Published in final edited form as:

Drug Alcohol Depend. 2016 September 1; 166: 268–271. doi:10.1016/j.drugalcdep.2016.07.008.

A delta-opioid receptor genetic variant is associated with abstinence prior to and during cocaine dependence treatment

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Abstract

Introduction—An intronic polymorphism in the delta-opioid receptor gene (*OPRD1*) was previously associated with cocaine dependence in African-Americans. However, it is not known if the polymorphism (rs678849) is associated with dependence-related phenotypes within the cocaine dependent population.

Methods—Cocaine and alcohol dependent subjects were randomized to either topiramate or placebo. Abstinence from cocaine use was confirmed by urine drug screens for benzoylecgonine three times per week. Cocaine withdrawal and craving was assessed at randomization using the Cocaine Selective Severity Assessment (CSSA) and Minnesota Cocaine Craving Scale (MCCS), respectively. Subjects were also interviewed using the Addiction Severity Index (ASI). Genotype at rs678849 was determined for 105 African-American subjects and compared to cocaine abstinence, as well as scores for CSSA, MCCS, and ASI.

Results—African-American patients with the C/T or T/T genotypes ($n=40$) were more likely to be abstinent at the first urine drug screen and more likely to be abstinent for the week prior to randomization compared to patients with the C/C genotype ($n=65$). Subjects carrying the T allele were also more likely to have abstinent weeks over the course of the trial compared to those with the C/C genotype ($RR=1.88$, 95% $CI=1.59-2.22$, $p=0.0035$). No effects of rs678849 genotype on withdrawal, craving, or addiction severity were observed.

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Author Contributions

The authors RCC, KMK, GAD, and WHB were responsible for the study concept and design. KMK, GAD, and WHB were responsible for sample acquisition and characterization. RCC conducted the bioinformatic and statistical analysis. RCC drafted the manuscript. All authors critically reviewed content and approved final version for publication.

Conflicts of Interest

Dr. Kampman receives grant support from Indivior and Braeburn Pharmaceuticals, and is on the advisory board for Opiant Pharmaceuticals. The other authors declare no potential conflicts of interest.

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Conclusions—A polymorphism in *OPRD1* appears to be associated with both cocaine dependence and cocaine use during treatment in African-Americans. Follow-up studies to confirm the effect on cocaine use are warranted.

Keywords

Cocaine; addiction; dependence; delta-opioid receptor; genetics

1. INTRODUCTION

Cocaine dependence affects millions of people worldwide and the underlying molecular mechanisms of the disorder are not completely understood. We previously reported an association analysis of six *OPRD1* variants in cocaine dependent individuals and healthy controls. A single intronic variant, rs678849, was associated with cocaine dependence in African-Americans but not European-Americans; the minor T allele was more common in controls than in cases (Crist et al., 2013a). Genotype at rs678849 has also been associated with other addiction phenotypes. The polymorphism predicts the efficacy of methadone and Suboxone in treating opioid dependence in African-Americans (Crist et al., 2013b). In an earlier study of European-Americans, haplotypes containing rs678849 were associated with opioid dependence (Zhang et al., 2008). Two other variants in *OPRD1* were also nominally associated with cocaine dependence in that population (Zhang et al., 2008).

Although rs678849 has been associated with cocaine dependence in African-Americans, the effect of the variant on addiction severity phenotypes has not been studied within a cocaine dependent population. To address this issue, we studied the effect of rs678849 genotype on cocaine abstinence as measured by urine drug screens in cocaine dependent African-Americans enrolled in a randomized trial of topiramate versus placebo.

2. MATERIALS AND METHODS

2.1 Subjects

The methods of the topiramate study have been previously described (Kampman et al., 2013). Briefly, subjects were treatment-seeking men and women with comorbid cocaine and alcohol dependence and were recruited at the University of Pennsylvania Treatment Research Center. Diagnoses of cocaine and alcohol dependence were made using the Structured Clinical Interview for DSM IV (SCID-IV; First et al., 1996). Drinking was assessed by Timeline Followback (TLFB; Sobell and Sobell, 1995) and subjects were required to meet the following drinking criteria: 1) drinking within 30 days of enrollment, 2) reporting a minimum of 48 standard alcoholic drinks for women and 60 standard drinks for men in a consecutive 30-day period over the 90-day period prior to enrollment, and 3) reporting 2 or more days of 5 or more drinks in males and 4 or more drinks in females in this same pre-treatment period. Subjects meeting DSM-IV criteria for dependence on additional drugs except nicotine and cannabis were excluded. Psychiatric exclusion criteria included psychosis, dementia, and the use of other psychotropic medications. All subjects signed informed consent prior to participation in the trial, after an investigator explained to them the

study procedures. The study was reviewed and approved by the Institutional Review Board (IRB) of the University of Pennsylvania.

