A genetic association study of the mu opioid receptor and severe opioid dependence

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Objectives **Twin, family and adoption studies have suggested that vulnerability to opioid dependence may be a partially inherited trait (Cadoret *et al.*, 1986; Merikangas *et al.*, 1998; Tsuang *et al.*, 1998, 2001). Studies using animal models also support a role for genetic factors in opioid dependence, and point to a locus of major effect on mouse chromosome 10 (Berrettini *et al.*, 1994; Alexander *et al.*, 1996), which harbors the mu opioid receptor gene (*Mor1*) (Kozak *et al.*, 1994). The gene encoding the human mu opioid receptor (OPRM1) is thus an obvious candidate gene for contributing to opioid dependence. A recent report (Hoehe *et al.*, 2000) found a significant association between a specific combination of OPRM1 single nucleotide polymorphisms (SNPs) and substance dependence.**

Methods **In the current study, we genotyped 213 subjects with severe opioid dependence (89 African-Americans, 124 European-Americans) and 196 carefully screened 'supercontrol' subjects (96 African-Americans, 100 European-Americans) at five SNPs residing in the OPRM1 gene. The polymorphisms include three in the promoter region ($T_{-1793}A$, $_{-1699}T$ insertion and $A_{-1320}G$) and two in exon 1 ($C_{+17}T$ [Ala6Val] and $A_{+118}G$ [Asp40Asn]).**

Results **Statistical analysis of the allele frequency differences between opioid-dependent and control subjects for each of the polymorphisms studied yielded P values in the range of 0.444–1.000. Haplotype analysis failed to identify any specific combination of SNPs associated with the phenotype.**

Conclusions **Despite reasonable statistical power we found no evidence of association between the five mu opioid receptor polymorphisms studied and severe opioid dependence in our sample. There were, however, significant allele frequency differences between African-Americans and European-Americans for all five polymorphisms, irrespective of drug-dependent status. Linkage disequilibrium analysis of the African-American genotypes indicated linkage disequilibrium ($P<0.0001$) across the five-polymorphism, 1911 base pair region. In addition, only four haplotypes of these five polymorphisms are predicted to exist in African-Americans. *Psychiatr Genet* 13:169–173**

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Introduction

The mu opioid receptor is the molecular target for endogenous opioid peptides and several exogenous opioid drugs, including morphine. The mu opioid receptor mediates several properties of morphine, including the rewarding effects and the development of tolerance and dependence (Matthes *et al.*, 1996). It also seems to be involved in the reinforcing effects of non-opioid drugs, including alcohol and cocaine (Kreek, 1996). Sequence variability in the gene encoding the human mu opioid receptor (OPRM1) may create a receptor with altered expression, structure or function, and as a consequence may increase or decrease an individual's susceptibility to substance dependence (Lichtermann *et al.*, 2000). Several case–control studies have investigated associations between OPRM1 sequence variability and substance dependence, with inconsistent results. Most of these studies have focused on two polymorphisms found in OPRM1 exon 1 that alter amino acid sequence, $A_{+118}G$ (Asn40Asp) and $C_{+17}T$ (Ala6Val).

The $A_{+118}G$ polymorphism is of particular interest since functional effects of the $A_{+118}G$ polymorphism have been demonstrated both *in vitro* and *in vivo*. Bond *et al.*

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(1998) showed that, in cell culture, mu opioid receptors coded by the G (Asp) variant bind beta-endorphin and activate inwardly-rectifying potassium channels with three times greater potency than receptors coded by the A (Asn) variant. Wand *et al.* (2002) found that individuals heterozygous for $A_{+118}G$ possessed altered HPA axis activation induced by opioid receptor blockade, while Smolka *et al.* (1999) showed that heterozygous individuals display greater dopaminergic sensitivity during acute alcohol withdrawal.

