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Low frequency genetic variants in the mu-opioid receptor (*OPRM1*) affect risk for addiction to heroin and cocaine

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Abstract

The μ -opioid receptor (MOR) binds exogenous and endogenous opioids and is known to mediate the rewarding effects of drugs of abuse. Numerous genetic studies have sought to identify common genetic variation in the gene encoding MOR (*OPRM1*) that affects risk for drug addiction. The purpose of this study was to examine the contribution of rare coding variants in *OPRM1* to the risk for addiction. Rare and low frequency variants were selected using the National Heart Lung and Blood Institute –Exome Sequencing Project (NHLBI-ESP) database, which has screened the exomes of over 6500 individuals. Two SNPs (rs62638690 and rs17174794) were selected for genotyping in 1377 European American individuals addicted to heroin and/or cocaine. Two different SNPs (rs1799971 and rs17174801) were genotyped in 1238 African American individuals addicted to heroin and/or cocaine. Using the minor allele frequencies from the NHLBI-ESP dataset as a comparison group, case-control association analyses were performed. Results revealed an association between rs62638690 and cocaine and heroin addiction in European Americans ($p=0.02$; 95% C.I. 0.47 [0.24–0.92]). This study suggests a potential role for rare *OPRM1* variants in addiction disorders and highlights an area worthy of future study.

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Disclosure

Author contributions are as follows: The authors TKC, RC and WB were responsible for the study concept and design. TKC, and RC were responsible for the acquisition of genotype data. TKC and RC conducted the statistical analysis. WHB, TNF, KMK, CPO, HMP, DWO, CAD, and FWL were responsible for sample acquisition and characterization. TKC, RC and WB drafted the manuscript. All authors report no biomedical financial interests or potential conflicts of interest. All authors critically reviewed content and approved final version for publication. This work has not been published previously and is not currently under consideration for publication elsewhere. If accepted, this manuscript will not be published elsewhere.

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Keywords

OPRM1; addiction; cocaine; heroin; rare variants; genetics

Introduction

In 2010, 1.7 million Americans used heroin or cocaine and about 1.35 million of these were dependent upon or abusing these substances (National Survey on Drug Use and Health, 2010). The risk for developing heroin or cocaine dependence is influenced by genetic factors, with heritability estimates ranging from 30–70% [21, 34, 35]. One gene that has been extensively studied in relation to drug addiction is *OPRM1*, the gene encoding the μ -opioid receptor (MOR). MOR is a seven transmembrane G-coupled protein receptor that exhibits high affinity for binding endogenous and exogenous opioids [30]. Mice lacking MOR have abolished therapeutic responses to opioids and display attenuated reward responses to cocaine [4, 16, 27]. In cocaine addicted men, positron emission tomography (PET) scans show increased MOR binding, which is associated with cocaine craving [42].

Numerous genetic association studies have sought to find variants in *OPRM1* that influence the risk for addiction [3, 20, 25, 28, 38–40]. One functional coding variant in *OPRM1* is the A118G polymorphism (rs1799971), which eliminates a glycosylation site in the extracellular domain of the protein. This SNP has been associated with heroin and cocaine dependence in several populations [13, 14, 23, 26, 33]. However, two meta-analyses that studied A118G in substance dependent populations did not find an overall significant association with addiction, nor did they find evidence of an association when ethnicity or drug type were analyzed [2, 15]. The C17T (rs1799972) variant in exon 1 of *OPRM1* is another putatively functional variant that changes from an alanine to a valine. Association studies have shown rs1799972 to be part of a haplotype associated with cocaine/heroin dependence in African Americans [19] and with quantitative drug abuse scores (KMSK scales) for cocaine, alcohol and tobacco use in African American women [12]. However, negative findings have also been reported [11].

The majority of the association studies analyzing *OPRM1* and drug addiction have focused on common variants. Common variants have an allele frequency greater than 5% in the general population and when associated with disease typically confer a small to moderate amount of risk. Most genetic variants associated with drug addiction-related phenotypes have odds ratios (OR) in the range of 1.1–1.3. Conversely, the rare variant hypothesis states that a significant proportion of disease risk arises from low frequency variants (<1%) that confer a much greater risk for disease. The mean OR for rare variants across a range of common diseases is estimated to be 3.74 [8].

