# Serotonin Transporter Gene Moderates the Development of Emotional Problems Among Children Following Bullying Victimization

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**Objective:** Bullying is the act of intentionally and repeatedly causing harm to someone who has difficulty defending him- or herself, and is a relatively widespread school-age phenomenon. Being the victim of bullying is associated with a broad spectrum of emotional problems; however, not all children who are bullied go on to develop such problems. **Method:** We tested the hypothesis that the relationship between bullying victimization and emotional problems was moderated by variation in the serotonin transporter (*5-HTT*) gene in 2,232 British children comprising the Environmental Risk (E-Risk) study cohort. **Results:** Our data supported the hypothesis that children's bullying victimization leads to their developing emotional problems, and that genetic variation in the *5-HTT*LPR moderates this relationship. Specifically, frequently bullied children with the SS genotype were at greater risk for developing emotional problems at age 12 than were children with the SL or LL genotype. Furthermore, we demonstrated that this genetic moderation persisted (a) after controlling for children's previctimization emotional problems by assessing intraindividual change in problems between ages 5 and 12 years, and (b) after controlling for other risk factors shared by children growing up in the same family by comparing emotional problems in twins discordant for bullying victimization. **Conclusions:** These findings are further evidence that the *5-HTT*LPR moderates the risk of emotional disturbance after exposure to stressful events. *J. Am. Acad. Child Adolesc. Psychiatry,* 2010;49(8):830–840. **Key Words:** bullying, emotional problems, gene–environment interaction, serotonin transporter, victimization

Bullying is the act of intentionally and repeatedly causing harm (through verbal harassment, coercive actions, or physical assault) to someone who has difficulty defending him- or herself.[1] Bullying victimization is widespread among school-aged children.[2] Although bullying is not a new problem, its consequences are not as benign as long presumed; Being the victim of bullying is associated with a broad spectrum of emotional problems,[3] and compromises the well-being and health of some children and adolescents.

A notable feature of research on the psychological effects of bullying victimization is the wide range of reactions observed among victims, raising the question of what accounts for response variability. Diathesis-stress models of psychopathology suggest the possibility that genetic differences may render some children more vulnerable than other children to the effects of bullying victimization.

In the present study, a functional polymorphism in the promoter region of the serotonin transporter gene (*5-HTT*) was used to characterize genetic vulnerability to bullying victimization and to test the hypothesis that *5-HTT* variation moderates the influence of bullying victimization on children's emotional problems. The *5-HTT* maps to chromosome 17, and transcriptional activity is modulated by variation in the length of the serotonin transporter linked polymorphic region (*5-HTT*LPR) within the gene's promoter. This regulatory region contains two common alleles, of which the

short (S) allele is associated with lower transcriptional efficiency of the promoter compared with the long (L) allele,[4] and it has been suggested that this polymorphism contributes to dysregulation of serotonergic neurotransmission.[5,6]

Studies of various stress-reactive endophenotypes suggest that S carriers should be most reactive to the effects of stressful experiences such as bullying victimization. We refer to a new generation of research in experimental psychopathology that exposes individuals with different genotypes to stress-inducing situations or affectively charged stimuli to examine genetic control of sensitivity to the environment by measuring their stress reactivity. Of these, five findings are of note.

First, functional magnetic resonance imaging (fMRI) studies have shown that the S allele is associated with exaggerated amygdala response to environmental threat.[7,8] This finding suggests that the S allele may influence risk for emotional problems by biasing the response of a key brain region mediating behavioral and physiologic arousal to environmental challenges.[6]

Second, research on fear conditioning—the neural mechanisms of which involve the amygdala—also reveals that variation in the 5-HTTLPR is implicated in how people learn to fear new stimuli. Compared with LL homozygotes, S-allele carriers acquired potentiated startle reactions to stimuli associated with an aversive event, and this acquired fear was more resistant to extinction. This research suggests that S-carriers are more likely pick up and retain fear of stimuli associated with threat.[9]

Third, research using acute stress-induction paradigms shows that 5-HTTLPR variation is associated with variations in cortisol response to a psychosocial challenge. Adolescents with two copies of the S allele showed a marked increase in cortisol immediately after exposure to stress and a slow return to baseline.[10] This research not only suggests that genetic susceptibility to hypothalamic–pituitary–adrenal axis dysregulation is detectable in SS carriers as early as adolescence, but also that those individuals have a higher reactivity to stressors than non-SS individuals.

Fourth, whereas much of the experimental research on 5-HTTLPR variation and stress reactivity has focused on mechanisms by which S-carriage confers risk, an investigation of biased attention provides evidence to suggest why LL homozygotes might be protected from negative events. When exposed to affectively charged images, LL homozygotes were characterized by selective avoidance of threat and selective attention to positive material.[11] This suggests that genetic variation in the tendency to "look on the bright side of life" may be a key mechanism underlying resilience.

