Research Article

CHRONIC FAMILY STRESS INTERACTS WITH 5-HTTLPR TO PREDICT PROSPECTIVE DEPRESSIVE SYMPTOMS AMONG YOUTH

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Background: Previous research, predominantly with adults, has shown that the serotonin transporter gene (5-HTTLPR) interacts with stress (G × E) to predict depressive symptoms; however, few G × E studies have been conducted with youth using rigorous methods, particularly a prospective design and contextual interview to assess stress. This study examined the interaction between 5-HTTLPR and stress, both chronic and episodic, to predict longitudinal change in depressive symptoms among children and adolescents. Methods: A general community sample of youth (N = 200; 57% girls; mean age: 12.09 years old) was genotyped for 5-HTTLPR (rs 25531) at baseline. They were interviewed via contextual stress procedures to ascertain chronic family stress and episodic stressors and completed depressive symptoms questionnaires at baseline and 6 months later. Results: A significant G × E showed that chronic family stress predicted prospective increases in depressive symptoms over 6 months among youth possessing the high-risk S allele. This G × E was not found for episodic stressors occurring in the last 6 months. There was no moderation by sex or pubertal status. Conclusions: These findings advance knowledge on G × E effects in depression among youth. This is the first study to show that chronic family stress, but not episodic stressors, when ascertained by rigorous stress interview, interacts with 5-HTTLPR to prospectively predict depressive symptoms among children and adolescents. Depression and Anxiety 28:1074–1080, 2011. © 2011 Wiley Periodicals, Inc.

Key words: adolescents; children; depression; environment; genetics; serotonin

INTRODUCTION

Depression in children and adolescents is a serious and debilitating disorder.[1] Developmental epidemiological research clearly shows symptoms and episodes of depression increase markedly from childhood into adolescence,[2–4] and the sex difference in depression emerges in early to middle adolescence.[5] Understanding etiological processes contributing to the development of youth depression is crucial as most individuals experience their first depressive episode in adolescence,[5,6,7] and adolescent onset depression substantially increases risk for continuity and recurrence of depression into adulthood.[8] Although many vulnerabilities to depression exist and have been studied,[9] much attention has been given to the study of gene–environment interactions (G × E). Interest in how G × E confers risk to depression has surged since the seminal publication

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by Caspi et al.\textsuperscript{[10]} demonstrating that those who both experienced major negative events and carried at least one short allele of the serotonin transporter promoter gene (5-HTTLPR) experienced elevated depression (both symptoms and disorder) over time in early adulthood. Since then, numerous G \times E studies of 5-HTTLPR and various environmental risks among adults have been conducted, with a recent and comprehensive meta-analysis showing a robust, significant G \times E in adult depression.\textsuperscript{[11]}

Despite this recent meta-analysis demonstrating an overall significant G \times E in adult depression, several limitations and unaddressed questions remain. First, many but not all adult studies demonstrated a significant G \times E.\textsuperscript{[12]} Before Karg et al.\textsuperscript{[5]} larger, more comprehensive, and positive meta-analysis, Risch et al.'s nonsignificant meta-analysis prompted several commentaries that may underlie the equivocal G \times E findings. Several reviews have noted the inconsistencies in the G \times E literature may stem from the use of environmental stress measures with unknown psychometric properties.\textsuperscript{[14,15]} Indeed, Uher and McGuffin\textsuperscript{[14,15]} have demonstrated that studies utilizing more specific or interview-based measures of stress were significantly more likely to obtain G \times E effects in depression. Clearly, careful and rigorous measurement of environmental stress is necessary to accurately test G \times E influences.

Additionally, there has been considerably less research investigating G \times E in youth in contrast to the preponderance of adult G \times E research. Initial studies have found evidence supporting an interaction between genes implicated in the 5HT system and environmental stress, such as maltreatment,\textsuperscript{[16,17]} family environment,\textsuperscript{[18–20]} and general stressors.\textsuperscript{[18,22]} However, there are particular limitations to most youth G \times E studies. First, the majority of studies utilized cross-sectional designs (see\textsuperscript{[18,22]} for exceptions), which cannot tease apart the directionality of G \times E on depression. Second, most studies measured environmental stress with potentially subjective self-report stress checklists (see\textsuperscript{[18]} as an exception). Reviewers of stress measurement have strongly advocated for contextual interviews as the gold-standard method to ascertain negative events more objectively relative to self-report checklists.\textsuperscript{[23–25,15]} Other studies examined relatively infrequent or highly specific stressors (e.g., maltreatment\textsuperscript{[16,17]} or included stressors that may not be developmentally appropriate for younger populations (e.g., financial difficulties\textsuperscript{[21]}).

