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Case-control association study of *WLS* variants in opioid and cocaine addicted populations

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ABSTRACT

The opioid receptor family is involved in the development and maintenance of drug addiction. The mu-opioid receptor (MOR) mediates the rewarding effects of multiple drugs, including opiates and cocaine. A number of proteins interact with MOR, potentially modulating MOR function and altering the physiological consequences of drug use. These mu-opioid receptor interacting proteins (MORIPs) are potential therapeutic targets for the treatment of addiction. The Wntless (WLS) protein was recently identified as a MORIP in a yeast two-hybrid screen. In this study, we conducted a case-control association analysis of 16 *WLS* genetic variants in opioid and cocaine addicted individuals of both African-American (opioid $n=336$, cocaine $n=908$) and European-American (opioid $n=335$, cocaine $n=336$) ancestry. Of the analyzed SNPs, three were nominally associated with opioid addiction and four were nominally associated with cocaine addiction. None of these associations were significant following multiple testing correction. These data suggest that the common variants of *WLS* analyzed in this study are not associated with opioid or cocaine addiction. However, this study does not exclude the possibilities that rare variants in WLS may affect susceptibility to drug addiction, or that common variants with small effect size may fall below the detection level of our analysis.

1. Introduction

In 2009, an estimated 1.1 million individuals in the United States were either addicted to cocaine or currently abusing the drug. An additional 399,000 people were addicted to or abusing opiates, including heroin (http://www.oas.samhsa.gov, 2010). Susceptibility to drug addiction is a multifactorial trait, combining both genetic and environmental factors. Twin studies have indicated that genetic factors explain 30–50% of susceptibility to both cocaine and opioid addiction (Arvidsson et al., 1995; Karkowski et al., 2000; Kendler et al., 2003, 2000; Kendler and Prescott, 1998; Merikangas et al., 1998; Tsuang et al., 1998; Zhang et al., 2006). Identification of genetic variants increasing risk for drug abuse and addiction would help assess individual risk and produce new therapeutic targets. However, discovery of relevant genes affecting susceptibility has proven difficult due to the polygenic nature of these phenotypes (Wong and Schumann, 2008).

A number of studies on drug addiction have focused on genes encoding opioid receptors (Becker et al., 2002; Hall et al., 2004; Hummel et al., 2006; Matthes et al., 1996; Sora et al., 1997). The opioid receptor family consists of several transmembrane proteins that mediate the cellular effects of opioid ligands through the activation of G-protein signaling cascades (reviewed in Law et al. (2000)). Most research on potential links between opioid receptors and drug addiction has been focused on the mu-opioid receptor (MOR), encoded by the gene *OPRM1*. MOR mediates reward pathways upon interaction with both endogenous opioid proteins and exogenous opiates (Di Chiara and Imperato, 1988; Herz, 1998). The rewarding effects of cocaine are also mediated by MOR, potentially through the release of endogenous opioid peptides following cocaine use (Soderman and Unterwald, 2008). Agonist binding of the MOR protein results in activation of the mesolimbic dopamine system and subsequent release of dopamine (reviewed in Le Merrer et al. (2009)). The downstream effects of dopamine release act as positive reinforcement, increasing the chance of future drug use (for review, see Le Merrer et al. 2009). Despite the link

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between MOR and reward following drug use, association studies have failed to find consistent connections between common genetic variants of *OPRM1* and addiction to either heroin or cocaine (Bart et al., 2004; Crowley et al., 2003; Hoehe et al., 2000; Smith et al., 2005; Szeto et al., 2001; Tan et al., 2003; Zhang et al., 2006).

Although polymorphisms in *OPRM1* may not alter susceptibility to drug addiction, other genes that encode proteins involved in reward pathways may still be relevant. MOR interacts with a variety of proteins that regulate the function of the opioid receptor and the downstream signaling caused by agonist binding (Milligan, 2005). These mu-opioid receptor interacting proteins (MORIPs) include opioid ligands and heterotrimeric G-proteins, which control receptor activation and downstream signaling, respectively (reviewed in Law et al. (2004)). Other MORIPs are known to prevent activation of MOR signaling or regulate the desensitization of MOR by blocking access to specific protein–protein binding domains (Gainetdinov et al., 2004; Guang et al., 2004; Milligan et al., 2004; Onoprishvili et al., 2003). The Wntless (WLS) homolog (Drosophila) protein was identified as a MORIP during a yeast two-hybrid screen (Jin et al., 2010).

WLS (a.k.a GPR177) is a transmembrane protein that is required for proper shuttling of WNT ligands from the Golgi apparatus to the cell surface for secretion into the extracellular space (Banziger et al., 2006; Bartscherer et al., 2006). During morphine exposure, WLS binds to MOR and is sequestered on the cell surface (Jin et al., 2010; Reyes et al., 2010). The sequestered WLS protein is unable to shuttle between the Golgi and the cell surface, resulting in decreased WNT secretion (Jin et al., 2010). We performed a case-control association analysis to determine if common variants in the *WLS* gene were associated with opioid or cocaine addiction in African-American and European-American populations.

