Published in final edited form as:

Genes Brain Behav. 2012 November ; 11(8): 1001–1008. doi:10.1111/j.1601-183X.2012.00839.x.

Modifying Role of Serotonergic 5-HTTLPR & TPH2 Variants on Disulfiram Treatment of Cocaine Addiction: A Preliminary Study

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Abstract

Disulfiram is a cocaine pharmacotherapy that may act through increasing serotonin, benefiting patients with genetically low serotonin transporter levels (5-HTTLPR S' allele carriers) and low serotonin synthesis (TPH2 A allele carriers). We stabilized 71 cocaine and opioid co-dependent patients on methadone for two weeks and randomized them into disulfiram and placebo groups for 10 weeks. We genotyped the SLC6A4 5-HTTLPR (rs4795541, rs25531) and TPH2 1125A>T (rs4290270) variants and evaluated their role in moderating disulfiram treatment for cocaine dependence. Cocaine positive urines dropped from 78% to 54% for the disulfiram group and from 77% to 76% for the placebo group among the 5-HTTLPR S' allele carriers ($F = 16.2$; $df = 1,301$; $P < 0.0001$). TPH2 A allele carriers responded better to disulfiram than placebo ($F = 16.0$; $df = 1,223$; $P < 0.0001$). Patients with both an S' allele and a TPH2 A allele reduced cocaine urines from 71% to 53% on disulfiram and had no change on placebo ($F = 21.6$; $df = 1,185$; $P < 0.00001$).

Keywords

Gene; disulfiram; polymorphism; cocaine; treatment; serotonin

Introduction

Cocaine dependence leads to substantial social and economic impacts on the more than 1.5 million afflicted with this disease (McLellan *et al.*, 2000, Samsha, 2011). Pharmacological approaches for cocaine dependence that target various neurobiological mechanisms have had limited success (e.g., (Lima *et al.*, 2002, Lima *et al.*, 2003)). However, disulfiram has shown promise for treating cocaine dependence including in opioid-dependent cocaine abusers (Carroll, 1993, Carroll *et al.*, 2004, Carroll *et al.*, 1998, George *et al.*, 2000, Kosten *et al.*, 2012, Petrakis *et al.*, 2000).

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

Disulfiram treatment increases serotonin's precursor tryptophan, its metabolite 5-hydroxyindole acetic acid, and the hydroxylated product of tryptophan, 5-hydroxytryptophan (Fukumori *et al.*, 1979, Fukumori *et al.*, 1980, Nagendra *et al.*, 1993, Nilsson & Tottmar, 1989). Since cocaine's mechanism of action is through blockade of dopamine, norepinephrine, and serotonin transporters (Han & Gu, 2006), it is possible that disulfiram may be effective for the treatment of cocaine dependence, in part, through its action on serotonin.

Making serotonin available for postsynaptic activity requires tryptophan hydroxylase and the serotonin transporter. Both genes coding for these proteins have functional variants that may make lower levels of serotonin available in the synapse. Tryptophan hydroxylase is the rate-limiting enzyme in the production of serotonin (Cooper & Melcer, 1961) and is expressed from *TPH2* in the brain (Zill *et al.*, 2007). A synonymous variant 1125A>T (rs4290270) in exon 9 of the *TPH2* gene is a marker for allelic expression imbalance (Lim *et al.*, 2007). The T allele is expressed at approximately 2-fold the levels of the A allele in heterozygous subjects. Individuals who are A allele carriers may produce less serotonin than those with a TT genotype.

The *SLC6A4* gene encodes the serotonin transporter that controls serotonin reuptake and inactivation from the synapse. In the promoter of the *serotonin transporter* gene is a repeat variant (*5-HTTLPR*) of either a short (S) or a long (L) form (Lesch *et al.*, 1996). Recently, a variant was identified within the repeat that alters transcriptional activity of the *serotonin transporter* gene. Combining this variant with the repeat yields a triallelic polymorphism that can be classified as a high transcriptional activity L' or a low activity S' allele (Hu *et al.*, 2006).

