Archival Report

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Cis-Expression Quantitative Trait Loci Mapping Reveals Replicable Associations with Heroin Addiction in *OPRM1*

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ABSTRACT

BACKGROUND: No opioid receptor, mu 1 (*OPRM1*) gene polymorphisms, including the functional single nucleotide polymorphism (SNP) rs1799971, have been conclusively associated with heroin/other opioid addiction, despite their biological plausibility. We used evidence of polymorphisms altering *OPRM1* expression in normal human brain tissue to nominate and then test associations with heroin addiction.

METHODS: We tested 103 *OPRM1* SNPs for association with *OPRM1* messenger RNA expression in prefrontal cortex from 224 European Americans and African Americans of the BrainCloud cohort. We then tested the 16 putative cis-expression quantitative trait loci (cis-eQTL) SNPs for association with heroin addiction in the Urban Health Study and two replication cohorts, totaling 16,729 European Americans, African Americans, and Australians of European ancestry.

RESULTS: Four putative cis-eQTL SNPs were significantly associated with heroin addiction in the Urban Health Study (smallest $p = 8.9 \times 10^{-5}$): rs9478495, rs3778150, rs9384169, and rs562859. Rs3778150, located in *OPRM1* intron 1, was significantly replicated ($p = 6.3 \times 10^{-5}$). Meta-analysis across all case-control cohorts resulted in $p = 4.3 \times 10^{-8}$: the rs3778150-C allele (frequency = 16%–19%) being associated with increased heroin addiction risk. Importantly, the functional SNP allele rs1799971-A was associated with heroin addiction only in the presence of rs3778150-C ($p = 1.48 \times 10^{-6}$ for rs1799971-A/rs3778150-C and $p = .79$ for rs1799971-A/rs3778150-T haplotypes). Lastly, replication was observed for six other intron 1 SNPs that had prior suggestive associations with heroin addiction (smallest $p = 2.7 \times 10^{-8}$ for rs3823010).

CONCLUSIONS: Our findings show that common *OPRM1* intron 1 SNPs have replicable associations with heroin addiction. The haplotype structure of rs3778150 and nearby SNPs may underlie the inconsistent associations between rs1799971 and heroin addiction.

Keywords: Genetic association study, Heroin, Multiancestry, Opioid, OPRM1, Prefrontal cortex

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According to the 2010 Global Burden of Disease Health Measurement Survey, around 15.5 million people worldwide were dependent on heroin and other opioid drugs (1). Three regions had prevalence rates significantly higher than the global rate: Australasia, western Europe, and North America (1). In the United States, the 2013 National Survey on Drug Use and Health estimated that 669,000 people aged 12 or older abused heroin in the past year, representing a 78% increase since 2007 (2). To address this public health burden, a better understanding of the pathogenesis leading to heroin addiction is needed. Genetic vulnerability is recognized as a major risk factor contributing to heroin and other opioid addiction, as evidenced by twin studies showing that genetic factors account for 40% to 60% of the population variability (3–6). A few genome-wide association (7–9) and numerous candidate gene studies (10–19) in humans have implicated genes encoding opioid receptors (*OPRM1*, *OPRD1*, and *OPRK1*) and potassium channels (*KCNG1* and *KCNG2*) and others as contributing to heroin/opioid addiction phenotypes. In supporting the biological plausibility of the candidate genes, particularly genes in the opioid system (20), knockout mouse models have been used to study behavioral effects resulting from genetic perturbations. However, conclusively identified associations of specific genetic variants remain elusive.

The current study focuses on the opioid receptor, mu 1 (*OPRM1*) gene, the most widely studied candidate gene (17), which encodes a predominant target for heroin and other opioid molecules. The *OPRM1* missense polymorphism rs1799971 has been widely studied for its functional consequences, including reduced signaling efficiency and reduced

SEE COMMENTARY ON PAGE 439

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expression of the receptor (21–25). However, rs1799971 associations with heroin and other drug addictions (13–17) have been modest and often inconsistent. Additional *OPRM1* single nucleotide polymorphisms (SNPs) have been tested for association with heroin/opioid addiction (10,12,13,18,19), but none of the findings have been independently replicated.

To enhance the detection of replicable *OPRM1* SNP associations with heroin addiction, our study focused only on SNPs with evidence for altering *OPRM1* messenger RNA expression in human brain, thereby reducing the multiple testing burden with a limited number of plausible regulatory SNPs carried forward for disease association testing. This approach was motivated by prior findings that psychiatric and other disease-associated SNPs tag expression quantitative trait loci (eQTL) more often than unassociated SNPs (26–28). Other studies have successfully used cis-eQTL mapping to nominate SNPs and consequently find associations with complex diseases, such as Crohn's disease (29), chronic obstructive pulmonary disease (30), and amyotrophic lateral sclerosis (31). We used cis-eQTL mapping to identify SNPs associated with *OPRM1* messenger RNA expression in human prefrontal cortex from 224 BrainCloud cohort participants (32) who had no evidence of drug use/abuse at the time of death and tested the putative cis-eQTL SNPs for association with heroin addiction across three independent cohorts totaling 16,729 (4287 cases and 12,442 control subjects). Our findings revealed replicable and highly significant SNP associations with heroin addiction and provided strong support of *OPRM1* as an important susceptibility gene for heroin addiction.