The $A_{+118}G$ polymorphism has been investigated in at least 10 substance dependence association studies, with mixed results. One group found a significant association between the G allele of $A_{+118}G$ and heroin addiction in a Chinese population (Szeto *et al.*, 2001), while another found a trend toward an increased G allele frequency in Caucasian alcoholics (Rommelspacher *et al.*, 2001). However, five similar studies have found no association between this polymorphism and opioid dependence (in Caucasians, Franke *et al.*, 2001), alcohol dependence (in Caucasians and Southwest American Indians, Bergen *et al.*, 1997; in Caucasians, Sander *et al.*, 1998; in Caucasians, Franke *et al.*, 2001), and alcohol and drug dependence (in European-Americans, African-Americans and Hispanics, Gelernter *et al.*, 1999). Furthermore, three additional reports have suggested that the G allele is actually protective against dependence upon alcohol and other substances (in Caucasians, Town *et al.*, 1999; Schinka *et al.*, 2002), and opioid dependence (in Hispanics but not in European-Americans and African-Americans, Bond *et al.*, 1998). These $A_{+118}G$ association studies have a range of total sample size ($n = 152$–1059) and the size of specific ethnic groups also varies considerably across studies.

Three groups have reported a trend toward an increased T allele frequency at the $C_{+17}T$ polymorphism in populations with substance dependence using case–control methods. A positive trend ($P = 0.05$) for increased T allele frequency was found in a pooled sample of European-American and African-American cocaine-dependent and/or opioid-dependent individuals (Berrettini *et al.*, 1997). Bond *et al.* (1998) also reported a marginal significance ($P = 0.054$) for increased T allele frequency in a combined sample of opioid-dependent European-Americans, African-Americans and Hispanics. Rommelspacher *et al.* (2001) found a similar trend ($P = 0.07$) when examining alcohol-dependent individuals of German descent. One additional case–control study failed to find this trend. Gelernter *et al.* (1999) compared European-Americans, African-Americans and Hispanics with a primary diagnosis of drug dependence or alcohol dependence, and found no evidence for an elevated T allele frequency in any ethnic group. These $C_{+17}T$ association studies also have a broad range of total sample sizes ($n = 106$–891) as well as considerable size variation of ethnic subsets.

The $A_{+118}G$ and $C_{+17}T$ exon 1 single nucleotide polymorphisms (SNPs) represent just a small fraction of OPRM1 sequence variability. A recent study identified 43 sequence variants in OPRM1 by sequencing all known functionally relevant regions of the gene in 172 African-American individuals (Hoehe *et al.*, 2000). Haplotype analysis and similarity clustering revealed a characteristic pattern of sequence variants ($T_{-1793}A$, $_{-1699}T$ insertion, $A_{-1320}G$, $C_{-111}T$ and $C_{+17}T$) significantly more frequent in substance-dependent individuals compared with matched controls. A recent report (Kraus *et al.*, 2001) has demonstrated that the region containing the $T_{-1793}A$, $_{-1699}T$ insertion and $A_{-1320}G$ SNPs functions as a promoter for the human OPRM1 gene.

In the current study, we further investigate this finding by genotyping the $T_{-1793}A$, $_{-1699}T$ insertion, $A_{-1320}G$, $C_{+17}T$ and $A_{+118}G$ SNPs in individuals with severe opioid dependence marked by early age of onset and a positive family history of substance dependence. The genotypes of the opioid-dependent subjects were compared with a 'supercontrol' group defined as individuals with no personal or family history of substance dependence.

