

Gene-by-Environment (Serotonin Transporter and Childhood Maltreatment) Interaction for Anxiety Sensitivity, an Intermediate Phenotype for Anxiety Disorders

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Anxiety sensitivity (AS) is a dispositional characteristic that predisposes to the development of anxiety disorders (eg, panic and post-traumatic stress disorder) and major depression. AS is subject to genetic and environmental influences, the former as yet unidentified and the latter known to include childhood maltreatment. The serotonin transporter gene (*SLC6A4*) promoter polymorphism (5-HTTLPR) has been associated with depression, but most consistently in the context of environmental stress. We tested the hypothesis that 5-HTTLPR genotype and childhood maltreatment would interact to increase susceptibility to AS in young adults. Subjects were European-American college undergraduates ($N = 150$, median age 18 years) characterized on a measures of AS (Anxiety Sensitivity Index) and retrospective childhood maltreatment (Childhood Trauma Questionnaire [CTQ]). 5-HTTLPR genotypes were obtained from blood-derived DNA. Linear regression was used to model relationships between 5-HTTLPR, childhood emotional abuse, and AS; covariates such as sex, neuroticism, and ancestral proportion scores were incorporated into some models in a larger, ethnically heterogeneous sample ($N = 247$) to evaluate robustness of the findings to model assumptions. A statistically significant interaction was observed between levels of childhood emotional (or physical) maltreatment and 5-HTTLPR genotype. Specifically, S/S individuals with higher levels of maltreatment had significantly higher levels of AS than subjects in other groups. No such relationship was found for neuroticism, attesting to the possible specificity of the findings for AS. Findings were consistently robust to the inclusion of covariates, and were not confounded by population stratification. In conclusion, these results provide evidence of a specific genetic influence on anxiety sensitivity—an intermediate phenotype for anxiety (and depressive) disorders; this effect is modified by severity of childhood maltreatment. These findings are consistent with the notion that 5-HTTLPR operates broadly to moderate emotional responsiveness to stress.

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INTRODUCTION

Anxiety sensitivity (AS) refers to the fear of anxiety-related sensations. AS is characterized by the belief that these sensations have harmful consequences (Reiss, 1991). For example, an individual may fear that the sensation of palpitations is indicative of a serious, life-threatening condition, such as a ‘heart attack.’ Some individuals are more prone than others to respond to anxiety symptoms in this fashion; the higher an individual’s level of AS, the more that individual is likely to experience anxiety symptoms as alarming, dangerous, and threatening. The phenotypic

structure of AS is stable across diverse samples (Bernstein *et al*, 2006), attesting to the generalizability of this construct.

AS is a well-established risk factor for the development of panic attacks. In a series of prospective studies of healthy young adults under stress (eg, basic military trainees), AS was found to predict the subsequent onset of panic attacks (as well as generalized anxiety and depressive symptoms) (Schmidt *et al*, 1997, 1999). Similar findings were obtained in a prospective study of healthy adolescents (Hayward *et al*, 2000). AS also predicts fearful responding to a number of biological challenge procedures (Stein and Rapee, 1998), notably carbon dioxide (CO₂) inhalation (Schmidt and Mallott, 2006), the anxious response which has also been noted to be moderately heritable (Battaglia *et al*, 2007). The role of AS may be most pronounced in panic disorder, but scores on the Anxiety Sensitivity Index (ASI) (Peterson and Reiss, 1992), the most frequently used measure of AS, are also elevated in other anxiety disorders, such as

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post-traumatic stress disorder (PTSD) (Bernstein *et al*, 2005; Lang *et al*, 2002). Furthermore, AS has also been associated with the severity of depressed mood (Cox *et al*, 1999b; Taylor *et al*, 1996; Zinbarg *et al*, 2001) suggesting that its influence may extend beyond the anxiety disorders to other Axis I forms of psychopathology (Schmidt *et al*, 2006).

Individual differences in AS are thought to arise from the combined influences of experiential and genetic factors. Twin research has shown that variation in AS (particularly the physical concerns aspect of AS) is explained by unique (as opposed to shared) environmental and additive genetic influences (Stein *et al*, 1999). Emotional maltreatment in childhood—particularly when it includes parental threatening, hostile, and rejecting behaviors—is associated with higher levels of AS in young adults (Scher and Stein, 2003). Thus, childhood emotional maltreatment is a plausible candidate to be an environmental risk factor for AS.

