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Case-control association analysis of polymorphisms in the delta-opioid receptor, *OPRD1*, with cocaine and opioid addicted populations*

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Abstract

BACKGROUND—Addiction susceptibility and treatment responsiveness are greatly influenced by genetic factors. Sequence variation in genes involved in the mechanisms of drug action have the potential to influence addiction risk and treatment outcome. The opioid receptor system is involved in mediating the rewarding effects of cocaine and opioids. The μ-opioid receptor (MOR) has traditionally been considered the primary target for opioid addiction. The MOR, however, interacts with and is regulated by many known MOR interacting proteins (MORIPs), including the δ-opioid receptor (DOR).

METHODS—The present study evaluated the contribution of *OPRD1*, the gene encoding the DOR, to the risk of addiction to opioids and cocaine. The association of *OPRD1* polymorphisms

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Author Contributions

The authors LMAL, MV, TKC, GAD, and WB were responsible for the study concept and design. LMAL, MV, AZ, CY were responsible for the acquisition of genotype data. LMAL, TKC, RC, GAD conducted the bioinformatic and statistical analysis. LMAL, WHB, TNF, FWL, GAD, KMK, CPO, HMP, DWO and HH were responsible for sample acquisition and characterization. RC, LMAL and WB drafted the manuscript. All authors critically reviewed content and approved final version for publication.

Conflicts of Interest

The authors report no biomedical financial interests or potential conflicts of interest.

with both opioid addiction (OA) and cocaine addiction (CA) was analyzed in African American (OA n=336, CA n=503) and European American (OA n=1007, CA n=336) populations.

RESULTS—The primary finding of this study is an association of rs678849 with cocaine addiction in African Americans (allelic p=0.0086). For replication purposes, this SNP was analyzed in a larger independent population of cocaine addicted African Americans and controls and the association was confirmed (allelic $p=4.53\times10^{-5}$; n=993). By performing a meta-analysis on the expanded populations, the statistical evidence for an association was substantially increased (allelic $p=8.5 \times 10^{-7}$) (p-values non-FDR corrected).

CONCLUSION—The present study suggests that polymorphisms in *OPRD1* are relevant for cocaine addiction in the African American population and provides additional support for a broad role for *OPRD1* variants in drug dependence.

Keywords

addiction; association study; delta-opioid receptor; OPRD1; genetics

1. INTRODUCTION

Twin and family studies suggest that a large percentage of risk for both opioid addiction (OA; Arvidsson et al., 1995; Karkowski et al., 2000; Kendler et al., 2003; Merikangas et al., 1998; Tsuang et al., 1998) and cocaine addiction (CA; Karkowski et al., 2000; Kendler et al., 2000; Kendler and Prescott, 1998; Zhang et al., 2006) is influenced by genetic factors (reviewed in Kreek et al., 2005; Saxon et al., 2005; Yuferov et al., 2010). Understanding which genes influence addiction susceptibility could improve treatment options and patient care. However, identification of causal genes has been difficult due to the polygenic inheritance underlying addictive behavior.

Opioid receptors have been the focus of addiction research due to the involvement of these receptors in drug reward pathways. The most widely studied members of the opioid receptor family are the μ-opioid receptor (MOR), δ-opioid receptor (DOR) and κ-opioid receptor (KOR), which bind endogenous opioid peptides such as β-endorphin, endomorphines, enkephalins, and dynorphins (reviewed in Zaki et al., 1996). Stimulation of opioid receptors inhibits adenylate cyclase, increases potassium conductance, decreases calcium conductance, and activates MAP Kinase pathways (Childers, 1991, reviewed in Law et al., 2000). The rewarding effects of drug use are mediated by MOR and DOR activation, whereas KOR activation is associated with aversion (Di Chiara and Imperato, 1988 ; Herz, 1998). Furthermore, DOR is thought to be involved in analgesia, morphine tolerance and mood regulation such as anxiety and depression (Filliol et al., 2000; Perrine et al., 2006; Zhu et al., 1999).

