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RESEARCH ARTICLE

Polymorphism in the serotonin transporter gene and response to treatment in African American cocaine and alcohol-abusing individuals

PAOLO MANNELLI,[1] ASHWIN A. PATKAR,[1] HEATHER W. MURRAY,[2] KENNETH CERTA,[2] KATHLEEN PEINDL,[1] MARJA MATTILA-EVENDEN[1] & WADE H. BERRETTINI[3]

[1]Department of Psychiatry, Duke University Medical Center, Durham, NC, [2]Department of Psychiatry, Thomas Jefferson University, Philadelphia, PA, and [3]Center for Neurobiology and Behavior, Department of Psychiatry, University of Pennsylvania, Philadelphia, PA, USA

Abstract

The serotonin transporter (5-HTT) regulates serotonin transmission and modulates behavioral effects of drug of abuse. A polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) yielding a short (S) and long (L) allele has been associated with severity of substance abuse. The aims of the study were to investigate whether 5-HTTLPR genotypes differed in their response to treatment in cocaine- and alcohol-abusing patients. Polymerase chain reaction-based genotyping of a 44 base pair insertion/deletion polymorphism was performed in 141 African American cocaine-dependent patients with concurrent alcohol use who were entering a 12-week behaviorally oriented outpatient treatment program. In treatment, end of treatment and 6-month follow-up outcome measures included changes in Addiction Severity Index (ASI) scores, urine drug screens, days in treatment, individual/group sessions, dropout and completion rates. As expected, there was a reduction in substance abuse by the end of treatment and follow-up ($F = 5.15$, $p = 0.000$). However, there were no differences in the reduction in cocaine use across the LL, LS and SS genotypes. Interestingly, individuals with the S allele showed greater severity of alcohol use at admission ($F = 4.84$, $p = 0.03$), and the SS genotype showed less improvement in alcohol measures than the LL at follow-up ($F = 3.68$, $p = 0.03$), after controlling for baseline variables. While we found no association of the 5-HTTLPR variants with severity of cocaine abuse or any cocaine-related outcome measures, the data suggested that the 5-HTTLPR polymorphism may distinguish responders from non-responders to behavioral treatment in terms of alcohol use. Further investigations are required to determine the role of the 5-HTTLPR polymorphism in influencing treatment – outcome among substance abusers.

Introduction

Deficits in central serotonin (5-HT) functions are implicated in the expression of substance abuse disorders (Koob *et al.*, 1998). Among the various components of the 5-HT system, the serotonin transporter (5-HTT) modulates central effects of drugs of abuse. In particular, the mechanism of action of cocaine involves blocking the uptake of both serotonin and dopamine by binding to the transporters specific for each neurotransmitter (Ravna *et al.*, 2003). Although the effect of cocaine on dopamine uptake is critical for reward (Dackis and O'Brien, 2001), animal experiments suggest that modifications in 5-HT transporter activity may significantly contribute to drug seeking (Burmeister *et al.*, 2003), self-administration (Arroyo *et al.*, 2000) and reinforcement (Sora *et al.*, 2001; Rocha, 2003). In addition, clinical studies have demonstrated serotonin involvement with the manifestation of cocaine use and craving (Satel *et al.*, 1995; Buydens-Branchey *et al.*, 1997), euphoria (Newton *et al.*, 2001), anxiety (Muller *et al.*, 2003) or aggression (Moeller *et al.*, 1994). However, clinical research examining the role of the 5-HTT in cocaine dependence is still limited.

Considerable evidence suggests that the serotonin transporter is critical in regulating 5-HT activity. Beside the fundamental role in serotonin reuptake, the transporter serves

Correspondence to: Ashwin A. Patkar MD, Associate Professor of Psychiatry Duke University Medical Center, 4323 Ben Franklin Blvd, Suite 700, Durham, NC 27704, USA. Tel: (919) 471 3826; Fax: (919) 620 0346; E-mail: ashwin.patkar@duke.edu

