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The dopamine receptor D2 *(DRD2)* SNP rs1076560 is associated with opioid addiction

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SUMMARY

The risk for drug addiction is partially heritable. Genes of the dopamine system are likely candidates to harbor risk variants, as dopamine neurotransmission is involved in mediating the rewarding effects of drugs of abuse. One functional SNP in *DRD2*, rs1076560, is involved in regulating splicing of the gene and alters the ratio of DRD2 isoforms located pre and post-synaptically. rs1076560 has been previously associated with cocaine abuse and we set out to confirm this association in a sample of European American (n=336) and African American (n=1034) cocaine addicts and European American (n=656) and African American (n=668) controls. We also analysed the role of rs1076560 in opioid dependence by genotyping European American (n=1041) and African American (n=284) opioid addicts. rs1076560 was found to be nominally associated with opioid dependence in European Americans (p=0.02, OR=1.27) and African Americans (p=0.03, OR=1.43). When both opioid addicted ancestral samples were combined, rs1076560 was significantly associated with increased risk for drug dependence (p=0.0038, OR=1.29). This association remained significant after correction for multiple testing. No association was found with cocaine dependence. These data demonstrate the importance of dopamine gene variants in the risk for opioid dependence and highlight a functional polymorphism which warrants further study.

Keywords

dopamine; genetics; drug addiction; rs1076560; *DRD2*

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INTRODUCTION

The dopaminergic system is known to mediate drug reward and reinforcement. As the genetic risk for dependence on drugs of abuse, such as heroin and cocaine, has been shown to be substantial (Kendler *et al.*, 2000, Kendler & Prescott, 1998, Tsuang *et al.*, 1996, Tsuang *et al.*, 1998), variants in genes of the dopamine system are potential candidates for drug dependence risk. The dopamine D2 receptor (DRD2) is coupled to inhibitory G proteins and its activation results in a decrease in intracellular cAMP (Strange, 1993). Human imaging studies have shown D2 receptor availability to be decreased in cocaine addicts, even after months of detoxification (Volkow et al., 1993). Striatal dopamine D2 and D3 receptor binding and dopamine release have also been found to be reduced in heroin dependent subjects when compared to healthy controls (Martinez et al., 2012). Furthermore, genetic association studies have found genetic variants in dopaminergic genes to be associated with cocaine dependence (Lohoff et al., 2010).

Two splice isoforms of DRD2 are known to exist: the short (D2S) and long (D2L) isoforms. These isoforms arise from the alternative splicing of exon 6. The D2S isoform is located presynaptically whereas the D2L is found mainly post-synaptically (Khan et al., 1998). These two receptor isoforms are functionally distinct. Studies of D2L-knockout mice have demonstrated that the D2S acts as an autoreceptor, inhibiting the function of the D1 receptors (Usiello et al., 2000). In humans, two intronic single nucleotide polymorphisms (SNPs) that influence D2S/D2L splicing have been discovered: rs1076560 and rs2283265 (Zhang et al., 2007). These two SNPs are in high LD (D'=1) and the minor alleles of both SNPs are associated with decreased expression of D2S relative to D2L in the pre-frontal cortex (PFC) and caudate putamen (Moyer et al., 2011). Furthermore, these SNPs have been found to be associated with cocaine abuse, as the minor alleles were significantly over-represented in cocaine abusers (n=119) compared to controls (n=95) (Moyer et al., 2011).

In the present study we set out to replicate the finding of Moyer *et al.* by genotyping rs1076560 in a large number of cocaine addicts and controls, in populations of European and African American ancestry. Furthermore, as animal studies have found that mice lacking the D2L receptor will not develop conditioned place preference for morphine, or place aversion to naloxone precipitated morphine withdrawal (Smith et al., 2002), we were also interested in examining the role of rs1076560 in opioid dependence. Therefore, association analyses of rs1076560 were also carried out in groups of opioid dependent individuals and controls of both European and African American ancestry.

As rs1076560 and rs2283265 are in perfect LD in Europeans and in (r^2=1, D'=1; HapMap release 27, CEU population) Yoruban Africans (r^2=1, D'=1; HapMap release 27, YRI population), only rs1076560 was selected for genotyping in this study.

