



## ORIGINAL ARTICLE

# Association of the $\kappa$ -opioid system with alcohol dependence

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**Opioid receptors and their endogenous peptide ligands play important roles in the reward and reinforcement of drugs such as heroin, cocaine, and alcohol. The binding of dynorphins to the  $\kappa$ -opioid receptor has been shown to produce aversive states, which may prevent the development of reinforcement. We genotyped SNPs throughout *OPRK1*, encoding the  $\kappa$ -opioid receptor, and *PDYN*, which encodes its ligand prodynorphin, in a group of 1860 European American individuals from 219 multiplex alcohol dependent families. Family-based analyses demonstrated associations between alcohol dependence and multiple SNPs in the promoter and 3' end of *PDYN*, and in intron 2 of *OPRK1*. Haplotype analyses further supported the association of *PDYN*. Thus, variations in the genes encoding both the  $\kappa$ -opioid receptor and its ligand, *OPRK1* and *PDYN*, are associated with the risk for alcohol dependence; this makes biological sense as variations in either should affect signaling through the  $\kappa$ -opioid system.**  
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## Introduction

Opioids function as neurotransmitters or neuro-modulators regulating many brain functions, including psychomotor stimulation, reward and reinforcement, mood and homeostatic adaptive processes like drinking, eating and thermoregulation.<sup>1–4</sup> The opioid system appears to play an important role in the reward system by influencing dopamine release in the nucleus accumbens.<sup>5</sup> There are three major groups of endogenous opioid peptides (dynorphins, endorphins and enkephalins) and three corresponding opioid receptors,  $\kappa$ -opioid receptor (KOR),  $\mu$ -opioid receptor (MOR) and  $\delta$ -opioid receptor (DOR).<sup>7</sup> Stimulation of MOR and DOR in mouse brain increases the release of dopamine in the nucleus accumbens, whereas stimulation of KOR reduces the release of dopamine and generates aversive states.<sup>8–11</sup>

Alcoholism (alcohol dependence) is a common complex genetic disease. Both twin and adoption studies show a strong heritable component involved in the risk for alcoholism.<sup>12–16</sup> The lack of a clear pattern of inheritance suggests that multiple genes contribute to the risk for alcoholism. Several genes have already been identified by the Collaborative Study on the Genetics of Alcoholism (COGA), including *GABRA2*,<sup>17,18</sup> *GABRG3*,<sup>19</sup> *CHRM2*,<sup>20,21</sup> and *hTAS2R16*.<sup>22</sup> Each of these genes has a modest effect on the risk for alcohol dependence and it appears likely that additional genes contribute to the genetic susceptibility for alcohol dependence.

There is evidence that the opioid system is involved in the vulnerability to various drug addictions, including alcoholism.<sup>23–25</sup> Naltrexone, a nonselective opioid receptor antagonist, decreases ethanol consumption in human alcoholics.<sup>26,27</sup> Naltrexone-treated alcohol-dependent subjects have lower levels of craving before and during ethanol self-administration compared to placebo-treated subjects.<sup>28</sup> Nonalcoholic offspring with a family history of alcohol dependence have greater adrenocorticotropin (ACTH) and cortisol responses to opioid receptor

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blockade induced by naloxone than do nonalcoholic offspring without a family history of alcohol dependence.<sup>29-31</sup> Treatment with buprenorphine, a partial agonist-antagonist of opioid receptors, in combination with naloxone, effectively reduces withdrawal symptoms in opioid-dependent individuals.<sup>32</sup> Evidence from animal models also suggests a possible relationship between the opioid system and alcohol preference.<sup>33-36</sup>

The dynorphin/KOR system may serve as a novel target for therapeutic treatment of alcohol and drug dependence. A selective KOR agonist U50,488H attenuates voluntary ethanol intake in rats.<sup>36</sup> Blocking the KOR in wild-type C57BL/6 mice with nor-binaltorphimine (nor-BNI), a  $\kappa$ -receptor-specific antagonist, increases alcohol self-administration.<sup>37</sup> Similarly, the endogenous KOR agonist dynorphin A (1-17) attenuates the cocaine-induced increases in dopamine levels in C57BL/6 mice brain, and this effect can be blocked by preinjection with the antagonist nor-BNI.<sup>38</sup> *OPRK1* knockout mice demonstrate an increased ethanol-evoked dopamine response in the nucleus accumbens<sup>37</sup> and show less oral alcohol self-administration than wild-type mice.<sup>39</sup>

