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Pharmacogenetic Randomized Trial for Cocaine Abuse: Disulfiram and *dopamine β-hydroxylase*

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Abstract

Background—Disulfiram has been an effective cocaine addiction pharmacotherapy, and one of its possible mechanisms of efficacy is through copper chelation and inhibition of an enzyme involved in catecholamine metabolism, dopamine β-hydroxylase (DβH), which converts dopamine to norepinephrine. A variant in the gene encoding DβH leads to reduced DβH activity and as such, disulfiram may not be an effective treatment of cocaine dependence for individuals with this variant. This study explored that potential matching.

Methods—Seventy-four cocaine and opioid co-dependent (DSM-V) subjects were stabilized on methadone for two weeks and subsequently randomized into disulfiram (250 mg/day, N=34) and placebo groups (N=40) for 10 weeks. We genotyped the *DBH* gene polymorphism, −1021C/T (rs1611115), that reduces DβH enzyme levels and evaluated its role for increasing cocaine free urines with disulfiram.

Results—Using repeated measures analysis of variance, corrected for population structure, disulfiram pharmacotherapy reduced cocaine positive urines from 80% to 62% (p = .0001), and this disulfiram efficacy differed by *DBH* genotype group. Patients with the normal DβH level genotype dropped from 84% to 56% on disulfiram (p = .0001), while those with the low *DBH* level genotype showed no disulfiram effect.

Conclusions—This study indicates that a patient's *DBH* genotype could be used to identify a subset of individuals for which disulfiram treatment may be an effective pharmacotherapy for cocaine dependence.

Keywords

Genes; disulfiram; polymorphism; cocaine; treatment; dependence

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Introduction

Cocaine dependence is common with over 1.5 million actively cocaine dependent people in 2011 who have substantial social and economic morbidity from it, but it has no FDA approved pharmacotherapy (1, 2)., In methadone maintenance programs rates of cocaine use range from 30% to 50% and lead to poorer outcomes and higher incidence of HIV risk behaviors (3–13). Although a number of innovative pharmacological approaches have had limited success in reducing cocaine use, (e.g.,(14–16), disulfiram has shown some initial promise in treating cocaine dependence in both non opioid-dependent (17–19) and opioid-dependent cocaine abusers (20, 21).

Tailoring pharmacological treatment to a person's genetic background can enhance therapeutic response (22), increase compliance (23) and decrease drug toxicity (24–26). Since cocaine addiction has a strong genetic basis, with the vulnerability to develop an addiction estimated to be as high as 72% (27), pharmacotherapy of this relapsing brain disease may be better treated using a molecular genetics approach (28–31). Applying a molecular genetics approach to disulfiram may involve its inhibitory action on the copper-containing glycoprotein enzyme dopamine β-hydroxylase (DβH), which transforms dopamine to norepinephrine (32). Inhibiting DβH decreases peripheral and central norepinephrine levels, and increases dopamine levels (33, 34).

Based on twin and family studies, plasma levels of DβH vary between unrelated individuals (35–37). Some of these differences are due to polymorphisms in close proximity to the *DBH* gene. Indeed, studies link the C-1021T (−1021C>T) variant to differences in circulating DβH levels (38–41). The variant C-1021T is positioned ~1000 nucleotides upstream from the initiation codon of the *DBH* gene (41). Several studies indicate that the C-1021T variant is a functional polymorphism, which alters transcription and decreases plasma levels of DβH (41–44). This variant accounts for up to 52% of overall variation in the enzyme levels (41, 43–45). Individuals that are homozygous for the T allele have the lowest levels of plasma DBH activity. Variable DβH enzyme levels or activity is linked with a number of psychiatric disorders ranging from psychotic (46) to conduct disorders (47–51). The cerebrospinal fluid level of the dopamine metabolite homovanillic acid (HVA) is an indirect measure of monoamine concentration in the brain and is correlated with the *DBH* C-1021T genotype (52). Several, complementary mechanisms probably contribute to disulfiram's efficacy and interact with this polymorphism: increased cocaine aversion by causing dopamine receptor hypersensitivity, reversal of a dopaminergic deficiency and dysphoria by increasing dopamine production in noradrenergic neurons during withdrawal, and preventing relapse by lowering norepinephrine levels and attenuating signaling via adrenergic receptors. All of these mechanisms might be enhanced by genetically determined baseline levels of the enzyme DβH and will be returned to in the Discussion. However, no simple genetic association can be ascertained *a priori* for enhancing disulfiram's efficacy, and it requires direct testing in a clinical trial, as we have done.

