Journal of Clinical Child & Adolescent Psychology

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/hcap20

Does 5HTTLPR Genotype Moderate the Association of Family Environment With Child Attention-Deficit Hyperactivity Disorder Symptomatology?

Alexis L. Elmore\textsuperscript{a}, Joel T. Nigg\textsuperscript{b}, Karen H. Friderici\textsuperscript{c}, Katherine Jernigan\textsuperscript{c} & Molly A. Nikolas\textsuperscript{a}

\textsuperscript{a} Department of Psychology, University of Iowa
\textsuperscript{b} Department of Psychiatry, Oregon Health and Science University
\textsuperscript{c} Department of Microbiology and Molecular Genetics, Michigan State University

Published online: 20 Jan 2015.

To cite this article: Alexis L. Elmore, Joel T. Nigg, Karen H. Friderici, Katherine Jernigan & Molly A. Nikolas (2015): Does 5HTTLPR Genotype Moderate the Association of Family Environment With Child Attention-Deficit Hyperactivity Disorder Symptomatology?, Journal of Clinical Child & Adolescent Psychology, DOI: 10.1080/15374416.2014.979935

To link to this article: http://dx.doi.org/10.1080/15374416.2014.979935

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the “Content”) contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions
Does 5HTTLPR Genotype Moderate the Association of Family Environment With Child Attention-Deficit Hyperactivity Disorder Symptomatology?

Alexis L. Elmore  
Department of Psychology, University of Iowa

Joel T. Nigg  
Department of Psychiatry, Oregon Health and Science University

Karen H. Friderici and Katherine Jernigan  
Department of Microbiology and Molecular Genetics, Michigan State University

Molly A. Nikolas  
Department of Psychology, University of Iowa

Problematic family dynamics are common among youth with attention-deficit hyperactivity disorder (ADHD). Multiple mechanisms, including diathesis-stress (vulnerability) and differential susceptibility Gene × Environment interaction effects (G × E), have been proposed to account for this association. G × E effects for ADHD were examined via interactions between a genetic marker hypothesized to influence sensitivity to the environment (the promoter polymorphism of the serotonin transporter gene −5HTTLPR) and family conflict and cohesion in predicting ADHD symptoms. There were 498 youth ages 6–17 years (251 ADHD, 213 non-ADHD) and their parents who completed a multi-stage, multi-informant assessment (including parent and youth reports on the Family Environment Scale), and saliva sample collection for genotyping. Linear regression analyses examined interactions between 5HTTLPR genotype and the Family Environment Scale scales of conflict and cohesion reported by parent and child. Criteria laid out by Roisman et al. (2012) were applied to evaluate diathesis stress versus differential susceptibility G × E mechanisms. Results demonstrated interactions between 5HTTLPR genotype and both conflict and cohesion in predicting inattentive but not hyperactivity-impulsivity. Both interactions were highly consistent with differential susceptibility models of G × E effects. 5HTTLPR genotype appeared to moderate the relationship between family conflict/cohesion and inattentive symptoms. Interactions highlight the role of 5HTTLPR genotype as a potential marker of environmental sensitivity and provide support for differential susceptibility models of G × E effects for ADHD.
difficulties related to their child’s functioning, including more stress, negative reactions to their children, and resorting to more maladaptive parenting methods than parents of non-ADHD youth (Ellis & Nigg, 2009; Lifford, Harold, & Thapar, 2008), whereas children with ADHD exhibit lower compliance, require greater caretaking, and engender increased parental stress and controlling or authoritarian parenting methods (Edwards, Barkley, Laneri, Fletcher, & Metevia, 2001; Lifford, Harold, & Thapar, 2008). Family functioning problems also tend to be amplified among youth with ADHD and comorbid symptom profiles (i.e., both ADHD and oppositional defiant disorder [ODD] or conduct disorder [CD]; Wymbs, Pelham, Molina, & Gnagy, 2008), although some research indicates that child ADHD symptoms are associated with parent-child conflict independent of comorbid externalizing behaviors (Wymbs et al., 2008). Past work regarding family processes and ADHD has focused largely on family conflict; consequently, less is known regarding the relationship between other (positive) aspects of family functioning and ADHD. However, prior work has suggested a protective effect of increased parental warmth in the development of comorbidity in children with ADHD (Boeldt et al., 2012), whereas decreases in ADHD symptoms lead to increased parental warmth and decreased negative parental responses (Edwards et al., 2001; Lifford et al., 2008).

The direction of effects for the association of ADHD and family functioning is unclear, with evidence supporting both that family functioning influences ADHD symptoms and that child’s ADHD symptoms influence family functioning (Ellis & Nigg, 2009; Nigg, Hinshaw, & Huang-Pollock, 2006). ADHD emergence is likely independent of parenting and may drive parenting effects to a large extent, whereas subsequent parenting difficulties may serve to maintain ADHD-related behavioral problems (Campbell, 2002; for a review, see Nigg et al., 2006). However, maladaptive parenting may primarily be involved in exacerbation of ADHD in terms of oppositional defiant and aggressive behaviors (Sonuga-Barke et al., 2013). Yet, child ADHD symptoms also continue to influence parental behaviors (Harold et al., 2013), suggesting a recursive relationship between child behavior and parenting over time.

At the same time, genetic effects are clearly involved in ADHD, and it is striking that little work has examined family functioning and ADHD in relation to genetic influences that may moderate this association. Genetic factors make large contributions to ADHD symptoms (Nikolas & Burt, 2010), play a role indirectly in shaping the family environment (Kendler & Baker, 2007), and influence parenting dimensions (Klahr & Burt, 2013). Thus, genetic and family environmental variables may operate synergistically, such that family environmental circumstances may differentially impact the development of ADHD symptoms based on child genetic factors (Nigg et al., 2010; Nikolas, Friderici, Waldman, Jernigan, & Nigg, 2010). Indeed, the potential importance of Gene × Environment interaction in shaping psychopathology is now widely appreciated, as exemplified in special sections and issues of major journals (e.g., Petrill, Bartlett, & Blair, 2013).

