HPA Axis Multilocus Genetic Profile Score Moderates the Impact of Interpersonal Stress on Prospective Increases in Depressive Symptoms for Offspring of Depressed Mothers

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Although offspring of depressed mothers are at an increased risk for depression themselves, not all of these children develop depression, highlighting the need to identify specific environmental and genetic moderators of risk. The goal of this study was to examine the aggregate influence of genetic polymorphisms associated with the regulation of the hypothalamic–pituitary–adrenal (HPA) axis as a potential moderator of the relation between environmental stress and prospective changes in depressive symptoms for offspring of depressed mothers. Participants were 238 mother–offspring dyads recruited from the community based on the mother's lifetime history of major depression during the youth's lifetime (present vs. absent). Mothers and youth completed assessments every 6 months for 2 years (5 total). Results indicated that offspring of depressed mothers showing the greatest increases in depressive symptoms during the follow up were those who had higher HPA multilocus genetic profile scores and who experienced the highest levels of interpersonal stress. These relations were significant for interpersonal stress and were not observed for noninterpersonal stress. These findings suggest that HPA multilocus genetic profile scores may be important genetic markers of stress reactivity and depression risk for offspring of depressed mothers. They also highlight interpersonal stress as a potentially modifiable risk factor for these high-risk youth.

General Scientific Summary
Although children of depressed mothers are at increased risk for depression themselves, most of these youth do not become depressed. This study provides evidence that the interaction of specific genetic and environmental influences may increase risk for depression in children of depressed mothers.

Keywords: intergenerational transmission, depression, youth, genetics, stress

Major depressive disorder (MDD) is one of the leading causes of disability worldwide (Ferrari et al., 2013). Epidemiological data suggest that over 15% of youth will experience MDD by the end of adolescence (Avenevoli, Swendsen, He, Burstein, & Merikangas, 2015), and approximately 40% of these individuals will experience a recurrence of depression by the age of 24 (Lewinsohn, Rohde, Seeley, Klein, & Gotlib, 2000). One subset of youth that are at particular risk for depression are children of depressed mothers, who are three to four times more likely to become depressed than offspring of nondepressed mothers (see Goodman, 2007, for review). This said, the majority of these individuals do not become depressed themselves. This highlights the importance of identifying additional moderating factors, which likely include both environmental and genetic influences.

Stressful life events can greatly increase one's risk for depression (Brown & Harris, 1978; Kessler, 1997; Mazure, 1998; Monroe, Slavich, & Georgiades, 2014) and there is evidence that children of depressed mothers experience more life stress than those of never-depressed mothers (Adrian & Hammen, 1993; Feurer, Hammen, & Gibb, 2016). One form of life stress that may be particularly important for depression risk in children and adolescents is interpersonal stress. Specifically, there is evidence that interpersonal negative life events are stronger predictors of depression risk in youth than are noninterpersonal events (Rudolph et al., 2000). This said, there are considerable individual differences in stress reactivity. Because of this, researchers have focused on understanding genetic influences that may help to identify which individuals may be most reactive to stressors in their environment. The starting point for much of this research was Caspi et al.'s (2003) seminal study showing that variation in the serotonin transporter gene (5-HTTLPR) moderated the impact of stressful life events on depression risk, though subsequent research on the role of 5-HTTLPR in depression risk has been more mixed (for reviews, see Culverhouse et al., 2017; Karg, Burmeister, Shedden, & Sen, 2011; Risch et al., 2009). Since this time, a number of studies have evaluated the potential moderating role of polymorphisms in various other candidate genes considered individually (e.g., BDNF, MAOA, CRHR1; for reviews, see Cicchetti, Rogosch, & Sturge-Apple, 2007; Gatt et al., 2009; Heim & Binder, 2012; Hosang, Shiles, Tansey, McGuffin, & Uher, 2014; Melas et al., 2013).

However, several concerns have been raised about the use of single candidate genes when conducting Gene × Environment (G × E) research (Dick et al., 2015). Indeed, recent research has begun to show that polygenic effects within a given biological pathway are stronger than the influence of any single candidate gene examined in isolation (Nikolova, Ferrell, Manuck, & Hariri, 2011; Owens et al., 2016; Pagliaccio et al., 2014). In line with this aggregate genetic approach, a few studies have begun to examine G × E relations utilizing multilocus genetic profile scores (MGPSs). For example, a MGPS examining the aggregate influence of serotonergic genes was found to moderate the impact of interpersonal life stress on increases in depressive symptoms (Vrshek-Schallhorn, Stroud, Mineka, Hammen et al., 2015). Additionally, two separate MGPSs comprised of genes associated with the body's stress-response system were found to interact with the experience of life stress to predict amygdala volume and function in youth and adults (Di Iorio et al., 2017; Pagliaccio et al., 2014, 2015). Importantly, in each of these studies, the MGPS exhibited greater predictive validity than any single polymorphism considered in isolation. This highlights the importance of considering the aggregate influence of multiple genes associated with a specific biological pathway.

Genes Impacting HPA Axis Functioning

Within this context, one reasonable approach is to focus on genes related to the functioning of the hypothalamic–pituitary–adrenal (HPA) axis, which coordinates the body's response to stress (see Gunnar & Quevedo, 2007, for review). Following a stressor, corticotropin releasing hormone (CRH) is released from the hypothalamus, causing adrenocorticotropin to be released by the pituitary, which then causes glucocorticoids, including cortisol, to be synthesized and released by the adrenal cortex. Although moderate HPA axis activation can be adaptive as it readies individuals to face threats in their environment, HPA axis hyperactivation is associated with increased risk for depression in children and adolescents. Depressed youth also have higher levels of basal cortisol than nondepressed youth (for a review, see Lopez-Duran, Kovacs, & George, 2009), and this hypercortisolism has been found to prospectively predict depression onset in youth (Adam et al., 2010; Goodyer, Herbert, & Tamplin, 2003). Additionally, compared to never-depressed youth, depressed children and adolescents have a heightened cortisol response to psychological stressors (for reviews, see Guerry & Hastings, 2011; Lopez-Duran et al., 2009). Building from these findings and using a biological systems approach to testing a G × E model of risk for depression, our goal was to examine the combined influence of genes known to affect HPA axis functioning.

The *CRHR1* gene codes for the CRH receptor and variation in *CRHR1* genotype has been shown to affect the level of cortisol released in response to laboratory-based stressors in adults (Mahon, Zandi, Potash, Nestadt, & Wand, 2013) and children (Sheikh, Kryski, Smith, Hayden, & Singh, 2013). There is evidence that three single nucleotide polymorphisms (SNPs) in *CRHR1* (rs7209436, rs110402, and rs242924) form a protective TAT haplotype. An individual SNP within the TAT haplotype (rs110402) has been found to predict greater cortisol reactivity for individuals exposed to childhood abuse (Heim et al., 2009; Tyrka et al., 2009). Furthermore, among individuals reporting a history of childhood abuse, those with no copies of the protective TAT haplotype, compared to those carrying one or two copies of the haplotype, reported significantly greater cortisol dysregulation (Cicchetti, Rogosch, & Oshri, 2011) and both higher current depressive symptoms and greater risk for depression (Bradley et al., 2008; Polanczyk et al., 2009; but see also Laucht et al., 2013), with risk appearing to decrease with each additional copy of the TAT haplotype present.

The *FKBP5* protein plays a key role in HPA axis activity by regulating the sensitivity of the glucocorticoid receptor, with higher levels of *FKPB5* expression associated with lower glucocorticoid activity (for a review, see Zannas & Binder, 2014). There is evidence that the minor T allele of the rs1360780 SNP within the *FKBP5* gene is associated with increased cortisol reactivity to laboratory stressors (Ising et al., 2008; Luijk et al., 2010; Zannas & Binder, 2014). Variation in this SNP has also been shown to moderate the impact of negative life events on risk for depression in adults (Appel et al., 2011; Comasco et al., 2015; Lahti et al.,

2016; Zimmermann et al., 2011), with risk higher among carriers of the minor allele.

Finally, the mineralocorticoid receptor gene (*NR3C2*) is also associated with HPA axis dysregulation (for a review, see DeRijk et al., 2006). For example, there is evidence that the minor G allele of the MRI180V (rs5522) SNP is associated with greater salivary and plasma cortisol and with greater heart rate reactivity to a stressor (DeRijk et al., 2006; but see also Bouma et al., 2011). There is also evidence that MRI180V forms a two-SNP haplotype with MR-2G/C (rs2070951) that predicts HPA axis functioning and risk for depression. Specifically, the CA haplotype formed by these two SNPs is associated with greater mineralocorticoid receptor protein expression (Klok, Giltay, et al., 2011; van Leeuwen et al., 2011) and decreased risk for depression in women (Klok, Giltay, et al., 2011; Vinkers et al., 2015), suggesting that, like the *CRHR1* TAT haplotype, the *NR3C2* CA haplotype may be protective.

