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Interaction between Childhood Trauma and Serotonin Transporter Gene Variation in Suicide

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Although the serotonin transporter promoter polymorphism (*5-HTTLPR*) contributes to depression and suicidality in a fashion modulated by environmental stress, *5-HTTLPR* has been little examined in relation to suicidal behavior in substance dependence. Recently, a third functional allele of *5-HTTLPR* was discovered enabling more of the interindividual variation in serotonin transporter expression to be predicted by genotype. We examined whether the *5-HTTLPR* gene alone, or interacting with childhood trauma, was predictive of suicidal behavior in substance-dependent patients, a clinical population that is at high risk of suicide, as well as childhood trauma and other stress. We interviewed 306 abstinent male African-American substance-dependent patients about whether they had ever attempted suicide and administered the 34-item Childhood Trauma Questionnaire (CTQ). Patients and 132 male African-American controls were genotyped to determine the S, L_G, and L_A *5-HTTLPR* alleles; some analyses grouped the S and L_G alleles on the basis of equivalent function. The distribution of *5-HTTLPR* genotypes did not differ between patients and controls, nor between suicide attempters and non-attempters. However, patients with low expression *5-HTTLPR* genotypes and above-median CTQ scores were more likely to have attempted suicide. Logistic regression showed increasing risk of a suicide attempt with increasing reports of childhood trauma scores; in addition, this increase was exaggerated among those with low expression forms of the *5-HTTLPR* genotype. Childhood trauma interacts with low expressing *5-HTTLPR* genotypes to increase the risk of suicidal behavior among patients with substance dependence.
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INTRODUCTION

Suicide and suicide attempts are a major problem in substance dependence (Hser *et al*, 2001). Harris and Barraclough (1997), in a meta-analysis of follow-up studies, found that both alcohol- and opiate-dependent patients had a standardized mortality ratio for suicide 14 times higher than expected. Patients with cocaine dependence also have a greater risk of suicide. Marzuk *et al* (1992) found that 29% of suicide victims, aged 21–30 years, in New York tested positive for cocaine. Data from the Epidemiologic Catchment Area Survey showed that cocaine abusers had a significantly increased risk of attempting suicide and clinical studies report that up to 40% of cocaine-dependent patients have attempted suicide at some time (Petronis *et al*, 1990; Roy, 2001). The risk for an attempt at suicide is also increased among alcohol- and opiate dependent patients (reviewed in Roy and Linnoila, 1986). Up to 40% of alcoholics and 50% of opiate dependent patients attempt suicide at some time in their life (Johnson and Fridell, 1997; Kosten and Rounsaville, 1988).

Clinical risk factors for suicidal behavior in alcohol- and drug-dependent patients have been studied (Darke and Ross, 2002; Roy *et al*, 1996; Roy, 2002, 2003). However, in a 5 year follow-up of alcoholics, clinical risk factors accounted for less than half of the variance for suicidal behavior, whereas genetic-epidemiologic studies indicate that up to half of the variance for attempting suicide is owing to genetic factors (Preuss *et al,* 2003; Statham *et al,* 1998; Glowinski *et al,* 2001; Fu *et al,* 2002). As low central serotonin function is implicated in suicidal behavior, we examine here the possible role of the serotonin transporter gene in suicidal behavior among substance dependent patients (Asberg, 1997). To do this we initially compare rates of suicide attempts and serotonin transporter promoter (*5-HTTLPR*) genotypes in substance dependent patients and controls.

The serotonin transporter (5-HTT) is located on presynaptic serotonin neurons and is responsible for the reuptake of serotonin. The protein is encoded by a single