Given these findings, multiple gene–environment interplay mechanisms must be differentiated. Common genes may influence both family functioning and ADHD via gene–environment correlation effects (rGE; Reiss, 2005), which can emerge as the result of shared genes between parents and children (passive rGE) as well as from environmental reactions elicited by a child’s genetically influenced traits and behavior (evocative rGE). Furthermore, family functioning may shape ADHD behavior via Gene × Environment interaction effects (G × E), such that individual differences in genetic makeup may moderate vulnerability to environmental risk or protective factors (e.g., level of family conflict or cohesion; see Nigg et al., 2010; Rutter, Moffitt, & Caspi, 2006, for reviews). Recent research has implicated both rGE and G × E with regard to relationships between ADHD and family conflict (e.g., Burt, Krueger, McGue, & Iacono, 2003; Lifford, Harold, & Thapar, 2009) as well as between parental involvement and ADHD (Nikolas, Klump, & Burt, 2014), suggesting the need for simultaneous consideration of both types of gene–environment interplay mechanisms.

Recent theoretical and empirical work has further advanced conceptualizations of G × E effects. The traditional diathesis-stress model, also called a vulnerability model, posits that genetic or biological diatheses exert risk for psychopathology in the context of environmental stressors (Rende & Plomin, 1992). By contrast, the differential susceptibility hypothesis suggests that genotype confers a general susceptibility (i.e., malleability) to environmental influences for good or for ill, indicating that susceptible individuals are more sensitive to both positive and negative environmental conditions (Belsky & Pluess, 2009). Therefore, individuals with a particular genetic liability may be particularly susceptible to deleterious consequences of family conflict (resulting in increased ADHD symptoms) and, conversely, these same individuals may be particularly amenable to the benefits of a supportive family environment, resulting in better than normative outcome for ADHD symptoms (see Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007).

In clarifying these effects, considering specific genes as well as specific environments is advantageous. The promoter polymorphism of the serotonin transporter gene in particular has been posited to be a potential marker of environmental susceptibility (e.g., Drury...
et al., 2012; Karg, Burmeister, Shedden, & Sen, 2011; Kent et al., 2002), specifically a 44 base-pair restriction-fragment length polymorphism found within the promoter region of the serotonin transporter gene (5HTTLPR). The serotonin transporter is primarily responsible for removal of serotonin from the synaptic cleft during neurotransmission. The presence of one or two “short” (s) alleles at 5HTTLPR (relative to the “long,” or l, alleles) adversely impacts the transcriptional efficiency of the gene, resulting in decreased reuptake of serotonin (Greenberg et al., 1999; Lesch et al., 1996). In addition, an A > G substitution contained within one of the repeat sequences of 5HTTLPR results in an additional allelic variant (Lg) with similar transcriptional functioning to that of the “short” allele (Hu et al., 2006). Therefore, there are three allelic variants of 5HTTLPR of interest in investigations of this gene as a hypothesized susceptibility marker: La, Lg, and short, forming six genotypes (La/La, La/s, La/Lg, Lg/Lg, Lg/s, and s/s), which can be further categorized based upon their probable influence on the functionality of the transporter (high functioning: La/La; intermediate functioning: La/Lg, La/s; low functioning: Lg/Lg, Lg/s, s/s).

There is ample reason to suspect that interactions between serotonergic functioning and family environment may be relevant for understanding the etiology of child behavior problems. Chronic stressful environments have predicted decreased serotonergic responsibility within the central nervous system (Manuck et al., 2005). In addition, prior work with rhesus monkeys supports an explicit link between rh5-HTTLPR genotypes and decreased transcriptional efficiency of the promoter, resulting in decreased concentrations of serotonin in the central nervous system, but only for those monkeys raised in a deleterious environment (Bennett et al., 2002). Taken together, these findings suggest that youth with specific 5HTTLPR genotypes may similarly exhibit decreased serotonergic functioning within the central nervous system as a result of a stressful rearing environment characterized by increased family conflict (or decreased cohesion). Further, serotonergic dysregulation may impact development of the ventromedial prefrontal cortex (vmPFC) and its projections to areas of the limbic system purported to underlie successful emotional and behavioral regulation skills. Within a conflictual environment, serotonergic dysregulation could therefore lead to a failure to develop appropriate behavioral and emotion regulation skills, resulting in increased ADHD symptoms. By contrast, in a more cohesive family environment, this same serotonergic dysregulation could increase some youths’ responsiveness to environmental input, resulting in the development of more appropriate regulation strategies. Of importance, in both conflictual and cohesive environments, the increased malleability resulting from serotonergic dysregulation may therefore set the stage for the development of either maladaptive behavior (in the face of conflictual environments) or adaptive behavior (in the face of cohesive environments).

Prior G × E investigations for ADHD have noted interactions between 5HTTLPR genotype and a variety of adverse environmental experiences. Retz et al. (2008) found that the l/l genotype provided a protective effect for ADHD individuals in the context of adverse childhood environments, although the l/l genotype has also been associated with decreased sensitivity in low-risk environments characterized by increased positive maternal expressed emotion (Sonuga-Barke et al., 2009). By contrast, deficient levels of serotonin associated with the s/s homozygous genotype may result in increased sensitivity to stress (Karg et al., 2011). Belsky and Beaver (2011) found that the more plasticity alleles (e.g., s/s genotype of 5HTTLPR, among others) that ADHD males carried, the more susceptible they were to both supportive and unsupportive parental relationships. In addition, we previously reported interactions between 5HTTLPR genotype and interparental conflict in predicting ADHD symptoms (Nikolas et al., 2010).

