An Interaction Between Perceived Stress and 5HTTLPR Genotype in the Prediction of Stable Depressive Symptomatology

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A significant amount of research has examined the interaction between a functional polymorphism in the serotonin transporter gene (5HTTLPR) and stressful life events in the prediction of depression and depressive symptomatology. The results of these studies have produced conflicting evidence, with some studies substantiating a significant interaction and others failing to detect a significant interaction. The purpose of the current study was to add to this line of research by testing for an interaction between 5HTTLPR and perceived stress in the prediction of stable depressive symptomatology. Analysis of data from the National Longitudinal Study of Adolescent Health (Add Health) indicates that the association between perceived stress and depression is moderated by 5HTTLPR genotype for females, but not for males. Specifically, females who were homozygous for the short allele were significantly more likely to report symptoms of depression in the face of perceived stress when compared to females who were homozygous or heterozygous for the long allele.

In recent years, there has been a tremendous amount of research examining gene–environment interactions and how they contribute to a range of psychopathologies (Kendler & Prescott, 2006; Rutter, 2006). The emphasis on gene–environment interactions has been particularly pronounced in studies attempting to uncover the etiological underpinnings to depression and depressive symptomatology. This focus on gene–environment interactions in depression research was spawned in large part by Caspi et al.’s (2003) study that analyzed data drawn from the Dunedin Multidisciplinary Health and Development Study to examine whether an interaction between stressful life events and a functional polymorphism in the serotonin transporter gene (5HTTLPR) predicted variation in depression. Analysis of their data supported the hypothesis by revealing that 5HTTLPR moderated the effect of stressful life events.

Caspí et al.’s (2003) study sparked a wave of research attempting to replicate the gene–environment interaction between 5HTTLPR and stressful life events in the prediction of depression. These replication studies have produced heterogeneous results, with some studies replicating the original gene–environment interaction and other studies failing to detect a significant gene–environment interaction (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Rutter, 2010). In an attempt to make sense of these disparate results, four reviews and meta-analyses were conducted that examined the literature testing for an interaction between 5HTTLPR and stressful life events in relation to depression (Clarke, Flint, Attwood, & Munafò, 2010; Munafò, Durrant, Lewis, & Flint, 2009; Risch et al., 2009; Uher & McGuffin, 2010). Rather than clarifying the true effect that the 5HTTLPR × stressful life events interaction had on depression, these studies actually created more uncertainty. For example, two meta-analyses indicated that the extant literature did not support a statistically significant gene–environment interaction in the prediction of depression (Munafò et al., 2009; Risch et al., 2009), but one meta-analysis did indicate a significant gene–environment interaction (Clarke et al., 2010). One review, moreover, did reveal that after taking into account the methodological rigor of pooled studies, there was a statistically significant interaction between 5HTTLPR and stressful life events across studies (Uher & McGuffin, 2010). Importantly, the results of this latter study suggested that higher quality studies were the same studies that were most likely to detect a significant gene–environment interaction. The null result of the gene–environment interaction reported in the two meta-analyses prompted some to advocate for a moratorium on research examining the interaction between 5HTTLPR and stressful life events in the etiology of depression.

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(Risch et al., 2009). Others, however, argued that the mixed results should lead to more research of better quality to further explore the merits of this gene–environment interaction (Caspi et al., 2010). The current study follows this latter recommendation and examines the interaction between 5HTTLPR and stress in the prediction of depression in a longitudinal sample of American adolescents and young adults. In doing so, we extend prior research in two important ways.