Methods

Subjects

Individuals from university-affiliated residential and non-residential addiction treatment programs were invited to participate in the study if an extensive history of substance dependence, particularly opioid dependence, was obtained during a preliminary medical interview. The study protocol was approved by the institutional review boards (IRB) of the University of Pennsylvania and Thomas Jefferson University. After obtaining written informed consents, individuals who elected to participate had modified Research Diagnostic Criteria diagnoses of opioid dependence established from medical records and urine drug screens by a Board Certified addiction psychiatrist. Individuals with neurological disorders, schizophrenia or bipolar disorders (by medical record) were excluded. Individuals with unipolar mood disorders or anxiety disorders were not excluded. All participants had serious multi-year drug dependence with an age of onset younger than 20 years and had at least one first-degree relative with a diagnosis of substance dependence by family history. Although a primary drug dependence on opioids was established for each subject, many patients fulfilled diagnostic criteria for abuse of multiple substances, including cocaine, nicotine and alcohol. Ethnic groups (89 African-Americans, 124 European Americans) were determined by self-identification. This clinical sample is distinct from a substance-dependent sample previously studied by our group (Berrettini *et al.*, 1997).

Control subjects (96 African-Americans, 100 European-Americans) were recruited from those responding to advertisements. Controls were matched by gender and ethnic background to the drug-dependent group. A SAD-L interview and systematic family history was obtained from each control subject. Individuals were excluded from the control group if they had a personal or first-degree relative history of drug abuse, addiction or alcoholism. Individuals who used nicotine chronically or caffeine were not excluded. Individuals with a major psychiatric disorder (schizophrenia, unipolar or bipolar illness) were excluded.

Twenty milliliters of ethylenediamine tetraacetic acid-treated venous blood was obtained for DNA extraction from cases and controls.

Genotyping

Genomic DNA was extracted from blood samples by standard methods (Lahiri and Schnabel, 1993). The $C_{+17}T$ and $A_{+118}G$ SNPs were genotyped using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method of Gelernter *et al.* (1999). The $T_{-1793}A$, $_{-1699}T$ insertion and $A_{-1320}G$ polymorphisms were genotyped by pyrosequencing (Ahmadian *et al.*, 2000), a non-electrophoretic sequencing system based on luminometric detection of pyrophosphate released upon nucleotide incorporation. The following pyrosequencing strategy was used: oligonucleotide primers (forward, 5′-GAGAATAGATGAACAGCAAG-3′; reverse, 5′-GTTCCTTTGTGCTTAGTT-3′) were used to amplify a 409 base pair fragment encompassing the $T_{-1793}A$ and $_{-1699}T$ insertion polymorphisms; a second set of primers (forward, 5′-CTCCCTGCTCCCTGAAAT-3′; reverse, 5′-CGTTTTTTGAATTTTATACCTTACT-3′) amplified a 243 base pair fragment containing the $A_{-1320}G$ SNP. The forward PCR primers were biotinylated on the 5′ end to allow capture of amplified product with streptavidin-coated beads. The PCR reaction conditions comprised an initial denaturing step at 94°C for 5 min, followed by 45 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 3 min. Polymorphisms were detected using a PSQ 96 pyrosequencing instrument and PSQ 96 SNP Reagent Kit (Pyrosequencing, Uppsala, Sweden; www.pyrosequencing.com) according to the manufacturer's instructions. Sequencing primers specific to each SNP were as follows: $T_{-1793}A$, 5′-GCCTACCCTCGCCTT-3′; $_{-1699}T$ insertion, 5′-AAGCTGATTTATAAAATGATT-3′; $A_{-1320}G$, 5′-AACATTGAAAATACATGTC-3′.

Statistical analysis

Allele frequency comparisons and agreement with Hardy–Weinberg equilibrium were tested by chi-squared analysis. Linkage disequilibrium analysis was performed using the EH program (Terwilliger and Ott, 1994). Statistical power was calculated (Schlesselman, 1982) under a dominant model (Sullivan *et al.*, 2001) with relative risk set to 2.5 and a significance level of 0.05 (two-sided test).

The NCBI accession numbers used were AJ000341 (OPRM1 promoter) and NM000914 (OPRM1 cDNA).