No specific genetic vulnerability factors for AS have yet been identified, but a strong candidate emerges from a convergence of findings with a functional length polymorphism in the promoter of the serotonin transporter gene (gene, *SLC6A4*; variant, 5-HTTLPR). This polymorphism has functional importance in that the ‘short’ (S) variant is less transcriptionally efficient than the ‘long’ (L) variant (Lesch *et al*, 1996). The 5-HTTLPR polymorphism has been shown in many (Caspi *et al*, 2003; Eley *et al*, 2004; Grabe *et al*, 2005; Jacobs *et al*, 2006; Kaufman *et al*, 2004, 2006b; Kendler *et al*, 2005; Wilhelm *et al*, 2006; Zalsman *et al*, 2006), but not all (Gillespie *et al*, 2005; Surtees *et al*, 2006) studies to interact with stressful life events to increase susceptibility to major depression or depressive symptoms. In one of these recent studies, the investigators surmised that this genotype-by-environment interaction might be best understood as involving mechanisms more proximal to psychological experience (eg, negative affectivity) than to a direct risk pathway to psychiatric illness (Jacobs *et al*, 2006). Following this line of reasoning, we hypothesized that a similar relationship would explain some of the interindividual variation in AS, which is itself a risk factor for anxiety and depressive disorders. This expectation was further bolstered by the finding of a gene (again, *SLC6A4* variant 5-HTTLPR)-by-environment (social support) interaction in the prediction of another anxiety-related trait—behavioral inhibition with unfamiliar peers in childhood (Fox *et al*, 2005), which is a risk factor for anxiety disorders such as social phobia (Biederman *et al*, 2001).

We therefore designed this study to test the hypothesis that self-reported emotional maltreatment in childhood would moderate the effects of 5-HTTLPR genotype on AS scores in young adults. Given preliminary evidence that it is the physical concerns component of AS that is heritable (Stein *et al*, 1999), we looked at this component in addition to overall AS scores. We chose to focus on emotional maltreatment (as opposed to other available indices of childhood maltreatment, such as sexual abuse) primarily for epidemiological reasons: it is not a rare event, and it has good variability in population-based samples (Scher *et al*, 2004; Thombs *et al*, 2006). But we also looked at physical abuse to test for specificity of the effect.

In light of the finding that an a/g single-nucleotide polymorphism (SNP; rs25531) in the variable region that

defines the variable number of tandem repeats (VNTR) S/L polymorphism divides L alleles of 5-HTTLPR into two functionally different variants, *La* and *Lg* (Hu *et al*, 2005; Wendland *et al*, 2006), the latter of which has been reported to behave equivalently to the S allele in terms of its low transcriptional efficiency, we also genotyped subjects for this SNP polymorphism and repeated analyses with this tetra-allelic (the SNP variant also occurs, rarely, on the S allele) reclassification. We also repeated analyses using the rs25531 SNP alone.

METHODS

Sample and Procedures

Participants ($N=247$) were 171 women and 76 men (mean age 18.8 years, SD 1.5 years) recruited from among undergraduate psychology students at San Diego State University (SDSU) who had, in a group testing session (at which some questionnaires were completed and demographic information provided), indicated their willingness to be contacted by research investigators to participate in future psychological experiments. A subset of subjects ($N=150$) was identified, consisting of all those who described themselves as being of European-American (Caucasian) ancestry; initial analyses were conducted on this subset in order to reduce the possibility of confounding by population stratification (Freedman *et al*, 2004).

Subjects each came for a scheduled appointment at which a blood sample (60 ml) was drawn for genetic studies and the subjects completed questionnaires and/or computerized tasks. Subjects gave informed, written consent to participate in this part of the study, which was approved by the Human Research Protection Programs at both SDSU and the University of California, San Diego. Subjects received \$25 for providing the blood sample.