The opioid receptor system, and particularly MOR, has been studied extensively for its role in OA (Matthes et al., 1996; Sora et al., 1997) and CA (Becker et al., 2002; Hall et al., 2004; Hummel et al., 2006). Several studies have analyzed the influence of single nucleotide polymorphisms (SNPs) in *OPRM1*, the gene encoding MOR, on risk for drug addiction, but the results have been inconclusive (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 MOR is considered the primary target for the rewarding effects of addiction, there are many known MOR interacting proteins (MORIPs) that may modulate MOR function. One of these MORIPs is DOR (Milligan, 2005), suggesting that genetic variation in *OPRD1*, the gene encoding DOR, may affect susceptibility to drug addiction.

Two coding variants of *OPRD1* have been studied for association with alcohol and drug addiction: rs1042114 (G80T) and rs2234918 (T921C). Mayer et al. found rs2234918 to be associated with heroin addiction in a German population (Mayer et al., 1997). rs1042114 was associated with OA and a 6 SNP haplotype which included rs1042114 and rs2234918 was associated with alcohol, cocaine, and opioid addiction in a cohort of European-Americans (Zhang et al., 2008). An additional study identified an association of three *OPRD1* intronic SNPs with heroin addiction in EA (412 cases vs. 184 controls) and also reported a combined effect of *OPRD1* and *OPRM1* on heroin addiction (Levran et al., 2008). A recent study of 1459 case and 1495 controls found two intronic *OPRD1* SNPs (rs2236857 and rs581111) to be associated with risk for heroin addiction (Nelson et al., 2012). However, no evidence was found for an association of either SNP in additional heroin- and alcohol-addicted German populations (Franke et al., 1999), heroin-addicted Chinese individuals (Xu et al., 2002), or alcohol-addicted Taiwanese Hans (Loh el et al., 2004). Xuei et al. also reported that *OPRD1* variants were not associated with alcohol addiction or OA in a study of 1,923 European-American subjects from 219 multiplex alcohol-addicted families (83 individuals demonstrating OA; Xuei et al., 2007).

Currently, additional research is needed to understand the role of *OPRD1* in both opioid and cocaine addiction. The present study was designed to genotype a comprehensive panel of SNPs within *OPRD1* and test for association with both OA and CA populations across European-American (EA) and African-American (AA) ancestries.

2. MATERIALS AND METHODS

2.1 Population Samples

Cases—DNA samples were requested and acquired through the NIDA Center for Genetic Studies in conjunction with Washington University and Rutgers University Cell and DNA Repository (RUCDR). OA (EA: n=1007; male 65.6%; AA: n=336 male 71.4%) and CA subjects (EA: n=336; male 50.3%; AA: n=503; male 52.1%) of EA and AA descent met *DSM-IV* criteria for addiction (Table 1). AA CA samples from RUCDR are labeled as "Group 1" in subsequent analysis. DNA samples were transferred to 96-well stock plates and diluted to a concentration of 1 ng/μl for genotyping.

A separate group of AA (n=993; male 66.9%) CA subjects (Group 2) were collected during clinical studies for CA treatment at the University of Pennsylvania Treatment Research Center and used for confirmation purposes (Table 1). 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 CA as defined by *DSM-IV*. Family history was not obtained and ethnicity was determined by self-report. All psychiatric axis I disorders except anxiety disorders, major depressive disorder, alcohol addiction/abuse and nicotine addiction were used as exclusion criteria. In addition, 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. For all samples, genomic DNA was extracted from peripheral leukocytes within obtained blood samples by standard protocols. All protocols were approved by the Institutional Review Boards at the University of Pennsylvania, and all subjects provided written informed consent before blood sample collection.

Controls—EA control individuals (n=656; male=50.8%) and AA control individuals (n=503; male= 38.0%) were acquired from the National Institute of Mental Health Genetics

Initiative (NIMH-GI; www.nimhgenetics.org, Table 1). Control individuals were screened for history of substance use disorders and other psychiatric illness. For rs678849 specifically, a second AA control group (n=875; male= 41 %) consisting of NIMH-GI samples and a small number of controls samples that were collected together with the cocaine-addicted patients at the University of Pennsylvania were also genotyped (Table 1).