MATERIALS AND METHODS

SNP GENOTYPING

SNP genotyping was performed using Taqman® SNP Genotyping Assays (Applied Biosystems Inc. (ABI); Foster City, CA, USA) as per manufacturer protocol. Quality control

was maintained by genotyping 10% duplicates which were checked for genotype concordance across the populations. Duplicate concordance was 100% across all populations genotyped. Genotypes were checked for deviation from Hardy-Weinberg Equilibrium (HWE) as a second measure of quality control. No significant deviations from HWE were observed in cases or controls in either population (all tests $p>0.51$).

SUBJECT INFORMATION

COCAINE AND OPIOID ADDICTED INDIVIDUALS—European American (EA) and African American (AA) DNA samples were requested and acquired through the NIDA Center for Genetic Studies in conjunction with Washington University and Rutgers University Cell & DNA Repository. Samples from opioid-dependent individuals were acquired from the NIDA Repository Studies 1 (PI: J. Gelernter et al.), 5 (PI: M.J.Kreek), and samples from cocaine-dependent individuals were acquired from Studies 7 (PI: L. Bierut) and 13 (PI: J. Cubells). Opioid-addicted (EA: n=1041; male 66.1%; AA: n=284 male 68%) and cocaine-addicted subjects (EA: n=336; male 50.3%; AA: n=336; male 62%) of EA and AA descent met DSM-IV criteria for dependence.

A portion of the AA cocaine addicted subjects (n=698) were collected during clinical studies for cocaine addiction treatment 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 dependence 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. 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 Thomas Jefferson University and the University of Pennsylvania, and all subjects provided written informed consent before blood sample collection.

Data on drug addiction co-morbidity was available for a subset of the individuals genotyped in this study. Amongst AA (N=336) and EA (N=336) cocaine dependents, 16.4% and 34.8% were also diagnosed as dependent on opioids respectively. In the opioid dependent populations, 68.4% of AA (N=225) and 34% of EA (N=394) also met criteria for cocaine dependence.

CONTROL INDIVIDUALS—EA control individuals (n=656; male=50.8%) and AA control individuals (n=668; male= 37.3%) were acquired from the National Institute of Mental Health Genetics Initiative (NIMH-GI) (www.nimhgenetics.org). Control subjects were screened using an online self-report clinical assessment which screens 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 disorders, including alcohol dependence, were excluded from this study. A portion of AA controls (n=82) were collected simultaneously with the cocaine-dependent patients at the University of Pennsylvania and were also genotyped.

STATISTICAL ANALYSIS—The allelic and genotypic association of SNPs with opioid and cocaine dependence were determined using the Fishers, Chi-square tests and model tests in the software package PLINK v1.04 (Purcell et al., 2007). Logistic regression analyses were also performed using PLINK, using ethnicity (EA/AA) as a covariate in the regression model. All p-values generated from these analyses were corrected for multiple comparisons using the false discovery rate (FDR) procedure (Benjamini et al., 2001).

A power analysis was conducted using Genetic Power Calculator (Purcell et al., 2003). Using a dominant test this study had 92% power to detect an association with an odds ratio of 1.3 and a minor allele frequency of 12% (α=0.05). Using recessive, genotypic and allelic tests the power to detect association was 12%, 87% and 90% respectively (α=0.05).

RESULTS

The minor allele frequency (MAF) of rs1076560 (A allele) was found to be 14% in the EA and 9% in the AA control populations. This is similar to the MAFs observed in the HapMap dataset for the European population (13% CEU) and the African American population (12% ASW).

Chi-square allelic association analysis found rs1076560 to be nominally associated with opioid dependence in the AA population (χ^2=4.85, 1 d.f., p=0.03, OR=1.43) with the minor allele over-represented in cases. rs1076560 was also found to be nominally associated with opioid dependence in the EA population; (χ^2=5.57, p=0.02, OR=1.27) however, a lower OR was observed (Table 1). No significant associations with cocaine dependence were found in the AA population (χ^2=1.75, p=0.19, OR=1.18) or the EA population (χ^2=1.42, p=0.23, OR=1.17) (Table 1).

