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HPA-Axis Multilocus Genetic Profile Score Moderates the Association Between Maternal Prenatal Stress and Offspring Depressive Symptoms in Young Adulthood

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Brooke G. McKenna
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An abstract of
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Abstract

HPA-Axis Multilocus Genetic Profile Score Moderates the Association Between Maternal Prenatal Stress and Offspring Depressive Symptoms in Young Adulthood

By Brooke G. McKenna

Studies have demonstrated that maternal stress during pregnancy can cause alterations to the fetal HPA-axis, a phenomenon known as *fetal programming,* that may have lasting impacts on offspring outcomes. Evidence suggests that maternal prenatal stress particularly confers risk for offspring depression, but few studies have examined depressive outcomes in adulthood. Moreover, animal studies indicate that the influence of prenatal stress exposure on depression may vary with respect to offspring's genetic risk. The present study builds upon the extant literature by examining the interaction of maternal prenatal stress and offspring HPA-axis polygenic risk to predict offspring depressive symptoms in early adulthood. 383 mother-child dyads participated in a prospective, longitudinal study spanning from pregnancy until offspring were 20 years of age. Polygenic risk was defined by a multilocus genetic profile score (MGPS) reflecting the additive risk of four HPA-axis candidate risk genes: CRHR1, FKBP5, NR3C1, and BDNF. Results indicated that the HPA-axis MGPS did not directly confer risk for offspring depressive symptoms at age 20, but the interaction of maternal prenatal stress with MGPS did. No individual risk genes were found to drive this effect. Further, our results supported the *differential susceptibility model* such that offspring at high genetic risk exhibited greater sensitivity to the presence or absence of maternal prenatal stress than offspring at low genetic risk. Finally, we demonstrated that this interaction was specific to maternal prenatal stress, as maternal stress during early childhood did not interact with genetic risk in the prediction of offspring depressive outcomes. Together, these findings provide the first evidence that genetic variants associated with the HPA-axis may act in a polygenic, additive fashion to moderate the association between fetal programming and offspring depression in early adulthood.

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Table of Contents

Introduction

Major depressive disorder (MDD) is a debilitating condition experienced by 9 to 12% of men and 20 to 25% of women across the lifespan (Kessler, Chiu, Demler & Walters, 2005). Decades of studies have indicated that environmental stress plays an indisputable role in the pathogenesis of MDD (Hammen, 2005). The majority of these studies focus on the impact of early life stress (for a review, see Heim and Binder, 2012) or stressful life events throughout adolescence and adulthood (e.g., Kessler, 1997). However, accruing evidence indicates that stress exposure *in utero* may also play an important role (Goodman & Gotlib, 1999). Recent studies have identified significant associations between maternal stress during pregnancy (i.e., maternal prenatal stress) and early childhood outcomes including altered immune function, anxious behaviors, reduced attention, and altered cardiovascular response to stress (Igosheva, Taylor, Poston, & Glover, 2007). These relationships are purported to be the result of *fetal programming*, in which prenatal exposures initiate an "adaptive" response in the fetus that carries forth to postnatal development, influencing both biology and behavior (Amiel-Tison et al., 2004; Barker, 2007; Gluckman & Hanson, 2005; Gluckman et al., 2009). In the case of maternal prenatal stress, the fetus may be programmed to anticipate a stressful postnatal environment through altered sensitivity to environmental stressors (Glover, O'Connor, & O'Donnell, 2010). Although this sensitivity may be adaptive in a threatening postnatal environment, it may serve as maladaptive in a nonthreatening environment, thus conferring risk for psychopathology.

To investigate the mechanisms underlying fetal programming theory, researchers have sought to identify pathways by which maternal stress may affect fetal development

(Starr, Hammen, Conway, Raposa, & Brennan, 2014). A key system that has been identified is the hypothalamic-pituitary-adrenal (HPA) axis (Glover, O'Connor, & O'Donnell, 2010). The HPA-axis involves a complex interaction of molecular signaling that ultimately produces the glucocorticoid *cortisol*, a hormone broadly responsible for regulating the body's response to stress (Davis et al., 2007). Due to its role in the stress response, examining alterations in the HPA-axis has become a primary focus of psychopathology research, particularly for major depression (Pariante and Lightman, 2008). This research has not only focused on individuals' own risk for depression, but also how alterations in a mother's HPA-axis may influence her offspring's risk. Evidence suggests that during pregnancy, roughly 10-20% of cortisol produced by a mother's stress-response is able to pass through the placenta into the blood of her fetus (Gitau, Cameron, Fisk, & Glover, 1998). Once cortisol crosses the fetal blood-brain barrier, it can then program the development of neural systems within the fetus (Davis et al., 2007). Both rodent and non-human primate studies (Dickerson et al., 2005; Schneider, 1992) suggest that neural networks involved in fear regulation and behavioral inhibition are particularly vulnerable to prenatal stress and cortisol exposures. For instance, one study of non-human primates found that offspring subjected to maternal prenatal stress exhibit increased irritability, fearfulness, and disturbance behaviors compared to offspring that are not (Schneider, 1992). In humans, elevated maternal cortisol levels during pregnancy have been associated with infant negative emotional reactivity, dysregulated temperament, and an increased fear response to novel stimuli (Davis et al., 2007; de Weerth, van Hees, & Buitelaar, 2003; Lundy et al., 1999). These infant outcomes are particularly salient because they predict maladaptive behaviors (e.g., heightened stress