Recently, Hammen et al.\textsuperscript{[18]} found chronic family stress at age 15, but not acute stressors, interacted with 5-HTTLPR to predict depressive symptoms at age 20. This study importantly advanced knowledge in the G \times E literature, as it utilized a reliable and valid contextual stress interview to ascertain both chronic and acute family stress and evaluated which type of stress (i.e., chronic versus acute) interacted with 5-HTTLPR to predict depressive symptoms at age 20 in a representative sample. However, additional research is needed in order to both replicate these findings using contextual stress interview procedures assessing chronic and acute family stress in a G \times E framework to predict depression and to address important remaining questions. In particular, chronic family stress was measured at age 15, whereas acute stressors occurring between ages 15 and 19 were assessed at age 20. Furthermore, baseline levels of depressive symptoms at age 15 were not controlled in G \times E analyses to predict symptoms at age 20. Controlling for initial levels of symptoms is essential given the strong continuity of depressive symptoms over time.\textsuperscript{[26,27]} This leaves open the possibility that processes other than G \times E may have contributed to the prediction of depressive symptoms at age 20.

Finally, G \times E research with youth has predominantly sampled and studied adolescents. Only Kaufman et al.\textsuperscript{[15]} studied preadolescents (ages 5–15), yet they controlled age which precludes an examination of whether and how G \times E in depression changes across development. Given the clear developmental trends and surge in depression from childhood into adolescence, the lack of G \times E research in samples of youth across different developmentally salient ages is a notable gap, as it is unknown whether developmental processes, such as puberty, moderate expected G \times E effects in depression. Pubertal status has been implicated in behavioral genetic studies as a possible moderator of G \times E predicting depression in youth\textsuperscript{[28,29]}; however, this has not yet been examined in molecular genetic research.

This study aimed to extend G \times E research among youth using a longitudinal design controlling baseline depressive symptoms to enable prospective prediction of depressive symptoms as a function of 5-HTTLPR interacting with stress among a community sample of youth. Specifically, we examined whether chronic family stress and recent acute (episodic) stressors interacted with 5-HTTLPR to predict youth depressive symptoms. We hypothesized that youth carrying a S/L\textsubscript{G} allele (in an additive genetic framework) and experiencing high levels of chronic family stress would exhibit the greatest prospective increase in depressive symptoms over time. Additionally, given the emergence of the sex difference in depression in early adolescence and mixed findings pertaining to G \times E in girls versus boys (e.g., sex moderation in\textsuperscript{[18,20,21]}; no moderation in the remaining studies), as well as a limited developmental focus in prior research, pubertal status and sex were examined as possible moderators.

**MATERIALS AND METHODS**

**PARTICIPANTS**

Participants included 200 children and adolescents who were recruited from metropolitan Denver, Colorado, school districts. Youth had to currently be in third (age 7–9 years old), sixth (age 10–12 years old), or ninth (age 13–15 years old) grade. They were
excluded if they had a severe learning or psychiatric problem (e.g., autism, psychosis) that was likely to interfere with the completion of an extensive laboratory protocol. The participation rate was 72%, which is above the rate recommended for having a representative sample of the target population[30,31] (see[32] for sampling details).

The sample was approximately evenly divided by sex (males: 43%, females: 57%), grade (third grade: 31%, sixth grade: 38%, ninth grade: 32%) and by mixed ethnic origin (Caucasian: 67%, African American: 7%, Latino: 7%, Asian/Pacific Islander: 4%, Other/Mixed Race: 14%). Youth ranged in age from 7 to 16 years old (mean age = 12.09 years old, SD = 2.32).

PROCEDURES

Each eligible parent and youth visited the laboratory for the baseline assessment. Parents provided informed written consent for their participation and for their child; youth provided written assent. Depressive symptoms were evaluated with a questionnaire and DNA was collected via saliva at the baseline assessment. Baseline episodic and chronic family stress was also evaluated. Follow-up assessment evaluating depressive symptoms and episodic stressors occurred 6 months after the baseline visit (retention rate of 96%). The Institutional Review Board approved all procedures. Youth were reimbursed for their participation.