Fifth, nonhuman primate studies also provide a hint as to why aspects of S-carriage may be linked to greater reactivity to bullying victimization. Bullying involves repeated hurtful actions between individuals where there is a power differential between the bully and the victim.[12] Research with rhesus macaques has uncovered different reactions among monkeys carrying the S allele versus LL homozygotes when they are confronted with high- versus low-status conspecifics. In particular, S-carrying monkeys were more likely to be threatened by and to avoid high-status dominant conspecifics (e.g., they displayed greater pupil diameter in response to high-status monkeys[13]). This research suggests that S-carriers may be more sensitive to the threat of confrontation in the context of power imbalance.

In previous research we empirically documented that being bullied is an environmentally mediated contributing factor to both boys' and girls' emotional problems in this same cohort.[14,15] In the present study we tested the hypothesis that genetic variation in the 5-HTTLPR would moderate the link between bullying victimization and the risk of developing these problems.

## METHOD

### Participants

Participants were members of the Environmental Risk (E-Risk) Study, which tracks the development of a birth cohort of 2,232 British children. The sample was drawn from a larger birth register of twins born in England and Wales in 1994-1995.[16] Details about the sample are reported elsewhere.[17] Briefly, the E-risk base sample was constructed in 1999-2000, when 1,116 families with same-sex 5-year-old twins (93% of those eligible) participated in home-visit assessments. The sample includes 55% monozygotic (MZ) and 45% dizygotic (DZ) twin pairs. Sex is evenly distributed within zygosity (49% male). Follow-up home visits were conducted when the children were aged 7 years (98% participation), 10 years (96% participation), and, most recently, 12 years (96% participation). The Maudsley Hospital Ethics Committee approved each phase of the study.

### Measures

Bullying victimization was assessed during private interviews with the children during home visits when they

were age 12 years. We explained to them that someone is being bullied when another child (1) says mean and hurtful things, makes fun, or calls a person mean and hurtful names; 2) completely ignores or excludes someone from their group of friends or leaves them out of things on purpose; 3) hits, kicks, or shoves a person, or locks them in a room; 4) tells lies or spreads rumors about them; and 5) does other hurtful things like these. We call it bullying when these things happen often and it is difficult for the person being bullied to stop it happening. We do not call it bullying when it is done in a friendly or playful way. Children indicated whether they had been bullied by another child "never," "sometimes," or "a lot." When a child reported being bullied, the interviewer asked the child to describe what happened. Notes taken by the interviewers were later checked by an independent rater to verify that the events described by the child relate to instances of bullying, operationally defined as evidence of repeated harmful actions between children where there is a power differential between the bully and the victim. This was done blind to data on emotional problems and genotype.

Emotional problems were assessed using the Child Behavior Checklist[18] for mothers and the Teacher's Report Form[19] for teachers, at age 5 years and again at age 12 years. The emotional problems scale is the sum of items on the Withdrawn and Anxious/Depressed scales, including items such as "cries a lot," "withdrawn, doesn't get involved with others," and "worries" (somatic complaints were not included, as this scale was not assessed at age 12). The internal consistency reliability of the mother and the teacher reports at age 5 years was 0.86 and 0.87, respectively. The internal consistency reliability of the mother and the teacher reports at age 12 was 0.87 and 0.89, respectively. Mother and teacher reports at each age were summed and standardized to create cross-informant scales for ages 5 and 12 years. For consistency across our research program on bullying, we use the same outcome measures as in previous reports.[15]