Arguments can be made for disulfiram's efficacy in cocaine dependence being enhanced in individuals who have the C-1021T allele that is associated with normal DβH or low levels. However, the potential importance of this functional variant in treatment outcome merits testing. Thus, we tested this hypothesis of its importance in a placebo controlled randomized clinical trial of disulfiram at 250 mg daily by comparing disulfiram's efficacy at reducing cocaine abuse in methadone maintained patients with the CC genotype and normal DBH levels to those carrying the T allele and lower DβH levels.

Methods and Materials

Subjects

Our sample of 74 opioid and cocaine dependent subjects (10 African American, 8 Hispanic, 56 Caucasian) were drawn from a sample of 93 candidates who entered into a two week screening period for stabilization on methadone maintenance between 2005 to 2006 at Yale University (n =40) and then from 2006 to 2008 at the Baylor College of Medicine (n = 53). During these two weeks, we obtained thrice-weekly urine toxicologies for opiates and cocaine metabolites, and subjects needed to have at least one urine sample showing cocaine use for entry into the randomized clinical trial. Eleven subjects had all six urines free of cocaine and were excluded. Another eight subjects dropped out during this screening. All subjects met DSM-IV criteria for opioid and cocaine dependence after interview by a psychiatrist or clinical psychologist. Exclusions included a current diagnosis of other drug or alcohol physical dependence (other than tobacco), current major medical illness unstablized on medications, a history of major psychiatric disorder (psychosis, schizophrenia, bipolar), current suicidality, and an inability to read and understand the consent form. Women of childbearing age were included provided they had a negative urine pregnancy test, agreed to use adequate contraception to prevent pregnancy during the study, and agreed to monthly pregnancy tests. All participants signed an informed consent approved by Yale University and the Baylor College of Medicine Institutional Review Boards that gave specific consent for genetic studies. Ethnicity was based self-report of ethnic/cultural background of the subjects.

Study Design and Medications

The 74 subjects were randomly assigned one to one by computer to disulfiram 250 mg daily or placebo while stabilized on methadone maintenance at 60 mg daily. We enrolled methadone maintained subjects in order to maximize our treatment retention during this study, since primary cocaine abusers not in methadone treatment have had poor treatment retention with over half of the subjects leaving treatment within 3 months (17–19). Subjects ingested methadone daily, 7 days per week. Methadone was administered orally in a colored liquid and ingested at the dispensing window under observation of the dispensing nurse (except on Sundays, for which take home doses were provided). During induction onto methadone, participants initially received 25 mg of methadone, which was increased by 5 mg at each subsequent daily dosing until participants received a 60 mg maintenance dose. During the 10 weeks after stabilization, subjects attended the clinic daily for oral methadone administration with either disulfiram or placebo (lactose) dissolved in their liquid methadone. Double blinding of patients, providers and clinical staff and treatment assignment were maintained through the research pharmacy, and the individual patient's bottles liquid methadone looked and tasted identical with lactose added to both active and placebo. Clinical staff enrolled participants and advised them not to drink alcohol or use alcohol-containing products during the study. Supervised urine samples were obtained thrice weekly and tested for the presence of opiates and cocaine metabolite (benzoylecgonine) using an Olympus AU 640 Emit system (Olympus America Inc., Melville, NY) with a cut-off concentration of 300 ng/ml. We obtained saliva samples for genotyping. At study entry, we completed the Addiction Severity Index (ASI) on all subjects to assess baseline characteristics and to compare them across treatment and genotype groups (53). The ASI includes seven interviewer ratings of problem severity in medical, employment, legal, drug, alcohol, family and psychological problems. All participants received weekly manual-driven individual cognitive behavioral therapy and had excellent participation with less than 10% missed sessions across all groups (54). At the end of the study, participants either transferred to a local opioid maintenance program or underwent detoxification from methadone over a 4–6 week period.