reactivity) in children and adolescents (Belsky et al., 1998; Carson & Bittner, 1994), which in turn confers risk for depression and other internalizing pathology in later life (Starr, Hammen, Conway, Raposa, & Brennan, 2014). Despite these associations, a dearth of studies has directly examined the association between maternal prenatal stress and offspring depressive outcomes into early adulthood. Given that rates of depression increase steadily throughout this time – with the average age of MDD onset between 15-29 years old (Lewinsohn et al., 1994; Fergusson et al. 2005; Weissman et al., 2006) – it is important to assess the role of prenatal exposures during this period of high developmental risk for depression. The present study focuses on offspring depressive outcomes at age 20 in order to take this risk period into consideration.

Moderating Factors

Although prenatal modifications to the HPA-axis have generally been shown to influence offspring outcomes, these effects appear to be moderated by a number of important factors. A review of animal studies conducted by Glover, O'Connor, & O'Donnell (2010) indicates that maternal stress during pregnancy does reprogram the function of a fetus' HPA-axis, but the consequences vary with respect to the fetus' genetics. This association remains understudied in humans, but evidence from human studies investigating the effects of *post*natal stress on mood disorders identifies a number of HPA-axis related genes that may play a moderating role. One such gene is the corticotrophin receptor 1 (CRHR1) gene, which codes for the corticotrophin-releasing hormone receptor and functions as a key regulator of the HPA-axis (Feurer et al., 2017). Studies have shown that variation in the CRHR1 genotype is associated with cortisol reactivity to laboratory stress tasks in both adults and children (Mahon, Zandi, Potash,

Nestadt, & Wand, 2013; Sheikh, Kryski, Smith, Hayden, & Singh, 2013). Polymorphisms in the CRHR1 gene have also been shown to interact with child abuse (Bradley et al., 2008; Heim and Binder, 2012; Polanczyk et al., 2009) and with physical neglect (Grabe et al., 2010) to predict adult depressive symptoms. Other studies have suggested that three single nucleotide polymorphisms (SNPs) in CRHR1 (rs7209436, rs110402, and rs242924) form a protective TAT haplotype, such that individuals who experience child maltreatment are less likely to develop adult depression if they possess the protective haplotype (Heim et al., 2009; Tyrka et al., 2009). Single SNPs within the TAT haplotype have been shown to independently moderate the relationship between child abuse and adult depression as well (Heim et al., 2009).

Another gene that plays a primary role in HPA-axis activity is the glucocorticoid receptor-regulating co-chaperone FK506 binding protein 5 (FKBP5) gene, which serves a regulatory role in the glucocorticoid, oxytocinergic, serotonergic, and dopaminergic systems (Bet et al., 2009; Conway et al., 2010; Gatt et al., 2009; Luijk et al., 2010; Roy, Gorodetsky, Yuan, Goldman, & Enoch., 2010; Thompson et al., 2010; Zimmerman et al., 2011). Polymorphisms of FKBP5 have been associated with increased cortisol reactivity in laboratory stress tasks (Ising et al., 2008, Luikjk et al., 2010; Zannas and Binder, 2014) and variation in the FKBP5 genotype has been shown to moderate the impact of negative life events on adult depression (Appel et al., 2011; Comasco et al., 2015; Lahti et al., 2016; Zimmerman et al., 2011). Multiple studies have found that a specific SNP within FKBP5, rs9296158, interacts with child abuse to predict adult PTSD symptoms (Binder et al., 2008). This SNP has also been shown to moderate the effects of adverse life events on adult depression (Zimmerman et al., 2011), such that individuals with two minor

alleles (AA) exhibit higher rates of depression after adverse events than heterozygous (AG) or homozygous major allele (GG) individuals.

The nuclear receptor subfamily 3 group C memory 1 (NR3C1) gene has also been implicated in life stress and depression. NR3C1, a glucocorticoid receptor, plays a vital role in stress regulation by modulating gene transcription related to the HPA-axis (DeRijk et al., 2002). Genetic variation in NR3C1 has been shown to predict baseline salivary cortisol levels (Palma-Gudiel, Córdova-Palomera, Leza, & Fañanás, 2015) and disruption in this gene can cause glucocorticoid resistance, a disorder that leads to increased plasma concentration and high urinary free cortisol (Gene, 2018). The SNP rs6198 has specifically been tied to this disease (Kumsta et al., 2008), and allelic variation at this locus has been shown to decrease glucocorticoid receptor sensitivity (van Rossum et al., 2004). This SNP has also been associated with MDD and depressive symptoms within bipolar disorder (Szczepankiewicz et al., 2011), such that individuals with at least one copy of the minor allele (CC or CT) are at higher risk than individuals with two copies of the major allele (TT). Epigenetic studies suggest that NR3C1 is implicated in the intergenerational transmission of depression as well. Oberlander et al. (2008) examined differential methylation at a prime CpG region of the NR3C1 gene and determined that infants exposed to maternal prenatal depression in the third trimester exhibited increased methylation of NR3C1 compared to infants of non-depressed mothers. This increased methylation was, in turn, associated with increased salivary cortisol reactivity in the infants.