Given that both the “long” and “short” alleles have been linked to increased ADHD symptoms within the context of adverse environmental circumstances, in addition to the hypothesized impact of differential susceptibility G × E effects on symptom outcomes, the role of 5HTTLPR in G × E effects on ADHD requires clarification. Specifically, careful evaluation of different functional 5HTTLPR genotype groups is needed within the context of analyses that can distinguish between diathesis-stress and differential susceptibility G × E mechanisms. Diathesis-stress and differential susceptibility both posit moderation between a given predictor and outcome but assign disparate theoretical meaning to the form of the moderator (i.e., in the case of differential susceptibility, the moderator is conceptualized as a mechanism of malleability, whereas for diathesis stress, the moderator is thought to be a mechanism of vulnerability). The mere presence of a statistically significant interaction cannot differentiate between these two different theoretical mechanisms. Instead, specific criteria, including quantitative metrics, have become crucial for distinguishing between diathesis-stress and differential susceptibility mechanisms (Roisman et al., 2012).

The present study therefore examined G × E effects involving 5HTTLPR genotype and family functioning in predicting ADHD symptom dimensions. Specifically, dimensions of family conflict as well as family cohesion were included in order to evaluate both diathesis-stress and differential susceptibility G × E mechanisms.
METHOD

Participants

Participants included 498 children and adolescents ages 6–17 years (M = 10.8 years, SD = 2.4 years, 55.0% male), including 205 sibling pairs and 88 singleton children from 293 families. Subjects were recruited for participation via mass mailings, public advertisements, and outreach to clinics in the area; multiple community-based recruitment methods were utilized in an effort to avoid biases associated with clinic-recruited samples. Written informed consent and informed assent were obtained from all participating parents and children, respectively. The current study received approval from the local Institutional Review Board.

Stage 1. There were 902 children of 762 parents screened via telephone to ascertain eligibility according to established exclusionary criteria, including physical handicap, non-native English speaking, history of intellectual disability, autistic disorder, prescription of non-stimulant psychiatric medication, and prescription of long-acting stimulant medications (e.g., atomoxetine, bupropion) to enable wash out for neuropsychological and cognitive assessments not reported on here (for a recent report, see Nikolas & Nigg, 2013).

Stage 2. There were 724 children from 588 families invited to complete the Stage 2 diagnostic assessment. Parents and teachers of participating children completed normative behavioral rating scales including the DSM-IV ADHD Rating Scale (DuPaul, Power, Anastopoulos, & Reid, 1998), the Conners’ (1997) Rating Scale, and the ADHD Rating Scale (DuPaul, Power, Anastopoulos, & Willcut, 2005). Exclusion criteria included intellectual disability (estimated full-scale IQ < 70), parent-reported head injury with a loss of consciousness, history of seizures as ascertained by parent report, autism spectrum disorders, and diagnostic-team-identified current major depressive episode, lifetime bipolar disorder, lifetime psychosis, or current substance abuse or dependence.

Measures

Family environment scale. Both participating children and their primary parent (most frequently the mother) completed the Family Environment Scale (FES; Moos & Moos, 2007). The FES consists of 90 statements requiring dichotomous responses (i.e., true or false). The FES comprises 10 subscales, each with nine items: Cohesion (e.g., “family members really help and support one another”), Expressiveness, Conflict (e.g., “we fight a lot in our family”), Independence, Achievement Orientation, Intellectual-Cultural Orientation, Active-Recreational Orientation, Moral-Religious Emphasis, Organization, and Control (Moos & Moos, 2007). Form R was used in the current study. Only the Cohesion and Conflict subscales of the FES

1 I standard deviation below the mean (or below standard score of 85) prompted consideration of potential learning disorder diagnosis by the diagnostic team (see next). According to this procedure, 17.5% of youth were classified as having a potential reading disorder, whereas 13.7% of parents reported a history of reading disorder on the KSADS-E.

Stage 3. A diagnostic team comprised of a board-certified child psychiatrist and a licensed child clinical psychologist examined data from KSADS-E, parent and teacher rating scales, IQ and achievement scores, interviewer notes and observations, and treatment history to implement a best estimate diagnostic procedure. Members of the diagnostic team independently reviewed files and assigned diagnostic opinions regarding ADHD status and comorbid disorders; in cases of disagreement, consensus was reached following discussion. Agreement rates were satisfactory (k > .80 for all diagnoses with base rate > 5%). All diagnoses were made in accordance with DSM-IV criteria. Therefore, to qualify for ADHD, youth were required to exhibit ADHD symptoms prior to 7 years of age, in at least two settings, and to have clinically significant impairment. Further, the ADHD diagnosis could not be better explained by another mental disorder. Youth carrying a current or previous diagnosis of ADHD-C were classified as Combined type for lifetime subtype diagnosis to account for diagnostic history (see Lahey, Pelham, Loney, Lee, & Willcut, 2005). Exclusion criteria included intellectual disability (estimated full-scale IQ < 70), parent-reported head injury with a loss of consciousness, history of seizures as ascertained by parent report, autism spectrum disorders, and diagnostic-team-identified current major depressive episode, lifetime bipolar disorder, lifetime psychosis, or current substance abuse or dependence.
were of interest here, because these scales assess the broad positive and negative domains of social functioning of the family unit. The Conflict subscale predicts reported frequency of disagreements within the family unit, whereas the Cohesion subscale is associated with measures of familial adjustment. Child report data were available for 491 youth, and parent report was available for 285 of 293 families (note that parents provided one set of ratings for both siblings in the family). Single-reporter internal consistency for both subscales was marginal (parent-reported cohesion: \( \alpha = .69 \); parent-reported conflict: \( \alpha = .73 \); child-reported cohesion: \( \alpha = .67 \); child-reported conflict: \( \alpha = .68 \)). The parent–child correlations were significant (conflict: \( p < .001 \); cohesion: \( p < .001 \)) though modest in size (\( r = .26 \) and \( r = .23 \), respectively), suggesting they might provide valuable convergent information. Therefore, to maximize information from parent and child reports on the FES and to improve internal consistency, mean composites of conflict and cohesion, respectively, were computed by averaging parent and child report. These composite ratings were retained for all subsequent analyses (composite cohesion: corrected \( \alpha = .82 \); composite conflict: corrected \( \alpha = .84 \); Nunnally, 1978). Creating mean composites of conflict and cohesion offered several important advantages. First, composite scores increased reliability to acceptable levels. Second, when removing variance in conflict and cohesion ratings due to informant (i.e., parent or child), a factor analysis indicated that a two-factor model, \( \chi^2(561) = 791.71 \) (CFI = .92, TLI = .91, RMSEA = .03), provided a superior fit to the data relative to one-factor model, \( \chi^2(562) = 1771.49 \) (CFI = .83, TLI = .82, RMSEA = .13; likelihood ratio: \( p < .05 \)).

