The Interaction of Stressful Life Events and a Serotonin Transporter Polymorphism in the Prediction of Episodes of Major Depression

A Replication

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Context: Prior evidence from twin studies suggested genetic moderation of the depressogenic effects of stressful life events (SLEs). Can the specific genes involved in this effect be identified?

Objective: To replicate and extend a recent study that a functional variant in the serotonin transporter (*5-HTT*) might in part explain these findings.

Design: Characterizing risk for major depression and generalized anxiety syndrome in the last year as a function of *5-HTT* genotype, sex, and the occurrence of SLEs and ratings of the SLE-associated level of threat.

Setting: A population-based sample of adult twins.

Participants: Five hundred forty-nine male and female twins with a mean age at participation of 34.9 years (SD 9.1).

Main Outcome Measure: Episodes of major depres-

sion and generalized anxiety syndrome in the last year with onset measured to the nearest month.

Results: Individuals with 2 short (S) alleles at the *5-HTT* locus were more sensitive to the depressogenic effects of all SLEs than were those with 1 or 2 long (L) alleles. When level of SLE-associated threat was examined, the interaction between genotype and SLE resulted from an increased sensitivity of SS individuals to the depressogenic effects of common low-threat events. These events had little impact on risk for those possessing the SL and LL genotypes. The *5-HTT* genotype did not modify the effects of SLEs on risk for generalized anxiety syndrome.

Conclusion: Variation at the *5-HTT* moderates the sensitivity of individuals to the depressogenic effects of SLEs largely by producing, in SS individuals, an increased sensitivity to the impact of mild stressors. Replication of these intriguing results is needed.

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S TRESSFUL LIFE EVENTS (SLEs) precede the onset of episodes of major depression (MD) more frequently than expected by chance,[1,2] and this relationship is probably causal.[3] However, humans display wide variation in response to adversity. Some individuals are stress-sensitive and prone to depression in response to modest stressors, while others are stress-resistant, remaining symptom free after severe adversity.[4] What is the source of this variation?

We previously demonstrated in an adult twin sample that genes, assessed in aggregate, affected sensitivity to the depressogenic effects of SLEs.[5] Similar results have been found in adolescent twins.[6,7] However, these studies did not examine the specific genes involved in this effect.

Recently, in a New Zealand birth cohort, Caspi et al[8] reported that a functional length polymorphism in the pro-

moter of the serotonin transporter (*5-HTT*) gene moderated the influence of SLEs on depressive symptoms and MD. They found that individuals with 1 or 2 "short" alleles at this polymorphism (hereafter SL and SS, respectively) were more stress-sensitive than those with 2 "long" alleles (hereafter LL). Their analyses had 3 potential methodologic limitations. First, they predicted past-year MD assessed at age 26 years from the sum of 14 possible SLEs in the preceding 5 years. However, the impact of SLEs on risk for MD is typically short-lived, usually 1 to 3 months.[9-13] Their results may reflect, at least in part, an indirect rather than a direct association between SLEs and MD. Second, the pathogenic effects of SLEs are highly variable[14] and related to their associated level of threat.[13,15,16] Examining the SLE-associated threat level along with the *5-HTT* genotype may better characterize the nature of genetic effects on stress re-

sponsivity. Third, their study did not address the specificity of the *5-HTT* effect. When assessed at the aggregate level, the genetic risk factors for MD and generalized anxiety disorder (GAD) are closely interrelated.[17,18] Given that SLEs also affect risk for GAD-like syndromes,[13,19] would the *5-HTT* polymorphism also modify the anxiogenic effects of SLEs?

In this report, we attempt to replicate the findings of Caspi et al[8] in a random sample of twins from a population-based registry. The SLEs and depressive onsets were measured to the nearest month, and for certain interviews, SLEs were rated on a 4-point scale of long-term contextual threat (LTCT). We address 3 questions:

1. Could we replicate the findings of Caspi et al that *5-HTT* promoter variation modifies the depressogenic effects of SLEs when the temporal proximity of the SLE to the depressive episode is assured?

