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Gene × Environment effects of serotonin transporter, dopamine receptor D4, and monoamine oxidase A genes with contextual and parenting risk factors on symptoms of oppositional defiant disorder, anxiety, and depression in a community sample of 4-year-old children

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Abstract

Genetic factors can play a key role in the multiple level of analyses approach to understanding the development of child psychopathology. The present study examined gene–environment correlations and Gene × Environment interactions for polymorphisms of three target genes, the serotonin transporter gene, the D4 dopamine receptor gene, and the monoamine oxidase A gene in relation to symptoms of anxiety, depression, and oppositional behavior. Saliva samples were collected from 175 non-Hispanic White, 4-year-old children. Psychosocial risk factors included socioeconomic status, life stress, caretaker depression, parental support, hostility, and scaffolding skills. In comparison with the short forms (s/s, s/l) of the serotonin transporter linked polymorphic repeat, the long form (l/l) was associated with greater increases in symptoms of oppositional defiant disorder in interaction with family stress and with greater increases in symptoms of child depression and anxiety in interaction with caretaker depression, family conflict, and socioeconomic status. In boys, low-activity monoamine oxidase A gene was associated with increases in child anxiety and depression in interaction with caretaker depression, hostility, family conflict, and family stress. The results highlight the important of gene–environment interplay in the development of symptoms of child psychopathology in young children.

A key aspect of the developmental psychopathology approach to the study of child disorders is the concept that psychopathology develops through the interactions of risk factors and psychological processes extending across multiple levels, from genetics to larger societal systems (Masten, 2006), a perspective known as a "multiple levels of analysis" approach (Cicchetti & Blender, 2006; Cicchetti & Curtis, 2007). Examining the role that genetic factors can play in the development of child psychopathology is an important aspect of this approach. Genetic factors may play a role in sequential processes known as developmental cascades, sequences of events in which interactions and transactions between specific variables, within and across domains or levels (e.g., genetic, physiological, cognitive, etc.) of functioning, have a cumulative effect on the developing organism (Masten & Cicchetti, 2010). The direction of these effects may vary, with some effects being direct and unidirectional, whereas others are bidirectional or indirect. The cumulative effect of these cascading processes can be significant. Explicating these cascading processes may help elucidate where prevention or intervention is needed to have maximum impact on altering a negative cascade or promoting a positive one.

Genetic factors may initiate the cascade process in multiple ways, by operating across levels and domains. Through their effects on early childhood temperamental and cognitive characteristics, genes may serve as early control parameters (Cox, Mills-Koonce, Propper, & Gariepy, 2010), influencing how infants and young children experience their surroundings as well as eliciting differences in parenting (evocative gene/environment correlations). Polymorphic or epigenetic variations also might lead to individual differences in the impact of environmental factors (Gene × Environment [G × E] interactions). As described more specifically below, certain allelic variations might potentiate the impact of risk factors in various domains or have a protective function. These genetic effects, operating in conjunction with contextual, family, parent, and child factors across domains and functional levels may then have long-term negative (or positive) cascading effects that contribute to the development of psychopathology, as well as its stability over time. In some cases, such genetic effects may begin to impact the child's function-

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ing in early childhood, but it is equally plausible that the expression of these genetic effects, and the cascading processes they initiate, could occur in any developmental period.

Currently, there is a greater appreciation of the multifactorial nature of psychological disorders and the influence of G×E effects on the development of disorder (Rutter, Moffitt, & Caspi, 2006). It now appears that single genetic mutations only account for rare cases of a few psychiatric disorders. Instead, it is more common that (a) genes involved in the susceptibility to psychological problems are common allelic variations; (b) direct effects of these allelic variations are small; (c) because the increased risk will be incremental, effects are more likely to be detected with dimensional rather than categorical outcome measures; and (d) genetic effects may increase the probability of developing a disorder through their exposure to, or sensitivity to, risk factors in the environment (Rutter et al., 2006). Moffitt, Caspi, and Rutter (2005) note that conditions for which there are modest direct genetic effects but large individual differences in developing psychopathology are most appropriate for studying G × E interactions.

Quantitative genetic designs, including twin studies, suggest that genetic factors make a substantial contribution to child and adolescent anxiety (Eaves et al., 1997; Sweeney & Pine, 2004), depression (Auerbach et al., 1999; Eaves et al., 1997; Gutierrez et al., 2004; Lakatos et al., 2003; Tadic et al., 2003; Tsuang, Taylor, & Faraone, 2004), and oppositional defiant behavior (Bartels et al., 2003; Burt, Alexandra, Krueger, McGue, & Iacono, 2001; Eaves et al., 1997; Hudziak, Derks, Althoff, Copeland, & Boomsma, 2005). Although twin studies can be used to estimate overall genetic contributions, there is increasing interest in identifying the specific genes associated with the development of disorders. Significant main effects for a few candidate genes associated with anxiety and depression have been found, mostly in adult studies, but they do not account for large proportions of variance in phenotypic expression. Following the criteria described above (Moffitt et al., 2005), it is clear that greater emphasis should now be placed on identifying G×E interactions for symptoms of these disorders. The best approach to studying G×E involves a molecular genetic approach (Rutter et al., 2006), and assessment of effects is enhanced when the outcomes are measured as continuous variables (Rutter et al., 2006). Key candidate genes that have been identified include the serotonin transporter gene (*5-HTT*, also known as solute carrier family C6, member 4 or *SLC6A4*), the dopamine receptor D4 gene (*DRD4*), and the monoamine oxidase A gene (*MAOA*), all of which have been associated with relevant symptomatology in prior studies as discussed below.

Gene × Environment Effects for *5-HTT*, *DRD4*, and *MAOA*

5-HTT: Main effects

The serotonin system plays an important role in emotional behavior (Munafo, Clark, & Flint, 2004). The short variant of the *5-HTT* linked polymorphic region (*5-HTTLPR*) long polymorphic repeat is associated with reduced transcriptional activity of the *5-HTT* gene (Collier et al., 1996), leading to reduced serotonin removal at the synaptic cleft.

Main effects for *5-HTTLPR* and anxiety/neuroticism have been found in three of four recent meta-analyses (Munafo et al., 2004; Munafo et al., 2003; Schinka, Busch, & Robichaux-Keene, 2004; Sen, Burmeister, & Ghosh, 2004). In studies of anxiety with children, main effects for *5-HTTLPR* have been somewhat inconsistent. Two studies found an association between the genotypes containing the short/long allele (s/l) or s/s allele and anxiety during stranger approach (Auerbach, Faroy, Ebstein, Kahana, & Levine, 2001), and for the s/s genotype with fear on laboratory tasks (Hayden et al., 2007). In contrast, Arbelle et al. (2003) found that the long form of *5-HTTLPR* was associated with shyness. For adult depression, one meta-analysis indicated a main effect of *5-HTTLPR* on suicidal behavior (Anguelova, Benkelfat, & Turecki, 2003), although two subsequent studies of adults suggested no association with depression (Kang, Namkoong, & Kim, 2008; Kunugi et al., 1997). There do not seem to be any studies of main effects of *5-HTTLPR* on child depression or externalizing problems, but Twitchell et al. (2001) found an association between the presence of a short allele (s/l or s/s) and increased behavioral disinhibition and negative affect in 7- to 16-year-old children. Given these weak associations G × E studies of *5-HTTLPR* are appropriate and needed.

G × E studies for *5-HTTLPR* effects on adult depression have primarily examined interactions with stressful life events and child maltreatment. Overall, the results for stressful life events are somewhat inconsistent. Two meta-analyses (Munafo, Durrant, Lewis, & Flint, 2009; Risch et al., 2009) contradict Caspi et al.'s (2003) early finding that the effects of stressful life events on depression was greater when a short allele was present. Although both of these reports have been criticized on methodological grounds (Karg, Burmeister, Shedden, & Sen, 2011; Kramer, 2009), a third meta-analysis also calls into question whether stressful life events increase the risk for depression when the short allele is present (Karg et al., 2011). Karg et al. examined three types of stressors: stressful life events, child maltreatment, and stressful life events among individuals with a medical condition. They found only marginal support for *5-HTTLPR* moderating the relationship between stressful life events and depression (i.e., the results were significant but not stable when any one of several different studies were removed from analysis). In contrast, there was strong support that *5-HTTLPR* moderates the relationship between child maltreatment and depression, as well as between stressful life events and depression in individuals with medical conditions. Thus, the evidence is stronger for interactions between *5-HTTLPR* and child maltreatment, and *5-HTTLPR* and life stress when a medical condition is present than for an interaction between *5-HTTLPR* and life stress alone.

In studies examining interactions of *5-HTTLPR* with other risk factors, the results are also somewhat contradictory. In

one report, the s/s form of *5-HTTLPR* coupled with poor social support increased behavioral inhibition, a precursor to social anxiety (Fox et al., 2005). In contrast, another report (Barry, Kochanska, & Philibert, 2008) examining the interaction effects of *5-HTTLPR* and maternal responsivity on child attachment security found that higher levels of maternal responsivity were associated with higher levels of attachment security in s/s and s/l infants but not in l/l infants. For externalizing problems, the short form of *5-HTTLPR* plus exposure to childhood adversity increased violent adult behavior (Reif et al., 2007), and the combination of low socioeconomic status (SES) with the short form of *5-HTTLPR* coupled with the long form of *DRD4* increased aggression (Nobile et al., 2007), but the interaction of SES and *5-HTTLPR* alone on externalizing behavior was not significant. Other contextual factors (e.g., SES), as well as parenting practices and their interaction with *5-HTTLPR*, have not been studied in children.

DRD4 gene

DRD4 codes for the D4 subtype of the dopamine receptor, which inhibits adenylyl cyclase. Consistent associations have been found between the 7-repeat allele of *DRD4* and attention-deficit/hyperactivity disorder (ADHD; Faraone, Doyle, Mick, & Biederman, 2001; Gizer, Ficks, & Waldman, 2009), and between *DRD4* and comorbid ADHD with conduct disorder (Holmes et al., 2002). The 7-repeat allele was associated with the presence of oppositional defiant disorder (ODD) among children with ADHD (Kirley et al., 2004). In addition, a relationship was found between *DRD4* and novelty seeking (a trait associated with externalizing problems) for the long form (5+ repeats) of *DRD4* (Schinka, Letsch, & Crawford, 2002), and poorer behavioral inhibition was observed on a laboratory stop-signal task for college students with the 7-repeat allele.

The results for the main effects of *DRD4* on internalizing problems are less consistent. One study reported an association between the short form (2 repeat) of *DRD4* and unipolar, but not bipolar, depression in adults (Leon et al., 2005). Other studies report no association between *DRD4* and depression (Kang et al., 2008) or suicide attempts (Persson et al., 1999; Zalsman et al., 2004). *DRD4* was not associated with shyness in 7-year-olds (Arbelle et al., 2003).