**Genotyping.** Saliva DNA samples were requested from all participating and purified using a method described in Meulenbelt, Droog, Trommelen, Boomsma, and Slagboom (1995). The 44-bp promoter polymorphism of the serotonin transporter gene (5HTTLPR) and the rs25531 A > G polymorphism were genotyped as follows. The “short” and “long” alleles of 5HTTLPR were genotyped according to previous methodology (Lesch et al., 1996) with the following modifications to the primer sets (5'-GACTGAG CTGGACAACCACG-3' and 5'-GGTTTGCGCTC TGAATGCCA-3'). Genomic DNA (40–60 ng) was amplified using the Taq DNA Polymerase kit (Qiagen Inc., Valencia, CA), standard kit protocol, including 1.5 mM MgCl\(_2\), 0.2 mM dNTPs, and 0.7 \( \mu \)M primer. Polymerase chain reaction conditions consisted of an initial denaturing step at 95°C for 3 min, followed by 35 cycles of: 95°C denaturation for 30 s, 63°C annealing for 30 s, and an extension at 72°C for 45 s, followed by a final extension step of 4 min at 72°C. A portion of the amplified DNA was analyzed using a 2% agarose gel to determine the l/s alleles. The remainder of the amplification reaction was digested with MspI endonuclease (New England Biolabs, Ipswich, MA) and examined by 3% agarose gel electrophoresis. The final products were (340, 120, and 64 bp) for (La), (174, 166, 120, and 64 bp) for (Lg), and 484 bp (short).

Based on previous work (Barr et al., 2004; Hu et al., 2006; Nikolas et al., 2010), we assigned youth to one of three groups that described the functionality of their genotype. These include the high-functioning group (youth homozygous for the La allele, \( n = 128 \)), the intermediate-functioning group (youth with heterozygote genotypes La/Lg and La/short, \( n = 209 \)), and the low-functioning group (youth with two copies of the low-functioning Lg or short alleles, \( n = 117 \)).

**Data Analytic Strategy**

All symptom dimension variables were Blom-transformed to alleviate skew (skewness after transformation ranging from .21 to .47). All variables were standardized to comply with recommendations to center variables for interaction tests and facilitate interpretation. Tests of rGE were conducted using a multivariate analysis of variance with genotype group (i.e., low, intermediate, and high functioning) as the fixed factor to avoid artificial finding of G × E (Rutter et al., 2006). Specifically, associations between 5HTTLPR genotype group and FES conflict and cohesion were examined to rule out possible rGE effects (i.e., differences in conflict and/or cohesion across genotype groups would suggest that passive and/or evocative rGE may be operating, which can falsely emerge as G × E if not controlled).

The main tests of G × E effects were conducted using linear regression procedures. Familial correlations (siblings) were accounted for using the CLUSTER option in Mplus (Muthén & Muthén, 1998-2012). Independent variables included 5HTTLPR genotype group, FES average scale scores (conflict and cohesion), and interactions among these variables. Parent-reported and teacher-reported ADHD symptom dimensions were examined as dependent variables in separate models to evaluate cross-setting generalizability of effects. Given previously established links between comorbid externalizing behaviors and family functioning (e.g., Wymbs et al., 2008), additional follow-up analyses were conducted in which ODD symptoms were not covaried and in which ODD was examined as an outcome to evaluate specificity of G × E effects to ADHD versus more general association with externalizing behavior problems.

Given conflicting findings from past work regarding the “risk” associated with different 5HTTLPR genotypes, two orthogonal contrast codes were used in G × E
analyses. The first was a “linear” code, which coded genotypes such that increased “low-functioning” alleles (short or Lg) conferred increased risk. The second “non-linear” code was designed to capture findings suggesting that both high- and low-functioning 5HTTLPR genotypes confer risk for ADHD (e.g., Belsky & Beaver, 2011; Retz et al., 2008), specifying both functional homozygote genotypes as higher risk relative to those with heterozygote genotypes. The main effects and interactions associated with both 5HTTLPR genotype codes were examined simultaneously in each model. Gender, age, ODD symptoms, and race were covaried in all analyses. Simple slopes analyses were used to clarify all significant interactions.

Comparison of Interaction Models

The recommendations of Roisman et al. (2012) were then applied to evaluate whether significant interactions between family functioning variables and 5HTTLPR genotype constituted differential susceptibility or diathesis-stress interactions (although see Belsky et al., 2007; Belsky & Pluess, 2009 for alternative criteria for evaluating G × E interactions). Both differential susceptibility and diathesis stress involve proposed moderations between predictor and outcome variables, in which the moderator is the intended mechanism of malleability (differential susceptibility) or vulnerability (diathesis stress), thus necessitating further analysis of interactions to differentiate between these two theoretical outcomes. These criteria require calculation of several metrics, including the Region of Significance (RoS) on X, the Proportion of Interaction (PoI), and the Proportion Affected (PA) index. The RoS on X, where X denotes the predictor variable (here, either family conflict or cohesion), yields upper and lower bounds of the values of the predictor for which different values of the proposed moderator (here, 5HTTLPR genotype group) result in significant differences in the outcome variable of interest (inattention or hyperactivity-impulsivity). When 5HTTLPR genotype and ADHD symptom dimension are significantly related at both high (2 or more SD above the mean) and low (2 or more SD below the mean) levels of FES conflict or cohesion, this provides strong evidence in favor of differential susceptibility.

