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Pharmacogenetic role of *dopamine transporter (SLC6A3)* variation on response to disulfiram treatment for cocaine addiction

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Abstract

Background and Objectives—Disulfiram has been beneficial in treating cocaine addiction in several studies. Patients with two *SLC6A3 (DAT)* rs28363170 10-repeat alleles who have with genetically high dopamine transporter (DAT) levels may benefit from increased dopamine levels resulting from disulfiram treatment.

Methods—After stabilization for two weeks on methadone, 70 cocaine and opioid co-dependent patients were randomized into disulfiram and placebo groups for 12 weeks of treatment. We genotyped the *SLC6A3 (DAT)* 40 bp 3' UTR VNTR variant and evaluated its role in moderating disulfiram efficacy for cocaine dependence.

Results—Among the 10,10-repeat genotype group, cocaine positive urines dropped from 78% to 48% and from 80% to 75% among the 9-repeat carrier group in the disulfiram group ($p = 0.0001$, with an effect size of 0.09). No difference was observed in cocaine positive urines in the placebo group between the 10,10-repeat genotype and the 9-allele carrier patients.

Conclusions and Scientific Significance—We found that patients with genetically higher DAT levels had better treatment outcomes with disulfiram pharmacotherapy of cocaine dependence than those with lower DAT levels.

INTRODUCTION

Substance abuse is a chronic and critical problem in the world today. Cocaine addiction has significant economic impact both on the individual affected and on society. Estimates for the number of cocaine users worldwide ranges from 14 to 21 million.^{1,2} Cocaine is one of the most commonly abused drugs in the United States with approximately 1.5 million

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

individuals age 12 and up currently using cocaine (0.6%; 1.4% in young adults aged 18–25) and 800,000 individuals dependent on cocaine in 2014.^{3,4} There is currently no FDA-approved pharmacotherapy for cocaine addiction.

Disulfiram (also known as Antabuse) is an FDA approved medication for treating alcohol use disorder and more recently has shown promise in treating cocaine use disorder.^{5–10} The mechanism behind the pharmacotherapeutic effect of disulfiram for cocaine is not entirely understood, but one potential mechanism is that disulfiram inhibits the oxidoreductase dopamine β -hydroxylase (DBH, encoded by the *DBH* gene) which converts dopamine (DA) to norepinephrine (NE).^{11,12} Inhibiting DBH decreases the amount of DA converted to NE, therefore increasing the amount of DA available for signaling in the brain.^{13,14}

The *SLC6A3 (DAT1)* gene encodes the membrane-spanning dopamine transporter (DAT) that performs re-uptake of dopamine from the synapse into the presynaptic neuron. Cocaine also binds to DAT in the brain.¹⁵ A functional polymorphism (rs28363170) is found in of *SLC6A3* in exon 15, the exon coding the 3'-untranslated region (UTR) that consists of a variable number tandem repeat (VNTR) of 40 base pair tandem repeats.¹⁶ The most common *SLC6A3* 3' UTR VNTR alleles are the 9- and 10-repeat alleles.^{17,18} In cellular reporter constructs and *in vivo* expression was greater for the 10-repeat allele than the 9-repeat allele, demonstrating that the VNTR polymorphism affects *SLC6A3 (DAT1)* expression.^{16,19–21}

In this study, we focus on the 3' UTR VNTR (rs28363170) of the *SLC6A3 (DAT1)* gene. We hypothesized that the increase in striatal dopamine from disulfiram would have the greatest effect on patients with high levels of dopamine transporter protein, that is those with the 10,10-repeat genotype, as these patients may have lower basal dopamine levels in the synapse. The 10,10-repeat genotype patients are predicted to experience a greater change in synaptic dopamine levels with the introduction of disulfiram and would display a greater therapeutic response to disulfiram treatment. Conversely, patients carrying a 9-repeat allele would have higher basal dopamine levels and would essentially have their dopamine transporter saturated. Therefore, disulfiram would not increase synaptic dopamine levels in the 9-repeat carrier patients as it would in patients with the 10,10 genotype, rendering disulfiram less efficacious in the 9-repeat carrier patients than in the 10,10 genotype patients.