Questionnaires

Psychiatric diagnostic interviews were not available for all subjects. All subjects completed a panel of self-report measures relevant to the study of personality and anxiety-related traits. Those measures that were examined for the purposes of this report are:

Anxiety Sensitivity Index (ASI). A 16-item self-report measure of anxiety sensitivity (‘fear of fear’) (Peterson and Reiss, 1992); scores range from 0 to 64. The ASI has three factors that deal with physical (eg, ‘It scares me when my heart beats rapidly’), psychological (eg, ‘It scares me when I am unable to keep my mind on a task’) and social (eg, ‘Other people notice when I feel shaky’) consequences of anxiety symptoms, respectively.

NEO personality inventory-revised (NEO-FFI). A widely used, 60-item self-report measure of personality, grouped into five major domains: neuroticism, extraversion, openness to experience, conscientiousness, and agreeableness (Costa and McCrae, 1992). *T* scores were calculated directly from college-age, sex-specific norms (Robert R McCrae PhD, personal communication, November 14, 2001). Neuroticism (as defined through the NEO) is a well-

established, nonspecific risk factor for anxiety and mood disorders (Middeldorp *et al*, 2005). Given the partial conceptual overlap between AS and N (Cox *et al*, 1999a; Smoller and Tsuang 1998; Zinbarg *et al*, 2001), we included neuroticism as a covariate in some models in order to isolate genetic effects more specific to AS.

Childhood trauma questionnaire (CTQ). A 28-item retrospective self-report questionnaire designed to assess five types of negative childhood experiences (Bernstein *et al*, 1994; Scher *et al*, 2001). Given the study hypotheses, and in an effort to minimize multiple comparisons, we focused on the Emotional Abuse (EA) component of this instrument. But, in order to evaluate specificity for EA, we also ran a parallel series of analyses with the Physical Abuse (PA) component of the CTQ.

Questionnaire scores for subjects in the three genotypic groups are shown in Table 1.

Genotype Analysis

Genomic DNA was extracted from whole blood and the 5-HTTLPR variant was analyzed by polymerase chain reaction (PCR) amplification, as described elsewhere for the diallelic L-S classification (Gelernter *et al*, 1997), and for the tetra-allelic La/Lg-Sa/Sg classification (Stein *et al*, 2006). Within the ethnically homogenous European-American sample ($N = 150$), distribution of 5-HTTLPR alleles for the diallelic classification was L 57% and S 43%; there was no significant deviation from HWE expectations ($\chi^2 = 0.81$, $df = 1$, $p = 0.37$). For the triallelic system, the distribution of

alleles was La 51%, Lg 6%, and S 43% with no significant deviation from HWE expectations ($\chi^2 = 1.87$, $df = 3$, $p = 0.60$).

Ancestral Proportion Scores

Subjects' ancestries were estimated using a set of unlinked genetic markers by Bayesian cluster analysis, using the procedures and software developed by Pritchard and colleagues (<http://pritch.bsd.uchicago.edu/software.html>) (Falush *et al*, 2003; Pritchard *et al*, 2000; Pritchard and Rosenberg, 1999). Pritchard's software program STRUCTURE implements Bayesian cluster modeling that can recognize cryptic population genetic patterns without prior information of population origins. Data were submitted to the program STRUCTURE using 35 markers with models specified as 'admixture' and 'allele frequencies correlated' and 500 000 burn-in and 500 000 MCMC (Markov Chain Monte Carlo) iterations. The markers were the set of STRs described previously (Yang *et al*, 2005). A two-cluster solution classified 149 of the 150 European American subjects in complete concordance with their reported ethnicity.

Statistical Analysis

Analyses were conducted using the biostatistical analysis program STATA (Version 9.2) (StataCorp, 2003). The tetra-allelic or, in the case of this sample, triallelic genotypes—we found no instances of Sg alleles—were reclassified into a biallelic model by their level of expression as follows: Lg/S, Lg/Lg, and S/S were reclassified as S'S', La/S and La/Lg were reclassified as L'S', and La/La was reclassified as L'L' (Parsey *et al*, 2006).