The Current Study

In the current study, our goal was to test an integrated model of risk for depression in offspring of depressed mothers within the context of a multiwave, 2-year prospective study. We chose to focus on children and adolescents who would be 8 to 16 years old during the course of the study because this is a key developmental window during which rates of depression increase dramatically (Hankin et al., 1998; Rudolph & Flynn, 2014). We utilized a MGPS approach focusing on the combined influence of polymorphisms in genes known to affect HPA axis functioning (*CRHR1*, *FKBP5*, and *NR3C2*). We chose to focus specifically on these three genes due to their known influence on HPA axis functioning and to replicate a previously established, theoretically and biologically driven MGPS utilized in previous research (Di Iorio et al., 2017).[1] As noted earlier, the MGPS approach is consistent with recent research highlighting the importance of examining the aggregate influence of multiple SNPs as opposed to examining candidate genes in isolation (Nikolova et al., 2011; Owens et al., 2016; Pagliaccio et al., 2014). In focusing on environmental influences, we examined interpersonal and noninterpersonal episodic life events separately, given evidence that interpersonal, compared to noninterpersonal, events are stronger predictors of future depression (Rudolph et al., 2000) and are associated with greater HPA axis reactivity, as measured via cortisol production (Dickerson & Kemeny, 2004; Stroud, Chen, Doane, & Granger, 2016). We predicted that offspring of depressed mothers would exhibit greater prospective increases in depressive symptoms following negative life events than offspring of never depressed mothers and that these symptom increases would be highest among youth of depressed mothers who carried genotypes associated with greater HPA axis reactivity. We also predicted that these results would be stronger for interpersonal than noninterpersonal stress. Finally, exploratory analyses were conducted to examine whether youth's age or sex moderated any of the relations.

Method

Participants

Participants were 238 mothers and their biological offspring recruited from the community for a study on the intergenerational transmission of depression. To qualify for the study, mothers were required to either have a history of MDD as defined by the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (*DSM–IV*; American Psychiatric Association, 1994) during their offspring's lifetime ($n = 122$) or have no lifetime diagnosis of any *DSM–IV* mood disorder and no current Axis I diagnosis ($n = 116$). Mothers were excluded if they exhibited symptoms of schizophrenia, alcohol or substance dependence in the last 6 months, or a history of bipolar disorder. Additionally, potential mothers needed to have a child between the ages of 8 and 14. If there was more than one child in this age range, one was randomly chosen for participation. For youth in our sample, the average age was 11.39 ($SD = 1.93$), and 51.7% were female. In terms of race, 81.9% were Caucasian, 4.6% were African American, 10.5% were biracial, and 3.0% identified as another race. For mothers in our sample, the average age was 40.32 ($SD = 6.72$), 87.4% were Caucasian, 4.6% were African American, 4.2% were biracial, and 3.8% identified as another race. The annual family income ranged from $0–5,000 to more than $115,000 and the median annual income was $50,001–55,000. Finally, 24.8% of children were from single parent homes.

Measures

Mothers' and children's histories of MDD and other Axis I disorders were assessed using the Structured Clinical Interview for *DSM–IV* Axis I Disorders (SCID-I; First, Spitzer, Gibbon, & Williams, 1995) and the Schedule for Affective Disorders and Schizophrenia for School Age Children–Present and Lifetime Version (K-SADS-PL; Kaufman et al., 1997), respectively. As previously stated, 122 mothers met criteria for MDD during their offspring's lifetime and 116 mothers reported no lifetime history of depression. In addition, 17 youth had a lifetime history of MDD at the initial assessment (15 of whom had mothers with a history of MDD). Lifetime rates of anxiety disorders in mothers were 26 with social phobia (25 from the MDD group), 25 with posttraumatic stress disorder (24 from the MDD group), 21 with panic disorder (20 from the MDD group), 12 with obsessive–compulsive disorder (10 from the MDD group), 10 with agoraphobia (8 from the MDD group), and 3 with generalized anxiety disorder (3 from the MDD group). Lifetime rates of anxiety disorders for youth were 12 with social phobia (10 children of mothers with MDD), 12 with separation anxiety disorder (10 children of mothers with MDD), 7 with generalized anxiety disorder (5 children of mothers with MDD), 4 with obsessive–compulsive disorder (4 children of mothers with MDD), 1 with panic disorder (whose mother had

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[1] We did, however, differ from Di Iorio et al.'s (2017) approach to creating the MGPS in two respects. First, for *CRHR1*, we focused on the established TAT haplotype, while Di Iorio et al. only included rs110402. Second, although Di Iorio and colleagues wanted to focus on *NR3C2* rs2070951 (as part of a haplotype with rs5522), their panel did not include this SNP so they included a different SNP (rs4635799) that is in high linkage disequilibrium with rs2070951 to form the *NR3C2* haplotype. To be consistent with the previous literature (Klok, Giltay, et al., 2011; Klok, Vreeburg, et al., 2011; van Leeuwen et al., 2011; Vinkers et al., 2015) and with the goal of the Di Iorio et al. (2017) article, we focused on the rs2070951 so that we could specifically examine the *NR3C2* rs2070951/rs5522 haplotype. This said, it is important to note that all significant findings in the current study were maintained when using a MGPS that precisely replicated the one used by Di Iorio et al.

MDD), and 1 with posttraumatic stress disorder (whose mother had MDD). A subset of 20 SCIDs and 20 K-SADS-PLs was coded by a separate interviewer to assess interrater reliability for diagnoses of MDD and anxiety disorders, yielding excellent kappa coefficients (all κs = 1.00).

Youth's depressive symptoms were assessed using the Children's Depression Inventory (CDI; Kovacs, 1981), which has shown excellent reliability and validity in previous research (Kovacs, 1981, 1985; Smucker, Craighead, Craighead, & Green, 1986). The CDI also exhibited good internal consistency across all five time points in this study (α ranged from .85 to .89).

Youth's exposure to interpersonal and noninterpersonal episodic life stress was assessed at each time point using the University of California, Los Angeles Life Stress Interview for Children (LSI-C; Adrian & Hammen, 1993), which is a semistructured interview and is modeled after contextual threat interviews (Brown & Harris, 1978). At the initial assessment, youth and their mothers were interviewed separately and were asked about any stressful life events across a variety of domains that may have occurred in the 6 months prior to the assessment. For the 6-, 12-, 18-, and 24-month follow-up assessments, participants were asked about any stressful events since the date of their last assessment. If the dyad missed an assessment, the LSI-C focused on stress experienced during the entire time between assessments instead of just the most recent 6-month interval. In these cases, any events reported before the date of their missed appointment were summed separately from the rest of the events reported at that time period, to back-date the events to the appropriate time point. For any reported events, the interviewer probed further to gain objective information about the timing, duration, context, and consequences of each event. Each reported event was then presented, devoid of any subjective information, to a team of four to seven coders who assigned a negative impact threat rating to each event. Any coding discrepancies across team members were discussed until consensus was achieved. These threat ratings ranged from 1 (*no stress*) to 5 (*severe stress and significant impact*). Additionally, the content of each reported event was categorized as either interpersonal or noninterpersonal. An event was categorized as "interpersonal" if the stressor had significant consequences for the youth's interpersonal relationships (e.g., a fight with a friend or the death of a family member). If the event did not have any impact on the youth's interpersonal relationships (e.g., the youth failed an exam or sustained a physical injury), the stressor was coded as "noninterpersonal." We calculated the total amount of interpersonal and noninterpersonal episodic stress reported at each time point by summing the stress scores separately for interpersonal and noninterpersonal events. To avoid inflation of stress scores by including reported events with a score of 1, and therefore no negative impact, we recoded the objective impact scores from 1–5 to 0–4 before summing the totals for interpersonal and noninterpersonal stress.[2]

Genotyping

Finally, youth's DNA was collected and isolated from buccal cells using established methods (Freeman et al., 1997; Lench, Stanier, & Williamson, 1988). For *CRHR1*, three SNPs were genotyped—rs7209436, rs110402, and rs242924—that form a TAT haplotype comprised of major alleles on each SNP (Bradley et al., 2008; Polanczyk et al., 2009). The Taqman assay IDs for

rs7209436, rs110402, and rs242924 were C___1570087_10, C___2544843_10, and C___2257689_10, respectively. The three *CRHR1* polymorphisms were genotyped using fluorogenic 5′ nuclease (Taqman, Applied Biosystems, Foster City, CA) method involving reagents (VIC and carboxyfluorescein [FAM], labeled probes, and TaqMan Universal Polymerase Chain Reaction [PCR] Master Mix without AMPerase uracil N-glycosylase [UNG]) obtained from Applied Biosystems (ABI). Genotype determination was performed using primers purchased from ABI or Integrated DNA Technologies (Coralville, IA). Genotypes were obtained using an ABI Prism 7300 Sequence Detection System using both absolute quantification and allelic discrimination modes (Livak, Flood, Marmaro, Giusti, & Deetz, 1995). All markers were found not to differ from Hardy–Weinberg equilibrium using default parameters in Haploview ($p < .001$; Barrett, 2009). To (a) maximize the amount of information provided by the multiple markers and (b) circumvent loss of power due to multiple testing, we utilized all of the available SNP data to identify haplotype blocks (i.e., the combinations of SNP markers that are statistically associated). Haploview was used to visualize haplotype blocks (Barrett, 2009; Barrett, Fry, Maller, & Daly, 2005). Marker-to-marker D' values were as follows: rs7209436 – rs110402 = 0.95, rs7209436 – rs242924 = 0.88, rs110402 – rs242924 = 0.93. Haplotypes for both chromosomes were then confirmed and extracted using PHASE (Version 2.1, Stephens & Donnelly, 2003; Stephens, Smith, & Donnelly, 2001), requiring that the probability of a haplotype be greater than or equal to 0.80. PHASE haplotypes were used to construct diplotypes (i.e., combination of haplotypes across the pair of homologous chromosomes) that were used in the analyses. *CRHR1* was coded as the number of copies of the protective TAT haplotype that were present.