An additional control group was used to confirm the association of rs678849 found in AA CA. In collaboration with Dr. Hakonarson, genotype data was obtained from the pediatric control group recruited by the Children's Hospital of Philadelphia (CHOP) clinicians, nursing and medical assistant staff within the CHOP Health Care Network. Recruitment of 12,299 AA subjects took place across the US. All control subjects were genotyped using the Illumina 550K (Illumina, San Diego, CA) SNP array which included rs678849. The Research Ethics Board of CHOP and other participating centers approved the study, and written informed consent was obtained from all subjects or their parents.

2.2 SNP selection and genotyping

SNPs were selected using the Tagger algorithm as part of Haploview software (http://www.broadinstitute.org/haploview; Barrett et al., 2005). Using the HapMap CEU population data (HapMap data release 28 phase II and III, August 10, www.hapmap.org), 7 SNPs (rs1042114;coding non-synonymous), rs678849 (intron), rs2236855 (intron), rs10753331 (intron), rs529520 (intron), rs581111 (intron) and rs2234918; coding synonymous) capture 85% of SNPs in *OPRD1* with a minor allele frequency cut-off of 10% and an r^2 of 0.8 (Figure 1A). rs2234918 was not genotyped in the HapMap population and is not included in the linkage disequilibrium (LD) analysis. In the HapMap ASW population data (HapMap data phase III/rel#2 Feb09), 7 SNPs (rs533123, intron; rs678849, intron; rs2236855, intron; 10753331, intron; rs529520, intron; rs581111, intron; and rs2234918, coding synonymous) capture 33% of SNPs in *OPRD1* with a minor allele frequency cut-off of 10% and an r^2 of 0.8 (Figure 1B). rs2234918 and rs533123 were not included in LD analysis since they were not genotyped in the HapMap population. Two additional SNPs, rs204047 and rs797397, were also genotyped in a subset of AA CA samples.

Genotyping reactions of 5 μl total volume (containing 2 μl of genomic DNA and 3 μl of Taqman Genotyping Master Mix plus Taqman Assay) were prepared in a 384-well plate format using a Biomek 3000 robotic workstation (Beckman Coulter, Inc.; Brea, CA). SNP genotyping was performed using an ABI 9700 Thermocycler and Taqman SNP Genotyping Assays (Life Technologies, Applied Biosystems Inc.; Foster City, CA, USA). Quality control was maintained by genotyping 10% duplicates for cases and controls, and the concordance rate for our genotyping was calculated to be 99.99%. Following amplification, genotypes were acquired using ABI Prism 7900 Sequence Detections System v2.2 software and Taqman Genotyper Software v1.0 (Life Technologies, Applied Biosystems Inc.; Foster City, CA, USA).

2.3 Statistical analysis

The allelic and genotypic association of SNPs with OA and CA were determined using the Chi-square 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 across populations were in Hardy-Weinberg Equilibrium ($p \geq 0.05$). Allelic and genotypic association meta-analyses for rs678849 were performed on the combined samples from Group 1 and Group 2, plus an additional 35 CA samples obtained from RUCDR. All p-values were corrected for multiple comparisons 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≤0.05 after FDR correction. Sliding-window haplotype analysis was performed in PLINK using the expectation maximization (EM) algorithm (http://pngu.mgh.harvard.edu/~purcell/plink/; Purcell et al., 2007).

3. RESULTS

3.1 European American Population Genotypic and Allelic Associations

In the EA population, no significant allelic associations were found for OA; however, rs10753331 was nominally significant for genotypic association with OA (p=0.02; Table 2). Sex-specific analyses showed nominal association in males with the non-synonymous SNPs rs1042114 (p=0.008), rs2236855 (p=0.02), and rs10753331 (p=0.01). Several haplotype blocks had nominally significant associations with OA as well (Supplemental Data Table 1[1]).

For CA, a nominally significant allelic association was observed for the synonymous SNP rs2234918 (p=0.009; Table 3). The genotypic p-values for this SNP (p=0.03), as well as rs581111 were also nominally significant (p=0.05; Table 3). Sex-specific analysis for allelic association showed nominal association in males for rs2236855 (p=0.0006), rs1075331 (p=0.002), rs529520 (p=0.02), and rs2234918 (p=0.04), and females for rs2234918 (p=0.04). None of the associations observed in the EA population were significant following FDR correction for multiple testing.