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gene on chromosome 17 (Ramamoorthy *et al,* 1993). A functional polymorphism in the 5′ regulatory promoter region of the 5-HTT gene (*5-HTTLPR*) involves two common alleles (0.4–0.6) that regulate transcription of the gene (Heils *et al,* 1996). The allele containing 14 copies of an imperfect 22 bp repeat (s allele) which reduces transcription efficiency of the 5-HTT gene as compared to the 16 repeat allele (l allele) (Lesch *et al,* 1996), and as also shown *in vivo* (Heinz *et al*, 2000) and in post-mortem brain (Little *et al*, 1998). Recently, a third functional allele was discovered which is an A > G single nucleotide polymorphism within the first of two extra repeats that characterize the l allele (Hu *et al,* 2006). The L_G allele is relatively common in Caucasian populations (0.09–0.15) and still more abundant in African-American populations (0.24) and it is associated with a low rate of HTT transcription, such that the L_G allele is functionally similar to the S allele (Hu *et al,* 2006). Because population stratification can produce spurious false positive results, and the fact that our controls and the majority of our patients are African American, we decided to restrict the subjects in this study to African Americans.

The many studies of the role of HTTLPR in suicidal behavior among patients with psychiatric disorders, only a few with substance dependence, have been reviewed by Lin and Tsai (2004). Not surprisingly for a complex behavior such as suicide, there is substantial between-study heterogeneity, with some studies reporting no linkage. However, Lin and Tsai concluded that the *5-HTTLPR* short allele occurred significantly more often among suicide attempters than among psychiatric patients who had never attempted suicide.

Caspi *et al* (2003) reported an interaction between *5-HTTLPR* and stressful life events for depression, suicidal ideation, and suicide attempts. The loss-of-function s allele and s-containing genotypes were again implicated. They carried out a prospective longitudinal study of 1037 Dunedin children assessed at regular intervals till the age of 26 years. They measured stressful life events occurring between the age of 21 and 26 years. Individuals with an s allele and with stressful life events after 21 years of age had an increase in depressive symptoms and suicidal behavior/ideation whereas l/l homozygotes did not. Furthermore, when Caspi *et al* (2003) examined childhood maltreatment that occurred during the first 10 years of age they similarly found that childhood trauma predicted depression as an adult—but again only among individuals with the s allele and not among l/l homozygotes.

Childhood maltreatment is a developmental risk factor that may predispose an individual to later suicidal behavior. Many general population and clinical studies have shown that childhood trauma like physical, sexual, and emotional abuse—and neglect—are associated with attempting suicide as an adult. As we had administered the 34-item Childhood Trauma Questionnaire (CTQ) to our sample of abstinent substance-dependent patients, who had been interviewed about whether or not they had ever attempted suicide and genotyped for *5-HTTLPR*, we reasoned that it would be especially important to evaluate the interaction between HTTLPR and environmental trauma in this clinical population in which suicide and suicidality are frequently seen.

SUBJECTS AND METHODS

A series of 306 male abstinent substance-dependent African-American patients was seen in the Substance Abuse Treatment Program at the Department of Veterans Affairs New Jersey Healthcare System (VANJHCS), East Orange Campus. Inclusion criteria: Patients were >18 years of age, met DSM-IV criteria for substance dependence, identified themselves as African American, and had been abstinent for at least 2 weeks in preparation for other biologic tests. Excluded were patients with mental retardation, dementia, or acute psychosis. African American male control subjects ($N = 132$) were recruited at the University of Medicine and Dentistry of New Jersey: New Jersey Medical School (UMDNJ), Newark, from among insulin-dependent diabetic outpatients seen at an ophthalmology clinic and in blood banks. After complete description of the study, all subjects gave written informed consent to protocols approved by the Institutional Review Boards of the VANJHCS and UMDNJ.

A semi-structured psychiatric interview, presented elsewhere, was conducted among the patients by a psychiatrist (AR), collecting socio-demographic variables, psychiatric history, and history of attempting suicide (Roy, 2001, 2002). Data from the patient, particularly for suicidal behavior, was supplemented by collateral information from mental health program staff, medical records, the program internist, and physician's assistant, and previous treating mental health professionals. A suicide attempt was defined as an act that was self-destructive with some intent to end one's life which was not self-mutilatory in nature. This definition was implemented by the first author, a psychiatrist, after interviewing the patient, and reviewing all of the collateral information detailed above. Psychiatric status of the controls was determined by a semi-structured screening interview. The CTQ (34-item version), which yields scores for childhood physical abuse, emotional abuse, sexual abuse, physical neglect, and emotional neglect, was completed only by patients (Bernstein *et al,* 1997). Reliability and validity of the CTQ have been demonstrated (Bernstein *et al,* 1994, 1997). These CTQ data have been previously reported in studies of clinical risk factors for suicidal behavior (Roy, 2001, 2002).