The PoI denotes the proportion of the upper and lower interaction regions to each side of the crossover point (i.e., the point at which the two interaction lines intersect in a graphical depiction) attributable to differential susceptibility and provides an indication of interaction type that is largely independent of sample size. Both diathesis stress and differential susceptibility models predict that genetically vulnerable (or malleable) individuals will exhibit worse than average outcomes in negative environments. However, differential susceptibility also predicts that malleable individuals will exhibit better than average outcomes in positive environments. Therefore, because the PoI represents the proportion of upper and lower interaction regions, a PoI approaching 0.00 is highly consistent with diathesis-stress, whereas a PoI between 0.40 and 0.60 provides strong evidence for differential susceptibility. Last, the PA index denotes the proportion of the population that should be differentially impacted by the predictor variable (X). A PA index of approximately 0.50 (indicating that 50% of the population should be differentially effected by the predictor) indicates a crossover point for the interaction at the mean value of the predictor and is highly consistent with a strong interpretation of differential susceptibility. All of the aforementioned indices are considered together in determining whether a given interaction most closely resembles differential susceptibility or diathesis-stress (Roisman et al., 2012).

RESULTS

Demographic and descriptive statistics for the sample are presented in Table 1. Examination of group differences indicated that diagnostic procedures were effective in discriminating ADHD from non-ADHD youth. Children with ADHD exhibited more inattentive (p < .001, d = 3.15) and hyperactive-impulsive symptoms (p < .001, d = 1.56) based on KSADS-E parent report. As expected, more children with ADHD also met criteria for current ODD compared to children without ADHD (44 vs. 16.6%, p < .001, d = .62). The ADHD group comprised significantly more males (consistent with population effects; p < .001) and was somewhat younger (p = .008) than the non-ADHD group. As noted, sex, age, and ODD symptoms were included as covariates in all G × E analyses (with the exception of some follow-up analyses, detailed next). Whereas differences between the groups in terms of race were trivial (ps ranging from .325 to .548), race was included as a covariate in G × E analysis given the potential for population stratification effects in case-control genetic studies (Cardon & Palmer, 2003).

FES Conflict and Cohesion

Consistent with past work, families of ADHD children reported more conflict (p = .004, d = .26; range = 0–9) and lower cohesion (p < .001, d = −.47; range = 1.5–9) compared to families of children without ADHD (cohesion: range = .5–9; conflict: range = 0–9). Cohesion did not differ by 5HTTLPR genotype group (p = .308), indicating that 5HTTLPR genotype did not produce significant differences in family cohesion, helping to rule
TABLE 1
Descriptive and Demographic Statistics

<table>
<thead>
<tr>
<th>N</th>
<th>Control 213</th>
<th>ADHD 251</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Male</td>
<td>42.9</td>
<td>66.9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>% Caucasian</td>
<td>74.5</td>
<td>72.1</td>
<td>.548</td>
</tr>
<tr>
<td>% African American</td>
<td>9.7</td>
<td>8.0</td>
<td>.492</td>
</tr>
<tr>
<td>% Latino</td>
<td>4.0</td>
<td>6.0</td>
<td>.325</td>
</tr>
<tr>
<td>% Mixed/Biracial</td>
<td>10.1</td>
<td>12.4</td>
<td>.431</td>
</tr>
<tr>
<td>Age (SD)</td>
<td>11.04 (2.37)</td>
<td>10.49 (2.28)</td>
<td>.008</td>
</tr>
<tr>
<td>Income*</td>
<td>76.66 (45.90)</td>
<td>64.49 (38.72)</td>
<td>.005</td>
</tr>
<tr>
<td>% Stimulant Medication</td>
<td>2.0</td>
<td>37.2</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Diagnostics

- Inattention Symptoms (SD): 1.18 (1.98) 7.23 (1.86) <.001
- Hyperactive Symptoms (SD): .79 (1.48) 4.32 (2.85) <.001
- % ODD (Current): 16.6 44.0 <.001
- % CD (Current): .40 4.4 .004
- ODD Symptoms (Current): .82 (1.43) 2.42 (2.33) <.001
- CD Symptoms (Current): .06 (.25) .29 (.66) <.001
- ADHD Rating Scale Parent Report Sum Score: 6.66 (7.53) 26.39 (10.20) <.001
- ADHD Rating Scale Parent Report Inattention Problems: 4.16 (4.81) 16.55 (5.81) <.001
- ADHD Rating Scale Parent Report Hyperactivity Problems: 2.54 (3.58) 9.99 (6.46) <.001
- ADHD Rating Scale Teacher Report Sum Score: 6.12 (8.76) 22.20 (12.61) <.001
- ADHD Rating Scale Teacher Report Inattention Problems: 3.89 (5.33) 14.02 (7.08) <.001
- ADHD Rating Scale Teacher Report Hyperactivity Problems: 6.12 (8.76) 22.20 (12.61) <.001

FES Scales

- FES Cohesion: 7.09 (1.50) 6.34 (1.68) <.001
- FES Conflict: 3.00 (1.72) 3.48 (1.90) .004

Note: Values reflect mean and standard deviation of key variables. ADHD = attention-deficit hyperactivity disorder; ODD = oppositional defiant disorder; CD = conduct disorder; FES = Family Environment Scale.

*Income reported in thousands.

Tests of G × E Effects With Conflict and Cohesion

Linear regression models were used to assess the main effects of 5HTTLPR genotype, family conflict and cohesion, and their interaction in predicting ADHD symptom dimensions of inattention and hyperactivity-impulsivity (parent and teacher report separately). Quadratic terms and interactions (e.g., conflict², genotype × conflict) were included in all models to capture any nonlinear effects between variables of interest. No higher order terms proved significant; these terms were therefore removed from the model, indicating that the effects discussed next are linear in nature. Unstandardized and standardized regression weights, standard errors, and p values are reported in Table 2.