G × E studies for DRD4 in children show interactions and contextual variables, parenting, and the presence of the *DRD4* 7-repeat polymorphism in relation to externalizing behavior, including ADHD. Maternal insensitivity was associated with higher externalizing behavior in toddlers only in the presence of the *DRD4* 7-repeat polymorphism (Bakermans-Kranenburg & van IJzendoorn, 2006), whereas race moderated the relationship of *DRD4* with parental warmth–responsiveness. In African Americans (Propper, Willoughby, Halpern, Carbone, & Cox, 2007), high parental warmth–responsiveness was associated with lower externalizing behavior when the long form of *DRD4* was absent. The long forms of *DRD4* along with low SES have been associated with aggression (Nobile et al., 2007).

MAOA gene: Main effects

MAO is an enzyme involved in the metabolism of biological amines, including the monoaminergic neurotransmitters serotonin, dopamine, and norepinephrine (Jacob et al., 2005). MAO contributes to controlling amine disposability at the synaptic cleft, deaminating serotonin and norepinephrine (Gutierrez et al., 2004). The short allele is associated with low activity. Because the *MAOA* gene is X linked, males (XY) carry only a single *MAOA* allele, whereas females (XX) carry two, one of which is subject to random X-chromosome inactivation in each cell. Thus, high-activity boys have a single long or "high-activity" allele, that is, high/–, whereas high-activity girls are high/high, with a single long allele active in each cell.

Studies examining the main effects for *MAOA* and adult internalizing disorders have shown mixed results, with *MAOA* associated with anxiety in some studies (Gutierrez et al., 2004; Tochigi et al., 2006) but not in others (Arbelle et al., 2003; Eley et al., 2003; Jacob et al., 2005; Syaglio et al., 2001). There are similar mixed results for depression, with depression (Tochigi et al., 2006) associated with *MAOA* in some studies and not in others (Gutierrez et al., 2004). Adult male suicide victims were more likely to carry the high-activity *MAOA* variant, but no association was found for females (Du et al., 2002).

Results for main effects on adult externalizing behaviors are also mixed. Although the low-activity alleles of *MAOA* are associated with greater violence (Reif et al., 2007), and carrying the low-activity variant is associated with cluster B personality problems (Jacob et al., 2005), no main effects for *MAOA* were found for criminal activity in male adolescents (Nilsson et al., 2006) or antisocial behavior (Huizinga et al., 2006; Widom & Brzustowicz, 2006). In 8- to 17-year-old girls, however, low-activity *MAOA* increased risk for conduct disorder (Prom-Wormley et al., 2009).

Interaction effects for *MAOA* and stressors, maltreatment, or early adversity have been reported, primarily in studies with adults. G × E interactions have been found for *MAOA* and adversity (Prom-Wormley et al., 2009), life trauma (Frazzetto et al., 2007), and child maltreatment (Taylor & Kim-Cohen, 2007). More complex interactions have also been noted, with high *MAOA* activity buffering the effects of early adversity on later antisocial behavior in Whites but not in non-Whites (Widom & Brzustowicz, 2006). Kinnally et al. (2009) reported that adult women having lower activity *MAOA* (s/s or s/l) who were exposed to early family stressors, but who had good parental care, showed lower impulsivity/aggression than when parental care was poor.

Among adolescents, Nilsson et al. (2006) found that low-activity *MAOA*, along with either poorer residence or violent victimization, was associated with greater criminal activity in male adolescents. Another study (Foley et al., 2004) found low-activity *MAOA* plus adverse environment was associated

with increased conduct disorder in 8- to 17-year-olds. However, Huizinga et al. (2006) found no main effect for *MAOA* on antisocial behavior, and no significant *MAOA* × Child Maltreatment interaction on antisocial behavior.

Most studies show an association among poor environment, poor mental health, and the presence of the low-activity *MAOA* variant. In women whose *MAOA* activity was low, those who had been exposed to early stressors even with good parental care had the highest level of depression (Kinnally et al., 2009). Cicchetti, Rogosch, and Sturge-Apple (2007) found that depressive symptoms increased for maltreated males only if they had the low-activity *MAOA* variant. Other data (Kim-Cohen et al., 2006) indicate that, in boys, low *MAOA* activity plus exposure to physical abuse predicted more mental health problems. Eley et al. (2004), however, found a nonsignificant relationship between *MAOA* and cumulative environmental risk on adolescent depression.

To summarize, for *5-HTT*, *DRD4*, and *MAOA*, there is some evidence for G × E interactions with externalizing and internalizing problems (anxiety, depression, or both). However, the range of environmental risk factors explored is still very limited, focusing on stress and child maltreatment but generally not examining a more comprehensive range of contextual or parenting behaviors that have been empirically shown to be associated with those disorders. In addition, the number of studies with children is relatively small; to date there are no G × E studies examining early onset of symptoms in preschool children, limiting our understanding of the timing at which the phenotypic symptoms of these disorders are manifest.

Environmental Risk Factors for G × E Analyses

General models of development such as Bronfenbrenner's (1979) bioecological model, as well as models of the development of psychopathology, such as Campbell's (1990) model of externalizing behaviors and Cicchetti and Toth's (1998) transactional model of childhood depression, all suggest that a variety of psychosocial factors, ranging from more distal to more proximal, can contribute to the development of psychopathology. This is consistent with the "multiple levels of analysis" concept. In reviewing relevant studies, Smeekens, Riksen-Walraven, and van Bakel (2007) identified four domains of risk: (a) contextual characteristics (e.g., stress), (b) parental characteristics (e.g., psychopathology), (c) parenting (e.g., hostility), and (d) child characteristics (e.g., temperament). Psychosocial factors from the first three categories may be appropriate for studying G × E effects.

Many of the contextual, parent, and parenting psychosocial risk factors have been found to be associated with more than one type of symptom; they have been associated with externalizing symptoms, such as symptoms of ODD, and internalizing symptoms, such as symptoms of anxiety and depression. Psychosocial factors contributing to multiple types of symptoms in both internalizing and externalizing categories may be components of a general diathesis for the development of psychopathology. It is also possible, however, that psychosocial risk factors contributing to different types of psychopathology may have specific effects when interacting with specific genes. G × E effects for these psychosocial risk factors may be different for symptoms of externalizing (e.g., symptoms of ODD) than internalizing (e.g., anxiety and depression), and they may differentiate between different symptoms of internalizing disorders as well. This further highlights the importance of expanding the range of psychosocial risk factors included in G × E studies to provide more specific models of these different types of symptoms.

We used the existing literature to identify a set of psychosocial risk factors associated with symptoms of both internalizing and externalizing disorders from each of the environmental domains noted by Smeekens et al. (2007), including contextual, parent, and parenting domains. Contextual variables associated with both internalizing and externalizing symptoms include SES (Evans, 2004), conflict (Zimet & Jacob, 2001), and stress (Grant, Compas, Thurm, McMahon, & Gipson, 2004). The association between maternal depression and child psychopathology is well documented for internalizing (Cummings, Keller, & Davies, 2005; Goodman et al., 2011) and externalizing disorders (Goodman et al., 2011; Kim-Cohen, Moffitt, Taylor, Pawlby, & Caspi, 2005). Among parenting variables, parental hostility (Heller & Baker, 2000) and lack of parental warmth or support (Hipwell et al., 2008) are associated with both externalizing and internalizing problems. Parental scaffolding skills are associated with effortful control (Lengua, 2006), components of which are associated with internalizing and externalizing symptoms (Hoffman, Crnic, & Baker, 2006).

In addition to examining the empirical literature to identify appropriate psychosocial variables to include, we also were mindful of the criteria outlined in several reports as being ideal for selecting plausible environmental pathogens (Moffitt et al., 2005; Moffitt, Caspi, & Rutter, 2006; Rutter et al., 2006). Given the present state of research in this area, not all of these criteria can be met, but selecting environmental pathogens meeting as many of these criteria as possible increases chances of finding significant G × E effects, while also reducing false positives. However, choosing only environmental pathogens meeting all of these criteria is likely to restrict the range of G × E effects to a very few possibilities and increase the chance that G × E effects important to a specific disorder will be missed. Thus, researchers must strike a balance in their choice of environmental pathogens to study, and adherence to these criteria may differ if the research goal is to establish that G × E interactions exist or to explicate factors contributing to a particular disorder.

Moffitt et al. (2006) and Rutter et al. (2006) suggest that disorders chosen should be ones for which there is (a) evidence of substantial environmentally mediated risk, (b) a clear association between the outcome variables and the risk factors, and (c) risk factors that show variability in their effects on those outcome variables. As noted above, the types of symptoms chosen to examine in the present report (symp-

toms of anxiety, depression, and oppositional behavior) have all been closely related to a number of environmental risk factors. The psychosocial variables (contextual, parent, and parenting) that we selected all have been demonstrated to be related to these disorders, thereby meeting the second criterion. The relationships between these risk factors and outcomes all show considerable interindividual variability, thereby meeting Rutter et al.'s (2006) third criterion. Moffitt et al. (2006) argue that the environmental factors studied must be measurable, and there are reasonable measures for each of the environmental factors in the present study.

Other criteria for the ideal environmental risk factors in G × E studies are harder to achieve. The ideal environmental risk factors are those showing some plausible effect on the biological systems associated with the disorder (Moffitt et al., 2006). Ideally, there would be sufficient information about the biological pathways that may be linking the genetic and environmental factors so that highly specific hypotheses could be examined. Unfortunately, however, more research is needed on most environmental factors to meet these criteria (Rutter et al., 2006), and this criterion is often not achievable (Moffitt et al., 2006). There are preliminary data, however, indicating that environmental risk factors of SES, stress, and conflict have some effects on the biological systems associated with disorder. That is, stress in early childhood can have an effect on neurotransmitter systems (DiMaio, Grizenki, & Joober, 2003), suggesting that early environmental characteristics associated with stress and its amelioration may be good candidates for G × E analyses. Caspi et al. (2002) demonstrated a G × E interaction for stress and *5-HTT*, and the dopamine system is also affected by stress (Caspi et al., 2002; Chinta & Anderson, 2005). In addition, significant deviations from ideal parenting in the form of child maltreatment have been found to be moderated by *5-HTTLPR* interacting with social support (Kaufman et al., 2004). Caspi et al. (2002) also showed a G × E interaction for *MAOA* and parental maltreatment of children. G × E interactions with other types of parenting behaviors that are less severe than child abuse have not been extensively examined, but exposure to problematic parenting practices such as hostile and insufficiently supportive parenting is associated with the development of psychopathology, shows variability in its impact on the development of child disorder, and is likely to be mildly to moderately stressful for the child. In such circumstances, genetic factors could play both a risk and a protective role in relationship to the development of child disorders. Because exposure to less than optimal parenting practices is very common, a fuller understanding of G × E with regard to those practices could have widespread public health implications.