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as a target site for certain antidepressants (Graham and Langer, 1992; Schloss and Williams, 1998), and binds with high affinity to cocaine analogs (Staley *et al.*, 1994). Changes of the transporter density and distribution have been demonstrated post-mortem (Little *et al.*, 1998) and in neuroimaging studies of cocaine abusers, in relation to the condition of dependence (Mash *et al.*, 2000) or during withdrawal (Jacobsen *et al.*, 2000). The human 5-HTT protein is encoded by a single gene, and a functional bi-allelic repeat polymorphism in the 5′ promotor region of 5-HTT (5-HTTLPR) yielding a short (S) and a long (L) variant of the allele has been described (Lesch *et al.*, 1994; Gelernter *et al.*, 1995). The S variant exerts a dominant effect and is associated with the reduction of transcriptional efficiency, resulting in reduced 5-HTT expression in lymphoblasts (Heils *et al.*, 1996; Lesch *et al.*, 1996). It has been hypothesized that the expression of the serotonin transporter may be influenced by the genotype in the case of alcohol and cocaine abuse (Little *et al.*, 1998). However, conflicting results have been reported; 5-HTTLPR variants have been associated with alcoholism in some studies (Sander *et al.*, 1997; Schuckit *et al.*, 1999; Lichtermann *et al.*, 2000), while other studies of alcohol and stimulant abusers have not confirmed the association (Berrettini and Persico, 1996; Patkar *et al.*, 2001; Kranzler *et al.*, 2002). Growing evidence indicates that the presence of the S allele is associated with negative clinical response in the pharmacological treatment of mood disorders (Arias *et al.*, 2003; Benedetti *et al.*, 2003) or in the prophylaxis of their recurrence (Serretti *et al.*, 2001). These recent findings support the hypothesis that a genetic variation of serotonin transporter may be involved in the clinical response to treatment of psychiatric disorders.

Most studies investigating the relationship of 5-HTTLPR genotypes with the expression of 5-HTT in drug addiction have primarily recruited individuals of European background with a primary diagnosis of alcohol dependence. Despite the existence of a major public health problem of cocaine addiction among minority populations, there are limited investigations focusing on cocaine dependent minorities.

We have previously identified an association between 5-HTT peripheral density and response to treatment (Patkar *et al.*, 2002a) and behaviors that are under serotonin influence, such as sensation seeking and impulsivity and aggression (Linnoila *et al.*, 1983; Patkar *et al.*, 2003) in African American cocaine abusers. However, we found no association of 5-HTT genetic variants with behavioral traits (Patkar *et al.*, 2002b) or with platelet serotonin transporter sites (Patkar *et al.*, in press).

Because markers of serotonin activity seem to interact with various clinical measures in predicting the outcome of cocaine treatment, we decided to examine the influence that 5-HTTLPR may exert on clinical and biological measures of outcome in a sample of African American cocaine and alcohol abusers undergoing outpatient behavioral treatment. To this end, we first investigated whether the characteristics of our sample at admission differed among the 5-HTTPLR genotypes and then identified objective treatment and follow-up outcome parameters. We finally analyzed the results to determine differences across alleles and genotypes.

Material and methods

Subjects and assessments

Two hundred and twelve subjects attending a state-licensed intensive outpatient cocaine treatment program in Philadelphia were screened. The study was approved by the Institutional Review Board of Thomas Jefferson University, Philadelphia. Following a description of the study, written informed consent was obtained from individuals who volunteered for the study. The Structured Clinical Interview (SCID) for *Diagnostic and Statistical Manual* version IV (DSM-IV) Axis I disorders (First *et al.*, 1997) was then administered to study participants. Individuals with a diagnosis of schizophrenia, major depression, bipolar disorder, schizoaffective disorder, a serious medical illness or those receiving psychotropic medications were excluded. If patients used more than one substance, they were included only if their primary drug was cocaine. As patients referred from the criminal justice system were court-mandated to attend treatment, these individuals were excluded from the study but not from regular treatment.