Analyses revealed a significant interaction between family cohesion and 5HTTLPR genotype (β = −.127, p = .002, AR² = .015) when predicting parent-reported inattention. Simple slope examination indicated that for individuals with the low-functioning genotype (Lg, Lg, Lg/s, s/s), cohesion was negatively related to inattention (β = −.461, p < .001). However, the relationship between cohesion and inattention was not significant for those with intermediate-functioning (β = −.117, p = .081) and high-functioning (β = −.093, p = .287) genotypes. The strength of the relationship between cohesion and inattention also appeared to strengthen with additional copies of 5HTTLPR low-functioning alleles (see Figure 1). However, significant G × E interaction effects for cohesion were not observed when predicting hyperactivity-impulsivity (AR² = .214, all ps > .432; see Table 2).

The interaction between 5HTTLPR genotype and conflict likewise predicted parent-reported inattention (β = .090, p = .017, AR² = .008). Examination of simple slopes indicated that for the low-functioning (Lg/Lg, Lg/s, s/s; β = .309, p = .001) genotype group, conflict
significantly predicted higher levels of inattention. However, for the high-functioning (La/La; \( \beta = .072, p = .398 \)) and intermediate functioning (La/s, La/Lg; \( \beta = .123, p = .072 \)) 5HTTLPR genotype groups, conflict did not significantly predict inattention. Of importance, the strength of this relationship appeared to increase for youth with two copies of the low-functioning alleles relative to those with just one copy, creating a dose-response effect (i.e., the relationship between conflict and inattention became increasingly stronger with more 5HTTLPR low-functioning alleles; see Figure 2). Notably, the overall pattern of results emerging for both conflict and cohesion in predicting parent-reported inattention were highly similar. Significant G \( \times \) E interaction effects for conflict did not emerge when predicting hyperactivity-impulsivity (all \( ps > .432 \); see Table 2).

### Teacher Report

Next, we examined whether interactions remained significant when examining teacher report. No significant G \( \times \) E effects emerged when examining teacher reports of ADHD symptoms (all \( ps > .108 \), see Table 2).

### Follow-Up Analyses

Follow-up analyses examining ODD symptoms as the dependent variable (while covarying ADHD symptoms) did not reveal a significant interaction between 5HTTLPR and cohesion or conflict when predicting parent-reported ODD symptoms (all \( ps > .144 \)), suggesting that differential susceptibility G \( \times \) E effects may be somewhat specific to ADHD. In line with this, removal of parent-reported ADHD symptoms from the model resulted in a significant interaction between 5HTTLPR genotype and cohesion (\( \beta = -.093, p = .016 \)). Additional analyses were also conducted without covarying parent-reported ODD symptoms to evaluate the impact of comorbid externalizing behaviors on constructs of interest. The majority of results did not change appreciably upon exclusion of ODD symptoms from the regression models.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>( b )</th>
<th>( SE )</th>
<th>( \beta )</th>
<th>( SE )</th>
<th>( p )</th>
<th>Total ( R^2 ) (( \Delta R^2 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parent-Report Inattention</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conflict</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear ( \times ) Conflict</td>
<td>.116</td>
<td>.048</td>
<td>.090</td>
<td>.038</td>
<td>.017</td>
<td>.192 (.008)</td>
</tr>
<tr>
<td>Nonlinear ( \times ) Conflict</td>
<td>-.001</td>
<td>.026</td>
<td>-.001</td>
<td>.044</td>
<td>.981</td>
<td>.192 (.000)</td>
</tr>
<tr>
<td>Cohesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear ( \times ) Cohesion</td>
<td>-.166</td>
<td>.052</td>
<td>-.127</td>
<td>.041</td>
<td>.002</td>
<td>.208 (.015)</td>
</tr>
<tr>
<td>Nonlinear ( \times ) Cohesion</td>
<td>.021</td>
<td>.027</td>
<td>.036</td>
<td>.046</td>
<td>.438</td>
<td>.208 (.001)</td>
</tr>
<tr>
<td><strong>Parent-Report Hyperactivity-Impulsivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conflict</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear ( \times ) Conflict</td>
<td>.028</td>
<td>.052</td>
<td>.022</td>
<td>.040</td>
<td>.584</td>
<td>.207 (.001)</td>
</tr>
<tr>
<td>Nonlinear ( \times ) Conflict</td>
<td>-.011</td>
<td>.027</td>
<td>-.017</td>
<td>.045</td>
<td>.699</td>
<td>.207 (.000)</td>
</tr>
<tr>
<td>Cohesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear ( \times ) Cohesion</td>
<td>-.037</td>
<td>.047</td>
<td>-.028</td>
<td>.036</td>
<td>.432</td>
<td>.214 (.001)</td>
</tr>
<tr>
<td>Nonlinear ( \times ) Cohesion</td>
<td>.011</td>
<td>.027</td>
<td>.018</td>
<td>.047</td>
<td>.694</td>
<td>.214 (.000)</td>
</tr>
<tr>
<td><strong>Teacher-Report Inattention</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conflict</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear ( \times ) Conflict</td>
<td>.038</td>
<td>.058</td>
<td>.030</td>
<td>.046</td>
<td>.512</td>
<td>.107 (.001)</td>
</tr>
<tr>
<td>Nonlinear ( \times ) Conflict</td>
<td>.045</td>
<td>.028</td>
<td>.075</td>
<td>.046</td>
<td>.108</td>
<td>.107 (.005)</td>
</tr>
<tr>
<td>Cohesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear ( \times ) Cohesion</td>
<td>-.011</td>
<td>.058</td>
<td>-.008</td>
<td>.045</td>
<td>.854</td>
<td>.114 (.000)</td>
</tr>
<tr>
<td>Nonlinear ( \times ) Cohesion</td>
<td>-.031</td>
<td>.028</td>
<td>-.053</td>
<td>.047</td>
<td>.265</td>
<td>.114 (.002)</td>
</tr>
<tr>
<td><strong>Teacher-Report Hyperactivity-Impulsivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conflict</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear ( \times ) Conflict</td>
<td>.019</td>
<td>.052</td>
<td>.015</td>
<td>.042</td>
<td>.712</td>
<td>.184 (.000)</td>
</tr>
<tr>
<td>Nonlinear ( \times ) Conflict</td>
<td>.010</td>
<td>.025</td>
<td>.017</td>
<td>.041</td>
<td>.682</td>
<td>.184 (.000)</td>
</tr>
<tr>
<td>Cohesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear ( \times ) Cohesion</td>
<td>.043</td>
<td>.049</td>
<td>.035</td>
<td>.039</td>
<td>.377</td>
<td>.181 (.002)</td>
</tr>
<tr>
<td>Nonlinear ( \times ) Cohesion</td>
<td>-.019</td>
<td>.024</td>
<td>-.033</td>
<td>.042</td>
<td>.434</td>
<td>.181 (.001)</td>
</tr>
</tbody>
</table>