### DNA Extraction and Genotyping
At ages 5 and 7 years, DNA was obtained from 2,161 (97%) of the children. DNA samples were obtained via buccal swabs and extracted using an established procedure.[20] Primer sequences for *5-HTT*LPR are described by Gelernter et al.[21] (forward primer: 5'-ATGCCAGCACCTAACCCCTAATGT-3'; reverse primer: 5'-GGACCGCAAGGTGGGCGGGA-3'). The forward primer was 5'-labeled with a HEX fluorophore. Polymerase chain reaction (PCR) was carried out on a PTC-225 DNA engine (MJ Research, Waltham, MA), using the following cycling conditions: initial 15-min denaturing step at 95°C, followed by 35 cycles of 94°C for 30 s, 66°C for 30 s, and 72°C for 40 s, and a final extension phase of 72°C for 15 min. Reactions were performed in 1X reaction Buffer IV (ABgene, Epsom, UK), 1.5 mM/l $MgCl_2$, 50 ng genomic DNA, 5 pmol of each prim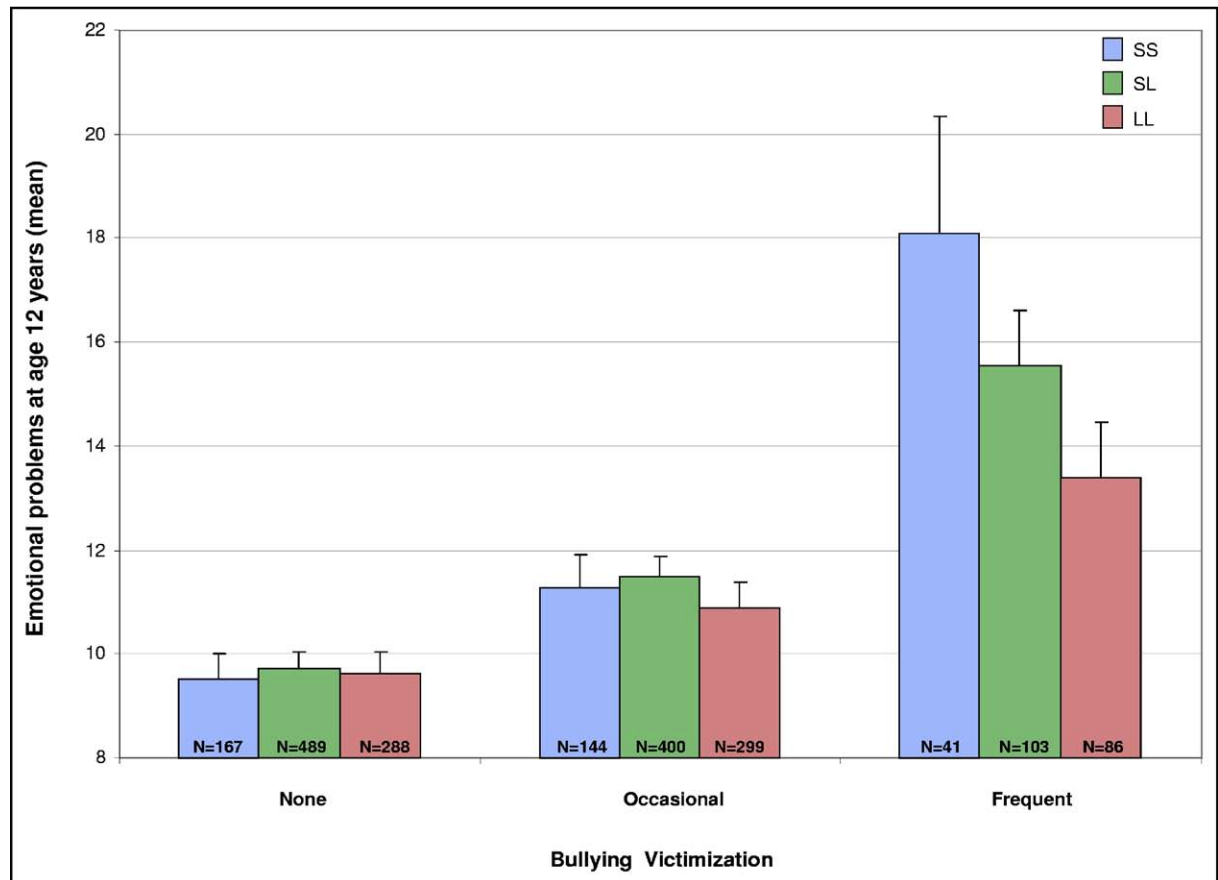er, 0.2 mM/l dNTPs, and 2 units of Native *Taq* (Promega, Madison, WI). This amplifies a 419–base pair product for the 16-repeat (L) allele and a 375–base pair product for the 14-repeat (S) allele. PCR products were denatured in highly deionized formamide and analyzed by electrophoresis on an Applied Biosystems 3100 genetic analyzer (Applied Biosystems, Foster City, CA), set up in genotyping mode, using POP4 polymer and ROX labeled GS500 size standard (Applied Biosystems). Results were analyzed using GeneScan v3.7 and genotypes called using Genotyper v3.6 software (Applied Biosystems). All genotype calls were reviewed manually. Samples were genotyped in birthdate order and blind to data on bullying victimization and emotional scores.

### Statistical Analyses
We tested the gene × environment interaction (GxE) in a hierarchical regression framework, with all main-effect terms entered on the first step of the model and interaction terms entered on the second step: Emotional problems = a+b1(*5-HTT*LPR)+b2(Occasional Bullying Victimization)+b3(Frequent Bullying Victimization)+b4(*5-HTT*LPR × Occasional Victimization)+b5(*5-HTT*LPR × Frequent Victimization)+e, where Occasional and Frequent Victimization were coded as dummy variables, respectively, and *5-HTT*LPR was coded as 0 = no S alleles, 1 = 1 S allele, and 2 = 2 S alleles. We tested for change in emotional problems between ages 5 and 12 by creating a variable that is the difference between the child's age-12 and age-5 scores, and performed the regression analysis as above. We also performed regression analyses of age-12 emotional problems, controlling for age-5 emotional scores. Reported significance tests are based on the sandwich, or Huber/White, variance estimator,[22] a method available in STATA 7.0 (StataCorp, College Station, TX). Application of this technique addresses the assumption of independence of observations; it adjusts estimated standard errors and therefore accounts for the dependence in the data that is due to analyzing sets of twins.

Within-family comparisons were conducted by correlating within-twin pair discordance in bullying victimization with within-twin pair discordance in age-12 emotional problems. To achieve this, we first created two categories of twin pairs: those who were concordant for bullying (i.e., same rate of bullying within twins) and those who were discordant (i.e., the twins experienced different rates of bullying). Twin pairs were selected only if they grew up in the same household. Second, we divided these twin pairs into three groups according to their *5-HTT*LPR genotype, selecting only pairs in which both twins shared the same *5-HTT*LPR genotype. Third, we correlated twin differences in bullying victimization with twin differences in both emotional problems at age 12 and change in emotional problems between ages 5 and 12 years.