We hypothesized that the increase in serotonin from disulfiram would have the greatest impact on patients who had low levels of both serotonin transporter (S'S' or L'S' genotypes) and serotonin biosynthesis (*TPH2* AA or AT genotypes). We first examined patients who were *5-HTTLPR* S' allele carriers expecting that it is associated low activity levels would not overcome disulfiram's increase in serotonin and lead to a better treatment response. To test this mechanism of increased serotonin as the basis for a pharmacogenetic association, we next examined patients having at least one low activity *TPH2A* allele. Lastly, we next examined patients having both the low activity *5-HTTLPR* (S'S' or L'S') and the low activity *TPH2* (AA or AT) genotypes. We expected these patients also to have a better therapeutic response to disulfiram than the remaining patients.

Materials and Methods

Patients

From 2005 to 2006 at Yale University (N = 40) and then from 2006 to 2008 at the Baylor College of Medicine (N = 53), 93 patients were enrolled in the clinical trial "Pharmacogenetics of Disulfiram for Cocaine" (NCT ID: NCT00149630, NIDA-18197-2, NCT00149630) (Tables 1 and 2). Subjects entered into a two-week screening period during which they were stabilized on methadone. During this two-week screening period, urine toxicologies for opiates and cocaine metabolites were obtained three times per week. Eight

patients dropped out during this two-week screening. To assure that only active cocaine users were included in our analyses, only those patients who had at least one cocaine positive urine sample during the 12-week study were retained (11 patients excluded) so that 74 cocaine and opioid dependent patients remained in the study. Indeterminate genotyping reduced this number to 71 for the *5-HTTLPR* group and 68 for the *TPH2* group. We completed the MINI (English Version 5.0.0, July 1, 2006) (Sheehan *et al.*, 1997) and the Addiction Severity Index (ASI) (McLellan *et al.*, 1992) on all subjects for the assessment of psychiatric and baseline characteristics. All patients met DSM-IV criteria for opioid and cocaine dependence after interview by a psychiatrist or clinical psychologist. Other exclusions included a current diagnosis of other drug or alcohol physical dependence (other than tobacco), current major medical illness unbalanced 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 signed an informed consent approved by the Yale University and the Baylor College of Medicine Institutional Review Boards, which gave specific consent for genetic studies. All experiments were undertaken with the understanding and written consent of each subject. Ethnicity was based self-report of ethnic/cultural background of the patients.

Study Design and Medications

The 71 patients were stabilized on methadone maintenance at 60 mg daily and were assigned randomly to placebo or disulfiram, 250 mg daily. Methadone dose increased from an initial 25 mg by 5 mg per day until patients reached a 60 mg per day maintenance dose. Individual manual-driven cognitive behavioral therapy (Carroll, 1997) was provided weekly to all patients.

Supervised urine samples were obtained thrice weekly and tested for the presence of cocaine metabolite (benzoylecgonine) and other drugs using an Olympus AU 640 Emit system (Olympus America Inc., Melville, NY, USA) with a cut-off concentration of 300 ng/ml. We obtained saliva samples for the isolation of DNA for genotyping.

Treatment retention

There was no difference between the percentage of subjects in the placebo (87% = 35/40) and in the disulfiram (77% = 26/34) groups who completed the full 12 week trial ($P = 0.3493$). Retention in the study was for a mean of 11.2 ± 3.6 weeks. Two patients were incarcerated, nine left for community treatment programs, and two withdrew due to adverse effects. No differences were found in the demographics for those who completed the trial and those that did not ($P > .05$).

Genotyping

DNA was purified as previously described (Kosten *et al.*, 2012). Briefly, DNA was isolated using the Gentra Puregene Buccal Cell Kit (Qiagen, Valencia, CA, USA) following the manufacturer's recommendations from pelleted buccal cells that were obtained by

centrifugation of 10 ml Scope mouthwash that was used to rinse the subject's mouth for 60 seconds.

The *serotonin transporter* gene *5-HTTLPR* variant was genotyped using a two-step process. The classic long "L" and short "S" alleles (rs4795541) (Lesch *et al.*, 1996) were determined by PCR amplification of DNA to yield a 181 base pair (bp) fragment for the L allele and a 138 bp fragment for the S allele (Hu *et al.*, 2005). The internal A→G single nucleotide polymorphism rs25531 in the L allele, which creates an *HpaII* restriction site, was determined by digestion of the amplified DNA with *HpaII* (New England Biolabs, Ipswich, MA, USA). The G allele, designated "L_G" (Stein *et al.*, 2006), is digested into 96 and 85 bp fragments, while the A ("L_A") allele remains undigested at 181 bp. Fragment sizes were determined by electrophoresis in a 4–20% polyacrylamide TBE gel. The genotype distribution of these *5-HTTLPR* genotypes among the two treatment groups is shown in Table 3. Since the L_G and S alleles have a lower transcriptional expression level than the L_A allele (Hu *et al.*, 2006), the functionally similar L_G and S alleles both were designated as S' and the L_A alleles as L'.