*Note:* Parameter estimates, standard errors, and \( p \) values are from linear regression analyses. In the interest of space, only interaction terms are reported. The linear 5HTTLPR code specified that increased numbers of short or Lg alleles conferred the greatest sensitivity to the environment (high functioning = 1, intermediate functioning = 0, low functioning = 1). The nonlinear code specifies that both high and low functioning genotypes are associated with risk for ADHD and increased sensitivity to the environment (high functioning = 1, intermediate functioning = 2, low functioning = -1).
Diathesis-Stress Versus Differential Susceptibility

Next, recommendations for quantifying interactions were applied to determine whether the interactions between conflict and cohesion and 5HTTLPR emerging in parent-rated data more closely resembled a differential susceptibility or diathesis-stress interaction (Roisman et al., 2012). For both interactions, the RoS on X, where X is defined as either conflict or cohesion, was calculated to yield upper and lower bounds of the values of these variables for which the genotype groups result in significant differences in inattention. Analyses revealed that for standardized values of conflict below \(-.421\) and above \(.428\), the 5HTTLPR genotype groups are significantly different from one another with respect to their inattention score (but not for values within these boundaries; see Figure 1). The PoI was calculated to be \(.51\), providing evidence for differential susceptibility. The PA index with respect to conflict of \(.505\) is also consistent with differential susceptibility, indicating that approximately 50.5% of the population should be differentially impacted by conflict, depending upon their 5HTTLPR genotype.

Similarly, analyses also indicate that for standardized values of cohesion below \(-.390\) and above \(.027\), the 5HTTLPR genotype groups are significantly different from one another on parent-reported inattention. The PoI for this interaction was \(.59\) and the PA index with respect to cohesion was \(.571\), indicating that approximately 57.1% of the population will be differentially impacted by family cohesion according to 5HTTLPR genotype. Both the PoI and PA indices provide optimal evidence for differential susceptibility. Further, taken together, the RoS, PoI, and PA all provide evidence in favor of the conclusion that differential susceptibility is operative in the relationship between both conflict and cohesion and 5HTTLPR with regard to inattention.

DISCUSSION

The present study investigated the hypothesis that youth with differing 5HTTLPR genotypes would be differentially susceptible to divergent family environments as indexed by FES cohesion and conflict. Significant interactions emerged involving family conflict and cohesion and 5HTTLPR in predicting parent-reported inattention, such that conflict predicted **increased** inattention scores and cohesion predicted **decreased** inattention.
scores specifically for individuals with low-functioning 5HTTLPR alleles. Further, interactions with the broad positive and negative dimensions of family functioning (i.e., cohesion and conflict, respectively) were clearly suggestive of differential susceptibility, according to the quantitative criteria proposed by Roisman et al. (2012).

Significant interactions between family functioning dimensions and 5HTTLPR genotype in predicting parent-reported inattentive symptoms did not replicate when examining teacher-reported inattentive symptoms. However, multiple factors may have contributed to this discrepancy. Cross-informant correlations, whereas moderate (parent- and teacher-reported inattention: \( r = .59 \); parent- and teacher-reported hyperactivity-impulsivity: \( r = .58 \)), were far short of unity. In addition, the wide age range of youth included in the current sample (6–17 years) may have served to increase differences between parent and teacher report of ADHD symptom dimensions. For example, teachers are more or less involved with students depending on children’s stage of education, such that teachers of younger children (e.g., 6–7 years) may possess more comprehensive knowledge of students’ ADHD symptoms compared to teachers of older children (e.g., 16–17 years) as a result of more extensive interaction. This difficulty may be especially pronounced with regard to inattentive symptoms, which may be less evident to teachers when interacting with students for relatively brief periods in restricted contexts. In the current sample, correlations between parent and teacher reports of both inattention and hyperactivity-impulsivity vary by age, with stronger cross-informant correlations for younger children (6–12 years; parent- and teacher-reported inattention: \( r = .62 \); parent- and teacher-reported hyperactivity-impulsivity: \( r = .60 \)) than for older children (12–17 years; parent and teacher reported inattention: \( r = .47 \); parent- and teacher-reported hyperactivity-impulsivity: \( r = .32 \)). More nuanced examination of age effects on interactions among 5HTTLPR genotype and family functioning variables is warranted in large samples including a similarly wide age range of youth or, alternatively, in large samples comprising youth with more restricted age ranges. An alternative explanation of the current lack of replication relates to the consistency and reliability of behavioral symptoms of ADHD, such that ratings of behavioral symptoms may not provide a stable outcome for examination of \( G \times E \) effects.