2. How does variation at the *5-HTT* polymorphism alter the dose-response relationship between severity of stress and risk for MD?

3. Does the *5-HTT* polymorphism modify the anxiogenic effects of SLEs?

METHODS

SAMPLE

Subjects in this study came from the Virginia Adult Twin Study of Psychiatric and Substance Use Disorders, a longitudinal study of twins drawn from the population-based Virginia Twin Registry.[20] For female-female (FF) twin pairs, entry criteria required that they be born between 1934 and 1974 and both members had previously responded to a mailed questionnaire between 1987 and 1988. These FF pairs have been approached for 4 subsequent waves of personal interviews from 1988 to 1997, with cooperation rates ranging from 85% to 92%, herein called, respectively, FF1, FF2, FF3, and FF4. For male-male/male-female (MMMF) twin pairs, they were eligible if they were born between 1940 and 1974 and had participated in our first wave interview (termed *MMMF1*; cooperation rate of 72.4% completed 1993-1996). They were later approached for a second interview (MMMF2, completed 1994-1998), which achieved an 82.6% cooperation rate. For the subsample used in this study, the relevant interwave intervals (mean±SD) were: FF3 to FF4, 29.1±5.8 months and MMMF1 to MMMF2, 18.5±7.6 months. After an explanation of the research protocol, informed consent was obtained prior to all interviews.

For this study, we first randomly selected 572 participants from our twin sample who had participated in the MMMF2 or FF4 interviews and had available DNA. Of these twins, 549 had complete data and were included in these analyses. The only selection rule was that we never took both members of a twin pair and we selected equal numbers of males and females. For every monozygotic twin in the sample with complete data on the cotwin (n=159), we then included phenotypic data from the cotwin assuming both twins had identical genotypes. The mean age and years of education of this subsample as of May 1996 were 34.9 years (SD 9.1) and 13.4 years (SD 2.4), respectively.

MEASURES

During each interview, we assessed the occurrence over the last year of 14 symptoms representing the disaggregated 9 "A criteria" for MD in *DSM-III-R*[21] (eg, 2 items for assessing, separately, insomnia and hypersomnia). For each reported symptom, interviewers probed to ensure that it was due neither to physical illness nor medication. The respondents then aggregated these symptoms into co-occurring syndromes, the dates of the onset and offset of which were recorded. The diagnosis of MD was made by computer algorithm incorporating the *DSM-III-R* criteria, except criterion B2 (excluding "uncomplicated bereavement"). In 375 twins interviewed twice by different interviewers with a mean (SD) inter-interview interval of 30 (9) days, the inter-interview reliability of the diagnosis of MD in the last year was: $\kappa^{22} = +0.66$ (95% confidence interval [CI], 0.58-0.74), tetrachoric correlation $= +0.88$ (95% CI, 0.82-0.93).

In addition, we inquired about times in the last year when subjects felt "anxious, nervous, or worried," their "muscles felt tense," or they "felt jumpy or shaky inside." Positive responses to these probes were followed by questions for all the individual symptoms of *DSM-III-R* GAD. For this report, we defined a disorder termed *generalized anxiety syndrome* (GAS) lasting 2 or more weeks with a minimum of 6 D criteria for GAD in *DSM-III-R*.[21,23] We use this definition so that we can focus on symptomatic differences between GAS and MD, rather than differences in duration. No diagnostic hierarchy was used between GAS and MD.

Our interviews assessed the occurrence, to the nearest month, of 11 personal SLEs: "assault," "divorce/separation," "major financial problem," "serious housing problems," "serious illness or injury," "job loss," "legal problems," "loss of confidant," "serious marital problems," "robbed," and "serious difficulties at work." We also assessed 4 classes of network events, affecting spouse, child, parent, sibling, other close relative, or "someone else close to you." These classes were: (1) "getting along with": serious trouble getting along with an individual in the network, (2) "crisis": a serious personal crisis of someone in the network, (3) "death": death of an individual in the network, and (4) "illness": serious illness of someone in the network.

