1.00	0.84	0.99
rs548646	154,510,261	intron 3	0.34	0.67	0.52	0.96
rs648007	154,514,725	intron 3	0.34	0.62	0.54	0.94
rs623956	154,522,139	intron 3	0.26	0.71	0.73	0.82
rs609148	154,523,128	intron 3	0.25	0.67	0.83	0.93
rs606148	154,528,100	intron 3	0.09	0.81	0.84	0.85
rs648893	154,530,742	intron 3	0.25	0.76	0.97	1.00
rs671531	154,532,855	downstream	0.35	0.83	0.78	0.83
OPRD1						
rs1042114	28,959,591	exon 1, Phe27Cys	0.14	0.62	0.93	0.82
rs533123	28,961,771	intron 1	0.21	0.40	0.85	0.97
rs797397	28,971,799	intron 1	0.48	0.46	0.23	0.77
rs204055	28,979,989	intron 1	0.49	0.37	0.31	0.95
rs2236857	28,982,225	intron 1	0.26	0.73	0.64	0.61
rs2236855	28,982,615	intron 1	0.26	0.75	0.60	0.61
rs760588	28,983,184	intron 1	0.33	0.60	0.64	0.94
rs10753331	28,985,198	intron 1	0.34	0.37	0.82	0.91
rs2298897	28,986,453	intron 1	0.27	0.53	0.49	0.49
rs4233254	28,988,670	intron 1	0.26	0.98	0.59	0.61
rs4654323	28,989,573	intron 1	0.26	1.00	0.59	0.61
rs421300	28,990,209	intron 1	0.34	0.81	0.66	0.94
rs595725	28,993,331	intron 1	0.46	0.98	0.81	0.57
rs12749204	28,996,829	intron 1	0.19	0.92	0.57	0.10
rs2298895	28,999,540	intron 1	0.06	0.42	0.30	0.90
rs508448	29,002,141	intron 1	0.46	0.08	0.12	0.80
rs12404612	29,005,709	intron 1	0.08	0.38	0.58	0.48
rs204076	29,011,006	downstream	0.40	0.74	0.32	0.68
PENK						
rs16920581	57,516,084	exon 2, 3'UTR	0.29	0.38	0.43	0.68
rs6474063	57,518,099	intron 1	0.24	0.48	0.32	0.07
rs1437277	57,519,288	intron 1	0.24	0.54	0.31	**0.03**
rs3808634	57,520,805	intron 1	0.30	0.09	0.24	0.49
rs1975285	57,521,236	upstream	0.24	0.58	0.29	**0.04**
rs2609998	57,522,588	upstream	0.44	0.13	0.26	0.06
rs2609997	57,523,039	upstream	0.44	0.15	0.17	**0.04**
POMC						
rs1042571	25,295,538	exon 3, 3'UTR	0.19	0.95	0.92	0.75
rs2071345	25,295,820	exon 3, Ala195	0.006	0.26	1.00	1.00
rs6713532	25,296,484	intron 2	0.24	0.79	0.84	0.81
rs12473543	25,298,832	intron 2	0.21	0.70	0.80	0.84
rs8192605	25,299,275	exon 2, Cys6	0.005	0.09	0.18	0.18
rs934778	25,300,875	intron 1	0.30	0.34	**0.05**	**0.04**
rs1009388	25,302,752	intron 1	0.27	0.46	0.29	**0.05**

[a]Chromosome positions are based on NCBI Human Genome Assembly v. 35.1.
[b]Minor allele frequency in European Americans.
[c]*P*-value of UNPHASED avg-PDT statistic for associations between the SNPs and alcohol, illicit drug, and opioid dependence, respectively. Significantly associated SNPs are shown in bold.

overtransmitted to the individuals who were not opioid dependent ($P = 0.04$).

DISCUSSION

This study reports the results of comprehensive analysis of SNPs covering the genes encoding the ligands and receptors of both the μ- and δ-opioid systems. Using a sample of 219 European-American families selected on the basis of multiple members diagnosed with alcohol dependence [Begleiter et al., 1995; Foroud et al., 2000; Reich et al., 1998], we have performed family-based association analysis with two primary phenotypes, alcohol and illicit drug dependence. We genotyped a sufficient number of SNPs to cover nearly all of the variation in each of these genes. We found no significant association

TABLE II. Association of *PENK* With Opioid Dependence Using 3-Consecutive-SNP Haplotype Analysis

3-SNP order	Opioid dependence (Global *P*-value)
rs16920581-rs6474063-rs1437277	0.02
rs6474063-rs1437277-rs3808634	0.02
rs1437277-rs3808634-rs1975285	0.02
rs3808634-rs1975285-rs2609998	0.18
rs1975285-rs2609998-rs2609997	0.04

between *OPRM1*, *OPRD1*, *PENK* or *POMC* and alcohol dependence. In contrast, previous studies of the same families demonstrated that genes encoding both the κ-opioid ligand (*PDYN*) and its receptor (*OPRK1)* were associated with the risk for alcoholism [Xuei et al., 2006]. In this group of COGA families, virtually all of the individuals with illicit drug dependence were also alcohol dependent. Therefore, given the association of the κ-opioid system with alcohol dependence, there was insufficient power to analyze whether illicit drug dependence was independently associated with either *PDYN* or *OPRK1*. In the current study there is no evidence of association between the genes in the μ- and δ-opioid systems and alcohol dependence; therefore, we can test for an effect of these genes on the risk for illicit drug dependence. We did not find significant association between *OPRM1*, *OPRD1*, *PENK* or *POMC* and illicit drug dependence.