Genomic DNA was extracted from blood. Genotyping of *5-HTTLPR* for S, L_G, and L_A alleles was performed using a two-stage 5′ exonuclease assay as described by Hu *et al* (2006). The high-expressing L allele is L_A. The two functionally equivalent low-expressing alleles are L_G and S. The *5-HTTLPR* allele function was determined by assays of mRNA levels in lymphoblastoid cell lines and a raphe neuron-derived cell line transfected with allele-specific expression constructs (Hu *et al*, 2006).

For statistical analyses, the S and L_G alleles were grouped based on their functional similarity as low expressing alleles. This yields three expression-defined genotype groups: low: SS, SL_G, and L_GL_G; intermediate: SL_A and L_GL_A; and high, L_AL_A. However, because of power considerations and because suicide may be a threshold behavior, we also compared two groups—a low-expression group defined as SS or SL_G, and high expression group comprised of all other genotypes. We first compared the distribution of genotypes between patients and controls by the χ^2 test. Then the rate of suicide attempts was compared

by χ^2 test between patients divided into groups using the median CTQ value, as well as by the three-category genotype grouping. Finally, the main effects and interaction of CTQ scores and the 2-category genotype grouping on suicide attempt were evaluated by logistic regression.

RESULTS

The sample consisted of 306 male African-American abstinent substance dependent patients in early remission and 132 male African-American controls (52 normal controls and 80 insulin-dependent diabetic patients). Table 1 shows similar distributions of *5-HTTLPR* genotypes in substance-dependent patients and controls.

Table 2 shows that suicide attempters reported significantly more childhood trauma than non-attempters. However, attempters were similar to non-attempters in age and in distribution of *5-HTTLPR* genotypes.

Figure 1 shows that suicide attempts are predicted by childhood trauma, with a potential interaction of HTTLPR. The sample is divided using the median split for CTQ scores, and using the three functional categories of HTTLPR genotypes: high, intermediate, and low-expressing. For CTQ scales of emotional abuse ($\chi^2(1) = 4.46$, $p = 0.035$), physical abuse ($\chi^2(1) = 8.58$, $p = 0.003$), emotional neglect ($\chi^2(1) = 8.58$, $p = 0.003$) and physical neglect ($\chi^2(1) = 5.84$, $p = 0.016$), higher levels of childhood trauma were associated with increased rates of suicide attempts in the low-expressing genotype group. Although not significant, higher attempter rates were also seen in the low-expressing group reporting higher levels of sexual abuse ($\chi^2(1) = 2.57$, $p = 0.11$). Rates of suicide attempts in the intermediate and high expression groups were unaffected by increasing levels of childhood trauma, except for emotional neglect in the high-expressing group ($\chi^2(1) = 6.06$, $p = 0.014$). These data suggest that childhood trauma interacts with the low-expressing *5-HTTLPR* genotypes to affect rates of suicide attempts.

Table 1 Rates of Suicide Attempts, Diagnoses and 5-HTTLPR Genotypes in Male African-American Abstinent Substance Dependent Patients and Controls

Variable	Controls $N = 132$	Patients $N = 306$	Group comparison
Suicide attempt	1.5%	30.7%	$\chi^2(1) = 45.6$, $p < 0.001$
Dependence			
Alcohol	8.3	52.6	$\chi^2(1) = 75.8$, $p < 0.001$
Cocaine	3.0	68.6	$\chi^2(1) = 158.8$, $p < 0.001$
Heroin	3.8	48.0	$\chi^2(1) = 79.7$, $p < 0.001$
Triallelic genotype			
SS	6.1	7.8	$\chi^2(5) = 4.7$, $p = 0.46$
SL_G	10.6	14.4	
SL_A	26.5	24.8	
L_GL_G	7.6	6.2	
L_GL_A	19.7	24.8	
L_AL_A	29.5	22.2	

