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Genetic moderation of child maltreatment effects on depression and internalizing symptoms by 5-HTTLPR, BDNF, NET, and CRHR1 genes in African-American children

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Abstract

Genetic moderation of the effects of child maltreatment on depression and internalizing symptoms was investigated in a sample of low-income maltreated and nonmaltreated African-American children ($N = 1,096$). Lifetime child maltreatment experiences were independently coded from Child Protective Services records and maternal report. Child depression and internalizing problems were assessed in the context of a summer research camp by self-report (*Children's Depression Inventory*, CDI) and adult counselor-report (*Teacher Report Form*, TRF). DNA was obtained from buccal cell or saliva samples and genotyped for polymorphisms of the following genes: 5-HTTLPR, BDNF, NET, and CRHR1. ANCOVAs with age and gender as covariates were conducted, with maltreatment status and respective polymorphism as main effects and their GxE interactions. Maltreatment consistently was associated with higher CDI and TRF symptoms. Results for child self-report symptoms indicated a GxE interaction for BDNF and maltreatment. Additionally, BDNF and tri-allelic 5-HTTLPR interacted with child maltreatment in a GxGxE interaction. Analyses for counselor-report of child anxiety/depression symptoms on the TRF indicated moderation of child maltreatment effects by tri-allelic 5-HTTLPR. These effects were elaborated based on variation in developmental timing of maltreatment experiences. NET was found to further moderate the GxE interaction of 5-HTTLPR and maltreatment status revealing a GxGxE interaction. This GxGxE was extended by consideration of variation in maltreatment subtype experiences. Finally, GxGxE effects were observed for the co-action of BDNF and the CRHR1 haplotype. The findings illustrate the variable influence of specific genotypes in GxE interactions based on variation in maltreatment experiences and the importance of a multi-genic approach for understanding influences on depression and internalizing symptoms among African-American children.

Child maltreatment represents a pathogenic relational environment that strikes at the core of children's stage-salient developmental issues and confers major risk for maladaptation across both biological and psychological domains of development. (Cicchetti & Lynch, 1995; Cicchetti & Toth, 1995, in press). The deleterious sequelae of child maltreatment not only result in adverse outcomes during childhood, but also often initiate a negative

developmental cascade that continues throughout the lifespan (Cicchetti & Tucker, 1994; Masten & Cicchetti; 2010).

Among the consequences of child maltreatment are diverse forms of psychopathology, including depression and internalizing problems (Cicchetti & Toth, in press; Cicchetti & Valentino, 2006; Toth, Manly, & Cicchetti, 1992; Widom, Dumont, & Czaja, 2007). Consistent with the developmental psychopathology principle of multifinality (Cicchetti & Rogosch, 1996), all maltreated children do not develop depression and internalizing problems. Thus, it is essential to discover and comprehend the processes and mechanisms that contribute to the development of depression and internalizing psychopathology in maltreated children.

In order to comprehend the pathways from child maltreatment to depression and internalizing psychopathology in their full complexity, it is important to examine systems operating at multiple levels of analysis (Cicchetti, 2006; Cicchetti & Dawson, 2002; Cicchetti & Toth, 2009). For over a decade, the molecular genetic level of analysis increasingly has been incorporated into studies examining the effects of stress and childhood adversity on the development of depression and internalizing psychopathology.

Gene variants may contribute to risk for depression in a number of ways. For example, such effects may be independent from maltreatment's effects on depression and constitute a genetic risk factor. In addition, because maltreated children often develop depression and internalizing psychopathology, there may be additive main effects of genes and of maltreatment on depression and internalizing psychopathology. Genes also may modify the developmental response to maltreatment experiences. Furthermore, maltreatment may intensify a main effect of genetic risk (i.e., maltreatment moderates genetic risk as in diathesis-stress theory (Gottesman & Shields, 1972). Alternatively, genetic variation may modify the main effect of the environmental pathogen (i.e., gene as moderator that is reflective of a protective effect of genetic variation). Finally, genetic variation may have opposite effects on outcome depending on the nature of the environmental pathogen, i.e., cross-over interactions as exemplified in differential susceptibility theory (Belsky & Pluess, 2009; Ellis et al., 2011).

Research in molecular genetics suggests that maltreated children's risk for psychopathology, including depression and internalizing problems, is not inevitable. GxE occurs when the effect of exposure to an environmental pathogen on a behavioral, health, or biological phenotype is conditional upon a person's genotype or, conversely, when the genotype's effect is moderated by the environment (Moffitt, Caspi, & Rutter, 2005). Reciprocal coactions between the environment and the individual result in differential expression of genetic material. Environmental conditions may interact with an individual's genetic constitution to alter processes such as the timing of the initiation of transcription and translation for a specific gene, the direction for which it does so, or whether the gene will ultimately be expressed (Grigorenko & Cicchetti, 2012; Meaney, 2010; Szyf & Bick, 2013).

Caspi et al. (2003) examined the prospective link between maltreatment and depression. In an ancestrally homogenous Caucasian sample, these investigators found that genetic

variation in a functional polymorphism (5-HTTLPR) in the promoter region of the serotonin transporter gene (5-HTT) plays a moderating role. Adults carrying the S allele (S/S or S/L) exhibited more depressive symptoms, diagnosable depression, and suicidality in response to stressful life events than individuals homozygous for the L (L/L) allele. Additionally, an examination of early life stress showed that a history of child maltreatment predicted depression in adulthood, but only among S carriers.

We next selectively review GxE research on maltreated children, adolescents, and adults. We focus on studies that examined the most commonly studied candidate genes in GxE research on child maltreatment and depressive symptomatology, namely 5-HTTLPR and corticotropin releasing hormone receptor gene (CRHR1).

In an ancestrally heterogeneous sample, Kaufman et al. (2004) found that maltreated children with the S/S genotype of 5-HTTLPR evinced depression scores that were almost twice as high as the depression scores of maltreated children with the S/L or L/L genotypes. In a subsequent multi-genic investigation, Kaufman et al. (2006) replicated the findings of their earlier study. Kaufman et al. (2006) also found a significant three-way GxGxE interaction. Specifically, the interaction among brain derived neurotrophic factor (BDNF), 5-HTTLPR, and maltreatment predicted heightened levels of depression scores. In another instance of GxGxE, Cicchetti, Rogosch, and Sturge-Apple (2007) examined an ancestrally heterogeneous sample and found that adolescents with a history of sexual abuse who carried the S/S genotype and the monoamine oxidase A (MAOA) low-activity genotype exhibited higher levels of depressive symptomatology than sexually abused adolescents with alternative combinations of the variants of the 5-HTTLPR and MAOA genes.

Banny, Cicchetti, Rogosch, Oshri, and Crick (2013) examined child maltreatment, peer victimization, and 5-HTTLPR as predictors of depressive symptomatology. Path analyses revealed that both relational and overt victimization mediated the association between child maltreatment and depressive symptoms. Bootstrapping procedures used to test moderated-mediation demonstrated that genotype moderated the indirect effects of relational and physical victimization on child depressive symptoms, such that victimized children with the L/L genotypic variant of 5-HTTLPR were at increased risk for depressive symptoms compared to victimized children carrying a short allele.

A number of investigations have demonstrated that individuals who had been maltreated possessed a significantly greater risk of experiencing suicidal behavior (i.e., suicidal ideation, suicide attempts, completed suicide) than comparable samples of non-maltreated persons from the same socioeconomic status. These studies have demonstrated that the experience of sexual and physical abuse are risk factors for suicidal behaviors in adolescents and adults (Brodsky & Stanley, 2008). Cicchetti, Rogosch, Sturge-Apple, and Toth (2010) investigated whether genotypic variation of 5-HTTLPR moderated the effect of maltreatment on suicidal ideation in an ancestrally heterogeneous sample of low-income maltreated and nonmaltreated school-age children. Higher suicidal ideation was found among maltreated school-age children. Higher suicidal ideation was found among maltreated than non-maltreated children. Children with one to two maltreatment subtypes and S/S or S/L genotypes had higher suicidal ideation than those with the L/L genotype;

suicidal ideation did not differ in non-maltreated children or in children with three to four maltreatment subtypes based on 5-HTTLPR variation. Results were applicable to emotionally maltreated/neglected and to physically abused/sexually abused children. Interestingly, for the more extensively maltreated (i.e., those with three to four subtypes), children expressed higher levels of suicidal ideation, irrespective of genetic variation. Thus, the pathogenic relational environment of children who experienced extensive maltreatment appears to have predominated over genotype variation in the risk for, or protection against, suicidal ideation.

In a final illustration of 5-HTTLPR as a moderator of depression in maltreated children, Uher et al. (2011) conducted a prospective longitudinal investigation of GxE interaction in two large ancestrally homogeneous samples, one in New Zealand, and one in England. The former sample was followed until age 32, the latter until age 40. The prospective nature of this study is unique in that the majority of studies of 5-HTTLPR $\times$ Maltreatment on depression have been cross-sectional. (For some exceptions, see Caspi et al., 2003; Cutuli, Raby, Cicchetti, Englund & Egeland, 2013). Uher and colleagues (2011) found that, in both longitudinal cohorts, statistical analyses of GxE interactions revealed positive results for depression that runs a persistent course in adulthood, but not for single-episode depression. Individuals with the SS 5-HTTLPR genotype and child maltreatment history had elevated risk of persistent but not single-episode depression.

Bradley et al. (2008) conducted an ancestrally homogeneous study of a predominately (97.4%) African American sample to test the hypothesis that genetic polymorphisms that alter the functionality of CRHR1 may moderate the effects of child maltreatment on adult depression. Bradley and colleagues (2008) conducted a study to test the hypothesis that genetic polymorphisms that alter the functionality of CRHR1 may moderate the effects of child maltreatment on adult depression. These investigators found that GxE interaction was important for the expression of depressive symptoms, or lack thereof, in adults who possess CRHR1 risk or protective alleles in conjunction with a history of child maltreatment. Specific CRHR1 polymorphisms appeared to moderate the effect of child abuse on the risk for adult depressive symptoms. These protective effects were substantiated with similar results in a second independent sample of Caucasians.

The findings of Bradley et al. (2008) underscore the importance of taking environmental experiences into account in genetic association and linkage studies that otherwise might miss many important genetic variants that are involved in the etiology of complex diseases. An extension and partial replication of the Bradley et al. (2008) study was conducted by Polanczyk et al. (2009). As in the investigation of Bradley et al. (2008), a CRHR1 haplotype was shown to exert a protective effect against depression in adults who were maltreated in their childhood; however, the replication only occurred when a retrospective, but not a prospective, measure of child maltreatment was used. The authors speculated that the protective effect of the CRHR1 haplotype was likely related to its function in consolidating memories of emotionally arousing experiences.