The sample was restricted to African American subjects as more than 90% of the population in the program was of African American background. Subjects were assessed using the Beck Depression Inventory (BDI, Beck and Steer, 1987), the Symptom Checklist-90 – Revised (SCL-90R) (Derogatis *et al.*, 1973) and the Addiction Severity Index (ASI, McLellan *et al.*, 1992). The BDI is a widely used, self-report questionnaire that assesses depressive symptomatology during the previous week and requires about 10 minutes to complete. The SCL-90 is a multidimensional self-report inventory designed to assess psychopathology in nine different dimensions including depression, and requires about 20 minutes to complete. Possible scores can range from 11 to 81. Reliability and validity has been used extensively for clinical and research purposes. The assessment tool captures problem severity in seven domains of functioning: drug use, alcohol use, employment/support, medical, legal, family/social and psychiatric. For each domain, a composite score that ranges from 0 (minimum) to 1 (maximum) indicates the level of functioning in these areas during the previous 30 days. ASI composite scores have been found to be highly specific and predictive measurements of addiction severity (Bovasso *et al.*, 2001).

Only subjects who had a history of substance abuse in one or more immediate family members (biological parents and full siblings), who reported cocaine use in the previous 30 days on the ASI and who had onset of regular cocaine use before 25 years of age were included. The family history was derived from items on the family history section of the ASI. This allowed us to define a subset of cocaine-dependent patients whose addiction may be more likely to be influenced by genetic factors (McGue *et al.*, 1992). The likelihood of detecting susceptibility genes is considered to be higher in such phenotypes (Berrettini and Persico, 1996).

Treatment approach

The study participants received the same treatment that was offered to the regular patients in the program. Treatment consisted of outpatient group counseling sessions three times a week, each session lasting for 3 hours for a total of 12 weeks.

In addition, patients participated in one 45-minute individual counseling session per week over the 12-week period. Patients completing the intensive program were offered an aftercare program which included one individual counseling session per week for up to 6 months. The treatment approach was problem oriented and focused on attaining well-defined goals. These objectives were delineated in a treatment plan that was prepared at admission and was updated monthly as treatment progressed. Treatment approaches were drawn from different treatment models including behavioral, supportive-expressive and relapse prevention strategies (Marlatt and Gordon, 1985; Rounsaville *et al*., 1985). Attendance at self-help meetings was encouraged, while pharmacotherapy was not used routinely and was available only when needed. Such a multimodal approach seems to be fairly typical of many outpatient addiction treatment programs. The treatment team was kept blind to the transporter polymorphism data.

Outcome measures

In-treatment, end-of-treatment and follow-up assessments were performed to provide objective estimates of abstinence from cocaine, as well as of retention in and attrition from treatment. The measures to assess outcome were as follows.

Number of negative urines. Urine drug screens (UDS) were obtained for all subjects on the day of admission to the treatment program, randomly every week during the treatment period and 6 months after treatment completion, using Accutests (Jant Pharmacal Corporation, CA, USA). The Accutest Drug Screen is a one-step immunoassay for the detection of cocaine, opiates, barbiturates and amphetamines. The results are obtained in a few minutes. The number of UDS negative for all tested substances was adopted as a measure of substance use during treatment. Missed urines were not taken into account for data analyses. A urine sample for considered positive or 'dirty' if it was positive for any illicit drug and negative or 'clean' if it tested negative for illicit drugs.

Days in treatment. This offers another estimate of treatment retention. Attendance was recorded as duration from date of first to date of last visit.

Number of treatment sessions. The total number of group and individual sessions attended by the patient was calculated. This reflects participation in the treatment process.

Dropouts. These were defined as individuals who stopped attending the treatment program within 14 days of admission.

Aftercare participants. These participants are described as patients completing treatment and continuing with aftercare programs. It should identify a group of individuals with well-defined clinical differences from the previous one.

Symptom reduction during treatment and at follow-up. The baseline (admission) composite score in each of the seven problem areas of the ASI, the last composite score obtained during the 12-week counseling period and the follow-up composite scores were employed as a measure of problem reduction during treatment.