**FIGURE 1** Children's emotional problems at age 12 years, as a function of their *5-HTT*LPR genotype and bullying victimization experiences. Note: Emotional problems at age 12 were elevated as a function of bullying victimization, but to a greater degree in SS homozygotes than in SL heterozygotes and LL homozygotes. Error bars represent standard errors of the mean. *5-HTT*LPR = serotonin transporter linked polymorphic region; LL = long/long; N = number of individuals; SL = short/long; SS = short/short.



## RESULTS

Bullying victimization and genotype data were available for 2,017 children (90.4% of the total sample); 46.8% of these individuals had never experienced any bullying victimization by age 12, 41.8% had experienced occasional victimization and 11.4% had experienced frequent victimization. Genotype frequencies were: SS = 17.5%, SL = 49.2%, and LL = 33.4% and genotypes were in Hardy-Weinberg equilibrium ($\chi^2 = 0.17$, $df = 2$, $p = .918$).

### Does *5-HTT*LPR Genotype Moderate the Association Between Bullying Victimization and Children's Emotional Problems?

Figure 1 shows children's emotional problems at age 12 years as a function of their *5-HTT*LPR genotype and bullying victimization experiences.

Children's genotype was not significantly associated with their emotional problems ($b = 0.38$, SE = 0.31, $t = 1.23$, $p = .218$) (Table 1). In contrast, children's victimization was significantly associated with their emotional problems; children victimized occasionally were significantly more likely to experience emotional problems than nonbullied children ($b = 1.60$, SE = 0.38, $t = 4.20$, $p < .001$), and children victimized frequently were at especially pronounced risk ($b = 5.56$, SE = 0.82, $t = 6.76$, $p < .001$). The association between bullying victimization and children's emotional problems was moderated, albeit at a trend level, by children's genotype, and this moderation was more pronounced among children who were frequently victimized ($b = 2.33$, SE = 1.23, $t = 1.89$, $p = .059$) rather than occasionally victimized ($b = 0.30$, SE = 0.54, $t =$

**TABLE 1** Results of Regression Analyses Testing Gene × Environment Interaction Effects on Emotional Problems at Age 12 Years and on Within-Individual Change in Emotional Problems Between Ages 5 and 12 Years

| Predictor Variable | Emotional Problems (Age 12 Years) | | | | Change in Emotional Problems (Ages 5–12 years) | | | |
|---|---|---|---|---|---|---|---|---|
| | *b* | SE | *t* | *p* | *b* | SE | *t* | *p* |
| **Genetic and environmental main effects** | | | | | | | | |
| *5-HTT*LPR | 0.38 | 0.31 | 1.23 | .218 | 0.49 | 0.35 | 1.40 | .160 |
| Occasional bullying | 1.60 | 0.38 | 4.20 | <.001 | 1.47 | 0.46 | 3.23 | .001 |
| Frequent bullying | 5.56 | 0.82 | 6.76 | <.001 | 4.09 | 0.77 | 5.28 | <.001 |
| **Gene × environment interactions** | | | | | | | | |
| *5-HTT*LPR × occasional bullying | 0.30 | 0.54 | 0.55 | .582 | −0.44 | 0.66 | −0.66 | .508 |
| *5-HTT*LPR × frequent bullying | 2.33 | 1.23 | 1.89 | .059 | 2.90 | 1.17 | 2.48 | .013 |

*Note: 5-HTTLPR = serotonin transporter linked polymorphic region.*

0.55, $p$ = .582). The statistical effect of frequent bullying victimization on children's emotional problems was strongest among SS homozygotes ($b$ = 8.56, SE = 2.42, $t$ = 3.53, $p$ <.001), followed by SL heterozygotes ($b$ = 5.81, SE = 1.16, $t$ = 5.00, $p$ < .001) and LL homozygotes ($b$ = 3.79, SE = 1.19, $t$ = 3.19, $p$ = .002). Within children who experienced frequent bullying, 31.7% of SS homozygotes had emotional problem scores in the clinical range ( i.e., emotional problem scores above 1.3 SD of the mean, the clinically relevant cut-off for the internalizing scale of the CBCL as suggested by Achenbach[18]), compared with 29.1% of SL heterozygotes and 15.1% of LL homozygotes.