MEASURES

Depression. The Children’s Depression Inventory (CDI)[33] was used to assess depressive symptoms in youth at both baseline (Time 1) and at the 6-month follow-up (Time 2). The CDI is the most commonly used measure of depressive symptoms in youth and possesses good reliability and validity.[34] Internal consistency (α) was above .80 at both Time 1 and Time 2. The range of scores from this community sample (Time 1: M = 6.64, SD = 5.47, range 0–35; Time 2: M = 4.0; SD = 3.71; range: 0–20) were comparable to published norms[34] and prior research with general community samples.[35]

Chronic family stress. The youth version of the UCLA Chronic Stress Interview (CSI),[36] a semi-structured contextual stress interview, assessed youths’ ongoing stress. The CSI has demonstrated excellent reliability and validity.[36–38] For this study, the parent–child and household domains were used to create an index for chronic family stress. The parent–child domain assesses the quality of the relationship between parent and parent figures. The household domain assesses the quality of the youths’ relationship with others in the household (e.g., siblings, grandparents). Interviewers ascertained from youth the duration that the quality of the parent–child and household relationships had been as described. Severity and duration information on parent–child and household stress were presented to a team of three or more blind raters, who came to an agreed upon severity score on a scale from 1 (little/no stress) to 5 (severe stress) and chronicity score on a scale from 1 (less than 6 months) to 5 (5 years or more). Severity and chronicity ratings were recoded and multiplied to create a composite stress score that weighted each severity score by its duration (see[39] for details). The parent–child and household domains’ combined severity/chronicity scores were moderately correlated (r = .57, P < .001), so they were averaged together to form the chronic family stress score.

Episodic stress. Episodic stressors were evaluated with the UCLA Life Stress Interview (LSI).[40,41] The LSI utilizes the contextual threat method pioneered by Brown and Harris.[42] At Times 1 and 2, interviewers obtained information on occurrence and circumstances (e.g., objective changes) of episodic stressors in the preceding 6 months. Information on spontaneously discussed events and specifically probed stressors was presented to the stress rating team (see above). Raters came to a consensus on the severity score of the event using a five-point scale (1 = no impact to 5 = extremely severe impact). Episodic stress scores were obtained by summing severity scores across all events coded for the particular time point. Measurement of episodic stress via the LSI procedures is reliable and valid.[19,43]

RESULTS

PRELIMINARY ANALYSES

Means and standard deviations for all primary variables overall and separated by sex and puberty are presented in Table 1. Postpubertal youth had significantly more episodic stress than prepubertal youth; no other sex or puberty differences were noted. Table 2 shows Pearson correlations among all primary variables. 5-HTTLPR polymorphisms were in the Hardy–Weinberg equilibrium. Genotype frequencies for 5-HTTLPR were 24% L4 homozygotes, 48% heterozygotes, and 27% S/L homozygotes. Genotype did not vary significantly by race (χ² < 6.62).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Full sample</th>
<th>Girls</th>
<th>Boys</th>
<th>Prepubertal</th>
<th>Postpubertal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M(SD)</td>
<td>M(SD)</td>
<td>M(SD)</td>
<td>M(SD)</td>
<td>M(SD)</td>
</tr>
<tr>
<td>CDI</td>
<td>4.00(3.71)</td>
<td>4.04(4.02)</td>
<td>3.78(4.03)</td>
<td>3.87(4.02)</td>
<td>4.26(4.42)</td>
</tr>
<tr>
<td>LSI</td>
<td>2.75(3.71)</td>
<td>3.55(4.24)</td>
<td>2.79(2.96)</td>
<td>2.41(3.38)</td>
<td>3.54(4.29)</td>
</tr>
<tr>
<td>CSI</td>
<td>1.01(1.51)</td>
<td>1.05(1.47)</td>
<td>0.97(1.56)</td>
<td>0.97(1.49)</td>
<td>1.08(1.56)</td>
</tr>
</tbody>
</table>

Note: CDI, Children’s Depression Inventory at Time 2; LSI, Life Stress Interview at Time 2; CSI = Chronic stress Interview at Time 1. Episodic Stress scores at Time 2 were computed by summing severity ratings across stressors and range from 0 to 24; Chronic family stress scores at Time 1 are a composite of severity and chronicity ratings and range from 0 to 7.50.

Table 1. Descriptive statistics overall and by sex and pubertal status.