3.2 African-American Population Genotypic and Allelic Associations

A nominal association was found for rs581111 in OA (allelic p=0.02; genotypic p=0.04; Table 4). In CA, nominally significant allelic associations were detected for rs2236855 in both the entire AA population (p=0.03) and AA females (p=0.003; Table 5). An association was also detected with CA for rs678849, which is located within intron one of *OPRD1* (allelic p=0.009; genotypic p=0.03; Table 5). The allelic association for rs678849 was still significant after correction for multiple testing. Sex-specific analysis for allelic association also identified a nominal association in females for rs678849 (p=0.01). Furthermore, multiple haplotype blocks including the rs678849 locus were nominally associated with CA (Supplemental Data Table 2[2]).

3.3 Confirmation of Allelic Association of rs678849 in Independent African-American Populations

Access to a large population of AA individuals with CA through the University of Pennsylvania Translational Research Center allowed us to confirm the initial association of rs678849 in an independent set of samples (Group 2). Group 2 (described in the methods) consists of an independent CA population and AA controls (cases n=993, controls =875). In Group 2, the significant association for rs678849 was confirmed (allelic $p=4.53\times10^{-5}$; genotypic p-value $=7.71\times10^{-6}$; Table 6). Additional CA samples (n=35) obtained from RUCDR were added to previously genotyped samples (Group 1) and a meta-analysis was performed on the combined samples from Group 1 and Group 2. The p-value for the association with CA was significantly strengthened in the meta-analysis (1527 cases vs. 1378 controls; allelic $p=8.50\times10^{-7}$; genotypic $p=3.65\times10^{-7}$; OR[95% C.I.]= 0.74 [.65–.83]), suggesting that the minor allele of rs678849 has a protective effect (Table 6). These p-values remained significant after FDR correction.

[1]Supplementary material can be found by accessing the online version of this paper at http://dx.doi.org and by entering doi:…
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Additional control sample data was obtained from 12,299 AA patients genotyped using the Illumina 550K platform. Chi-square analysis was performed on the allele frequencies of this control population (0.254) compared to the total AA population with CA (0.223). The difference in the minor allele frequencies between the combined cases from Group1 and Group 2 and this large control population was again statistically significant (p= 0.00027), although the p-value is less significant than the previous meta-analysis. This p-value remained significant after FDR correction for multiple testing.

3.4 Analysis of Neighboring SNP

To confirm that the significant association signal was coming from the rs678849 locus, Group 1 samples were genotyped for two SNPs that are in high D' with rs678849: rs204047 (D'=1; r^2=0.092) and rs797397 (D'=.885; r^2=0.638). Allelic and genotypic p-values for the association of rs204047 and rs797397 were not as low as those for rs678849, and neither SNP was significantly associated with CA after FDR correction (Table 7). Furthermore, haplotype analysis of these 3 SNPs did not increase the level of statistical significance (omnibus p=0.02).

4. DISCUSSION

The present study focused on the association between *OPRD1* genetic variants and cocaine and opioid addiction in EA and AA populations. In the EA population, a nominally significant association of the synonymous SNP rs2234918 with CA was observed, as well as sex-specific nominal associations for several other *OPRD1* SNPs. In the EA population with OA, a nominal sex-specific association of the non-synonymous SNP rs1042114 was identified. Associations of rs581111 with OA and rs2236855 with CA in the AA population were also nominally significant. Further, the intronic SNP, rs678849, was found to be strongly associated with CA and confirmed in an independent set of samples. This is the first study to report an association of *OPRD1* with CA in an AA population.