Does *5-HTT*LPR Genotype Confer Heightened Risk for Emotional Problems After Bullying, Even After Controlling for Children's Previctimization Emotional Problems?
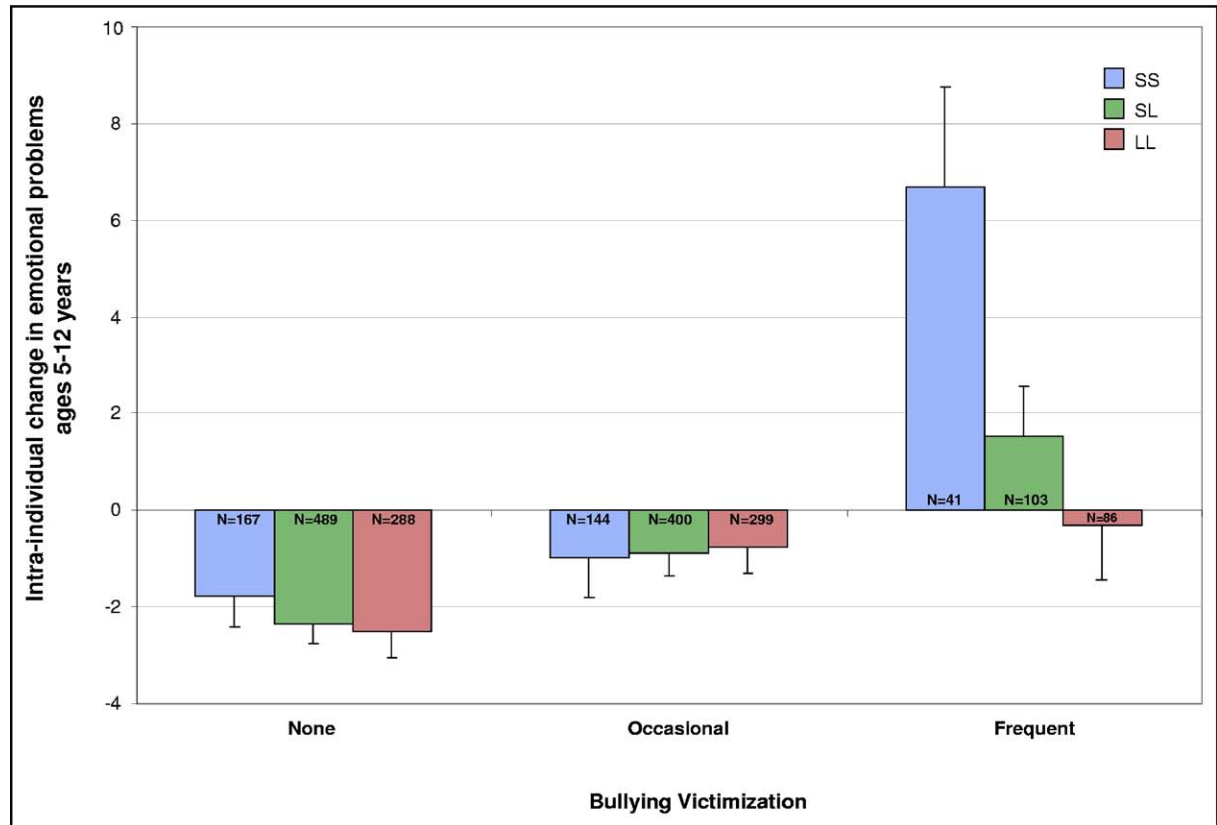
This evidence that *5-HTT*LPR variation moderates the statistical effect of bullying victimization on children's emotional problems does not constitute unambiguous evidence of GxE, because some children may evoke bullying victimization; such evocation could be a function of children's *5-HTT*LPR genotype or, more generally, of children's pre-existing and partially heritable emotional problems.[23] To rule out the possibility of such gene–environment correlations,[24] we conducted two tests. First, we tested whether *5-HTT*LPR genotype was associated with risk of bullying victimization. There was no significant association between *5-HTT*LPR genotype and risk of being bullied ($\chi^2$ = 7.25, df = 4, $p$ = .123). The rates of occasional and frequent victimization were 40.9% and 11.6% among SS homozygotes, 40.3% and 10.4% among SL heterozygotes, and 44.4% and 12.8% among LL homozygotes.

Second, we performed longitudinal analyses to test whether genotype moderated the effect of bullying victimization on within-individual change in children's emotional problems from age 5 to age 12. This analysis is important, because our longitudinal study revealed that children who were bullied had more emotional problems already at age 5 years, before they entered primary school (the mean age-5 emotional problem scores for children who were not bullied, occasionally bullied, and frequently bullied were 11.88 [SD = 8.31], 12.07 [SD = 8.16], and 13.29 [SD = 8.90], respectively). A multinomial logistic regression (a model that generalizes logistic regression by allowing two discrete outcomes) showed that children's emotional problems at age 5 years predicted their subsequent risk of experiencing frequent bullying victimization (relative risk ratio [RRR] = 1.019, SE = .009, z = 2.12, $p$ = .034, confidence interval [CI] = 1.001-1.036), although not occasional bullying victimization (RRR = 1.002, SE = .006, z = 0.37, $p$ = .710, CI = 0.99-1.01). As such, we sought to test (a) whether bullying victimization was associated with the emergence of more emotional problems among children from age 5 to age 12 years and (b) whether *5-HTT*LPR genotype moderated the risk of these newly emerging emotional problems, using each child as his or her own control.