To evaluate further the association between childhood trauma and the *5-HTTLPR* genotype on risk of suicide attempt, logistic regression was used to model risk as a function of each of the (continuous) childhood trauma scores, high- *vs* low-expression genotype, and their interaction. Table 3 shows that each model including main effects of different types of trauma and HTTLPR genotype significantly predicted attempter status (Step 1). In these logistic regression models, the different types of childhood trauma were associated with ORs for risk of making a suicide attempt ranging from 1.04 to 1.10. Thus, a one point increase in childhood trauma score was associated with a 4% (for emotional neglect) to 10% (for physical abuse) increase in risk of making a suicide attempt. Genotype alone did not predict attempter status.

In step 2, a G × E interaction was seen for physical abuse and for emotional neglect. For both of these environmental predictors of suicide, there was a significant increment in risk in the context of the low-expressing *5-HTTLPR* genotype. Although the interaction was significant for physical abuse and emotional neglect, we note that G × E interaction effects were of similar magnitude for other childhood traumas. Thus, childhood trauma interacted with low-expressing *5-HTTLPR* genotype to increase the risk of a suicide attempt, over and above the risk associated with childhood trauma alone.

Table 2 Age, Childhood Trauma, Dependence Diagnosis, and 5-HTTLPR Genotypes as Risk Factors for Suicide Attempts in Abstinent African-American Male Substance Dependent Patients Who Completed the CTQ

	Ever attempted suicide		
	Yes $N = 81$	No $N = 176$	Group comparison
Age (years) mean (SD)	46.5 (7.2)	44.9 (7.5)	$p = 0.09$
CTQ mean (SD)			
Physical abuse	13.1 (6.5)	10.2 (4.7)	$p < 0.001$
Emotional abuse	12.1 (5.7)	10.2 (5.1)	$p = 0.001$
Sexual abuse	10.4 (6.1)	8.7 (4.4)	$p = 0.011$
Physical neglect	15.2 (6.2)	13.5 (5.3)	$p = 0.025$
Emotional neglect	26.0 (9.5)	22.4 (8.8)	$p = 0.003$
Dependence			
Alcohol only	10.6%	9.0%	NS
Cocaine only	14.9	18.4	NS
Heroin only	6.4	15.6	NS
Multiple	67.9	55.2	$p = 0.05$
Triallelic genotype			
SS	11.7	6.1	NS
SL_G	13.7	14.9	
SL_A	22.3	25.9	
L_GL_G	6.4	6.1	
L_GL_A	23.4	25.5	
L_AL_A	21.3	22.6	

To illustrate the difference in risk of a suicide attempt attributable to these interactions, Figures 2 and 3 show scatter plots relating predicted risk of a suicide attempt (from the 3-variable logistic regression modeling) for the CTQ scales of physical abuse and emotional neglect, separately for those with and without the low-expressing *5-HTTLPR* genotype. The figures show that increasing childhood trauma was associated with increasing risk of attempting suicide, regardless of genotype (the main effect). Second, the slope of the effect for the group defined by low-expression HTTLPR genotypes is steeper than that for the group with high expressing genotypes (the interaction). Thus, although increasing levels of childhood trauma generally increase the risk of attempting suicide, the rate of increase is greater in those with the low-expressing *5-HTTLPR* genotype.

To explore the question of whether these associations might be mediated by major depressive disorder (MDD), further regression modeled MDD as an additional step. (Because 32 subjects were not assessed for lifetime MDD, Step 1, which would otherwise be redundant with Table 3, is repeated). Table 4 shows very strong effects of MDD on attempter status. However, although controlling for MDD status, reduces the ORs for risk attributable to CTQ scores and the interaction slightly, those effects were not fully erased. Although preliminary, this analysis is consistent with the conclusion that CTQ scores and *5-HTTLPR* status represent risks for suicide attempts that are independent of those associated with depression.