MATERIALS AND METHODS

Participants

Patients were enrolled at Yale University ($n = 40$) from 2005 to 2006 and at Baylor College of Medicine ($n = 53$) from 2006 to 2008 in a 12-week clinical trial “Pharmacogenetics of Disulfiram for Cocaine” (NCT ID: NCT00149630, NIDA-18197–2, NCT00149630) (Table 1). This is the same patient cohort as reported by us in previous studies.^{22–26} There was an initial two-week screening period in which patients were assessed for cocaine and opioid dependence based upon DSM-IV criteria. Patients were stabilized on methadone and urine toxicologies for cocaine and opioid metabolites were obtained three times per week. Eight patients dropped out during the two-week screening period. For assurance that only active cocaine users were included in the analyses, only patients who had at least one cocaine

positive urine sample during the 12-week study were included, thus excluding 11 patients, leaving 74 patients remaining in the study. Indeterminate genotyping for 4 patients reduced the number of patients to 70. Two patients with an 8,10-repeat genotype and one 10,11-repeat genotype subject were excluded from the study as the expression levels of the 8 and 11-repeat alleles have not been determined, leaving 67 patients for this study.

All patients met DSM-IV criteria for opioid and cocaine use disorders based on interview by a clinical psychologist or psychiatrist, and baseline psychiatric assessment using the MINI.²⁷ All patients also completed the Addiction Severity Index at baseline.²⁸ Women of childbearing age were included in the study so long as the initial urine pregnancy test was negative, appropriate contraception was used to prevent pregnancy, and the subject agreed to monthly pregnancy tests while in the study. Further exclusions were other potential comorbidities such as major psychiatric disorders (bipolar, schizophrenia, psychosis), major medical illness not controlled by pharmacotherapy, other comorbid substance abuse/dependence (not including tobacco use), current suicidality, and inability to consent to the study. Ethnicity was recorded based on self-report by the patients. All patients signed the administered informed consent, which was approved by both the Yale and Baylor College of Medicine IRBs and the Research and Development Committee of the MEDVAMC, to participate in the study and consent specifically to genetic testing.

Study Design and Medications

All patients were stabilized on methadone maintenance at 60 mg daily. Patients were and randomly assigned to placebo or disulfiram treatment groups. Supervised urine samples from all patients in the study were collected three times weekly and tested for the cocaine metabolite, benzoylecgonine, to gauge cocaine usage during the 12-week trial. Disulfiram was administered at a dose of 250 mg per day for the treatment group. Both groups (disulfiram and placebo) were provided with weekly cognitive behavioral therapy.

Genotyping

DNA was purified from saliva samples as previously described.²² The dopamine transporter gene *SLC6A3* (*DAT1*) 40 bp VNTR polymorphism (rs28363170) was genotyped using PCR to determine the number of repeats on each allele.²⁹ PCR amplification yielded fragment sizes ranging from 208 bp (3 repeats) to 528 bp (11 repeats). PCR was performed using Platinum® Taq DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA) on an Eppendorf Mastercycler Pro S (Eppendorf, Hamburg, Germany). Samples were amplified at 95° for 5 minutes, followed by 25 cycles of 95° for 30 seconds, 60° for 30 seconds, and 72° for 30 seconds, with a final extension step of 72° for 7 minutes. DNA fragment size was determined by electrophoresis in a 4–20% polyacrylamide TBE gel.

The sex of each subject was confirmed by a PCR assay of the SRY gene on the Y chromosome.³⁰ Population substructure was determined using TaqMan® SNP genotyping of ancestry informative markers (AIMs).²² Genotype analysis was performed by staff who were unaware of the patients' clinical status.