Genotyping

Genomic DNA was isolated from 20 ml of ethylenediamine tetraacetic acid (EDTA)-treated venous blood obtained by each individual using standard techniques (Miller *et al*., 1988). Genotyping for a 44 base pair (bp) insertion/deletion polymorphism in the 5′ promotor region was performed as described by Heils *et al*. (1996). Briefly, the 5-HTTLPR region was amplified using polymerase chain reaction (PCR) with oligonucleotide primers (5'-GGCGTTGCCGCTCT-GAATTGC and 5′-GAGGGACTGAGCTGGACAACC-CAC) to generate a 484 bp (S) or a 528 bp (L) fragment. The amplified fragments were separated on agarose gels, and bands were visualized by ethidium bromide staining and ultraviolet illumination. Genotypes were evaluated by investigators who were blind to the status of the subject and any discrepancies were resolved by test replication.

Statistical analyses

The allele frequencies and genotype distributions of the 5-HTTLPR variants were calculated for the cocaine-dependent patients and χ^2 tests were used to compare the genotype frequency in our population with the predicted Hardy–Weinberg equilibrium (Lesch *et al*., 1996). Demographic characteristics, cocaine severity use and treatment outcomes, measured on a continuous scale, were compared among the three genotypes using one-way ANOVA and Fisher's exact tests. χ^2 tests were used for differences between the dichotomous demographic, clinical and outcome variables and genotypes. To examine the relation between treatment outcome measures and genotypes one-way ANOVAs were employed, while the differences among genotypes in the seven ASI composite scores at baseline, end of treatment and follow-up were investigated using MANOVA. This test analyzes the differences between genotypes for multiple dependent variables across time-points. Repeated-measures ANCOVA analysis was used to covary for admission variables while examining the relationship between genotypes and ASI scores. Comparisons between time-points of the ASI composite scores for each genotype were performed using Student's *t*-test (paired). The Spearman correlation coefficient was calculated to determine the relationship between alleles and demographic information and outcome measures. All calculations were performed using the SPSS11.0 software. Bonferroni corrections were applied for multiple comparisons.

Results

Sample

A total of 141 African-American cocaine-dependent patients fulfilled the inclusion criteria and consented to participate in the study. Their demographic and clinical characteristics are summarized in Table 1.

A significant proportion of the subjects had additional current or past abuse and dependence diagnoses: 82% were nicotine dependent, 53% had abuse dependence from alcohol, 31% from marijuana, and about 15% had life-time opioid abuse or dependence.

Table 1. *Demographic and clinical information for total group of cocaine-dependent individuals and for each genotype*

	Genotypes Total ($n = 141$)	35% LL ($n = 50$)	47% LS ($n = 66$)	18% SS ($n = 25$)
Men	70%	70%	70%	72%
Age	36.29 (6.35)	35.97 (6.28)	36.86 (6.51)	35.45 (6.13)
Never married	53%	56%	55%	44%
Unemployed	69%	64%	70%	76%
Highest grade	11.60 (1.77)	11.38 (1.94)	11.66 (1.55)	11.90 (1.97)
BDI	11.84 (7.79)	11.76 (8.76)	11.35 (6.96)	13.32 (7.99)
Drug use				
Age of onset (years)	20.41 (4.03)	19.92 (4.75)	20.79 (3.40)	20.40 (4.01)
Duration (years)	1.94 (0.84)	1.98 (0.84)	1.88 (0.81)	2.00 (0.91)
Quantity (wraps/day)	2.90 (0.82)	3.02 (0.82)	2.81 (0.78)	2.88 (0.93)
Frequency (days/week)	3.59 (0.63)	3.56 (0.73)	3.61 (0.55)	3.60 (0.65)
Positive admit UDS	(35)	32%	36%	40%

Values represent means (SD).

5-HTTLPR genotypes and cocaine-dependent patients

5-HTTLPR genotype distributions were consistent with the Hardy–Weinberg equilibrium ($\chi^2 = 6.0$, df = 4, $p = 0.19$). The allele frequencies were L = 166(58.6%) and S = 16 (41.1%). The genotype distributions were LL (35%) LS (47%) and SS (18%).

We performed three sets of comparisons for admission, end of treatment and follow-up measures. These included comparing LL, LS and SS genotypes, comparing LL genotype with combination of LS and SS genotypes assuming a dominant effect of S, and comparing L and S alleles. Bonferroni correction was applied for all ANOVAs. The analyses are summarized in Table 2.