Most studies on rare variants in the field of addiction have been conducted in nicotine dependent groups. Genome wide association studies (GWAS) have identified common variants within the cholinergic nicotinic receptor genes to be associated with nicotine addiction [7, 10, 32]. Rare variants within the same gene cluster (*CHRNA5*, *CHRNA2*, *CHRNA3* and *CHRNA4*) are also associated with nicotine addiction with OR as low as 0.29 observed [17, 36, 37]. As MOR has an important role in mediating the rewarding effects of drugs of abuse, we were interested to see whether rare variants within *OPRM1* would be associated with addiction to opioids or cocaine. In order to address this we combined cocaine addicted individuals and heroin addicted individuals together to create a ‘drug-addicted’ cohort.

Materials and Methods

Subject Information

DNA samples were acquired through the NIDA Center for Genetic Studies in conjunction with Washington University and Rutgers University Cell & DNA Repository. Samples from opioid-dependent subjects were acquired from the NIDA Repository Studies 1 (PI: J. Gelernter *et al.*)[N=313], 5 (PI: M.J. Kreek)[N=491], 17 (PI: W.H. Berrettini)[N=47] and 24(PI: W.H. Berrettini)[N=668] and samples from cocaine dependent subjects were acquired from Studies 7 (PI: L. Bierut)[N=541] and 13 (PI: J. Cubells)[N=133]. Opioid addicted (EA: n=1008; male 66.1%; AA: n=336 male 68%) and cocaine addicted subjects (EA: n=336; male 50.3%; AA: n=681; male 62%) of EA and AA descent met DSM-IV criteria for dependence and were genotyped for this study.

A portion of the AA cocaine addicted population (n=336) was collected during clinical studies on treatment for cocaine at the University of Pennsylvania Treatment Research Center. Subjects were at least 18 years of age. All were assessed with the Structured Clinical Interview for DSM Disorders (SCID) and urine drug screens were obtained. All patients had a clinical diagnosis of cocaine addiction as defined by DSM-IV. Family history was not obtained and ethnicity was determined by self-report. All psychiatric axis I disorders except alcohol dependence/abuse and nicotine dependence were used as exclusion criteria. Participants were excluded if they had a history of a seizure disorder (except cocaine-induced seizures) or a severe medical illness, including a history of AIDS (but not merely of HIV+ status). Individuals currently being treated with psychotropic medications or with psychiatric symptoms, including psychosis, dementia, suicidal or homicidal ideation, mania or depression requiring antidepressant therapy were also excluded. All studies were approved by the Institutional Review Boards at the University of Pennsylvania, and all subjects provided written informed consent before blood sample collection.

SNP selection

Rare variants in *OPRM1* were selected from the National Lung Heart and Blood Institute - Exome Sequencing Project (NHLBI-ESP) database (ESP6500 data release), which includes exome sequencing for 6503 European and African American individuals.

SNPs were selected using a MAF range of 0.5–5% in either the EA or AA population. Missense and nonsense SNPs located within the main *OPRM1* isoform (MOR-1) were prioritized for genotyping. This led to the identification of 4 SNPs for genotyping in our addicted populations: rs62638690 and rs17174794 in EA's and rs1799971 and rs17174801 in AA's.

SNP genotyping

All SNPs were genotyped in cocaine and opioid addicted subjects using Taqman® SNP Genotyping Assays (Applied Biosystems Inc. (ABI); Foster City, CA, USA). In a modification of the protocol recommended by manufacturers, we increased template DNA in the reaction from 2.5ng to 50ng. The standard Applied Biosystems protocol was otherwise followed. Quality control was maintained by genotyping 10% duplicates, which were checked for genotype concordance across the populations. The duplicate concordance rate for all 4 SNPs was 100%.

Statistical Analyses

To perform case-control association analyses, the allele frequencies from the NHLBI-ESP were used to represent a control population. The allelic association of SNPs with opioid and cocaine addiction was determined using the Fisher's exact test. Due to the different minor

allele frequencies of the polymorphisms in EA and AA populations, the two populations were analyzed separately. Heroin and cocaine addicts were combined and analyzed as a 'drug addicted' cohort. The false discovery rate (FDR) correction was used to adjust for multiple testing for p-values in each ethnicity separately [6].

Power analyses were carried out using QUANTO 1.2.4 assuming an unmatched case-control design, a population risk for addiction of 0.005, a log additive model and a 2 sided p-value of 0.05. For variants with a MAF of 0.5% we had 77% power to detect association in the EA population and 64% power in the AA population.