Results

In this clinical sample of severe opioid-dependent cases and highly screened supercontrols, no association was detected between any of the OPRM1 polymorphisms and opioid dependence (Table 1). The results do, however, indicate significant allele frequency differences between African-American and European-American subjects at each of these loci ($\chi^2 = 24.8$–44.5, degrees of freedom = 1, $P < 0.0001$). All SNPs are in Hardy–Weinberg equilibrium. The statistical power to detect associations in the African-American sample was 0.79 for the $T_{-1793}A$, $_{-1699}T$ insertion and $A_{-1320}G$ SNPs, 0.98 for the $C_{+17}T$ and 0.58 for the $A_{+118}G$ variant. In the European-American sample, the $A_{+118}G$ SNP had a power of 0.96 while the other SNPs had only minimal power due to the low minor allele frequencies.

Linkage disequilibrium analysis of the African-American genotypes using the EH program (Terwilliger and Ott, 1994) indicated linkage disequilibrium ($P < 0.0001$) across the five-polymorphism, 1911 base pair region. Pairwise analysis (Devlin and Risch, 1995) using predicted haplotypes indicated strong linkage disequilibrium between all adjacent SNP markers (see Figure 1). The four predicted haplotypes consist of chromosomes with the following alleles of $T_{-1793}A$, $_{-1699}T$ insertion, $A_{-1320}G$, $C_{+17}T$ and $A_{+118}G$, respectively: T_{-1793}, $_{-1699}$no insertion, A_{-1320}, C_{+17}, A_{+118} (all common alleles); T_{-1793}, $_{-1699}$no insertion, A_{-1320}, C_{+17}, $_{+118}G$ (rare allele at $A_{+118}G$); T_{-1793}, $_{-1699}$no insertion, A_{-1320}, $_{+17}T$, A_{+118} (rare allele at $C_{+17}T$); $_{-1793}A$, $_{-1699}T$ insertion, $_{-1320}G$, $_{+17}T$, A_{+118} (common allele at $A_{+118}G$). Linkage disequilibrium determination could not be performed with the European-American genotypes because only one of the five loci had a minor allele frequency $> 1\%$.

Discussion

The allele frequencies and ethnic differences displayed for the $A_{+118}G$ SNP are in good agreement with previous studies of this polymorphism. For the other four SNPs, however, some discrepancies with the literature were detected. In this study, significant ethnic differences were seen for the $C_{+17}T$ polymorphism (African-American, 20% minor allele frequency; European-American, 1%), agreeing closely with several previously published reports (Bergen *et al.*, 1997; Bond *et al.*, 1998; Gelernter *et al.*, 1999; Hoehe *et al.*, 2000; Rommelspacher *et al.*, 2001). Berrettini *et al.* (1997) found no evidence of allele frequency differences for $C_{+17}T$ between African-

Table 1 Allele frequencies of human mu opioid receptor gene (OPRM1) variants in African-American and European-American cases and controls

Polymorphism	Ethnicity	Group	Genotypes			Alleles		Minor allele frequency (%)	χ^{2a}	*P* value
$T_{-1793}A$	AA	Case	77 T/T	15 T/A	1 A/A	169 T	17A	9.1	0.006	1.000
		Control	81 T/T	13 T/A	3 A/A	175 T	19A	9.8		
	EA	Case	124 T/T	0 T/A	0 A/A	248 T	0A	0.0	0.000	1.000
		Control	101 T/T	0 T/A	0 A/A	202 T	0A	0.0		
$_{-1699}$T Ins	AA	Case	73–/–	15+/–	1+/+	161 –	17+	9.6	0.006	1.000
		Control	80 –/–	13+/–	3+/+	173 –	19+	9.9		
	EA	Case	123 –/–	2+/–	0+/+	248 –	2+	0.8	0.156	1.000
		Control	99 –/–	1+/–	0+/+	199 –	1+	0.5		
$A_{-1320}G$	AA	Case	81 A/A	13 A/G	2 G/G	175 A	17G	8.9	0.006	1.000
		Control	82 A/A	13 A/G	3 G/G	177 A	19G	9.7		
	EA	Case	129 A/A	0 A/G	0G/G	258 A	0A	0.0	1.243	0.444
		Control	100 A/A	1 A/G	0G/G	201 A	1G	0.5		
$C_{+17}T$	AA	Case	62 C/C	31 C/T	3 T/T	155 C	37T	19.3	0.008	1.000
		Control	65 C/C	27 C/T	7 T/T	157 C	41T	20.7		
	EA	Case	126 C/C	3 C/T	0 T/T	255 C	3T	1.2	0.630	0.629
		Control	100 C/C	1 C/T	0 T/T	201 C	1T	0.5		
$A_{+118}G$	AA	Case	87 A/A	9 A/G	0 G/G	183 A	9G	4.7	0.084	0.790
		Control	90 A/A	8 A/G	1 G/G	188 A	10G	5.1		
	EA	Case	90 A/A	38 A/G	1 G/G	218 A	40G	15.5	0.084	0.791
		Control	71 A/A	29 A/G	1 G/G	171 A	31G	15.3		