Figure 2 shows the mean change in children's emotional problems from age 5 to age 12 years (calculated as the difference between each child's

**FIGURE 2** Change in children's emotional problems from age 5 to age 12 years, as a function of their *5-HTT*LPR genotype and bullying victimization experiences. Note: Frequent victimization led to significant increases in emotional problems, and this statistical effect is strongest among SS homozygotes, followed by SL heterozygotes. Error bars represent standard errors of the mean. *5-HTT*LPR = serotonin transporter linked polymorphic region; LL = long/long; N = number of individuals; SL= short/long; SS = short/short.



age-5 and age-12 emotional problem score), as a function of their *5-HTT*LPR genotype and bullying victimization experiences. Emotional problems remained fairly stable from age 5 to 12. However, one group of children, namely, children who were frequently victimized by bullying, showed significant increases in emotional problems (Fig. 2). Moreover, within the group of frequently victimized children, the results point to significant genetic moderation ($b = 2.90$, SE $= 1.17$, $t = 2.48$, $p = .013$) (Table 1). The statistical effect of frequent bullying victimization on increases in children's emotional problems was strongest among SS homozygotes ($b = 8.47$, SE $= 2.22$, $t = 3.82$, $p <.001$), followed by SL heterozygotes ($b = 3.90$, SE $= 1.04$, $t = 3.73$, $p <.001$), and was weakest among LL homozygotes ($b = 2.18$, SE $= 1.20$, $t = 1.82$, $p = .070$). We also tested whether this genetic moderation was observed when predicting age-12 emotional problems after

statistically controlling for age-5 emotional problems. In agreement with the change-score analyses, we found a significant interaction effect between *5-HTT*LPR and frequency bullying victimization ($b = 2.52$, SE $= 1.14$, $t = 2.22$, $p = .027$).

Does *5-HTT*LPR Genotype Confer Heightened Risk for Emotional Problems After Bullying, Even After Controlling for Other Common Family Experiences?
The longitudinal analyses suggest that the experience of being frequently victimized by bullying leads to increases in children's emotional problems from age 5 to age 12 years, and that this increase is especially pronounced among genetically vulnerable SS homozygotes. However, it is also possible that other factors in children's environments could place them at risk both for being victimized by bullies and for developing emotional problems. These family-wide factors—

i.e., factors common to members of a family—might include circumstances such as living in a deprived neighborhood or attending a school where bullying is widely accepted, or having neglectful parents who do not teach their children how to avoid bullies.[25] This suggests the possibility that it is not the effects of bullying *per se* that are being genetically moderated, but the effects of other environmental risk factors that are correlated with bullying victimization and that can also affect children's emotional problems. To address this possibility, we used our twin design to test whether genotype confers heightened risk to bullying, even after controlling for other family-wide experiences that are shared by the twins. We did this in three steps. First, we studied twin pairs who grew up in the same family, and for each pair we identified whether the twins were concordant (N = 471 pairs) or discordant (N = 314 pairs) for bullying. Second, we divided these twin pairs into three groups according to their *5-HTT*LPR genotype, pairs in which both twins were SS (N = 125 pairs, 73% MZ), SL (N = 390 pairs, 66% MZ), or LL (N = 270 pairs, 67% MZ), excluding pairs with different genotypes. (Ideally, we would have restricted our analysis to MZ twin pairs, but we had to include both MZ and DZ pairs to ensure sufficient power for this analysis.) Genotype groups did not differ on their rates of discordance of bullying (percent discordance: SS = 42%, SL = 38%, LL = 42%). Third, we correlated twin differences in bullying victimization with twin differences in emotional problems at age 12. If bullying is associated with emotional problems independently of other risk factors shared by children growing up in the same family, the twin who is bullied should have more emotional problems than the nonbullied twin. Moreover, if genotype moderates the association between bullying victimization and emotional problems, the twin who is bullied should have more emotional problems than the nonbullied cotwin if he or she is also genetically stress reactive (i.e., if they carry the SS genotype). The results support this prediction. The correlation between discordance in bullying victimization and corresponding discordance in emotional problems was positive and significant among twin pairs who were SS homozygotes (r = .21, $p$ = .017), but not significant among SL heterozygotes (r = .03, $p$ = .565) or among LL homozygotes (r = .06, $p$ = .333). Both groups of L-carri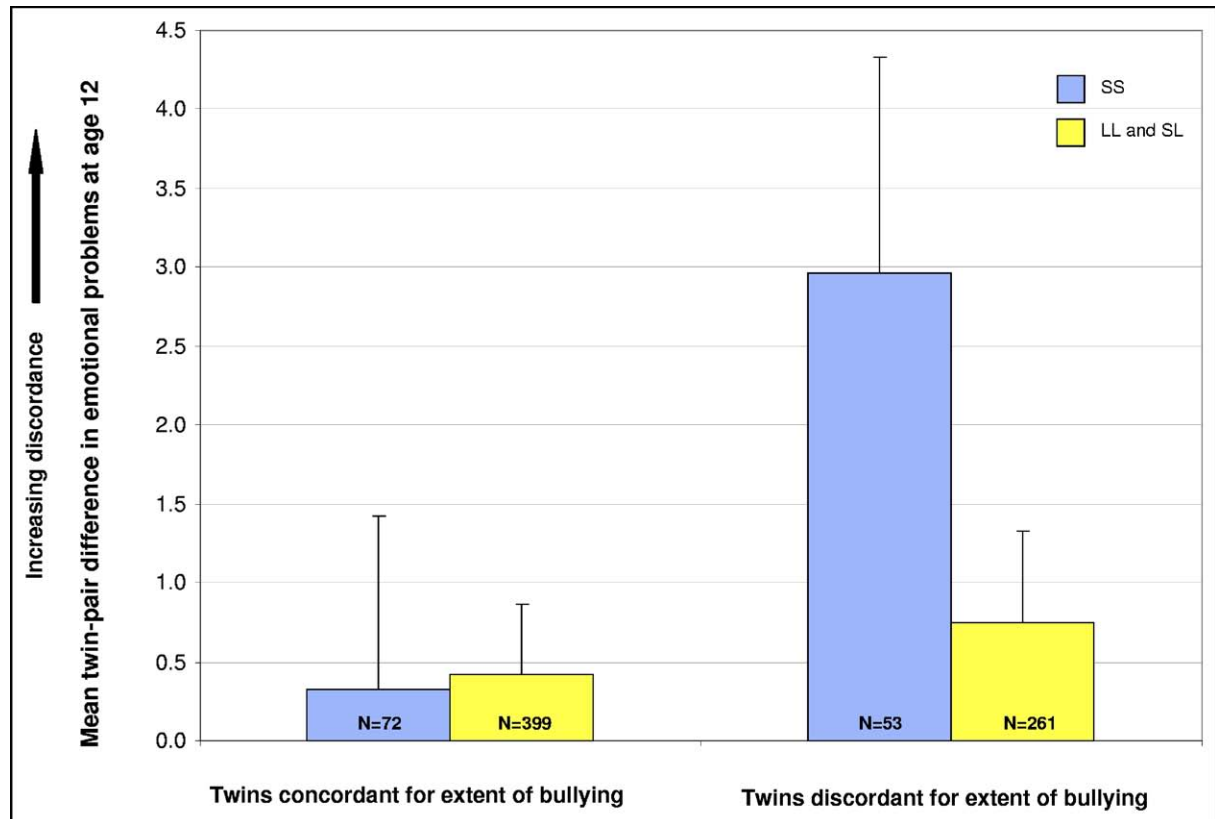ers appear to be protected from the effect of bullying victimization. Figure 3 shows the twin-pair differences in emotional problems as a function of twin-pair differences in bullying victimization. Among twin pairs discordant for bullying, there were corresponding twin-pair differences in emotional problems, and these twin-pair differences were larger among children carrying two copies of the *5-HTT*LPR S alleles than among L-carriers. We repeated this analysis, correlating the discordance in bullying victimization with corresponding discordance in change in emotional problems between ages 5 to 12 years. In agreement with the previous analysis, we found that bullied twins were more likely to experience increases in emotional problems than their nonbullied cotwins, but this was conditioned by genotype. Specifically, the correlation was positive and significant among twin pairs who were SS homozygotes (r = 0.20, $p$ = .024), less so among SL heterozygotes (r = 0.10, $p$ = .046), but not significant among LL homozygotes (r = −0.01, $p$ = .868). These within-family correlations suggest that genotype has a moderating effect on the association between bullying victimization and children's emotional problems, independent of other risk factors that are shared by children growing up in the same family.