Each SLE in the FF3, FF4, and MMMF2 interviews was rated by the interviewer on the level of LTCT, where *long-term* means persisting at least 10 to 14 days. Following Brown, we instructed our interviewers to rate "what most people would be expected to feel about an event in a particular set of circumstances and biography, taking no account either of what the respondent says about his or her reaction or about any psychiatric or physical symptoms that followed it."[24(p24)]

The LTCT was rated on a 4-point scale: minor, low moderate, high moderate, and severe.[24] Reliability of our ratings of LTCT was determined by interrater and test-retest designs. Interrater reliability was assessed by having experienced interviewers review tape recordings of the interview sections in which 92 randomly selected individual SLEs were evaluated. Interrater reliability was $r_s = +0.69$ and $\kappa = +0.67$. Test-retest reliability was obtained by repeating the interview with 191 respondents at a mean interval of 4 weeks. We obtained 173 scored life events that were reported to have occurred within 1 month of one another and we assumed represented the same event. We assessed reliability by Spearman correlation (r_s) and weighted κ.[25] The test-retest reliability for LTCT was $r_s = +0.60$ and $\kappa = +0.41$.

For this study, we used 2 different data sets. First, we examined only the presence or absence of SLEs in each month using our FF3, FF4, MMMF1, and MMMF2 waves. (We did not use the FF1 and FF2 waves because of differences in the ways in which SLEs were coded.) This sample contained 2 strata, the first made up of the FF3 and MMMF1 samples and containing 662 observations or "periods of wellness" of which 46 ended in a depressive episode. A *period of wellness* is defined as a period of observation that either begins at the start of a 1-year prevalence window or at the time of recovery from an episode and ends either at the conclusion of that 1-year win-

dow or at the time of an onset of an episode. The second strata consisted of the FF4 and the MMMF2 samples containing 710 periods of wellness, 44 of which ended in an episode of MD.

For the second series of analyses, we used the LTCT ratings and were therefore restricted to the use of the FF3, FF4, and MMMF2 waves. In these analyses, the first strata was represented by the FF3 and MMMF2 waves, which contained 662 periods of wellness, 44 of which ended in episodes of MD. Strata 2 was represented solely by the FF4 wave, which contained 299 periods of wellness of which 20 ended in a depressive episode. For GAS, only 1 strata was necessary because coded data on onsets was not available from the FF4 interview, consisting of 662 periods of wellness of which 53 resulted in an episode of GAS.

GENOTYPING

Cytology brushes were used to obtain a sample of buccal cells from the subjects for DNA analysis. Genomic DNA was isolated using the Instagene Matrix (Biorad, Hercules, Calif) kit protocol for cell lysis product absorption. Each sample was diluted to a working concentration of 5 to 20 ng/µL. We used primer sequences described previously,[26] HTTLPR-F (5′-tgaatgccagcacctaaccc-3′) and HTTLPR-R (5′-ttctggtgc-cacctagacgc-3′). We amplified polymerase chain reaction products in 96-well microtiter plates in 20-µL volume containing 50 to 200 ng of human genomic DNA; 0.5µM each forward and reverse primer; 0.3mM each deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), and deoxythymidine triphosphate (dTTP); 0.15mM deoxyguanosine triphosphate (dGTP); 0.15mM 7-deaza-dGTP (Amersham, Piscataway, NJ); 0.4 units of HotMaster Taq (Eppendorf, Westbury, NY); 1× HotMaster buffer (Eppendorf); and 1.5mM magnesium chloride . Polymerase chain reaction was carried out in a PTC 225 DNA Engine (MJ Research, Waltham, Mass). Cycling conditions were 5 minutes initial denaturation at 95°C followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 58°C, and 30 seconds at 72°C, with a final extension of 10 minutes at 72°C. HTTLPR long allele (insertion) of 528 base pair (bp) and short allele (deletion) of 484 bp were resolved on 2% agarose gels and visualized with ethidium bromide. The frequencies of the 3 genotypes in the sample, which was entirely white, were: SS, 23.3%; SL, 47.5%; and LL, 29.2%.