TPH2 genotype was determined using a 5'-fluorogenic exonuclease assay (TaqMan®, Applied Biosystems, Foster City, CA, USA). The *TPH2* 1125A>T rs4290270 variant was genotyped using the TaqMan® primer-probe set (Applied Biosystems), Assay ID C_26385365_10. PCR amplifications were performed using Platinum® quantitative PCR SuperMix-UDG (Invitrogen, Carlsbad, CA) on a GeneAmp® PCR system 9700 (Applied Biosystems). 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. Amplification products were analyzed using an Applied Biosystems Prism® 7900 sequence detection system and SDS 2.2 software (Applied Biosystems).

A PCR assay that identifies the presence of the Y chromosome-specific SRY gene was used to confirm the subject's sex (Kosten *et al.*, 2012). Ten ancestry informative markers (TaqMan® primer-probe sets rs1876482, C_11640969_10; rs722869, C_7566096_20; rs1858465, C_11417706_10; rs1344870, C_8767848_10; rs1363448, C_3169933_1_; rs2352476, C__26357333_20; rs1823718, C__12080106_10; rs735612, C___2043758_10, rs952718, C_8844929_10; rs714857, custom order, Applied Biosystems) were evaluated. Our cohort was compared against CEPH-HGDP samples (1,035 subjects of 51 populations) to determine population structure. The CEPH-HGDP cohort is a compilation of 1,035 subjects derived from 51 populations from America, Central and East Asia, Oceania, Europe, the Middle East, and sub-Saharan Africa. Genotypic data for the ancestral informative markers for this cohort were thoughtfully provided by Dr. Lao (Lao *et al.*, 2006). The STRUCTURE 2.3.3 software (Hubisz *et al.*, 2009, Pritchard *et al.*, 2000) was run using a burnin period of 100,000 iterations, 1 million MCMC replications after burnin, and four ancestral populations (K = 4) to determine population substructure.

The TaqMan® assays were performed in duplicate and had a concordance of 100%. All genotype analyses were performed by an individual unaware of the clinical status of the subjects.

Statistical Analysis

The two treatment groups were compared for baseline differences in cocaine use history and demographics using χ^2 or t-test as appropriate. As previously described (Kosten *et al.*, 2012), we used R version 2.9.1 (R_Development_Core_Team, 2009) with a repeated measures analysis of variance to compare disulfiram to placebo over time on the number of cocaine positive urines over the total number of visits (six) for each two-week period. We also determined whether the effect of disulfiram was modulated by genotype or genotype pattern. The explanatory variables were condition (whether a patient was taking disulfiram or placebo), genotype group (0 = S'S' or L'L' genotype, 1 = L'L' genotype for 5-HTTLPR; 0 = AA or AT genotype, 1 = TT genotype for TPH2 rs4290270), time (each two week period), the interaction between condition and time, and interaction between condition and genotype. A repeated measures ANOVA was performed using the data on all individuals who had complete data (N = 57) and unbalanced repeated measures ANOVA for all individuals (N = 71). Similar results were obtained from each analysis. Effect size was calculated as a partial eta-squared statistic using a ratio of condition or SNP variance to the residual variance. There are three general levels of cutoffs for effect size: 0.01 is a small effect, 0.06 is medium effect, and 0.14 is a large effect.

We used a similar statistical approach to test the hypotheses about the effects of baseline serotonin supply on the efficacy of disulfiram for cocaine dependence. We compared the response to disulfiram for the 5-HTTLPR S' allele carriers (S'S' or L'S', i.e., low serotonin transporter) when paired with TPH2 A allele carriers (AT or TT genotypes, i.e., low serotonin production) or with TT (high serotonin production) versus the rest of the patients in each of these two comparisons. We expected disulfiram to be effective in the low transporter group when paired with low serotonin production, but disulfiram to be ineffective in the remaining patients. This was our test for the mechanism of disulfiram action and included 68 patients, because we had insufficient sample to determine the genotype for three subjects.