First, we include a measure of stress that focuses on perceived stress in contrast to the more widely used measurement strategy of quantifying exposure to stressful life events. The extent literature typically employs measures of stressful life events or childhood maltreatment as a way of capturing exposure to stress. These types of scales act as proxy indicators that are designed to measure the amount of stress that is experienced by the individual. In other words, stress is quantified by measuring exposure to life events and then extrapolating that those events hold the potential to create the same amounts of stress across people. This may or may not be true, as people interpret, perceive, and experience many of the same events in very different ways depending on a suite of psychological traits (Cole et al., 2010; Rhodewalt & Agustsdottir, 1984). Regardless of genotype, ostensible stressful life events may produce varying degrees of stress. Even life stressors, such as the death of a close family member, may produce varying amounts of stress depending on the meaning and context attached to those particular events (Rhodewalt & Agustsdottir, 1984). Measuring stress via a stressful life events scale may be far too distally removed from the individual and, as a result, attenuate the effect that stress has on the individual. One way to overcome this limitation would be to assess stress by employing a more proximal measure of stress that centers on perceptions of stress. Such a measurement strategy allows for a more direct assessment of the degree to which a person is affected by stress. A perceived stress scale is likely to capture not only sensitivity to major life events, including the death of a family member, but also the extent to which day-to-day stressors, such as work and family duties, affect them. Moreover, there is some evidence to suggest that these daily life stressors may be more salient than major life events (Kanner, Coyne, Schaefer, & Lazarus, 1981).

The second way that the current study builds on prior research is by measuring depression as a single phenotype that is assessed across multiple points in adolescence and early adulthood. Prior research typically measured depression that occurred during the past year, month, or week. This approach allows for a cross-sectional assessment of state depression and depressive symptomatology, but it does not permit a longitudinal assessment of stable trait-like depressive symptomatology that cuts across sections of the life course. There are at least two reasons why using a longitudinal measurement strategy is useful in gene–environment research on depression. First, a longitudinal assessment strategy aggregates depression scales across time into a single depression phenotype, which necessarily means that it is not as prone to random and nonrandom fluctuations as cross-sectional depression scales. Second, research suggests that the heritability of depression tends to be strongest for those who are plagued by serious depression that is characterized by multiple bouts of depression across long periods of time (Kendler & Prescott, 2006). A relatively high heritability for chronic depression tentatively suggests that gene–environment interactions may be more influential among the chronically depressed as opposed to those who are depressed for only short periods of time.

Method

Participants

Data for this study come from the DNA subsample of the National Longitudinal Study of Adolescent Health (Add Health; Udry, 2003). Extensive discussions of the Add Health data have been published previously (Harris et al., 2003; Harris, Tucker Halpern, Smolen, & Haberstick, 2006; Resnick et al., 1997). Briefly, the Add Health is a four-wave, prospective study of a nationally representative sample of American youths enrolled in 7th through 12th grade. The first wave of data was collected during a specified school day during the 1994–1995 academic school year. More than 90,000 youths completed the Wave 1 in-school self-report surveys. Students were asked a wide range of questions about their demographic characteristics, their family life, and their school experiences. Then, a subsample of respondents was selected to be reinterviewed at their homes as part of the Wave 1 in-home component to the study. These interviews were designed to gather more in-depth information about the respondents, including information about sensitive topics, such as sexual activities. A total of 20,745 adolescents and 17,700 of their primary caregivers were included in this wave of data collection. Approximately 1 and 1/2 years later, the second round of surveys was administered with 14,197 of the original respondents. Because relatively little time had passed since Wave 1, most of the respondents were still youths. As a result, the questions contained on the survey instruments remained very similar. The third wave of data was collected between 2001 and 2002, when most of the respondents were young adults. Given that the age range of the respondents changed so dramatically since Waves 1 and 2, the survey instruments also were changed to include questions more appropriate for young adults. For example, respondents were asked about their marital status, their employment history, and their lifetime contact with the criminal justice system. The fourth and final round of interviews was completed between 2007 and 2008 when the respondents were 24–32 years old. Questions asked at Wave 4 pertained to adulthood outcomes, such as the highest level of education received and factors associated with parenthood. In total, 15,701 respondents were included in Wave 4 of the Add Health study (Harris et al., 2003).