## DISCUSSION

The current study provides evidence (a) that children's bullying victimization leads to their developing emotional problems, and (b) that genetic variation in the *5-HTT*LPR is a moderator of the link between bullying victimization and children's risk of developing these problems. Specifically, frequently bullied children with the SS genotype are at greater risk for developing emotional problems at age 12 than children with the SL or LL genotype. This genetic moderation persists after controlling for children's previctimization emotional problems and for other risk factors shared by children growing up within the same family environment.

These findings confirm and add to the body of evidence that victims of bullying are at risk for developing emotional problems.[14-26] However, not every bullied child develops emotional problems, and the present findings offer new clues as to why this might be. First, genetic differences (in the *5-HTT*LPR) interact with bullying victimization to exacerbate emotional problems. Second, the strength of this genetically influenced re-

**FIGURE 3** Twin-pair differences in emotional problems as a function of twin-pair discordance in bullying victimization. Note: Within twin pairs concordant for the extent of bully victimization, both twins had similar levels of emotional problems; that is, the mean twin-pair difference in emotional problems was near zero regardless of genotype. Within twin pairs discordant for bullying victimization, there were corresponding twin-pair differences in emotional problems; that is, the more victimized twin had more emotional problems than the less victimized twin. These twin-pair differences were noticeable among children carrying two copies of the *5-HTT*LPR S alleles but not among L-carriers. Error bars represent standard errors of the mean. *5-HTT*LPR = serotonin transporter linked polymorphic region; LL = long/long; N = number of twin pairs; SL = short/long; SS = short/short.



sponse is related to the frequency of the bullying experience (i.e., the GxE was strongest for frequently bullied children). The present findings are consistent with the recent report that SS genotype victims of relational aggression are prone to depression.[27] Because early-onset emotional problems constitute a risk for developing later mental health problems,[28] strategies to reduce bullying victimization in school-aged children, especially those individuals with specific genetic vulnerabilities, could help to reduce both childhood emotional problems and subsequent psychiatric difficulties.