[a]Chi-squared values are for allele frequency comparisons. AA, African-American. EA, European-American. Ins, insertion.

Fig. 1

Pairwise linkage disequilibrium (D′) was calculated between adjacent single nucleotide polymorphisms using predicted haplotypes of the African-American cases and controls. D′ ranges from 1 to 0, with 1 indicating complete linkage disequilibrium and 0 representing free association. Marker positions are given relative to the translation start site.

Americans and European-Americans, perhaps due to the single-strand conformational polymorphism (SSCP) genotyping method used in this study, in which the $C_{+17}T$ variant causes a gel shift similar to the shift associated with the $A_{+118}G$ variant. Thus, in Berrettini *et al.* (1997), the two genotypes might have been confounded, since the amplicon genotyped by SSCP analysis contained both the $C_{+17}T$ and the $A_{+118}G$ SNPs.

In our study, the $T_{-1793}A$, $_{-1699}T$ insertion and $A_{-1320}G$ SNPs showed minor allele frequencies of 8.9–9.9% in African-Americans with no differences between cases and controls. Furthermore, we found no haplotype that distinguished cases from controls. In contrast, Hoehe *et al.* (2000) identified a haplotype that differed between cases and controls in African-Americans.

The statistical procedures used by Hoehe *et al.* (2000) to define haplotypes that may increase risk for opioid dependence are similar to a discriminant function analysis, in that the haplotypes were merged with a goal of maximizing differences between affected individuals and controls. As with other discriminant function analyses, the results must be tested in a second population to prove validity. Our results suggest that the putative opioid-dependence risk haplotypes nominated by Hoehe *et al.* (2000) are not influencing susceptibility to risk in our sample.

We have demonstrated linkage disequilibrium between all five of these polymorphisms in African-American individuals and found that only four haplotypes of these SNPs (none of which associated with the disorder) exist in African-Americans. As described in Results, the minor alleles of $T_{-1793}A$, $_{-1699}T$ insertion, $A_{-1320}G$ and $C_{+17}T$ are not predicted to reside on the same chromosome (haplotype) with the minor allele of $A_{+118}G$. This may indicate that the $A_{+118}G$ SNP arose independently at a separate evolutionary time and so is not in the same haplotypes with the other variants examined.

The previous studies of the $A_{+118}G$ and $C_{+17}T$ OPRM1 polymorphisms have yielded promising, yet mixed, results. A total of eight out of 14 reports, including one by our group (Berrettini *et al.*, 1997), have indicated a positive association, or a trend towards a positive association, between one of these SNPs and substance dependence. Failure to replicate a case–control study result can occur for several reasons, including chance, population stratification, different definition of 'cases' and an improper control group (Sullivan *et al.*, 2001). The strength of this study was its attempt to maximize the inclusion of cases with highly heritable forms of opioid dependence (by requiring age of onset younger than 20 years and at least one first-degree relative with a history of substance dependence) and highly screened 'super-controls' lacking such genetic predisposition (by requiring no personal or family history of substance dependence). One limitation of this study was its exclusion of many other SNPs present in OPRM1, the inclusion of which would have allowed a more powerful haplotype analysis (Hoehe *et al.*, 2000). In addition, a more robust sample size would have yielded greater power to detect allele frequency differences.