STATISTICAL METHODS

Our unit of analysis was a period of wellness. Using these periods, our analyses were conducted with a Cox proportional hazards regression model operationalized in the SAS procedure PHREG.[27,28] Three predictor variables were used: 5-HTT genotype (LL, SL, or SS), sex, and either the presence or absence of an SLE or the level of LTCT. When multiple events occurred in the same month, LTCT was coded as the highest recorded threat level. The 2 dependent variables were onset of an episode of MD and onset of an episode of GAS.

For our analyses incorporating threat levels, LTCT was coded so that 0 meant no SLE occurrence in the month and 1 through 4 meant the occurrence of an SLE with minor, low-moderate, high-moderate, and severe LTCT. To incorporate the ordinal structure and to simplify interpretation of the interaction, LTCT was coded as follows: 4 dummy variables, X1, X2, X3, and X4, were used. If there was no life event, all 4 were coded as zero. If there was an SLE with an LTCT of 1 or more, X1 was coded as 1. If LTCT was 2 or more, X2 was also coded as 1. If LTCT was 3 or more, then X3 was coded as 1. For an event with a LTCT of 4, all 4 dummy variables were coded to 1. Thus, the coding for a month with an event with a LTCT of 2 was: X1=1, X2=1, X3=0, X4=0. This method of dummy variable coding

is often referred to as *thermometer coding*.[29] Finally, they were incorporated as a time-dependent covariate with a linear decay, which abated after 2 months.

The 5-HTT genotype was coded so that 0 meant 2 long alleles, 1 meant 1 long and 1 short allele, and 2 meant 2 short alleles. To incorporate this into the model, 2 thermometer-type dummy variables, H1 and H2, were used. If there were no short alleles, H1 and H2 were both coded as 0. If there was 1 short and 1 long allele, H1 was coded as 1 and H2 was coded as 0. Only when both alleles were short was H2 coded as 1. This allowed easy comparison of 2 to 1 short alleles and 1 short allele to none.

Thermometer coding does not alter model results and is simpler yet mathematically equivalent to contrasts. Compared with typical indicator variables, it greatly simplifies the model selection process. Removal of a level of a variable with standard indicator variables requires a recoding of the data and a likelihood ratio test. With thermometer coding, the same task is no different than removing other independent variables.

The model was produced using 2 strata to accommodate the first wellness period of 2 different 13-month periods for the subjects. At most, 1 onset of MD was used for each period. If 2 or more onsets did occur for a subject in the same period, only the first was analyzed. This stratification is a conservative way to deal with within-subject correlation.

Model selection began with the 5-HTT genotype, LTCT, sex, and all 2-way interactions. The final model, which consisted of only significant interactions and main effects that were either significant or were a part of a significant interaction, was obtained by removing nonsignificant interactions and main effects from the full model. To verify the final model, a random selection of nonsignificant interactions and main effects was added to the final model to verify that the same model emerged.

The same methods were used to pursue models where LTCT ratings were not available. In these situations, we set all SLEs as though they had an LTCT level of 1 so that our analytic model was constructed similarly for data that included LTCT and data that did not.

We examined whether, in our data, 5-HTT genotype (along with sex as a covariate) predicted the occurrence of 1 or more SLEs. The effect did not approach significance (H1 χ^2_1=1.64; P=.20 and H2 χ^2_1=0.20; P=.65). We then repeated these analyses for SLEs with levels of LTCT of 2 or more, 3 or more, and 4 or more. In none of these analyses were the results significant.