The *OPRK1* gene is located on human chromosome 8. The 22 kb *OPRK1* gene contains four exons, including a newly identified exon that encodes the 5' untranslated region.<sup>40,41</sup> Yuferov *et al.*<sup>41</sup> suggested possible association of a coding SNP (G36T) in exon 2 with opiate addiction. Loh *et al.*<sup>42</sup> genotyped three *OPRK1* coding SNPs, G36T, C459T and A843G, in 307 Taiwanese Han subjects, but found no association between the SNPs and alcohol dependence.

Dynorphins are derived proteolytically from prodynorphin, which is encoded by *PDYN*.<sup>43</sup> Dynorphins bind selectively to the KOR, encoded by *OPRK1*.<sup>44-46</sup> The human *PDYN* gene contains four exons, covering 15.3 kb on chromosome 20. Variation in *PDYN* has been studied for its role in addiction to opiates, cocaine and alcohol. A 68-bp repeat variant located about 1.5 kb upstream of the promoter region was identified in the initial sequencing of the gene.<sup>43</sup> The level of gene expression appeared to be affected by the number of copies of the 68-bp repeats.<sup>47,48</sup> Longer alleles appear to increase gene expression while the shorter alleles appear to decrease expression. Longer alleles may be protective against cocaine addiction; alleles containing three or four copies of the repeat were more common in control subjects than in cocaine-addicted subjects.<sup>47</sup> Geijer *et al.*<sup>49</sup> identified a GC/AT base pair exchange 301 bp upstream of the exon 1/intron 1 boundary of *PDYN*, but found no association of the variant with alcoholism in a Scandinavian population.

Based on the biological evidence noted above, we hypothesized that genetic differences in the  $\kappa$ -opioid system may be linked to differences in the risk for alcoholism. The present study is designed to test that hypothesis in a large sample of families selected for the presence of multiple alcoholic members.

## 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 US: 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.<sup>50,51</sup> 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 1860 European American individuals from 219 alcoholic families was used in this study.

Phenotypic diagnoses were based on interview data from the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA).<sup>52,53</sup> Alcoholism was defined as meeting criteria for both DSM-III-R alcohol dependence<sup>54</sup> and Feighner definition of alcoholism.<sup>55</sup> As a component of the genetic risk might be common to other drugs, individuals who did not meet criteria for alcoholism but did meet criteria for dependence on an illicit drug (marijuana, cocaine, stimulant, sedative or opioid) were defined as unknown rather than unaffected.

### SNP selection and genotyping

SNPs distributed throughout the *OPRK1* and *PDYN* genes and extending to the 5' and 3' flanking regions 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 SNPs encoding *PDYN* and *OPRK1* coding variants and the *PDYN* promoter variants were genotyped regardless of their minor allele frequency (Tables 1 and 2). 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, USA). The success rate of all genotypes was 95% or higher. All SNP genotypes were checked for Mendelian inheritance using the program PEDCHECK.<sup>56</sup> Marker allele frequencies and heterozygosities were computed in the COGA sample using the program USERM13, part of the MENDEL