Baseline differences across genotypes

At admission there were no differences in demographic variables, depression measures (BDI, SCL-90R depression scores and ASI-psychological scores) or measures of cocaine use. However, assuming a dominant effect of S, a significant difference in ASI-alcohol scores was observed with those with carrying the S allele having the highest alcohol severity scores ($p < 0.05$). Consistent with this difference a trend effect ($p = 0.09$) was observed in alcohol severity scores in allele-wise and genotype comparisons (Table 2), with the SS genotype and S allele showing highest ASI-alcohol composite scores.

Differences in end-of-treatment outcome across genotypes

We explored the relationship between genotypes and cocaine-related outcome measures and found no significant association with length of treatment, number of negative UDS, counseling sessions attended, dropouts, aftercare participants and ASI-drug scores. The data are summarized in Tables 2 and 3. However, there was a significant difference in ASI alcohol scores at end of treatment in the three sets of comparisons, with ASI-alcohol scores being higher in patients carrying the S allele. The strongest difference was observed assuming a dominant effect of S (Table 2).

Differences in follow-up measures across genotypes

Consistent with the negative results observed for cocaine-related end-of-treatment measures, follow-up comparisons did not reveal any differences in ASI-drug scores or urine samples across genotypes. However, differences in ASI-composite scores between genotypes that were found at end of treatment continued to remain significant at follow-up (Table 2). The ASI-scores at follow-up continued to be higher in those carrying the S allele ($p < 0.01$).

Changes in drug and alcohol use within 5-HTTLPR genotypes

Within-subject repeated-measure ANOVA in the entire sample showed that both alcohol and drug composite scores were significantly reduced over time ($F_{10.96}$, $p = 0.000$ and $F_{10.39}$, $p = 0.001$, respectively). After correction for multiple comparisons, ASI drug composite score decreased significantly from baseline to end of treatment for the LL ($p = 0.04$), LS ($p = 0.02$) and SS ($p = 0.03$) genotypes. Similar reductions were observed at follow-up in each genotype ($p < 0.05$ in each case). However, no significant differences were detected between genotypes in reduction in drug use ($F_{0.01}$, $p = 0.98$). The findings are summarized in Fig. 1.

Interestingly, multivariate analyses found that alcohol use was significantly different between genotypes over the treatment and follow-up periods ($F_{3.68}$, $p = 0.03$). In particular, *post-hoc* comparisons showed that SS subjects showed increased alcohol use compared to LL ($p = 0.02$) and LS ($p = 0.001$) at end of treatment and at follow-up ($p = 0.03$ for the LL genotype and $p = 0.002$ for the LS genotype). Controlling for admission alcohol scores did not change the strength of significance (ANCOVA $F_{3.11}$, $p = 0.04$). Because depression has been associated with alcohol use (Brown *et al.*, 1998), the data were reanalyzed using ANCOVA controlling for depression. The differences in alcohol use between genotypes continued to remain significant after controlling for BDI ($F_{3.46}$, $p = 0.04$), SCL-90R depression ($F_{2.44}$, $p = 0.04$) and ASI-psychological ($F_{3.92}$, $p = 0.03$) scores (Fig. 2).

We then investigated the association between changes in drug and alcohol use and found an inverse correlation for the SS genotype ($r = -0.58$, $p = 0.015$). In these patients cocaine

use decreased, while alcohol consumption increased over time.

Discussion

To the best of our knowledge, this is the first published study that examined the relationship between the 5-HTTLPR polymorphism and response to standard behavioral treatment in African American substance-abusing individuals. While we found no association of the 5-HTTLPR variants with severity of cocaine abuse or any cocaine-related outcome measures, the data suggested that the 5-HTTLPR polymorphism may be associated with measures of alcohol use. Subjects with the S allele had the highest alcohol severity scores at admission and

Table 2. Comparisons of the demographics, clinical variables and outcome measures across genotypes and alleles