DeYoung, Cicchetti, and Rogosch (2011) examined the influence of CRHR1 variation on neuroticism, in interaction with child maltreatment, in a large ancestrally heterogeneous

sample of maltreated children and a well-matched non-maltreated comparison group. Neuroticism, one of the Big Five personality characteristics, is a risk factor for mood and anxiety disorders. The biological systems involved in neuroticism are thus of great import in the etiology of internalizing disorders. Genes that are involved in the systems that are stress responsive, such as CRHR1, are thus important candidates for studies of the genetic moderation of the effects of major stressors like maltreatment.

DeYoung et al. (2011) found that the CRHR1 TAT haplotype significantly moderated the association of child maltreatment with neuroticism. Having two copies of the TAT haplotype of CRHR1 was associated with higher levels of neuroticism among maltreated children relative to non-maltreated children, with the exception of sexually abused children and children who had experienced three or four types of maltreatment. The findings of this study are also important because they contribute to a growing body of evidence that variation in the CRHR1 gene moderates the effects of child maltreatment on affective functioning.

Research reviewed above shows that GxE interactions mediating depressive symptomatology have been identified in both stress-sensitive serotonergic (5-HTTLPR) and cortico-releasing-hormone (CRHR1) systems. In an investigation of an ancestrally homogenous sample of African Americans from low-socioeconomic stress backgrounds, Ressler et al. (2010) sought to determine whether the effects of child maltreatment are moderated by GxGxE interactions between CRHR1 and 5-HTTLPR polymorphisms. Ressler et al. (2010) first replicated the interaction of child maltreatment and 5-HTTLPR on lifetime major depressive disorder. They next replicated earlier work in their laboratory by Bradley et al. (2008) and Polanczyk et al. (2009), again finding that an interaction between a CRHR1 haplotype and child maltreatment predicted greater depressive symptoms. In addition, Ressler et al. (2010) discovered that a G (5-HTTLPR, S allele) $\times$ G (CRHR1 haplotype) interaction with child maltreatment predicted heightened current depressive symptoms.

In the present multi-genic investigation, we examined GxE and GxGxE to test whether variation in these genes moderates the influence of child maltreatment on depressive symptomatology and internalizing problems. We chose to include four candidate genes that have been shown to be of interest to research on depressive symptomatology and/or internalizing problems: the serotonin transporter gene – linked polymorphic region (5-HTTLPR), the corticotropin releasing hormone receptor 1 (CRHR1), brain derived neurotrophic factor (BDNF), and the norepinephrine transporter (NET). Below, we discuss why each of these genes is worthwhile for investigation.

1. 5-HTTLPR The neurotransmitter serotonin has been shown to be involved in the development of diverse forms of psychopathology, including anxiety problems and depression (Cicchetti & Toth, 1995; McGrath, Weill, Robinson, MacRae, & Smoller, 2012). Differences in the promoter region of the serotonin transporter gene (5-HTTLPR) have been conceptualized as a marker for a stress-vulnerable phenotype through the contribution of 5-HTTLPR on serotonin functioning (Cutuli et al., 2013). The serotonin transporter has received a significant amount of

attention because it is involved in the reuptake of serotonin at brain synapses (Caspi et al., 2003). There are two forms of a functional insertion/deletion polymorphism that have been studied with respect to psychopathological outcomes: a 16 unit repeat “long” L form and a 14 unit repeat “short” S form (bi-allelic 5-HTTLPR). The short variant has been linked to decreased transcriptional efficiency and reduced levels of serotonin (Caspi et al., 2010). There also is an A/G single-nucleotide polymorphism in the long form (rs25531) (tri-allelic 5-HTTLPR). The more common L_A allele is associated with the reported higher basal activity, whereas the less common L_G allele has transcriptional activity no greater than the S. Thus, L_G carriers are considered effectively to be S carriers with functioning similar to the short form (Hu et al., 2005). Given the links with the serotonin system, and links between reduced serotonin functioning and depression, 5-HTTLPR polymorphisms are a good candidate gene for etiological models of depressive symptomatology and internalizing problems.

2. BDNF is expressed in the brain. It is active in the hippocampus, cerebral cortex, and the basal forebrain. Additionally, BDNF is found in diverse tissue and cell types. Despite the fact that the majority of neurons in the human brain are formed prenatally, areas of the adult brain retain the ability to grow new neurons through the process of neurogenesis. BDNF is one of the most active neurotrophins that stimulate and control neurogenesis. Most research of the role of the BDNF genotype in moderating early adversity has focused on risk for depression (Aguilera et al., 2009; Gunnar et al., 2012; Kaufman et al., 2006) or on depression endophenotypes).
3. CRHR1 – This gene has been shown to be a viable candidate gene that influences vulnerability to depression. Corticotropin-releasing hormone is the key activator of the hypothalamic-pituitary-adrenal (HPA) axis, binding to receptors that initiate the stress response, culminating with release of cortisol from the adrenal cortex. Over-activity of the HPA-axis has been shown to be partially caused by hyperactivity of CRH neurons. Additionally, CRH activity at the CRH type 1 receptor (CRHR1) in extra-hypothalamic regions also are thought to bring about internalizing disorders, such as depression and anxiety (Bradley et al., 2008). Published investigations to date suggest that atypical activity of the HPA axis may be a function of early life stress (Cicchetti, Rogosch, Gunnar & Toth, 2010; Heim, Newport, Mletzko, Miller, & Nemeroff, 2008).
4. NET (also known as SLC6A2) – This SNP is located on the gene that encodes for the norepinephrine transporter. These transporters are located along the cell bodies, axons, and dendrites of the noradrenergic neurons. Animal models have shown that NET is related to fear and anxiety. Human studies reveal that females who have ADHD often exhibit more comorbid anxiety disorders than males.

We chose to conduct this investigation with an ancestrally homogenous population of African American children from low socioeconomic backgrounds. There are challenges inherent to conducting molecular genetic research with low income maltreated populations. There is great racial diversity and high minority representation. The vast majority of

molecular genetic research has been conducted with Caucasian samples (Odgerel et al., 2013). The results of this molecular genetic research may not generalize to African Americans, given known variation in allelic distributions in different ancestral groups. Molecular genetic research conducted with African Americans by Brody and Beach (Bradley et al., 2008, 2011), and (Brody et al., 2013, 2014), and Ressler et al., (2010) are important exceptions to the paucity of genetic research with African Americans. In order to understand the contributions that genetic variation may make to depressive symptomatology and internalizing problems in African American children, it is essential that research of this nature be conducted.

Hypotheses

1. We expect genetic variation in 5-HTTLPR, CRHR1, BDNF, and NET to moderate the effects of child maltreatment on depressive symptomatology and internalizing problems in African-American children.
2. We expect to obtain interactions among multiple genes and the environmental pathogen of child maltreatment (GxGxE) to further heighten and explain the effects of child maltreatment on depressive symptomatology and internalizing problems.

Method

The participants in this investigation included 1,096 African-American children aged seven to twelve (M age = 10.24, SD = 1.38) who attended a summer camp research program designed for school-aged low-income children. The sample was comprised of both maltreated children (n = 545) and nonmaltreated children (n = 551). The subsample of African-American children was drawn from a larger racially and ethnically diverse sample of camp participants that included Caucasian, Latino, and other groups. African-American children were selected in order to have a uniform ancestral group for genetic analysis purposes and because of limited genetic research on African-American samples during the school-age years. The maltreated and nonmaltreated children did not differ significantly in age, $t(1094) = 1.04$, ns). Among the participants, 49.5 % were girls. The gender distribution evidenced a difference between the maltreated and nonmaltreated groups, $\chi^2(1, N = 1096) = 5.01$, $p = .03$, with 52.8% girls in the maltreated group and 46.1% girls in the nonmaltreated group. Gender was controlled in subsequent analyses. The families of the children were low income, with over 97% of the families in both the maltreated and nonmaltreated groups having a history of receiving public assistance benefits. Marital status in the families of the two groups was not significantly different, $\chi^2(6, N = 1026) = 10.69$, $p = .10$. Nonmarried mothers headed 83.5% of the families.

Recruitment and Classification Procedures

Parents of all maltreated and nonmaltreated children provided informed consent for their child's participation, as well as consent for examination of any Department of Human Services (DHS) records pertaining to the family. The research was approved by the Research Subjects Review Board of the University of Rochester. Children in the maltreated group had been identified by the county DHS as having experienced child abuse and/or

neglect, and the sample was representative of the children in families receiving services from the DHS. A recruitment liaison from DHS contacted eligible maltreating families, explained the study, and if parents were interested, then their names were released to the project team for recruitment. Families were free to choose whether or not to participate. Comprehensive searches of DHS records were completed, and maltreatment information was coded utilizing operational criteria from maltreatment nosology specified in the *Maltreatment Classification System* (MCS: Barnett, Manly, & Cicchetti, 1993), as discussed below.

Consistent with national demographic characteristics of maltreating families (National Incidence Study – NIS-4; Sedlak et al., 2010), the maltreated children were predominantly from low socioeconomic status families. Consequently, demographically comparable nonmaltreated children were recruited from families receiving Temporary Assistance for Needy Families (TANF). A DHS recruitment liaison contacted eligible nonmaltreating families, described the project, and if interested, parents signed a release for their names to be given to the project for recruitment. DHS record searches were completed for these families to verify the absence of any record of child maltreatment. Trained research assistants also interviewed mothers of children recruited for the nonmaltreatment group to confirm a lack of DHS involvement and prior maltreatment experiences. Subsequently, record searches were conducted in the year following camp attendance to verify that all available information had been accessed. Only children from families without any history of documented abuse or neglect were retained in the nonmaltreatment group. In addition, families who had received preventive services through DHS due to concerns over risk for maltreatment were excluded from the sample to reduce the potential for unidentified maltreatment existing within this group.

The MCS is a reliable and valid method for classifying maltreatment (Bolger, Patterson, & Kupersmidt, 1998; English et al., 2005; Manly, 2005) that utilizes DHS records detailing investigations and findings involving maltreatment in identified families over time. Rather than relying on official designations and case dispositions, the MCS codes all available information from DHS records, making independent determinations of maltreatment experiences. Based on operational criteria, the MCS designates all of the subtypes of maltreatment children have experienced (i.e., neglect, emotional maltreatment, physical abuse, sexual abuse). Coding of the DHS records was conducted by trained research assistants, doctoral students, and clinical psychologists. Coders were required to meet acceptable reliability with criterion standards before coding actual records for the study; weighted κ 's with the criterion ranged from .86 to .98. Reliabilities (κ 's) for the presence vs. absence of maltreatment subtypes ranged from .90 to 1.00.