**Table 1** *PDYN* SNPs: minor allele frequency and association with alcohol dependence

SNP number	SNP_ID	Chromosome position <sup>a</sup>	SNP location <sup>b</sup>	MAF <sup>c</sup>	Alcohol dependence <sup>d</sup>
1	<b>rs6045784</b>	1 904 663	Downstream	0.11	<b>0.02</b>
2	<b>rs2235749</b>	1 907 939	3'UTR	0.27	<b>0.007</b>
3	<b>rs910080</b>	1 908 226	3'UTR	0.26	<b>0.02</b>
4	<b>rs10485703</b>	1 908 313	3'UTR	0.11	<b>0.03</b>
5	<b>rs6045819</b>	1 909 134	H200, exon 4	0.11	<b>0.007</b>
6	<b>rs6035222</b>	1 911 413	Intron 3	0.12	<b>0.01</b>
7	rs2179617	1 914 169	Intron 2	0.14	0.27
8	rs1418038	1 914 947	Intron 2	0.14	0.20
9	<b>rs6045868</b>	1 915 278	Intron 2	0.26	<b>0.03</b>
10	rs1883723	1 916 843	Intron 2	0.09	0.81
11	rs2235751	1 917 934	Intron 2	0.25	0.09
12	rs2235756	1 919 060	Intron 2	0.09	0.82
13	rs2281285	1 920 460	Intron 2	0.15	0.19
14	rs6045912	1 922 008	Intron 1	0.25	0.15
15	<b>rs1997794</b>	1 922 858	Promoter	0.36	<b>0.004</b>
16	rs3830064	1 923 679	Promoter	0.16	0.18
17	<b>rs10854244</b>	1 925 045	Upstream	0.25	<b>0.02</b>
18	rs6136667	1 926 301	Upstream	0.15	0.10

<sup>a</sup>Chromosome positions are based on NCBI Human Genome Assembly vs 35.1.

<sup>b</sup>The gene is transcribed in the opposite direction.

<sup>c</sup>Minor allele frequency in European Americans.

<sup>d</sup>P-value of UNPHASED avg-PDT statistic for association between the SNPs and alcohol dependence. Significantly associated SNPs are shown in bold.

**Table 2** *OPRK1* SNPs: minor allele frequency and association with alcohol dependence

SNP number	SNP_ID	Chromosome position <sup>a</sup>	SNP location <sup>b</sup>	MAF <sup>c</sup>	Alcohol dependence <sup>d</sup>
1	rs963549	54 304 377	3'UTR	0.14	0.46
2	rs702764	54 304 710	A281, exon 4	0.09	0.53
3	rs7815824	54 310 023	S153, exon 3	0.04	0.12
4	<b>rs997917</b>	54 314 931	Intron 2	0.27	<b>0.03</b>
5	<b>rs6473797</b>	54 315 535	Intron 2	0.22	<b>0.05</b>
6	<b>rs12548098</b>	54 318 008	Intron 2	0.16	<b>0.01</b>
7	rs16918931	54 318 029	Intron 2	0.08	0.08
8	rs7826614	54 319 979	Intron 2	0.16	0.08
9	<b>rs16918941</b>	54 323 255	Intron 2	0.08	<b>0.04</b>
10	<b>rs6985606</b>	54 323 669	Intron 2	0.49	<b>0.007</b>
11	rs6982096	54 323 978	Intron 2	0.08	0.66
12	rs1051660	54 326 115	P12, exon 2	0.08	0.25
13	rs3808627	54 327 355	Promoter	0.21	0.63

<sup>a</sup>Chromosome positions are based on NCBI Human Genome Assembly vs 35.1.

<sup>b</sup>The gene is transcribed in the opposite direction. The SNP location in the promoter, exon 1 and exon 2 of *OPRK1* is based on Yulifov *et al.*<sup>51</sup>

<sup>c</sup>Minor allele frequency in European Americans.

<sup>d</sup>P-value of UNPHASED avg-PDT statistic for association between the SNPs and alcohol dependence. Significantly associated SNPs are shown in bold.

linkage computer programs.<sup>57</sup> Only SNPs with a minimal allele frequency of 4% (Tables 1 and 2) were included in subsequent analyses.

#### Resequencing

To determine if there were any additional coding variations within the two genes, all exons of *OPRK1* and *PDYN* were sequenced in 16 individuals. For each gene, eight individuals with the high-risk

haplotype and eight individuals with the low-risk haplotype were chosen based on the tag SNPs from the most significant haplotype block of each gene; four of the individuals were sequenced for both genes.

Sequencing was performed using the ABI PRISM 3100 Genetic Analyzer capillary DNA sequencer with Big Dye chemistry (Applied Biosystems). The sequencing covered the exons plus approximately 200 bp flanking intronic regions on each side, plus 1.1–1.6 kb

upstream and 600–900 bp downstream. A total of 12 new SNPs were found, seven in *OPRK1* and five in *PDYN*. These were submitted to dbSNP (see Electronic-Database Information).

