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

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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 μ-opioid receptor for opioids.

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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.

1. 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% to 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

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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*, *CHRNB2, 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.

2. Materials and methods

2.1. 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: Gelernter et al.) [$N = 313$], 5 (PI: Kreek) [$N = 491$], 17 (PI: Berrettini) [$N = 47$] and 24(PI: Berrettini) [$N = 668$] and samples from cocaine dependent subjects were acquired from studies 7 (PI: Bierut) [$N = 541$] and 13 (PI: 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.

2.2. 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.

2.3. 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 2.5 ng to 50 ng. 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%.

2.4. 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.

3. Results

3.1. 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.