During Wave 3 data collection, a subsample of participants was selected to submit buccal cells to be genotyped. Only respondents who had a sibling who was also participating in the Add Health study were eligible to be included in the DNA subsample of the Add Health study. In total, more than 2,500 respondents provided samples of their DNA, creating one of the largest samples to include genotypic and phenotypic measures. Additional information about the DNA subsample of the Add Health has been published elsewhere (Harris et al., 2006).
Assessments

Lifetime depressive symptomatology. At each of the four waves of data collection, respondents were screened for symptoms of depression using items drawn from the Center for Epidemiological Studies Depression Scale (CES-D). The CES-D is a widely used instrument for assessing depressive symptomology, and psychometric research has indicated that it is both valid and reliable (Radloff, 1977). Not all of the items from the CES-D were asked at each of the waves, and across waves, there were differences in the items that were asked. To create the depression scales for each wave, all of the CES-D items that were available were factor analyzed and subjected to internal reliability assessments. CES-D items were retained for inclusion in the scale if (a) the item loaded on the same factor as the other CES-D items and (b) if the inclusion of the item did not decrease internal reliability as assessed via Cronbach’s alpha. Items that did not meet these two criteria were removed from that wave’s depression scale.

During Wave 1 interviews, 18 questions were asked about various symptoms associated with depression that were included in the depression scale. For example, adolescents were asked how frequently during the past week they felt bothered by things that usually do not bother them, they felt that they could not shake off the blues even with the help of family and friends, and they felt depressed. Responses to these items were coded as follows: 1 (rarely or none of the time; <1 day), 2 (some or a little of the time; 1–2 days), 3 (occasionally or a moderate amount of time; 3–4 days), and 4 (most or all of the time; 5–7 days). Similarly, the Wave 2 depression scale was developed from 14 items drawn from the CES-D (α = .85). During Wave 3 interviews, a much smaller subset of questions from the CES-D was asked of the respondents. As a result, eight items were included in the Wave 3 depression scale (α = .80). Similar to Wave 3, only a small subset of questions from the CES-D was asked of respondents at Wave 4, and, consequentially, the Wave 4 depression scale included four items (α = .80).

The four wave-specific depression scales were then used to create a global measure of depression that tapped stability in lifetime depressive symptomatology. To do this, each of the four depression scales was transformed into z-scores to create a common weighting system. Bivariate correlations revealed that all four depression scales were significantly intercorrelated, with correlation coefficients ranging between r = .304 and .538 (p < .05, two-tailed tests). The four z-transformed depression scales were then subjected to a principal components factor analysis with varimax rotation. The results of the analysis indicated that the variance-covariance structure of the four scales could be accounted for by a unitary factor. Specifically, the factor accounted for 54.03% of the total variance, and the eigenvalue was 2.161. The next factor only accounted for 18.87% of the total variance, with an associated eigenvalue of .755. As a result, the four z-transformed depression scales were averaged by summing them together and dividing by four. The resulting value indexed the average depression score across approximately 13 years of the life course. Using a lifetime measure of depression represents an improvement over using the wave-specific depression scales in two main respects. First, a lifetime depression scale is not as sensitive to time-specific fluctuations in depression that may attenuate the psychometric properties of the scale. Second, a lifetime depression scale is better able to delineate persons who have a predisposition toward depression from those whose depression is more the result of immediate environmental pathogens.

Perceived Stress. Perceived stress was measured with items drawn from Cohen’s Perceived Stress Scale (PSS). The PSS has been shown to be a reliable and valid measure of stress, and it has been shown to correlate significantly with exposure to stressful life events and with depressive symptomatology (Cohen, Kamarck, & Mermelstein, 1983). During Wave 4 interviews, respondents were asked how often in the past 30 days they (a) were unable to control important things in their lives, (b) felt confident in their ability to handle their personal problems (reverse coded), (c) felt things were going their way (reverse coded), and (d) felt that difficulties were piling up so high that they were unable to overcome them. The response set to these items was as follows: 0 (never), 1 (almost never), 2 (sometimes), 3 (fairly often), and 4 (very often). Responses to the four items were summed together to create the PSS, with higher values representing more perceived stress (α = .73). Because this scale does not include all of the items from the original PSS, we calculated a series of additional statistics to help establish reliability and validity. First, a factor analysis revealed that all the four items were loaded together on a single factor. Second, internal reliability analysis revealed that removing any of the four items would decrease Cronbach’s alpha. Third, the bivariate correlation between the perceived stress scale and the lifetime depression scale was statistically significant (r = .51, p < .05, two-tailed test) with an effect size that is similar to what others have reported when using the full PSS (e.g., Kuiper, Olinger, & Lyons, 1986). This latter analysis helps to establish predictive validity of the scale used in the current analyses.