Other studies have analyzed *OPRD1* polymorphisms and substance dependence. A large candidate gene study of Australian heroin addicted cases and controls analyzed SNPs in *OPRD1*. They found rs2236857 to be associated with heroin addiction ($p=2.9\times10^{-4}$, OR=1.25; Nelson et al., 2012). The present study genotyped rs2236855, which is in complete linkage disequilibrium with rs2236857. We observed no association between rs2236855 and OA in the EA population, although rs2236855 was nominally associated with CA in the AA population (allelic p=0.03, OR=1.24). The Australian study also identified an association between a haplotype block of rs2236857 and rs581111 and heroin addiction (Nelson et al., 2012). Interestingly, rs581111 was found to be nominally associated with CA in EA (genotypic p=0.05, OR=1.16) and OA in AA (allelic p=0.02, OR=1.26) in our study. Zhang et al. genotyped 1063 EA substance addicted cases and 443 controls and found a 6 SNP haplotype to be associated with alcohol, cocaine and opioid addiction (Zhang et al., 2008). This haplotype contained rs1042114 and rs2234918, both of which displayed nominal significance with opioid and cocaine addiction in our study. A large scale candidate gene study analyzing 1350 variants in 412 cases and 184 controls found 3 intronic SNPs in *OPRD1* to be associated with heroin addiction (Levran et al., 2008). Although the same SNPs were not genotyped in our study, tag SNPs rs1075331 and rs2236855 are in LD with these variants and they were found to be nominally associated with cocaine and opioid addiction, respectively.

The strongest association reported in the present study was between rs678849 and CA in the AA population. The minor allele (T) was overrepresented in the controls compared to cases, indicative of a protective effect. Following the initial association of rs678849 with CA in the Group 1 samples, the association was confirmed in an independent set of samples (Group 2).

Two additional SNPs in high LD with rs678849 (D'>0.88) were genotyped to determine the source of the association signal. The results revealed that rs678849 had the strongest association with CA, supporting the surrounding intronic segment as a genomic region of interest in addiction. At this time, we cannot conclude whether rs678849 is driving the association as un-typed variants genetically linked to the SNP may also be responsible for the signal. However, previous studies have implicated rs678849 in addiction susceptibility and treatment efficacy. In a pharmacogenetic study of naltrexone treatment in alcohol addiction, individuals carrying the "T" allele of rs678849 had a significantly lower relapse rate after treatment compared to matched placebo controls (Gelernter et al., 2007). Another recent study by Luo et al. reported an association between a haplotype including rs678849 and drug addiction in a mixed population of EA and AA cases (Luo et al., 2008). In conjunction with our findings, these results suggest that future studies designed to test the pharmacogenetic effects of rs678849 in AA CA populations are warranted.

We cannot exclude the possibility that associations in the AA population are due to population stratification. According to data from the International Hapmap Project (www.hapmap.org), the minor allele of rs678849 (T) has a frequency of 26% in a population with African ancestry from the Southwest USA (ASW). In a population of Utah residents with Northern and Western European ancestry (CEU), however, the 'T' allele has a frequency of 53%. Due to this large difference in minor allele frequency between the two populations, different degrees of population admixture between AA cases and controls may have contributed to the association found in the present study. In support of our findings, African genetic heritage was not found to be associated with OA or CA in a previous study (Ducci et al., 2009) and the significance level of the rs678849 allele remains significant when comparing our control data to a larger independent control group. Further work using ancestry informative markers is needed to determine whether rs678849 is relevant for CA in AA.

The present study, in combination with the current literature, suggests that DOR may be a potential therapeutic target for addiction. *OPRD1* knock-out mice demonstrate anxiogenic-like and depressive-like behaviors, suggesting a role for DOR in modulating emotional state (Filliol et al., 2000; Konig et al., 1996; Ragnauth et al., 2001). Decreased DOR signaling resulting from treatment with the DOR antagonist naltrindole also causes anxiogenic-like effects (Marin et al., 2003). Mediation of anxiety makes DOR an appealing target for alleviation of cocaine withdrawal-induced anxiety. SNC-80, a DOR agonist, produced anxiolytic-like effects in males similar to the effects of classical therapeutics for anxiety (Perrine et al., 2006) and was able to decrease anxiety-like behavior following withdrawal from chronic cocaine in male (Perrine et al., 2008) and female rats (Ambrose-Lanci, 2009). Non-peptide DOR agonists, such as SNC-80, have greater bioavailability than peptide agonists and more readily cross the blood-brain-barrier. These non-peptide agonists may have clinical potential for the treatment of not only anxiety but also depression, pain and other neurological disorders. Despite some potential limitations related to convulsant effects, the use of DOR agonists is encouraging since they are reported to have fewer negative side effects and lower abuse potential compared to MOR agonists (reviewed in Jutkiewicz, 2006; Pradhan et al., 2011).