Linear regression techniques in STATA ('rreg') (StataCorp, 2003) that are considered robust to outliers were used to model the relationships between 5-HTTLPR genotype (coded as 0 for LL or L'L', 1 for LS or L'S', and 2 for SS or S'S'), level of emotional abuse (CTQ-EA), and AS (Basic Model); additional covariates included in some models were sex (Model 1); and sex and ancestral proportion scores (Model 2). (Models were rerun with the AS-Physical Concerns subscale (rather than total AS score) as the outcome.) A third model added neuroticism as a covariate with the aim of isolating effects of AS that are not attributable to shared variance with neuroticism (Model 3). Models 1 and 2 were also applied to neuroticism rather than AS to determine specificity of effects for AS. Analogously, Models 1 and 2 were run with physical abuse (CTQ-PA) rather than emotional abuse (CTQ-EA) to determine specificity of the G \times E interaction for the latter. Multivariate regression analyses were run in the full sample ($N = 247$) to take advantage of this larger, more diverse sample, but were also rerun constrained to the European-American sample ($N = 150$); in light of recent findings that there may be stratification even among self-identified European-American samples (Campbell *et al*, 2005), these analyses also included the ancestral proportion scores as covariates (Models 2 and 3).

An additional set of analyses, as described above, was conducted using rs25531 to classify individuals ($N = 237$ subjects: aa = 209, ag = 25, gg = 3). There were no signifi-

Table 1 Characteristics of Study Participants by 5-HTTLPR Diallelic Genotype ($N = 247$)

Variable	Obs	Mean	Std. dev.	Min	Max
<i>5-HTTLPR = L/L</i>					
Age (years)	71	18.58	1.06	18	24
ASI total	71	19.80	11.61	3	62
NEO-N	71	47.30	10.78	25	78
CTQ-EA	71	8.18	3.95	5	25
CTQ-PA	71	6.11	2.88	5	22
<i>5-HTTLPR = L/S</i>					
Age (years)	108	18.82	1.82	18	31
ASI total	108	17.36	9.52	1	47
NEO-N	108	47.97	9.98	24	75
CTQ-EA	108	8.27	4.09	5	24
CTQ-PA	108	6.71	3.29	5	24
<i>5-HTTLPR = S/S</i>					
Age (years)	68	18.89	1.11	18	22
ASI total	68	21.32	11.95	3	50
NEO-N	68	48.62	9.87	22	77
CTQ-EA	68	8.62	4.01	5	25
CTQ-PA	68	7.69	3.54	5	24

cant main effects found for rs25531 and no significant interactions with the childhood maltreatment variables. These data are not shown, but are available upon request from the corresponding author.

RESULTS

Overall AS Levels in European-American Subjects (N = 150)

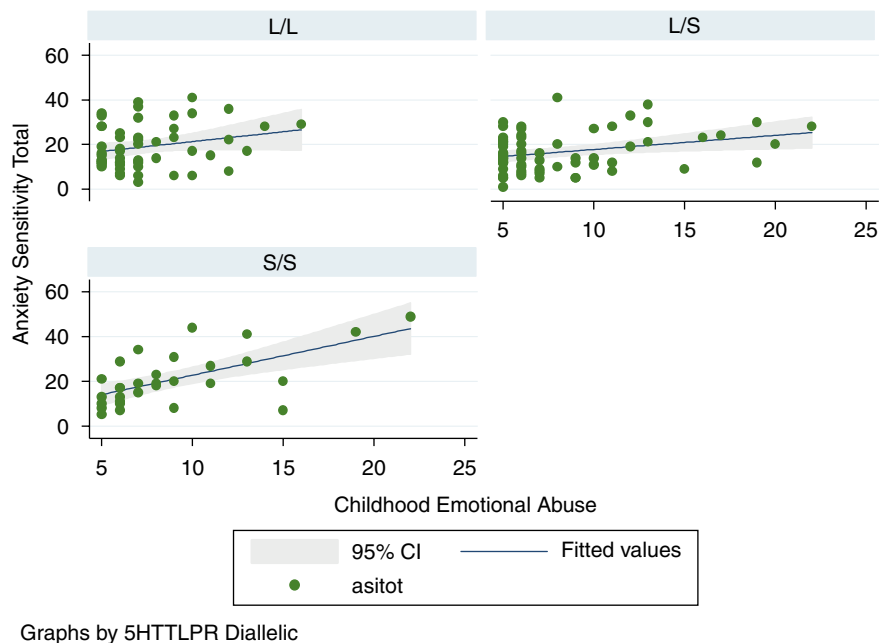
There were no significant main effects of 5-HTTLPR genotype (either diallelic or triallelic) on emotional abuse scores (CTQ-EA) in regression models that incorporated sex and the ancestral proportion scores as covariates.