Population structure was determined as previously described. 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. P-values were similar to those obtained when we did not correct for these covariates. Analyses were performed with the total group then within the two genotype subgroups. Results are presented for all subjects meeting our criteria above (N = 71), except where it is stated that results are only for those patients who completed the 12 weeks of treatment (N = 58).

Results

Baseline characteristics by medication and 5-HTTLPR plus TPH2 genetics

We were unable to genotype three patients for the serotonin transporter thereby providing a sample of 71 patients who had been randomized: 33 to disulfiram and 38 to placebo. The patients included 17 with the S'S', 40 with the S'L', and 14 with the L'L' 5-HTTLPR genotype and 10 with the AA, 34 with the AT, and 24 with the TT TPH2 genotype. The 71 patients were mostly Caucasian males with a mean age of 39 years and 10 years of opiate

abuse. Forty (54%) patients had been previously treated with methadone maintenance. They used cocaine for a mean of 11 years and for 17 days in the month before entering the study. Only 30 patients (42%) reported any alcohol abuse history reflecting our exclusion criteria, and 32 patients (45%) reported marijuana use. As shown in Table 2 for three different types of analyses, we found no significant baseline differences among the six treatment by genotype groups in any clinical characteristics ($P > 0.05$). The left hand four columns are for the serotonin transporter alone, the center four columns are for the *TPH2* alone, while the four right hand columns are for the combined serotonin transporter and *TPH2* groups. Those patients with both genetically low serotonin transporter (S'/S'/L'/S') and low (AA/AT) *TPH2* levels were contrasted to the rest of the patients without both of these characteristics.

Cocaine Treatment Outcomes and Serotonin Transporter Genotype

Similar to findings we previously reported, cocaine positive urine screens showed a significant difference between treatment groups as the positive cocaine urine rates decreased from 80% during the baseline two weeks to 62% for the disulfiram group and to 75% for the placebo group during the last two weeks of treatment ($F = 12.4$; $df = 1,73$; $P < 0.0005$) (Kosten *et al.*, 2012).

We divided the 71 patients who were genotyped for the *serotonin transporter 5-HTTLPR* allele into two groups based on their *5-HTTLPR* genotype: those patients with one or two S' alleles (S'/S'/L'/S' genotype group) and those without an S' allele (L'/L' genotype group). The cocaine positive urine rates across the 12-week clinical trial differed based on treatment for patients in the S'/S'/L'/S' group ($F = 16.2$; $df = 1,301$; $P < 0.0001$) accounting for 5.1% of the total variance (Fig. 1, upper panel), but not for those in the L'/L' group ($F = 0.958$; $df = 1,69$; $P = 0.3311$). The rate of cocaine positive urines for the patients in the S'/S'/L'/S' group were 78% for those receiving disulfiram and 77% for placebo during the two baseline weeks. Positive urine cocaine rates in this group decreased to 54% in the last two-week period for the group treated with disulfiram and remained relatively constant at 76% in patients in the placebo group. During the initial two-week baseline period, the mean rate of cocaine positive urines differed between the S'/S'/L'/S' and L'/L' genotype groups ($t = 2.218$, $p = 0.0304$; 78% versus 92%). If we included only the 58 patients who completed the study, disulfiram treatment remained effective only in the S'/S'/L'/S' group ($F = 20.0$; $df = 1,266$; $p < 0.0001$).

Cocaine Treatment Outcomes by Tryptophan Hydroxylase 2 Genotype

Next, we divided the 68 subjects who had been genotyped at the *TPH2* rs4290270 allele into two genotype groups based on their *tryptophan hydroxylase 2* genotypes; one group was composed of subjects with at least one A allele (AA/AT genotype group) while the other group were without an A allele (TT genotype group). The rates of cocaine positive urine across the 12-week clinical trial differed for subjects in the AA/AT group based on treatment ($F = 16.0$; $df = 1,223$; $P < 0.0001$) and accounted for 6.7% of the variance (Fig. 1, middle panel). These did not differ for those in the TT group ($F = 3.21$; $df = 1,128$; $P = 0.0752$). During the two baseline weeks, the rate of cocaine positive urines in the AA/AT group was 80% for those receiving disulfiram and 83% for placebo. The rates of positive urine cocaine rates for the AA/AT group decreased to 64% in the last two-week period for

the group treated with disulfiram and increased slightly to 85% for subjects in the placebo group. The mean rate of cocaine positive urines during the initial two-week baseline period did not differ between the AA/AT' and TT genotype groups ($t=.2531$, $P = 0.8034$; 82% versus 80%). If we included only the 55 subjects who completed the study, disulfiram treatment remained effective only among the AA/AT patients ($F = 16.0$; $df = 1,194$; $P < 0.0001$).