Table 3 Logistic Regressions Relating High and Low Expressing Forms of the 5-HTTLPR Genotype and CTQ Scores to Attempter Status

Variable	Step 1 OR (95% CL)	Step 2 OR (95% CL)
HTTLPR	1.42 (0.76, 2.67)	0.28 (0.05, 1.55)
CTQ physical abuse	**1.10 (1.05, 1.15)**	**1.07 (1.01, 1.13)**
G X E interaction	—	**1.15 (1.01, 1.31)**
Goodness of fit	$\chi^2(2) = 16.0$, $p < 0.001$	$\chi^2(1) = 4.64$, $p = 0.03$
HTTLPR	1.46 (0.79, 2.71)	0.55 (0.11, 2.60)
CTQ emotional abuse	**1.07 (1.02, 1.12)**	1.05 (0.995, 1.11)
G X E interaction	—	1.09 (0.96, 1.24)
Goodness of fit	$\chi^2(2) = 8.47$, $p = 0.014$	$\chi^2(1) = 1.9$, $p = 0.16$
HTTLPR	1.37 (0.74, 2.53)	0.74 (0.18, 3.0)
CTQ sexual abuse	**1.06 (1.01, 1.12)**	1.05 (0.99, 1.11)
G X E interaction	—	1.06 (0.93, 1.21)
Goodness of fit	$\chi^2(2) = 7.1$, $p = 0.03$	$\chi^2(1) = 0.9$, $p = 0.33$
HTTLPR	1.34 (0.73, 2.49)	0.38 (0.07, 2.19)
CTQ physical neglect	**1.05 (1.01, 1.10)**	1.03 (0.97, 1.08)
G X E interaction	—	1.09 (0.98, 1.21)
Goodness of fit	$\chi^2(2) = 5.75$, $p = 0.06$	$\chi^2(1) = 2.42$, $p = 0.12$
HTTLPR	1.40 (0.75, 2.61)	0.22 (0.03, 1.57)
CTQ emotional neglect	**1.04 (1.01, 1.08)**	1.03 (0.99, 1.06)
G X E interaction	—	**1.08 (1.01, 1.16)**
Goodness of fit	$\chi^2(2) = 10.0$, $p = 0.007$	$\chi^2(1) = 4.27$, $p = 0.04$

For each regression, Step 1 shows OR (95% confidence limits) for the main effects of 5-HTTLPR status and each measure of childhood trauma. Step 2 shows the model with that interaction. χ^2 statistics indicate significance of the full model for Step 1, and for the significance of the addition of the interaction on Step 2. Bold indicates $p < 0.05$.

DISCUSSION

In this study childhood trauma—without any consideration of genotype—was associated with an increased risk of a suicide attempt, as previously reported in these patients (Roy, 2001, 2002). However, the results of the present study advance these previous reports as a serotonin transporter genotype interacted with childhood trauma to increase the