2. Methods

2.1. Population information

2.1.1. Cases

DNA samples were requested and acquired through the NIDA Center for Genetic Studies in conjunction with Washington University and Rutgers University Cell & DNA Repository. Opioid addicted (European-American: $n=335$, male 63.3%; African-American: $n=336$, male 71.4%) and cocaine addicted subjects (European-American: $n=336$; male 50.3%) met DSM-IV criteria for dependence. DNA samples from African-American cocaine addicted subjects ($n=908$, male 66.7%) were collected during clinical studies for cocaine addiction treatment at the University of Pennsylvania Treatment Research Center as previously described (Crist et al., 2013). All protocols were approved by the Institutional Review Boards at the University of Pennsylvania. All subjects provided written informed consent before blood sample collection.

2.1.2. Controls

European-American control individuals ($n=656$; male=50.8%) and African-American control individuals ($n=671$; male= 38.6%) were acquired from the National Institute of Mental Health Genetics Initiative (NIMH-GI) (www.nimhgenetics.org). Control subjects were screened for adult psychiatric diseases using a modified version of the Composite International Diagnostic Interview—Short Form (CIDI-SF). Individuals who self-identified as having an axis-I psychiatric disorder were excluded from this study.

2.2. Genotyping

SNPs were selected using the Tagger algorithm as part of Haploview software (http://www.broadinstitute.org/haploview) (Barrett et al., 2005). HapMap data from the CEU population (Hapmap data release 28 phase II & III, August 10, www.hapmap.org) and ASW population (Hapmap data phase III/rel#2 Feb09) was used to identify tag SNPs with an r^2 of 0.8 and a minor allele frequency cut-off of 9% and 5.7%, respectively. The cut-off was lowered in the African-American population to include rs2820487, which was previously associated with substance dependence (Drgon et al., 2010). The boundaries of the WLS gene for SNP selection were defined as 68,615,965 bp—68,659,904 bp on chromosome 1, which includes both the 3′ and 5′ UTR's but not the promoter region. At these frequency cut-offs, 13 SNPs captured 39% of alleles in the European-American population and 8 SNPs captured 8% of alleles in the African-American population. rs2033349 and rs2772297 were genotyped but not used in the coverage calculation as they are not part of the HapMap dataset. SNP genotyping was performed and analyzed as previously described (Crist et al., 2013). The number of cases and controls with each genotype are included in Supplementary Tables 1 and 2

2.3. Statistical analysis

The allelic and genotypic associations of SNPs with opioid and cocaine addictions were determined using the χ^2 test in the software package PLINK v1.07 (Purcell et al., 2007). For each SNP, deviation from Hardy–Weinberg was assessed in the total population and also in cases and controls individually. All SNPs were in Hardy–Weinberg Equilibrium in both cases and controls. Sliding window haplotype analysis was performed in PLINK using the expectation maximization algorithm included in the software packages (data not shown). Minor allele frequencies and the frequencies of all three genotypic groups were compared between cases and ethnicity-matched controls by χ^2 test for all SNPs. Sex was used as a covariate in all association analyses. p-Values for all analyses were corrected for multiple testing using the false discovery rate (FDR) procedure (Benjamini et al. 2001). The FDR procedure allows control of the average fraction of false rejections made out of the number of false rejections performed. The cut-off for statistical significance for this study was $p\leq 0.05$ after FDR correction. Power to detect significant associations was calculated using Quanto Version 1.2.4 (Gauderman, 2006). In the cocaine addicted African-American population, the average power to detect an association were ~76% and ~100% for associations with odds ratios of 1.3 and 1.5, respectively. In all other addicted populations, the average power to detect an association were ~42% and ~90%, respectively. Power calculations for all populations using an additive model are included in Supplemental Tables 3 and 4. The power to detect associations in dominant and recessive models was lower than the additive model (data not shown).

3. Results

Three SNPs were found to have nominally significant associations between their allelic or genotypic frequencies and cocaine addiction (Tables 1 and 2). rs1367444 (allelic $p=0.024$, genotypic $p=0.011$), rs1430753 (allelic $p=0.020$), and rs2183269 (allelic $p=0.047$) were nominally associated in European-Americans. In the African-American population, rs1337406 (genotypic $p=0.043$) was nominally associated with cocaine addiction. None of these associations were statistically significant ($p<0.05$) following FDR correction for multiple testing.

Nominally significant associations were also found between an additional 3 SNPs and opioid addiction (Tables 1 and 2). In the African-American population, rs3748705 (allelic $p=0.025$) was associated with addiction. rs983034 and rs1036066 (allelic $p=0.043$; genotypic $p=0.045$) were associated in the European-American population. None of these SNPs were significant after FDR correction for multiple testing.

No significant haplotypes were identified in African-Americans or European-Americans for either cocaine or opioid addiction (data not shown).

4. Discussion

The rewarding effect of drug use is one of the underlying causes leading to abuse and addiction (reviewed in Le Merrer et al. (2009)). MOR is one of the primary mediators of this effect, activating dopamine release in response to both cocaine and opioid use (Di Chiara and Imperato, 1988; Herz, 1998). MOR antagonists, such as naltrexone and naloxone, have been shown to reduce the rewarding effects of drug use and are currently used to treat addiction to a variety of different drugs (reviewed in Goodman et al. (2007)). Polymorphisms in the *OPRM1* gene may modulate the ability of MOR to activate downstream signaling, potentially explaining some of the variability in drug addiction susceptibility. Association studies of *OPRM1* in opioid and cocaine