FKBP5 rs1360780 was genotyped following the procedures used for *CRHR1* SNPs above. The Taqman assay ID for rs1360780 was C___8852038_10. Results of an exact test for Hardy Weinberg proportions using Markov chain–Monte Carlo implementation (Engels, 2009; Guo & Thompson, 1992) yielded a *p* value of .0436 ± .0002, suggesting the presence of excess homozygosity in our sample. *FKBP5* was coded as the number of rs1360780 minor (T) alleles.

For *NR3C2*, two SNPs (rs2070951 and rs5522) were genotyped using PsychArray (Illumina, San Diego, CA) and scanned using a HiScan (Illumina) using standard manufacturer's protocols. Quality control was performed sequentially on the PsychArray genetic data using PLINK Version 1.9 (Purcell et al., 2007). First markers

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[2] The current study focused on the impact of stressful life events on increases in youth's depressive symptoms. However, according to stress generation theory (Hammen, 1991), depressed individuals may generate additional stress in their lives, which can lead to questions of causality when examining the relation between stress and depression. Therefore, coders also assigned a dependence score to each event to signify the extent to which the occurrence of the event was due to the actions of the participant. A dependence score of 1 indicated that the event was entirely independent of the child, a score of 3 indicated mixed or indeterminate dependence, and a score of 5 indicated that the event was completely dependent on the participant. To address concerns about stress generation, all analyses were reconducted with independent stress as a predictor variable (i.e., the sum of the impact ratings of all events coded as a 1 or 2 for dependence). Both the MDD × MGPS × Stress interaction and the MGPS × Stress interaction for children of depressed mothers remained significant.

with a genotyping rate of less than 90% and individuals with more than 10% of their data were missing were removed. Then, markers with minor allele frequency of less than 1% and those that failed Hardy–Weinberg equilibrium ($p < .001$) were removed. Genotypes for rs5522 were taken directly from the PsychArray data whereas genotypes for rs2070951 were imputed using the Michigan Imputation Server (https://imputationserver.sph.umich.edu). Similar to *CRHR1*, Haploview was used to visualize haplotype blocks (Barrett, 2009; Barrett et al., 2005). Haplotypes for both chromosomes were then confirmed and extracted using PHASE (Version 2.1, Stephens & Donnelly, 2003; Stephens et al., 2001), requiring that the probability of a haplotype be greater than or equal to 0.80. PHASE haplotypes were used to construct diplotypes (i.e., combination of haplotypes across the pair of homologous chromosomes) that were used in the analyses. Marker to marker D' values were as follows: rs5522 – rs2070951 = 1.00. *NR3C2* was coded as the number of CA haplotypes.

Multilocus genetic profile scores were then calculated by summing the number of copies of the *CRHR1* TAT haplotype, rs1360780 T allele, and the *NR3C2* CA haplotype present. Because the *CRHR1* TAT and *NR3C2* CA haplotypes are considered to be protective, they were reverse scored so that higher scores reflect greater HPA axis reactivity. Coding for each gene is described in Table 1. MGPSs ranged from 0 to 6, with larger scores reflecting more copies of genotypes associated greater HPA axis reactivity.

Procedure

Potential participants were recruited through a variety of means (e.g., newspaper and bus ads, flyers) and were screened over the telephone to determine their eligibility. At the initial assessment, the SCID and K-SADS-PL were administered to all mothers and youth to assess for their lifetime history of MDD and other Axis I disorders. Additionally, at the initial time point, youth were administered the CDI to assess for their current levels of depressive symptoms. DNA samples were also obtained from the youth through the collection of genetic samples at this initial assessment. Finally, at the first time point the LSI-C was conducted with both mothers and their offspring separately to assess for the amount of episodic life stress occurring in the youth's life during the 6 months before the start of the study.

Table 1
Descriptive Statistics for the Polymorphisms Included in the Multilocus Genetic Profile Score

Polymorphism	Coding	N
CRHR1 TAT haplotype	Two copies = 0	25
(rs7209436, rs110402, rs242924)	One copy = 1	122
	Zero copies = 2	91
FKPB5	CC = 0	109
(rs1360780)	CT = 1	99
	TT = 2	30
NR3C2 CA haplotype	Two copies = 0	38
(rs2070951, rs5522)	One copy = 1	125
	Zero copies = 2	75

Note. The hypothalamic–pituitary–adrenal axis multilocus genetic profile score was created by summing the number of "high-risk" polymorphisms across these three genes.

After the initial assessment, mothers and their offspring came back to the lab for 6-, 12-, 18-, and 24-month follow-up assessments. Of the 238 participants who completed the Time 1 assessment, 202 (84.9%) completed Time 2, 187 (78.6%) completed Time 3, 161 (67.6%) completed Time 4, and 166 (69.7%) completed Time 5. Completion rates did not differ across the two mother MDD groups at any time point (lowest $p = .15$). At each time point, youth were readministered the CDI to assess for depressive symptoms. Additionally, mothers were readministered the depression supplement of the SCID to assess for the onset of any new episodes of MDD since their last assessment. Finally, at each follow-up appointment the LSI-C was readministered to mothers and their offspring to assess for the amount of episodic life stress the youth experienced since the last assessment. Participants were paid $75 for their participation in the initial assessment, and an additional $50 for the completion of each follow-up assessment. All study procedures were approved by the Institutional Review Board at Binghamton University, The State University of New York (Protocol Number 2013–09).

Analytic Plan

We used hierarchical linear modeling (HLM; Raudenbush & Bryk, 2002; Raudenbush, Bryk, Cheong, & Congdon, 2004) to examine the impact of maternal MDD, youth's MGPS, episodic stress, and their interactions on changes in youth's depressive symptoms during the study. We conducted separate analyses for interpersonal and noninterpersonal episodic stress. The Level 1 model for these HLM analyses was as follows:

$$CDI_{tij} = \pi_{0j} + \pi_{1j}(CDI_{t-1ij}) + \pi_{2j}(\text{Episodic Stress}_{tij}) + e_{ij},$$

where CDI_{tij} represents the youth's CDI score at time t for assessment i and participant j, CDI_{t-1ij} represents the youth's CDI score at Time $t - 1$ for assessment i and participant j, and Episodic Stress$_{tij}$ represents the youth's level of stress between Time $t - 1$ and time t for assessment i and participant j. In addition, π_{0j} is the CDI_{tij} intercept, π_{1j} is the slope of the relation between youth's CDI score between Time t and Time $t - 1$ at each assessment i for participant j (i.e., the autocorrelation), π_{2j} is the slope of the relation between youth's episodic stress (interpersonal or noninterpersonal) and CDI scores at Time t, and e$_{ij}$ represents the error term. Importantly, including CDI at Time $t - 1$ in the model while predicting CDI at Time t allows us to determine whether episodic stress occurring between assessments predicts prospective change in youth's depressive symptom levels between those assessments.

The level 2 model was as follows:

$$\pi_{0j} = \beta_{00} + \beta_{01}(MDD) + \beta_{02}(MGPS) + \beta_{03}(MDD \times MGPS) + r_{0j},$$

$$\pi_{1j} = \beta_{10} + \beta_{11}(MDD) + \beta_{12}(MGPS) + \beta_{13}(MDD \times MGPS) + r_{1j}, \text{ and}$$

$$\pi_{2j} = \beta_{20} + \beta_{21}(MDD) + \beta_{22}(MGPS) + \beta_{23}(MDD \times MGPS) + r_{2j},$$

where β_{01} is the cross-level interaction term representing the effect of maternal MDD history (absent vs. present in youth's life) on the CDI intercept, β_{11} is the cross-level interaction term representing

the effect of maternal MDD history on the slope of the relation between youth's lagged and current CDI scores, and β_{21} is the cross-level interaction representing the effect of maternal MDD on the slope of the relation between youth's episodic stress (interpersonal and noninterpersonal) and CDI scores. Similarly, β_{02}, β_{12}, and β_{22} are the cross-level interaction terms representing the effect of the MGPS on the CDI intercept, the slope of the relation between youth's current and lagged CDI scores, and the slope of the relation between episodic stress and CDI scores, respectively. β_{03}, β_{13}, and β_{23} are the cross-level interaction terms representing the effect of the mother MDD $\times$ MGPS interaction on the CDI intercept, the slope of the relation between youth's current and lagged CDI scores, and the slope of the relation between episodic stress and CDI scores, respectively. Finally, β_{00}, β_{10}, and β_{20} are the intercept terms for each of their respective equations, and r_{0j}, r_{1j}, and r_{2j}, are the error terms.