In terms of the subtypes of maltreatment, *neglect* involves failure to provide for the child's basic physical needs for adequate food, clothing, shelter, and medical treatment. In addition to inadequate attention to physical needs, forms of this subtype include lack of supervision, moral-legal neglect, and education neglect. *Emotional maltreatment* involves extreme thwarting of children's basic emotional needs for psychological safety and security, acceptance and self-esteem, and age-appropriate autonomy. Examples of emotional maltreatment of increasing severity include belittling and ridiculing the child, extreme

negativity and hostility, exposure to severe marital violence, abandoning the child, and suicidal or homicidal threats. *Physical abuse* involves the non-accidental infliction of physical injury on the child (e.g., bruises, welts, burns, choking, broken bones). Injuries range from minor and temporary to permanently disfiguring. Finally, *sexual abuse* involves attempted or actual sexual contact between the child and caregiver for purposes of the caregiver's sexual satisfaction or financial benefit. Events range from exposure to pornography or adult sexual activity, to sexual touching and fondling, to forced intercourse with the child.

Children in the maltreatment group all had documented histories of abuse and/or neglect. Among the maltreated children, 82.2% had experienced neglect, 56.5% had experienced emotional maltreatment, 30.3% had experienced physical abuse, and 8.1% had experienced sexual abuse. As is typical in maltreated populations (Bolger et al., 1998; Manly et al., 1994; 2001), the majority of children had experienced multiple subtypes of maltreatment. Specifically, 58.4% of the maltreated children had experienced two or more maltreatment subtypes. Among maltreated children, we derived a variable to characterize maltreatment subtype experiences. Given the overlap among subtypes and the relatively lower rates of physical and sexual abuse as compared to neglect and emotional maltreatment, we identified children who had experience neglect and/or emotional maltreatment (PNEM; 64.6%) without physical or sexual abuse versus children who had experience physical and/or sexual abuse (PASA; 35.4%). The PASA group also may have experienced neglect or emotional maltreatment.

The MCS also determines when in the course of development maltreatment events occurred, providing indices of developmental timing. Events were coded as occurring during five developmental periods, including infancy (0–18 months), toddlerhood (19–36 months), preschool (36 to 59 months), early school age (age 5 to 7), and later school age (age 8 to 12). The timing information allows for the determination of whether maltreatment occurred within each of the developmental periods. The developmental periods of *onset* of maltreatment and the *recency* of maltreatment were determined. In the current investigation, we classified maltreated children in terms of early onset (infancy through preschool, 76.3%) vs. later onset (early and later school age, 23.7%), and in terms of recency during school-age (49.9%) vs. recency prior to school-age (51.1%). The onset and recency of maltreatment variables were combined to generate *onset-recency* classifications, including early onset only (onset and recency in the infancy through preschool periods, 51.3%), early and recent (onset in the infancy through preschool periods and recency in the school age years, 25.0%) and late onset (onset and recency in the school age periods, 23.7%). These groups were compared to nonmaltreated children.

Procedure

Children attended a week-long day camp program and participated in research assessments. At the camp, children were assigned to groups of eight to ten same-age and same-sex peers; half of the children assigned to each group were maltreated. Each group was conducted by three trained camp counselors, who were unaware of the maltreatment status of children and the hypotheses of the study. Camp lasted 7 hrs/day for five days, providing 35 hours of

interaction between children and counselors. In addition to the recreational activities, after providing assent, children participated in various research assessments (see Cicchetti & Manly, 1990, for detailed descriptions of camp procedures). Trained research assistants, who also were unaware of research hypotheses and maltreatment status, conducted individual research sessions with children, in which questionnaires and other research measures were administered. Clinical consultation and intervention occurred if any concerns over danger to self or others emerged during research sessions. At the end of the week, the counselors, who had been trained extensively for two weeks prior to the camp, also completed assessment measures on individual children, based on their observations and interactions with children in their respective groups. DNA samples also were obtained from the camp participants, as described below.

Measures

The measures described below constitute a subset of assessments conducted during the research camp. The camp context and associated measurement battery provide a multi-informant, multi-perspective view of child adaptive functioning. In the current analyses, self-report and adult counselor-report measures of child depressive/internalizing symptomatology were used.

Children's Depression Inventory (CDI; Kovacs, 1982, 1992)—The CDI is a widely used self-report questionnaire to assess depressive symptomatology in school-age children. For each item, children chose from among three option statements, depicting increasing levels of depressive symptoms, in order to characterize their experiences in the past two weeks. Kovacs (1992) reports that internal consistency for the total scale has ranged from .71 to .89, and validity has been well established.

Teacher Report Form (TRF; Achenbach, 1991)—Behavioral symptomatology was evaluated at the end of each week by counselors' completion of the TRF. The TRF is a widely used and validated instrument to assess behavioral disturbance from the perspective of teachers, and the measure was used in the present study, because camp counselors are able to observe similar behaviors to that of teachers. The TRF, containing 118 items rated for frequency, assesses two broadband dimensions of child symptomatology, externalizing and internalizing, as well as total behavior problems. In the current analyses, we focus on the Anxiety/Depression subscale. In the present study, interrater reliability for the internalizing scale based on average intraclass correlations among pairs of raters was .70. The counselors' scores for each child were averaged to obtain individual child scores.

DNA Collection, Extraction, and Genotyping

Trained research assistants obtained DNA samples from participants by collecting buccal cells using the Epicentre Catch-All Collection Swabs or by collecting saliva using the Oragene DNA Self-Collection kits. For buccal cells, DNA was extracted and prepared for polymerase chain reaction (PCR) amplification using the Epicentre BuccalAmp DNA Extraction Kit (Epicentre, Cat. No. BQ090155C). For saliva samples, DNA was purified from 0.5 ml of Oragene-DNA solution using the DNAgenotek protocol for manual sample purification using prepIT-L2P. Sample concentrations were determined using the Quant-iT

PicoGreen dsDNA Assay Kit (P7589, Invitrogen). Genotyping was then performed using established protocols. SNP genotyping was conducted using Applied Biosystems Custom Taqman SNP Genotyping Assays. The products of these analyses were then analyzed using endpoint allelic discrimination. Genotypes were identified and sequenced with the Beckman-Coulter CEQ8000 semiautomated fluorescent sequencing system, which utilizes Fragment Analysis Application and associated software. All samples were genotyped twice for quality control. Human DNA from cell lines were purchased from Coriell Cell Repositories for each genotype and used as control samples using DTCS chemistry on an ABI 3130xl. These cell lines and a no template control were run with study samples representing 9% of the total data output. Samples that were not able to be genotyped to a 95% or greater confidence level were repeated under the same procedures up to four times.

Call rates for individual SNP determinations ranged from 96.5 to 100%. Genotype distributions for all SNPs included in the analyses are presented in Table 1. All genetic polymorphisms were in Hardy-Weinberg equilibrium.

Serotonin Transporter (5-HTTLPR: Biallelic and Triallelic)

The 5-HTT gene has a polymorphism in the linked polymorphic region (5-HTTLPR) in the 5' regulatory region due to a 44-base pair deletion that eventuates in either the short (s) or long (l) allele (Lesch et al., 1996). 5-HTTLPR samples were genotyped for fragment length polymorphisms of 5-HTTLPR with Hot Star Taq PCR Mix (Qiagen, Catalog No. 203205) and previously described primers (Gelernter, Kranzler, & Cubells, 1997), followed by fragment analysis using a CEQ 8000 (Beckman-Coulter, Inc.).

SNP Genotyping for 5-HTTLPR (rs25531)—The SNP located within the 5-HTTLPR L/S region, rs25531 (NC_000017.11:g.30237328T>C), was genotyped using previously reported TaqMan probes². Individual allele determinations were made using TaqMan Genotyping Master Mix (Life Technologies, Catalog 4371357) with amplification on a GeneAmp 9700 (Applied Biosystems) and analyzing the endpoint fluorescence using a Tecan M200 and data analyzed with JMP 10.0 (SAS, Inc.). Human DNA from cell lines was purchased from Coriell Cell Repositories for all representative genotypes in duplicate and genotypes confirmed by sequencing using DTCS on an ABI 3130xl. These and no template controls were run alongside study samples representing 9% of the total data output. Any samples that were not able to be genotyped to a 95% or greater confidence were repeated under the same conditions. Hu et al., (2005).

Fragment length polymorphism genotyping for 5HTTLPR—Human Genomic DNA was collected using the Buccal Amp Kit (Epicentre, Cat. No. BQ0901SSC) and amplified using the Repli-g kit (Qiagen, Catalog No. 150043) per the kit instructions. Amplified samples were then diluted to a working concentration and PCR amplified with HotStar Taq PCR Mix (Qiagen, Cat. No. 203205) and previous described primers¹, followed by fragment analysis using a CEQ8000 (Beckman-Coulter, Inc.). (Gelernter, Kranzler & Cubells, 1997).

Brain Derived Neurotrophic Factor (BDNF)

SNP Genotyping for BDNF (rs6265, rs4923461)—Amplified samples were then diluted to a working concentration and genotyped for 2 in the BDNF gene rs6265 (C_000011.10:g.27658369C>T) and rs4923461 (NC_000011.10:g.27635363A>G) using TaqMan SNP genotyping assay C_11592758_10 and C____50562_10 (Applied Biosystems, Inc) respectively. Individual allele determinations were made using TaqMan Genotyping Master Mix (Life Technologies, Catalog 4371357) with amplification on a GeneAmp 9700 (Applied Biosystems) and analyzing the endpoint fluorescence using a Tecan M200 and JMP 10.0 (SAS, Inc.). Human DNA from cell lines was purchased from Coriell Cell Repositories for all representative genotypes in duplicate using previously reported genotyped from NCBI. These and no template controls were run alongside study samples representing 9% of the total data output. Any samples that were not able to be genotyped to a 95% or greater confidence were repeated under the same conditions.

SNP Genotyping for BDNF (rs925946, rs7103411)—Repli-g amplified samples were then diluted to a working concentration and genotyped for 2 in the BDNF gene rs925946 (NC_000011.10:g.27645655T>G) and rs7103411 (NC_000011.10:g.27678578C>T). DNA from study subjects was submitted to the BioMedical Genomics Center at the University of Minnesota for quantity and quality testing then subsequent SNP genotyping. Sample quantity was measured using a non-allelic real-time PCR reaction and a standard TaqMan probe and DNA quantity measured using the Quant-iT PicoGreen dsDNA Assay Kit (P11496, Life Technologies). Once samples were determined to be of sufficient quantity and quality, they were subjected to single base primer extension (SBE) with fluorophore labeled nucleotides from primers designed for SNPs of interest. Genotyping was then carried out on the iPLEX platform from Sequenom Bioscience, Inc. using the Sequenom MassArray by MALDI-TOF. Duplicate samples were used to ensure reproducibly and no template controls were run alongside study samples representing 2% of the total data output.

The frequencies of the minor homozygote genotype for BDNF SNPs rs7103411 and rs4923461 were very rare. (See Table 1). Accordingly, genotype groups combining the minor homozygote with the heterozygote genotype were used in analyses (rs7103411: TT vs. TC or CC; rs4923461: AA vs. AG or GG).