Genotypic tests for association revealed a nominal association of rs1076560 in the EA opioid population (χ^2=6.15, 2 d.f., p=0.046); however, the genotypic association in the AA opioid population only displayed a trend towards significance (2 d.f, p=0.051, Fisher's Exact test) (Table 2). Genotypic tests for association did not reveal significant associations for either cocaine population (data not shown). All association statistics and observed allele frequencies are summarized in Table 1. After correction for multiple testing none of the associations of rs1076560 with opioid dependence remained significant (FDR p<0.06).

As the association analyses involving the opioid dependent samples yielded statistically significant results for both EA and AA, these samples were combined (n=2578) and a logistic regression analysis was performed. In the regression model, ethnicity was used as a covariate since the minor allele frequency of the SNP in the two populations was different (14% EA vs 9% AA). A significant association was found between opioid dependence and rs1076560 (p=0.0038, OR=1.29) (Table 3). This association remained significant after correction for multiple testing (FDR p=0.034).

DISCUSSION

This study aimed to replicate the findings of Moyer *et al* (2011), who found rs1076560 to be associated with cocaine abuse in a mixed population of AA and EA individuals (N=214). They found the minor allele of rs1076560 to have a frequency of 18% in cases and 10% in controls. We did not find rs1076560 to be associated with cocaine dependence in either EA or AA populations; however, we did observe the same trend as the minor allele was increased in cases compared to controls. However, rs1076560 was found to be associated with opioid dependence in both the EA and AA populations. In the EA population the MAF in cases compared to controls was 17% vs 14% and in the AA population the MAF was 12% in cases and 9% in controls. The odds ratios associated with these findings are relatively modest: 1.27 for EA opioid dependence and 1.43 in AA opioid dependence.

This is in contrast to the findings of Moyer *et al* (2011), who found an odds ratio of 1.94 in their population of cocaine abusers. However, this may be an artifact of the small sample size used by Moyer *et al* (N=214), as random sampling can inflate odds ratios. An odds ratio of 1.3-1.4 is more consistent with the impact of risk variants reported in previous studies on polygenic diseases such as addiction. Furthermore, Moyer and colleagues used surrogate measures for defining chronic cocaine abuse such as hospital admission and arrest records. The participants used in this study were addicted to cocaine and diagnosed according to the DSM-IV. As Moyer *et al* analyzed cocaine abusers rather than cocaine addicts, the differences in the two samples may account for the lack of replication. It should also be noted that the samples genotyped as part of this study were not genotyped for ancestry informative markers. Given that population stratification can influence the results of association studies, it is a limitation of this study that these data were not available.

A number of recent studies have associated rs1076560 with brain function and cognitive phenotypes in healthy controls. rs1076560 is associated with neuronal connectivity in the amygdala, dorsolateral prefrontal cortex and striatal regions (Blasi *et al.*, 2009, Sambataro *et al.*, 2011), and differences in resting posterior minus frontal electroencephalographic (EEG) slow oscillations (Koehler et al., 2011). Brain activity in the left basal ganglia, thalamus, supplementary motor area, and primary motor cortex was also found to be associated with rs1076560 (Blasi *et al.*). These differences in brain function have been found to correlate with alterations in working memory, reaction time and impairments in negative decision making (Bertolino *et al.*, 2009, Bertolino *et al.*, 2010, Fazio *et al.*, 2011, Frank & Hutchison, 2009).

As rs1076560 is linked to differences in brain function, it is perhaps not surprising that the SNP is associated with various psychiatric disorders. The minor allele of rs1076560 has been associated with working memory and schizophrenia phenotypes (Bertolino *et al.*, 2009, Blasi *et al.*, 2011). rs1076560 has been associated with schizophrenia in two independent cohorts of Chinese patients (Zheng et al., 2012) and an interaction between rs1076560 and a SNP in *AKT1* is associated with the response to olanzapine in schizophrenic patients (Blasi et al., 2011). rs1076560 is also relevant for addiction to alcohol, as its minor allele is found at a greater frequency in Japanese alcoholic patients compared to healthy controls (P=0.03,

OR=1.3) (Sasabe et al., 2007). Furthermore, D2 receptor availability is associated with rs1076560 in alcoholic patients (Lucht et al., 2010).