Statistical Analysis

The four treatment by genotype groups were compared for baseline differences demographics using χ^2 or t-test as appropriate. The 9-repeat carrier group consisted of 25 patients with a 9,10-repeat genotype and eight patients with a 9,9-repeat genotype. The 10,10-genotype group was comprised of 34 10,10-repeat genotype participants. We compared the two *SLC6A3* (*DAT1*) genotype groups (0 = 9,9, 9,10 genotypes, 1 = 10,10 genotype) using R version 2.9.1 (R_Development_Core_Team, 2009). An analysis of variance (ANOVA) was used to analyze the continuous variables in the demographic data, while a Fisher's exact test was used to analyze any categorical variables. To examine the response to disulfiram to placebo moderated by *SLC6A3* (*DAT1*) genotype over time, in our primary analyses a repeated measures analysis of variance (ANOVA) was used to analyze the number of cocaine-positive urines per total number samples collected for each two-week period. Missing cocaine urines were considered as negative urines. We compared condition (whether a patient was taking disulfiram or placebo), *SLC6A3* (*DAT1*) genotype groups (9-repeat carrier group versus 10,10-repeat genotype group), time (each two-week period), the interaction between condition and time, and the interaction between condition and *SLC6A3* (*DAT1*) genotype groups. An unbalanced repeated measures ANOVA was performed for all individuals ($n = 67$). In addition, we performed a repeated measures ANOVA on all the individuals who had complete data ($n = 54$; data not shown). Similar results were obtained from each analysis. As a secondary analysis, the number of cocaine-negative urines per total possible urines over each two-week period was used to derive percent negative urines using gender and population structure as covariates in the statistical model. Missing cocaine urines were considered as positive in this analysis. There were no differences in the number of missing urines for each participant between the two genotype groups for any of the time periods not overall for the 12-week study.

The cohort was compared as described³¹ against the Centre d'Etude du Polymorphisme Humain–Human Genome Diversity Panel (CEPH-HGDP) samples (1035 patients of 51 populations; kindly provided by Oscar Lao.³² Population structure was calculated by genotyping ten ancestry informative markers (AIMs). The STRUCTURE 2.3.3 software (58,59) was run with four ancestral populations ($K = 4$), a burnin period of 100,000 iterations, and 1 million Markov chain Monte Carlo replications after burnin to determine population substructure. Possible confounding effects were corrected for by including the proportion of each subject from the founder populations as well as gender and site effects as covariates in the model. Previously, it was demonstrated that 94.6% of the maximum informativity value is obtained using these ten AIMs.³²

All analyses were corrected for any possible confounding effects, by including the proportion of each subject from the founder populations as well as gender as covariates in the model. Analyses were performed with the two *SLC6A3* (*DAT1*) groups. Results are presented for all patients meeting our inclusion criteria ($n = 67$). Corrections for multiple testing were performed to evaluate experiment-wise significance by applying the Bonferroni correction for the 25 variants for which this data set has been examined thus far^{22,24,26,33,34}, excluding the SRY assay and the ten ancestry informative markers, we have examined using this data set ($p < 0.05/25 = 0.0020$). We calculated effect size as a partial eta-squared

statistic using SNP variance over the residual variance. The three general levels of cutoffs for effect size are as follows: 0.01 is a small effect, 0.06 is a medium effect, and 0.14 is a large effect.³⁵ Hardy-Weinberg equilibrium was not violated for the *SLC6A3* (*DAT1*) genotypes.

RESULTS

Baseline characteristics by treatment and *SLC6A3* (*DAT1*) genetics

The 67 patients for whom *SLC6A3* (*DAT1*) 40 bp VNTR genotypes were available were randomized into two groups: 32 to disulfiram and 35 to placebo. Nine of the 34 patients in the 9-repeat carrier group had a 9,9-repeat genotype and 25 the 9,10-repeat genotype. Thirty-four patients had the 10,10-repeat genotype. The total group of 67 patients consisted of primarily Caucasian males (76.1%) with a mean age of 38.9 ± 10.4 (SD) years. Thirty-seven patients (55.2%) had been previously treated with methadone maintenance and patients had a mean of 13.3 ± 8.3 (SD) years of heroin use. The duration of cocaine usage amongst the patients was a mean of 12.7 ± 8.0 (SD) years and patients used cocaine a mean of 19.2 ± 8.1 (SD) days in the month before entering the study. Twenty-eight patients (42%) reported a history of alcohol abuse and 36 (53.7%) reported marijuana use. As shown in Table 1, the only two differences among the demographic variables were for sex in both the placebo and disulfiram groups (placebo: point-wise $p = 0.015$; disulfiram, point-wise $p = 0.014$) and employment ($p = 0.003$) in the placebo group. No significant baseline differences in any other clinical characteristics were observed ($p < 0.05$).