Results

Preliminary Analyses

An initial inspection of the data revealed that several variables exhibited significant skew ($z > 3.29$; cf. Tabachnick & Fidell, 2007). These variables were transformed prior to further analysis to satisfy assumptions of normality (square root: T1–T5 interpersonal stress, T1–T5 independent stress, T1–T5 CDI; inverse: T1–T5 noninterpersonal stress).[3] Additionally, given the presence of missing data (T2 stress: 10.5%; T3 stress: 18.5%; T4 stress: 22.7%; T5 stress: 30.3%; CDI T1: 2.1%; CDI T2: 16.4%; CDI T3: 23.9%; CDI T4: 34.0%; CDI T5: 31.9%; *NR3C2* haplotype: 2.9%), we examined whether the data were missing at random, thereby justifying the use of data imputation methods for estimating missing values (cf. Schafer & Graham, 2002). We found that Little's missing-completely-at-random test, for which the null hypothesis is that the data are missing completely at random, was nonsignificant, $\chi^2(966) = 1,003.01$, $p = .20$. Given this, maximum likelihood estimates of missing data were created and used for all analyses. Descriptive statistics for all study variables are presented in Table 2. To facilitate comparisons with other studies, we present untransformed values for these variables. Preliminary analyses were then conducted to determine if any of the study variables were significantly related to youth's MGPS. There were no significant differences in youth's MGPS based on mothers' history of MDD, $t(236) = -0.59$, $p = .56$, $r_{effect-size} = -.04$, or youths' sex, $t(236) = 1.26$, $p = .21$, $r_{effect-size} = .08$. Additionally, youth's MGPS was not significantly associated with average levels of interpersonal, $r = .06$, $p = .39$, or noninterpersonal, $r = .04$, $p = .58$, stress across the study.

Vulnerability–Stress Analyses

Next, we tested the vulnerability-stress models using HLM as described earlier. The results of these analyses are presented in Table 3. As can be seen in the table, although none of the main or interaction effects for mother MDD or MGPS were significant for noninterpersonal stress, the mother MDD $\times$ MGPS $\times$ Interpersonal Stress interaction was significant in predicting prospective changes in youth's depression symptoms. To explore the form of this interaction, we examined the MGPS $\times$ Interpersonal Stress

interaction separately for youth of mothers with and without a history of MDD. We found that MGPS significantly moderated the relation between interpersonal stress and prospective changes in youth's depressive symptoms among offspring of mothers with a history of MDD, $t(120) = 2.55$, $p = .01$, $r_{effect-size} = .23$ (95% confidence interval [CI] [.05, .38]), but not among offspring of never depressed mothers, $t(114) = -0.86$, $p = .39$, $r_{effect-size} = -.08$ (95% CI [$-.26$, .10]). The significant MGPS $\times$ Interpersonal Stress interaction for offspring of depressed mothers is presented in Figure 1. Conducting a region of significance analysis using Preacher, Curran, and Bauer's (2006) online computational tools, we found that the relation between interpersonal stress and prospective increases in youth's depressive symptoms was significant for youth with a MGPS of 3 or greater but not among those with a lower MGPS.

Next, we conducted a series of follow-up tests to examine the robustness of these results. Focusing on offspring of depressed mothers with a MGPS of 3 or more, we first examined whether the relation between interpersonal stress and prospective increases in depressive symptoms was maintained when we excluded youth with a lifetime history of MDD at the initial assessment. Our results were maintained, $t(68) = 3.26$, $p < .01$, $r_{effect-size} = .37$ (95% CI [.15, .54]). Next, we examined whether our results were maintained when statistically controlling for the influence of mothers' depressive episodes during the course of the 2-year follow-up. Specifically, we included the number of weeks that each mother was in a major depressive episode between each assessment as a time-varying covariate in our HLM analyses. Again, our results were maintained, $t(71) = 2.40$, $p = .02$, $r_{effect-size} = .27$ (95% CI [.05, .46]). Third, given potential concerns about population stratification, we examined whether our results were maintained when focusing only on Caucasians, and they were, $t(61) = 3.03$, $p < .01$, $r_{effect-size} = .36$ (95% CI [.13, .54]). Exploratory analyses were then conducted to determine if any of these effects were moderated by youth's age or sex. None of these analyses were significant.

Finally, we reconducted our main analyses using individual SNPs/haplotypes instead of the MGPS to determine whether the MDD $\times$ SNP/Haplotype $\times$ Stress interaction was significant for any of the individual SNPs or haplotypes that make up the MGPS. None of these interactions was significant for interpersonal or noninterpersonal stress, although the MDD $\times$ *CRHR1* TAT $\times$ Interpersonal Stress interaction approached significance, $t(234) = 1.76$, $p = .08$, $r_{effect-size} = .11$ (95% CI [$-.01$, .24]). These results suggest that the MGPS results were not driven by any single haplotype/SNP acting in isolation.

Discussion

The primary aim of this study was to examine whether specific genetic and environmental influences could help to identify which children of depressed mothers are at greatest risk for depression themselves. In terms of genetic influences, we focused on a MGPS reflecting variants in genes known to be associated with greater HPA axis reactivity (Derijk, 2009; Ising et al., 2008; Luijk et al.,

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[3] The T1, T3, T4, and T5 noninterpersonal stress variables did not reach our criteria for normality ($z < 3.29$) following inverse transformations (zs = 4.53, 3.43, 3.46, and 3.42, respectively). However, because the inverse transformations resulted in lower skew statistics than any of the other transformations, these are what were used for all analyses.

Table 2
Descriptive Statistics for Study Variables

Variable	Depressed mothers (n = 122)	Nondepressed mothers (n = 116)	r_{effect_size}
Youth age	11.40 (2.03)	11.38 (1.82)	.01
Youth sex (% girls)	50.0%	53.4%	−.03
Youth race (% Caucasian)	73.8%	90.5%	−.22**
Multilocus genetic profile score	3.06 (1.14)	3.15 (1.24)	−.04
Children's Depression Inventory			
T1	7.26 (5.77)	4.74 (5.23)	.25**
T2	6.40 (5.77)	3.53 (4.19)	.29**
T3	5.76 (4.62)	3.64 (4.15)	.25**
T4	5.07 (4.60)	2.90 (3.68)	.28**
T5	6.02 (5.71)	3.65 (4.86)	.25**
Interpersonal stress			
T1	2.63 (2.25)	1.54 (1.57)	.26**
T2	2.39 (2.44)	1.00 (1.16)	.34**
T3	2.40 (1.97)	1.38 (1.50)	.27**
T4	1.99 (1.74)	1.13 (1.12)	.25**
T5	2.07 (1.89)	1.36 (1.41)	.22**
Noninterpersonal stress			
T1	.39 (.59)	.35 (.50)	.01
T2	.48 (.63)	.49 (.70)	.00
T3	.75 (1.04)	.36 (.71)	.25**
T4	.50 (.77)	.43 (.61)	.02
T5	.68 (.92)	.37 (.64)	.23**

** $p < .01$.

2010; Mahon et al., 2013; Pagliaccio et al., 2014; Sheikh et al., 2013; van Leeuwen et al., 2011; Zannas & Binder, 2014). In terms of environmental influences, we focused on interpersonal and noninterpersonal events separately given evidence that interpersonal events are stronger contributors to depression risk (Rudolph et al., 2000) and may be stronger predictors of HPA axis reactivity (Dickerson & Kemeny, 2004; Stroud et al., 2016) than noninterpersonal stressors. Supporting our hypotheses, we found that,

among children of depressed mothers, those with a higher MGPS who also experienced higher levels of interpersonal stress experienced the greatest increases in depressive symptoms between assessments. The G × E findings were significant for interpersonal, but not noninterpersonal, stress and were not observed among children of never depressed mothers.