*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<sup>58</sup> was employed to examine the extent of linkage disequilibrium between pairs of SNPs as well as the haplotype block structure within each gene. Blocks were defined using the criteria proposed by Gabriel *et al.*<sup>59</sup> Tag SNPs were selected to capture haplotypes with frequency of  $\geq 5\%$ .<sup>60</sup>

The Pedigree Disequilibrium Test (PDT),<sup>61</sup> as implemented in the program UNPHASED,<sup>62</sup> 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 discordant sibships. Evidence for association is assessed based

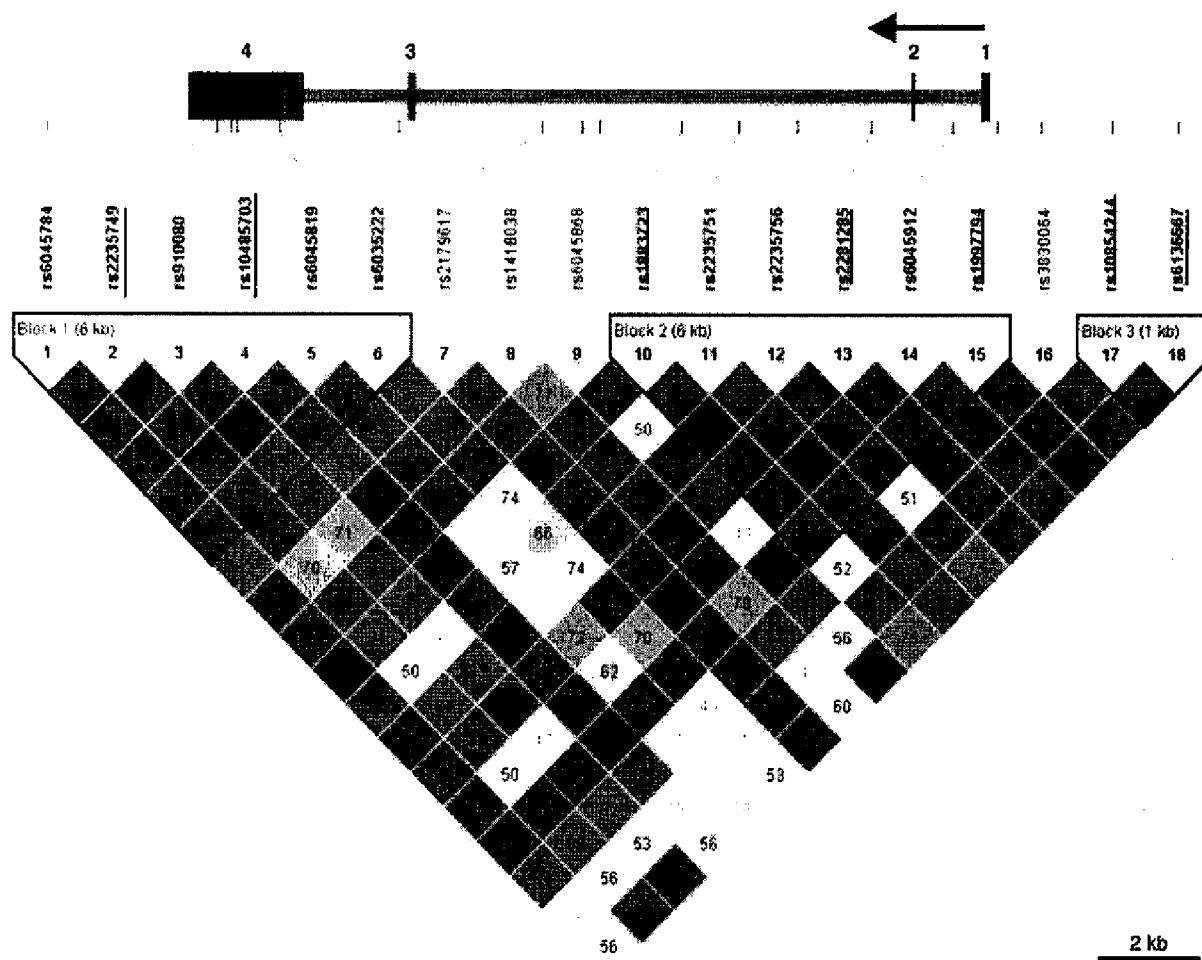
on overtransmission of a particular allele to affected individuals, and greater frequency of the allele in affected individuals as compared to their unaffected siblings. The ‘avg-PDT’ statistic, which averages the association statistic across all families, was used to test for association between the SNPs and alcohol dependence.<sup>63</sup>

Association results were compared to the SNP disequilibrium pattern of each gene. Haplotypes were constructed using consecutive sets of three adjacent SNPs<sup>17</sup> as well as employing the tag SNPs of the haplotype blocks containing SNPs associated with alcohol dependence. PDT analysis results using these two complementary haplotyping methods were compared to evaluate the pattern of association.