The relationships between the variables of interest were first modeled (Basic Model) as a main effect of level of emotional abuse (CTQ-EA) ($\beta = 0.30$ (SE $\beta = 0.40$), $t = 0.75$, $p = 0.46$), 5-HTTLPR genotype ($\beta = -5.18$ (SE $\beta = 2.79$), $t = -1.86$, $p = 0.065$), and the interaction of these two factors ($\beta = 0.64$ [SE $\beta = 0.32$], $t = 2.02$, $p = 0.045$). To discern the nature of this statistically significant interaction, we stratified by 5-HTTLPR genotype and compared the main effects of emotional abuse level across genotypes (Figure 1): L/L ($\beta = 0.881$, SE $\beta = 0.515$, $N = 52$); L/S ($\beta = 0.634$, SE $\beta = 0.240$, $N = 68$); S/S ($\beta = 1.743$, SE $\beta = 0.407$, $N = 30$). S/S homozygotes had significantly greater slopes than S/L heterozygotes ($t = 2.46$, $df = 96$, $p = 0.016$) but not L/L homozygotes ($t = 1.16$, $df = 80$, $p = 0.251$). We therefore also contrasted S/S homozygous genotypes vs L/L or L/S genotypes grouped together ($\beta = 0.665$, SE $\beta = 0.228$) and found that the slopes of the latter were also statistically significantly greater ($t = 2.18$, $df = 148$, $p = 0.03$). This difference in slopes reflects the interaction between genotype and childhood maltreatment such that at the upper end of the emotional abuse range, S/S homozygotes had higher AS scores than other subjects.

Very similar results (Basic Model) were obtained for the triallelic 5-HTTLPR classification (interaction term: $\beta = 0.530$ (SE $\beta = 0.309$), $t = 1.72$, $p = 0.088$), although the comparison of the beta weights of La/La, La/Lg, or La/S genotypes ($\beta = 0.657$ (SE $\beta = 0.254$, $N = 107$) vs Lg/Lg, Lg/S, or S/S (ie, S'/S') genotypes ($\beta = 1.44$ (SE $\beta = 0.340$), $N = 36$) fell short of statistical significance ($t = -1.629$, $df = 141$, $p = 0.106$).