Corticotropin Releasing Hormone 1 (CRHR1)

CRHR1 was genotyped using assays for SNPs rs110402, rs242924, and rs7209436 purchased from Applied Biosystems, Inc. (ABI) as C2544843 10, C2257689 10, and C1570087 10, respectively. Individual allele discriminations were made using Taq Man Genotyping Master Mix (Applied Biosystems, Inc. (ABI), Catalog No. 4371357) with amplification in an ABI 9700 thermal cycler and analyzing the endpoint fluorescence using a Tecan M200

Arlequin v3.5.1.3 was used to form haplotypes using a pseudo-Bayesian approach to estimate phase (Escoffier & Lischer, 2011). All samples were haplotyped with greater than 98% confidence, with the exception of four samples, which were subsequently excluded from analyses. Over 92% of the sample was represented by either the TAT or CGG

haplotype. The distribution of copies for the TAT haplotype, as shown in Table 1, was 0 copies (52.8%), 1 copy (40.0%), and 2 copies (7.2%).

Norepinephrine Transporter (NET)

SNP Genotyping for SLC6A2 (rs168924) (i.e., NET – 1014A/G)—Amplified samples were then diluted to a working concentration and genotyped for rs168924 a SNP in the SLC6A2 gene which has also been identified in literature as the NET – 1014A/G polymorphism. This SNP is located at NC_000016.10:g.55655632A>G and was genotyped using C____581568_10 (Life Technologies, Inc.). Individual allele determinators were made using TaqMan Genotyping Master Mix (Life Technologies, Catalog 4371357) with amplification on a GeneAmp 9700 (Applied Biosystems) and analyzing the endpoint fluorescence using a Tecan M200 and JMP 10.0 (SAS, Inc.). Human DNA from cell lines was purchased from Coriell Cell Repositories for all representative genotypes in duplicate using previously reported genotypes from NCBI. These as no template controls were run alongside study samples representing 9% of the total data output. Any samples that were not able to be genotyped to a 95% or greater confidence were repeated under the same conditions.

Because of the low frequency of the GG genotype ($n = 74$), it was combined with the AG heterozygote, and compared to the AA group in statistical analyses.

Ancestral Proportion Determinations

DNA from the study participants was submitted to the BioMedical Genomics Center at the University of Minnesota for quantity and quality testing and subsequent SNP genotyping. Acceptable samples (99.0%, 10 cases were excluded) were subjected to SNP genotyping of the Burchard et al. panel of 106 SNPs (Lai et al., 2009; Yaeger et al., 2008), known to be informative for ancestry from Africa, Europe, and Native America. The SNPs were genotyped using the iPLEX platform from Sequenom Bioscience, Inc which uses the Sequenom MassArray. The SNP genotyping results were then recoded and uploaded into STRUCTURE v2.3.4 which uses algorithms developed by Pritchard et al. (Falush Stephens, & Pritchard, 2003, 2007; Hubisz, Falush, Stephens, & Pritchard, 2009). Three SNP tests were excluded based on high allele call rates of the non-DNA containing wells. The data from the remaining 103 loci were uploaded into the software and set to analyze with an Admixture model of ancestry and initialization of the simulation on the GALA cohort. The simulation was set to run with a Burn-in of 10,000, MCMC Reps of 1,000 and assuming 3 populations within the group. The results of the simulations were subsequently identified as percent association to each ancestry group, African, Native American, and European, based on the known ancestry of the GALA cohort.

Logistic regression procedures were used to classify individuals into distinct ancestral groups, utilizing the continuous proportion scores of the ancestrally important markers to predict parent-reported child race in the larger sample of camp participants. The resulting classification for the African-American group used in this study ($n = 1,096$) was highly homogeneous for the African ancestral markers, $M = .92$, $SD = .10$.

Results

Plan of Analysis

We examined a series of models to investigate the coactions of genetic variants and maltreatment status and associated maltreatment parameters in relation to child self-report of depressive symptoms (CDI total scores) and adult-report of child anxiety/depression symptoms (TRF anxiety/depression subscale). Allelic variants of SNPs linked to the serotonin transporter, brain derived neurotrophic factor, norepinephrine transporter, and corticotropin releasing hormone receptor 1 genes were evaluated. Analyses for each genetic variant involved ANCOVAs, with gender and child age included as covariates. Gender was controlled because of the significant difference in gender composition between the maltreated and nonmaltreated samples, $p = .03$, as indicated above). Gender correlated, $r = .07$, $p = .02$, with CDI scores, indicating higher scores among boys relative to girls. Gender was not associated with anxiety/depression TRF symptoms, $r = -.01$, *ns*. Age was correlated with CDI, $r = -.09$, $p = .002$, and TRF anxiety/depression, $r = -.07$, $p = .02$, with younger children self-reporting and being rated as exhibiting higher symptomatology. In the ANCOVA models, the genetic variant and maltreatment status were included as main effects, along with their interaction. Additional analyses involving maltreatment parameters were considered when cell sizes were sufficient to undertake such analyses. Bonferroni-corrected contrasts were used in follow-up analyses to identify significant group differences and the pattern of GxE interaction effects. Additionally, analyses involving two gene systems were evaluated for GxGxE interaction effects.

Following the recommendations of Keller (2014), rather than control for gender as a covariate, we initially evaluated models that included gender main effects and interactions with maltreatment status and with genotypes. Except for one analysis, all of these gender interaction effects were nonsignificant and results involving other main effects and interaction effects were consistent with those without the gender interaction. Accordingly, for parsimony and because of a lack of hypotheses about gender interactions, we maintained gender as a covariate in all models. However, in the analysis of BDNF, tri-allelic 5-HTTLPR, and maltreatment status, which did have a significant gender interaction, we maintained all gender interactions in the model, as presented below.

Initial analyses indicated that bi-allelic 5-HTTLPR in models of CDI and TRF anxiety/depression symptoms did not result in significant G or GxE effects. However, important findings were found when tri-allelic 5-HTTLPR was examined, and results with tri-allelic 5-HTTLPR are reported.

Evidence for rGE effects

For each of the genotypes determined for each of the targeted genes, bi-allelic 5-HTTLPR, tri-allelic 5-HTTLPR, BDNF SNPs, NET, and CRHR1 TAT haplotype comparisons were conducted between the distribution of genotype groups in the maltreated and nonmaltreated samples with χ^2 tests. These comparisons are shown in Table 2. All maltreatment group differences were nonsignificant, indicating that maltreatment status was not associated with genetic variation in any of the respective genes.

Models for Children's Depression Inventory Outcomes

First, BDNF SNP rs7103411 was examined in an ANCOVA model for CDI symptoms. After controlling for covariates, although the main effects for the BDNF, $F(1, 1037) = 1.31$, $p = .25$, and for maltreatment status, $F(1, 1037) = .99$, $p = .32$, were nonsignificant, the GxE interaction was significant, $F(1, 1037) = 4.48$, $p = .03$. As shown in Figure 1, for children with the TT genotype, maltreated children had significantly higher depressive symptoms than nonmaltreated children, whereas the difference between maltreated and nonmaltreated children was not significant in the TC-CC group. Among nonmaltreated children, no differences in depressive symptoms were found for the two BDNF genotype groups. In contrast, among maltreated children, those with the TT BDNF genotype had significantly higher symptoms than those with the TC-CC genotype.

A similar analysis was conducted with the rs49233461 BDNF SNP. Neither G nor GxE effects were significant. However, we further examined this BDNF SNP in GxGxE analyses with tri-allelic 5-HTTLPR, given the precedence in the literature for GxGxE effects of 5-HTTLPR, BDNF, and maltreatment history (cf., Kaufman et al., 2006). This was the one analysis in which gender interactions were observed. In this ANCOVA analysis, after controlling for age and including gender main effects and interactions, with CDI scores as the dependent variable, a significant main effect for maltreatment status was observed, $F(1, 1043) = 8.92$, $p = .003$, with maltreated children having higher scores than nonmaltreated children. Other significant main and main effects and two-way interactions were nonsignificant. However, a significant three-way interaction of maltreatment, gender, and 5-HTTLPR was observed, $F(2, 1043) = 3.27$, $p = .01$. More importantly, the GxGxE three-way interaction was significant, $F(2, 1043) = 3.00$, $p = .05$. Analysis of this three-way interaction is depicted in Figures 4a, 4b, and 4c. These figures show the pattern of the interaction effects of maltreatment status and the BDNF SNP separately for the 3 tri-allelic 5-HTTLPR genotypes. For children with the tri-allelic 5-HTTLPR SS genotype (Figure 2a), within BDNF genotype groups, among children with AG or GG genotypes, maltreated children had significantly higher CDI scores than nonmaltreated children. Among children with AA genotypes, maltreatment group differences were nonsignificant. Further, among maltreatment status groups, for nonmaltreated children, those with the AA BDNF genotype had significantly higher CDI scores than those in the AG_GG genotype group.

A different pattern emerged for children with the tri-allelic 5-HTTLPR SL genotype (Figure 2b). For children with AA genotypes, maltreated children had significantly higher CDI symptoms than nonmaltreated children; differences were not significant for maltreatment groups for those with AG-GG genotypes. Additionally, differences among nonmaltreated and among maltreated children were not significantly different depending on whether they had AA or AG-GG BDNF genotypes.

Finally, for children with the tri-allelic 5-HTTLPR LL genotype (Figure 2c), the only significant group contrast was for children with the AA BDNF genotype, with maltreated children having higher CDI scores than nonmaltreated children.

Thus, significant differences between maltreated and nonmaltreated children in CDI scores were observed for children with SL or LL genotypes of tri-allelic 5-HTTLPR and BDNF

AA genotypes (major alleles are prominent), whereas for children with SS genotypes, maltreated children had higher CDI symptoms than nonmaltreated children among those with AG-GG BDNF genotypes (minor alleles are predominant).

Additional analyses examining the CRHR1 TAT haplotype and NET in relation to CDI symptoms did not result in G or GxE effects. However, these genetic variants were found to operate in models for TRF anxiety/depression.

Models for TRF Anxiety-Depression Outcomes

Tri-allelic 5-HTTLPR—In the first analysis, we examined tri-allelic 5-HTTLPR and maltreatment status with TRF anxiety/depression symptoms as the dependent variable. In this ANCOVA model, the effect of maltreatment status was significant, $F(1, 1087) = 11.78$, $p = .001$, whereas the effect of genotype was not, $F(1, 1087) = 1.38$, $p = .25$. However, the GxE interaction effect was significant, $F(1, 1087) = 3.43$, $p = .03$. This interaction is depicted in Figure 3. Follow up Bonferroni contrasts indicated that maltreated children had significantly higher anxiety/depression symptoms in both the LL genotype group and in the SS genotype group, whereas there was no difference among maltreated and nonmaltreated children for those with the SL genotype. Furthermore, among the nonmaltreated children, those with the SL genotype had significantly higher symptoms than those with the SS genotype. Other contrasts were not significant. Among nonmaltreated children, none of the contrasts among genotype groups was significant.