Results

SNP summary

Two SNPs were selected for genotyping in the EA heroin and cocaine addicted population: rs17174794 and rs62638690. rs17174794 changes the amino acid sequence of MOR from a cysteine to a serine at position 147 in the protein (S147C), while the minor allele of rs62638690 changes a cysteine to a phenylalanine at position 192 (C192F). The two SNPs selected for genotyping in the AA drug addicted population are rs17174801 and rs1799971. rs17174801 changes the amino acid sequence from an asparagine to an aspartic acid at position 152 (N152D). rs1799971 is the well-studied A118G SNP which changes an asparagine to an aspartic acid at position 40 (N40D). Polyphen [1] predicts each of these 4 SNPs to have a 'probably damaging' effect on protein function.

Association analyses

Of the 2 SNPs that were genotyped in EA cases, only rs62638690 was significantly associated with drug addiction. The minor allele frequency in cases was 0.38% compared to 0.79% in the control population ($p=0.02$, $OR=0.47[0.24-0.92]$), suggesting a protective effect against drug addiction. This association did withstand correction for multiple testing (Bonferroni correction = 0.04). rs17174794 was not found to be significantly associated with drug addiction in EA's (Table 1). The total rare variant burden in cases compared to control in the EA population was not found to be statistically different ($p=0.796$, $\chi^2=0.07$, $OR=0.94[0.64-1.36]$).

In AA's, neither rs1799971 nor rs17174801 were associated with drug addiction when compared to the frequencies reported in the NHLBI-ESP database (Table 1). The total rare variant burden in cases compared to controls in the AA population was not found to be statistically different ($p=0.74$, $\chi^2=0.11$, $OR=0.94[0.71-1.25]$).

Discussion

Rare coding variants in *OPRM1* were assessed for their contribution to risk for addiction to heroin and cocaine. Our findings show that rs62638690 is associated with drug addiction in EAs, as the minor allele is found at a higher frequency in controls (0.38% vs 0.79%). Therefore, the common allele of rs62638690 may increase risk for drug addiction ($OR=0.47$). However, the finding needs to be replicated in an independent cohort in order to confirm its association with addiction. The function of rs62638690 has previously been determined by stably expressing MOR carrying the rs62638690 (192F) variant in HEK293 cells. When expressed on a MOR-1A (lacking the 12 amino acids encoded by exon 4) background, a reduced potency for [D-Ala²,N-MePhe⁴,Gly⁵-ol]enkephalin (DAMGO) and morphine was observed [31].

Interestingly, the S147C (rs17174794) variant genotyped in EAs was previously found to have increased potency for morphine [31]; however, our association was not statistically

significant ($p=0.19$). The function of rs17174801 (N152D) has also been assessed in mammalian cell lines and the mutant allele leads to reduced expression of the receptor [5]. However, N152D does not appear to increase risk for drug addiction, since no significant association was observed in our AA population ($p=0.65$). rs1799971 (N40D) leads to the loss of a glycosylation site in the extracellular N-terminal domain of the MOR. Functional studies have found differential expression, receptor binding, signaling efficiency, and altered intracellular effects to be associated with this variant [9, 14, 22, 29, 41]. While rs1799971 has been associated with heroin and cocaine addiction [13, 14, 23, 26, 33], negative findings have also been reported [2, 15]. In support of the latter findings, we did not find rs1799971 to be associated with the risk for drug addiction ($p=0.57$).

Although all of the variants genotyped in this study have some biological function ascribed to them, it should be noted that three of the four SNPs are rare in the population and the rare variant carriers in our drug addicted populations were all heterozygotes. The functional consequences of carrying a single mutant allele may be different to those observed when homozygous mutants are expressed in cell lines. If the 'wild-type' allele is dominant, then heterozygous [13, 14, 23, 26, 33] individuals would have no distinct phenotype. This effect may account for the lack of association observed between these alleles and drug addiction; however, it is also plausible that they are simply not relevant for addiction risk.

Not all rare coding variants in the MOR were genotyped in this study. We focused our efforts on coding variants with a frequency of 0.5–5% located in the main *OPRM1* isoform (MOR-1). The minor allele frequency cut-off was set at 5% to find variants with a strong effect on phenotype; these are typically kept at a low frequency in the population due to purifying selection. The lower threshold for our SNP selection criteria was set at 0.5% to provide sufficient power to detect associations. By focusing on these variants we did not assess the contribution of 'personal' SNPs to the risk for addiction. There are 34 'personal' SNPs recorded in the NHLBI-ESP database for the *OPRM1* gene. It is likely that a subset of these variants increase the risk for addiction; however, it is not possible to test the effects of these variants using the classical case-control association approach. Furthermore, coding variants in the less well characterized isoforms of *OPRM1* may also be relevant for drug addiction risk and these variants could be of interest for future studies.