RESULTS

### INTERACTION BETWEEN EVENT OCCURRENCE AND 5-HTT GENOTYPE IN THE PREDICTION OF MAJOR DEPRESSION

In our initial analyses, which included only the presence or absence of SLEs, we began with a full model containing the SS, SL, and LL genotypes, sex, and the occurrence of an SLE. We simplified the model by combining the effects of SL and LL genotypes with an improvement in fit. This best-fit model, the results of which are shown with CIs in the **Table** and illustrated in **Figure 1**, found, for the prediction of episodes of MD, significant main effects for sex (χ^2_1=6.19; P=.01) and SLE occurrence (χ^2_1=7.36; P=.02) but not for genotype (χ^2_1=1.15; P=.28). However, a significant genotype × SLE interaction was seen (χ^2_1=4.34; P=.04). Estimates based on this model indicate that, averaged across sexes, event exposure increased the hazard ratio (HR) for MD in individuals with an SL/LL and SS genotype, respectively, 2.13-fold and 6.68-fold.

	Male		Female	
Genotype	LS/LL	SS	LS/LL	SS
No SLE	1 (reference)	0.7 (0.1-1.1)	1.7 (1.0-3.8)	1.2 (0.2-2.9)
Any SLE	2.1 (1.3-4.6)	4.4 (2.8-12.2)	3.7 (1.7-13.2)	7.7 (4.0-33.9)
No threat	1 (reference)	0.5 (0.1-1.1)	1.4 (0.7-2.8)	0.6 (0.1-1.9)
Minor/low-moderate threat	0.2 (0-0.5)	4.0 (1.0-8.9)	0.2 (0-0.9)	5.5 (1.1-18.0)
High-moderate threat	4.2 (1.5-8.7)	5.1 (1.1-12.7)	5.7 (1.8-15.2)	7.0 (1.8-21.0)
Severe threat	29.1 (12.0-64.5)	35.7 (8.3-104.1)	40.0 (14.4-110.6)	49.0 (10.5-170.6)

Abbreviations: L, long; S, short, SLE, stressful life event.
*Values are expressed as hazard ratio (95% confidence level) unless otherwise specified.
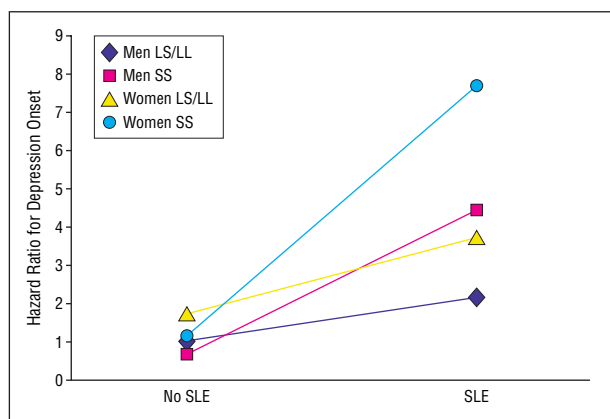
Figure 1. The hazard ratio of onset of major depression within a 2-month period as a result of (1) sex (men vs women), (2) genotype at the *5-HTT* polymorphism (SS vs LS and LL), (3) the occurrence, in the first month, of a stressful life event (SLE). A hazard rate of unity was defined as the risk level for a male with an SS genotype and no life-event exposure. S indicates short allele; L, long allele.

INTERACTION BETWEEN LTCT RATINGS AND *5-HTT* GENOTYPE IN PREDICTION OF MAJOR DEPRESSION

Given evidence for an interaction between *5-HTT* genotype and event exposure in the prediction of MD, we explored how this polymorphism altered the dose-response relationship between severity of stress and risk for depressive onset.

In our sample containing LTCT ratings, we again simplified the model by collapsing our genotypic data into the 2 classes of SS vs SL/LL. We also found no significant difference between the effects of LTCT levels 1 and 2. Sex was retained in the model, although its effect fell short of significance. Of the 3 possible interactions with genotype and LTCT level, 2 were retained. The final model for the prediction of MD contained sex, the main effects of *5-HTT* genotype (coded as SS vs SL/LL), the main effects of 3 levels of LTCT (coded as LTCT levels ≥ 1, ≥ 3, or 4), and the interactions between genotype and LTCT value of 1 or more and LTCT; value of 3 or more.