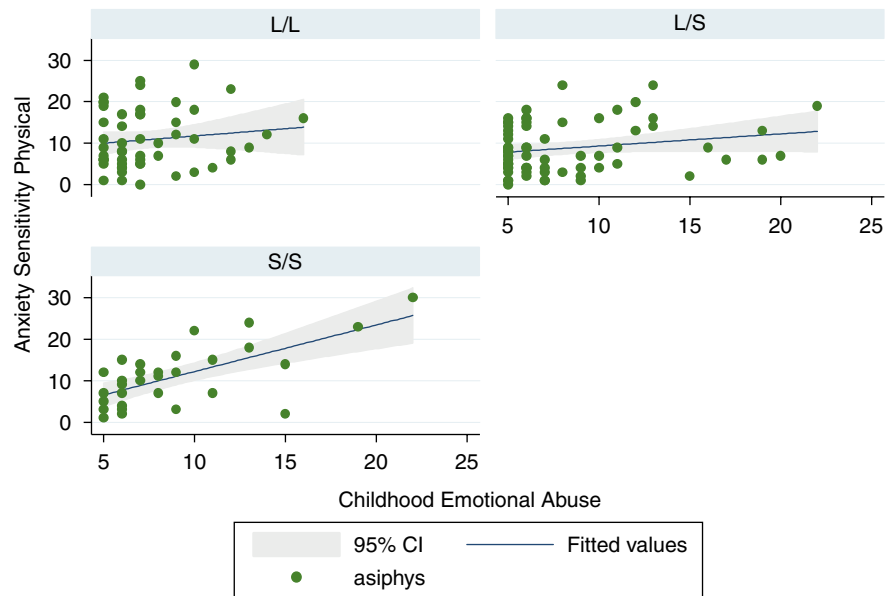
AS Physical Concerns

In light of observations that only the physical concerns subscale of the ASI (ie, the fear of physical sensations such as shortness of breath and tachycardia) is known to be heritable (Stein *et al*, 1999), we repeated these analyses (Basic Model) exclusively for this particular component of AS. The results were comparable, but somewhat more robust. Specifically, the interaction term for the diallelic 5-HTTLPR classification and level of emotional abuse was statistically significant: $\beta = 0.549$, SE $\beta = 0.222$, $t = 2.47$, $p = 0.015$. When stratified by genotype (Figure 2), the mean β weight for the S/S genotype ($\beta = 1.126$, SE $\beta = 0.237$, $N = 30$) was once again significantly larger than the β weight of the L/L or L/S genotypes grouped together ($\beta = 0.268$, SE $\beta = 0.172$, $N = 120$; $t = -2.44$, $df = 148$, $p = 0.016$). Using the triallelic classification, nearly identical results were obtained: the interaction term for the 5-HTTLPR classification and level of emotional abuse was statistically significant: $\beta = 0.565$, SE $\beta = 0.214$, $t = 2.64$, $p = 0.009$. When stratified by genotype, the mean β weights for the Lg/Lg, Lg/S, or S/S genotypes ($\beta = 0.981$, SE $\beta = 0.201$, $N = 36$) was statistically significantly larger than that of the La/La, La/Lg, or La/S combined group ($\beta = 0.223$, SE $\beta = 0.182$, $N = 107$; $t = -2.26$, $df = 141$, $p = 0.025$). No significant G \times E interactions were found for the other two subscales—psychological or social concerns—of the AS (data not shown).



Graphs by 5HTTLPR Diallelic

Figure 1 Robust linear regression analysis of childhood emotional abuse (CTQ-EA; x-axis) on anxiety sensitivity level (ASI total score; y-axis) stratified by diallelic 5-HTTLPR genotype. The slope of S/S is significantly ($p = 0.030$) higher than that of L/L or L/S subjects combined. Y-axis is ASI total score. X-axis is Childhood Trauma Questionnaire Emotional Abuse score.



Graphs by 5HTTLPR Diallelic

Figure 2 Robust linear regression analysis of childhood emotional abuse (CTQ-EA; x-axis) on anxiety sensitivity physical concerns subscale scores (ASI Physical Subscale; y-axis) stratified by diallelic 5-HTTLPR genotype. The slope of S/S is significantly ($p=0.016$) higher than that of L/L or L/S subjects combined. Y-axis is ASI Physical Symptoms Subscale score. X-axis is Childhood Trauma Questionnaire Emotional Abuse score.

Multivariate Models

A series of multivariate regression models, incorporating additional covariates (Model 1: sex; Model 2: sex and ancestral proportion scores; Model 3: sex and ancestral proportion scores and neuroticism), were evaluated in the larger, ethnically diverse sample ($N=247$). A summary of the β weights (and their standard errors) for the interaction term (diallelic 5-HTTLPR genotype * CTQ-EA level) in each of these models is shown in Table 2. The interaction term was statistically significant in each of these models, with Model 2, which included the ancestral proportion scores, yielding larger β weights than when not so adjusted (Model 1). Results were entirely consistent when confined to the European-American (EA) subsample (Table 2). In all models, S/S subjects had significantly higher AS at a given level of CTQ-EA than either L homozygotes or heterozygotes. Nearly identical results were obtained for the triallelic classification (data for Model 3 shown).

Specificity of Maltreatment Type as the Stressor

When the models in Table 2 were rerun using physical abuse (CTQ-PA) scores rather than emotional abuse (CTQ-EA) scores, very similar results were found for the interaction term of CTQ-PA*5-HTTLPR (Model 1: $\beta=0.632$, SE $\beta=0.269$, $p=0.020$; Model 2: $\beta=0.745$, SE $\beta=0.274$, $p=0.007$; Model 3: $\beta=0.404$, SE $\beta=0.243$, $p=0.097$) with AS as the outcome.