Cocaine Treatment Outcomes by Serotonin Transporter Genotypes and Tryptophan Hydroxylase 2 Genotype Patterns: Combined Efficacy

We had only 68 patients to test the treatment response by combining the low activity 5-*HTTLPR* group (S'S' or L'S') with the low activity *TPH2* A allele, because three patients could not be genotyped for the *TPH2* polymorphism. We hypothesized that those patients with the low activity genotype patterns would respond to disulfiram, while the remaining patients would not respond. We compared the 37 patients having both the low activity 5-*HTTLPR* genotype (S'S' or L'S') and the low activity *TPH2* rs4290270 genotype (AA or AT) to the remaining 31 patients with genotypes coding for higher levels of either the 5-*HTTLPR* (L/L') or *TPH2* (TT) genotypes ($N = 24$), or both genotypes ($N = 7$). As shown in the lower panel of Fig. 1, patients who carried a low activity 5-*HTTLPR* S' allele and a low activity *TPH2* A allele had a significant reduction in cocaine urines from 71% to 53% on disulfiram and no reduction on placebo ($F = 21.6$; $df = 1,185$; $P < 0.00001$) accounting for 11% of the variance. The remaining patients showed no disulfiram effect ($F = 0.832$; $df = 1,166$; $P = 0.3630$). During the initial two-week baseline period, the mean rate of cocaine positive urines did not differ between the 5-*HTTLPR* S' and *TPH2* A allele carrier group and the group without these alleles ($t=-0.3157$, $P = 0.7533$; 80% versus 82%). If we included only the 55 subjects who completed the study, disulfiram treatment remained effective only in the 5-*HTTLPR* S' and *TPH2* A allele carrier patients ($F = 26.43$; $df = 1,158$; $P < 0.0001$).

Discussion

We have found that those patients with specific genetic polymorphisms and genotype patterns of the *serotonin transporter* and *tryptophan hydroxylase 2* genes are associated with better treatment outcome during the pharmacotherapy of cocaine dependence with disulfiram. This supports our hypotheses that we developed since disulfiram raises serotonin levels (Fukumori *et al.*, 1979, Fukumori *et al.*, 1980, Nagendra *et al.*, 1993, Nilsson & Tottmar, 1989). Subjects with at least one copy of the 5-*HTTLPR* S' allele or at least one *TPH2* A allele had decreased cocaine positive urines compared to those without these alleles. The combination of both low transporter levels and low TPH2 activity appeared to have an additive effect with the overall effect as reflected in the F statistic going from 12 for no genetic selection, to 16 for either the *serotonin transporter* or *TPH2*, to 24 when both polymorphisms were combined.

The mechanism for this improved outcome with genetic selection may involve serotonin availability in the synapse. The serotonin transporter controls synaptic serotonin levels by pumping serotonin back into the presynaptic neuron after release. This refreshes the synapse following action potential activity. In patients treated with disulfiram who have increased

levels of serotonin transporter, as found in those subjects with the L/L' genotype, the disulfiram-induced increase in serotonin may not be physiologically effective. This may be due to the presynaptic neuron having an adequate level of serotonin transporter to maintain a basal serotonin level that is minimally affected when serotonin is increased by disulfiram. In contrast, the serotonin transporter may not efficiently remove serotonin from the synapse in patients with low serotonin transporter levels, when serotonin is increased due to disulfiram treatment. Thus, synaptic serotonin levels are effectively increased and then can bind more effectively to serotonin receptors stimulating downstream events. For example, serotonin modulates the response to drug-paired cues through binding to its post-synaptic receptors, some of which are involved in behaviors related to cocaine and the priming effects of cocaine cues [e.g., (Fletcher *et al.*, 2002, Liu & Cunningham, 2006, Nic Dhonnchadha *et al.*, 2009)].