These results add to a growing body of research suggesting that variants in genes that help to regulate the HPA axis may moderate

Table 3
Summary of Hierarchical Linear Modeling Analyses Examining Maternal Major Depressive Disorder (MDD) × Multilocus Genetic Profile Scores (MGPS) × Stress Interactions Predicting Increases in Youth's Depressive Symptoms

Variable	Interpersonal stress		Noninterpersonal stress	
	t	r_{effect_size} [95% CI]	t	r_{effect_size} [95% CI]
CDI$_t$ intercept (π_{0j})				
Intercept (β_{00})	1.69	.11 [−.02, .23]	1.66	.11 [−.02, .23]
MDD[a] (β_{01})	0.65	.04 [−.09, .17]	−0.20	−.01 [−.14, .12]
MGPS (β_{02})	−0.11	−.01 [−.13, .12]	−0.13	−.01 [−.14, .12]
MDD × MGPS (β_{03})	−0.67	−.04 [−.17, .08]	0.37	.02 [−.10, .15]
CDI$_{t-1}$ slope (π_{1j})				
Intercept (β_{10})	2.40*	.15 [.03, .27]	2.09*	.14 [.01, .26]
MDD (β_{11})	1.11	.07 [−.06, .20]	0.84	.05 [−.07, .18]
MGPS (β_{12})	0.81	.05 [−.08, .18]	0.62	.04 [.09, .17]
MDD × MGPS (β_{13})	−0.96	−.06 [−.19, .07]	−0.54	−.04 [−.16, .09]
Episodic stress slope (π_{2j})				
Intercept (β_{20})	1.67	.11 [−.02, .23]	0.07	.00 [−.12, .13]
MDD (β_{21})	−1.80	−.12 [−.24, .01]	−0.28	−.02 [−.15, .11]
MGPS (β_{22})	−0.84	−.06 [−.18, .07]	0.15	.01 [−.12, .14]
MDD × MGPS (β_{23})	2.15*	.14 [.01, .26]	0.27	.02 [−.11, .15]

Note. CI = confidence interval; CDI = Children's Depression Inventory.
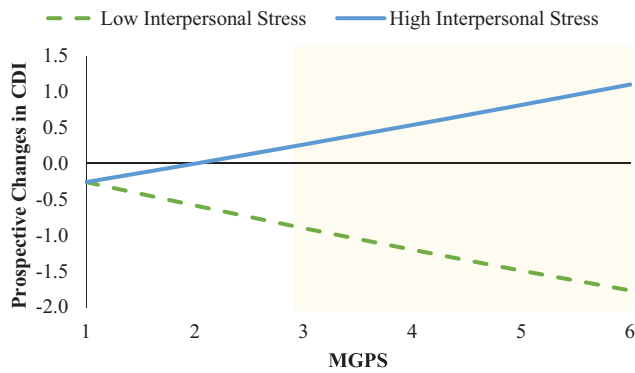[a] Mother history of MDD (yes, no).
* $p < .05$.

Figure 1. Interpersonal Stress × Multilocus Genetic Profile Score (MGPS) interaction for offspring of depressed mothers. The shaded region is the region of the MGPS at which the slope of the relation between stress and youth's depressive symptoms becomes significant ($p < .05$). CDI = Children's Depression Inventory. See the online article for the color version of this figure.

the impact of life stress on individuals' risk for depression (e.g., Appel et al., 2011; Bet et al., 2009; Bradley et al., 2008). The current study extends this previous research in several important ways. It is the first study to examine whether variation in these genes moderate the impact of negative life events on prospective changes in depressive symptoms among children and adolescents. It is also the first to test this type of integrated G × E model of risk for the intergenerational transmission of depression. Although previous studies have examined whether variation in these and other genes may moderate the link between mother and child depression (e.g., Lewis, Collishaw, Harold, Rice, & Thapar, 2012), the current findings highlight the important additional role played by interpersonal stressors in understanding youths' risk for depression. Finally, the current study is one of the first to examine an HPA axis MGPS within the context of a G × E model of depression risk. In contrast, the majority of previous research has examined variation in each gene individually and it is increasingly recognized that complex phenotypes such as depression likely result from the combined influence of multiple genes within specific biological pathways rather than any single gene acting in isolation. This approach is also supported by a recent study showing that although an HPA axis MGPS significantly predicted cortisol reactivity to a stressor, none of the individual SNPs were significant when examined in isolation (Pagliaccio et al., 2014).

We should also highlight that the pattern of the G × E observed in our study is consistent with differential susceptibility models of genetic influence (Belsky, 1997; Belsky, Bakermans-Kranenburg, & Van Ijzendoorn, 2007). Specifically, although a higher MGPS combined with elevated levels of interpersonal stress predicted increases in depressive symptoms for children of depressed mothers, a higher MGPS was associated with lower levels of depression in the context of low stress. Consistent with differential susceptibility models, therefore, it appears that this MGPS is associated with worse outcomes in negative environments but better outcomes in positive environments. These results add to a growing body of research suggesting that these genetic influences may be better characterized as increasing "plasticity" rather than only risk (see Bakermans-Kranenburg & van IJzendoorn, 2015; Belsky et al., 2007).

Finally, we should note that, although significant, our model only explained 12% of the variance in depressive symptom increases among the highest risk youth (offspring of depressed moms with MGPS of 3 or greater) and relatively modest increases in depressive symptoms between adjacent assessments. This said, we believe that the current results are an important first step in utilizing a MGPS approach to Test G × E models of risk for the intergenerational transmission of depression. It will be important for future research to incorporate other potential influences to develop more comprehensive, unified models of risk. For example, because maternal depression is associated with both a decreased likelihood of developing a secure attachment with one's infant (for a review, see Martins & Gaffan, 2000) and lower levels of peer support for their offspring (Lewinsohn, Olino, & Klein, 2005), social support and attachment styles may be important influences. Furthermore, although we focused on episodic stress, children of depressed mothers also experienced elevated levels of chronic stress compared to children of never depressed mothers (Feurer et al., 2016; Gershon et al., 2011; Hammen, Shih, & Brennan, 2004) and there is evidence that chronic and episodic stressors may be unique predictors of depression for adolescents (Vrshek-Schallhorn, Stroud, Mineka, Zinbarg, et al., 2015). Therefore, future studies should examine whether these different forms of stress independently interact with MGPSs to predict increases in depressive symptoms in high-risk youth. Finally, we focused on genetic variation in a select number of genes associated with HPA axis activity. There are a number of other factors that may influence the relevance of these genes to responses to interpersonal stress including epigenetic modification and other influences that modify transcription and/or translation of the gene products. However, variability in these mechanisms raises additional questions about possible tissue-specificity that are beyond the scope of these data (i.e., in contrast to epigenetic, and expression approaches, genetic variation is assumed to be largely consistent in all tissues).

The current study exhibited several strengths including the use of a theoretically derived, biological pathway based MGPS established in previous research (Di Iorio et al., 2017), semistructured life stress interview assessments, and a multiwave prospective study design. However, there were also some limitations which should be acknowledged. First, our study only examined the depressive history of mothers and its impact on offspring depressive symptoms. Because paternal history of depression also increases depression risk in offspring (Lieb, Isensee, Höfler, Pfister, & Wittchen, 2002), future studies should examine the history of MDD in both parents. Second, because we only focused on prospective changes in depressive symptoms, future research is needed to determine whether these results generalize to the prediction of depressive episodes in youth. Third, we did not assess for youth's exposure to early life stress. There is evidence that early life stress may contribute to depression risk in adolescence through the mediating role of continued stress exposure (Hazel, Hammen, Brennan, & Najman, 2008). Although many studies have shown that early life stress interacts with HPA regulatory genes to predict depression in adults (e.g., Appel et al., 2011; Polanczyk et al., 2009), it may be that genetic influences associated with greater HPA axis reactivity do not exacerbate the impact of early life stress, per se, but rather moderate the impact of more recent life stressors that are associated with this early stress exposure. Furthermore, as early life stress may sensitize individuals to

the impact of more recent stressors (Hammen, Henry, & Daley, 2000; McLaughlin, Conron, Koenen, & Gilman, 2010), it may be that individuals with high MGPS who experienced high levels of both early and recent life stress (G × Early Life Stress × Recent Life Stress) are at greatest risk for depression. Future studies should examine both early life and recent stressors together when considering G × E interactions and risk for depression in youth. Fourth, it is possible that the lack of support for noninterpersonal stress may have been due to the extreme skew of these variables. Therefore, although our results are consistent with prior research highlighting the importance of interpersonal stress specifically for youth depression risk (Flynn & Rudolph, 2011), future research is needed to more definitively examine the potential role of noninterpersonal stress in risk for depression among children of depressed mothers. Fifth, although our focus on a MGPS within a theory-driven biological pathway represents an advance beyond the traditional focus on a single polymorphism, the number of included variants was still rather small and it is likely that greater coverage of genes associated with HPA axis reactivity will yield stronger results. In addition, we used an additive approach in creating our MGPS, with each variant given equal weight and it is likely that future advances will allow us to provide weighted scores that better account for the individual influence of each variant. Another limitation is that our sample size is relatively small for a genetic association study. Therefore, replication with large samples are needed. Finally, because we were not able to include a replication sample as part of this study, conclusions must remain tentative pending replication.

In summary, the current study contributes to the literature on mechanisms underlying the intergenerational transmission of depression by highlighting specific genetic and environmental influences that may increase risk in children of depressed mothers. As such, it not only highlights a biological pathway underlying this risk (genes associated with HPA axis reactivity) but also a modifiable risk factor in at-risk youth (heightened levels of interpersonal stress). If replicated, these results suggest that focusing specifically on reducing levels of interpersonal stress among children of depressed mothers may help to reduce their risk for developing depression themselves.