It is more difficult to establish that the environmental pathogen has causal effects. Since random assignment of participants to studies of putative risk factors is unethical, Moffitt et al. (2006) suggest that support for a causal relationship can only be indirect, that is, derived from treatment studies (a) showing that altering the risk factor improves the disorder, or (b) capitalizing on naturally occurring experiments demonstrating that putative risk factors alter the prevalence of disorder, or (c) adoption or twin studies indicating that an environmental factor for which the siblings were discordant influences outcome. Establishing that each of the risk factors included in the present study has a specific causal effect is particularly difficult. There is ample evidence from treatment studies that altering parenting practices improves outcomes (Beauchaine, Webster-Stratton, & Reid, 2005; Kazdin, 1997; Webster-Stratton, Reid, & Hammond, 2004). Such studies, however, tend to focus on changing a number of parental attitudes and behaviors at once, so treatment studies generally do not provide causal evidence specific to particular parental practices. This is also true with regard to naturally occurring experiments or twin/adoption studies. There is support from studies of naturally occurring events to support poverty as having a causal relationship to child psychopathology (Costello, Compton, Keeler, & Angold, 2003) although the mechanisms through which changes in income (reduced stress, conflict, etc.) has effects are less clear.

Rutter et al. (2006) maintain that proximal measures of environmental pathogens are likely to be more productive in demonstrating G × E effects than are distal factors. This assertion, however, can be tested empirically, and the present report includes both proximal and distal factors.

Moffitt et al. (2006) argue that the effect of a single environmental factor is likely to be small, so examining cumulative effects may be more productive in G × E studies. This guideline, of course, must be considered in the context of the overall purpose of the study. If the goal is to demonstrate that there are G × E effects, then cumulative indices of environmental risk are valuable. If the goal, however, is to determine if there are specific effects associated with different risk factors, then a cumulative index is not appropriate. Finally, Moffitt et al. (2006) caution that it is possible that G × E effects could be age specific, and we were mindful in drawing inferences from the findings of this study that the findings may be specific to the preschool period, the age group of the study participants.

Gene–environment correlations (rGEs)

Although awareness of possible *r*GEs is not new (Plomin, DeFries, & Loehlin, 1977), the first *r*GE for a specific gene was not reported until 2006 (Dick et al., 2006). Although attention to the role of *r*GE as part of the gene–environment "interplay" in developmental psychopathology is increasing (Belsky, Bakermans-Kranenburg, & Van IJzendoon, 2007; Kim-Cohen et al., 2006; Rutter et al., 2006), studies measuring *r*GEs are still uncommon (Jaffee & Price, 2007), particularly with children. G × E effects involve differences in the effects of one variable on an outcome as function of the level of the other variable; *r*GE occurs when genetic factors influence the individual's exposure to an environmental risk (Lau & Eley, 2008). Typically, *r*GE and G × E effects are studied independently, although awareness is growing that they should

be studied jointly because the presence of *r*GEs could increase apparent G × E effects (Lau & Eley, 2008).

The present study

The aims of the present study were to examine G × E interactions across a range of theoretically relevant (contextual, parent, and parenting) factors that have been associated with symptoms of both internalizing (e.g., anxiety and depression) and externalizing disorders (e.g., ODD); identify G × E effects; and determine whether these interactions are specific to, or generalized across, disorders. We examined main effects, *r*GEs, and G × Es for three target genes, *5-HTT*, *DRD4*, and *MAOA*, which are involved in pathways thought to be important in the domains under study and that had been associated with internalizing, externalizing, or both kinds of symptoms in prior analyses. Rather than being exclusively causal, we expect that variants in these genes contribute to pathway disruption and that positive associations will eventually permit the identification of additional pathway-associated genetic factors that can likewise lead to disruption and the emergence of behavioral phenotypes. Consistent with the "multiple level of analysis" approach, we included risk factors across multiple domains, including contextual (e.g., SES, conflict, and stress), parental psychopathology (e.g., depression), and parenting (e.g., supportive-engagement, hostility, and scaffolding skills). Risk factors selected have been found to be related to the outcome measures of symptoms of psychopathology and met most guidelines suggested by Moffitt et al. (2006) for environmental factors worth studying in G × E analyses.

Studying several risk factors and outcomes simultaneously offers advantages over studies focusing on only one or two risk factors and single types of symptoms. First, a review of the specificity of contextual risk factors across child and adolescent disorders suggests that most studies have found that such risk factors are nonspecific and are associated with more than one form of psychopathology (Shanahan, Copeland, Costello, & Angold, 2008), a pattern consistent with the concept of multifinality. Such studies, however, typically included few risk factors and often only one disorder. Examining G × E interactions may help identify interaction effects specific to disorders that would not be detected when examining either genetic main effects or environmental main effects alone. Second, we sought to examine whether individual risk factors had specific effects. Studies examining a single environmental risk factor may encounter the omitted variables problem in which variables correlated with the risk factor being studied are not included in the tested model. When variables correlated with the environmental factor under study are omitted, the effects of the omitted variables on the outcome measure may be attributed to that environmental factor, possibly biasing estimates of causal parameters and affecting the significance levels of the specific relationships included in the model (Cicchetti & Curtis, 2007; Tomarken & Waller, 2003). For example, when Fox and colleagues (2005) examined the interaction of *5-HTT* and maternal social support on behavioral inhibition, the environmental effects were attributed to social support. If social support was associated with parenting style, which was not assessed in that study, the effects attributed to social support actually may have been due to parenting attitudes and behavior.

For the variables examined in the present study: (a) SES effects on child psychopathology are associated with maternal depression, parenting practices such as harsh discipline, and family conflict (Grant et al., 2006); (b) the effects of family conflict and stressful life events on the development of psychopathology may be mediated by parenting (Grant et al., 2004) and parental depression (Monroe, Slavich, & Georgiades, 2009); and (c) maternal depression may increase child psychopathology through its association with parental hostility (Bor & Sanders, 2004) and poorer maternal scaffolding skills (Hoffman et al., 2006). If, say, a G × E effect was found for a study that examined only parental depression as the environmental risk factor for externalizing problems, effects that might be due to correlates of depression (e.g., SES, conflict, and stress) might be misattributed to parental depression per se; if parental hostility was the only risk factor, effects attributable to parental depression might be misattributed to parental hostility, and so on. Such misattributions could have profound effects on intervention planning.

Although it is virtually impossible to include all potential correlates from a model, when few or none are included, the chances of identifying specific effects associated with that variable of interest decrease. Presently, the study of single environmental factors is common in G × E studies (Bakermans-Kranenburg & van IJzendoorn, 2006; Barry et al., 2008; Caspi et al., 2002; Caspi et al., 2002, 2003, 2005; Cicchetti et al., 2007; Fox et al., 2005; Haberstick et al., 2005; Huizinga et al., 2006; Kochanska, Philbert, & Barry, 2009; Nobile et al., 2007; Sheese, Voelker, Rothbart, & Posner, 2007; Widom & Brzustowicz, 2006), albeit not in all (Kaufman et al., 2004; Propper et al., 2007; Wakschlag et al., 2009). In the present study, we examined the potential effects of omitted variables by comparing G × E effects resulting from analysis of a single interaction versus those in which multiple G × E interactions were examined simultaneously.

Method

Participants

Participants were part of the first wave of a longitudinal study of risk factors for oppositional–defiant behavior, anxiety, and depression in preschoolers. The main study was funded by the National Institute of Mental Health to examine a large set of psychosocial risk factors for these disorders. The first wave of data collection occurred when the children were 4 years old, with a community sample of 796 children. Additional funding was obtained to assess G × E interactions in a subsample of participants, and saliva samples were collected from a consecutive sample of 175 non-Hispanic White

children (59.90%). The sample included 97 boys (55.4%) and 78 girls (44.6%). The mean age was 4.40 years (range = 3.87–5.08 years) at the time of assessment. Although all SES groups were represented, the sample was skewed in the direction of the higher Hollingshead SES groups (90.9% higher two SES groups). Children were excluded from the study if (a) the child exhibited an autism spectrum disorder, (b) the parent did not speak Spanish or English, (c) the child had not lived with the same primary caretaker for the prior 6 months (because otherwise the caretaker may not have had sufficient experience to report on the child's functioning), (d) the child obtained a standard score on a language screen less than 70 at baseline, or (e) the child was enrolled in a classroom for the intellectually disabled. Because the sample sizes were smaller and differences in allelic distributions make combining race/ethnic groups difficult, only data on the single largest subsample, non-Hispanic Whites, are included in the present report.

Genetic factors

For the *5-HTT* promoter variant *5-HTTLPR*, most prior studies have grouped genotypes according to a dominant short allele (s/s, s/l versus l/l) model (Reif et al., 2007; Schmidt, Fox, & Hamer, 2007) or a dominant long allele (s/s versus s/l, l/l) model (Auerbach et al., 2001; Hayden et al., 2007), with short and long representing 14 or 16 repeat lengths, respectively, usually without providing a clear rationale for the choice made. Others have examined the three different genotypes (s/s, s/l, l/l) separately (Caspi et al., 2003; Chipman et al., 2007). Choosing the three-group approach in the present study was problematic because using three groups required dummy coding, increased the number of G × E analyses because of the dummy coding, and made understanding the meaning of the interactions difficult. Thus, this approach was not feasible in the present study. The dominant short allele model (s/s, s/l vs. l/l) was chosen and examined in the present report.

For *DRD4*, most studies have classified the *DRD4* exon III VNTR genotypes into two groups based upon the presence or absence of the 7-repeat allele (Bakermans-Kranenburg & van IJzendoorn, 2006; Faraone et al., 2001; Leon et al., 2005), whereas a few others (Schmidt et al., 2007) have grouped *DRD4* genotypes as short (s/s; 2–5 repeats) and long (s/l, l/l; 7–8 repeats). In the present study, agreement between the two approaches was high ($\kappa = 0.75$), and the former approach was used. Both genders were combined for *5-HTT* and *DRD4* analyses.

Since the work of Deckert et al. (1999), many studies (Cicchetti et al., 2007; Eley et al., 2004; Nilsson et al., 2006; Reif et al., 2007; Sjoberg et al., 2007; Syaglio et al., 2001; Widom & Brzustowicz, 2006) have classified *MAOA* alleles with 3 or fewer repeats as low activity (2.6 and 3 repeats) and those with more than 3 (3.5, 4, 5) repeats as high activity. This classification approach is straightforward for boys, who have only one *MAOA* allele. The approach, however, is more problematic for girls. Because they have two *MAOA* alleles, girls can be homozygous (low/low or high/high) or heterozygous (low/high), with heterozygous girls comprising a mosaic of cells expressing either the low or high allele due to X-inactivation. Studies have differed in their approach to heterozygosity, with some studies excluding heterozygous females (Cicchetti et al., 2007; Nilsson et al., 2006; Widom & Brzustowicz, 2006) and others not describing how heterozygous females were dealt with (Syaglio et al., 2001). For *MAOA*, Sjoberg et al. (2007) found that patterns for heterozygous females are similar to those for females homozygous for the low-activity allele. More recently, Wakschlag et al. (2009) combined the heterozygotes (low/high) with the low-activity (low/low) group and found significant effects for *MAOA* × Prenatal Exposure to Cigarette Smoke. In that study, a three-level genotype classification for girls showed similar patterns for homozygous low-activity girls and heterozygous girls, supporting the approach of classifying these together as a lower activity group. Although the functional consequences of *MAOA* expression-level mosaicism are unknown, it is thus possible to hypothesize that a threshold percentage of high-expressing cells, presumably greater than 50%, is necessary for the system to perform in a manner that is functionally distinct from that of low/low individuals. Following Wakschlag et al. (2009), for *MAOA*, genotypes were classified as low versus high activity, reflecting the presence of a single high-activity 3.5- or 4-repeat *MAOA*-VNTR allele for boys (i.e., low-activity boys had a single 2.6 or 3 allele; high-activity boys, 3.5 or 4) or two high-activity alleles for girls (low-activity girls had 2.6/3, 2.6/3.5, 2.6/4, 3/3, 3/3.5, 3/4; high-activity girls, 3.5/3.5, 3.5/4, 4/4).