Genotyping

DNA was purified from buccal cells. Briefly, 10 ml Scope mouthwash was swished in the subject's mouth for 60 seconds and recovered. Cells were isolated by centrifugation at 2,000×g for 5 min. DNA was isolated from the pellet using the Gentra Puregene Buccal Cell Kit (Qiagen, Valencia, CA) following the manufacturer's recommendations. DNA was rehydrated in 300 μl DNA hydration solution.

Genotypes were determined using 5'-fluorogenic exonuclease assays (TaqMan®, Applied Biosystems, Foster City, CA). The *DBH* −1021C/T genetic variant was genotyped using the TaqMan® primer-probe sets (Applied Biosystems) *DBH* rs1611115, Assay ID C_2535786_10. PCR amplifications were performed using Platinum® quantitative PCR SuperMix-UDG (Invitrogen, Carlsbad, CA) on a GeneAmp® PCR system 9700. Samples were amplified at 50°C for 2 min, 95° C for 10 min, and then 50 cycles of 95°C for 15s and 60°C for 1 min. The amplification products were analyzed using an Applied Biosystems Prism® 7900 sequence detection system and SDS 2.2 software (Applied Biosystems). All genotype analyses were performed by an individual unaware of the clinical status of the subjects. The *DBH* genotypes did not show significant evidence for deviation from Hardy-Weinberg Equilibrium ($\chi^2 = 0.686$, $p = 0.4074$). An SRY PCR assay that identifies the presence of the Y chromosome-specific SRY gene was used to confirm the subject's sex (55). Ten ancestry informative markers were evaluated using the TaqMan® primer-probe sets (rs722869, C_7566096_20; rs1858465, C_11417706_10; rs1876482, C_11640969_10; rs1344870, C_8767848_10; rs1363448, C_3169933_1_; rs952718, C_8844929_10; rs2352476, C__26357333_20; rs714857, custom order; rs1823718, C__12080106_10; rs735612, C___2043758_10, Applied Biosystems). The TaqMan® assays were performed in duplicate and had a concordance of 100%.

Statistical Analysis

Sample size of 35 per group, which was met, used a power of 0.8 with alpha 0.05 based on effect sizes from three previous Yale studies of disulfiram for cocaine. We compared baseline differences in demographics and drug use history using chi squared or t-test. A repeated measures analysis of variance (ANOVA) used the number of cocaine positive urines over the total number of samples (six) for each two week period to compare disulfiram to placebo over time and to determine if the effect of disulfiram is modulated by the *DBH* locus using R version 2.9.1 (56). We compared condition (disulfiram or placebo), *DBH* genotype (0 = CT/TT genotype, 1 = CC genotype), time (each two week period), and interactions between condition and time, and between condition and *DBH*. We analyzed all individuals who had complete data (n = 61) and unbalanced repeated measures ANOVA for all individuals (n = 74). The two analyses yielded almost identical results.

To determine population structure, our cohort was compared against CEPH-HGDP samples (1,035 subjects of 51 populations). The CEPH-HGDP cohort is a collection of 1,035 subjects derived from 51 populations from America, Europe, the Middle East, Central and East Asia, and Oceania, and sub-Saharan Africa. Genotypic data for the ancestral informative markers and population codes for this cohort were kindly provided by Oscar Lao (57). The STRUCTURE 2.3.3 software (58, 59) was run using four ancestral populations (K = 4), a burnin period of 100,000 iterations, and 1 million MCMC replications after burnin to determine population substructure. For all analyses, we corrected for any possible confounding effects by including the proportion of each subject from the founder populations as well as gender and site effects as covariates in the model. The obtained p-values were very similar to those obtained when we did not correct for these covariates. Furthermore, analyses were performed with the total group then within the two *DBH* subgroups.

Results

Baseline characteristics by treatment and *DBH* genetics

We enrolled 74 patients from the 93 screened for this study and randomized 34 to disulfiram and 40 to placebo. The patients included 38 with the CC, 32 with the CT, and 4 with the TT genotype. The patients were mostly Caucasian males with a mean age of 39 years and 13 years of opiate abuse. Forty (54%) patients had been previously treated with methadone maintenance. They used cocaine for a mean of 12 years and for 19 days in the month before entering the study. Only 29 patients (39%) reported any alcohol abuse history reflecting our exclusion criteria, and 39 patients (53%) reported marijuana use. As shown in Table 1, we found no significant baseline differences among the four treatment by genotype groups in any clinical characteristics including the ASI interviewer problem severity ratings ($p > .05$).

