HPA Axis Reactivity: A Mechanism Underlying the Associations Among 5-HTTLPR, Stress, and Depression

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Background: Recent evidence indicates that individuals who are homozygous for the short (*s*) allele in the promoter region of the serotonin transporter gene have higher rates of depression and other psychiatric disorders as a function of exposure to increasing levels of stressful life events than do individuals who have one or two copies of the long (*l*) allele. Despite the reliability of this association, the mechanism by which this polymorphism confers risk for psychopathology in the presence of stress is not understood. This study was designed to examine the formulation that individuals who are homozygous for the *s* allele are characterized by a greater biological reactivity to stress than are their counterparts who have one or two copies of the *l* allele.

Methods: Girls at high ($n = 25$) and low ($n = 42$) risk for depression by virtue of the presence or absence of a family history of this disorder were genotyped and exposed to a standardized laboratory stress task. Cortisol levels were assessed before the stressor, after the stressor, and during an extended recovery period.

Results: Girls who were homozygous for the *s* allele produced higher and more prolonged levels of cortisol in response to the stressor than did girls with an *l* allele.

Conclusions: These findings indicate that the 5-HTTLPR polymorphism is associated with biological stress reactivity, which may increase susceptibility to depression in the face of stressful life events.

Key Words: Depression, HPA-axis reactivity, risk for depression, serotonin transporter gene, stress

Major depressive disorder (MDD) is one of the most common and debilitating of all psychiatric disorders (1). The high chronicity and recurrence of depression, combined with its significant prevalence, personal loss, and societal costs make it imperative that we identify factors that are involved in the onset of this disorder. Consistent findings that a family history of depression is one of the strongest predictors of the development of this disorder have led investigators to examine the heritability of depression. Indeed, there is now considerable evidence from twin, adoption, and pedigree investigations, and from genomewide linkage studies, indicating that there is a significant genetic contribution to MDD (2). It is important to note, however, that the majority of individuals with a positive family history of depression do not develop the disorder. Thus most contemporary theories concerning the role of genes in the onset of depression do not postulate that genes affect depression directly; rather, they are explicitly diathesis-stress theories, positing that a genetic vulnerability interacts with major life stressors to produce depression (3). Given the importance of the serotonin system in depression and the effectiveness of selective serotonin reuptake inhibitors in the treatment of this disorder, it is not surprising that investigators examining this formulation have focused on the serotonin transporter (5-HTT) gene (SLC6A4) and, in particular, on a polymorphism in the promoter region of this gene (5-HTTLPR). The short (*s*) and long (*l*) alleles in the 5-HTTLPR have been shown to affect transcriptional rates of the 5-HTT gene (4). Perhaps most notably, Caspi *et al.* (5) recently found that individuals with one or two copies of the *s* allele of the 5-HTTLPR polymorphism exhibited higher levels of depressive symptoms, higher rates of diagnosable depression, and more suicidality as a function of exposure to increasing levels of stressful life events than did individuals who were homozygous for the *l* allele.

Several investigators have now replicated Caspi *et al.*'s (5) results (6–8). Despite growing evidence that the 5-HTT gene moderates the association between life stress and depression, however, the mechanisms underlying this moderation are not well understood. Findings from animal research suggest that one possible mechanism involves the construct of stress reactivity. Li *et al.* (9), for example, found that mice with diminished or absent function of the 5-HTT gene exhibited greater increases in the stress hormone adrenocorticotropin (ACTH) in response to stress than did their control littermates. Indirect support for the involvement of stress reactivity also comes from studies with humans. Kendler *et al.* (10) found that individuals with two *s* alleles in the 5-HTTLPR were more likely to become depressed in response to "common, low-threat events" and hypothesized that this genetic polymorphism produces an increased sensitivity to the impact of stressful events. Perhaps reflecting this "sensitivity," investigators have found that, compared with individuals who have at least one *l* allele in the promoter region of the 5-HTT gene, individuals homozygous for the *s* allele exhibit greater amygdala activation in response to fearful stimuli (11,12).

Consistent with this "stress reactivity" formulation, investigators have found cortisol, a reliable indicator of hypothalamic-pituitary-adrenocortical (HPA) axis functioning and stress reactivity not only to have a hereditary component (13) but also to be elevated in 40%–60% of adults diagnosed with MDD (14). Indeed, hypercortisolemia has been postulated to lead to hippocampal neuronal loss, which in turn has been posited to be involved in the pathogenesis of depression (15). Given the research just described, it is possible that depressed individuals, many of whom are likely to be 5-HTTLPR "s-carriers" (5), are characterized by hypercortisolemia not only because they have

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been exposed to a *greater number* of stressful life events than are nondepressed persons (16) but also because they are biologically *more reactive* to stressors. Indeed, the results of a recent meta-analysis indicate that MDD patients have higher cortisol levels following exposure to a stressor than do nondepressed individuals (17). The formulation that s-carriers are more biologically reactive to stress than are individuals who are homozygous for the *l* allele may explain both Caspi *et al.*'s (5) finding of an increased likelihood of developing depression in response to stressful events among s-carriers and Kendler *et al.*'s (10) finding of the importance of low-threat events in predicting the onset of depression.