Genotyping the 5HTTLPR Polymorphism

A coordinated effort between the Institute for Behavioral Genetics in Boulder, Colorado, and the Add Health research team was responsible for genotyping the subjects at Wave 3. One of the polymorphisms that subjects were genotyped for was found in the serotonin transporter (5-HTT) gene. The 5-HTT gene is located on chromosome 17 and has a 43-base-pair insertion/deletion polymorphism in the 5’ regulatory section of the gene (5HTTLPR; Heils et al., 1996). The 5HTTLPR polymorphism contains common alleles of different lengths: a short (s) allele and a long (l) allele. This polymorphism is considered to be functional in that compared to the l allele, the s allele has been found to suppress transcription of the serotonin transporter protein (Hu et al., 2006; Lesch et al., 1996), which may translate into the s allele creating lower levels of serotonin in the brain. Prior research has identified the s allele as the putative risk allele for depression and depressive symptomatology (Caspil et al., 2003, 2010).

Overall, 17.3% of the sample was homozygous for the s allele, 45.5% of the sample was heterozygous for the s allele, and 37.3% of the sample was homozygous for the l allele. These allelic distributions are similar to those reported in previous
studies (e.g., Caspi et al., 2003). An initial analysis of the data revealed that a recessive coding of the s allele resulted in a better fit to the data (i.e., the mean values on depression for the l/l and s/l genotypes were not significantly different from each other across scores of the perceived stress scale, but the s/s genotype was significantly different). The substantive results were identical regardless of how 5HTTLPR was coded, but given that the fit to the data was better with the recessive coding scheme, we opted to report the results generated from that measurement strategy. There were not any significant differences in the allelic distributions by gender ($\chi^2 = .554, df = 1, p = .457$).

**Procedure**

Ordinary least squares (OLS) regression was used to examine the effects of 5HTTLPR, perceived stress, and 5HTTLPR × perceived stress on depression. OLS is appropriate to employ because the distribution of the depression scale approximated normality. However, some of the observations lacked independence (i.e., more than one sibling from the household was genotyped), which can produce downwardly biased standard errors and artificially inflated t values for tests of statistical significance for the regression coefficients. This problem was corrected in two ways. First, all models were estimated using Huber/White standard errors, which corrects for the clustering of observations. Second, in cases where monozygotic (MZ) twin pairs were included in the sample, one was randomly removed from the final analytical sample (Haberstick et al., 2005). The models were calculated for the full sample and separately for men and women because of prior research indicating that 5HTTLPR may be differentially related to depression in men and women. All of the models controlled for race (0 = Caucasian, 1 = African American) to help avoid population stratification effects. The analyses were conducted using STATA 10.1.

**Results**

We began our analysis by first examining whether there was an association between 5HTTLPR and the perceived stress scale. If there is a statistically significant association, then any interaction between 5HTTLPR and perceived stress could be the result of the correlation between these two measures. The results of a bivariate correlation revealed no association between 5HTTLPR and perceived stress ($r = -.04, p = .082$).

We next turned our attention to the OLS models predicting scores on the lifetime depression symptomatology scale for the full sample and separately for women and men. The results are presented in Table 1. The first column of this table reveals a statistically significant interaction between 5HTTLPR and perceived stress in the prediction of depression. Similarly, a statistically significant interaction between 5HTTLPR and perceived stress was observed for women. For men, however, there was not a statistically significant interaction, but it is worth noting that the effect is trending toward significance.