2.2 Data Collection

Subjects began psychosocial therapy for up to three weeks and were then screened for abstinence from both cocaine and alcohol three times during the week prior to randomization (week 1). Abstinence during this period was defined as three consecutive days of abstinence determined by TLFB self-report and confirmed by urine drug screens for benzoylecgonine using fluorescent polarization assays for cocaine and breathalyzer tests for alcohol. Samples containing equal to or greater than 300 ng/ml of benzoylecgonine were considered positive. Eligible subjects were randomized to either topiramate or placebo at the start of week 2 and continued pharmacological treatment for 13 weeks. Weekly individual cognitive-behavioral therapy (CBT) was also provided for both treatment groups. Cocaine abstinence was assessed three times per week during treatment as described above. Abstinent weeks were defined as weeks in which a patient was abstinent at all three assessments. Alcohol use during the trial was assessed by TLFB self-report.

The Addiction Severity Index (ASI; McLellan et al., 1992) was used to assess the severity of addiction-related problems at randomization and three other times during the trial. The Minnesota Cocaine Craving Scale (MCCS) was used to measure cocaine craving intensity (MCCS-I), cocaine craving frequency (MCCS-F) and cocaine craving duration (MCCS-D) at the start of treatment (Halikas et al., 1991). Cocaine withdrawal symptoms were also measured using the Cocaine Selective Severity Assessment (CSSA) at the start of treatment (Kampman et al., 1998).

2.3 Genotyping and statistical analysis

Genotyping of rs678849 was performed using a Taqman SNP Genotyping Assay as previously described (Crist et al., 2013a). Due to the small number of individuals with the T/T genotype ($n = 3$), the T/T and C/T genotype groups were combined and compared to patients with the C/C genotype. Chi-square tests were used to compare the percentages of male patients, the most frequent route of cocaine administration, cocaine positive baseline urine tests, abstinence from cocaine over the week prior to randomization, and the percentage of days spent drinking during the trial. The Kolmogorov-Smirnov test was used to determine if the data sets for the ASI components, MCCS, CSSA, age, abstinent weeks, years of cocaine use, and days of cocaine use in the last 30 days were normally distributed (Massey, 1951). Age, years of cocaine use, years of alcohol use to intoxication, days of alcohol use to intoxication in the last 30 days, MCCS, ASI-medical, and ASI-alcohol were normally distributed and analyzed by student's t-tests. All other data were analyzed by Wilcoxon rank-sum tests. A generalized estimating equation (GEE) was used to determine the associations between rs678849 genotype and abstinence when binary cocaine abstinence data for weeks 1 through 14 were taken as repeated measures. The GEE also included the effects of time, age, sex, and treatment group. Results are reported as relative risks (RRs) with 95% confidence intervals. In all analyses, missing urine tests were counted as positive.

3. RESULTS

Only African-Americans from the topiramate study were analyzed since the previous associations between rs678849 and cocaine dependence were observed in that ethnic group but not European-Americans. DNA samples were available for 105 African-American patients. The demographic information for patients carrying the T allele (C/T or T/T genotypes) and patients with the C/C genotype is provided in Table 1. The two genotypic groups did not have statistically significant differences in the percentage of male patients, age, days of alcohol use to intoxication in the last 30 days, years of alcohol use to intoxication, most frequent route of cocaine administration, days of cocaine use in the last 30 days, or years of cocaine use.