In conclusion, despite reasonable statistical power, we found no association between five mu opioid receptor gene polymorphisms and opioid dependence. In addition, we have demonstrated linkage disequilibrium between all five of these polymorphisms in African-American individuals and found that only four haplotypes of these SNPs exist in African-Americans. Future association studies of opioid dependence might benefit from genotyping SNPs in numerous genes involved in opioid pharmacology (e.g. genes for beta-endorphin, PKA, CREB, and cytochrome P450 2D6). This approach would take into account the multigenic nature of the disease and may help to identify combinations of variants associated with the phenotype.

References

Ahmadian A, Gharizadeh B, Gustafsson AC, Sterky F, Nyrén P, Uhlén M, Lundeberg J (2000). Single-nucleotide polymorphism analysis by pyrosequencing. *Anal Biochem* **280**:103–110.

Alexander RC, Heydt D, Ferraro TN, Vogel WH, Berrettini WH (1996). Further evidence for a quantitative trait locus on murine chromosome 10 controlling morphine preference in inbred mice. *Psychiatr Genet* **6**:29–31.

Bergen AW, Kokoszka J, Peterson R, Long JC, Virkkunen M, Linnoila M, Goldman D (1997). Mu-opioid receptor gene variants: lack of association with alcohol dependence. *Mol Psychiatry* **2**:490–494.

Berrettini WH, Ferraro TN, Alexander RC, Buchberg AM, Vogel WH (1994). Quantitative trait loci mapping of three loci controlling morphine preference using inbred mouse strains. *Nat Genet* **7**:54–57.

Berrettini WH, Hoehe MR, Ferraro TN, DeMaria PA, Gottheil E (1997). Human mu opioid receptor gene polymorphisms and vulnerability to substance abuse. *Addict Biol* **2**:303–308.

Bond C, LaForge KS, Tian MT, Melia D, Zhang SW, Borg L, *et al.* (1998). Single-nucleotide polymorphism in the human mu-opioid receptor gene alters beta-endorphin binding and activity: possible implications for opioid addiction. *Proc Natl Acad Sci USA* **95**:9608–9613.

Cadoret TJ, Troughton E, O'Gorman TW, Heywood E (1986). An adoption study of genetic and environmental factors in drug abuse. *Arch Gen Psychiatry* **43**:1131–1136.

Devlin B, Risch N (1995). A comparison of linkage disequilibrium measures for fine-scale mapping. *Genomics* **29**:311–322.

Franke P, Wang T, Nöthen MM, Knapp M, Neidt H, Albrecht S, *et al.* (2001). Nonreplication of association between mu-opioid-receptor gene (OPRM1) A118G polymorphism and substance dependence. *Am J Med Genet* **105**:114–119.

Gelernter J, Kranzler H, Cubells J (1999). Genetics of two mu opioid receptor gene (OPRM1) exon 1 polymorphisms: population studies, and allele frequencies in alcohol- and drug-dependent subjects. *Mol Psychiatry* **4**:476–483.

Hoehe MR, Köpke K, Wendel B, Rohde K, Flachmeier C, Kidd KK, *et al.* (2000). Sequence variability and candidate gene analysis in complex disease: association of mu opioid receptor gene variation with substance dependence. *Hum Mol Genet* **9**:2895–2908.

Kozak C, Filie J, Adamson C, Chen Y, Yu L (1994). Murine chromosomal location of the mu and kappa opioid receptor genes. *Genomics* **21**:659–661.