References

Adam, E. K., Doane, L. D., Zinbarg, R. E., Mineka, S., Craske, M. G., & Griffith, J. W. (2010). Prospective prediction of major depressive disorder from cortisol awakening responses in adolescence. *Psychoneuroendocrinology, 35,* 921–931. http://dx.doi.org/10.1016/j.psyneuen.2009.12.007

Adrian, C., & Hammen, C. (1993). Stress exposure and stress generation in children of depressed mothers. *Journal of Consulting and Clinical Psychology, 61,* 354–359. http://dx.doi.org/10.1037/0022-006X.61.2.354

American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders* (4th ed.). Washington, DC: Author.

Appel, K., Schwahn, C., Mahler, J., Schulz, A., Spitzer, C., Fenske, K., . . . Grabe, H. J. (2011). Moderation of adult depression by a polymorphism in the FKBP5 gene and childhood physical abuse in the general population. *Neuropsychopharmacology, 36,* 1982–1991. http://dx.doi.org/10.1038/npp.2011.81

Avenevoli, S., Swendsen, J., He, J.-P., Burstein, M., & Merikangas, K. R. (2015). Major depression in the national comorbidity survey-adolescent supplement: Prevalence, correlates, and treatment. *Journal of the American Academy of Child & Adolescent Psychiatry, 54,* 37–44.e2. http://dx.doi.org/10.1016/j.jaac.2014.10.010

Bakermans-Kranenburg, M. J., & van IJzendoorn, M. H. (2015). The hidden efficacy of interventions: Gene × Environment experiments from a differential susceptibility perspective. *Annual Review of Psychology, 66,* 381–409. http://dx.doi.org/10.1146/annurev-psych-010814-015407

Barrett, J. C. (2009). Haploview: Visualization and analysis of SNP genotype data. *Cold Spring Harbor Protocols, 2009,* pdb ip71. http://dx.doi.org/10.1101/pdb.ip71

Barrett, J. C., Fry, B., Maller, J., & Daly, M. J. (2005). Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics, 21,* 263–265. http://dx.doi.org/10.1093/bioinformatics/bth457

Belsky, J. (1997). Theory testing, effect-size evaluation, and differential susceptibility to rearing influence: The case of mothering and attachment. *Child Development, 68,* 598–600. http://dx.doi.org/10.2307/1132110

Belsky, J., Bakermans-Kranenburg, M. J., & Van Ijzendoorn, M. H. (2007). For better and for worse: Differential susceptibility to environmental influences. *Current Directions in Psychological Science, 16,* 300–304. http://dx.doi.org/10.1111/j.1467-8721.2007.00525.x

Bet, P. M., Penninx, B. W. J. H. J. H., Bochdanovits, Z., Uitterlinden, A. G., Beekman, A. T. F. F., van Schoor, N. M., . . . Hoogendijk, W. J. G. G. (2009). Glucocorticoid receptor gene polymorphisms and childhood adversity are associated with depression: New evidence for a gene-environment interaction. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 150B,* 660–669. http://dx.doi.org/10.1002/ajmg.b.30886

Bouma, E. M. C., Riese, H., Nolte, I. M., Oosterom, E., Verhulst, F. C., Ormel, J., & Oldehinkel, A. J. (2011). No associations between single nucleotide polymorphisms in corticoid receptor genes and heart rate and cortisol responses to a standardized social stress test in adolescents: The TRAILS study. *Behavior Genetics, 41,* 253–261. http://dx.doi.org/10.1007/s10519-010-9385-6

Bradley, R. G., Binder, E. B., Epstein, M. P., Tang, Y., Nair, H. P., Liu, W., . . . Ressler, K. J. (2008). Influence of child abuse on adult depression: Moderation by the corticotropin-releasing hormone receptor gene. *Archives of General Psychiatry, 65,* 190–200. http://dx.doi.org/10.1001/archgenpsychiatry.2007.26

Brown, G. W., & Harris, T. (1978). *Social origins of depression.* London, United Kingdom: Free Press.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., . . . Poulton, R. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science, 301,* 386–389.

Cicchetti, D., Rogosch, F. A., & Oshri, A. (2011). Interactive effects of CRHR1, 5-HTTLPR, and child maltreatment on diurnal cortisol regulation and internalizing symptomatology. *Development and Psychopathology, 23,* 1125–1138. http://dx.doi.org/10.1017/S0954579411000599

Cicchetti, D., Rogosch, F. A., & Sturge-Apple, M. L. (2007). Interactions of child maltreatment and serotonin transporter and monoamine oxidase A polymorphisms: Depressive symptomatology among adolescents from low socioeconomic status backgrounds. *Development and Psychopathology, 19,* 1161–1180. http://dx.doi.org/10.1017/S0954579407000600

Comasco, E., Gustafsson, P. A., Sydsjö, G., Agnafors, S., Aho, N., & Svedin, C. G. (2015). Psychiatric symptoms in adolescents: FKBP5 genotype—early life adversity interaction effects. *European Child & Adolescent Psychiatry, 24,* 1473–1483.

Culverhouse, R. C., Saccone, N. L., Horton, A. C., Ma, Y., Anstey, K. J., Banaschewski, T., . . . Bierut, L. J. (2017). Collaborative meta-analysis finds no evidence of a strong interaction between stress and 5-HTTLPR genotype contributing to the development of depression. *Molecular Psychiatry.* Advance online publication. http://dx.doi.org/10.1038/mp.2017.44

Derijk, R. H. (2009). Single nucleotide polymorphisms related to HPA axis reactivity. *Neuroimmunomodulation, 16,* 340–352. http://dx.doi.org/10.1159/000216192

DeRijk, R. H., Wüst, S., Meijer, O. C., Zennaro, M. C., Federenko, I. S., Hellhammer, D. H., . . . de Kloet, E. R. (2006). A common polymor-

phism in the mineralocorticoid receptor modulates stress responsiveness. *The Journal of Clinical Endocrinology and Metabolism, 91,* 5083–5089. http://dx.doi.org/10.1210/jc.2006-0915

Dick, D. M., Agrawal, A., Keller, M. C., Adkins, A., Aliev, F., Monroe, S., . . . Sher, K. J. (2015). Candidate gene-environment interaction research: Reflections and recommendations. *Perspectives on Psychological Science, 10,* 37–59. http://dx.doi.org/10.1177/1745691614556682

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin, 130,* 355–391. http://dx.doi.org/10.1037/0033-2909.130.3.355

Di Iorio, C. R., Carey, C. E., Michalski, L. J., Corral-Frias, N. S., Conley, E. D., Hariri, A. R., & Bogdan, R. (2017). Hypothalamic-pituitary-adrenal axis genetic variation and early stress moderates amygdala function. *Psychoneuroendocrinology, 80,* 170–178. http://dx.doi.org/10.1016/j.psyneuen.2017.03.016

Engels, W. R. (2009). Exact tests for Hardy–Weinberg proportions. *Genetics, 183,* 1431–1441. http://dx.doi.org/10.1534/genetics.109.108977

Ferrari, A. J., Charlson, F. J., Norman, R. E., Patten, S. B., Freedman, G., Murray, C. J. L., . . . Whiteford, H. A. (2013). Burden of depressive disorders by country, sex, age, and year: Findings from the global burden of disease study 2010. *PLoS Medicine, 10,* e1001547. http://dx.doi.org/10.1371/journal.pmed.1001547

Feurer, C., Hammen, C. L., & Gibb, B. E. (2016). Chronic and episodic stress in children of depressed mothers. *Journal of Clinical Child and Adolescent Psychology, 45,* 270–278. http://dx.doi.org/10.1080/15374416.2014.963859

First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. W. (1995). *Structured clinical interview for DSM–IV Axis I disorders-Patient edition (SCID-I/P).* New York, NY: Biometrics Research Department, NY State Psychiatric Institute.