This study was designed, in part, to examine this formulation. To ensure that we had participants in this study who might go on to develop an episode of depression, we assessed genotype and stress reactivity in young girls at high and low risk for this disorder by virtue of a the presence or absence of a family history of recurrent depression (18). On the basis of the literature just reviewed, we predicted that girls who were homozygous for the *s* allele 5-HTTLPR polymorphism would exhibit greater and more prolonged cortisol production in response to a laboratory stressor than would girls with one or two *l* alleles.

Methods and Materials

Participants

Participants were 67 girls aged 9 to 14 with no current or past Axis I disorder. Forty-two girls had biological mothers with no current or past Axis I disorder (low-risk daughters), and 25 girls had biological mothers with a history of recurrent episodes of MDD during their daughter's lifetime (high-risk daughters). Participants were recruited through advertisements posted in numerous locations within the local community (e.g., Internet bulletin boards, university kiosks, supermarkets) and through the Department of Psychiatry and Behavioral Sciences at Stanford University. The mothers' responses to a telephone interview provided initial selection information. This phone screen established that both mothers and daughters were fluent in English and that daughters were between 9 and 14 years of age. This telephone interview was also used to identify mothers who were likely either to have no psychiatric history or to meet criteria for recurrent depression during their daughter's lifetime, and daughters who were likely to have no past or current psychiatric disorder. Those mother and daughter pairs who were considered likely to be eligible for participation were invited to come to the laboratory for more extensive interviews.

Assessment of Depression and Psychopathology

Interviews. All mothers and daughters were administered structured clinical interviews by different trained interviewers to diagnose the presence of at least two distinct episodes of depression in the MDD mothers since the birth of their daughters (Structured Clinical Interview for DSM-IV-TR [SCID]) (19) and a lifetime absence of psychopathology in the daughters (Kiddie Schedule for Affective Disorders and Schizophrenia [K-SADS]) (20) and in the control mothers (SCID). Both the daughters and the mothers were administered the K-SADS to assess the daughters' functioning, and both informants had to report an absence of any past or current Axis I disorder in the daughter. To assess interrater reliability, an independent trained rater who was blind to group membership randomly evaluated 10% of the SCID and K-SAD-PL interviews. In all cases, diagnoses of former depressive episodes in mothers, no history of depressive episodes in mothers, and absence of any current or previous Axis I disorder in the girls matched the diagnosis made by the original interviewer, $\kappa = 1.00$. Eligible participants were invited to come back to the lab within 1 week of their interview session to take part in the stress task, and saliva samples for the DNA analyses were taken.

Questionnaires. Daughters completed the 10-item version of the Children's Depression Inventory (CDI-S) (21), a self-report measure of depressive symptomatology for children between the ages of 8 and 17. Mothers completed the Beck Depression Inventory-II (BDI) (22), a 21-item self-report measure of the severity of depressive symptoms.

Verbal Intelligence

The vocabulary section of the verbal subtest of the Wechsler Intelligence Scale for Children—third edition (23) was administered to the daughters to ensure that any group differences in response to the stressor are not a function of differences in intellectual ability.

Cortisol Collection and Stress Task

Participants refrained from eating and drinking (except water) 1 hour before arrival at the laboratory and during the experimental procedures. Participants were first instructed to rest and relax for 30 min. They were allowed to read magazines and listen to music, and saliva samples were collected just before they were given instructions for the task. They then underwent a 15-min laboratory session during which they were stressed by an experimenter and salivary cortisol was collected at regular intervals. More specifically, daughters completed a 3-min serial subtraction task in which they were instructed to begin at 400 and count backward by sevens as quickly and accurately as possible. If they made a mistake, they were interrupted by the experimenter and were told to start over. Daughters who moved quickly and easily through this procedure were stopped and told to start over at 4000 and count backward by 17s. Following this task, daughters were administered the 12-min Ewart Social Competence Interview (24), a semistructured interview developed to induce emotional stress and arousal in adolescents by discussing details of stressful life situations.

Four saliva samples were collected from each girl using salivettes (Sarstedt, Nümbrecht, Germany) during the laboratory stress paradigm: one sample immediately before task instructions and three samples at 15, 30, and 45 min post-onset of stressor. These collection times are based on meta-analytic findings indicating that peak cortisol response occurs 21–40 min following the onset of an acute stressor and that complete recovery to baseline values occurs within 41–60 min (25). Following the laboratory stressor (i.e., during collection of the final two samples), participants watched a neutral videotape about Denali National Park. On average, the first sample was collected at 12:15 PM; groups did not differ in their collection times, $t(65) < 1$. Saliva samples were stored in a freezer chest until they were transferred to a −20° freezer located at the General Clinical Research Center at Stanford University, where they were maintained until radioimmunoassays were performed. Samples were assayed together to control for interassay error, and control samples were included to evaluate variability.

A minimum of .2 mL of liquid saliva was absorbed into a small cotton roll and expressed through a plastic tube into a sterile vial (Salivette Sarstedt without the additives device). Cortisol was assayed by luminescence immunoassay reagents using a commercial kit from Immuno-Biological Laboratories (Hamburg,