Previous studies of the association of the A118G polymorphism in *OPRM1* (rs1799971) and alcoholism and other drug dependence have primarily employed a case control design. Results have been mixed. A118G has been associated with alcohol dependence in diverse ethnic populations including Japanese, Korean, Swedish, Russian and other Caucasian samples [Town et al., 1999; Luo et al., 2003; Kim et al., 2004; Bart et al., 2005; Nishizawa et al., 2006; Zhang et al., 2006]. However, other studies in populations of Southwest Indians, Jewish-, German-, Finnish-, and US-Caucasians have been negative [Bergen et al., 1997; Sander et al., 1998; Gelernter et al., 1999; Franke et al., 2001]. Franke et al. [2001] examined the A118G polymorphism in both case control and family (trio) studies in Germans with alcohol dependence and found no association; they also found no association with opioid dependence. The Franke et al. [2001] results are consistent with ours.

Zhang et al. [2006] examined 13 SNPs throughout the *OPRM1* region in case control studies with European-American and Russian populations and found that both haplotype blocks they identified were associated with alcohol and/or drug dependence. Although we identified the same two haplotype blocks in our study of European-American families, neither block provided evidence of association with either alcohol or illicit drug dependence ($P \geq 0.28$). Some of the discrepancies could be due to differences in the nature of the study populations. The COGA families were ascertained on the basis of alcohol dependent probands in treatment who had at least two alcohol dependent first degree relatives (meeting criteria for both DSMIII-R and Feighner diagnoses of alcohol dependence). Therefore, drug dependence is nearly always comorbid with alcohol dependence. Thus, the sample of drug dependent individuals, and more specifically those who were addicted to opioids, may represent a unique subset of individuals with drug dependence. In contrast, Zhang et al. ascertained cases who met criteria for lifetime diagnosis (using either DSM-IIIR or DSM-IV criteria) of alcohol (83%), cocaine or opioid dependence (52%; 35% were dependent on both alcohol and drugs) and controls who did not have substance dependence or major Axis I disorders; family history was not a criterion for recruitment.

Previous studies of two coding SNPs in *OPRD1,* G80T (rs1042114) and T921C (rs2234918), showed no significant association with alcohol dependence, heroin abuse or methamphetamine addiction [Franke et al., 1999; Xu et al., 2002; Loh et al., 2004; Kobayashi et al., 2006]. Our analysis, which included G80T and also covered nearly all the variation across this gene, are consistent with these results in showing no significant association of *OPRD1* with either alcohol or illicit drug dependence.

Secondary analyses of the narrower phenotype of opioid dependence, which utilized 83 affected individuals in family based analyses, did not find evidence of association with *OPRM1* or *OPRD1*. This result is consistent with many previous findings [Li et al., 2000; Franke et al., 2001; Schinka et al., 2002; Crowley et al., 2003; Ide et al., 2004; Arias et al., 2006], although some studies have found evidence of association of these genes with heroin dependence [Hoehe et al., 2000; Szeto et al., 2001; Bart et al., 2004; Drakenberg et al., 2006]. However, we did demonstrate consistent evidence of association with *PENK* and *POMC*. Our results are consistent with an association study of *PENK* with opiate dependence in a sample of non-Hispanic Caucasians [Comings et al., 1999]. Because our data on opioid dependence were based on a small sample of affected individuals within these families selected for alcohol dependence, further investigation is warranted to confirm these results.

To our knowledge, these analyses represent the most comprehensive assessment to date of the role of the opioid system with alcohol and illicit drug dependence. We have analyzed many SNPs across the genes encoding both receptors and ligands for the μ- and δ-opioid systems, covering most of the known variations in these genes. We have shown that in a large group of European-American families selected on the basis of multiple alcohol-dependent members, there is no association between variations in *OPRM1*, *OPRD1*, *PENK* and *POMC* and either alcohol dependence or dependence on illicit drugs in general. We have previously demonstrated that variations in the κ-opioid system, both the gene encoding the ligand (*PDYN*) and the κ-opioid receptor (*OPRK1*), are associated with alcoholism [Xuei et al., 2006]. In a secondary analysis, we did find evidence that *PENK* and *POMC* are associated with opioid dependence.

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