5-HTTLPR Moderates the Relation between Changes in Depressive and Bulimic Symptoms in Adolescent Girls: A Longitudinal Study

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ABSTRACT
Objective: Depression and bulimia both are associated with low serotonin levels. We examined whether the serotonin transporter gene (5-HTTLPR) moderates the relation between depressive and bulimic symptoms over time.

Method: Fifty adolescent girls with no current or past Axis I disorder were genotyped for the 5-HTTLPR gene. Twice, 6 months apart, participants completed self-report measures of depressive symptoms and bulimic symptoms.

Results: The association between change in depressive symptoms and change in bulimic symptoms over time was significantly stronger in girls who are homozygous for the short 5-HTTLPR allele than for girls with at least one long allele.

Discussion: This finding is consistent with previous studies documenting a relation between depressive and bulimic symptoms in adolescents. Few studies, however, considered the possible role of serotonin linking both disorders. Gaining a better understanding of developmental effects of low serotonin could help to identify high-risk individuals and provide effective prevention and intervention.

Keywords: 5-HTTLPR; depression; bulimia; binging; adolescents

Introduction
Depressive symptoms in adolescence have been shown to predict eating disorders, such as binge eating and bulimia. Importantly, investigators have demonstrated that serotonin, a monoamine neurotransmitter, plays a critical role in both depression and bulimia. For example, studies showed decreased serotonin transporter binding in the midbrain in both obese women with binge eating disorder and suicide victims with major depression. Moreover, several investigators have documented anomalous serotonin activity in patients with eating disorders (e.g., Refs. 6,7 for reviews), mirroring the low levels of serotonin that have been found to be associated with depression. In fact, acute perturbation of serotonergic tone by dietary depletion of tryptophan, an amino acid that synthesizes serotonin (5-HT), has been linked to increased food intake and mood irritability in individuals with bulimia nervosa. Finally, treatments that decrease serotonin activity have been found to increase the likelihood of binge eating in both animals and humans (e.g., Refs. 11,12), whereas medications that increase serotonin activity, such as selective serotonin uptake inhibitors have been demonstrated to be effective in the treatment of both bulimia and depression.

Both expression of the serotonin transporter (5-HTT) and serotonin uptake are reduced in individuals who carry a short (s) allele of the serotonin transporter gene (5-HTTLPR), compared to long-allele homozygotes (ll; Ref. 15). The 5-HTTLPR s allele has been associated both with bulimia symptoms and disorder (Refs. 19,20 for a review, but see Ref. 21 for a recent meta-analysis, and, Ref. 22 for a commentary). Perhaps not surprisingly, therefore, a number of investigators have documented significant comorbidity between depression and bulimia (e.g., Refs. 23,24); fewer studies, however, have attempted to examine mechanisms that may underlie this association.

The present study was designed to gain a better understanding of the role of 5-HTTLPR in both depressive and bulimic symptoms by examining...
their association over time. Using a longitudinal design, we assessed the temporal dynamics between symptoms of depression and symptoms of bulimia and examined the moderating influence of the 5-HTTLPR gene. We assessed adolescent girls because adolescence is a critical developmental period that is associated with the onset of a number of psychiatric disorders, including depression and bulimia. Approximately twice as many females as males experience depression, and the ratio for eating disorders is even more extreme: adolescent girls are 30 times more likely than are boys to develop bulimia. To ensure that the obtained results were not confounded by a history of either clinically significant depression or bulimia, we included in this study only girls who, on structured interview, were free of current or past diagnosable psychopathology. We examined whether change in depressive symptoms is associated with change in bulimic symptoms, and whether this association is stronger in girls who carry a 5-HTTLPR s allele than it is in their homozygous l-allele counterparts. We predicted that a greater increase in depressive symptoms would be associated with a greater increase in bulimic symptoms for girls with one or two s alleles than for girls who are homozygous for the l allele.