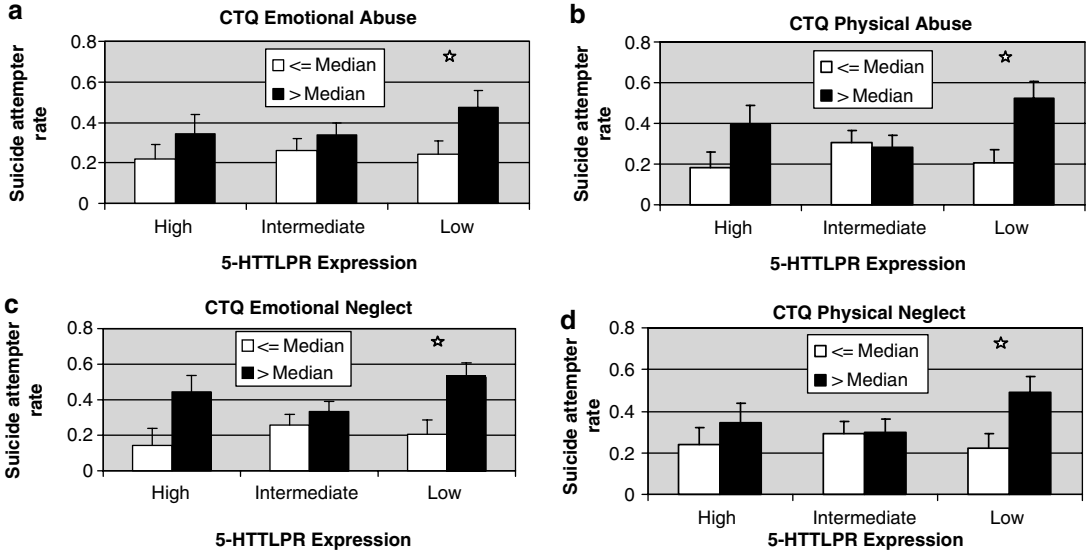

Figure 1 Suicide attempter rates (±SD) as a function of median CTQ scores (a), emotional abuse, (b), physical abuse, (c), emotional neglect, and (d), physical neglect; and high, intermediate, and low expression groupings of the *5-HTTLPR* genotype. *Significantly increased attempt rate in low expression group with higher CTQ scores, $p = 0.035$ for emotional abuse, $p = 0.003$ for physical abuse and emotional neglect, and $p = 0.016$ for physical neglect.

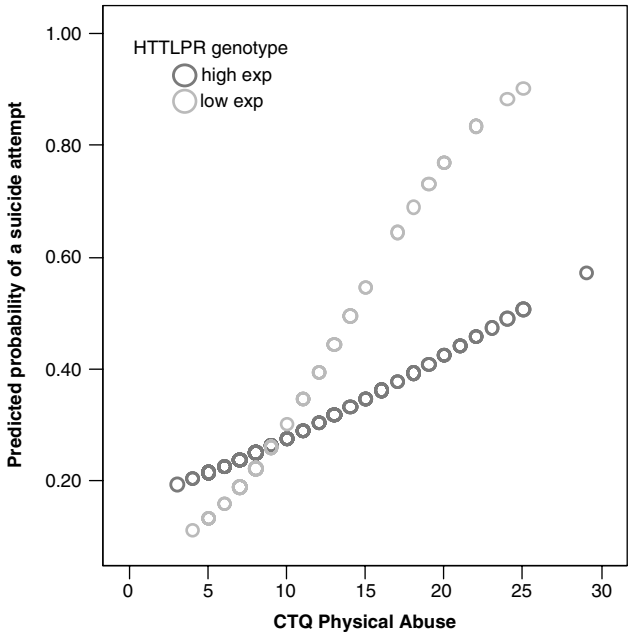

Figure 2 Computed probability of a suicide attempt as a function of childhood physical abuse score, in those with low-expressing, and high-expressing forms of the *5-HTTLPR* gene.

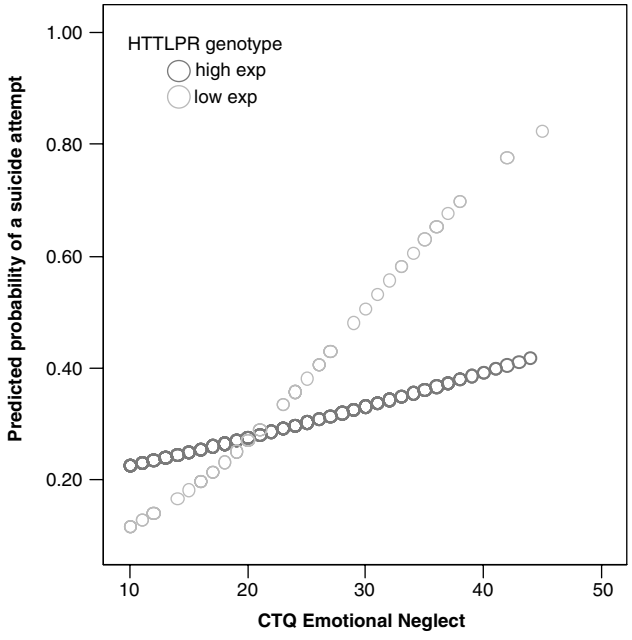

Figure 3 Computed probability of a suicide attempt as a function of childhood emotional neglect score, in those with low-expressing, and high-expressing forms of the *5-HTTLPR* gene.

likelihood of a suicide attempt. Specifically, among those with the low-expressing *5-HTTLPR* genotype, but not among those with intermediate or high-expressing genotypes, above median CTQ scores were associated with a significant additional risk of a suicide attempt. Logistic regression showed that a one-point increase in CTQ scale score for childhood physical abuse and childhood emotional neglect were associated with 7 and 3% increases, respectively, in risk of a suicide attempt in all patients, as well an additional 15 and 8% increases, respectively, among those with the low-expressing genotype. Thus, the low-expression *5-HTTLPR* genotype amplified the risk of suicide attempt associated with childhood trauma.