Retention by Treatment Condition

Treatment retention for the full 12 weeks was 82% (61/74) with no significant difference between disulfiram (77% = 26/34) and placebo (87% = 35/40) ($p > .05$). The mean numbers of weeks completed was 11.2 ± 3.6. The reasons for dropout were incarceration in two patients (both disulfiram) and nine others left for community treatment programs mostly near the end of the study (four disulfiram). Only two disulfiram patients left the study for adverse effects. Subjects who completed the full 12 week trial did not differ demographically from the 13 who did not complete ($p > .05$).

Adverse Events

We only had four significant adverse events, and no patient reported an adverse interaction with alcohol, although some patients did report drinking alcohol. One disulfiram patient left the study for reduced sexual functioning, but this was considered as related to the methadone. Other adverse events were two disulfiram cases of arm numbness that resolved spontaneously during the trial without any changes in medication. One of them completed 12 weeks, but the other patient also had a back rash and left at week 6. One placebo patient also made a suicide gesture of superficially cutting his wrist, but was not hospitalized and completed the 12 weeks.

Cocaine Treatment Outcomes by Genotype

Cocaine positive urine screens showed a significant difference between treatment groups as the overall cocaine urine rates decreased from 80% during the baseline two weeks to 69% during the last two weeks of treatment ($F = 12.4$; $df = 1,440$; $p < .0005$). As shown in Figure 1 with SEM of 3.4% to 5%, the mean cocaine rates during the two baseline weeks were 80% for disulfiram and 80% for placebo. These rates dropped during the last two weeks of treatment to 62% for disulfiram and 75% for placebo. When we only included the 61 subjects who completed the study, the disulfiram treatment effect remained highly significant ($F = 15.2$; $df = 1,364$; $p < .0002$). The interaction between treatment and the SNP was also significant ($F=2.6$; $df=2, 440$; $P<0.05$), although the SNP × weeks and treatment × weeks were not significant.

Subjects were divided into two *DBH* genotype groups: those subjects without a T allele (CC genotype group) and those with a T allele (CT/TT genotype group). When separated into these two genotype groups, cocaine positive urine rates differed between the treatment groups for patients having the CC genotype ($F = 17.2$; $df = 1,236$; $p < .00005$), but did not differ for those having the CT or TT genotypes ($F = 1.12$; $df = 1,234$; $p > .05$). As shown in Figure 2, cocaine positive urines for the CC patients during the two baseline weeks were 84% for disulfiram and 84% for placebo. These rates dropped during the last two weeks of treatment to 56% for disulfiram and were unchanged at 84% for placebo. In Figure 2 for

comparison, cocaine urines for the CT/TT patients during the two baseline weeks were 77% for disulfiram and 76% for placebo. These rates dropped during the last two weeks of treatment to 67% for disulfiram and to 68% for placebo. When we only included the 61 subjects who completed the study, the disulfiram treatment effect remained highly significant only among the CC patients (F = 24.2; df = 1,176; $p < .0002$).

Opioid Treatment Outcomes by Genotype

Opioid positive urine screens decreased over time, but did not significantly differ between treatment groups. The mean opioid positive rates during the two baseline weeks were 50% for disulfiram and 49% for placebo. These rates dropped during the last two weeks of treatment to 35% for disulfiram and 25% for placebo. When separated into the two genotype groups, patients in neither group showed a difference between the treatment regimens. We also found no significant correlation between the rates of opiate and cocaine positive urines (r=0.08).

Discussion

We found a significant reduction in cocaine positive urines with 250 mg of disulfiram compared to placebo, which is consistent with several other previous studies in cocaine abusers (17–21). This reduction in cocaine use was associated with a specific functional genetic polymorphism in the gene that codes for the enzyme dopamine β-hydroxylase (DβH) (rs1611115). We found that patients having two of the alleles associated with normal levels of DβH (CC) responded to disulfiram, while those with the genotypes encoding lower levels (CT and TT) showed no difference from placebo. Genotype made no difference in the reduction in opioid use.