Method

Participants were 50 girls between 10 and 16 years (M = 13.9, SD = 1.9) with no current or past history of Axis I disorder. Thirty-five of the girls self-identified as Caucasian, four as Hispanic, seven as Asian American, and four as biracial. They were recruited (through their mothers) using advertisements in newspapers and via the internet, and were paid for participating. The mean body mass index (BMI; kg/m²) for the 20 daughters who gave their height and weight was in the normal range (M = 19.5, SD = 2.9); eight of the girls had a BMI lower than 18.5 and, therefore, would be considered underweight, and one girl had a BMI over 25 (BMI = 26.3) and would be considered overweight.

The Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (K-SADS-PL) was used to confirm that girls had no current or lifetime diagnosable psychopathology. In addition, depressive symptoms were assessed with the 10-item version of the Children’s Depression Inventory (CDI-S). For 10 groups of 3 statements each (e.g., “I am sad once in a while,” “I am sad many times,” “I am sad all the time”), participants are asked to mark the statement that describes them best. Bulimic symptoms were measured at the same times as the CDI-S with the Eating Disorders Inventory for children and adolescents (EDI-C), using the items of the factor overeating based on the validated factor structure for this instrument. The overeating factor correlates highly (r = .96) with the bulimia scale of the Eating Disorders Inventory for adults, therefore, we refer to the construct it measures as “bulimic symptoms” throughout the manuscript. The eight items on this factor assess thoughts of engaging in bouts of uncontrollable eating or vomiting (e.g., “I have gone on eating binges where I have felt that I could not stop.” “I have the thought of trying to vomit in order to lose weight”). Both the CDI-S and the EDI-C were administered twice, 6 months apart, using the software dynQuest. Cronbach’s α for the CDI-S at the first measurement point was α = 0.61 and α = 0.75 at the second measurement occasion; reliability for the EDI-C subscale at the first assessment was α = 0.77 and α = 0.84 at the second assessment.

Participants were genotyped from saliva collected using the Oragene Kit an all-in-one system for the collection, preservation, transportation, and purification of DNA from saliva. Oligonucleotide primers flanking the 5-HTTLPR-linked polymorphic region and corresponding to the nucleotide positions −1416 to −1397 (strp5, 5'-GGC GTT GCC GCT CTG AAT GC) and −910 to −888 (strp3, 5'-GAG GGA CTG AGC TGG ACA ACC AC) of the 5-HTT gene 5'-flanking regulatory region were used to generate 484-bp or 528-bp fragments. The polymerase chain reaction products were electrophoresed through 5% polyacrylamide gel (Acrylamide/bis-Acrylamide ratio 19:1) at 60 V for 60 min. Fourteen of the 50 girls were homozygous for the l allele (ll genotype), 14 were homozygous for the s allele (ss genotype), and 22 were heterozygous (ls genotype). The allele frequencies of 5-HTTLPR were in the Hardy–Weinberg equilibrium, χ²(2,50) = 0.50, p = .786.

Results

For the full sample, CDI-S scores ranged from 0 to 7 (M = 1.20, SD = 1.59) at the first assessment and

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*a Recently, investigators have suggested that a polymorphism (rs25531) modulates the functionality of 5-HTTLPR, leading to a reordering of the 5-HTTLPR long alleles into Ls and Ll. The Lc variant and the s-allele have similar levels of 5-HTT mRNA expression, and both are lower than that of La (Wendland et al., 2006). It is not known, however, whether the in vitro differences in 5-HTT expression are also responsible for the differences in the gene-environment interaction encountered at the population level; thus, it is unclear whether the reordering of the 5-HTTLPR is warranted (for a review, see Uher and McGuffin, 2008). Forty-six of the girls in our study were genotyped for these subtypes of the long allele (La and Lc); seven girls were homozygous for the high-expressing La allele, 22 girls had either one copy of the s-allele or the low-expressing Lc allele and one copy of the La allele, and 17 girls had two s-alleles, one s-allele and one Lc allele, or two Lc alleles. Given the small number of girls in the homozygous Lc group and the resultant low statistical power, it is difficult to obtain reliable group differences. Nevertheless, graphing the relations in these three groups between the change in depressive symptoms and change in bulimic symptoms over 6 months yields a figure identical to that presented in Figure 1.
from 0 to 10 (M = 1.84, SD = 2.17) at the second assessment. The mean CDI-S score at both time points was well below a score of 10, the recommended cut-off for possible depression. Twenty girls had higher CDI-S scores at the second assessment than at the first assessment (difference range = 1–7; M = 2.40, SD = 1.73), 22 girls had the same CDI-S score at both assessments, and eight girls had lower scores at the second assessment (difference range = 1–4; M = 2.0, SD = 1.20).