Measures

Risk factors. As part of the longitudinal study design, multiple measures were used for several risk factors. To reduce the number of predictors in the present report, composite measures were created for those risk factor and outcome measures by converting each measure to standard scores and calculating the sum of the standard scores to create composite measures. All measures were questionnaires completed by a parent except the National Institute of Child Health and Human Development Three Boxes task, which was observer rated.

SES and demographics. A demographic questionnaire providing information concerning education and employment to be coded into the Hollingshead Four-Factor Index of Social Status (Hollingshead, 1975) was completed by the parent.

Life stress. The parental life stress composite measure ($\alpha = 0.80$) was derived from: (a) the Perceived Stress Scale (Cohen, Kamarck, & Mermelstein, 1983), which correlates well with other measures of life stress and shows high internal consistency (0.84) and test–retest reliability (0.86); (b) the total stress score of the Parenting Stress Index Short Form (Abidin, 1995), with high internal consistency (i.e., $\alpha s > 0.90$) and

test–retest reliability coefficients of 0.65–0.96 (Lessenberry & Rehfeldt, 2004); and (c) the McCubbin Family Changes & Strains Scale,' a measure with an α of 0.79 (McCubbin, Thompson, & McCubbin, 1996).

Family conflict. A composite family conflict scale (α = 0.71) was created from: (a) the McCubbin Family Distress Index (McCubbin et al., 1996), α = 0.87; (b) the well-known Family Environment Scale conflict scale (Moos & Moos, 1981); and (c) the McCubbin Family Problem Solving/Communication Scales, α = 0.89 (McCubbin et al., 1996).

Caretaker depression. The composite caretaker depression measure (α = 0.89) was derived from (a) the Center for Epidemiological Studies–Depression Scale (Radloff, 1977), with high internal consistency (>0.85), moderate reliability (0.45–0.70), and high correlations with other depression scales (Radloff, 1977); and (b) the widely used Beck Depression Inventory (Beck & Steer, 1987; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), average internal consistency (α) of 0.86 in clinical samples (Beck, Steer, & Garbin, 1988).

Parental support and hostility. The Parent Behavior Inventory (Lovejoy, Weis, O'Hare, & Rubin, 1999), used to assess parental support and hostility, includes factor-analytically derived subscales of support/engagement (α = 0.83) and hostility/coercion (α = 0.87).

Support/scaffolding. The National Institute of Child Health and Human Development Three Boxes task (NICHD Early Childhood Research Network, 1999) was used to assess parental support/scaffolding skills. This task assesses the parent's skill in helping the child complete tasks that are too difficult to accomplish without parental assistance, as well as a free-play, parent–child activity. The 15-min interaction is videotaped and coded by independent observers. Ratings are made on 7-point Likert scales of caretaker supportive presence, quality of assistance, cognitive stimulation, respect for autonomy, caretaker confidence, and hostility. A composite measure of caretaker support/ scaffolding (α = 0.81) was created by summing scores on these scales (with hostility reverse-coded). Coders were trained to 80% reliability with two master coders, and a random sample of 20% of the tapes was double-coded to assess interrater reliability (mean reliability of 0.74).

Measures of internalizing and externalizing symptoms

Three measures assessed symptoms of ODD: the Diagnostic Interview Schedule for Children–Parent Scale—Young Child (DISC-YC), the Child Symptom Inventory (CSI) ODD Scale, and the Eyberg Child Behavior Inventory (ECBI). Anxiety symptoms were measured by the DISC-YC generalized anxiety scale, the CSI generalized anxiety scale, and the CSI separation anxiety scale, and depressive symptoms were measured by the DISC-YC major depression scale, the CSI major depression scale, and the CSI dysthymia scale. The αs for the composite measures were 0.95 for symptoms of ODD, 0.70 for depression, and 0.78 for anxiety.

DISC-YC. The young children's DISC-YC (Fisher & Lucas, 2006) is a developmentally appropriate adaptation of the DSM-IV based, structured parent interview, the DISC-YC parent self-report. Symptom counts for each disorder were used. High levels of agreement are obtained for concrete, observable symptoms, and test–retest reliabilities for the DISC-YC are high. Overall reliability of symptom scales is acceptable to high (test–retest reliability for ODD = 0.88; for anxiety and depression scales = 0.57–0.81; C. Lucas, personal communication, 2006).

CSI. The CSI parent inventory (Gadow & Sprafkin, 1997, 2000) is a DSM-IV derived behavior problem checklist. Overall reliability of symptom scales is acceptable to high for the ODD scale (α = 0.70; test–retest, 0.90); anxiety scales (α = 0.70–0.83; test–retest, 0.65–0.77); and depression (α = 0.70–0.74; test–retest, 0.51–0.53).

ECBI. The ECBI assesses parental report of ODD symptoms (test–retest reliability = 0.86–0.88; interrater reliability = 0.79–0.86; internal consistency = 0.88–0.95; Eyberg & Pincus, 1999).

Procedure

Children were recruited from 23 pediatric practices in Cook County, Illinois, and 13 Chicago Public Schools with preschool programs. Recruitment occurred when the children were approximately 4 years old. Parents who expressed interest at initial contact were subsequently contacted by telephone, with 47.8% of those expressing interest agreeing to participate in the longitudinal study. When funding was obtained to collect the DNA samples, a consecutive sample of children and parents were also asked to provide DNA samples. There were 82.3% of the non-Hispanic White families approached to provide the DNA sample who agreed to do so (N = 175).

All data were collected during home visits. After scheduling the visit, questionnaires were mailed to the family. At the visit, there was an observation period followed by administering the Three Boxes task followed by the DISC-YC interview, either in English or Spanish. Parents then completed the remainder of the questionnaires. Subsequently, a saliva sample was obtained. The child expectorated approximately 2 ml into an Oragene sample container (DNA Genotek, Ontario); in resealing the container, 2 ml of preservation buffer was released, allowing for long-term, room temperature storage. The total duration of the home visit was about 2.5 hr. Procedures were approved by the institutional review boards of the authors' institutions.

In the laboratory, DNA was extracted from 2 ml of the sample using standard Oragene protocols and reagents.

DNA quality and quantity were ascertained using a NanoDrop spectrophotometer. For each locus, a polymerase chain reaction (PCR) was carried out in a 10 ml volume containing 50 ng of genomic template, 1 m*M* deoxyribonucleotide triphosphates, and 0.5 m*M* of each primer, one of which was 5′ fluorescently labeled, using reaction and amplification conditions, with slight modifications, as described for *5-HTTLPR* and *DRD4* variable number tandem repeat (VNTR; Kim et al., 2005). PCR for *MAOA* upstream VNTR utilized TaqGold (Applied Biosystems) polymerase and included 1% DMSO in the manufacturer's buffer with 2.5 m*M* $MgCl_2$. Following a 2-min hot start at 94°C, cycling conditions were 1 min at 95°C, 1 min at 62°C, and 1.5 min at 72°C for 35 cycles. Primer sequences were as follows: for *5-HTTLPR*, 5′-NED-CTGAATGCCAGCACCTAACCCCTAATGT-3′ and 5′-GTTTCTTGGGGAATACTGGTAGGGTGCAAGGAGA A-3′; for *DRD4*-VNTR, 5′-FAM-GCGACTACGTGGTC TACTCG-3′ and 5′-GTTTCTTGGTCTGCGGTGGAGTCT G-3′; for *MAOA*-uVNTR, 5′-PET-ACAGCCTGACCGTG GAGAAG-3′ and 5′-GTTTCTTGAACGTGACGCTCCAT TCGGA-3′ (Haberstick et al., 2005). Post-PCR products were diluted in deionized formamide and added to 0.5 ml of ROX-labeled size standard. PCR products were injected and detected by laser-induced fluorescence on an Applied Biosystems 3730xl Genetic Analyzer at the Children's Memorial Research Center Sequencing Core Facility. Approximately 20% of samples were confirmed for accuracy of allele calls by gel electrophoresis and/or reextraction of DNA, including but not exclusively any with low signal intensity, unusual allele size, or those with limited initial saliva collection volume. Sample numbers were blinded for subject phenotype.

Data analyses. Hardy–Weinberg equilibrium was assessed using likelihood ratio tests. Univariate analyses of variance were used to assess *r*GEs. G × E effects were examined using hierarchical multiple regression analyses. In the multiple interaction analyses, genotype and main effects for each risk factor were entered at the first step, and the interaction of genotype with each risk factor was entered at the second step. In the single interaction analyses, genotype and main effect for a specific risk factor were entered at the first step, and the interaction of genotype with that specific risk factor was entered at the second step. A separate analysis was then conducted for each risk factor. In the multiple interaction analyses, for the analyses of *5HTT* and *DRD4*, there was power of 0.80 to detect a medium (0.12) effect size with $p = .05$; for *MAOA*, power was 0.80 to detect a medium effect size (boys, 0.23; girls, 0.30) with $p = .05$. In analyses of single G × E effects for *5HTTLPR* and *DRD4*, power was 0.80 to detect a small to medium (0.07) effect size with $p = .05$; for *MAOA*, power was 0.80 to detect a medium effect (boys, 0.12; girls, 0.15). The Statistical Package for the Social Sciences 18.0 was used for analyses.

Because the interactions of several risk factors and target genes were examined, and both *r*GE and G × E were investigated, the experiment-wise number of comparisons was large, and corrections were made for multiple comparisons. Bonferroni comparisons are highly conservative, so the somewhat less conservative Holm (1979) procedure was used to achieve more balance between Type I and II errors; for the present study, the Holm procedure requires $p < .008$ for each set of single/multiple interactions. We note all comparisons achieving traditional significance levels but only consider findings to be statistically significant if they remained significant after correcting for multiple comparisons. We describe the relationship between variables for each variable for which the interaction was significant at the $p < .05$ level, but conduct formal post hoc probing only for those interactions significant after correcting for multiple comparisons. Post hoc probing and figures presenting G × E effects were prepared following Aiken and West's (1991) procedures.

Results

Genotypes and allele frequencies

Genotypes and allele frequencies are presented in Table 1. For *5-HTTLPR* (96.0% genotyped successfully), cell frequencies were as follows: s/s ($n = 32$); s/l ($n = 93$); l/l ($n = 43$). For *DRD4* (98.8% genotyped successfully), cell frequencies were 7-repeat present ($n = 37$) and 7-repeat absent ($n = 136$). For *MAOA* (98.3% genotyped successfully), cell frequencies for boys were low activity ($n = 33$) and high activity ($n = 61$); for girls, low activity ($n = 45$) and high activity ($n = 33$).