The different treatment response to disulfiram between those patients with low and high DβH activity may reflect differences in brain dopamine receptors. Minimal DβH activity reduces norepinephrine, but also reduces basal extracellular dopamine in the nucleus accumbens and caudate-putamen (34, 60). This reduction upregulates high-affinity postsynaptic dopamine receptors as much as six-fold and produces behavioral hypersensitivity to psychostimulants (61). Psychostimulant induced locomotor, reinforcing, and aversive effects are enhanced in *DBH* knockout mice (34, 62). These findings suggest that modest reductions of norepinephrine and dopamine transmission from disulfiram may not attenuate the behavioral responses to psychostimulants in those individuals who have upregulated dopamine receptors because of their genetically low DβH levels.

The number of *DBH* alleles affects dopamine and norepinephrine levels in the prefrontal cortex of mice when they are treated with disulfiram (Bourdelat-Parks et al., 2005). Disulfiram increased dopamine and decreased norepinephrine levels in their prefrontal cortex of mice with two normal alleles, while disulfiram showed relatively little effect on these levels in mice with null alleles (33). Like these mice, our human study participants with low DβH appeared less affected by disulfiram-induced inhibition of DβH than those with high DβH activity.

Lowering DβH activity through disulfiram may increase aversive symptoms from acute cocaine use, as one mechanism for its efficacy. Although none of these outpatients reported aversive symptoms from cocaine as an adverse event, disulfiram has increased cocaine-associated negative effects including anxiety and paranoia and reduced positive subjective effects during acute laboratory cocaine administration in humans (63–66). Low DBH levels have been associated with psychotic symptoms in psychiatric disorders (see review (67)). For instance, schizophrenic or depressed patients who have low plasma or cerebrospinal fluid levels of DβH exhibit more positive psychotic symptoms compared to those with

higher levels of DβH (68–72). Moreover, patients diagnosed with unipolar depression plus psychotic features have lower DβH levels than those without psychotic features (73). In addition, the genetic predisposition for lower levels of DβH protein is associated with cocaine-induced paranoia (39).