The scores for the bulimic symptoms ranged from 1 to 4 (M = 1.77, SD = 0.66) on a 6-point scale from 1 = never to 6 = always at the first assessment and from 1 to 4.5 (M = 1.84, SD = 0.73) at the second measurement point, indicating that, on average, the girls have low bulimic symptoms. Twenty-six girls had higher scores at the second assessment than they did at the first assessment (difference range = 0.13 to 1.38; M = 0.43, SD = 0.33), seven girls did not change, and 17 girls had lower scores at the second assessment (difference range = 0.13 to 1.00; M = 0.44, SD = 0.29). Change scores for both CDI-S and EDI-C were calculated as the standardized residual from regressing T2 scores on T1 scores. This procedure is preferable to using difference scores, which often lead to overcorrection of the postscore by the prescore.

The three 5-HTTLPR genotype groups did not differ in age, change in depressive symptoms, or change in bulimic symptoms (see Table 1 for means and standard deviations). To examine whether the 5-HTTLPR polymorphism moderated the relation between change in depressive symptoms and change in bulimic symptoms, we conducted an analysis of variance (ANOVA) on change in bulimic symptoms with 5-HTTLPR genotype group (s/s, s/l, l/l) and change in depressive symptoms as predictor variables. Throughout the analyses, change in depressive symptoms was used as a continuous variable. Neither genotype group, F(2, 44) = 1.76, p = .19, nor change in depressive symptoms, F(1, 44) = 3.23, p = .08, individually predicted change in bulimic symptoms. The interaction of these two variables, however, significantly predicted change in bulimic symptoms, F(2, 44) = 4.15, p = .02, indicating that change in depressive symptoms had differential effects on change in bulimic symptoms as a function of genotype group.

To examine which of the genotype groups differed from the others in its association with the relation between change in bulimic symptoms and change in depressive symptoms, we conducted three pairwise group comparisons, again conducting ANOVAs with change in bulimic symptoms as the dependent variable and 5-HTTLPR group, change in depressive symptoms, and their interaction as independent variables. The results of these analyses indicated that girls who are homozygous for the s allele differed significantly from girls in the other two genotype groups with respect to the interaction of 5-HTTLPR genotype and change in depressive symptoms (heterozygous girls: F(1, 32) = 5.22, p = .03; homozygous l-allele girls: F(1, 24) = 4.87, p = .04); the heterozygous girls did not differ significantly from the homozygous l-allele girls, F(1, 32) = 1.12, p = .30. Neither of the main effects of genotype group or the change in depressive symptoms was significant in any of the three ANOVAs.

These analyses indicate that the association between change in depression and change in bulimic symptoms is different for girls who are homozygous for the s allele than it is for girls with at least one l allele. Computing the strength of the association between these two variables in the three groups of girls yielded correlations of r = .68, p = .01, for s-allele homozygotes, r = .25, p = .26, for heterozygous girls, and r = .15, p = .61, for girls homozygous for the l allele (see Fig. 1). Thus, girls with two s alleles experienced a stronger relation between depressive symptoms and bulimic symptoms than did girls with at least one l allele.