We next probed the interaction between 5HTTLPR and perceived stress by plotting scores on the lifetime depression scale (z score transformed to facilitate interpretation of the effects) as a function of scores on the perceived stress scale and genotype. Figure 1 displays the results for the full sample. As can be seen, the s/s genotype group was the most susceptible to perceived stress. In comparison with the l/l or s/l genotypes, the s/s genotype group scored the lowest on depression when their perceived stress was the lowest, and they also scored the highest on depression when their perceived stress was the highest. The difference between the two groups was relatively moderate in magnitude. For example, respondents with the l/l or s/l genotype scored 0.75 standard deviations lower on the depression scale than respondents with the s/s genotype when perceived stress was 3 standard deviation units above the mean. Z tests (Paternoster, Brane, Mazero, & Piquero, 1998) confirmed that the difference in slopes between the two genotypes was statistically significant ($z = 2.21, p < .05$).

The graphical depiction of the interaction between 5HTTLPR and perceived stress in the prediction of depression for women is contained in Figure 2. The pattern of results is virtually identical to the one generated with the full sample. Once again, there is a crossover interaction where the s/s genotype is the most susceptible to perceived stress, and respondents who are homozygous for the s genotype tend to score the lowest on depression when perceived stress is low and the highest on depression when perceived stress is high. The magnitude of the effect is also relatively moderate in magnitude. For example, women with the l/l or s/l genotype scored 0.89 standard deviations lower on the depression scale than women with the s/s genotype when perceived stress was 3 standard deviation units above the mean. Z tests (Paternoster et al., 1998) confirmed that the difference in slopes between the two genotypes was statistically significant ($z = 2.63, p < .05$).

Last, we plotted the depression scores for men across different genotypes and scores on the perceived stress scale. Recall that

| Table 1. Results of OLS Regression Analyses Predicting Self-Reported Depression for the Full Sample and by Gender |
|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| **Full Sample**                                               | **Females**                                                  | **Males**                                                    |
|                                                              | $b$   | $\beta$ | $SE$ | $p$    | $b$   | $\beta$ | $SE$ | $p$    | $b$   | $\beta$ | $SE$ | $p$    |
| 5HTTLPR × Stress                                             | .23   | .15     | .07  | .001   | .29   | .18     | .09  | .002   | .13   | .09     | .09  | .151   |
| 5HTTLPR                                                     | -.68  | -.08    | .30  | .023   | -.92  | -.10    | .43  | .032   | -.33  | -.05    | .38  | .391   |
| Stress                                                      | .50   | .47     | .02  | <.001  | .29   | .50     | .03  | <.001  | .39   | .41     | .03  | <.001  |
| Race                                                        | .69   | .09     | .17  | <.001  | .55   | .07     | .23  | .018   | .88   | .13     | .23  | <.001  |
| $R^2$                                                        | .27   |         | .31  |        |       |         |      |        |       |         |      |        |
| $N$                                                         | 1,702 |         |      |        | 925   |         |      |        | 777   |         |      |        |

*Note. All models were estimated using Huber/White standard errors.*
the results generated from the OLS model did not reveal a significant interaction between 5HTTLPR and perceived stress in the prediction of depression. Nonetheless, it is important to note that Figure 3 indicates a trend that is similar to women, although the difference between the genotype groups is not statistically significant ($z = 1.00, p > .05$).

**Discussion**

There has been a significant amount of debate surrounding the degree to which an interaction between 5HTTLPR and stressful life events contributes to the development of depression and depressive symptomatology (Kaufman, Gelernter, Kaffman, Caspi, & Moffitt, 2010; Clark et al., 2010; Rutter, 2010; Rutter, Thapar, & Pickles, 2009). The results of more than 30 studies and of four meta-analyses and reviews have produced divergent results regarding this debate (Caspi et al., 2010; Clarke et al., 2010; Munafò et al., 2009; Risch et al., 2009; Uher & McGuffin, 2010). The goal of the current study was to add to this body of literature in two important ways. First, instead of assessing stress through a stressful life events scale, we measured stress through a perceived stress scale. Second, instead of measuring depression at one point in time, we created a longitudinal depression scale that was employed to measure stable depressive symptomatology. Analysis of data drawn from the National Longitudinal Study of Adolescent Health (Add Health) revealed a statistically significant interaction between 5HTTLPR and perceived stress in the prediction of lifetime depressive symptomatology for women. That the effects were demonstrated for women but not men is consistent with the sheer sex differences shown in the epidemiology of internalizing disorders (Kendler & Prescott, 2006, pp. 169–180)—differences that have an important neural basis (Hines, 2010). Future research is needed to unpack the underlying mechanisms that can explain how stress moderates the effect of 5HTTLPR and how 5HTTLPR moderates the effect of stress. Prior studies, however, have identified a number of different ways in which environments moderate genetic effects (Seabrook & Avison, 2010). These different models will prove useful in the elucidation of how and why there is an interaction between 5HTTLPR and stress in the creation of depressive symptomatology.