**FIGURE 2** Significant interaction between conflict and 5HTTLPR in predicting parent-reported inattention. *Note:* The reference lines denote the upper and lower bounds of the Region of Significance on conflict, indicating the upper and lower bounds for the values of conflict for which the genotype groups result in significant differences in inattention. For values of conflict below –.421 and above .428, 5HTTLPR genotype and inattention are significantly related. (Family Environment Scale [FES] conflict represents the unstandardized residuals retained after regressing conflict onto 5HTTLPR genotype to eliminate gene-environment correlation effects.)
Examination of functional impairment stemming from ADHD symptoms may provide an outcome that is more stable across time and contexts, suggesting that future work incorporating functional impairment outcomes may prove valuable.

The current study adds to the growing body of literature suggesting that 5HTTLPR may be one genetic marker indexing sensitivity to various environmental contexts. Previous work investigating interactions between 5HTTLPR and family environment has suggested that individuals with the low-functioning 5HTTLPR genotype (i.e., s/s) exhibit increased malleability in the presence of either adverse (e.g., Bakermans-Kranenburg, Dobrova-Krol, & van Ijzendoorn, 2011) or improved (e.g., Drury et al., 2012) environmental circumstance. Current findings support previously established associations between the homozygous short genotype (s/s) and more pronounced adverse reactions to negative environments (e.g., Karg et al., 2011; Nikolas et al., 2010). Nikolas et al. (2010) found that children with both low-functioning (Lg/Lg, s/s, Lg/s) and high-functioning (La/La) genotypes exhibited greater malleability to environmental influences (conceptualized as interactions between ADHD symptom dimensions and youth ratings of self-blame related to interparental conflict according to genotype), whereas youth with intermediate genotypes evinced an absence of plasticity (Nikolas et al., 2010), a potential example of heterozygote advantage. A similar pattern of findings emerged in the current work among ADHD youth with low-functioning genotypes exhibiting differing levels of inattention symptoms according to the level of conflict and cohesion.

The current results highlighted interactions between 5HTTLPR genotype and family functioning in predicting inattention but not hyperactivity-impulsivity. Of importance, this pattern held when excluding ODD symptoms as a covariate in follow-up G × E analyses. Prior research has supported a connection between family conflict or disorganization and child psychopathology, including inattention, hyperactivity-impulsivity, depression, and conduct problems (George, Herman, & Ostrander, 2006). In the current study, we found associations between both family conflict and cohesion when predicting parent-reported inattention symptoms, even when controlling for comorbid disruptive behavior problems. The specific interactive effect predicting inattention may reflect the notion that maladaptive family functioning may serve to exacerbate youth difficulties with self-regulation, broadly, including behavioral and emotion regulation as well as cognitive control (Nigg et al., 2006). Statistically, our inclusion of all ADHD subtypes/presentations may have also impacted findings, in that the overall predictable variance was higher for inattention than for hyperactivity-impulsivity in the current sample. Future research examining specificity of effects to each symptom dimension is needed to further tease apart these possibilities.

The current work has several limitations. Only a single candidate gene was considered, although other genes that have previously been associated with ADHD (e.g., DRD4, see Martel et al., 2011) may be important to consider in future investigations of ADHD and family environment. Only child genotype was examined in relation to the environmental variables of interest here, such that future work incorporating both parent and child genetic information will be important in quantifying G × E and rGE as they relate to family functioning. The current data are also cross-sectional in nature, thus limiting the ability to posit causal relationships among the variables of interest here. Additional work examining differential susceptibility G × E effects for ADHD and family environment within a prospective longitudinal framework is necessary. Covarying for race in all analyses and examining frequencies of 5HTTLPR genotypes by ethnic group addresses population stratification (but see Cardon & Palmer, 2003), although other concerns relevant to case control designs (e.g., complexity of the phenotype of interest) may still be relevant, highlighting the importance of replication of the current results. In addition, future work would benefit from specific examination of G × E interactions involving family cohesion and 5HTTLPR genotype to further elucidate potential mechanisms.

The measure of family environment employed here is limited and likely does not assess other important aspects of family environment (i.e., multidimensional factors capturing complex interplay among positive and negative components of family dynamics), such that future efforts to improve extant measures of environmental factors are required for more precise assessment of G × E effects for child psychopathology. Combining across informants with respect to the FES may have masked important differences between parent and child report of the family environment. Although follow-up analyses examining parent and child report separately partially address this limitation, reporter-specific effects constitute an important domain for future research. The current study was not explicitly designed to test differential susceptibility models of G × E effects, such that examination of positive outcomes was somewhat limited (i.e., absence of ADHD symptoms), though the combined use of FES cohesion (indexing positive family environment) and FES conflict (indexing negative family environment) allowed examination of primary aspects of the differential susceptibility distribution.

Despite these limitations, we found compelling evidence of differential susceptibility G × E effects for ADHD, such that the relationship between both positive and negative family environments and ADHD
symptoms varied across youth based on 5HTTLPR genotype. The current findings have potential clinical significance in that youth with 5HTTLPR genotypes conferring malleability may reap greater benefits from treatment efforts targeted at improving the family environment in ways that promote adjustment and adaptation. Moreover, future work examining treatment effects may benefit by considering etiological factors, including genetic influences, while tests of these hypotheses could be incorporated into treatment settings to evaluate differential benefits experienced by youth with malleable genotypes.

ACKNOWLEDGMENTS

We thank all of the participating children and their families for making this work possible. The authors declare they have no conflict of interest.

FUNDING

We acknowledge the National Institute of Mental Health (MH070004-01A2 to K. H. Friderici, Michigan State University) for funding this research.

REFERENCES


---

[Downloaded by [University of Iowa Libraries] at 10:30 16 June 2015]
transporter gene influences susceptibility to attention deficit hyperactivity disorder (ADHD): Analysis and pooled analysis. Molecular Psychiatry, 7, 908–912. doi:10.1038/sj.mp.4001100