METHODS AND MATERIALS

Figure 1 outlines our overall study design of conducting cis-eQTL mapping for *OPRM1* in nonaddicted BrainCloud participants and testing the nominated SNPs for association in heroin addiction case-control cohorts. All study protocols received Institutional Review Board approval at their respective sites, and all study participants or their legal next of kin provided informed consent.

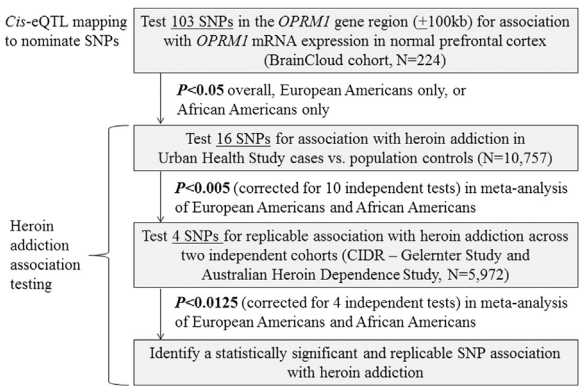

Figure 1. Overview of study design. CIDR, Center for Inherited Disease Research; eQTL, expression quantitative trait loci; mRNA, messenger RNA; SNP, single nucleotide polymorphism.

Cis-eQTL Mapping Using Human Prefrontal Cortex in the BrainCloud Cohort

To identify putative cis-eQTL SNPs for *OPRM1*, we utilized SNP genotype and gene expression data (Supplement 1) available on postmortem dorsolateral prefrontal cortex samples from 110 European Americans and 114 African Americans, ranging in age from 0 to 78 years old, who had no neuropathologic or neuropsychiatric diagnoses, no drug or alcohol abuse, and no positive toxicology result (32–34).

Normalized ratios of gene expression levels from the single *OPRM1* probe available in BrainCloud (Figure S1 in Supplement 1) were log-transformed and tested for association with additive three-level SNP genotypes using the BrainCloud software (32). Genotyped SNP association results were generated from the best fit general linear model with race, sex, age, life stage (infant, child, teen, and adult), and an age by life stage interaction included as the range of covariates. Inclusion of age, life stage, and the corresponding interaction was used to account for nonlinear trajectories of age-dependent gene expression over the life span (33). We evaluated associations overall and stratified by ancestry for the 103 SNPs genotyped across *OPRM1* and its 100 kilobase flanking regions. A p value threshold of .00125, which takes into account the correlations among the 103 *OPRM1* SNPs ($\alpha = .05/40$ independent tests) (35,36), was used to declare statistical significance. Given the hypothesis-generating nature of our cis-eQTL mapping, SNPs associated with *OPRM1* expression at the nominal significance threshold of $p < .05$ were carried forward for association testing with heroin addiction.

Discovery Cohort for Heroin Addiction Association Testing: Urban Health Study Cases versus Population Control Subjects

European American and African American cases were drawn from the Urban Health Study (UHS), one of the largest studies of street-recruited injection drug users in North America (37). See Supplement 1 for further details on the UHS design. Stored serum samples from 3227 UHS participants were selected for genotyping on the Illumina Omni1-Quad BeadChip (Illumina, Inc, San Diego, California) (38). Over 60% of the genotyped UHS participants met the Office of National Drug Control Policy definition of heroin abuse (injecting 10+ times in the past 30 days) (39,40), which is highly correlated with clinical levels of dependence on the Severity of Dependence Scale (41,42) and with DSM-IV (43) heroin abuse/dependence in analyses of the National Household Survey on Drug Use and Health data (87% positive predictive value; see Supplemental Methods and Table S1 in Supplement 1). These UHS participants, who abused heroin an average of 80.9 times in the past month and were very likely dependent on it, are henceforth referred to as heroin addiction cases. The remaining genotyped UHS participants who were addicted to cocaine or other substances but not addicted to heroin were not included in the current study.

For comparison with the UHS heroin addiction cases, we used six study cohorts from the database of Genotypes and Phenotypes (dbGaP) as a source of control subjects. Several prior studies have been reliably conducted using a similar design with study cases and population control subjects (44–49). See Supplemental Methods, Table S2, and Figure S2 in