Flynn, M., & Rudolph, K. D. (2011). Stress generation and adolescent depression: Contribution of interpersonal stress responses. *Journal of Abnormal Child Psychology, 39,* 1187–1198. http://dx.doi.org/10.1007/s10802-011-9527-1

Freeman, B., Powell, J., Ball, D., Hill, L., Craig, I., & Plomin, R. (1997). DNA by mail: An inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. *Behavior Genetics, 27,* 251–257. http://dx.doi.org/10.1023/A:1025614231190

Gatt, J. M., Nemeroff, C. B., Dobson-Stone, C., Paul, R. H., Bryant, R. A., Schofield, P. R., . . . Williams, L. M. (2009). Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety. *Molecular Psychiatry, 14,* 681–695. http://dx.doi.org/10.1038/mp.2008.143

Gershon, A., Hayward, C., Schraedley-Desmond, P., Rudolph, K. D., Booster, G. D., & Gotlib, I. H. (2011). Life stress and first onset of psychiatric disorders in daughters of depressed mothers. *Journal of Psychiatric Research, 45,* 855–862. http://dx.doi.org/10.1016/j.jpsychires.2011.03.016

Goodman, S. H. (2007). Depression in mothers. *Annual Review of Clinical Psychology, 3,* 107–135. http://dx.doi.org/10.1146/annurev.clinpsy.3.022806.091401

Goodyer, I. M., Herbert, J., & Tamplin, A. (2003). Psychoendocrine antecedents of persistent first-episode major depression in adolescents: A community-based longitudinal enquiry. *Psychological Medicine, 33,* 601–610. http://dx.doi.org/10.1017/S0033291702007286

Guerry, J. D., & Hastings, P. D. (2011). In search of HPA axis dysregulation in child and adolescent depression. *Clinical Child and Family Psychology Review, 14,* 135–160. http://dx.doi.org/10.1007/s10567-011-0084-5

Gunnar, M., & Quevedo, K. (2007). The neurobiology of stress and development. *Annual Review of Psychology, 58,* 145–173. http://dx.doi.org/10.1146/annurev.psych.58.110405.085605

Guo, S. W., & Thompson, E. A. (1992). Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics, 48,* 361–372. http://dx.doi.org/10.2307/2532296

Hammen, C. (1991). Generation of stress in the course of unipolar depression. *Journal of Abnormal Psychology, 100,* 555–561. http://dx.doi.org/10.1037/0021-843X.100.4.555

Hammen, C., Henry, R., & Daley, S. E. (2000). Depression and sensitization to stressors among young women as a function of childhood adversity. *Journal of Consulting and Clinical Psychology, 68,* 782–787. http://dx.doi.org/10.1037/0022-006X.68.5.782

Hammen, C., Shih, J. H., & Brennan, P. A. (2004). Intergenerational transmission of depression: Test of an interpersonal stress model in a community sample. *Journal of Consulting and Clinical Psychology, 72,* 511–522. http://dx.doi.org/10.1037/0022-006X.72.3.511

Hankin, B. L., Abramson, L. Y., Moffitt, T. E., Silva, P. A., McGee, R., & Angell, K. E. (1998). Development of depression from preadolescence to young adulthood: Emerging gender differences in a 10-year longitudinal study. *Journal of Abnormal Psychology, 107,* 128–140. http://dx.doi.org/10.1037/0021-843X.107.1.128

Hazel, N. A., Hammen, C., Brennan, P. A., & Najman, J. (2008). Early childhood adversity and adolescent depression: The mediating role of continued stress. *Psychological Medicine, 38,* 581–589. http://dx.doi.org/10.1017/S0033291708002857

Heim, C., & Binder, E. B. (2012). Current research trends in early life stress and depression: Review of human studies on sensitive periods, gene-environment interactions, and epigenetics. *Experimental Neurology, 233,* 102–111. http://dx.doi.org/10.1016/j.expneurol.2011.10.032

Heim, C., Bradley, B., Mletzko, T. C., Deveau, T. C., Musselman, D. L., Nemeroff, C. B., . . . Binder, E. B. (2009). Effect of childhood trauma on adult depression and neuroendocrine function: Sex-specific moderation by CRH receptor 1 gene. *Frontiers in Behavioral Neuroscience, 3,* 41. http://dx.doi.org/10.3389/neuro.08.041.2009

Hosang, G. M., Shiles, C., Tansey, K. E., McGuffin, P., & Uher, R. (2014). Interaction between stress and the BDNF Val66Met polymorphism in depression: A systematic review and meta-analysis. *BMC Medicine, 12,* 7. http://dx.doi.org/10.1186/1741-7015-12-7

Ising, M., Depping, A. M., Siebertz, A., Lucae, S., Unschuld, P. G., Kloiber, S., . . . Holsboer, F. (2008). Polymorphisms in the FKBP5 gene region modulate recovery from psychosocial stress in healthy controls. *The European Journal of Neuroscience, 28,* 389–398. http://dx.doi.org/10.1111/j.1460-9568.2008.06332.x

Karg, K., Burmeister, M., Shedden, K., & Sen, S. (2011). The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: Evidence of genetic moderation. *Archives of General Psychiatry, 68,* 444–454. http://dx.doi.org/10.1001/archgenpsychiatry.2010.189

Kaufman, J., Birmaher, B., Brent, D., Rao, U., Flynn, C., Moreci, P., . . . Ryan, N. (1997). Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADS-PL): Initial reliability and validity data. *Journal of the American Academy of Child & Adolescent Psychiatry, 36,* 980–988. http://dx.doi.org/10.1097/00004583-199707000-00021

Kessler, R. C. (1997). The effects of stressful life events on depression. *Annual Review of Psychology, 48,* 191–214. http://dx.doi.org/10.1146/annurev.psych.48.1.191

Klok, M. D., Giltay, E. J., Van der Does, A. J., Geleijnse, J. M., Antypa, N., Penninx, B. W., . . . DeRijk, R. H. (2011). A common and functional mineralocorticoid receptor haplotype enhances optimism and protects against depression in females. *Translational Psychiatry, 1,* e62. http://dx.doi.org/10.1038/tp.2011.59

Klok, M. D., Vreeburg, S. A., Penninx, B. W. J. H., Zitman, F. G., de Kloet, E. R., & DeRijk, R. H. (2011). Common functional mineralocorticoid receptor polymorphisms modulate the cortisol awakening re-

sponse: Interaction with SSRIs. *Psychoneuroendocrinology, 36,* 484–494. http://dx.doi.org/10.1016/j.psyneuen.2010.07.024

Kovacs, M. (1981). Rating scales to assess depression in school-aged children. *Acta Paedopsychiatrica, 46,* 305–315.

Kovacs, M. (1985). The children's depression inventory. *Psychopharmacology Bulletin, 21,* 995–998.

Lahti, J., Ala-Mikkula, H., Kajantie, E., Haljas, K., Eriksson, J. G., & Räikkönen, K. (2016). Associations between self-reported and objectively recorded early life stress, FKBP5 polymorphisms, and depressive symptoms in midlife. *Biological Psychiatry, 80,* 869–877.

Laucht, M., Treutlein, J., Blomeyer, D., Buchmann, A. F., Schmidt, M. H., Esser, G., . . . Banaschewski, T. (2013). Interactive effects of corticotropin-releasing hormone receptor 1 gene and childhood adversity on depressive symptoms in young adults: Findings from a longitudinal study. *European Neuropsychopharmacology, 23,* 358–367. http://dx.doi.org/10.1016/j.euroneuro.2012.06.002

Lench, N., Stanier, P., & Williamson, R. (1988). Simple non-invasive method to obtain DNA for gene analysis. *Lancet, 1,* 1356–1358. http://dx.doi.org/10.1016/S0140-6736(88)92178-2

Lewinsohn, P. M., Olino, T. M., & Klein, D. N. (2005). Psychosocial impairment in offspring of depressed parents. *Psychological Medicine, 35,* 1493–1503. http://dx.doi.org/10.1017/S0033291705005350

Lewinsohn, P. M., Rohde, P., Seeley, J. R., Klein, D. N., & Gotlib, I. H. (2000). Natural course of adolescent major depressive disorder in a community sample: Predictors of recurrence in young adults. *The American Journal of Psychiatry, 157,* 1584–1591. http://dx.doi.org/10.1176/appi.ajp.157.10.1584

Lewis, G., Collishaw, S., Harold, G., Rice, F., & Thapar, A. (2012). Maternal depression and child and adolescent depression symptoms: An exploratory test for moderation by CRHR1, FKBP5 and NR3C1 gene variants. *Behavior Genetics, 42,* 121–132. http://dx.doi.org/10.1007/s10519-011-9482-1

Lieb, R., Isensee, B., Höfler, M., Pfister, H., & Wittchen, H. U. (2002). Parental major depression and the risk of depression and other mental disorders in offspring: A prospective-longitudinal community study. *Archives of General Psychiatry, 59,* 365–374. http://dx.doi.org/10.1001/archpsyc.59.4.365

Livak, K. J., Flood, S. J. A., Marmaro, J., Giusti, W., & Deetz, K. (1995). Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *Genome Research, 4,* 357–362. http://dx.doi.org/10.1101/gr.4.6.357

Lopez-Duran, N. L., Kovacs, M., & George, C. J. (2009). Hypothalamic–pituitary–adrenal axis dysregulation in depressed children and adolescents: A meta-analysis. *Psychoneuroendocrinology, 34,* 1272–1283. http://dx.doi.org/10.1016/j.psyneuen.2009.03.016

Luijk, M. P. C. M., Velders, F. P., Tharner, A., van IJzendoorn, M. H., Bakermans-Kranenburg, M. J., Jaddoe, V. W. V., . . . Tiemeier, H. (2010). FKBP5 and resistant attachment predict cortisol reactivity in infants: Gene-environment interaction. *Psychoneuroendocrinology, 35,* 1454–1461. http://dx.doi.org/10.1016/j.psyneuen.2010.04.012

Mahon, P. B., Zandi, P. P., Potash, J. B., Nestadt, G., & Wand, G. S. (2013). Genetic association of FKBP5 and CRHR1 with cortisol response to acute psychosocial stress in healthy adults. *Psychopharmacology, 227,* 231–241. http://dx.doi.org/10.1007/s00213-012-2956-x