**Results**

*PDYN*

Eighteen SNPs were genotyped across *PDYN* from 4 kb upstream of the transcription initiation site to



**Figure 1** Gene structure of *PDYN* and linkage Disequilibrium ( $D'$ ) between the genotyped SNPs (HAPLOVIEW). The gene structure is across the top with the untranslated regions in blue/black, coding regions in red/gray, and exons as numbered. The direction of transcription is represented by an arrow. Tag SNPs for each haplotype block are underlined. The size of the gene is indicated at the lower right side.

3 kb downstream of the 3' end (Table 1; Figure 1). The extent of linkage disequilibrium as measured by  $D'$  between pairs of adjacent SNPs ranged from 0.77 to 1.00. The overall coverage for the gene was compared to the data in HapMap using Tagger.<sup>64</sup> Fifteen of the 18 SNPs showed an average  $r^2$  of 0.95 with 40 SNPs (MAF > 0.05) in a region of 30 kb; 88% of the HapMap alleles had  $r^2 > 0.8$  with at least one of our SNPs. Therefore, the association analyses of the selected SNPs on *PDYN* captured a very large fraction of untested variations for the gene and its immediate flanking regions. Three haplotype blocks were found (Figure 1); blocks 1 and 2 were similar to those defined by HapMap for the CEU population (www.HapMap.com). Block 3, identified in our data, appeared to be part of block 2 in the HapMap data.

There was significant evidence of association between alcohol dependence and multiple SNPs in *PDYN* (Table 1). Association was observed for multiple SNPs in a 7 kb region at the 3' end of the gene, including rs6045819, a synonymous variation in exon 4, and additional SNPs at the promoter region. The haplotype constructed from the two SNPs that tag block 1 (rs2235749 and rs10485703; Figure 1) provided further support for the association with alcohol dependence (global  $\chi^2 P = 0.008$ ). Haplotype block 2, tagged by rs1883723, rs2281285 and rs1997794, was also significantly associated (global  $P$ -value = 0.004). To further examine the association results, haplotypes were constructed using consecutive sets of 3-SNP sliding windows; five of six consecutive haplotypes ( $P \leq 0.05$ ) in the 3' region of the gene (Table 3)

**Table 3** *PDYN*: haplotype analysis of 3-SNP sliding window for association of alcohol dependence

3-SNP order	Alcohol dependence (global $P$ -value)	Overtransmitted haplotype to	
		Alcohol dependence	Nonalcohol dependence
SNP [1-3]	<b>0.015</b>	<b>CAG</b>	<b>TGA</b>
SNP [2-4]	<b>0.010</b>	<b>AGG</b>	<b>GAA</b>
SNP [3-5]	<b>0.044</b>	<b>GGG</b>	<b>AAA</b>
SNP [4-6]	<b>0.005</b>	<b>GGA</b>	<b>AAG</b>
SNP [5-7]	0.065	GAA	AGA*
SNP [6-8]	<b>0.027</b>	<b>AAC</b>	—
SNP [7-9]	0.062	ACA*	—
SNP [8-10]	0.09	CAT**	—
SNP [9-11]	0.08	—	—
SNP [10-12]	0.28	—	—
SNP [11-13]	0.37	—	—
SNP [12-14]	0.60	—	—
SNP [13-15]	<b>0.031</b>	<b>TGC</b>	<b>TGT</b>
SNP [14-16]	0.057	GCA	GTA
SNP [15-17]	0.074	CCT**	TAA
SNP [16-18]	0.30	—	—

Individual overtransmitted haplotypes for alcohol dependence are indicated in bold when  $P < 0.05$ ; \* $P = 0.06$ , and \*\* $P < 0.10$ .

supported association with alcohol dependence. The haplotype C-A-G-G-G-A (SNPs 1-6) was overtransmitted ( $P = 0.045$ ) to alcohol-dependent individuals, while its complementary haplotype T-G-A-A-A-G was overtransmitted ( $P = 0.038$ ) to nonalcohol-dependent individuals (global  $P = 0.075$ ).