The results of the current study point to the potential importance of assessing stress with measures that capture perceived stress. Indexing stress with a simple raw count of the total number of stress life events that are encountered may not be the most reliable or valid way to measure individual levels of stress for three main reasons. First, there is evidence indicating that baseline levels of stress vary significantly across people (Pruessner, Hellhammer, Priessner, & Lupien, 2003), with some people experiencing relatively high levels of stress and others experiencing relatively low levels of stress. A stressful life events scale ignores these individual differences in stress reaction (Rhodewalt & Aguistsdottir, 1984). Second, people respond very differently to the same types of life stressors, such that an ostensibly life stressor may produce higher levels of stress in one person versus another, something which has been found for criminal victimization (DeLisi, Jones-Johnson, Johnson, & Hochstetler, 2010). A stressful life events scale is unable to capture this heterogeneity. Third, stressful life events scales typically do not...
include more mundane forms of stress, such as stress that emanates in daily activities at work, school, and home (Kanner et al., 1981). It is quite possible that these daily cumulative life stressors are even more critically involved in the pathogenesis of depression than are stressful yet episodic life events.

**Limitations**

The findings reported in this study should be interpreted with caution in light of at least four study limitations. First, although the Add Health data are nationally representative, the DNA subsample of the Add Health data is not. As a result, the degree to which the results reported here would generalize to all Americans or to citizens of other countries is an issue that needs to be addressed by future research. Second, the measures of perceived stress and the depression scales were all based on self-reports. The significant associations between these two measures, therefore, could be partially or fully because of shared methods variance. Third, the direction of the association between depression and the 5HTTLPR × perceived stress remains unknown. Recall that perceived stress was measured at Wave 4, whereas depression was a single phenotypic measure that was assessed through data gathered at Waves 1 through 4. Unfortunately, the Add Health data did not include measures of perceived stress at the previous waves, and thus we were forced to examine perceived stress through a measure at Wave 4. Fourth, the interaction between 5HTTLPR and perceived stress explained only a modest amount of variance in depressive symptomatology for women.

**Conclusions**

Gene–environment research has come under attack for the inconsistent results that are generated from replication studies (Risch et al., 2009). Nonreplication, however, does not necessarily mean that the original effect was a methodological artifact or simply a chance finding. Instead, nonreplication may have its roots in a range of methodological issues, including differences in the measurement of the environmental stressor or in the measurement of the phenotype of interest. To address these issues more directly, we also examined whether the 5HTTLPR × perceived stress interaction predicted variation in any of the wave-specific depression scales. The results of these supplementary analyses revealed that the interaction term was not a consistent predictor of the individual depression scales. The Add Health data did not include a measure of stressful life events, but it did include a very limited measure of early childhood maltreatment. As a result, we examined whether 5HTTLPR interacted with a childhood maltreatment scale to predict variation in the depressive symptomatology phenotype. The results of these models failed to detect a significant interaction. Against this backdrop, the current study tentatively suggests that differences in measurement strategies may contribute to some of the mixed results in regard to the literature examining the interaction between 5HTTLPR and stressful life events in the prediction of depression.

**Keywords:** adolescents; young adults; depression; perceived stress; gene–environment interaction; serotonin transporter gene

**References**