This trial has several limitations. First, the sample size is small for the genetic association studies and larger replications are needed of this preliminary study. Second, the genetic associations reflect a modest reduction in cocaine use to a mean proportion of 0.56 cocaine positive urines. However, this reduction for the normal DβH (CC genotype) patients treated with disulfiram was a 33% reduction compared to no change with placebo. The low DβH patients showed only a 13% reduction, which was the same as the 13% reduction with placebo. Thus, we had at least a doubling in efficacy with this genetic selection. Third, most cocaine abusers are not also opioid dependent, which limits the generalization of our findings. Fourth, alcohol abuse can be common among cocaine abusers and our rates of alcohol abuse were low reflecting our exclusion criteria. Fifth, an alternative rationale that may also explain the effectiveness of disulfiram involves ALDH-2 inhibition leading to generation of tetrahydropapaveroline (THP). This chemical inhibits activated tyrosine hydroxylase and suppresses cocaine induced dopamine production and release (74). Future studies might examine the polymorphisms in the gene coding for ALDH-2 as well as the gene for tyrosine hydroxylase and the role of these as potential pharmacogenetic targets. Finally, disulfiram may not be the optimal medication for attaining DβH inhibition, but another DβH inhibitor, nepicastat is being developed that does not inhibit aldehyde dehydrogenase or produce aversive interactions with alcohol (75). Future studies should investigate this more selective DβH inhibitor's efficacy, since this compound attenuates cocaine-seeking during relapse-like behavior in rats (76) and reduces some positive subjective effects of cocaine in humans (77).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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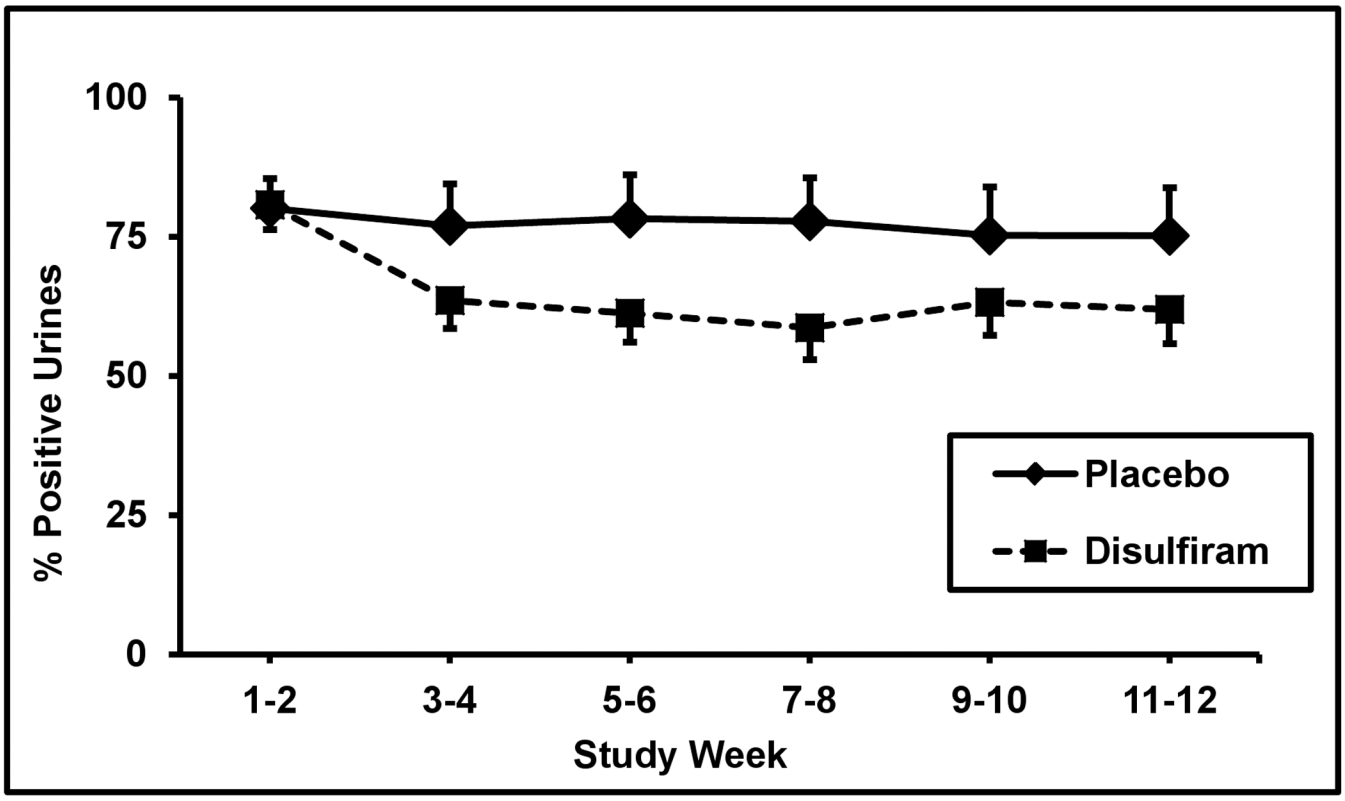

Figure 1.
Percentage of cocaine positive urine toxicology screens for two-week time blocks across the 12-week trial for the placebo (solid line, n = 40) versus disulfiram (250 mg/day) (dashed line, n = 34) treatment groups. Standard error bars are shown at each time point.