### Discussion

This study was designed to examine whether the 5-HTTLPR genotype moderates the relation between change in depressive and bulimic symptoms. Consistent with our hypothesis, we found that an increase in depressive symptoms over time was
more strongly associated with an increase in bulimic symptoms for girls who have two 5-HTTLPR s alleles than it was for girls who have at least one l allele. It is noteworthy that although girls with s/l and l/l genotypes had a weaker association between change in depressive symptoms and bulimic symptoms than did homozygous s-allele carriers, their magnitude of association did not differ significantly from each other. Although this finding could be due to low power associated with the relatively small sample size, it is also possible that s/l carriers are less vulnerable to life stressors that might initiate or exacerbate depressive symptoms and bulimic symptoms. Indeed, this formulation is consistent with Kendler et al.'s findings that individuals with two copies of the s allele were more likely than were their counterparts with two l alleles to become depressed in response to "common low-threat events," and with Gotlib et al.'s finding that girls who were homozygous for the s allele produced higher and more prolonged levels of cortisol in response to a laboratory stressor than did girls with either one or two l alleles.

Our finding of an association between depressive and bulimic symptoms over time is consistent with the results of a number of studies that have documented a relation between depressive and bulimic symptoms in adolescents, both cross-sectionally and longitudinally (e.g., Refs. 23,37). Few investigators, however, have considered the possible role of serotonin linking both disorders. By examining the serotonin transporter gene polymorphism, we were able to identify a group of individuals that appears to be particularly vulnerable to experiencing comorbidity of bulimia and depression. It is noteworthy that we found that 5-HTTLPR moderates the association between depressive and bulimic symptoms over time in adolescents who had no clinically significant symptoms of either disorder. The significantly stronger link between symptoms of these two disorders in adolescents with two s alleles and, thus, the lowest serotonin uptake, is especially important in attempting to gain a better understanding of the development of this comorbidity, for example, by considering reduced serotonin as a plausible common underlying mechanism.

We were not able in this study to examine statistically the issue of directionality of symptom change, that is, whether depressive symptoms lead to symptoms of bulimia or vice versa. Studies examining this issue have yielded inconsistent findings. Whereas some studies have found that adolescent girls with bulimic symptoms are more likely than are girls without bulimic symptoms to develop depression 4 years later, the results of other investigations indicate that depressive symptoms or negative affect can precede bulimic pathology and an increase in bulimic symptoms, and still other studies have reported a reciprocal relation between symptoms of depression and bulimic symptoms. Given the finding in the present study that the relation between change in depressive symptoms and change in bulimic symptoms over a 6-month period is moderated by the 5-HTTLPR gene, it will be important in future research to examine longitudinally the role of serotonin and the serotonin transporter gene in the development, and comorbidity of depression and bulimia. Moreover, given the current debate about the reliability of the interaction of 5-HTTLPR and life stress in predicting depression (e.g., Refs. 21,43), it will also be important in future research to replicate the present findings concerning the role of the 5-HTTLPR gene in moderating the interaction of changes in depressive and bulimic symptoms.

In this study we examined individuals at high risk for both depression and bulimia—adolescent girls. Our results suggest that serotonin is relevant in understanding the co-occurrence of these disorders. It is particularly noteworthy that the effects of the 5-HTTLPR s/s genotype on the strength of association between bulimic and depressive symptoms over time were evident at subclinical symptom levels in participants without past or current diagnosable psychopathology. Given, however, that sub-syndromal depressive symptoms have been found to be associated with impaired psychosocial functioning (e.g., Ref. 44) and to predict the subsequent

FIGURE 1. Change in depressive symptoms (standardized residuals) and change in bulimic symptoms (standardized residuals) between the first (T1) and second (T2) assessment by 5-HTTLPR polymorphism.
onset of Major Depressive Disorder (e.g., Ref. 45), it is likely that our findings obtained with individuals experiencing subsyndromal levels of symptoms are relevant for clinical populations as well. Elucidating the developmental effects of lower serotonin on depressive symptoms and risk factors for bulimia nervosa, such as binge eating, will be important in the early identification of groups at risk for these disorders, and in providing effective treatment for affected individuals.

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