Hardy–Weinberg equilibrium

The distribution of genotypes was in Hardy–Weinberg equilibrium for *5-HTTLPR*, c^2 (1) = 2.1, $p = .16$; for *DRD4*, c^2 (36) = 47.03, $p = .49$; and for *MAOA*, c^2 (6) = 2.82, $p = .67$.

rGE

The association between *DRD4* and caretaker hostility was significant, $F(1, 171) = 4.12$, $p = .044$, with the 7-repeat present variant associated with greater caretaker hostility. *DRD4* was not significantly associated with any of the other environmental factors. For *5-HTTLPR*, only the association of *5-HTTLPR* with caretaker depression was significant, F (1, 167) = 5.87, $p = .017$, with higher levels of depression associated with the l/l variant. For boys, only the association of *MAOA* with SES was significant, F (1, 93) = 7.39, $p = .008$, with lower activity associated with lower SES. *MAOA* for boys was not associated with stress, conflict, caretaker depression, support/engagement, caretaker hostility, or scaffolding. For girls, none of the associations between *MAOA* and environmental risk factors were significant. After correcting for multiple comparisons, none of the *r*GEs was significant.

Table 1. *Genotypes and allele frequencies for each target gene*

	Genotypes			Alleles											
5-HTT	s/s	s/l	l/l	14/14	14/16	16/16									
White	32 (19.0%)	93 (55.4%)	43 (25.6%)	32	93	43									
DRD4	7-Repeat absent	7-Repeat present		2/2	2/3	2/4	3/4	4/4	4/5	4/6	4/8	2/7	3/7	4/7	6/7	7/7
White	136 (78.6%)	37 (21.4%)		3	1	29	7	85	5	2	4	3	2	29		3
MAOA boys	Low activity	High activity		2.6	3	3.5	4									
White	33 (35.1%)	61 (64.9%)		1	32	1	60									
MAOA girls				2.6/3	2.6/4	3/3	3/4	3.5/4	4/4							
White	45 (57.7%)	33 (42.3%)			1	16	28	3	30							

Note: 5-HTT, serotonin transporter gene; s, short; l, long; *DRD4*, dopamine receptor D4 gene; *MAOA*, monoamine oxidase A gene.

Correlations between risk and outcome variables

Bivariate correlations between environmental risk factors and outcome variables are presented in Table 2. Significant correlations were noted between risk factors for stress, conflict, and caretaker depression. A moderately high correlation was found for stress and caretaker depression. Outcome measures were moderately correlated with one another. Multicollinearity statistics were reviewed; there were no indications of multicollinearity.

DRD4

Main effects. None of the main effects for *DRD4* were significant for symptoms of ODD, depression, or anxiety (effect sizes for all main effects and G × E are available online).

G × E for ODD, depression, and anxiety symptoms. There were no significant multiple or single G × E interactions for *DRD4* for any type of symptom.

5-HTTLPR

Main effects. None of the main effects for *5-HTTLPR* was significant for symptoms of ODD, depression, or anxiety.

G × E for ODD symptoms. In the multiple interaction analyses, none of the G ×E interactions were significant for ODD symptoms. In single interaction analyses, there were significant interactions for *5-HTTLPR* × SES, t (167) = 2.25, p = .026, with an increase in ODD symptoms as SES increased for the l/l group); *5-HTTLPR* × Conflict, t (167) = 2.44, p = .016, with a greater increase in ODD symptoms as conflict increased for the l/l group; *5-HTTLPR* × Caretaker Depression, t (167) = 2.66, p = .009, with a greater increase in ODD symptoms as caretaker depression increased for the l/l group; and *5-HTTLPR* × Stress, t (167) = 2.79, p = .006. Only the *5-HTTLPR* × Stress interaction was significant after correcting for multiple comparisons (Figure 1a; see Table 3 for a summary of the regression analyses). In post hoc probing, the slopes of the lines for the s/s, s/l group and the l/l group both differed significantly from zero. There was an increase in ODD symptoms as stress increased for both the s/s, s/l and l/l groups but with a significantly greater increase for the l/l groups.

G × E for depressive symptoms. In the multiple interaction comparisons, the *5-HTTLPR* × Caretaker Depression interaction was significant, t (167) = 3.07, p = .003 after correcting for multiple comparisons (Figure 1b). In post hoc probing, the slopes of the lines for the s/s, s/l group and the l/l group did not differ from zero, but the slopes of the two lines were significantly different from one another, indicating that there was a greater increase in child depression symptoms as caretaker depression increased for the l/l group than for the s/s, s/l group. In this interaction, and in others for which one or both

Table 2. *Correlations of risk factors and measures of child psychopathology (N = 175)*

	SES	Family Stress	Family Conflict	Caretaker Depression	Parental SE	Parental Hostility	Parental Scaffolding	Child ODD	Child MDD
SES									
Family stress	.00								
Family conflict	.08	.38***							
Caretaker depression	−.13	.62***	.42***						
Parental SE	−.13	−.26***	−.11	−.09					
Parental hostility	.00	.13	.32***	.13	−.05				
Parental scaffolding	.03	−.08	−.17*	−.12	.13	−.18*			
Child ODD symptoms	.01	.33***	.57***	.39***	−.13	.42***	−.21**		
Child depression symptoms	.08	.28***	.46***	.41***	−.07	.26***	−.01	.56***	
Child anxiety symptoms	−.06	.29***	.42***	.46***	−.05	.17*	−.14	.49***	.65***

Note: SES, socioeconomic status; SE, supportive engagement; ODD, oppositional defiant disorder; MDD, major depressive disorder.
*$p < .05$. **$p < .01$. ***$p < .001$.

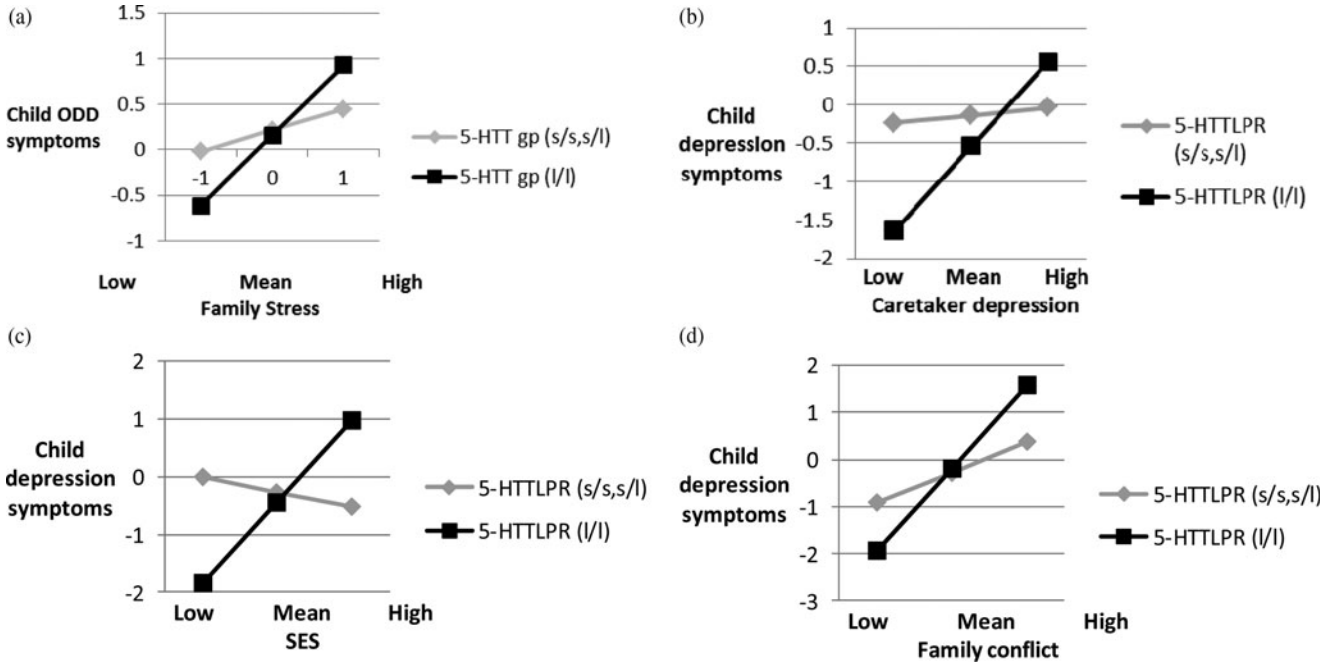

Figure 1. Serotonin transporter linked polymorphic region gene (*5-HTTLPR*) interaction effects on symptoms of oppositional defiant disorder (ODD) and depression in children: (a) *5-HTTLPR* × Family Stress, multiple interaction analysis; (b) *5-HTTLPR* × Caretaker Depression, multiple interaction analysis; (c) *5-HTTLPR* × Socioeconomic Status, single interaction analysis; (d) *5-HTTLPR* × Family Conflict, single interaction analysis. For the environmental risk factors, the low value is –1 *SD* and the high is +1 *SD*.

of the slopes of each regression line did not differ from zero, but the difference between the slopes was significant, the standard error was large enough that the slope did not differ from zero, but the differences in the two slopes were large enough to differ significantly.

In single interaction analyses, the *5-HTTLPR* × SES interaction was significant $t\ (167) = 2.88$, $p = .005$, after correcting for multiple comparisons (Figure 1c). The slopes of the lines for neither the s/s, s/l group nor the l/l group differed from zero, but the slopes of the two lines were significantly different from one another, $t\ (167) = 4.97$, $p < .0001$. There was an increase in child depression symptoms as SES increased for the l/l group in comparison to the s/s, s/l group.

The *5-HTTLPR* × Conflict interaction was also significant, $t\ (167) = 3.12$, $p = .002$ (Figure 1d), after correcting for multiple interactions. The slopes for both groups differed from zero and were significantly different from one another. There was a greater increase in child depression symptoms as a function of family conflict in the l/l than in the s/s, s/l group. The *5-HTTLPR* × Caretaker Depression interaction, $t\ (167) = 3.83$ $p = .001$, was also significant after correcting for multiple comparisons, closely following the pattern in Figure 1b.