During the trial, patients received psychosocial therapy for up to three weeks and then cocaine use was assessed by three urine drug screens in the week before randomization. Patients carrying the T allele were significantly more likely to submit a cocaine negative urine (70.0%) for the first drug screen compared to patients with the C/C genotype (47.7%, $p = 0.026$). T allele carriers were also more likely than C/C patients to abstain from cocaine for the entire week proceeding treatment randomization (55.0% vs 24.6%, $p = 0.0017$) and had more abstinent weeks overall (3.2 ± 3.9 vs 5.6 ± 4.6 , $p = 0.0085$). A generalized estimating equation was used to determine the effect of rs678849 genotype on cocaine use when weekly abstinence data were taken as repeated measures. Patients carrying the T allele were more likely to have abstinent weeks than patients with the C/C genotype (RR=1.88, 95% CI=1.59–2.22, $p = 0.0035$) (Figure 1). The effect of time was also significant; the percentage of patients with abstinent weeks decreased over the course of the trial ($p = 1.4 \times 10^{-5}$) (Figure 1). No significant effects were observed for age, sex, or treatment group. There was also no effect of genotype on the percentage of days spent drinking during the trial.

Prior to randomization, patients were interviewed using the ASI, which encompasses seven different problem domains. No significant differences were found between the genotype groups for the composite scores in any of these domains (Table 1). Craving and withdrawal measurements were also measured by the MCCS and CSSA, respectively. No significant effect of rs678849 genotype was observed for either of these metrics (Table 1).

4. DISCUSSION

Although rs678849 has been associated with a number of phenotypes, the underlying effects of the polymorphism are unknown. Intronic variants may affect gene expression by altering *cis* regulatory elements or they may be associated with alternative splicing. The gene expression hypothesis for rs678849 has indirect support from studies of DOR and neurodegeneration markers. The rs678849 variant has been associated with both regional brain volume and the ratio of tau to beta-amyloid in cerebral spinal fluid from people of European descent (Roussotte et al., 2014). Beta-amyloid is a marker of neurodegeneration and mice treated with a DOR antagonist demonstrate reduced beta-amyloid accumulation (Teng et al., 2010). Treatment of cell lines with DADLE, a DOR agonist, has the opposite effect (Ni et al., 2006). *In vitro* analyses have also shown that the minor allele of rs1042114,

a common non-synonymous variant in *OPRD1*, results in reduced levels of DOR at the cell surface (Leskela et al., 2009; Sarajarvi et al., 2011). The allele was also associated with a decrease in beta-amyloid, supporting the idea that a decrease in the receptor at the cell surface leads to a reduction in amyloid precursor protein metabolism (Sarajarvi et al., 2011). These data suggest that the connection between rs678849 genotype and tau/beta-amyloid ratio, as well as associations with cocaine dependence phenotypes, could be the result of changes in DOR expression levels. Additional research will be necessary to test these hypotheses *in vivo*. The rs678849 polymorphism has also been identified as an expression-QTL for *PHACTR4*, suggesting that this gene may also be involved in the downstream effects of rs678849 (<http://eqtl.uchicago.edu>).

While the direct functional consequences of rs678849 genotype are not clear, our results show an association between the SNP and abstinence from cocaine during a trial of topiramate. One potential caveat of these findings is the relatively small number of patients ($n = 105$) included in the analysis. Smaller sample sizes increase the risk of differences based on genotype being the result of random chance. Although the smaller of the two genotypic groups still contained 40 patients, replication studies in larger populations will be needed to verify the effect of the variant on cocaine use during treatment. Another potential caveat would be the presence of comorbid psychiatric disorders and addictions that were not part of the exclusion criteria (e.g., cannabis dependence, nicotine dependence, depression, anxiety). Although none of these disorders have been associated with rs678849 to date, there is still the possibility that they occur at different rates in the two genotypic groups and could affect the rates of cocaine abstinence.

One explanation for the observed effect is that rs678849 affects levels of either craving or withdrawal in cocaine addicts. Both factors are integral in driving continued use of the drug and relapse following periods of abstinence. Reductions in one or both of these symptoms in patients carrying the T allele might explain why they were able to use less cocaine over the 14 week trial than patients with the C/C genotype. However, there was no statistical difference in the measurements of either craving or withdrawal at the start of treatment based on rs678849 genotype. The lack of significance may be the result of sample size. These scoring systems also may not adequately assess certain types of symptoms (e.g. cue-induced craving) that may still be affected by rs678849. Another possibility is that T allele carriers do not receive the same amount of reward from cocaine use as other users and are therefore less driven to use cocaine overall.