Since the original report of an interaction between life stress and the *5-HTT* gene,[29] there have been multiple positive replications of this GxE in prospective-cohort,[30] cross-sectional,[31] and case-only[32] designs. Additional GxE studies have documented that variation in the *5-HTT*LPR is related to other stress-reactive phenotypes, including PTSD[33] and anxious mood.[34] There have also been failures to replicate.[35] This body of research has been difficult to summarize because of cross-study inconsistency in measurement; in particular, practically every study has measured stress exposure differently. Unfortunately, this has not stopped some meta-analysts from failing to take differences in exposure measurement seriously, and from generating uninterpretable summary statistics by averaging findings across different studies of uneven quality.[36,37] Some reviewers have cautioned that many of these different studies cannot be treated as replications, positive or negative, because of their *ad hoc* approaches to measuring life stress.[38] For example, whereas some researchers have stud-

ied maltreated children,[39] others have treated large family size as a chronic stressor.[40] As an analogy, would two independent genetic association studies be considered replications if the positive finding were with different markers of the same gene? Superficially, both markers measure the same thing (i.e., the gene in question), but each could be representative of two completely different pieces of information (e.g., different haplotypes that have diverse biological consequences). In this example, the claim of valid replication would be met with some caution. If one were to consider different methods of measuring and defining stress analogous to the two different markers in the previous example, then we should consider claims of replicating 5-HTTLPR × stress interactions with equal prudence.

As such, we do not claim the present study to be a replication of earlier study designs. Instead, we focused on a specific childhood stressor (i.e., bullying victimization) and tested the hypothesis that its effects on children's emotional problems are modified by variation in the 5-HTTLPR. Following the lead of experimental research,[41] we think that focusing on a specific, developmentally relevant, and clearly operationalized stressor (rather than on *ad hoc* measures of stress) offers a valuable opportunity to study the genetics of stress reactivity and stress resistance.[38] Furthermore, using a precise, well-operationalized stressful experience decreases between-subject heterogeneity in the stressful event and thereby increases the internal validity of the study design. In turn, understanding genetic sensitivity to a particular stressor may offer insights about GxE more generally, although it must be understood that generalization to other stressors must be demonstrated rather than assumed.

With a focus on bullying victimization, we used a number of strategies to ensure the robustness of the observed GxE. First, we used independent assessments of stress exposure and outcome (i.e., we obtained reports about victimization experiences from the children themselves and reports about emotional problems from adults who knew the children). In most previous studies of GxE, the same person who has been the source for acquiring stress data has also been the source for acquiring outcome data.[38] In the present study, we used independent sources of measurement to control for subjective bias and reduce shared method variance, which inflates associations between measures of stress exposure and emotional problems.

Second, we established a temporal relation-ship between the stressor (bullying victimization) and the outcome (emotional problems) and evaluated the GxE in relation to within-individual change in emotional problems between ages 5 and 12 years. Specifically, we documented that frequent bullying is associated with increases in emotional problems between ages 5 and 12 years among children with the SS genotype, independent of previctimization emotional problems among these genetically at-risk children. Temporal precision coupled with analysis of developmental change helped to rule out the possibility of reverse causation (i.e., that emotional problems led children to be victimized by bullies[42]) and the potential confounding of GxE by gene–environment correlation.[24]

Third, we used within-family comparisons to assess whether the genetic moderation of bullying victimization was independent of other environmental risk factors shared by children growing up in the same family. We correlated twin-pair discordance in bullying experience (the difference in the amount of bullying each twin experiences) with twin-pair discordance in emotional problems. We found that the bullied twin had more emotional problems, but only among those twin pairs in which both twins carried the SS genotype. These results suggest that variation in 5-HTTLPR moderates the association between bullying victimization and emotional problems independent of other risk factors shared by twins.

The present study also has limitations. First, although we carried out within-family comparisons of twins discordant for bullying victimization to control for family-wide factors that might influence the observed GxE, we are not able to rule out the residual influence of child-specific environmental experiences. That is, we established that the GxE persists irrespective of experiences shared by children in the same family, but it remains possible that the interaction may depend on experiences unique to the bullied versus nonbullied child in the family. Furthermore, the influence of shared environmental factors may not be stable throughout childhood and early adolescence.[43,44] Second, the interaction between 5-HTTLPR and bullying victimization observed here may be modified by other unmeasured genetic characteristics.[45,46] One way to test this possibility is to conduct within-family tests using exposure-discordant but genetically identical (MZ) twin pairs; however, we did not have

sufficient power or variation to carry out analyses on MZ twin pairs alone. Third, we cannot entirely rule out the possibility that children with emotional problems overreported victimization experiences. However, we attempted to control for this by using independent assessments of exposure and outcome, as recommended in stress research.[38]

In conclusion, this study adds to an accumulating body of observational studies showing that emotional disturbance is jointly influenced by stressful events and *5-HTT*LPR genotype, and that this GxE is observable in childhood. This observational evidence is buttressed by a wide range of emerging experimental GxE studies of stress reactivity (highlighted in the introduction) and GxE animal models of stress exposure,[47-50] all of which underscore the need for studies that can elucidate the mechanism of the contribution of *5-HTT*LPR variation to stress reactivity in development. &

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