The main effects of *5-HTT* genotype ($\chi^2_1 = 2.04$; $P = .15$) and LTCT of 1 or more ($\chi^2_1 = 3.31$; $P = .07$) were nonsignificant in this final model. By contrast, the main effects of both levels of stress remained significant: LTCT of 3 or more ($\chi^2_1 = 9.89$; $P = .002$) and LTCT of 4 ($\chi^2_1 = 18.66$; $P < .001$). Most importantly, we observed a significant positive interaction between genotype and LTCT of 1 or more ($\chi^2_1 = 10.74$; $P = .001$) such that individuals with the SS genotype had greater sensitivity to the depressogenic effects of SLEs with LTCT levels of mild or greater than did individuals with the SL or LL genotypes. Furthermore, we also saw a significant, negative, and nearly balancing interaction between genotype and LTCT of 3 or more ($\chi^2_1 = 6.47$; $P = .001$). That is, high levels of LTCT were associated with a large increase in risk for MD in all genotypes, whereas low levels of LTCT were associated with increased risk only among individuals with the SS genotype.

These results, along with 95% CIs, are presented in the Table and illustrated in **Figure 2**A (the overall results of the best-fit model) and Figure 2B (which "zooms in" at the critical part of the curve at the mild level of LTCT). Four findings from the best-fit model are noteworthy. First, as seen previously,[4] the HR for MD increases with higher levels of LTCT with the effect being particularly marked when moving from high-moderate to severe levels of LTCT. Second, at every level of threat and genotype, the HR for MD is greater in females than in males. Third, at LTCT levels of 3 and 4, in both males and females, the HR is greater for those with the SS than with the SL or LL genotypes, but the difference is small. Fourth (as most clearly seen in Figure 2B), at mild and low-moderate levels of threat (LTCT = 1 in Figure 2B), the differences in risk between those with SS vs SL or LL genotypes is substantial. The risk for a depressive onset is actually decreased for individuals with SL or LL genotypes when they experienced an SLE with a mild level of threat compared with no life event at all. However, for individuals with an SS genotype, the risk for an episode of MD is more than 8 times greater in the presence of a mild or low-moderate threat event compared with months with no reported SLE.

PREDICTION OF GAS

We initially applied the full model, including levels of LTCT, to predict onsets of GAS. *5-HTT* genotype had no effect on risk for GAS either as a main effect or in interaction with levels of LTCT. We reduced the model in an attempt to reveal a significant genetic effect without success. We then applied to the prediction of onsets of GAS the final best-fit model for MD and present these results.

The main effects of *5-HTT* genotype ($\chi^2_1 = 0.05$; $P = .82$), sex ($\chi^2_1 = 1.22$; $P = .27$), and LTCT of 4 or more ($\chi^2_1 = 0.06$; $P = .81$) were all nonsignificant. By contrast, the remaining 2 main effects of levels of stress were both significant: LTCT of 1 or more ($\chi^2_1 = 5.41$; $P = .02$) and LTCT of 3 or more ($\chi^2_1 = 10.68$; $P = .001$). No significant interactions were observed between genotype and LTCT of 1 or more ($\chi^2_1 = 0.02$; $P = .89$) or LTCT of 3 or more ($\chi^2_1 = 0.75$; $P = .39$).

COMMENT

We sought, in these analyses, to address 3 questions, which we review in turn.

REPLICATION OF INTERACTION

Our first goal was to attempt to replicate the key prior finding that the length polymorphism in the *5-HTT* promoter modified the depressogenic effects of SLEs.[8] Using different measures of SLEs and different analytic methods, we broadly confirmed this finding with a greater degree of temporal resolution than was possible in the original report.

Our genotype results differed in 1 way. In the original analyses,[8] the largest differences in stress responsivity were between those with the LL genotype and those with the SS and SL genotype.[8] By contrast, we found significant differences only between those with the SS vs the SL or LL genotype.