Kraus J, Borner C, Giannini E, Hickfang K, Braun H, Mayer P, *et al.* (2001). Regulation of mu opioid receptor gene transcription by interleukin-4 and influence of an allelic variation within a STAT6 transcription factor binding site. *J Biol Chem* **276**:43901–43908.

Kreek MJ (1996). Opiates, opioids and addiction. *Mol Psychiatry* **1**:232–254.

Lahiri DK, Schnabel B (1993). DNA isolation by a rapid method from human blood samples: effects of $MgCl_2$, EDTA, storage time, and temperature on DNA yield and quality. *Biochem Genet* **31**:321–328.

Lichtermann D, Franke P, Maier W, Rao ML (2000). Pharmacogenics and addiction to opiates. *Eur J Pharmacol* **410**:269–279.

Matthes HW, Maldonado R, Simonin V, Valverde O, Slowe S, Kitchen I, *et al.* (1996). Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature* **383**: 819–823.

Merikangas KR, Stolar M, Stevens DE, Goulet J, Preisig MA, Fenton B, *et al.* (1998). Familial transmission of substance use disorders. *Arch Gen Psychiatry* **55**:973–979.

Rommelspacher H, Smolka M, Schmidt LG, Samochowiec J, Hoehe MR (2001). Genetic analysis of the mu-opioid receptor in alcohol-dependent individuals. *Alcohol* **24**:129–135.

Sander T, Gscheidel N, Wendel B, Samochowiec J, Smolka M, Rommelspacher H, *et al.* (1998). Human mu-opioid receptor variation and alcohol dependence. *Alcohol Clin Exp Res* **22**:2108–2110.

Schinka JA, Town T, Abdullah L, Crawford FC, Ordorica PI, Francis E, *et al.* (2002). A functional polymorphism within the mu-opioid receptor gene and risk for abuse of alcohol and other substances. *Mol Psychiatry* **7**:224–228.

Schlesselman JJ (1982). *Case–Control Studies*: *Design, Conduct, Analysis*. New York: Oxford University Press.

Smolka M, Sander T, Schmidt LG, Samochowiec J, Rommelspacher H, Gschiedel N, *et al.* (1999). Mu-opioid receptor variants and dopaminergic sensitivity in alcohol withdrawal. *Psychoneuroendocrinology* **24**:629–638.

Sullivan PF, Eaves LJ, Kendler KS, Neale MC (2001). Genetic case–control association studies in neuropsychiatry. *Arch Gen Psychiatry* **58**:1015–1024.

Szeto CYK, Tang NLS, Lee DTS, Stadlin A (2001). Association between mu opioid receptor gene polymorphisms and Chinese heroin addicts. *Neuroreport* **12**:1103–1106.

Terwilliger JD, Ott J (1994). Linkage disequilibrium and disease loci. In: *Handbook of Genetic Linkage*. Baltimore, MD: The Johns Hopkins University Press; pp. 199–210.

Town T, Abdullah L, Crawford F, Schinka J, Ordorica PI, Francis E, *et al.* (1999). Association of a functional mu-opioid receptor allele (+118A) with alcohol dependency. *Am J Med Genet* **88**:458–461.

Tsuang MT, Bar JL, Harley RM, Lyons MJ (2001). The Harvard Twin Study of Substance Abuse: what we have learned. *Harv Rev Psychiatry* **6**:267–279.

Tsuang MT, Lyons MJ, Meyer JM, Doyle T, Eisen SA, Goldberg J, *et al.* (1998). Co-occurrence of abuse of different drugs in men: the role of drug-specific and shared vulnerabilities. *Arch Gen Psychiatry* **55**:967–972.

Wand GS, McCaul M, Yang XJ, Reynolds J, Gotjen D, Lee S, Ali A (2002). The mu-opioid receptor gene polymorphism (A118G) alters HPA axis activation induced by opioid receptor blockade. *Neuropsychopharmacology* **26**: 106–114.