Consideration of variation in developmental timing of maltreatment experiences further elaborated the tri-allelic 5-HTTLPR $\times$ maltreatment interaction effect. In terms of onset-recency groups, after controlling for covariates, the ANCOVA resulted in a significant effect for maltreatment onset/recency group, $F(3, 1072) = 5.64$, $p = .001$, a nonsignificant genotype effect, $F(2, 107) = .13$, $p = .88$, and a significant GxE interaction, $F(6, 1072) = 3.51$, $p = .002$. See Figure 4. Follow-up Bonferroni contrasts indicated that among children with the SS genotype, children with early and recent maltreatment experiences had significantly higher symptoms than nonmaltreated children and children with only recent maltreatment. In contrast, for the LL genotype group, early only maltreatment had significantly higher symptoms than nonmaltreated children; no other contrasts were significant. As in the prior analysis with maltreatment status, among children with the SL genotype, no significant differences across onset-recency groups were observed.

When genotype differences were examined within onset-recency groups, as before, among nonmaltreated children those with SL genotypes had significantly higher symptoms on the TRF than nonmaltreated children with SS genotypes. Among children with early only maltreatment, those with LL genotypes had significantly higher symptoms than those with SL genotypes. Significant symptom differences among genotype groups were not found for children in the early and recent group and the recent only group.

Norepinephrine Transporter (NET)—Variation in the norepinephrine transporter gene (NET) was examined in relation to maltreatment status for TRF anxiety/depression scores. The initial model examining NET and maltreatment status did not indicate significant G or GxE effects. However, relations were observed when NET was added to a model including

tri-allelic 5-HTTLPR In this ANCOVA model after controlling for covariates, the main effect of maltreatment was significant, $F(1, 1063) = 12.47, p < .001$. Additionally, a three-way interaction of NET $\times$ 5-HTTLPR $\times$ maltreatment status was observed, $F(2, 1063) = 3.48, p = .03$. See Figures 5a and 5b for illustration of this interaction effect. The interaction effects were examined with Bonferroni contrasts. In Figure 5a, children with the AA genotype of NET are shown. The interactive effects of tri-allelic 5-HTTLPR and maltreatment status are presented. Maltreated children had higher symptom scores than nonmaltreated children when they had LL and the SS 5-HTTLPR genotypes. For children with SL genotypes, the maltreated and nonmaltreated children did not significantly differ.

Within maltreatment status groups, among nonmaltreated children with the SL genotype had significantly higher symptoms than those with the SS genotype; for maltreated children, those with the LL genotype had significantly higher symptom scores than those children with the SL genotype.

In contrast, relations were different for children with a minor allele of NET, AG or GG genotypes, as shown in Figure 5b. For these children there were no main effects of 5-HTTLPR or interaction effects of 5-HTTLPR and maltreatment status. The main effect of maltreatment status on symptoms for the AG-GG subgroup was significant, with maltreated children having higher symptoms than nonmaltreated children, irrespective of genetic variation.

The findings for the NET $\times$ 5-HTTLPR $\times$ maltreatment status interaction were further elaborated through consideration of maltreatment subtype effects. The ANCOVA utilizing subtype group, with NET and 5-HTTLPR genotypes resulted in a significant effect for maltreatment subtype group, $F(2, 1057) = 7.38, p = .001$, as well as significant three-way interaction of NET, 5-HTTLPR, and subtype group, $F(4, 1057) = 2.72, p = .03$. Figures 6a and 6b present this three-way interaction effect. In comparison to the analysis above with maltreatment status (Figure 5a), Figure 6a shows the interaction effect among children with the NET AA genotype and illustrates variation between the EMPN and PASA subgroups. Bonferroni contrasts indicated that among children with the SS 5-HTTLPR genotype, children in the PASA group had higher TRF anxiety/depression scores than nonmaltreated children. Among children with the LL 5-HTTLPR genotype, the EMPN group had significantly higher anxiety/depression scores than nonmaltreated children. For children with the SL 5-HTTLPR genotype, maltreatment status group differences were not significant.

As in prior analyses, among the nonmaltreated children, those with the SL genotype had significantly higher scores than those with the SS genotype. In contrast, among children in the EMPN group, those with the LL 5-HTTLPR genotype had significantly higher symptom scores than those with the SL genotype and those with the SS genotype. For children in the PASA group, difference among children in the different 5-HTTLPR genotype groups did not differ significantly.

Figure 6b shows the different effects for children with the NET AG or GG genotypes. As in the analysis with maltreatment status, contrasts indicated a significant effect overall for

subtype group, with children in the PASA group having significantly higher symptoms than nonmaltreated children. However, no subtype group contrasts were significant within 5-HTTLPR genotype groups, and no genotype group effects were significant within maltreatment subtype groups. Thus, genetic effects were not present in the subgroup of children with NET AG or GG genotypes.

Brain Derived Neurotrophic Factor (BDNF)—Analyses were conducted with BDNF SNPs rs7103411 and rs4923461 in modeling individual differences in TRF anxiety/depression scores. G, GxE, and GxGxE effects were found for these two SNPs.

Although significant G and GxE effects were not obtained for BDNF rs71043, ANCOVA analyses with BDNF SNP rs4923461 were informative. For TRF anxiety/depression symptoms, the ANCOVA indicated a main effect for maltreatment status, $F(1, 1078) = 4.24$, $p = .04$, and a marginally significant gene main effect, $F(1, 1078) = 3.23$, $p = .07$, with children having the major allele homozygote, AA, tending to have higher symptomatology than those with a minor allele, AG or GG. The GxE interaction was not significant, $F(1, 1078) = .86$, $p = .35$.

We followed up these gene main effects in ANCOVAs examining maltreatment parameters. In these analyses, significant gene main effects were observed for analyses with maltreatment subtype, $p = .01$, number of subtypes, $p = .01$, and onset-recency, $p = .03$. In each of these ANCOVAs, the gene main effect was significant, with children with the AA major allele genotype having higher scores on TRF anxiety/depression symptoms than those with a G allele.

Corticotropin Releasing Hormone Receptor 1 (CRHR1) TAT Haplotype—The CRHR1 TAT haplotype did contribute G or GxE effects to the model for TRF anxiety/depression symptoms. However, given the GxE interaction effect for maltreatment status and BDNF rs7103411 presented above, we further considered whether CRHR1 would contribute to the model in conjunction with BDNF. In the ANCOVA model for TRF anxiety/depression symptoms, after controlling for covariates, a significant main effect was found for CRHR1 haplotype, $F(2, 1036) = 3.79$, $p = .02$, as well as two significant two-way interactions: BDNF $\times$ maltreatment status, $F(1, 1036) = 12.49$, $p < .001$, and BDNF $\times$ CRHR1 haplotype, $F(2, 1050) = 3.52$, $p = .03$. However, these effects were clarified by a significant three-way, GxGxE, interaction, $F(2, 1036) = 5.61$, $p = .004$. This interaction effect is shown in Figures 7a and 7b. In figure Figure 5a, the interaction of CRHR1 TAT haplotype and maltreatment status is depicted for children with the BDNF TT genotype. Among children with two copies of the TAT haplotype, maltreated children had significantly higher anxiety/depression symptoms than nonmaltreated children. This maltreatment status effect was also significant for children with 0 copies of the TAT haplotype, but not for those with one copy. Within maltreatment status groups, there were no significant differences between haplotype groups for maltreated or for nonmaltreated children.

When children with the BDNF TC or CT genotypes were examined (Figure 5b), a very different pattern emerged. Nonmaltreated children with 2 copies of the TAT haplotype were

significantly higher in anxiety/depression symptoms than nonmaltreated children with 0 copies or 1 copy, $p = .002$. Thus, the role of CRHR1 and maltreatment is very different among children with the BDNF TT genotype vs. those with the CT or CC genotypes.

Discussion

Evidence for the experience of child maltreatment being associated with polymorphic variation in any of the genes included in this investigation was not found. Thus, the potential for rGE effects to influence the probability of children experiencing abuse and neglect was not supported. Child maltreatment had a significant independent main effect in predicting CDI depressive symptomatology and TRF anxiety/depression problems. Among the genes examined, 5-HTTLPR, BDNF (4 SNPs), CRHR1 TAT haplotype, and NET, only one SNP of BDNF (rs49233461) evidenced a main effect on TRF anxiety/depression in the context of maltreatment parameters. This was an additive effect, in that maltreatment parameters had a direct effect on symptoms; BDNF added to that effect, but did not diminish the effects of maltreatment. The remaining genes, while not significantly directly related to outcomes, were found to be variously involved in moderating the main effects of child maltreatment on depression and internalizing symptoms.

We found support for a GxE interaction with the triallelic 5-HTTLPR moderating the effect of maltreatment on TRF anxiety/depression. However, the pattern of the genotype differences was not typically reported in the literature. Specifically, both SS and LL genotypes of 5-HTTLPR were associated with greater anxiety/depression symptoms for maltreated children. For children with the SL genotype, maltreated and nonmaltreated children did not differ in symptom level. Moreover, within the group of nonmaltreated children, those with SL genotypes had significantly higher anxiety/depression symptoms than nonmaltreated children who possessed the SS genotype.

These findings demonstrate that researchers should not presume that a particular genotype is uniquely associated with plasticity. Plasticity may vary based on a number of factors, including culture, racial composition, and variation in the parameters of the risk construct (e.g., developmental timing, subtype differences, and individual differences in experiencing, or exposure to, the environmental pathogen). Furthermore, the findings of this investigation illustrate the importance of analyzing SS and SL genotypes independently. Combining them, as often is the case in studies with small sample sizes, would have missed important differences in this African American sample.

The triallelic 5-HTTLPR $\times$ Maltreatment GxE picture became expanded when maltreatment parameters were considered. We examined GxE findings for onset-recency in maltreatment groups. The effects of the various triallelic 5-HTTLPR genotypes further varied based on children's developmental experiences of maltreatment. Specifically, the highest TRF anxiety/depression symptoms were found for children in the SS genotype group who had experienced both early onset and recent maltreatment. The highest anxiety/depression symptoms were found in the LL genotype group for children who had experienced only early onset maltreatment. Finally, children who experienced only recent onset maltreatment were similar to, but not significantly different from, the nonmaltreated children, who had

their highest symptoms with SL genotypes. These results reveal that variation in the developmental timing of child maltreatment is associated with differential genetic vulnerability to anxiety/depression symptomatology. All three 5-HTTLPR genotypes were associated with greater risk for symptomatology, depending on variation in the pattern of maltreatment exposure across development.

The interaction of BDNF SNP rs7103411 and child maltreatment produced a statistically significant GxE such that maltreated children who had the TT genotype had higher depressive symptomatology on the CDI than nonmaltreated children with the TT genotype (cf. Kaufman, et al., 2004). Whereas nonmaltreated children did not differ in symptom level dependent on genotype variation, maltreated children with the TT genotype had significantly higher CDI depressive symptoms than maltreated children in the TC-CC genotype group. Thus, maltreated children who were minor allele carriers appeared protected from depressive symptoms.