Any of these hypotheses may also explain our previous finding that T allele carriers have reduced risk of cocaine dependence. The idea that genetic variation might be able to stratify patient drug use and also be associated with case-control status is not new. Polygenic risk for alcoholism is strongly correlated with the number of alcoholic drinks consumed per week across the general population (Clarke et al., 2016). A polymorphism in the *CHRNA3/CHRNA5* locus is also associated with both nicotine dependence and the number of cigarettes smoked per day (Caporaso et al., 2009). Our findings suggest that similar effects may be present in regards to cocaine dependence. The T allele of rs678849 may reduce susceptibility to cocaine dependence, while also being associated with reduced use of cocaine during treatment.

Acknowledgments

Role of Funding Sources

Financial support is gratefully acknowledged from NIDA career development award K01DA036751 (P.I.: R. Crist) and NIDA grant R21DA036808 (P.I.: W. Berrettini). NIDA had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

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Highlights

- Cocaine dependent African-Americans were randomized to topiramate or placebo.
- Cocaine use in both groups was associated with rs678849, an *OPRD1* polymorphism.
- Rs678849 was previously associated with cocaine dependence in African-Americans.

Figure 1.

The percentage of cocaine dependent African-American subjects who were abstinent from cocaine during each week of a 14-week randomized trial of topiramate versus placebo. Subjects are separated based on rs678849 genotype. T/T subjects were combined with C/T subjects due to the low number of T/T subjects ($n = 3$). Abstinence was assessed three times per week by urine drug screens for benzoylecgonine. Samples containing equal to or greater than 300 ng/ml of benzoylecgonine were considered positive. Abstinent weeks were defined as weeks in which a subject had negative urines at all three assessments. Missing tests were counted as positive. Time, age, sex, and treatment group were analyzed as covariates. Patients carrying the T allele ($n = 40$) were more likely to have abstinent weeks than patients with the C/C genotype ($n = 65$; $RR=1.88$, $95\% \text{ CI}=1.59\text{--}2.22$, $p = 0.0035$).

Table 1

Comparison of demographic and addiction-related characteristics by rs678849 genotype in cocaine dependent African-Americans treated with either topiramate or placebo.

		rs678849 Genotype		
		C/C	C/T+T/T	p-value
n (% male) *		65 (76.9%)	40 (82.5%)	0.50
Age †		45.6 ± 6.9	44.0 ± 5.5	0.20
Route of Administration *	Smoking	81.5%	92.5%	0.33
	Nasal	15.4%	7.5%	
	Other	3.1%	0%	
Days of Cocaine Use – Last 30 Days ‡		12.8 ± 7.5	11.5 ± 7.6	0.35
Years of Cocaine Use †		14.7 ± 7.9	15.0 ± 6.2	0.88
Days of Alcohol Use to Intoxication – Last 30 Days †		13.1 ± 8.1	15.3 ± 8.5	0.19
Years of Alcohol Use to Intoxication †		20.9 ± 10.0	20.9 ± 7.8	0.99
ASI – Alcohol †		0.51 ± 0.17	0.51 ± 0.20	0.64
ASI – Drugs ‡		0.25 ± 0.06	0.24 ± 0.08	0.33
ASI – Employment/Support ‡		0.76 ± 0.26	0.72 ± 0.27	0.46
ASI – Family/Social ‡		0.16 ± 0.19	0.15 ± 0.21	0.51
ASI – Legal ‡		0.03 ± 0.11	0.08 ± 0.16	0.19
ASI – Medical †		0.30 ± 0.38	0.20 ± 0.31	0.34
ASI – Psychiatric ‡		0.17 ± 0.20	0.11 ± 0.17	0.15
MCCS †		8.0 ± 4.9	9.8 ± 5.7	0.091
CSSA ‡		16.5 ± 12.6	18.5 ± 12.4	0.35
Cocaine Abstinent First Urine (%) *		47.7%	70.0%	0.026
Cocaine Abstinent First Week (%) *		24.6%	55.0%	0.0017
Cocaine Abstinent Weeks ‡		3.2 ± 3.9	5.6 ± 4.6	0.0027
Days of Alcohol Use (%) *		14.5%	13.9%	0.94

ASI = addiction severity index;

* χ^2 test;

† Student's t-test;

‡ Wilcoxon rank-sum test.

Other routes of cocaine administration include patients who predominantly administered cocaine orally (n = 1) or by intravenous injection (n = 1).