Specificity of AS as the Outcome

All multivariate models were applied to Neuroticism as the dependent variable, rather than AS. None of these models yielded a statistically significant effect for the $G \times E$

Table 2 Parameter Estimates for Interaction Term (5-HTTLPR*CTQ-EA) from Robust Multivariate Linear Regression Analyses Predicting ASI Total Scores

	β	SE β	p -value
<i>Diallelic classification</i>			
<i>Any ethnicity</i>			
Model 1 ^a ($N=247$)	0.533	0.211	0.012
Model 2 ^b ($N=247$)	0.655	0.209	0.002
Model 3 ^c ($N=247$)	0.713	0.187	0.0005
Model 3 ($N=247$) ^d	0.676	0.193	0.001
<i>European-American only</i>			
Model 1 ($N=150$ EA)	0.644	0.310	0.039
Model 2 ($N=150$ EA)	0.634	0.311	0.043
Model 3 ($N=150$ EA)	0.697	0.275	0.012
Model 3 ($N=150$ EA) ^d	0.753	0.265	0.005

^aModel 1: covariates: sex.

^bModel 2: covariates: sex, ancestral proportion coefficients (2).

^cModel 3: covariates: sex, ancestral proportion coefficients (2), neuroticism.

^dTriallelic classification.

All models included 5-HTTLPR, CTQ-EA, and the interaction term (shown).

interaction between 5-HTTLPR genotype (diallelic or triallelic) and level of childhood maltreatment (EA or PA).

DISCUSSION

Anxiety sensitivity is an extremely well-replicated risk factor for anxiety (and depressive) psychopathology. The present results provide evidence for modification by severity of childhood abuse of a specific genetic influence on AS—which may be considered an intermediate pheno-

type for anxiety and depressive disorders (Schmidt *et al*, 2006). These observations, along the lines of those finding effect modification by 5-HTTLPR of various forms of stress, may be yet another example of genetic influences that ‘control sensitivity to the pathogenic effects of the environment’ (Kendler, 2005). In terms of the particular type(s) of stress that may be relevant to the development of AS, a focus on early childhood environment—and psychosocial moderators thereof (Kaufman *et al*, 2004, 2006b)—may be crucial for identifying gene-by-environment interactions that influence risk for later psychopathology.

It is noteworthy that only the physical concerns component of AS—the only component known to be heritable (Stein *et al*, 1999)—appeared subject to this gene-by-environment interaction. The possibility that fear of physical sensations is moderated by this conjoint effect of 5-HTTLPR and early emotional maltreatment suggests a possible effect on *interoception* as a mechanism for increased anxiety vulnerability. As noted in a recent review of brain mechanisms involved in proneness to anxiety, pathological interoceptive processing points to involvement of the insular cortex (Paulus and Stein, 2006). We have shown that individuals prone to anxiety do, indeed, have increased involvement of the insular cortex during the processing of emotional information (Stein *et al*, 2007). Findings from the present study should now enable the testing of specific hypotheses about gene–environment interactions and anxiety- and depressive-related endophenotypes. Several studies have documented an effect of 5-HTTLPR on amygdala function during the processing of emotional faces as measured with BOLD fMRI (Hariri *et al*, 2005, 2006). Based on these results, we would expect, for example, that 5-HTTLPR would modify the effects of childhood emotional abuse on amygdala (and, perhaps, insular cortical) function.

This study has several limitations. First, the sample is relatively small. As a result, the number of subjects with the S/S (or S'/S') genotype, which drives the main findings, is also small. A robust regression package (included in STATA) was used to limit the influence of outliers, and multivariate analyses that upheld—indeed strengthened—the main findings lend additional credence to their veracity, but replication is needed. Second, although the sample is drawn from an undergraduate college student sample, it is not population-based, raising questions about generalizability; once again, replication in larger, more diverse samples is needed. Lacking psychiatric diagnostic information about the sample, we also cannot comment with confidence that the findings relate to AS as an intermediate phenotype, as opposed to the possibility that many of the subjects have DSM disorders that AS is merely tracking. Third, the assessment of maltreatment in childhood was retrospective, and assessed cross-sectionally at the same time as anxiety sensitivity levels. This limitation is mitigated somewhat by the fact that subjects were young and within a relatively narrow age range, that is, not too far temporally from the time they were asked to recall. These methodological constraints nonetheless may raise problems related to biased recall, as well as mutual heritability of both sets of factors (ie, levels of emotional abuse and anxiety sensitivity) (Moffitt *et al*, 2005). Consistency with 5-HTTLPR findings in depression is encouraging and suggests

our observations are not spurious. Nonetheless, replication in additional (ideally, longitudinal) data sets will be needed to confirm them.