Initially, the 67 patients who had been genotyped for the *SLC6A3* (*DAT1*) 40 bp VNTR polymorphism were divided regardless of genotype into two treatment groups, those that received disulfiram and those that had received placebo (Figure 1). The reduction in cocaine use between the disulfiram versus placebo patients over the 12-week trial was significant point-wise ($F = 8.68$; $df = 1, 348$; $p = 0.0034$, with an effect size of 0.02). The disulfiram group had a reduction in cocaine positive urines over the 12-week study from 79% at study week 1–2 to 63% at study week 11–12, while the placebo group only decreased slightly from 80 to 75% over the same time period.

When treatment outcome was evaluated using the number of cocaine-negative urines over the course of the study, similar results were obtained as when using cocaine-positive urines. The increase in cocaine abstinence between the disulfiram versus placebo patients over the 12-week trial was significant point-wise ($F = 7.82$; $df = 1, 362$; $p = 0.0053$, with an effect size of 0.02). The disulfiram group had an increase in cocaine-negative urines over the 12-week study from 22% at study week 1–2 to 33% at study week 11–12, while the placebo group only increased slightly from 19 to 23% over the same time period.

Cocaine treatment outcomes by *SLC6A3* (*DAT1*) genotype

The 67 patients were divided into two genotype groups: the 34 patients in the 9-repeat carrier group, and the 33 patients in the 10,10-repeat genotype group. After grouping based on genotype, the genotype groups were divided based on treatment group (disulfiram or placebo). Cocaine positive urines decreased from 78% to 48% among the 10,10-repeat

genotype group and from 80% to 75% for the 9-repeat carrier group in the disulfiram group ($F = 15.23$ df = 1, 169; $p = 0.0001$, with an effect size of 0.09; Figure 2). No difference was found between the 10,10-repeat genotype group and the 9-repeat carrier group in the placebo group, with cocaine positive urines decreasing from 78% to 70% and from 85% to 81% for the two genotype groups, respectively.

The evaluation of treatment outcome evaluated by the number of cocaine-negative urines also gave similar results to that obtained using cocaine-positive urines. Cocaine-negative urines increased from 26% to 39% among the 10,10-repeat genotype group and increased from 19% to 26% for the 9-repeat carrier group in the disulfiram group ($F = 14.8$; df = 1, 183; $p < 0.0001$, with an effect size of 0.08). No difference was found between the 10,10-repeat genotype group and the 9-repeat carrier group in the placebo group, with cocaine-negative urines increasing from 22% to 26% and increased from 16% to 19% for the two genotype groups, respectively.

DISCUSSION

Similar to our previous reports^{22–26} and others^{5–7,36–38}, we found a reduction in cocaine-positive urines after pharmacotherapy with disulfiram compared to placebo. Since cocaine users may be hypodopaminergic, disulfiram may have been efficacious since it increases dopamine levels at the synapse.³⁹

Our group and others previously have shown that the response to disulfiram therapy for cocaine dependence can be moderated by genetic variation in specific genes of the dopaminergic pathway^{22,26,37}, as well as by several other genes hypothesized to be involved in disulfiram pharmacotherapy.^{22–26} In the present study, we have demonstrated that patients with the 10,10-repeat genotype of the *dopamine transporter SLC6A3 (DAT1)* gene had better treatment outcomes with disulfiram pharmacotherapy of cocaine use disorder than did those patients carrying a 9-repeat allele. This supports our hypotheses that the 10,10-repeat genotype subjects would respond to disulfiram treatment to a greater extent than the 9-repeat carrier subjects.