To date, association studies for complex human behavioral traits have been problematic, producing low rates of replication. This has arisen from many causes, including low a priori probability, low power, and use of a liberal α level.[30,31] These cautions are probably less relevant to our findings because we replicated a prior report and performed our analyses on a single marker. However, neither our findings nor those reported by Caspi et al[8] are typical association studies. Instead of a main effect of genotype on phenotype, these reports examine genotype-environment interactions. Since interactions are harder to detect than main effects,[32,33] replications might be expected to be rarer and hence of particular value when they occur. In reality, we have little to guide us on the degree of replication required before such a finding should be accepted as likely correct.

We are aware of 3 studies that have examined interactions between *5-HTT* genotype, stress, and depression. Gillespie et al[34] failed to replicate either a direct effect of the *5-HTT* polymorphism on depression or an interaction with SLEs.[34] The SLEs were assessed during a 1-year period using self-reported measures so that, like the Caspi et al report, a close temporal resolution for the association between SLEs and depressive onsets was not possible. Eley et al[35] studied self-reported depressive symptoms in adolescents and found a trend for an interaction between *5-HTT* genotype and a composite measure of environmental risk in the prediction of depression that reached significance in female subjects. Examining a very different outcome, Grabe et al[36] found, in a general adult sample, a significant interaction between unemployment and the *5-HTT* genotype in the prediction of chronic disease burden in women
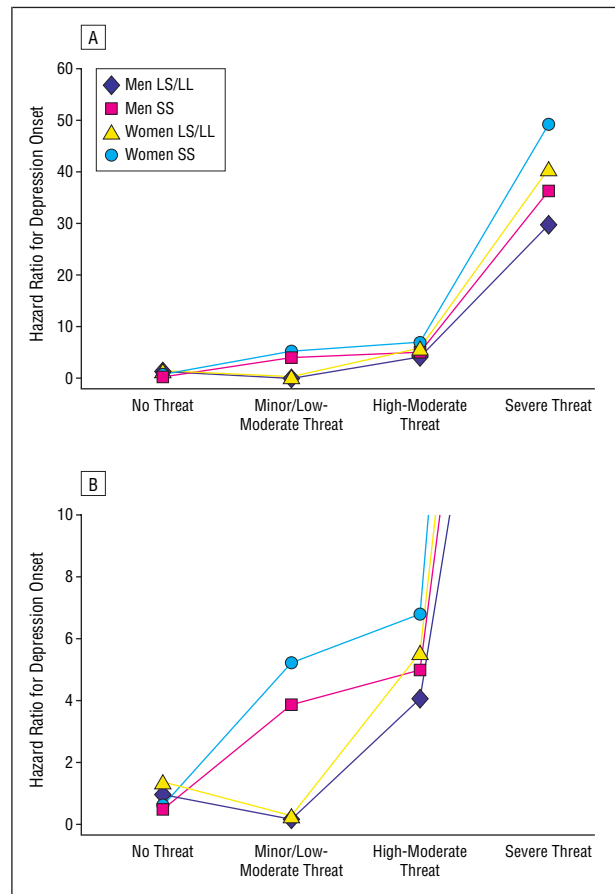
Figure 2. The hazard ratio of onset of major depression within a 2-month period as a result of (1) sex (men vs women), (2) genotype at the *5-HTT* polymorphism (SS vs LS and LL), (3) the level of long-term contextual threat experienced in the first month, broken down into 4 levels: (1) no threat (no stressful life event [SLE] exposure); (2) exposure to an SLE rated as having minor or low-moderate threat; (3) exposure to an SLE rated as having high-moderate threat; and (4) exposure to an SLE rated as having severe long-term contextual threat. A hazard rate of unity was defined as the risk level for a male with an SS genotype and no life-event exposure. A, The full results. B, A "zoom in" on the crucial part of the curve reflecting changes in response to minor and low-moderate threat. S indicates short allele; L, long allele.

but not in men. Further work will be needed to clarify whether and how the *5-HTT* gene modulates the pathogenic effects of SLEs and other social stressors.