Among the questions raised by our findings is the issue of specificity for particular forms of psychopathology. In a recent study replicating the gene-by-environment interaction between 5-HTTLPR and stressful life events on liability for episodes of major depression, investigators noted specificity of effects wherein they did not see this interaction increasing liability for generalized anxiety disorder (GAD) (Kendler *et al*, 2005). As reviewed earlier in this paper, AS is a risk factor for more than panic attacks; it is thought to increase susceptibility to a range of anxiety- and depressive disorders. Keeping in mind that DSM-IV categories only imperfectly reflect the nature of psychopathology, it should be expected that genetic and experiential risk factors may at times cross diagnostic borders. Other investigators have recently commented on the likelihood that the 5-HTTLPR interaction with life stress to increase depressive symptoms reflects an impact on more proximate psychological processes, rather than on any psychiatric diagnostic entity, *per se* (Jacobs *et al*, 2006). To the best of our knowledge, this is the first report of a gene-by-environment interaction that is associated with increased levels of a trait (ie, AS) that serves as a broad susceptibility factor (‘intermediate phenotype’) for anxiety-depressive psychopathology. Importantly, in this data set, there was some specificity for AS, as similar findings were not detected for another related trait, Neuroticism—although we must consider the possibility that this failure to find an association with neuroticism is a false negative, owing to sample size or the particular measure of neuroticism used in this study (Sen *et al*, 2004). On the other hand, specificity for emotional abuse was not confirmed, as similar (although somewhat less consistent) effects were seen for physical abuse, suggesting that this pathway may not discriminate between different types of stressors.

The *SLC6A4* VNTR, 5-HTTLPR, is one of the best-studied genetic polymorphisms in psychiatry, and its status as a functional variant is well supported in the literature, both directly (through functional studies) and indirectly (through observed effects on phenotype). The description of an SNP variant in the VNTR region that also alters function was therefore of great interest (Hu *et al*, 2005; Parsey *et al*, 2006). Specifically, the ‘g’ SNP allele on the background of the VNTR L allele is functionally similar to the VNTR S allele—that is, this additional genotyping allows reclassification of some L alleles that function like S alleles. Making this reclassification in genetic association studies should increase power by reducing the ‘noise’ introduced by the presence of L alleles that are functionally equivalent to S alleles. In our sample, this resulted in the reclassification of 16 L alleles as S' alleles and, in the case of the heritable physical sensitivity component of AS, slightly improved statistical significance for the relevant comparison, although it did not differ substantively from the original diallelic ‘L/S’ classification. We should also point out that, contrary to some other studies which find that presence of one or more S alleles seems to confer increased stress-related vulnerability (with no dose–response relationship for number of S alleles), our findings centered on the

impact of S homozygosity. Thus, although a very consistent literature is emerging with regard to a role for SLC6A4 and stress-related psychopathology, the precise identification of the risk-conferring variant(s) remains to be determined.

This report adds to the growing literature documenting specific examples of gene-by-environment interaction involving the 5-HTTLPR variant at the SLC6A4 locus; we now add AS to previously studied phenotypes that already include depression and alcohol use (Kaufman *et al*, 2006a). These results are surprising in their consistency (although, as noted above, some studies have not been consistent), and together, they suggest that this one variant plays a very substantive role in modulating an individual's stress sensitivity or resilience. We hypothesize that this variant regulates some aspect of brain function very relevant for buffering stress, which could be through a very complex mechanism, but which could also involve such theoretically quantifiable aspects of brain function as available pools of serotonin. Testing this hypothesis, and identifying other mechanisms by which constitutional variations in brain serotonin function can influence emotional resiliency to stress, are challenges for the future as we work to identify endophenotypes for stress-related psychopathology.

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DISCLOSURE/CONFLICT OF INTEREST

There are no conflicts for any of the authors relating to this submission.

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