In cellular reporter constructs, expression was higher when the construct contained the 10-repeat allele of the *SLC6A3 (DAT1)* 3'-UTR than when it contained the 9-repeat, demonstrating that the VNTR polymorphism affects *DAT1* expression.¹⁹ Additionally, two *in vitro* studies demonstrated that the 10-repeat allele is associated with increased DAT expression.^{16,19} Similarly, two *in vivo* studies showed that the 10,10 genotype was associated with greater *SLC6A3 (DAT1)* mRNA expression²¹ and higher DAT protein availability in the striatum.⁴⁰ In contrast, one *in vivo* study observed the opposite; that 9-tandem repeat allele carriers had higher DAT availability compared with the 10,10 homozygotes.

The better treatment outcome among the 10,10-repeat genotype group may be due to dopamine availability at the level of the synapse. A higher level of dopamine transporter protein is associated with the 10,10-repeat genotype in comparison to the 9-repeat carrier genotypes⁴⁰, which may lead to less dopamine availability at the synapse in 10,10-repeat

genotype patients. Therefore, disulfiram, which would increase levels of dopamine in the synapse, may have been effective only in those patients with lower basal dopamine availability within the synapse. In contrast, disulfiram may have shown no response amongst the 9-repeat carrier group since the 9-repeat carrier group potentially may have a higher basal dopamine due to lower reuptake, causing disulfiram to have a smaller effect due to higher basal levels of dopamine within the synapse. A small reduction in cocaine positive urines was found in the 9-repeat carriers treated with disulfiram, however this was a much smaller decrease than was found with 10,10-repeat genotype group.

Genetic variation of *SLC6A3 (DAT1)* has also been associated with cocaine abuse^{41,42} although other studies have failed to confirm these findings [e.g., ⁴³]. Additional studies have shown that genetic variation of *SLC6A3 (DAT1)* has been associated with differing subjective effects. For instance, *SLC6A3 (DAT1)* 9-repeat carriers have an increased susceptibility to cocaine-induced paranoia.⁴⁴ Individuals with the 10,10 *SLC6A3 (DAT1)* genotype reported lower subjective responses to cocaine administration for “high”, “any drug effects”, “anxious”, and “stimulated” than did 9-repeat carriers.²⁹ Furthermore, the 3’ UTR 9,9-repeat genotype was associated with protection against risky behaviors including illicit drug use⁴⁵, and with reduced subjective response to amphetamine administration⁴⁶ compared to the other *SLC6A3 (DAT1)* genotypes.

Cocaine-induced paranoia in whites has been associated with allelic variation in the *SLC6A3 (DAT1)* gene, with the 9-repeat carriers having an increased susceptibility to cocaine-induced paranoia.⁴⁴ This aversive effect in the 9-repeat carriers was hypothesized be due to lower levels of dopamine transporter protein as more basal dopamine would have been available within the synapse, which would contribute to the cocaine-induced paranoia. It may be possible that disulfiram treatment of 10,10-repeat genotype patients may augment the lower basal dopamine availability to levels similar to that found in 9-repeat carriers, leading to increased paranoia among the 10,10-repeat genotype group and, therefore, decreased usage of cocaine. However, this relationship would have to be examined further.

The functional VNTR polymorphism in the 3’UTR of the *SLC6A3 (DAT1)* gene has been associated with several neuropsychiatric disorders and with pharmacotherapy response. The disorders include attention deficit hyperactivity disorder, Parkinson’s disease, alcoholism, and schizophrenia, although the relationship between this gene and these disorders remains unclear.¹⁹ There also is an increased prevalence of the 9-repeat allele in alcoholics displaying withdrawal seizures or delirium compared to non-alcoholics.⁴⁷ For pharmacotherapy response, antidepressant therapy response is less rapid in carriers of the 9/9-repeat genotype compared to those with the 9/10 or 10,10-repeat genotypes, perhaps due to lower basal dopamine levels.⁴⁸

In our primary analysis, treatment effectiveness was evaluated in a similar fashion to be congruent with our earlier studies using the percentage of cocaine-positive urines over the course of the clinical trial in our previous studies^{22,25,31,49,50} As a secondary analysis, we evaluated the number of cocaine-negative urines during each time period. The evaluation of treatment effectiveness using cocaine-positive urines effectively assigns missing urines as

being “cocaine-negative”, while the use of cocaine-negative urines implies the missing sample to be “cocaine-positive”.