The interpretation of genotype-environment interaction can be confounded by genotype-environment correlation. However, this is unlikely to be a concern herein as we found that the *5-HTT* genotype predicted neither exposure to SLEs in general nor specifically to SLEs with high threat levels.

CLARIFICATION OF THE DOSE-RESPONSE CURVE

Our second goal was to clarify the impact of the *5-HTT* polymorphism on the dose-response relationship between stress and risk for MD. Examining the results presented by Caspi et al, we expected that differences in risk as a function of genotype would grow larger as stress levels increased. We did not observe this. Instead, we saw an increased sensitivity of individuals with the SS genotype to the depressogenic effects of SLEs with mild or low-

moderate LTCT. Our initial evidence for the interaction between *5-HTT* genotype and the presence or absence of SLEs was due to this effect and was so robust because events at these mild levels of threat are more common than events with severe LTCT.

Our finding that the genotype-environment interaction is due to a "left-ward" shift in the dose-response curve such that SS individuals have increased sensitivity only to mild SLEs is intriguing. If correct, it would suggest that understanding the pathway from genetic variation to clinical disorder in psychiatry may require refined measures of environmental risk factors. Increasing evidence of genetic involvement in the etiology of psychiatric disorders can be interpreted as supportive of the reductionist agenda in psychiatry, which seeks to develop etiologic theories for psychiatric disorders in purely molecular terms. Our results argue against this as they suggest that understanding gene action in depression requires us to both "go down" to individual genetic polymorphisms and "go out" into the environment with detailed measurements of stressful experiences. However, replication of this specific feature of our results is clearly needed before further speculation about its meaning is warranted.

DIAGNOSTIC SPECIFICITY

Our last goal was to clarify the specificity of the action of *5-HTT* in its modification of the pathogenic effects of SLEs. Twin studies have suggested a high degree of overlap of genetic risk factors for MD and GAD[17,18] and the anxiogenic and depressogenic effects of SLEs are only partially distinct.[13,19,37] Therefore, we expected that the *5-HTT* polymorphism would also modulate the anxiogenic effects of SLEs. However, we found no such effect. While highly preliminary, and possibly limited because of modest power, this suggests some specificity in the modulation of the effects of stress by functional variation in the serotonin transporter.

LIMITATIONS

These results should be considered in the context of 2 potentially significant methodologic limitations. First, these findings were based on twins from 1 racial and geographical region and might not extrapolate to other groups. Second, our analyses assumed that when SLEs occurred in the same month as depressive onsets, the SLE preceded the onset. In 2 prior studies, we used additional interview material to determine, when SLEs and depressive onsets co-occurred in the same month, that in nearly all instances, the SLE preceded rather than followed the onset.[5,13]

OVERALL SIGNIFICANCE

A recent meta-analysis of the *5-HTT* polymorphism and MD, including 11 studies with 941 patients and 2110 controls, concluded that the studies were homogeneous and the association was not significant (with a pooled odds ratio and 95% CI of 1.08 and 0.96-1.22[38]). Another recent meta-analysis examined the association between this polymorphism and "avoidance-related" personality traits, which include neuroticism and related constructs that have been shown in both genetic and prospective designs to be strongly related to risk for MD.[39-42] Their analyses of 22 studies suggest a quite modest relationship with a mean difference of 0.11 SD units (95% CI, 0.06-0.17).[43] These results suggest that the straightforward association between variation in the 44 bp insertion/deletion polymorphism in the *5-HTT* gene and risk for the clinical syndrome of MD or associated personality traits is subtle at best. If our results and those of the original report by Caspi et al are correct, the *5-HTT* may be an example of a gene that influences liability to MD not by a main effect on risk but rather by control of sensitivity to the pathogenic effects of the environment.[44]

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