In addition, we conducted a GxGxE analysis with a three-way interaction among the tri-allelic 5-HTTLPR, BDNF rs4923461, and child maltreatment in relation to depressive symptomatology on the CDI. Maltreated children with the SS genotype of the tri-allelic 5-HTTLPR and either the AG or GG genotypes of BDNF had significantly higher CDI depression scores than nonmaltreated children. Interestingly, another pattern was found for maltreated children who possessed the tri-allelic 5-HTTLPR SL genotype and the AA genotype of BDNF. These maltreated children had higher depressive symptoms on the CDI than the nonmaltreated children with the same genotypes. A similar pattern was found for maltreated children with the LL 5-HTTLPR genotype and AA genotype of BDNF. These findings further underscore the importance of examining each of the triallelic 5-HTTLPR genotypes, and illustrate variation in outcomes with consideration of multi-genic influences.

The triallelic 5-HTTLPR $\times$ BDNF $\times$ Maltreatment results bear striking similarity to those obtained by Kaufman and colleagues (2006) who reported a GxGxE interaction among the bi-allelic 5-HTTLPR, BDNF VAL66MET, and child maltreatment on child-reported depressive symptoms. Kaufman et al.'s (2006) sample was comprised of 196 children. Slightly over 100 were residing in foster care for recent (within the past six months) child maltreatment. The remainder of the participants were from comparable low-socioeconomic (SES) backgrounds, living in their natural homes, and had not experienced maltreatment. Although the sample was ancestrally heterogeneous, Kaufman and colleagues utilized ancestral informative markers (AIMS) to covary for racial differences. The environmental pathogen of child maltreatment utilized was carefully defined.

The current study was comprised of an ancestrally homogenous sample of 1096 African American 7–12 year old children. The sample enrolled was over 5 times larger than that of Kaufman et al. (2006). The tri-allelic 5-HTTLPR was utilized and a different BDNF SNP was used than in the Kaufman et al. (2006) study because of the very low rate of bi-allelic 5-HTTLPR SS genotypes (6.3%) and of Val/Met or Met/Met BDNF genotypes (5.8%) in our African-American sample. Maltreatment and nonmaltreatment status were documented using objective, reliable, and valid criteria. All children lived at home and were from low-SES families. Similar to the Kaufman et al. (2006) study which controlled for ancestral

markers, in the present study ancestral markers were used to identify a homogeneous African-American group. Despite the differences in sample size, racial composition, and contextual characteristics, our results provide confirmatory evidence for Kaufman et al.'s BDNF $\times$ (bi-allelic) 5-HTTLPR $\times$ child maltreatment three-way interaction finding on depression symptoms. The present study was not designed as a literal replication of Kaufman et al (2006), nor was our sole focus on confirming or disconfirming Kaufman et al.'s (2006) findings. Nonetheless, the similarity between the results of the present study to their GxGxE results is striking and important.

The NET and CRHR1 genes did not evince GxE effects with either TRF or CDI; however, each of these genes was found to be important in GxGxE interactions. For example, the GxGxE effect for the triallelic 5-HTTLPR, NET genotypes, and maltreatment suggests that NET moderates the GxE effects of 5-HTTLPR and child maltreatment on anxiety/depression scores. Specifically, for children with the NET AA genotype, the pattern of GxE effects were very similar to the pattern for 5-HTTLPR for the entire sample. In contrast, for children with NET AG-GG genotypes, the interactive effects of 5-HTTLPR and maltreatment were no longer observed. Thus, variation in NET influenced where 5-HTTLPR had a moderating influence on the effects of child maltreatment on anxiety/depression symptoms.

These results were further elucidated through examination of variation in children's maltreatment experiences by evaluating the effects of different subtypes of maltreatment. Again, the moderating effects of 5-HTTLPR were observed only among children with NET AA genotypes. Striking here was that the highest anxiety/depression symptoms were found among children in the emotional maltreatment/physical neglect group with LL genotypes, whereas among children with the SS 5-HTTLPR genotype, those children who had experienced physical/sexual abuse were the most symptomatic. These differential 5-HTTLPR/maltreatment subtype effects were not present among children in the NET AG-GG genotype group; only subtype appeared to influence symptom outcomes for these children.

Another pattern of GxGxE interaction was observed when the influences of CRHR1 and BDNF rs7103411 were examined together to predict TRF anxiety/depression symptoms. In isolation, neither gene was found to interact with maltreatment to influence symptom outcomes. However, when they were considered together, a significant GxGxE interaction was observed. Specifically, among children who had two copies of the CRHR1 haplotype and the TT genotype of BDNF, maltreated children had more symptoms than nonmaltreated children. Maltreated children with 0 copies of the TAT haplotype also had a significantly greater number of anxiety/depression symptoms than nonmaltreated children. In contrast, for children with the BDNF TC or CC genotypes and two copies of the TAT haplotype of CRHR1, it was the nonmaltreated children who had higher anxiety and depression scores than nonmaltreated children with 0 or 1 copy; not CRHR1 haplotype differences were observed among maltreated children with BDNF TC or CC genotypes. Thus, it appears that the co-action of both genes together was important in revealing genetic moderation of the effects of child maltreatment on symptoms and would have not been detected had the genes only been considered separately.

This multi-genic study of the genetic moderation of the effects of child maltreatment on depression and internalizing symptomatology in school-aged African American children is an important addition to the molecular genetic literature. Multi-level research such as conducted with this sample of African-American children contributes to reducing the gap in molecular genetic studies with African-American children. The ancestrally homogeneous sample was comprised of a large number of children, many of whom were in transition from preadolescence to adolescence – a developmental period characterized by marked changes in neurobiological, hormonal, psychological, and social systems and great risk for depression and internalizing psychopathology.

Another positive aspect of this investigation is that it was conceived to address issues raised by Duncan (Duncan & Keller, 2011; Duncan, Pollastri, & Smoller, 2014) and Keller (2014) regarding the current state of gene $\times$ environment research. The replication and extension of Kaufman et al. (2006) with a substantially larger sample size is important. The low-income population from which the majority of maltreated children come (NIS-4; Sedlak et al, 2010) is very racially and ethnically diverse. Keller (2014) has cautioned the need to not only covary race in GxE analyses, but also to evaluate interaction effects of race with genetic variation and race with environmental risk factors. In the present study of African-Americans with ancestral marker homogeneity, we have eliminated the effect of race interactions influencing results. Keller (2014) also suggests gender interactions should similarly be evaluated. We evaluated all such gender interactions in our analyses and found only one such effect. Thus, this analysis retained these more thorough gender interaction controls. These features of our approach all serve to decrease the probability that the present findings are false positives.

The experience of child maltreatment is a major risk factor for the development of depression and internalizing problems, even in the absence of genetic moderation. Delineation of how genetic variation influences symptomatology adds to the understanding of how psychopathology emerges in African-American children exposed to child abuse and neglect. The targeted genes we investigated influence neurotransmitter systems, stress regulation, and synaptic plasticity, thus implicating multiple components that may contribute to differential pathways to depression and internalizing outcomes. Several different patterns of GxE and GxGxE interactions emerged between maltreated and nonmaltreated African-American children. Moreover, variation in developmental timing and subtypes of maltreatment also are important considerations in understanding developmental pathways. Future multilevel research that examines gene – brain – HPA axis dysregulation in relation to depression will enable the construction of relatively homogeneous subgroups of African-American children that may show greater improvement with different types of intervention.

Differential pathways may suggest implications for preventing depression in African-American children and for intervening with individuals where depression has occurred. Depression among African-American adolescents is an important public health concern (Byck, Bolland, Dick, Asbeck, & Mustanski, 2013), and distressed African-American youth are less likely than other racial/ethnic groups to receive treatment (Carson, Cook, & Alegria, 2010; Kodjo & Audinger, 2004). Moreover, racial discrimination contributes to the development of depressive symptoms (English, Lambert, & Ialongo, 2014). Thus, cultural

considerations will be important in any intervention initiatives. Multiple prevention and intervention strategies are likely necessary. The design and implementation of effective intervention approaches will be advanced by attention to the diversity of multi-level processes involved in differential pathways to depressive outcomes among African-American children and adolescents.

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REFERENCES

- Achenbach, TM. Manual for the Teacher's Report Form and 1991 Profile. Burlington, VT: University of Vermont, Department of Psychiatry; 1991.
- Aguilera M, Arias B, Wichers M, Barrantes-Vidal N, Moya J, Villa H, Fañanás L. Early adversity and 5-HTT/BDNF genes: new evidence of gene-environment interactions on depressive symptoms in a general population. *Psychological Medicine*. 2009; 39:1425–1432. [PubMed: 19215635]
- Banny A, Cicchetti D, Rogosch FA, Crick NR, Oshri A. Vulnerability to depression: A moderated mediation model of the roles of child maltreatment, peer victimization, and genetic variation among children from low-SES backgrounds. *Development and Psychopathology*. 2013; 25:599–614. [PubMed: 23880379]
- Barnett, D.; Manly, JT.; Cicchetti, D. Defining child maltreatment: The interface between policy and research. In: Cicchetti, D.; Toth, SL., editors. *Child abuse, child development, and social policy*. Norwood, NJ: Ablex; 1993. p. 7-73.
- Bolger KE, Patterson CJ. Pathways from child maltreatment to internalizing problems: Perceptions of control as mediators and moderators. *Development and Psychopathology*. 2001; 13:913–940. [PubMed: 11771914]
- Bradley RG, Binder EB, Epstein MP, Tang Y, Nair HP, Liu W, Ressler KJ. Influence of child abuse on adult depression: Moderation by the corticotrophin-releasing hormone receptor gene. *Archives of General Psychiatry*. 2008; 65:190–200. [PubMed: 18250257]
- Brodsky B, Stanley B. Adverse childhood experiences and suicidal behavior. *Psychiatric Clinics of North America*. 2008; 31:223–235. [PubMed: 18439446]
- Brody GH, Yu T, Beach SRH, Windle M, Kogan SM. Harsh parenting and adolescent health: A longitudinal analysis with genetic moderation. *Health Psychology*. 2014; 33:401–409. [PubMed: 23668852]
- Brody GH, Yu T, Chen Y, Evans GW, Beach SRH, Windle M, Simons RL, Gerrard M, Gibbons FX, Philbert RA. Cumulative socioeconomic status risk, allostatic load, and adjustment: A prospective latent profile analysis with contextual and genetic protective factors. *Developmental Psychology*. 2013; 49:913–927. [PubMed: 22709130]
- Byck GR, Bolland J, Dick D, Ashbeck AW, Mustanski BS. Prevalence of mental health disorders among low-income African American adolescents. *Social Psychiatry and Psychiatric Epidemiology*. 2013; 48:1555–1567. [PubMed: 23385803]
- Carson N, Cook B, Alegria M. Social determinants of mental health treatment among Haitian, African American, and White youth in community mental health centers. *Journal of Health Care for the Poor and Underserved*. 2010; 21(2 Suppl):32–48. [PubMed: 20453375]
- Caspi A, Hariri A, Holmes A, Uher R, Moffitt TE. Genetic sensitivity to the environment: The case of the serotonin transporter gene (5-HTT) and its implications for studying complex diseases and traits. *American Journal of Psychiatry*. 2010; 167:509–527. [PubMed: 20231323]
- Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington HL, Poulton R. Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*. 2003; 301:386–389. [PubMed: 12869766]