The present study provides an important step in understanding the genetic influence of *OPRD1* on both CA and OA. In the *OPRD1* gene, nominally significant associations were reported as well as a highly statistically significant association an intronic SNP (rs678849) and CA in AA. In light of previous findings implicating the DOR system in both emotional state and addiction withdrawal, our data support further research to fully determine the role of DOR in cocaine addiction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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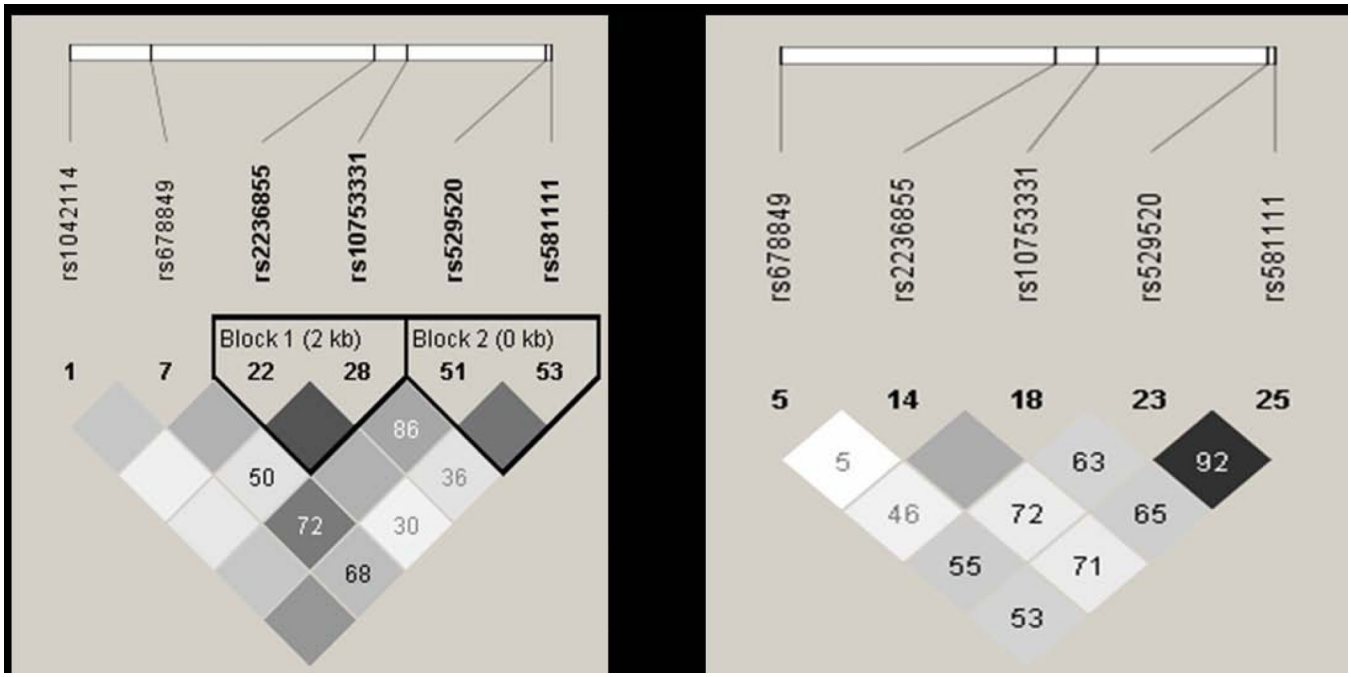

Figure 1.