G × E for anxiety symptoms. In multiple interaction analyses, both the *5-HTTLPR* × Stress interaction, $t\ (167) = 2.56$, $p = .025$, with a greater increase in anxiety symptoms as stress

Table 3. *Slopes, standard errors, t- and p values for post-hoc probing of interactions significant after correcting for multiple comparisons*

			Overall		Post Hoc Probing					
Symptoms	Risk Factor	Interactions	*B*	*SE B*	Slope	*SE*	Slope	*SE*	Slope Diff. *t*	*df*
5-HTTLPR					s/s, s/l		l/l			
Child ODD symptoms	Family stress	Single	0.54***	0.193	0.23*	0.11	0.77*	0.35	14.57***	
Child depression (Fig. 1a)	Caretaker depression	Multiple	1.00***	0.33	0.1	0.19	1.1	0.6	9.37***	151
Child depression (Fig. 1c)	SES	Single	1.67***	0.58	−0.25	0.34	0.62	1.06	4.97***	151
Child depression (Fig. 1d)	Family conflict	Single	1.10***	0.38	0.65*	0.26	1.76*	0.71	7.6***	151
Child anxiety (Fig. 2a)	Caretaker depression	Multiple	1.51***	0.3	−0.08	0.17	1.43**	0.54	17.36***	151
Child anxiety (Fig. 2c)	SES	Single	1.67***	0.26	1.43***	0.33	3.09***	1.03	5.28***	151
Child anxiety (Fig. 2d)	Family conflict	Single	1.47***	0.36	0.44	0.23	1.91***	0.67	11.14***	151
Child anxiety (Fig. 2e)	Caretaker depression	Single	1.41***	0.21	0.21	0.14	1.36***	0.39	25.48***	151
MAOA					Low activity		High activity			
Child depression (Fig. 4c)	Caretaker depression	Multiple	−1.66***	0.59	1.54***	0.48	−0.1	1.13	4.76***	78
Child depression (Fig. 4d)	Family conflict	Single	−1.48***	0.54	2.22***	0.37	0.74	1.01	5.01***	78
Child anxiety (Fig. 4a)	Family stress	Single	−0.87***	0.26	1.09***	0.22	0.22	0.49	13.18***	94
Child anxiety (Fig. 4b)	Family conflict	Single	−1.53***	0.52	2.15***	0.36	0.62	0.97	5.64***	94
Child anxiety (Fig. 4c)	Caretaker depression	Single	−0.78***	0.24	1.35***	0.18	0.61	0.45	13.18***	94

Note: 5-HTTLPR, serotonin transporter linked polymorphic region gene; s, short; l, long; ODD, oppositional defiant disorder; SES, socioeconomic status; *MAOA*, monoamine oxidase A gene.
*$p < .05$. **$p < .01$. ***$p < .008$.

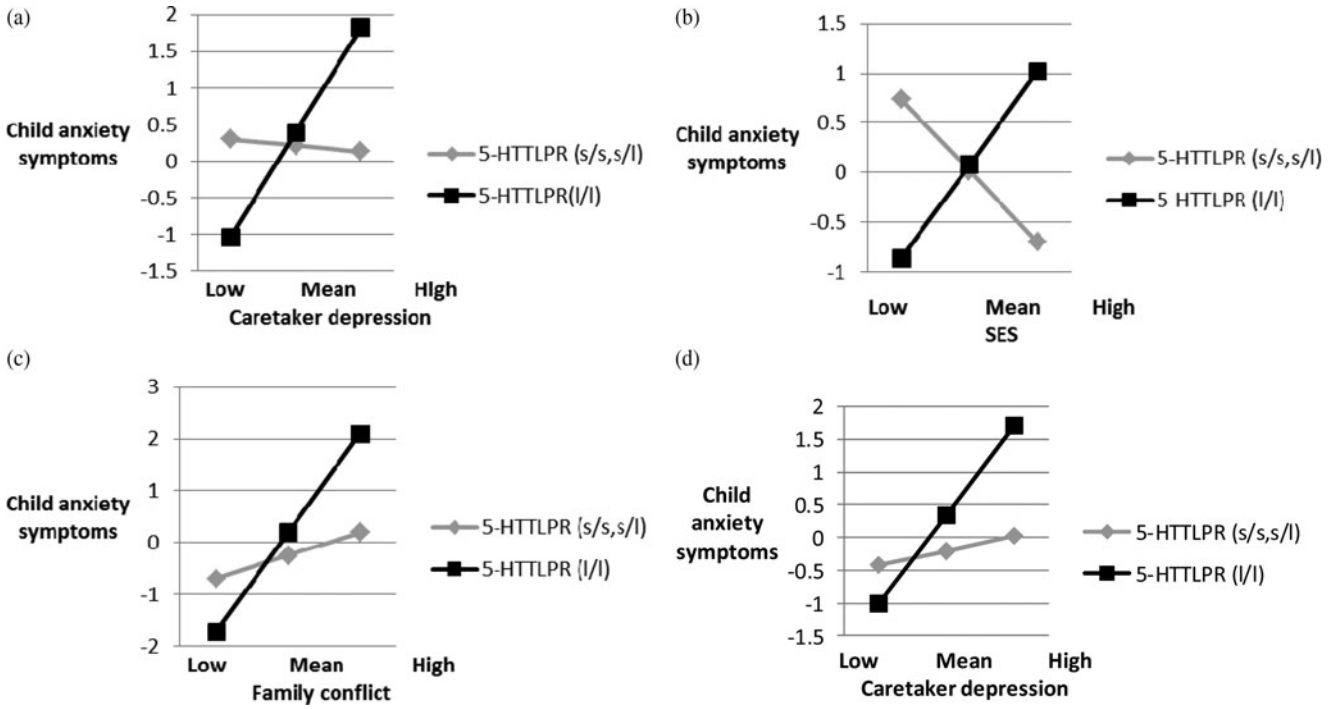

Figure 2. Serotonin transporter linked polymorphic region gene (*5-HTTLPR*) interaction on child anxiety symptoms: (a) *5-HTTLPR* × Caretaker Depression, multiple interaction analysis; (b) *5-HTTLPR* × Socioeconomic Status, single interaction analysis; (c) *5-HTTLPR* × Family Conflict, single interaction analysis; (d) *5-HTTLPR* × Caretaker Depression, single interaction analysis. For the environmental risk factors, the low value is –1 *SD* and the high is +1 *SD*.

increased for the l/l group, and the *5-HTTLPR* × Caretaker Depression interaction, t (168) = 5.12, p =.001, were significant, but only the *5-HTTLPR* × Caretaker Depression interaction was significant after correcting for multiple comparisons (Figure 2a). The slope for the s/s, s/l group was not significantly different from zero but was significant for the l/l group. The two slopes were significantly different from one another. Again, there was a greater increase in child anxiety symptoms in the l/l group than in the s/s, s/l group as a function of caretaker depression.

In single interaction analyses, the *5-HTTLPR* × Stress interaction, t (167) = 2.26, p = .025, showed a greater increase in anxiety symptoms as stress increased for the l/l group, but it was not significant after correcting for multiple comparisons. However, there were significant interactions for the *5-HTTLPR* × SES interaction, t (167) = 2.96, p = .004; the *5-HTTLPR* × Conflict interaction, t (167) = 3.12, p = .002; and the *5-HTTLPR* × Caretaker Depression interaction, t (167) = 5.42, p = .001, that remained significant after correcting for multiple comparisons. In the *5-HTTLPR* × SES interaction (Figure 2b), slopes for both groups differed from zero and were significantly different from one another. The pattern was similar to that for the interaction with SES and child depression, with child anxiety symptoms increasing as a function of SES for the l/l group and declining for the s/s, s/l group. In the *5-HTTLPR* × Conflict interaction (Figure 2c), the slope for the s/s, s/l groups did not differ from zero, whereas the slope for the l/l group was significantly different from zero. The two slopes were significantly different from one another. There was a more rapid increase in child anxiety symptoms as a function of family conflict in the l/l group. In the *5-HTTLPR* × Caretaker Depression interaction (Figure 2d), the slope for the s/s, s/l group did not differ from zero, whereas the slope for the l/l group was significant, and the two slopes were significantly different from one another. There was a greater increase in child anxiety symptoms as a function of caretaker depression in the l/l group.

MAOA

Main effects. None of the main effects for *MAOA* for boys or girls were significant for ODD, depression, or anxiety symptoms.

G × E for ODD symptoms. For ODD, none of the interactions were significant in either multiple or single interaction analyses for boys or girls.

G × E for depressive symptoms: Boys. In the multiple interaction analyses, the *MAOA* × Caretaker Depression interaction was significant after correcting for multiple comparisons, t (94) = 2.81, p = .006 (Figure 3a). The slope for the low-activity *MAOA* group differed significantly from zero, whereas the slope for the high-activity group did not. The two slopes differed significantly from one another, with child depression

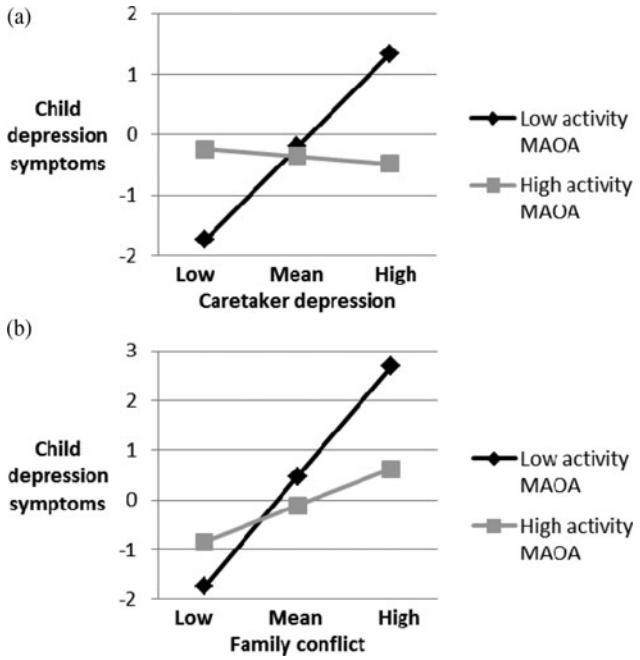

Figure 3. Monoamine oxidase A gene (*MAOA*) interaction effects on child depression symptoms for boys: (a) *MAOA* × Caretaker Depression, multiple interaction analysis; (b) *MAOA* × Family Conflict, single interaction analysis. For the environmental risk factors, the low value is –1 *SD* and the high is +1 *SD*.

symptoms increasing as caretaker depression increased only for the low-activity *MAOA* group.

In single interaction analyses, the MAOA × Conflict interaction was significant, t (94) = 2.72, p = .008 (Figure 3b) after correcting for multiple interactions. The slope for the low-activity *MAOA* group differed significantly from zero, whereas the slope for the high-activity group did not. The two slopes differed significantly from one another. There was a more rapid increase in child depression symptoms as a function of family conflict in the low-activity *MAOA* group.

G × E for depression symptoms: Girls. In both multiple interaction and single interaction analyses, none of the interactions were significant.

G × E for anxiety: Boys. In multiple interaction analysis, none of the interactions were significant. In single interaction analyses, however, there were three interactions that were significant after correcting for multiple comparisons. For the *MAOA* × Stress interaction, t (94) = 3.39, p = .001 (Figure 4a), the slope for the low-activity *MAOA* group differed significantly from zero, with a greater increase in child anxiety symptoms as a function of family stress in the low-activity *MAOA* group. The slope for the high-activity group was not significant. The two slopes differed significantly from one another. Similarly, for the *MAOA* × Family Conflict interaction, t (94) = 2.94, p = .004 (Figure 4b), the slope for the low-activity *MAOA* group differed significantly from zero, indicating an increase in child anxiety symptoms as a function of family conflict in the low-activity *MAOA* group. The slope for the high-activity group was not significant. The two slopes differed significantly from one another. The pattern recurred for the *MAOA* × Caretaker Depression interaction, t (94) = 3.12, p = .002 (Figure 4c). The slope for the low-activity *MAOA* group differed significantly from zero, with an increase in child anxiety symptoms as a function of caretaker depression in the low-activity *MAOA* group. The slope for the high-activity *MAOA* group was not significant. The two slopes differed significantly from one another.