- Cicchetti, D. Development and Psychopathology. In: Cicchetti, D.; Cohen, DJ., editors. Developmental psychopathology. 2nd. Vol. 1. New York: Wiley; 2006. p. 1-23.
- Cicchetti D, Dawson G. Multiple levels of analysis. Development and Psychopathology. 2002; 14:417–420. [PubMed: 12349866]
- Cicchetti, D.; Lynch, M. Failures in the expectable environment and their impact on individual development: The case of child maltreatment. In: Cicchetti, D.; Cohen, DJ., editors. Developmental psychopathology: Risk, disorder, and adaptation. Vol. 2. New York: Wiley; 1995. p. 32-71.
- Cicchetti, D.; Manly, JT. A personal perspective on conducting research with maltreating families: Problems and solutions. In: Brody, G.; Sigel, I., editors. Methods of family research: at Families risk. Vol. 2. Hillsdale, NJ: Erlbaum; 1990. p. 87-133.
- Cicchetti D, Rogosch FA. Equifinality and multifinality in developmental psychopathology. Development and Psychopathology. 1996; 8:597–600.
- Cicchetti D, Rogosch FA, Gunnar MR, Toth SL. The differential impacts of early abuse on internalizing problems and diurnal cortisol activity in school-aged children. Child Development. 2010; 25:252–269. [PubMed: 20331666]
- Cicchetti D, Rogosch FA, Sturge-Apple ML. Interactions of child maltreatment and 5-HTT and monoamine oxidase A polymorphisms: Depressive symptomatology among adolescents from low-socioeconomic status backgrounds. Development and Psychopathology. 2007; 19:1161–1180. [PubMed: 17931441]
- Cicchetti D, Rogosch FA, Sturge-Apple M, Toth SL. Interaction of child maltreatment and 5-HTT polymorphisms: Suicidal ideation among children from low SES backgrounds. Journal of Pediatric Psychology. 2010; 35:536–546. [PubMed: 19779024]
- Cicchetti D, Toth SL. A developmental psychopathology perspective on child abuse and neglect. Journal of the American Academy of Child and Adolescent Psychiatry. 1995; 34:541–565. [PubMed: 7775351]
- Cicchetti D, Toth SL. The past achievements and future promises of developmental psychopathology: The coming of age of a discipline. Journal of Child Psychology and Psychiatry. 2009; 50:16–25. [PubMed: 19175810]
- Cicchetti, D.; Toth, SL. A multilevel perspective on child maltreatment. In: Lamb, M.; Garcia Coll, C., editors. Handbook of child psychology and developmental science, 7th Vol. 3: Socioemotional process. New York: Wiley; (in press).
- Cicchetti, D.; Toth, SL.; Manly, JT. Maternal Classification Maltreatment Interview. Mt. Hope Family Center; 2003. Unpublished manuscript
- Cicchetti D, Tucker D. Neural plasticity, sensitive periods, and psychopathology [Special Issue]. Development and Psychopathology. 1994; 6:531–814.
- Cicchetti, D.; Valentino, K. An ecological transactional perspective on child maltreatment: Failure of the average expectable environment and its influence upon child development. In: Cicchetti, D.; Cohen, DJ., editors. Developmental Psychopathology. 2nd. Vol. 3. New York: Wiley; 2006. p. 129-201.
- Cutuli JJ, Raby KL, Cicchetti D, Englund MM, Egeland B. Contributions of maltreatment and serotonin transporter genotype to depression in childhood, adolescence, and early adulthood. Journal of Affective Disorders. 2013; 149:30–37. [PubMed: 22951355]
- DeYoung C, Cicchetti D, Rogosch FA. Moderation of the association between childhood maltreatment and neuroticism by the corticotropin-releasing hormone receptor 1 gene. Journal of Child Psychology and Psychiatry. 2011; 52:898–906. [PubMed: 21438878]
- Duncan LE, Keller MC. A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. American Journal of Psychiatry. 2011; 168:1041–1049. [PubMed: 21890791]
- Duncan LE, Pollastri AR, Smoller JW. Mind the gap: Why many geneticists and psychological scientists have discrepant views about gene–environment interaction (G×E) research. American Psychologist. 2014; 69:249. [PubMed: 24750075]

- Ellis BJ, Boyce WT, Belsky J, Bakermans-Kranenburg MJ, van IJzendoorn MH. Differential susceptibility to the environment: An evolutionary-neurodevelopmental theory. *Development and Psychopathology*. 2011; 23:7–28. [PubMed: 21262036]
- English D, Lambert SF, Ialongo N. Longitudinal associations between experienced racial discrimination and depressive symptoms in African American adolescents. *Developmental Psychology*. 2014; 50:1190–1196. [PubMed: 24188037]
- English DJ, Upadhyaya MP, Litrownik AJ, Marshall JM, Runyan DK, Graham JC, et al. Maltreatment's wake: The relationship of maltreatment dimensions to child outcomes. *Child Abuse & Neglect*. 2005; 29:594–619.
- Escoffier, L.; Lischer, H. Arlequin version 3. 5 [Computer software]. Bern, Switzerland: Swiss Institute of Bioinformatics; 2011.
- Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*. 2003; 164:1567–1587. [PubMed: 12930761]
- Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: Dominant and null alleles. *Molecular Ecology Notes*. 2007; 7:574–578. [PubMed: 18784791]
- Gelernter J, Kranzler H, Cubells JF. Serotonin transporter protein (SLC6A4) allele and haplotype frequencies and linkage disequilibria in African- and European-American and Japanese populations and in alcohol-dependent subjects. *Human Genetics*. 1997; 101:243–246. [PubMed: 9402979]
- Gottesman, II.; Shields, J. Schizophrenia and genetics: A twin study vantage point. Oxford, England: Academic Press; 1972.
- Grigorenko EL, Cicchetti D. Genomic sciences for developmentalists: The current state of affairs. *Development and Psychopathology*. 2012; 24:1157–1164. [PubMed: 23062288]
- Gunnar MR, Wenner JA, Thomas KM, Glatt CE, McKenna MC, Clark AG. The brain-derived neurotrophic factor Val66Met polymorphism moderates early deprivation effects on attention problems. *Development and Psychopathology*. 2012; 24:1215–1223. [PubMed: 23062292]
- Heim C, Newport JD, Mletzko T, Miller AH, Nemeroff CB. The link between childhood trauma and depression: Insights from HPA axis studies in humans. *Psychoneuroendocrinology*. 2008; 33:693–710. [PubMed: 18602762]
- Hu X, Oroszi G, Chun J, Smith TL, Goldman D, Schuckit MA. An expanded evaluation of the relationship of four alleles to the level of response to alcohol and the alcoholism risk. *Alcoholism: Clinical and Experimental Research*. 2005; 29:8–16.
- Hubisz MJ, Falush D, Stephens M, Pritchard JK. Inferring weak population structure with assistance of sample group information. *Molecular Ecology Resources*. 2009; 5:1322–1332. [PubMed: 21564903]
- Kaufman J, Yang B, Douglas-Palumberi H, Grasso D, Lipschitz D, Houshyar S, Gelernter J. Brain-derived neurotrophic factor-5-HTTLPR gene interactions and environmental modifiers of depression in children. *Biological Psychiatry*. 2006; 59:673–680. [PubMed: 16458264]
- Kaufman J, Yang B, Douglas-Palumberi H, Houshyar S, Lipschitz D, Krystal J. Social supports and serotonin transporter gene moderate depression in maltreated children. *Proceedings of the National Academy of Sciences, USA*. 2004; 101:17316–17321.
- Keller MC. Gene $\times$ Environment interaction studies have not properly controlled for potential confounders: The problem and the (simple) solution. *Biological Psychiatry*. 2014; 75:18–24. [PubMed: 24135711]
- Kodjo CM, Audinger P. Predictors for emotionally distressed adolescents to receive mental health care. *Journal of Adolescent Health*. 2004; 35:369–373.
- Kovacs, M. The Children's Depression Inventory: A self-rated depression scale for school-aged youngsters. University of Pittsburgh; 1982. Unpublished manuscript
- Kovacs, M. Children's Depression Inventory manual. North Tonawanda, NY: Multi-Health Systems; 1992.
- Lai CQ, Tucker KL, Choudhry S, Parnell LD, Mattei J, Garcia-Bailo B, et al. Population admixture associated with disease prevalence in the Boston Puerto Rican health study. *Human Genetics*. 2009; 125:199–205. [PubMed: 19107526]

- Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*. 1996; 274:1527–1531. [PubMed: 8929413]
- Manly JT. Advances in research definitions of child maltreatment. *Child Abuse & Neglect*. 2005; 29:425–439. [PubMed: 15970318]
- Manly JT, Cicchetti D, Barnett D. The impact of subtype, frequency, chronicity, and severity of child maltreatment on social competence and behavior problems. *Development and Psychopathology*. 1994; 6:121–143.
- Manly JT, Kim JE, Rogosch FA, Cicchetti D. Dimensions of child maltreatment and children's adjustment: Contributions of developmental timing and subtype. *Development and Psychopathology*. 2001; 13:759–782. [PubMed: 11771907]
- Masten AS, Cicchetti D. Developmental cascades. *Developmental Cascades* [Special Issue, Part 1], *Development and Psychopathology*. 2010; 22:491–495. [PubMed: 20576173]
- McGrath LM, Weill S, Robinson EB, Macrae R, Smoller JW. Bringing a developmental perspective to anxiety genetics. *Development and Psychopathology*. 2012; 24:1179–1193. [PubMed: 23062290]
- Meaney MJ. Epigenetics and the biological definition of gene $\times$ environment interactions. *Child Development*. 2010; 81:41–79. [PubMed: 20331654]
- Moffitt TE, Caspi A, Rutter M. Measured gene-environment interactions in psychopathology. *Perspectives in Psychological Science*. 2005; 1:5–27.
- Polanczyk G, Caspi A, Williams B, Price TS, Danese A, Sugden K, Moffitt TE. Protective effects of CRHR1 gene variants on the development of adult depression following childhood maltreatment. *Archives of General Psychiatry*. 2009; 66:978–985. [PubMed: 19736354]
- Ressler KJ, Bradley B, Mercer KB, Deveau TC, Smith AK, Gillespie CF, Binder EB. Polymorphisms in CRHR1 and the serotonin transporter loci: Gene $\times$ gene $\times$ environment interactions on depressive symptoms. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics*. 2010; 153B:812–824.
- Rogers J, Raveendran M, Fawcett GL, Fox AS, Shelton SE, Oler JA, Kalin NH. CRHR1 genotypes, neural circuits and the diathesis for anxiety and depression. *Molecular Psychiatry*. 2012; 18:700–707. [PubMed: 23147386]
- Sedlak AJ.; Mettenburg, J.; Basena, M.; Petta, I.; McPherson, K.; Greene, A.; Li, S. Fourth National Incidence Study of Child Abuse and Neglect (NIS-4): Report to Congress, Executive Summary. Washington, DC: U. S. Department of Health and Human Services, Administration for Children and Families; 2010.
- Szyf M, Bick J. DNA methylation: A mechanism for embedding early life experiences in the genome. *Child Development*. 2013; 84:49–57. [PubMed: 22880724]
- Toth SL, Manly JT, Cicchetti D. Child maltreatment and vulnerability to depression. *Development and Psychopathology*. 1992; 4:97–112.
- Uher R, Mors O, Rietschel M, Rajewska-Rager A, Petrovic A, Zobel A, McGuffin P. Early and delayed onset of response to antidepressants in individual trajectories of change during treatment of major depression: A secondary analysis of data from the Genome-Based Therapeutic Drugs for Depression (GENDEP) study. *Journal of Clinical Psychiatry*. 2011; 72:1478–1484. [PubMed: 22127194]
- Widom CS, DuMont K, Czaja SJ. A prospective investigation of major depressive disorder and comorbidity in abused and neglected grown up children (grown up). *Archives of General Psychiatry*. 2007; 64:49–56. [PubMed: 17199054]
- Yaeger R, Alvial-Bront A, Abdul K, Nolan PC, Grann VR, Birchette MG, et al. Comparing genetic ancestry and self-described race in African Americans born in the United States and in Africa. *Cancer Epidemiology, Biomarkers & Prevention*. 2008; 17:1329–1338.

Note:
Group Contrasts:

Maltreatment Status Contrasts
Within BDNF Genotype Groups

TT: Mal > NMal***

CT-TT: ns

BDNF Genotype Contrasts
Within Maltreatment Status

NMal: ns

Mal: TT > CT-TT*

* $p < .05$; *** $p < .001$

Figure 1.

BDNF rs7103411 $\times$ maltreatment status interaction for CDI depressive symptoms

a.

Note:

Group Contrasts:

Maltreatment Status Contrasts
Within BDNF Genotype Groups

AA: ns

AG-GG: Mal > NMal *

BDNF Genotype Contrasts
Within Maltreatment Status

NMal: AA > AG-GG *

Mal: ns

* $p < .05$

b.

Note:

Group Contrasts:

Maltreatment Status Contrasts
Within BDNF Genotype Groups

AA: Mal > NMal *

AG-GG: ns

BDNF Genotype Contrasts
Within Maltreatment Status

NMal: ns

Mal: ns

* $p < .05$

c.

Note:

Group Contrasts:

Maltreatment Status Contrasts
Within BDNF Genotype Groups

AA: Mal > NMal **

AG-GG: ns

BDNF Genotype Contrasts
Within Maltreatment Status

NMal: ns

Mal: ns

** $p < .01$

Figure 2.

a. BDNF rs4923461 $\times$ maltreatment status within the tri-allelic 5-HTTLPR SS genotype group: CDI depressive symptoms

b. BDNF rs4923461 $\times$ maltreatment status within the tri-allelic 5-HTTLPR SL genotype group: CDI depressive symptoms

c. BDNF rs4923461 $\times$ maltreatment status within the tri-allelic 5-HTTLPR LL genotype group: CDI depressive symptoms

Note:

Group Contrasts:

Maltreatment Status Contrasts
Within 5-HTTLPR Genotype Groups

LL: Mal > NMal**

SL: ns

SS: Mal > NMal**

5-HTTLPR Genotype Contrasts
Within Maltreatment Status

NMal: SL > SS **

Mal: ns

** $p < .01$

Figure 3.

Tri-allelic 5-HTTLR $\times$ maltreatment status interaction for TRF anxiety/depression symptoms

Note:

Group Contrasts:

Onset-Recency Group Contrasts
Within 5-HTTLPR Genotype Groups

LL: EO > NMal**

SL: ns

SS: ER > RO**

ER > NMal***

5-HTTLPR Genotype Group Contrasts
Within Onset-Recency Groups

NMal: SL > SS**

RO: ns

EO: LL > SL*

ER: ns

* $p < .05$; ** $p < .01$; *** $p < .001$

Figure 4.

Tri-allelic 5-HTTLR $\times$ maltreatment onset-recency group interaction for TRF anxiety/depression symptoms

a.

Note:

Group Contrasts:

Maltreatment Status Contrasts
Within 5-HTTLPR Genotype Groups

LL: Mal > NMal**

SL: ns

SS: Mal > NMal**

5-HTTLPR Genotype Group Contrasts
Within Maltreatment Status

NMal: SL > SS**

Mal: LL > SL*

* $p < .05$; ** $p < .01$

b.

Note:

Group Contrasts:

Maltreatment Contrasts
Within 5-HTTLPR Genotype Groups

LL: ns

SL: ns

SS: ns

5-HTTLPR Genotype Contrasts
Within Maltreatment Status

NMal: ns

Mal: ns

Overall, there is a Mal > NMal effect, not different by genotype.

Figure 5.

a. Tri-allelic 5-HTTLPR × maltreatment status interaction within the NET AA genotype group: TRF anxiety/depression symptoms

b. Tri-allelic 5-HTTLPR × maltreatment subtype group interaction within the NET AG-GG genotype group: TRF anxiety/depression symptoms

a.

Note:

EMPN= Emotional Maltreatment/Physical Neglect Only

PASA = Physical Abuse/Sexual Abuse

Group Contrasts:

Maltreatment Subtype Contrasts
Within 5-HTTLPR Genotype Groups

LL: EMPN > NMal*

SL: ns

SS: PASA > NMal**

5-HTTLPR Genotype Contrasts
Within Maltreatment Subtype Groups

NMal: SL > SS**

EMPN: LL > SS*; LL > SL ***

PASA: ns

* $p < .05$; ** $p < .01$; *** $p < .001$

b.

Note:

EMPN= Emotional Maltreatment/Physical Neglect Only

PASA = Physical Abuse/Sexual Abuse

Group Contrasts:

Maltreatment Subtype Group Contrasts
Within 5-HTTLPR Genotype Groups

LL: ns

SL: ns

SS: ns

5-HTTLPR Genotype Group Contrasts
Within Maltreatment Subtype Groups

NMal: ns

EMPN: ns

PASA: ns

Overall, there is a PASA > NMal effect, not different by genotype.

Figure 6.

a. Tri-allelic 5-HTTLPR × maltreatment subtype group interaction within the NET AA genotype group: TRF anxiety/depression symptoms

b. Tri-allelic 5-HTTLPR × maltreatment subtype group interaction within the NET AG-GG genotype group: TRF anxiety/depression symptoms

a.

Note:

Group Contrasts:

Maltreatment Status Contrasts

Within CRHR1 TAT Haplotype Groups

0 copies: Mal > NMal*

1 copy: ns

2 copies: Mal > NMal**

CRHR1 TAT Haplotype Contrasts

Within Maltreatment Status

NMal: ns

Mal: ns

* $p < .05$; ** $p < .01$

b.

Note:

Group Contrasts:

Maltreatment Status Contrasts
Within CRHR1 TAT Haplotype Groups

0 copies: ns

1 copy: ns

2 copies: ns

CRHR1 TAT Haplotype Contrasts
Within Maltreatment Status

NMal: 2 copies > 1 copy**; 2 copies > 0 copies***

Mal: ns

** $p < .01$; *** $p < .001$ **Figure 7.**

a. CRHR1 TAT haplotype $\times$ maltreatment status interaction within the BDNF rs7103411 TT genotype group: TRF anxiety/depression symptoms

b. CRHR1 TAT haplotype $\times$ maltreatment status interaction within the BDNF rs7103411 TC-CC genotype group: TRF anxiety/depression symptoms

Table 1

Call rate, genotype frequencies, and Hardy-Weinberg equilibrium for selected genes and associated SNPs

Gene	Call Rate	Major Allele Homozygote	Heterozygote	Minor Allele Homozygote	HWE χ^2	p
		N	N	N		
5-HTTLPR		LL	SL	SS		
Bi-Allelic	99.5	620	403	68	.05	.82
5-HTTLPR		LL	SL	SS		
Tri-Allelic	99.5	290	549	252	.06	.81
BDNF		Val	Val/Met	Met		
rs6265	99.9	1032	62	1	.00	1.00
BDNF		GG	GT	TT		
rs925946	96.5	561	422	75	.13	.72
BDNF		TT	TC	CC		
rs7103411	96.5	919	125	4	.16	.69
BDNF		AA	AG	GG		
rs4923461	99.3	761	291	36	1.56	.21
NET		AA	AG	GG		
rs168924	99.0	624	387	74	1.73	.19
CRHR1		CC	CT	TT		
rs7209436	97.9	509	475	111	.00	1.00
CRHR1		GG	GA	AA		
rs110402	99.8	520	455	119	1.67	.20
CRHR1		GG	GT	TT		
rs242924	97.9	561	446	88	.00	1.00
CRHR1		0 copies	1 copy	2 copies		
TAT Haplotype		577	437	78		

Table 2

Comparison of maltreated and nonmaltreated children on genotype group distributions.

Polymorphism	Genotype/Percentage			χ^2	<i>p</i>
Bi-allelic 5-HTTLPR	LL	SL	SS		
Maltreated	57.7	36.0	6.3	.40	.82
Nonmaltreated	55.9	37.8	6.3		
Tri-allelic 5-HTTLPR	LL	SL	SS		
Maltreated	24.1	51.5	24.4	2.60	.27
Nonmaltreated	22.1	49.2	28.7		
BDNF rs9295946	GG	GT	TT		
Maltreated	55.2	37.3	7.5	2.84	.24
Nonmaltreated	50.8	42.4	6.8		
BDNF rs7103411	TT	TC/CC			
Maltreated	87.8	12.2		.82	.42
Nonmaltreated	85.9	14.1			
BDNF rs4923461	AA	AG/GG			
Maltreated	68.5	31.5		1.06	.32
Nonmaltreated	71.4	28.6			
NET	AA	AG/GG			
Maltreated	58.0	42.0		.09	.81
Nonmaltreated	57.1	42.9			
CRHR1 TAT Haplotype	0	1	2		
Maltreated	55.1	38.1	6.8	2.15	.34
Nonmaltreated	50.6	41.9	7.5		