There are a number of limitations to this study. Our heroin and cocaine dependent populations were grouped together in the association analyses to make an 'addicted' cohort. It is possible that the variants which were not associated with drug addiction still affect risk for heroin or cocaine addiction. We were not able to split our cohort for heroin or cocaine specific analyses while maintaining sufficient statistical power. However, there is a substantial co-morbidity between heroin and cocaine addiction; 50% of intravenous cocaine users report using heroin on a regular basis [24] and 92% of heroin users also use cocaine [18]. Splitting our sample into heroin and cocaine dependents would not adequately assess the contribution of these variants to 'pure' cocaine or heroin addiction as such individuals are rare in the population.

The use of the minor allele frequencies in the NHLBI-ESP database as a 'control' cohort for our association analyses could also be a limitation of this study. The individuals which comprise this sample are taken from population based cohorts, only some of which are screened for drug addiction. In 2008–2009 only 1.9% of the United States population was classed as dependent on an illicit drug (National Survey on Drug Use and Health, 2010) and only a subset were addicted to heroin or cocaine. The NHLBI-ESP controls may, therefore, include some drug addicted individuals. However, the addicted cohort will be enriched for addiction risk alleles compared to population based cohorts.

There are additional limitations pertaining to the case samples used in this study. Drug addicted individuals were obtained from various NIDA genetics study populations, each of which has different inclusion and exclusion criteria for drug addicted participants. This may have led to subtle differences between the case populations; however, it should be noted that all drug addicted individuals genotyped in this study do meet DSM-IV criteria for opioid or cocaine addiction. Furthermore, while the NHLBI-ESP removes all exomes from their database which demonstrate first to third degree relatedness, the cases used in this study were not analyzed for cryptic relatedness and this may have impacted the frequency of rare variants reported in this study. Population stratification may also have affected the associations reported in this study as differences in population substructure were not controlled for between the cases and the NHLBI-ESP population. Without access to the raw genotype data for the NHLBI-ESP population it is not possible to test for differences in population admixture between the cases and controls and this should be recognized as a limitation of this study.

This study analyzes the contribution of rare functional variants in *OPRM1* to the risk for addiction. By studying variants with known function we can increase our understanding of addiction processes at the molecular level. rs62638690 has been shown to reduce the potency of MOR for opioids [31] and these data show a significant association of this SNP with drug addiction. A reduced sensitivity to opioids, therefore, may protect against addiction to illicit drugs. In order to confirm this nominally significant finding, additional genotyping in independent cohorts of individuals addicted to illicit drugs or prescription opioids is warranted.

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Highlights

- We performed case-control analyses of low frequency genetic variants in *OPRM1* in drug addicted individuals
- 1377 European Americans and 1238 African Americans addicted to heroin or cocaine were genotyped for 4 SNPs in *OPRM1*
- One SNP, rs rs62638690, was significantly associated with cocaine and/or heroin addiction in European Americans.
- Previous studies have found this SNP to reduce the potency of the mu-opioid receptor for opioids.

Allelic association of rare non-synonymous SNPs in *OPRM1* with heroin or cocaine addiction. Addicted alleles show the minor allele count in the heroin/cocaine addicted population and the NHLBI-ESP alleles show the allele count according to the NHLBI-ESP database.

Table 1

SNP	Location	Addicted Alleles	MAF	NHLBI-ESP Alleles	MAF	P-Value	OR (95% C.I.)
European Americans							
rs17174794	S147C	26/2628	0.99%	56/8456	0.66%	0.19	1.49 (0.94–2.38)
rs62638690	C192F	10/2644	0.38%	67/8389	0.79%	0.02	0.47 (0.24–0.92)
African Americans							
rs17174801	N152D	17/1865	0.9%	34/4234	0.80%	0.65	1.14 (0.63–2.04)
rs1799971	N40D	52/1934	2.62%	122/3862	3.06%	0.57	0.92(0.66–1.26)

MAF= minor allele frequencies, OR = odds ratio, 95% C.I. = confidence intervals.