G × E for anxiety symptoms: Girls. In both single interaction and multiple interaction analyses, none of the interactions were significant.

Co-occurrence of rGE and G × E. Although none of the *r*GEs were significant after correcting for multiple comparisons, the association of *5-HTTLPR* with caretaker depression was significant prior to that correction. This raises the possibility that the *5-HTTLPR* × Caretaker Depression effects on child depression and child anxiety symptoms were due to the association of *5-HTTLPR* with caretaker depression. To try to disentangle these effects as much as possible, we followed the suggestion of Jaffee and Price (2007) and the procedure of Dick et al. (2006) by examining the effects separately for each genetic subgroup, thereby reducing the effects of *r*GE. That approach involved examining the slopes of each of the genotypes. Essentially, Dick et al. argue that if the two genotypes are correlated with an environmental factor, then any G × E effect that was found could be attributed to the correlation between the genotypes and that environmental factor. However, if each group is examined separately, the effects of the correlation will be reduced because (a) if the correlation was entirely responsible for the effect, then the slopes of the lines for each group would not differ significantly from zero; and (b) if the slope did differ from zero for either (or both) genotypes, then the effects could not be due solely to the *r*GE effect. Thus, finding a significant slope suggests that the effects are not entirely due to *r*GE and that some G × E is likely to be present. Although this approach is not entirely definitive because the relative contributions of *r*GE and G × E cannot be quantified, it is an improvement over ignoring the possibility that *r*GE can completely account for the G × E effect.

Following the Dick et al. (206) procedure, we examined the slopes for each genotype separately. For the child outcome of depression, in the multiple interaction analysis (Figure 1a), neither slope was significant (Table 3). Thus, the G × E interaction in the multiple analysis may have been due to the *r*GE effects. For the child outcome of anxiety, however, the l/l slope was significant for both the single (Figure 2e) and the multiple (Figure 2a) analysis, and the G× E effect cannot be fully explained by *r*GE effects. Since the *r*GE effects were not significant for the other G × E effects that were significant after correcting for multiple comparisons, *r*GE was unrelated to those G × E effects.

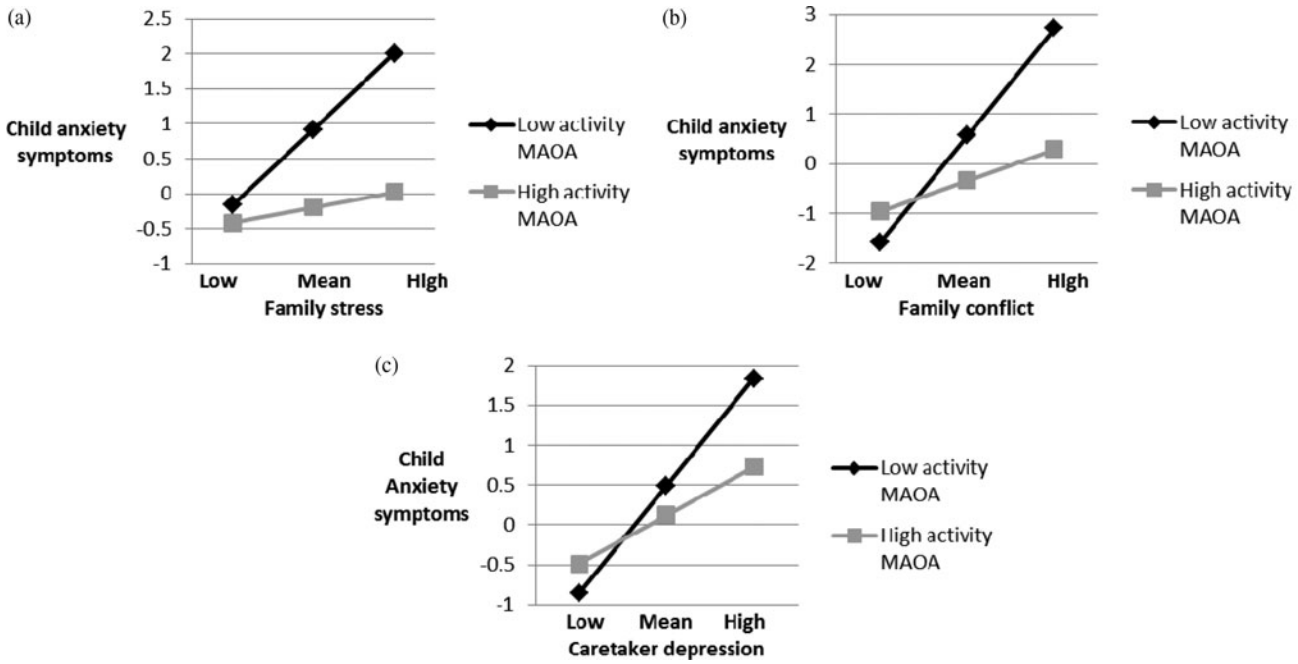

Figure 4. Monoamine oxidase A gene (*MAOA*) interaction effects on child anxiety symptoms for boys: (a) *MAOA* × Family Stress, single interaction analysis; (b) *MAOA* × Family Conflict, single interaction analysis; (c) *MAOA* × Caretaker Depression, single interaction analysis. For the environmental risk factors, the low value is –1 *SD* and the high is +1 *SD*.

Discussion

The approach to studying the role of genetic factors in the development of psychopathology has evolved from a focus on the main effects of genes to the interactions between genotypes and psychosocial variables. The gene–environment "interplay" is complex and can involve *r*GEs of various types (passive, active, or evocative) as well as true G × E interactions. Studying the relationships between these factors is still in a nascent state: Studies of main effects of various genes have been occurring with adults for many years and the number of such studies with children are increasing; studies of G × E interactions in children are also increasing, but still relatively uncommon; and concept papers discussing *r*GE in developmental psychopathology have emerged, but there are still very few studies that have measured *r*GE or attempted to show how it is related to G × E in children. Even more unusual are studies of these phenomena in preschoolers. Studies with older children afford researchers the opportunity to investigate genetic factors when certain disorders like anxiety or depression have become relatively common. Studies of younger children allow for the examination of problems, such as the development of symptoms of ODD, that are relatively common in that age group, as well as the early emergence of symptoms of disorders, such as anxiety and depression, which are likely to increase in severity over time. Furthermore, studying G × E in young children may shed light on how genetic factors may initiate a cascading process that operates across levels and domains, and how risk factors associated with both externalizing and internalizing disorders may contribute differentially to the occurrence of such symptoms in interaction with genetic factors.

Main effects

None of the main effects for the three target genes were significant for anxiety, depression, or ODD symptoms. This is not entirely surprising since good candidate genes for G × E studies should not have very powerful main effects. Although each of the target genes had shown main effects on psychopathology in prior work, the results were often inconsistent, a pattern suggesting that G × E effects may be particularly relevant for that gene and environmental factors related to that disorder. In addition, main effects have typically been identified in samples with older children and adults rather than the preschool age group included in this study. This suggests that absence of significant main effects in the present study may reflect a developmental phenomenon, with main effects emerging later, or alternatively, that what may have appeared to be main effect in studies with older children may be because these studies did not include G × E effects that may have appeared earlier in development.

G × E effects

5-HTTLPR. There was a consistent pattern of effects among the significant findings. For each significant G × E effect for symptoms of anxiety, depression, and oppositional behavior, there were significant differences in the slopes of the lines for the two groups, s/s, s/l versus l/l, with greater increases in

symptoms as a function of the environmental risk factor for the l/l group. In comparison to the s/s, s/l group, the l/l genotype appeared to be both a risk and a protective factor, with fewer symptoms in children with the s allele when the environmental risk was low and more problems at higher levels of environmental risk.

There have been many studies examining G × E effects for family adversity, stress, or child maltreatment on depression, and these studies yield conflicting results. Almost all of these studies have been conducted with adult or older adolescents (Uher & McGuffin, 2008). Caspi et al.'s (2003) early study examining stress in young adults found that the s/s, s/l genotypes were more strongly related to depression than was the l/l genotype. Two meta-analyses (Munafo et al., 2009; Risch et al., 2009) did not find support for this relationship. A third meta-analysis (Karg et al., 2011) also failed to support Caspi et al.'s original finding for *5-HTTLPR* × Life Stress but did support such an interaction for *5-HTTLPR* × Child Maltreatment and *5-HTTLPR* × Life Stress among adults with a medical problem.

In reviewing the available studies on G × E interactions of *5-HTTLPR* with stress and environmental adversity (Uher & McGuffin, 2008, 2010), Uher and McGuffin concluded that G × E interactions for *5-HTT* may differ across developmental levels. They note that the interaction of s/s, s/l with increased stress resulting in higher levels of depression seems to be prominent in studies of young adults, but that pattern has not been found in studies with adolescents (Uher & McGuffin, 2008). Along with the results of the Arbelle et al. (2003) report, the results of the present study suggest that the effects on behavior problems in young children may be quite different than that in young adults, with the l/l variant in conjunction with higher levels of stress, conflict, and adversity.

Potential developmental differences in *5-HTTLPR* × E interaction patterns and underlying mechanisms are only beginning to be investigated. A recent report (Wiggins et al., 2011) indicates that there may be developmental differences in the influence that *5-HTTLPR* has on resting-state default network connectivity. That study found that connectivity strengthens the most with increasing age in a sample of 8- to 19-year-olds among individuals with particular variants of the l/l genotype and strengthens the least in the s/s group. At age 8, the groups showed similar levels of connectivity. Further research is needed to determine whether the level of connectivity is similar, or even lower, in preschool children with the l/l genotype. Such developmental changes in neural networks would contribute to the developmental differences in G × E that both Moffit et al. (2006) and Uher and McGuffin (2008) suggest may exist.

Uher and McGuffin (2008) also argue that studies are needed that simultaneously explore a number of adverse outcomes. The present study identifies an association of increased risk for the l/l genotype across different types of adversity as well as different types of child behavior and emotional problems in young children.