LD between OPRD1 SNPs genotyped in HapMap CEU population and ASW population (www.hapmap.org). **A:** HapMap data release 28, phase II and III, August 10. In the CEU population, 7 SNPs capture 85% of SNPs in OPRD1 with a minor allele frequency cut-off of 10% and an r^2 of 0.8. (rs2234918 not included in LD analysis as HapMap data not available). **B:** HapMap data phase III/rel#2 Feb09. In the ASW population, 7 SNPs capture 33% of SNPs in OPRD1 with a minor allele frequency cut-off of 10% and an r^2 of 0.8. (rs2234918 and rs533123 not included in LD analysis as HapMap data not available). Numbers inside the squares represent D' and the shading is representative of r^2. When no number is displayed D' =1.

Table 1

Total number of CA cases, OA cases, and controls in each of the analyzed populations. The percentage of male individuals for each group is included in parentheses. Average age and standard deviation for each population is in provided in brackets.

Population	Cocaine Addicted Cases	Opioid Addicted Cases	Controls
European Americans	336 (50.3%)[36.1 ± 8.5]	1007 (65.6%)[36.9 ± 11.4]	656 (50.8%)[53.0 ± 17.6]
African Americans (Group 1)	503 (52.1%)[40.9 ± 7.0]	336 (71.4%)[48.2 ± 9.2]	503 (38.0%)[45.9 ± 14.0]
African Americans (Group 2)	993 (66.9%)[43.4 ± 6.5]	-	875 (41.0%)[44.9 ± 12.7]

Table 2

Comparison of genotype and allele frequencies in an EA population of OA cases and controls. P-values represent PLINK-generated X^2 tests for association between EA opioid-addicted cases and unrelated controls. C.I. =95% Confidence Interval; MA = minor allele; MAF = minor allele frequency; OR=odds ratio.

SNP ID (MA)	Case MAF (N)	Control MAF (N)	Allelic p-value	Odds Ratio (C.I.)	Genotypic p-value
rs1042114 (G)	0.12 (996)	0.14 (643)	0.09	0.83 (0.68–1.03)	0.22
rs678849 (C)	0.49 (995)	0.48 (654)	0.32	1.07 (0.93–1.23)	0.48
rs2236855 (T)	0.30 (998)	0.28 (652)	0.17	1.11 (0.95–1.30)	0.13
rs10753331 (A)	0.37 (994)	0.34 (647)	0.08	1.14 (0.99–1.32)	0.02
rs529520 (T)	0.48 (1001)	0.49 (654)	0.78	0.98 (0.85–1.13)	0.29
rs581111 (T)	0.26 (998)	0.28 (654)	0.41	0.94 (0.80–1.10)	0.70
rs2234918 (C)	0.45 (999)	0.44 (654)	0.88	1.01 (0.88–1.16)	0.71

Table 3

Comparison of genotype and allele frequencies in an EA population of CA cases and controls. P-values represent PLINK-generated X^2 tests for association between EA cocaine-addicted cases and unrelated controls. C.I. =95% Confidence Interval; MA = minor allele; MAF = minor allele frequency; OR=odds ratio.

SNP ID (MA)	Case MAF (N)	Control MAF (N)	Allelic p-value	Odds Ratio (C.I.)	Genotypic p-value
rs1042114 (G)	0.15 (331)	0.14 (643)	0.69	1.06 (0.81–1.38)	0.75
rs678849 (C)	0.51 (331)	0.48 (654)	0.19	1.13 (0.94–1.37)	0.42
rs2236855 (T)	0.30 (330)	0.28 (652)	0.32	1.11 (0.90–1.37)	0.53
rs10753331 (A)	0.37 (328)	0.34 (647)	0.20	1.14 (0.94–1.38)	0.39
rs529520 (T)	0.52 (330)	0.49 (654)	0.21	1.13 (0.93–1.36)	0.46
rs581111 (T)	0.31 (330)	0.28 (654)	0.16	1.16 (0.94–1.42)	0.05
rs2234918 (C)	0.51 (329)	0.44 (654)	0.009	1.28 (1.06–1.55)	0.03

Table 4

Comparison of genotype and allele frequencies in an AA population of OA cases and controls. P-values represent PLINK-generated X^2 tests for association between AA opioid-addicted cases and unrelated controls. C.I. =95% Confidence Interval; MA = minor allele; MAF = minor allele frequency; OR=odds ratio.