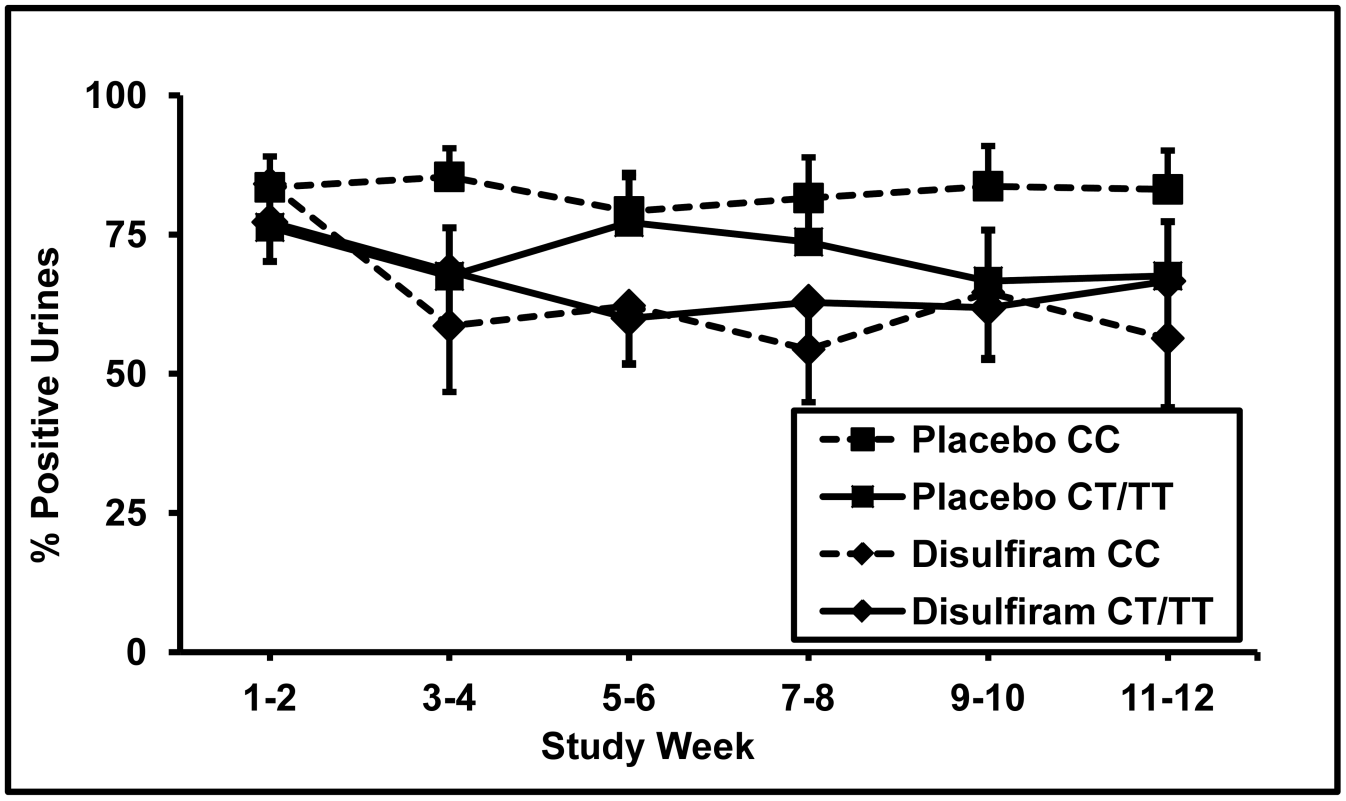

Figure 2.
Percentage of cocaine positive urine toxicology screens for two-week time blocks across the 12-week trial for the placebo versus disulfiram (250 mg/day) treatment groups. Subjects with the CC genotype (square symbols, dashed line, n = 21) and those with CT/TT genotypes (square symbols, solid lines, n = 19) in the placebo group, and subjects the CC genotype (diamond symbols, dashed line, n = 17) and the CT/TT genotypes (diamond symbols, dashed line, n = 17) in the disulfiram group are shown. Standard error bars are shown at each time point.

Table 1

Demographic and clinical characteristics by treatment and *DBH* genotype

Characteristic	Placebo		Disulfiram	
	CC	CT/TT	CC	CT/TT
N	21	19	17	17
% Male	71	63	70	53
% Caucasian	67	79	82	77
% Employed	67	53	47	71
Age years (s.d.)	43 (10)	37 (10)	38 (11)	37 (10)
Cocaine last 30 days	14 (7)	18 (8)	17 (9)	18 (9)
Cocaine years	14 (10)	11 (7)	9 (7)	9 (8)
Heroin years	11 (11)	7 (6)	9 (7)	9 (8)
% Alcohol abuse	33	42	40	47
% Marijuana abuse	33	37	70	35% past
Methadone	71	48	47	47
ASI medical	2.4 (1.6)	2.8 (1.9)	4.2 (2.1)	2.3 (1.3)
ASI employment	2.4 (4.2)	0.8 (2.6)	0.9 (2.0)	1.4 (3.1)
ASI alcohol	0 (0)	0 (0)	0 (0)	0 (0)
ASI drug	8.8 (0.6)	8.8 (0.7)	8.8 (0.4)	8.8 (0.4)
ASI legal	0 (0)	0 (0)	0 (0)	0.3 (1.0)
ASI family	0.4 (1.0)	1.7 (3.2)	0 (0)	1.4 (3.1)
ASI psychological	0 (0)	0.2 (0.6)	1.1 (2.3)	0.1 (0.3)