rs1076560 has been associated previously with cocaine, alcohol and in the present study, opioid dependence (Moyer *et al.*, 2011, Sasabe *et al.*, 2007). There is considerable overlap between these addictions and it is rare to find individuals solely dependent on one substance. The prevalence of alcohol use disorders was estimated to be 89% amongst cocaine dependent individuals and 74% in opioid dependent individuals (Stinson et al., 2005). Various studies of polydrug use have reported that 50% of intravenous cocaine users also use heroin on a regular basis (Lauzon et al., 1994) and that 92% of heroin users were also taking cocaine (Hasin et al., 1988). It is possible that the original finding by Moyer *et al* reflects an indirect association with opioid or alcohol dependence, explaining our failure to detect an association with cocaine dependence in the present study. In our sample, a proportion of heroin addicts report the abuse of cocaine. This makes our positive association with opioid addiction and the lack of association with cocaine addiction difficult to interpret. Considering that the OR in cocaine addiction was similar to that observed in the opioid addicted cohort, it is possible that with a larger sample size an effect of the SNP on cocaine addiction would have been detected. However, whether rs1076560 increases risk for drug dependence in general or has specific effects depending on the drug type remains to be elucidated.

Other dopaminergic gene variants have been associated with substance addiction with varying success (Doehring *et al.*, 2009, Gelernter *et al.*, 1994, Guindalini *et al.*, 2006, Hou & Li, 2009). These include a variable nucleotide tandem repeat (VNTR) polymorphism in the 3'UTR (rs28363170) of the dopamine transporter gene (*DAT1*) and the Taq1A allele now known to reside upstream of *DRD2* in ANKK1 . A recent study found evidence of an epistatic interaction between the splice polymorphism in *DRD2* and a VNTR in intron 8 of *DAT1* (rs3836790) and cocaine use that contributes to risk for lethal cocaine intoxication (Sullivan *et al.*, 2013). Therefore, future studies of the role of dopaminergic gene variants and drug dependence would benefit from studying the epistatic effects of polymorphism on the risk for addiction.

rs1076560 is associated with brain anatomy and function in healthy controls and this is likely due to its role in *DRD2* splicing, which alters the ratio of D2S and D2L isoforms and subsequently impacts pre- and post-synaptic dopamine signaling. rs1076560 is also found to increase risk for psychiatric disorders such as schizophrenia and also the risk for developing substance dependence. We confirm the role of rs1076560 in drug dependence by documenting an association of the minor allele with opioid dependence in both AA and EA populations. Future studies in drug dependent populations should seek to determine the specificity of this finding for opioid, cocaine and alcohol dependence and also whether minor allele carriers of rs1076560 may be amenable to certain pharmacotherapies for the treatment of drug addiction.

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Table 1

Chi-square allelic association of rs1076560 with cocaine/opioid addiction in African Americans and European Americans.

Population	Phenotype (N)	Minor Allele Frequency	Chi-Square	P-value	Odds Ratio (95% C.I.)
African American	Controls (750)	0.09			
	Opioid addicts (278)	0.12	4.84	0.03	1.43 (1.04-1.97)
	Cocaine addicts (923)	0.10	1.75	0.19	1.18
European American	Controls (656)	0.14			
	Opioid addicts (999)	0.17	5.57	0.02	1.27 (1.04-1.54)
	Cocaine addicts (336)	0.16	1.42	0.23	1.17

Minor allele frequencies in cocaine and opioid addicted populations are compared to controls. 95% C.I. = confidence intervals.

Table 2

Detailed results of genotypic association (2 d.f. test) of rs1076560 and opioid addiction in African Americans and European Americans.

Population	Phenotype	CC	CA	AA	P-value
African American	Cases	77.1%	21.3%	1.6%	0.051
	Controls	82.8%	16.7%	0.5%	
European American	Cases	68.5%	28.7%	2.8%	0.046 (0.013*)
	Controls	74.3%	23.4%	2.3%	

*Dominant test for association p-value. The Chi-square test was performed for European Americans and the Fisher's exact test for African Americans.

Table 3

Results of logistic regression performed on AA and EA opioid cases vs AA and EA controls using ethnicity as a covariate in the regression model.

Test	N	OR (95% C.I.)	Co-efficient t statistic	P-value
ADD	2578	1.29 (1.09-1.53)	2.898	0.0038
ETHNICITY		4.09 (3.43-4.87)	15.81	2.8×10^{-56}

ADD p-value represents the test for the SNP-phenotype association after controlling for ethnicity.