Variable	Genotypes LL vs. LS vs. SS	Dominant S LL vs. LS + SS	Alleles L vs. S
Admission ($n = 141$)	ANOVA/ χ^2/Fisher's exact tests		
Gender	0.05	0.02	0.19
Age	0.54	0.20	0.01
BDI	0.58	0.01	0.49
SCL-90 depression	1.45	0.15	1.37
Negative urine	0.51	0.41	0.49
Cocaine use			
Age of onset	0.66	1.16	0.15
Duration	0.29	0.21	0.01
Quantity	0.87	1.64	0.88
Frequency	0.08	0.16	0.01
ASI			
Medical	0.18	0.07	0.00
Employment	0.01	0.01	0.04
Alcohol	2.44	4.84*	2.79
Drug	0.84	1.37	0.1.61
Legal	0.26	0.05	0.27
Family	0.53	0.11	0.14
Psychological	0.03	0.06	0.04
12 weeks ($n = 101$)			
Days in treatment	0.83	0.18	0.79
Sessions completed	1.13	0.19	0.20
Negative urines	0.30	0.16	0.31
Dropout	0.941	0.42	0.36
ASI-drug	0.28	0.23	0.49
ASI-alcohol	4.05*	8.16***	4.68*
Follow-up ($n = 92$)			
ASI-drug	0.34	0.19	0.74
ASI-alcohol	4.93**	8.61***	8.71***
Negative urine	3.40	0.07	0.06

*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$; all other comparisons not significant. Degrees of freedom (df) for ANOVA ranged from 1,84 to 1,140 for genotype comparisons and from 1,168 to 1,281 for allele comparisons. Bonferroni correction was performed for multiple comparisons.

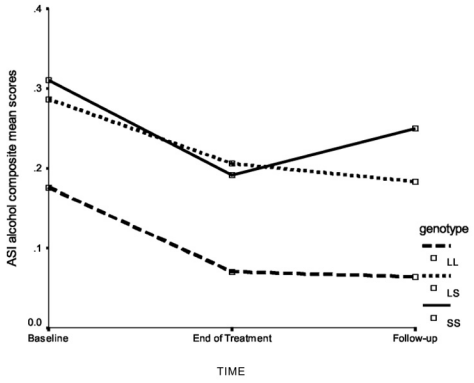

Figure 2. *Mean ASI alcohol composite scores for each genotype during treatment and at follow-up. Alcohol scores reduced significantly over time within each genotype (ANOVA $F = 10.96$, $p < 0.01$). Also significant differences were observed between genotype post-hoc with SS group showing higher scores at end of treatment and at follow-up compared to LL and LS genotypes (p ranged from < 0.05 to < 0.01).*

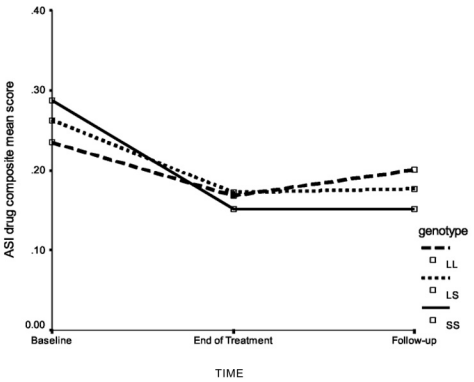

Figure 1. *Mean ASI drug composite scores for each genotype during treatment and at follow-up. While drug scores reduced significantly over time within each genotype (ANOVA $F = 10.39$, $p = 0.001$), no significant differences were detected between genotypes (ANOVA $F = 0.01$, $p = 0.98$).*

Table 2. In-treatment and end of treatment outcome measures across genotypes in cocaine-dependent patients

	Total ($n = 141$) LL ($n = 50$)		LS ($n = 60$)	SS ($n = 25$)
	Genotypes	35%	47%	18%
Number of sessions	21.2(20.7)	20.1 (22.6)	25.1(20.1)	16.0 (17.0)
Number of negative urines	5.3 (6.4)	5.1 (6.1)	6.24(6.96)	3.76 (5.01)
Days in treatment	62.9(55.9)	65.6 (62.7)	65.8 (52.4)	49.8 (50.9)
Dropouts	40 (28%)	14 (28%)	17 (28%)	9 (36%)
Aftercare participants	41 (29%)	15 (30%)	20 (30%)	6 (24%)