The functional polymorphism that we studied in the promoter of the serotonin transporter gene has been associated with a number of psychiatric disorders and behaviors, although some of these findings have lacked replication [e.g. (Risch *et al.*, 2009)]. The serotonin transporter is expressed in the raphe and thalamus, and in cortical and limbic areas such as the cingulate cortex, and hippocampal pyramidal cells, brain regions that process emotional components of behavior (Zhuang *et al.*, 2005). Hence, emotional regulation, anxiety, and impulsivity may be modified by specific alterations of serotonin function (Courtet *et al.*, 2005). An increase in anxiety related traits has been found in healthy volunteers with an S allele (Lesch *et al.*, 1996). It has been suggested in studies on coping ability that S allele carriers are more cognitively vulnerable to anxiety when making decisions (Szily *et al.*, 2008). Amygdala activation while making decisions as measured by fMRI was found to be lower in those with an L_AL_A genotype (Roiser *et al.*, 2009). Thus, when taking disulfiram, S' allele carriers may be more sensitive than L/L' individuals to the anxiety provoking effects from decisions related to taking cocaine or anxiety induced by cocaine. A number of other studies have reported associations of response to therapy with the serotonin 5-HTTLPR genotype. For example, a meta-analysis of 15 studies of the response to selective reuptake inhibitors for depression found an association with the S allele (Serretti *et al.*, 2007).

Since our serotonin transporter hypothesis relied on serotonin levels to explain the role of the serotonin transporter in response to disulfiram treatment, we also evaluated the role of a functional variant in the *TPH2* gene. We found that that patients with the low activity *TPH2* A allele responded to disulfiram treatment better than those patients without this allele. This may be due to disulfiram having a greater effect in raising serotonin levels in those with genetically low serotonin biosynthesis. This same variant in *TPH2* also has been found to be associated with heroin addiction (Nielsen *et al.*, 2008).

Cocaine directly inhibits reuptake of dopamine, norepinephrine and serotonin (Han & Gu, 2006), and disulfiram can affect all three, including increasing serotonin levels. Cocaine administration to rats increases serotonin, as well as dopamine, within seconds (Broderick *et al.*, 2004). In cultured serotonergic neurons, cocaine treatment has been shown to enhance surface expression of the serotonin transporter, whereas serotonin down-regulated serotonin transporter expression (Kittler *et al.*, 2010). In post-mortem brains, cocaine use was associated with increased serotonin transporter levels in the midbrain and striatum (Little *et*

al., 1993a, Little *et al.*, 1993b). Therefore, long-term cocaine use may accentuate these genetic differences in the efficacy of disulfiram, since these enhanced transporter levels will distinguish further the patients with the high activity serotonin variant. Our patients' long histories of cocaine use (mean of 13 years) did not allow us to examine shorter term cocaine users, which may be of interest in future studies of disulfiram.

This study has several limitations including its relatively small sample size. Replication using larger populations will be required to substantiate these findings. Other limitations of this study were that all our subjects were opioid dependent. We cannot speculate as to how this co-dependency would affect our findings. In addition, no subjects had current alcohol dependence, but many had alcohol abuse. While the 250 mg dose is unlikely to produce significant adverse reactions with one or two alcohol drinks in many individuals, a disulfiram-induced decrease in alcohol use might also drive some reduction in cocaine use. Baseline rates of alcohol abuse were unusually low in the TPH2 TT genotype patients, and these patients were relatively non-responsive to disulfiram. Finally, disulfiram may increase serotonin through a variety of mechanisms not directly studied here, including inhibition of aldehyde dehydrogenase (Vallari & Pietruszko, 1982) and monoamine oxidase (Schurr *et al.*, 1978), both of which metabolize serotonin. Variants in these genes may also be important in disulfiram pharmacotherapeutic action and should be investigated in future studies.

Acknowledgements

We thank G. Wu and J. Lappalainen for recruiting, screening, and assessment of study subjects. This study was supported by NIH/NIDA 5 P50 DA018197-05 (TK), the Veterans Health Administration, and the Toomim Family Fund.

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Figure 1. Interaction of the 5-HTTLPR S'S'/L'S' genotype, the TPH2 AA/AT genotype, and the 5-HTTLPR S'S'/L'S':TPH2 AA/TT genotype pattern with percentage of cocaine positive urines
 Urine toxicology screens are shown for each two-week time period across the 12-week trial for the placebo (solid line) versus disulfiram (250 mg/day) (dashed line) treatment groups. Panel A: patients with the 5-HTTLPR S'S' or L'S' genotype (disulfiram, N = 27; placebo, N = 30), B: patients with the 5-HTTLPR L'L' genotype (disulfiram, N = 6; placebo, N = 8), C: patients with the TPH2 AA or AT genotypes (disulfiram N = 22; placebo, N = 22), D: patients with the TPH2 TT genotype (disulfiram, N = 9; placebo, N = 15), E: those with the HTTLPR S'S' or L'S':TPH2 AA or AT genotype pattern (disulfiram, N = 10; placebo, N = 18), F: patients in the Other genotype patterns group (not having the HTTLPR S'S' or L'S' (N = 18)).