Table 4 Odds Ratios from Logistic Regression Relating 5-HTTLPR Genotype Status ($SS+SL_G$ *vs* Others) and CTQ Physical Abuse and Emotional Neglect Scores to Attempter Status

	Step 1	Step 2
HTTLPR	0.40 (0.07, 2.29)	0.38 (0.05, 2.65)
Physical abuse	**1.07 (1.01, 1.13)**	1.04 (0.98, 1.11)
G × E interaction	1.11 (0.97, 1.27)	1.15 (0.99, 1.35)
MDD		**7.46 (3.57, 15.57)**
HTTLPR	0.42 (0.05, 3.23)	0.83 (0.09, 7.27)
Emotional neglect	**1.04 (1.00, 1.08)**	1.03 (0.99, 1.07)
G × E interaction	1.05 (0.97, 1.13)	1.03 (0.95, 1.12)
MDD		**6.97 (3.42, 14.21)**

In step 1, OR (95% confidence limits) are shown for the main effect of 5-HTTLPR status and trauma, as well as their interaction. In step 2, the model is controlled for the lifetime diagnosis of MDD. Bold indicates $p<0.05$.

A gene–environment interaction between life events and *5-HTTLPR* polymorphism was first reported by Caspi *et al* (2003). They found a significant interaction between the s allele and life events occurring after 21 years of age in producing depressive symptoms from the age of 21–26 years. There are now both replications and extensions of this finding as well as failures to confirm (Gillespie *et al,* 2005; Kaufman *et al*, 2004; Eley *et al,* 2004; Kendler *et al*, 2005; Surtees *et al,* 2006; Wilhelm *et al,* 2006; Zalsman *et al*, 2006). Interestingly, and akin to the childhood trauma results of the present study, Caspi *et al* (2003), also found that a significant interaction between stressful life events and *5-HTTLPR* genotype predicted suicidal ideation or suicide attempts, but again only among subjects with an s allele.

As in the present study an interaction of *5-HTTLPR* genotype with early experience has been reported to affect central serotonin functioning in primates Bennett *et al* (2002), compared monkeys reared with their mothers with monkeys separated from their mothers at birth and peer-reared. The measure of central serotonin function used was cisternal CSF 5-HIAA concentrations, which they and others had previously shown were influenced by both genetic and environmental factors (Higley *et al,* 1991, 1992, 1993; Rogers *et al,* 2004). They found that CSF 5-HIAA concentrations were significantly influenced by genotype but only in the peer-reared monkeys. Rhesus macaques have an orthologous serotonin transporter promoter polymorphism that is known as rh-HTTLPR, and that exerts similar effects on transporter expression and behavior. Peer-reared s/l heterozygous monkeys had significantly lower CSF 5-HIAA concentrations than peer raised homozygous monkeys with two l alleles. However, there was no difference in CSF 5-HIAA concentrations between the s/l or l/l genotypes in mother-reared monkeys. Only monkeys with early deleterious rearing experiences showed the genotype-predicted difference in CSF 5-HIAA concentrations. These CSF 5-HIAA data are relevant as low CSF 5-HIAA concentrations are found among patients attempting suicide (reviewed in Asberg, 1997).

Also relevant are earlier findings by Higley *et al* (1992, 1996, 1998) and others, that monkeys with low CSF 5-HIAA

concentrations were significantly more impulsive and aggressive than monkeys with CSF 5-HIAA concentrations within the normal range (Higley *et al*, 1992, 1996, 1998; Mehlman *et al,* 1994; Westergaard *et al,* 1999). These data are relevant as the trait of impulsive aggression is associated with low CSF 5-HIAA concentrations in humans and is thought to be an intermediate phenotype for suicidal behavior (Roy and Linnoila, 1988; Higley and Linnoila, 1997; Williams *et al,* 2003; Zhou *et al,* 2005).