Resequencing the 4 *PDYN* exons uncovered five new variations in the region (see Electronic-Database Information). However, none of the new variations altered amino-acid sequence.

#### *OPRK1*

Thirteen SNPs were genotyped across *OPRK1*, extending from 800 bp into the 5' promoter region to the 3' end (Table 2; Figure 2). Estimates of  $D'$  were > 0.89 for 92% of the adjacent SNPs. The overall coverage of the gene was compared to the data in HapMap using Tagger.<sup>64</sup> Twelve of the 13 SNPs showed an average  $r^2$  of 0.94 with 40 SNPs (MAF > 0.05) in a region of 30 kb; 95% of the HapMap alleles in the region had  $r^2 > 0.8$  with at least one of our SNPs. Thus, the selected SNPs also captured a very large fraction of untested variations contained within *OPRK1* and its promoter region. The *OPRK1* gene resides in two haplotype blocks, which encompassed 12 SNPs (Figure 2); SNP 5, rs6473797, was not included in either block. The haplotype block pattern was similar to that derived by HapMap for the CEU population (www.HapMap.com).

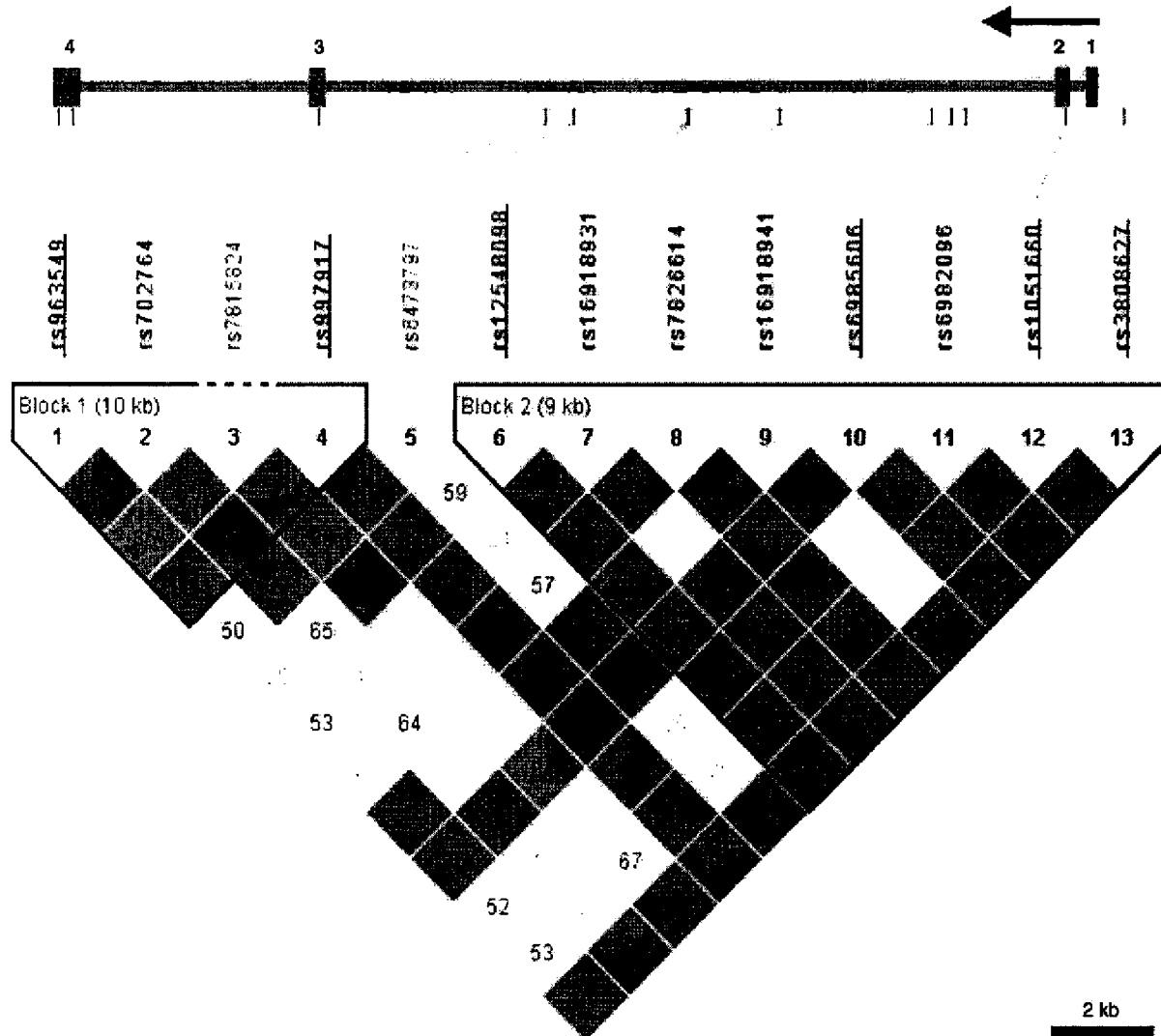
There was significant evidence of association between alcohol dependence and multiple SNPs across a 9 kb region located in intron 2 of *OPRK1* ( $P$ -value  $\leq 0.05$ ; Table 2). None of the three coding SNPs (rs702764, rs7815824, and rs1051660) demonstrated evidence of association ( $P > 0.11$ ). Haplotype analyses employing tag SNPs (Figure 2) were not significant, nor were haplotypes constructed with three consecutive SNPs.