¹:*TPH2* AA or AT genotype pattern (disulfiram, N = 13; placebo, N = 14) in their response to disulfiram and placebo treatments. Standard error bars are shown at each time point.

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Table 1

CONSORT description.

Study Phase	Subjects (n)	Description
Enrollment	93	Subjects assessed for eligibility
Excluded	11	Not meeting inclusion criteria
	0	Declined to participate
	8	Dropped out
	19	Total
Randomization	34	Disulfiram group
	40	Placebo group
	74	Total
Disulfiram group	34	Received allocated intervention
	5	Left for other treatment programs
	0	Discontinued intervention
	34	Subjects analyzed
Placebo group	40	Received allocated intervention
	4	Left for other treatment programs
	2	Incarceration
	2	Side effects
	40	Subjects analyzed

Table 2

Clinical and demographic characterization by treatment and genotype.

Genotype:	Treatment									
	Disulfiram		Placebo		Disulfiram		Placebo		Disulfiram	
	<i>S/S/L/S'</i>	<i>L/L'</i>	<i>S/S/L/S'</i>	<i>L/L'</i>	<i>AA/AT</i>	<i>TT</i>	<i>AA/AT</i>	<i>TT</i>	<i>AA/AT</i>	<i>TT</i>
5-HTTLPR:	<i>S/S/L/S'</i>	<i>L/L'</i>	<i>S/S/L/S'</i>	<i>L/L'</i>	Other		Other		Other	
TPH2:	<i>S/S/L/S'</i>	<i>L/L'</i>	<i>S/S/L/S'</i>	<i>L/L'</i>	<i>AA/AT</i>	<i>TT</i>	<i>AA/AT</i>	<i>TT</i>	<i>AA/AT</i>	<i>AA/AT</i>
N	27	6	30	8	22	9	22	15	17	20
% Male	67	50	63	75	59	67	73	60	64	75
% Caucasian	78	83	70	75	73	89	59	87	71	86
% African American	4	17	20	25	5	11	27	13	0	14
% Hispanic	19	0	10	0	23	0	14	0	29	0
% Employed	74	50	53	75	68	67	50	67	65	50
Age years (s.d.)	39 (9)	33 (14)	40 (11)	40 (12)	39 (11)	38 (7)	42 (12)	37 (9)	40 (10)	42 (12)
Cocaine last 30 days	19 (9)	13 (10)	20 (8)	17 (8)	16 (8)	22 (11)	16 (8)	18 (9)	17 (11)	16 (9)
Cocaine years	9 (7)	7 (7)	13 (9)	14 (9)	9 (6)	9 (8)	13 (10)	15 (7)	10 (6)	13 (11)
Heroin years	10 (9)	7 (8)	8 (10)	15 (11)	14 (8)	7 (8)	9 (11)	11 (10)	11 (8)	9 (11)
% Alcohol abuse	44	50	37	50	59	11	32	53	59	29
% Marijuana abuse	48	83	37	38	55	56	36	40	41	71
% Past Methadone	48	50	60	50	50	56	77	27	53	50
										80
										29

No significant baseline differences among the six treatment by genotype groups in any clinical characteristics ($P > 0.05$).

Table 3

Genotype distribution of the triallelic 5-HTTLPR alleles.

5-HTTLPR genotype:	SS (N, %)	SL _G (N, %)	L _G L _G (N, %)	SL _A (N, %)	L _A L _G (N, %)	L _A L _A (N, %)
Disulfiram	8 (24)	0 (0)	1 (3)	15 (45)	3 (9)	6 (18)
Placebo	4 (11)	3 (8)	1 (3)	19 (50)	3 (8)	8 (21)
Total	12 (17)	3 (4)	2 (3)	34 (48)	6 (8)	14 (20)

In the text, the L_G and S alleles are designated S' and the L_A allele as L'.