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over time. Given work questioning functionality of genetic model with youth possessing the S allele the L/L genotype group was not significant (but less steep slope for those with the S/L genotype the steepest slope for those with the S/S genotype above and below the mean. Post hoc analyses showed 5-HTTLPR (rs25531). This G E effect is shown in Table 2, which shows there was no significant gene–environment correlation (rGE) between 5-HTTLPR and chronic family stress. To test G × E, we first included both puberty (pre- and postpuberty) and sex as possible moderators of the chronic family stress by 5-HTTLPR interaction. Neither puberty \((b = .68, SE = 1.26, t = 0.54, P = .59)\) nor sex \((b = .38, SE = 0.60, t = 0.58, P = .56)\) nor the interaction between sex and puberty \((b = .39, SE = 1.45, t = -0.27, P = .79)\) moderated chronic family stress × 5-HTTLPR, so all higher order interactions were removed. Analyses (Table 3) revealed a significant interaction between chronic family stress and 5-HTTLPR (rs25531). This G × E effect is shown in Figure 1, with chronic family stress depicted at 1 SD above and below the mean. Post hoc analyses showed the steepest slope for those with the S/S genotype \((b = .98, SE = 0.35, t = 2.82, P = .005)\) and a significant but less steep slope for those with the S/L genotype \((b = .66, SE = 0.20, t = 3.27, P = .001)\). The slope for the L/L genotype group was not significant \((b = .34, SE = 0.37, t = 0.91, P = .37)\). This supports an additive genetic model with youth possessing the S allele exhibiting prospective elevations in depressive symptoms over time. Given work questioning functionality of rs25531[52] and many studies[10] utilizing the standard VNTR for 5-HTTLPR (i.e., coding for S and L versus S, L_{A}, and L_{C}), we also analyzed the data in this manner. This significant G × E was comparable when the standard VNTR for 5-HTTLPR × chronic family stress was analyzed \((b = .53)\). Last, the G × E effect was comparable when the largest ethnic group of only White youth was analyzed \((b = .53)\).

**Effect of chronic family stress.** Table 2 shows the interaction between chronic family stress and 5-HTTLPR (final statistics) Table 3. Prediction of depressive symptoms from genotype and chronic family stress (final statistics)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>(\Delta R^2)</th>
<th>(b (SE, t))</th>
<th>(\beta)</th>
<th>(t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDI Time 1</td>
<td>.31</td>
<td>.42(0.04)</td>
<td>.57</td>
<td>9.56**</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td>.02(0.13)</td>
<td>.01</td>
<td>0.13</td>
</tr>
<tr>
<td>Step 2</td>
<td></td>
<td>.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puberty</td>
<td></td>
<td>.01(0.50)</td>
<td>.001</td>
<td>0.02</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td>-.93(0.46)</td>
<td>-.12</td>
<td>-2.02</td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td></td>
<td>.29(0.37)</td>
<td>.05</td>
<td>0.90</td>
</tr>
<tr>
<td>Chronic family stress</td>
<td></td>
<td>-.03(0.30)</td>
<td>-.01</td>
<td>-0.11</td>
</tr>
<tr>
<td>Step 3</td>
<td></td>
<td>.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HTTLPR × Chronic Family Stress</td>
<td></td>
<td>.58(0.24)</td>
<td>.26</td>
<td>2.41*</td>
</tr>
</tbody>
</table>

***P < .001, *P = .02. Model \(R^2 = .39, F(7, 188) = 16.42, P < .001.\)

Figure 1. Interaction between 5-HTTLPR and chronic family stress predicting prospective elevations in depressive symptoms over time.
between episodic stress and 5-HTTLPR, so all higher order interactions were removed. Episodic stress in the last 6 months did not interact with 5-HTTLPR to predict prospective change in depressive symptoms ($b = -.01, SE = 0.09, t = -0.15, P = .88$). Likewise, there was no significant interaction between 5-HTTLPR and episodic stress when Time 1 episodic stress was not controlled ($b = -.06, SE = 0.10, t = -0.57, P = .57$). The $G \times E$ effect was comparable when 5-HTTLPR was analyzed with the standard VNTR method ($b = -.04$).

**DISCUSSION**

Recent meta-analytic research supports a $G \times E$ predicting depression,[11] yet there still remain several questions regarding $G \times E$ in depression, specifically of stress type using rigorous stress measurement, prospective prediction of symptoms among youth samples, and possible moderation by sex and pubertal status from childhood into adolescence. This study utilized a validated and developmentally appropriate measure of environmental stress, directly compared recent episodic stressors and chronic family stress, and sampled youth from the community across developmentally salient periods to test a $G \times E$ predicting prospective increases in depressive symptoms. Results demonstrated that chronic family stress, but not recent episodic stressors, predicted prospective elevations in depressive symptoms over 6 months among youth who possessed the S allele of the 5-HTTLPR gene (in an additive manner). This effect was found to be equivalent in both boys and girls and across pubertal development.