Resequencing the four *OPRK1* exons uncovered seven new variations in the region (see Electronic-Database Information). None of the new variations altered amino-acid sequence. The only known coding SNP that alters amino-acid sequence (rs9282808; Asn374Asp) had zero allele frequency in a sample of 40 unrelated European-American samples from Coriell and was not genotyped.

#### Discussion

We have demonstrated in a large, family-based study of European-Americans that variations in both parts of the  $\kappa$ -opioid system, the *OPRK1* gene that encodes the receptor and the *PDYN* gene that encodes its ligand prodynorphin, are associated with alcohol dependence. The fact that both receptor and its ligand are associated makes biological sense, as variations in either should affect the overall level of signaling through the system.

For both *OPRK1* and *PDYN* the evidence for association was from SNPs that did not change the encoded protein (Tables 1 and 2). In neither case did



**Figure 2** Gene structure of *OPRK1* and linkage Disequilibrium ( $D'$ ) between the genotyped SNPs (HAPLOVIEW). The gene structure is across the top with the untranslated regions in blue/black, coding regions in red/gray, and exons as numbered. Exons 1 and 2 are based on Yuferov *et al.*<sup>41</sup> The direction of transcription is represented by an arrow. Tag SNPs for each haplotype block are underlined. The size of the gene is indicated at the lower right side.

resequencing of individuals with high- and low-risk haplotypes reveal additional coding variations, suggesting that it is differences in some aspect of gene regulation that contribute to the risk for alcoholism. The strongest association in *PDYN* came from SNPs in the 3' region, including exons 3 and 4, as well as two markers at the 5' promoter region. The strongest association in *OPRK1* was with multiple SNPs in intron 2. In *PDYN*, analyses of haplotypes provided additional consistent evidence of association with alcohol dependence. In contrast, haplotype analyses in *OPRK1* did not yield further support for association. There are several possible explanations for these differing results. For example, the variation(s) associated with alcohol dependence in *PDYN* may have a

single ancestral origin while the variation(s) in *OPRK1* associated with alcohol dependence may have multiple ancestral origins. In the former instance, haplotypic analyses would provide greater genetic information while in the latter case, they would not.

In a study among the Taiwanese Han population,<sup>42</sup> no association between alcoholism and *OPRK1* was detected; the study examined only the three synonymous variations in the coding region (G36T, rs1051660; C459T, rs7815824; A843G, rs702764). Our results for these three markers were also negative (Table 2). However, we hypothesized that regulatory variations were likely to influence complex genetic diseases, so we genotyped many more SNPs to ensure

coverage throughout *OPRK1*, including the promoter and intronic regions. We found the strongest association was in intron 2.