SNP ID (MA)	Case MAF (N)	Control MAF (N)	Allelic p-value	Odds Ratio (C.I.)	Genotypic p-value
rs533123 (G)	0.48 (333)	0.49 (502)	0.76	0.97 (0.80–1.18)	0.24
rs678849 (T)	0.28 (332)	0.28 (500)	0.84	1.02 (0.82–1.27)	0.25
rs2236855 (T)	0.30 (336)	0.29 (499)	0.88	1.02 (0.82–1.26)	0.71
rs10753331 (G)	0.50 (336)	0.45 (501)	0.10	1.18 (0.97–1.44)	0.23
rs529520 (C)	0.32 (336)	0.28 (502)	0.12	1.19 (0.96–1.47)	0.23
rs581111 (G)	0.41 (336)	0.35 (502)	0.02	1.26 (1.03–1.54)	0.04
rs2234918 (T)	0.37 (336)	0.35 (493)	0.45	1.08 (0.88–1.33)	0.69

Table 5

Comparison of genotype and allele frequencies in an AA population of CA cases and controls. P-values represent PLINK-generated X^2 tests for association between AA cocaine-addicted cases and unrelated controls. C.I. =95% Confidence Interval; MA = minor allele; MAF = minor allele frequency; OR=odds ratio.

SNP ID (MA)	Case MAF (N)	Control MAF (N)	Allelic p-value	Odds Ratio (C.I.)	Genotypic p-value
rs533123 (G)	0.47 (498)	0.49 (502)	0.47	0.94 (0.79–1.12)	0.36
rs678849 (T)	0.23 (500)	0.28 (500)	0.009	0.76 (0.62–0.93)	0.03
rs2236855 (T)	0.34 (497)	0.29 (499)	0.03	1.24 (1.02–1.49)	0.09
rs10753331 (G)	0.43 (499)	0.45 (501)	0.22	0.90 (0.75–1.07)	0.48
rs529520 (C)	0.25 (492)	0.28 (502)	0.12	0.85 (0.70–1.04)	0.28
rs581111 (G)	0.35 (497)	0.35 (502)	0.76	0.97 (0.81–1.17)	0.36
rs2234918 (T)	0.34 (493)	0.35 (493)	0.60	0.95 (0.79–1.15)	0.80

Table 6

Confirmation of rs678849 association in an independent AA population of CA cases. For Group 2, P-values represent PLINK-generated X^2 tests for association between AA cocaine-addicted cases and unrelated controls. C.I. =95% Confidence Interval; MAF = minor allele frequency; OR=odds ratio. Meta-analysis was conducted and the combined p-value for both genotype and association tests remained significant after correction for multiple testing.

	Case MAF (N)	Control MAF (N)	Allelic p-value	Odds Ratio (C.I.)	Genotypic p-value
Group 2	0.22 (993)	0.28 (875)	4.53×10^{-5}	0.73 (.63–.85)	7.71×10^{-6}
Groups 1 & 2	0.22 (1527)	0.28 (1378)	8.49×10^{-7}	0.74 (.65–.83)	3.65×10^{-7}

Table 7

Analysis of SNPs Neighboring rs678849 in an AA population of CA cases. Two SNPs that were in high D' with rs678849 were genotyped in the AA CA Group 1: rs204047 (D'=1; r2=.092) and rs797397 (D'=.885; r2=.638). Table shows the allele and genotypic frequencies for rs204047 and rs797397 compared to rs678849. P-values represent PLINK-generated X^2 tests for association between AA cocaine-addicted cases and unrelated controls. C.I. =95% Confidence Interval; MA = minor allele; MAF = minor allele frequency; OR=odds ratio

SNP ID (MA)	Case MAF (N)	Control MAF (N)	Allelic p-value	Odds Ratio (C.I.)	Genotypic p-value
rs204047 (T)	0.23 (496)	0.20 (501)	0.11	1.19 (0.96–1.47)	0.26
rs678849 (T)	0.23 (500)	0.28 (500)	0.009	0.76 (0.622–0.93)	0.03
rs797397 (T)	0.19 (486)	0.23 (457)	0.02	0.77 (0.62–0.96)	0.09