In addition to developmental differences, epigenetic factors may also contribute to differences in outcomes in G × E studies. Van IJzendoorn, Caspers, Bakermans-Kranenburg, Beach and Philbert (2010) found that the association of the *5-HTTLPR* genotype with unresolved loss or trauma in adults was moderated by the level of methylation of the *5-HTT* promoter-associated CpG islands. Methylation of the alleles that carried the long (l/l) variant of *5-HTT* seemed to hamper the protective effect sometimes associated with that variant, such that higher levels of methylation of that variant were marginally related to more unresolved loss or trauma. In addition, Although the short variant of *5-HTT* may be associated with increased risk, this was only true when levels of methylation of that allele were low, perhaps indicating that high methylation of the s/s genotype drives individuals into a separate mechanism of coping with trauma (van IJzendoorn et al., 2010). Caveats to interpretation of such studies, however, include (a) patterns of methylation may differ across samples, and differences in methylation may contribute to differences in G × E findings across studies (van IJzendoorn et al., 2010); (b) patterns of methylation may differ throughout development and may mask, or amplify, the impact of genetic variation or G × E response on phenotype differentially depending on developmental stage; and (c) environmental factors can themselves cause changes in DNA methylation or other chromatin modifications, leading to altered gene expression in a way which might itself be dependent on underlying genetic variation (de Rooij et al., 2011). Greater attention to epigenetic modifications may contribute to our understanding of G × E effects in future research (Schroeder, Krebs, Bleich, & Frieling, 2010). Similarly, meta-analytic reviews need to be cognizant that sample differences in underlying methylation or other epigenetic patterns may affect study homogeneity when combining studies for analyses.

MAOA. In previous studies, main effects for *MAOA* have been inconsistent for anxiety (Arbelle et al., 2003; Eley et al., 2003; Gutierrez et al., 2004; Jacob et al., 2005; Syaglio et al., 2001; Tochigi et al., 2006), depression (Gutierrez et al., 2004; Tochigi et al., 2006), and externalizing behaviors (Huizinga et al., 2006; Jacob et al., 2005; Nilsson et al., 2006; Prom-Wormley et al., 2009; Reif et al., 2007; Widom & Brzustowicz, 2006). The present study extends these findings, showing no main effects for *MAOA* on internalizing or externalizing behavior in young children.

A number of significant interactions were found for *MAOA* in boys. For child anxiety, the interactions for *MAOA* × Caretaker Depression, *MAOA* × Family Stress, and *MAOA* × Family Conflict were all significant. There was also a significant *MAOA* × Family Conflict effect in the single interaction analysis for child depression.

For child depression symptoms, the *MAOA* × Caretaker Depression interaction was significant. However, this interaction was significant only in the multiple interaction analysis and not in the single interaction analyses, a pattern that may be consistent with suppression effects. As a result, this finding must be interpreted with caution.

In prior studies of G × E interactions for *MAOA*, low-activity *MAOA* has been associated with more psychopathology, and significant findings occur more often in samples of males than females. Low *MAOA* activity in 7-year-old boys, plus exposure to physical abuse, predicted more mental health problems (Kim-Cohen et al., 2006). Symptoms of depression increased for maltreated males only if they had the low-activity *MAOA* gene (Cicchetti et al., 2007). Low-activity *MAOA* interacting with adversity (poorer residence or victimization) was associated with higher rates of criminal activity in adolescent males (Nilsson et al., 2006), and low-activity *MAOA* plus adverse environment was associated with increased conduct disorder in adolescents (Foley et al., 2004). The present study found significant, nonspecific effects in boys on depression and anxiety, for *MAOA* × Caretaker Depression, *MAOA* × Stress, and *MAOA* × Family Conflict. Consistent with most prior studies, there was a greater increase in symptoms for the low-activity *MAOA* group. These G × E interactions affecting symptoms of anxiety and depression in preschoolers may have long-term cascading effects that contribute to the presence of these symptoms in later childhood, adolescence, and adulthood. Therefore, a potentially effective way to prevent these negative cascading effects in boys may be to develop intervention strategies that focus on decreasing family conflict, caregiver stress, and depression.

The present study found no significant G × E interactions for *MAOA* for girls, and previous studies have shown inconsistent G × E effects for *MAOA* with girls. Although Kinnally et al. (2009) found a significant G × E interaction, with women whose *MAOA* activity was low, and who were exposed to early stressors showing higher levels of depression, Eley et al. (2004), found a nonsignificant relationship between *MAOA* and cumulative environmental risk on depression for adolescent girls. Du et al. (2002) found that, Although adult male suicide victims were more likely to carry the high-activity *MAOA* variant, no association was found for females, and Jacob et al. (2005) found a weaker relationship between *MAOA* and cluster B personality types for women than for men. It is difficult to account for these gender differences in G × E effects, except to speculate that there may be a third, unmeasured factor that is more prevalent in boys that interacts with the low-activity *MAOA* gene and/or stress, family conflict, and caregiver depression. It is possible, for example, that higher activity level in boys exacerbates family conflict and caregiver depression, thereby potentiating the G × E effect.

DRD4. Although *MAOA* and *5-HTTLPR* show some significant G × E effects for symptoms of anxiety, depression, and oppositional behavior, *DRD4* showed no effect for any type of symptoms or environmental risk factor in either multiple interaction or single interaction analyses. Previous data indicate a main effect for *DRD4* and ADHD, which made it a likely candidate for study, but the lack of significant findings in the current study suggests that this gene may be linked specifically to ADHD in young children and not to internalizing or ODD type externalizing symptoms.

Results specific to each disorder

As the correlations indicate, stress, family conflict, caretaker depression, and caretaker hostility were all associated with all three symptom types. This is consistent with a common diathesis model. The pattern differs for G × E, however, suggesting that certain G × E interactions may be specific to internalizing or externalizing symptoms in young children.

Only one interaction was significant for symptoms of ODD, the *5-HTTLPR* × Stress interaction. The significant interactions for conflict and caretaker depression were associated with anxiety, depression, or both, but not with symptoms of ODD. Thus, although all three variables are correlated with the three symptom types, the G × E interactions of *5-HTTLPR* with conflict and caretaker depression may contribute to the specificity of the occurrence of anxiety and depression rather than symptoms of ODD. Similarly, the *5-HTTLPR* × SES interaction was also significant for anxiety and depression and not ODD, and may be specific to those two internalizing disorders.

For boys, there were significant *MAOA* × Conflict and *MAOA* × Caretaker Depression interactions that were significant for both depression and anxiety but not for symptoms of ODD, and they may have contributed to the development of those internalizing but not externalizing symptoms. However, the *MAOA* × Family Stress interaction was significant for anxiety but not for depression.

Thus, most of the significant interactions for *5-HTTLPR* and *MAOA* were consistent with a common diatheses model for the internalizing disorders of anxiety and depression but not for ODD symptoms, whereas the *MAOA* × Conflict interactions might lead to differences in the presence of anxiety and depression in young children.

rGE. A number of *r*GEs were significant, but none remained significant after correcting for multiple comparisons. Taking a conservative approach, we examined whether *r*GE effects that were not significant after correcting for multiple comparisons could explain the G × E effects that were significant. Among all the G × E effects that were significant, only the *5-HTTLPR* × Caretaker Depression effect for child depression symptoms may have been attributed to *r*GE.

Relatively specific and nonspecific effects

The present study included a broad range of theoretically relevant and empirically based environmental risk factors. When studies examine only one or two risk factors in isolation, the "omitted variable" problem is likely to be present, in which the effect size of the particular variable included in the model may be inflated because it is correlated with other, unmeasured variables. We addressed this problem by conducting analyses that included G × E interactions for multiple G × E interactions simultaneously in the regression analyses and comparing the results to those in analyses when one G × E effect was examined at a time. This allows for a com-

parison of specific effects for an environment risk factor with the constellation of effects for that risk factor, plus any correlates of that risk factor that may influence the outcome measure but were not included in the model. If results are significant in single interaction analyses but not in multiple interaction analyses, then it is possible that the effects of the environmental risk factor are present but not specific, that is, are influenced by the correlates of that risk factor that are not included in the regression model. Of course, no model can include all possible relevant variables, so it is best to conceptualize the significant effects as relatively specific or nonspecific.

For *5-HTTLPR* effects on both child depression and child anxiety symptoms, there was a specific G × E effect for *5-HTTLPR* × Caretaker Depression, whereas interactions of *5-HTTLPR* with SES and family conflict were nonspecific. For *5-HTTLPR* effects on symptoms of ODD, the *5-HTTLPR* × Stress effect was nonspecific. For *MAOA* in boys, the G × E effect of *MAOA* × Caretaker Depression on child depression symptoms was relatively specific, whereas interaction of *MAOA* × Family Conflict on child depression symptoms was nonspecific. For anxiety symptoms, the interactions of *MAOA* with family stress, family conflict, and caretaker depression were all nonspecific. For the nonspecific effects, correlates of the risk factor (Table 1) may have also been contributing to the G × E effect (e.g., effects of stress also may reflect the influence of family conflict, caretaker depression, and parental support, etc).

As Moffit et al. (2006) note, G × E effects may be developmentally specific. The present study utilized a cross-sectional design with young children and provides a "snapshot" of the role of the gene–environment interplay in a community sample of 4-year-olds. Early experience is likely to have important long-term effects, psychopathology can vary over time, and it is likely that the gene–environmental effects will change as the children develop. Longitudinal investigations will be important to assess such changes in the gene–environment relationships over time. Nevertheless, these early manifestations of the multidomain interplay of gene and environmental effects may have long-term cascading influences on the development and stability of psychopathology in older children, adolescents, and adults that needs further exploration.

There were some important limitations to this study. Because of the large number of analyses that were performed, other analyses of interest involving gene–gene interactions and G × G × E interactions could not be performed. In addition, it is not always clear how genotypes should best be grouped, and different grouping patterns (e.g., recessive pattern for *5-HTTLPR*) may have led to different results, as may further refinement of grouping based on putative allele-variant activity levels, such as subtyping the *5-HTTLPR* long allele into high (l_A) and low (l_G) activity and grouping accordingly (Hu et al., 2006) or inclusion of other functional polymorphic variants within the *MAOA* gene (Rosenberg et al., 2006). The significant finding in this study to the *5-HTTLPR* l/l group without such subtyping, however, speaks toward the strength of this association.

The present study extends our knowledge of the gene–environment interplay in developmental psychopathology, extending our understanding of both *r*GE and G × E effects. It demonstrates the role that genetic factors can play in potentiating and protecting the child from specific environmental risks, setting in motion a cascade of events that may have lasting positive or negative effects. Increasing our understanding of the role that genetic factors play in the development of psychopathology can have important implications for preventive and intervention work in the future. The pathways by which differences in genotypes for these target genes, in interaction with different environmental risk factors, lead to differences in phenotypic expression of psychopathology are not well established (Kinnally et al., 2009). The MAOA enzyme, for example, is known to affect oxidation and inactivation of the monoamines serotonin, dopamine, and norepinephrine in the central nervous system (Kinnally et al., 2009). This process may be differentially affected in interaction with environmental events, but it is not clear how the resulting biochemical changes, in turn, affect psychological processes resulting in increased psychopathology. Further study of the G × E effects on endophenotypes associated with the development of psychopathology is an important direction for future research (Uher & McGuffin, 2008). In addition, the results of genetic and G × E findings may be specific to race/ethnic groups, and future studies are needed to determine if these specific findings can be replicated in samples with different races and ethnicities (Bakermans-Kranenburg & van IJzendoorn, 2011).

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