Martins, C., & Gaffan, E. A. (2000). Effects of early maternal depression on patterns of infant-mother attachment: A meta-analytic investigation. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 41,* 737–746. http://dx.doi.org/10.1111/1469-7610.00661

Mazure, C. M. (1998). Life stressors as risk factors in depression. *Clinical Psychology: Science and Practice, 5,* 291–313. http://dx.doi.org/10.1111/j.1468-2850.1998.tb00151.x

McLaughlin, K. A., Conron, K. J., Koenen, K. C., & Gilman, S. E. (2010). Childhood adversity, adult stressful life events, and risk of past-year

psychiatric disorder: A test of the stress sensitization hypothesis in a population-based sample of adults. *Psychological Medicine, 40,* 1647–1658. http://dx.doi.org/10.1017/S0033291709992121

Melas, P. A., Wei, Y., Wong, C. C. Y., Sjöholm, L. K., Åberg, E., Mill, J., . . . Lavebratt, C. (2013). Genetic and epigenetic associations of MAOA and NR3C1 with depression and childhood adversities. *The International Journal of Neuropsychopharmacology, 16,* 1513–1528. http://dx.doi.org/10.1017/S1461145713000102

Monroe, S. M., Slavich, G. M., & Georgiades, K. (2014). The social environment and life stress in depression. In I. H. Gotlib & C. L. Hammen (Eds.), *Handbook of depression* (3rd ed., pp. 340–360). New York, NY: Guilford Press.

Nikolova, Y. S., Ferrell, R. E., Manuck, S. B., & Hariri, A. R. (2011). Multilocus genetic profile for dopamine signaling predicts ventral striatum reactivity. *Neuropsychopharmacology, 36,* 1940–1947. http://dx.doi.org/10.1038/npp.2011.82

Owens, M., Harrison, A. J., Burkhouse, K. L., McGeary, J. E., Knopik, V. S., Palmer, R. H. C., & Gibb, B. E. (2016). Eye tracking indices of attentional bias in children of depressed mothers: Polygenic influences help to clarify previous mixed findings. *Development and Psychopathology, 28,* 385–397. http://dx.doi.org/10.1017/S0954579415000462

Pagliaccio, D., Luby, J. L., Bogdan, R., Agrawal, A., Gaffrey, M. S., Belden, A. C., . . . Barch, D. M. (2014). Stress-system genes and life stress predict cortisol levels and amygdala and hippocampal volumes in children. *Neuropsychopharmacology, 39,* 1245–1253. http://dx.doi.org/10.1038/npp.2013.327

Pagliaccio, D., Luby, J. L., Bogdan, R., Agrawal, A., Gaffrey, M. S., Belden, A. C., . . . Barch, D. M. (2015). Amygdala functional connectivity, HPA axis genetic variation, and life stress in children and relations to anxiety and emotion regulation. *Journal of Abnormal Psychology, 124,* 817–833. http://dx.doi.org/10.1037/abn0000094

Polanczyk, G., Caspi, A., Williams, B., Price, T. S., Danese, A., Sugden, K., . . . Moffitt, T. E. (2009). Protective effect of CRHR1 gene variants on the development of adult depression following childhood maltreatment: Replication and extension. *Archives of General Psychiatry, 66,* 978–985. http://dx.doi.org/10.1001/archgenpsychiatry.2009.114

Preacher, K. J., Curran, P. J., & Bauer, D. J. (2006). Computational tools for probing interactions in multiple linear regression, multilevel modeling, and latent curve analysis. *Journal of Educational and Behavioral Statistics, 31,* 437–448. http://dx.doi.org/10.3102/10769986031004437

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., . . . Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics, 81,* 559–575. http://dx.doi.org/10.1086/519795

Raudenbush, S. W., & Bryk, A. S. (2002). *Hierarchical linear models: Applications and data analysis methods* (2nd ed.). Thousand Oaks, CA: SAGE.

Raudenbush, S. W., Bryk, A. S., Cheong, Y. F., & Congdon, R. T. (2004). *HLM 6: Hierarchical linear and nonlinear modeling.* Lincolnwood, IL: Scientific Software International.

Risch, N., Herrell, R., Lehner, T., Liang, K.-Y., Eaves, L., Hoh, J., . . . Merikangas, K. R. (2009). Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: A meta-analysis. *Journal of the American Medical Association, 301,* 2462–2471. http://dx.doi.org/10.1001/jama.2009.878

Rudolph, K. D., & Flynn, M. (2014). Depression in adolescents. In I. H. Gotlib & C. L. Hammen (Eds.), *Handbook of depression* (3rd ed., pp. 391–409). New York, NY: Guilford Press.

Rudolph, K. D., Hammen, C., Burge, D., Lindberg, N., Herzberg, D., & Daley, S. E. (2000). Toward an interpersonal life-stress model of depression: The developmental context of stress generation. *Development and Psychopathology, 12,* 215–234. http://dx.doi.org/10.1017/S0954579400002066

Schafer, J. L., & Graham, J. W. (2002). Missing data: Our view of the state of the art. *Psychological Methods, 7,* 147–177. http://dx.doi.org/10.1037/1082-989X.7.2.147

Sheikh, H. I., Kryski, K. R., Smith, H. J., Hayden, E. P., & Singh, S. M. (2013). Corticotropin-releasing hormone system polymorphisms are associated with children's cortisol reactivity. *Neuroscience, 229,* 1–11. http://dx.doi.org/10.1016/j.neuroscience.2012.10.056

Smucker, M. R., Craighead, W. E., Craighead, L. W., & Green, B. J. (1986). Normative and reliability data for the children's depression inventory. *Journal of Abnormal Child Psychology, 14,* 25–39. http://dx.doi.org/10.1007/BF00917219

Stephens, M., & Donnelly, P. (2003). A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *American Journal of Human Genetics, 73,* 1162–1169. http://dx.doi.org/10.1086/379378

Stephens, M., Smith, N. J., & Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics, 68,* 978–989. http://dx.doi.org/10.1086/319501

Stroud, C. B., Chen, F. R., Doane, L. D., & Granger, D. A. (2016). Individual differences in early adolescents' latent trait cortisol (LTC): Relation to recent acute and chronic stress. *Psychoneuroendocrinology, 70,* 38–46. http://dx.doi.org/10.1016/j.psyneuen.2016.04.015

Tabachnick, B. G., & Fidell, L. S. (2007). *Using multivariate statistics* (5th ed.). New York, NY: Pearson.

Tyrka, A. R., Price, L. H., Gelernter, J., Schepker, C., Anderson, G. M., & Carpenter, L. L. (2009). Interaction of childhood maltreatment with the corticotropin-releasing hormone receptor gene: Effects on hypothalamic-pituitary-adrenal axis reactivity. *Biological Psychiatry, 66,* 681–685. http://dx.doi.org/10.1016/j.biopsych.2009.05.012

van Leeuwen, N., Bellingrath, S., de Kloet, E. R., Zitman, F. G., DeRijk, R. H., Kudielka, B. M., & Wüst, S. (2011). Human mineralocorticoid receptor (MR) gene haplotypes modulate MR expression and transactivation: Implication for the stress response. *Psychoneuroendocrinology, 36,* 699–709. http://dx.doi.org/10.1016/j.psyneuen.2010.10.003

Vinkers, C. H., Joëls, M., Milaneschi, Y., Gerritsen, L., Kahn, R. S., Penninx, B. W. J. H., & Boks, M. P. M. (2015). Mineralocorticoid receptor haplotypes sex-dependently moderate depression susceptibility following childhood maltreatment. *Psychoneuroendocrinology, 54,* 90–102. http://dx.doi.org/10.1016/j.psyneuen.2015.01.018

Vrshek-Schallhorn, S., Stroud, C. B., Mineka, S., Hammen, C., Zinbarg, R. E., Wolitzky-Taylor, K., & Craske, M. G. (2015). Chronic and episodic interpersonal stress as statistically unique predictors of depression in two samples of emerging adults. *Abnormal Psychology, 124,* 918–932. http://dx.doi.org/10.1037/abn0000088

Vrshek-Schallhorn, S., Stroud, C. B., Mineka, S., Zinbarg, R. E., Adam, E. K., Redei, E. E., . . . Craske, M. G. (2015). Additive genetic risk from five serotonin system polymorphisms interacts with interpersonal stress to predict depression. *Journal of Abnormal Psychology, 124,* 776–790. http://dx.doi.org/10.1037/abn0000098

Zannas, A. S., & Binder, E. B. (2014). Gene-environment interactions at the FKBP5 locus: Sensitive periods, mechanisms and pleiotropism. *Genes Brain & Behavior, 13,* 25–37. http://dx.doi.org/10.1111/gbb.12104

Zimmermann, P., Brückl, T., Nocon, A., Pfister, H., Binder, E. B., Uhr, M., . . . Ising, M. (2011). Interaction of FKBP5 gene variants and adverse life events in predicting depression onset: Results from a 10-year prospective community study. *The American Journal of Psychiatry, 168,* 1107–1116. http://dx.doi.org/10.1176/appi.ajp.2011.10111577