In the present study, there was no significant difference between patients attempting suicide and controls for the frequency of low- or high-expressing alleles of *5-HTTLPR*. This negative case–control result is in accord with findings reported by others, as reviewed by Lin and Tsai (2004). Evaluating 18 case–control studies, they found no significant difference in the distribution of *5-HTTLPR* genotypes between 1521 cases exhibiting suicidal behavior and 2429 normal controls. Although those studies were composed primarily of Caucasian subjects, the negative case–control result in the present study of African American males confirms that result. Furthermore, we have genotyped the S, L_A, and L_G alleles, the L_G allele being especially important to distinguish in African-Americans, because of its high frequency in this population (0.24). Lin and Tsai also observed significant heterogeneity among the odds ratios of the 18 studies they reviewed, and suggested this might be attributed to the presence of some moderating variables. The present results suggest the possibility that two such moderating variables may include childhood trauma and unrecognized *5-HTTLPR* allelic variation.

In case–control association studies, population stratification can produce spurious false-positive results owing to ethnic stratification of diagnostic groups and a difference in allele (haplotype) frequencies between populations. This latter point could be a special concern for the African-American population we studied because of its genetic diversity and history of admixture. To formally evaluate the role of population stratification in the present study, we genotyped 186 ancestry informative markers (AIMS). Each is a single nucleotide polymorphism (SNP) tested for allele frequency in Hapmap in four source populations: Caucasians, Chinese, Japanese, and Yoruban Africans, and as verified by us in Chinese, Caucasian, and African populations, as well as in American Indians. Each SNP selected was a highly informative AIM, having an allele frequency difference of at least 0.7 between two of the ethnic populations and a 10-fold allele frequency difference between two of the ethnic populations. We used Structure 2 (Pritchard) to define ethnic variation factors present within populations and to determine the factor membership of each individual in the dataset. Three populations we tested (Finnish Caucasians, Han Chinese, and American Indians) showed very little admixture, but those individuals who were admixed were readily identifiable. Thus far, we have genotyped 50 of the African-Americans in the present sample for the 186 AIMS. The average degree of Caucasian admixture is 0.11, insufficient to account for the present association in which the low-expression HTTLPR alleles are substantially modulating the effect of childhood trauma on the risk of suicide attempt. In the context of concern about ethnic stratification, it is also important to recognize that we did not find that HTTLPR predicted suicide attempts or substance dependence. The effect of HTTLPR was to modulate the effect of trauma on suicide attempts, and it did so in a fashion coherent with the HTTLPR/trauma gene–environment effect also seen in the Dunedin study of a Caucasian cohort.

Limitations of the present study include that not all of the patients completed the CTQ, that none of the controls completed the CTQ as most were studied in a busy medical clinic, that the definition used for a suicide attempt could encompass heterogeneous phenomena, and that recent stressful life events were not also measured. The controls were mainly a convenience group of African-American insulin-dependent patients and future studies might involve normal controls free of medical as well as psychiatric disorder. Also, self-report studies in substance dependent patients may be confounded by state and trait issues. However, this is not an issue in the present study as all the patients were studied in the third week of an inpatient rehabilitation program when they participated in other biologic tests, which required that they be free of medication and substance abuse for at least 2 weeks. We had no systematic information on duration or severity of dependence, both lifetime and before admission, and thus were unable to evaluate their potential role in the results or as a mediator. Future studies might examine changes in substance availability or habits as they might influence the prevalence of suicidal behavior.

In summary, although substance-dependent patients are a high-risk group for suicidal behavior, present results suggest that the combination of risk genotype and environmental risk further increases that risk. The low-expressing *5-HTTLPR* genotype was found to interact with childhood stress/trauma to increase the risk of suicidal behavior in substance-dependent patients, a clinical group in whom suicide ideation, attempts, and completion is a serious problem.

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