The variation GC/AT in the 5' promoter region of *PDYN*, reported by Geijer *et al.*,<sup>49</sup> which showed no evidence of association with alcoholism in the Scandinavian population, was significantly associated with alcoholism in our study (rs1997794;  $P=0.004$ ). Bakalkin *et al.*<sup>65</sup> described an 11-nucleotide consensus sequence for NF- $\kappa$ B (GGGGCTTCT) located in exon 4 of *PDYN*, within the coding sequence of the dynorphin peptide, and found that the expression of the gene was regulated by binding of NF- $\kappa$ B to the intragenic sequences. Interestingly, the synonymous marker rs6045819 in exon 4, which was significantly associated with alcohol dependence in our data ( $P=0.007$ ), was only 22 bp upstream of the  $\kappa$ B-element; the same SNP was also within several nucleotides of the initiating Met198 of an N-terminally truncated T2 transcript<sup>66</sup> that encodes dynorphin A and B.

In our sample, there were only 85 individuals who were dependent on one or more illicit drugs (cocaine, opiate, marijuana, stimulants or sedatives) but who did not meet criteria for alcohol dependence. Therefore, there was insufficient power to test the hypothesis that SNPs in these genes are associated with illicit drug dependence independent of the association observed with alcohol dependence. Adding these 85 individuals to the 869 alcohol-dependent individuals to analyze a phenotype of dependence on alcohol or illicit drugs would not substantially change the results because virtually all of the power in the sample would still be from the alcohol dependence phenotype, and therefore such an analysis would not effectively address issues of general drug dependence.

Dynorphin primarily modulates KOR responses, although it also acts through MOR in alcohol and drug addictions. Significantly higher basal levels of KOR mRNA were found in alcohol-avoiding mice (DBA/2) than in alcohol-preferring mice (C57BL/6) in septum and hypothalamus.<sup>67</sup> Similar results were also described in rats: the density of KOR in the ventromedial hypothalamus was much lower in alcohol-avoiding AA rats than in alcohol-preferring ANA rats.<sup>39</sup> Difference in basal amount of KOR mRNA could lead to differences in KOR activity and subsequently to behavioral differences between the strains of animals in alcohol preferences. The application of a KOR-specific agonist U50,488H dose-dependently decreased voluntary ethanol intake in AA rat.<sup>36</sup> A complete knockout of *OPRK1* significantly reduced alcohol preference and consumption in mice.<sup>39</sup>

Alteration of the  $\kappa$ -opioid system expression was further investigated in Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) mice,<sup>68</sup> using *in situ* hybridization (ISH) and KOR autoradiography. Ethanol withdrawal increased *PDYN* mRNA in multiple brain regions of WSP, but

not WSR mice;<sup>68</sup> basal KOR binding was higher in WSR mice than in WSP mice. Furthermore, increased KOR density was shown to be present during ethanol withdrawal in WSP mice.<sup>68</sup> Thus differences in the  $\kappa$ -opioid system might contribute to the selected differences in ethanol withdrawal severity. This evidence supports the role of differential expression of *PDYN* and its receptor *OPRK1* in alcohol dependence.

In summary, we have demonstrated that variations in the  $\kappa$ -opioid system, in genes encoding both the receptor (*OPRK1*) and its ligand (*PDYN*), are associated with risk for alcoholism. Association of variations in both receptor and its ligand makes biological sense, since both should affect the overall signaling. There is considerable biological evidence for the involvement of the  $\kappa$ -opioid system in alcohol preference, alcohol intake, and withdrawal. Genes encoding other opioid receptors and their endogenous ligands have also been implicated as playing a role in alcohol dependence, and we are in the process of conducting further genotyping and analysis to explore possible association of these genes.

#### Electronic-database information

Online Mendelian Inheritance in Man (OMIM):  
<http://www.ncbi.nlm.nih.gov/Omim>

dbSNP: <http://www.ncbi.nlm.nih.gov/SNP>

#### New SNPs submitted

<i>OPRK1</i>	ss# 49785917
	ss# 49785918
	ss# 49785919
	ss# 49785920
	ss# 49785921
	ss# 49785922
<i>PDYN</i>	ss# 49785912
	ss# 49785913
	ss# 49785914
	ss# 49785915
	ss# 49785916

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