This study contributes to the $G \times E$ literature in depression and replicated Hammen et al.'s findings.[11] We directly examined the differential impact of chronic versus episodic stress in youth with a well-established assessment of stress (i.e., contextual stress interview), which has been validated in prior research[11,36] and allowed for more precise measurement and understanding of each participant’s life circumstances. This is the preferred approach to assessing stress and has shown stronger $G \times E$ effects in depression. Prior reviews of the $G \times E$ literature in depression noted concerns with appropriate measurement of environmental stress, which reduces sensitivity when testing $G \times E$.[15,53]

Our results are consistent with previous $G \times E$ findings in youth that used measures of chronic family stress.[16–18] Chronic social stress is a major risk factor for depression,[54] especially when experienced during susceptible developmental periods. Additionally, chronic stress may be a critical factor at the cellular level, as suggested by recent research reviewing the link between stress and epigenetics.[55] Chronic stress may contribute to epigenetic changes which may endure via altered cellular “memory.” Chronic stress has a marked impact on 5-HTT gene expression, especially in those possessing an S allele.[56,57] Although previous $G \times E$ studies have found significant results when measuring acute and/or recent stressors,[10,21,22] it is possible that these studies were, in fact, capturing chronic stress exposure given they did not explicitly ascertain and separately test chronic and acute stress. The use of a contextual stress interview in this study and Hammen et al.’s work enables more precise measurement and distinction between chronic and episodic stress as well as direct comparison of the two types of stress exposure.

Furthermore, this study explicitly sampled youth across different developmentally salient periods to begin to study whether the $G \times E$ was moderated by pubertal status. Most previous studies did not examine whether their findings differed across developmental periods. Results showed that pubertal status did not moderate the $G \times E$.

Also, there was no sex moderation of the $G \times E$ effect. Prior research has been mixed regarding sex moderation of $G \times E$ in youth.[16–18,20,21] One possible explanation for this discrepancy may be that the three studies finding sex moderation utilized samples of older adolescents or young adults.[16,18,20] Developmental epidemiological research shows that sex differences in depression emerge after age 13.[5,38] As this study’s average age was approximately 12 years old, this may not have been the optimal age range for investigating sex moderation in the context of $G \times E$ in youth.

There were various strengths and limitations to this study. In addition to the contextual stress interview to carefully ascertain important aspects of stress, the longitudinal design enabled a more stringent test of $G \times E$ in depression. We controlled initial levels of depressive symptoms that overlap with both stress and genetic risk to enable prediction of prospective elevations of depressive symptoms and establish temporal precedence of $G \times E$ predicting depressive symptoms. Finally, we examined $G \times E$ effects with a community sample of youth, which have been shown to be more generalizable and provide more accurate statistical tests compared with clinical samples.[59,60]

Limitations of this study provide opportunities and suggestions for future research. The relatively small sample size could have affected the ability to detect sex and puberty moderation of the $G \times E$ effect; therefore, future studies should aim to use larger sample sizes to examine these effects. Additionally, this study investigated elevations in depressive symptoms and not clinical depression. We assessed depressive symptoms given research demonstrating that subclinical depressive symptoms predict later disorder[61,62] and are on a continuum with clinical depression.[63] Nevertheless, utilizing diagnostic interviews in future research would clarify whether these findings would apply to clinical levels of depression. Although the longitudinal aspect of this study is a strength given that very few studies have examined the effects of $G \times E$ prospectively (see[18] and[22] for exceptions), future...
studies should examine change in depression over longer follow-up time frames, as this study’s timeframe was relatively short (6 months). As the youth were of mixed ethnic background, population stratification may be a concern. However, ethnicity was controlled in analyses providing a more rigorous statistical test of the G × E\(^4\) and the G × E effect was comparable among the subsample of White youth. Finally, although there was no evidence of an rGE between 5-HTTLPR and episodic or chronic family stress, future studies should investigate the possibility of other parent or youth rGE or perhaps even gene by gene interactions (G×G) that could be related to 5-HTTLPR and environmental stress.

In sum, this study demonstrated that youth possessing the S allele who experienced more chronic family stress exhibited greater increases in depressive symptoms over time. This G × E effect was not seen when utilizing episodic stressors occurring in the preceding 6 months as a measure of environment. These findings suggest that chronic exposure to family stress has a significant impact on the development of depressive symptoms, especially in youth at measured genetic risk.

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