All comparisons non-significant.

the SS genotype group benefited the least from treatment in terms of reduction of alcohol severity at end of treatment and at follow-up. This relationship was observed even after controlling for baseline depression and drug severity scores. The relationship between severity of alcohol use and SS genotype observed prior to treatment is consistent with several reports that susceptibility to alcohol dependence may be linked to the 5-HTTPLR SS variant (Sander *et al*., 1997; Schuckit *et al*., 1999; Lichtermann *et al*., 2000; Stoltenberg 2003; Herman *et al*., 2003; Nellissery *et al*., 2003). Interestingly, we observed that individuals with the SS genotype failed to alter their drinking behavior with behavioral treatment significantly as opposed to the LL and LS genotypes. The SS genotype is reported to be associated with reduced 5-HTT expression in cell lines (Lesch *et al*., 1996) and it can be argued that individuals with such a 'serotonergic impairment' are less likely to respond to a behavioral approach. It seems logical to explore whether these patients would respond to a pharmacological agent such as a selective serotonin uptake inhibitor, a question we could not address in the present study. In this context impaired serotonergic neurotransmission was found to predict poor response to citalopram in alcoholic patients (Berggren *et al*., 2001). From a clinical standpoint, whether the 5-HTTLPR polymorphism may distinguish between responders and non-responders to treatment among alcohol abusers merits further investigation.

As expected, there was a reduction in severity of cocaine use with behavioral treatment; however, all three genotypes showed comparable reduction. This suggests that the 5-HTTLPR polymorphism does not appear to be associated with cocaine-related outcome measures in a behaviorally oriented treatment program. We had previously failed to observe an association of the 5-HTTLPR polymorphism with susceptibility to cocaine dependence (Patkar *et al*., 2001). The findings from the present study add to the negative data regarding the role of the 5-HTTLPR gene in cocaine dependence. At follow-up, there was a noticeable but statistically non-significant reduction in cocaine use among the SS genotype compared to the LL and LS genotypes. Interestingly, an inverse correlation between cocaine and alcohol use was observed among the SS genotype. It is difficult to draw any definitive conclusions about the link between reduced cocaine use and increased alcohol use among the SS genotype at follow-up, but it is worth noting that substitution of cocaine with alcohol is reported commonly among substance abusers (Magura and Rosenblum, 2000).

A few other findings deserve comment. First, African American substance abusers in our study showed a lower frequency of the L allele (58.6%), compared to healthy African American individuals from a recent investigation (77–87%) (Lotrich *et al*., 2003). We have no explanation for these differences, although our results are similar to those reported across ethnic groups in substance abusers (Sander *et al*., 1998; Edenberg *et al*., 1998; Heinz *et al*., 2000) or healthy controls from either western Europe or the United States (Lesch *et al*., 1996; Hoehe *et al*., 1998; Heinz *et al*., 2000). Secondly, the computed power to detect differences was low. Assuming a moderate effect size of 0.30 (Cohen, 1988), the power to detect differences between genotypes in a $\times$ 1 ANOVA design was about 0.2 in our sample. In other words, to detect genotype differences for changes in drug severity between baseline and follow-up, a sample size of nearly 2500 cocaine patients would be required, suggesting that the 5-HTTLPR polymorphism does not exert a clinically meaningful effect on treatment response among cocaine abusers. Thirdly, other limitations of the study need to be acknowledged. These include a mixed population of cocaine and alcohol abusers, rather than a 'pure' alcohol or cocaine abusing subset; lack of objective measures of alcohol use, a 30% dropout rate and an exclusively African American population. Therefore our findings should be considered preliminary and deserve replication in large samples, preferably of primary alcohol users and across other ethnic groups. It also seems timely to extend the study of the 5-HTTLPR genotype response to pharmacological agents.

In conclusion, the 5-HTT genotypes are not associated with any outcome measures of cocaine use. On the other hand, our data suggest that the 5-HTTPLR polymorphism may distinguish responders from non-responders to behavioral treatment in terms of alcohol use. Further investigations are required to determine the role of the 5-HTTLPR polymorphism in influencing treatment–outcome among substance abusers.

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