This study has several limitations. The sample size was small and confirmative studies with larger cohorts will be necessary to validate these findings. Although these patients did not have current alcohol dependence, some of the patients had a history of alcohol abuse, which may have contributed to their cocaine usage. A 250 mg dose of disulfiram is unlikely to produce an aversive response to alcohol use in most individuals, but a decrease in alcohol use secondary to disulfiram treatment may have contributed to decreased cocaine use. Disulfiram may affect opioid usage and therefor may moderate cocaine usage; however, this is unlikely as disulfiram has not been shown to moderate opioid use in previous studies^{22,51}

In addition, disulfiram may increase dopamine through a variety of mechanisms not directly studied here. Diethyldithiocarbamate, a metabolite of disulfiram, is a potent copper chelator and disulfiram may affect the activity of other copper-dependent enzymes involved in the metabolism of dopamine. These would include tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of dopamine, DBH that coverts dopamine into norepinephrine in adrenergic neurons⁵², and the catabolic monoamine oxidases.⁵³

In summary, we interrogated the 3’UTR VNTR of the *dopamine transporter* gene (*SLC6A3* (*DAT1*)) as a potential mediator of response to disulfiram pharmacotherapy in patients with comorbid cocaine and opioid use disorders. We found that patients with the *SLC6A3* (*DAT1*) 10,10-repeat genotype responded to disulfiram treatment and had significantly reduced cocaine positive urines compared to patients carrying the 9-repeat allele. These findings suggest that a patient’s *SLC6A3* (*DAT1*) genotype may identify individuals with cocaine use disorder for whom disulfiram may be an effective pharmacotherapy. These findings may improve personalized medicine and help develop hypotheses for future therapies for cocaine use disorder.

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FIGURE 1.

Response to disulfiram and placebo treatment ($n = 70$). Cocaine urine toxicology screens across the 12-week trial are presented for each two-week time period (percent positive urines). The disulfiram (250 mg/day) treatment group (dashed line; $n = 32$) and the placebo treatment group (solid line; $n = 35$) are shown and at each time point errors are displayed as standard error bars. Data is from all study participants.

FIGURE 2.

Interaction of the *SLC6A3* (*DAT1*) genotype with percentage of cocaine positive urines for the placebo versus disulfiram treatment groups. Urine toxicology screens are shown for each two-week time period across the 12-week trial for the placebo (dashed lines) versus disulfiram (250 mg/day) (solid lines) treatment groups. 9-repeat carrier groups (squares; disulfiram, $n = 17$; placebo, $n = 17$) and 10,10-repeat genotype groups (diamonds; disulfiram, $n = 15$; placebo, $n = 18$) and their response to disulfiram and placebo treatments are displayed. Standard error bars are shown for each time point.

TABLE 1. Demographic and clinical characteristics by treatment group and *SLC6A3 (DAT1)* genotype

Characteristic	Placebo		Disulfiram	
	9,9/9,10	10,10	9,9/9,10	10,10
<i>n</i>	17	18	17	15
Male (%)	88	50 ^a	82	40 ^b
Caucasian (%)	76	72	76	80
Employed (%)	82	33 ^c	76	60
Age years (SD)	41.4 (10.7)	37.6 (10.7)	39.8 (8.8)	36.7 (11.6)
Cocaine last 30 days (SD)	20.2 (7.7)	19.9 (8.0)	19.5 (7.3)	16.9 (9.8)
Cocaine years (SD)	14.6 (7.9)	11.9 (8.5)	13.7 (7.4)	10.40 (7.1)
Heroin years (SD)	14.25 (8.7)	12.2 (9.6)	15.1 (6.07)	11.1 (8.7)
Alcohol use (%)	47	33	35	40
Marijuana use (%)	59	50	53	67
Past Methadone (%)	65	56	59	40

Statistical analyses were performed within treatment groups by genotype.

^aPlacebo x Sex: point-wise $p = 0.015$.

^bDisulfiram x Sex: point-wise $p = 0.014$.

^cPlacebo x employed: point-wise $p = 0.003$.