Finally, genetic variation in the brain-derived neurotrophic factor (BDNF) gene has also been shown to impact HPA-axis functioning and susceptibility to depression

(Alexander et al., 2010; Shalev et al., 2009). BDNF is directly involved in neurogenesis and plasticity within brain regions associated with depressive symptoms, such as the amygdala and the frontohippocampus (Gatt et al., 2009), and reduced serum concentrations of BDNF have been tied to MDD (Karege et al., 2005; Shimizu et al., 2003). Alterations in BDNF secretion have specifically been tied to a valine-methionine substitution at codon 66 [Val66Met] (Egan et al., 2003), which has also been associated with depression symptoms (Chen et al., 2006). Studies have shown that individuals with a Met allele exhibit reduced secretion of BDNF (Egan et al., 2003) and some studies suggest that Met carriers are at a higher risk for depression (Verhagen et al., 2010). Evidence for the latter is mixed, likely because the effects of this variant are moderated by environmental stress. For example, a recent meta-analysis concluded that the Met allele significantly interacts with both early life adversity and stressful life events to predict depression (Hosang, Shiles, Tansey, McGuffin, & Uher, 2014).

Multilocus Genetic Profile Scores

Recent research has shown that the additive effects of multiple genes within a biological pathway contribute significantly more to an interaction than any single gene (Gatt et al., 2010). As such, researchers have begun to utilize a new measure of polygenic risk, known as a multilocus genetic profile score (MGPS), to examine the interaction of genetics and environmental stress (G x E). Unlike a polygenic risk score (PGR), which employs an exploratory approach to identifying risk genes, MGPSs approach polygenic risk in a hypothesis-driven manner such that a score reflects the unweighted summed effect of previously-identified risk SNPs (Nikolova, Ferrell, Manuck, & Hariri, 2011). The score can then be used to examine the additive influence of those polymorphisms on

outcomes of interest. For example, a recent study used an HPA-axis MGPS comprised of polymorphisms in FKBP5, NR3C2 and the CRHR1 TAT haplotype to identify a moderating effect of HPA-axis genes on the association between interpersonal stress and depressive symptoms in early childhood (Feurer et al., 2017). Others used an MGPS comprised of stress response-related genes to determine whether that score moderated the impact of life stress on amygdala volume and function (di Iorio et al., 2017; Pagliaccio et al., 2014), which in turn has been associated with MDD in adulthood (Hastings et al., 2004; Beesdo et al., 2009).

Several studies have found that the cumulative effect of allelic variation within an MGPS exhibits greater predictive power than any single SNP within the score. One study examined five polymorphisms in dopaminergic genes and found that while none of the individual SNPs predicted reward-related brain activity (which is often disrupted in the context of depression), the MGPS did (Nikolova, Ferrell, Manuck, & Hariri, 2011). Another investigated the interaction of a serotonergic MGPS with interpersonal stress to predict depressive symptoms and found similar results – the MGPS exhibited greater predictive utility than any single SNP considered alone (Vrshek-Schallhorn et al., 2015). These results again highlight the potential advantage of applying a polygenic approach to disentangling G x E interactions.

The Present Study

Despite the evidence that 1) prenatal stress can influence the HPA-axis, 2) alterations in the HPA-axis have been closely tied to stress reactivity and depression, and 3) genes related to the HPA-axis have been shown to moderate environmental risk for these outcomes, few studies have addressed the potential interaction between prenatal

stress and HPA-axis genes to predict offspring stress reactivity and depressive outcomes. Moreover, no studies to our knowledge have examined these associations in adult offspring. The present study aims to fill these gaps by utilizing offspring genetic data on four loci of interest: CRHR1 (rs242924), FKBP5 (rs9296158), NR3C1 (rs6198), and BDNF (Val66Met). We hypothesize that an offspring's genetic risk – as defined by an HPA-axis MGPS – will moderate the effect of maternal prenatal stress on early adult outcomes. Specifically, increases in the HPA-axis MGPS will be associated with stronger associations between maternal prenatal stress and offspring stress reactivity, as well as between maternal prenatal stress and offspring depressive symptoms. We further hypothesize that these GxE effects will be specific to maternal stress reported during pregnancy, and will not be evident for maternal stress reported during early childhood.

Methods

Participants

Participants were 383 women and their children, drawn from a sample of 815 mother-child dyads enrolled in the Mater-University of Queensland Study of Pregnancy (MUSP; Keeping et al., 1989). The original MUSP cohort included over 7,000 women and offspring who were studied longitudinally to investigate the effects of pregnancy conditions on children's physical, cognitive, and psychological development. The subsample of 815 dyads was enriched for mothers with depressive symptomatology, based on self-reported depressive symptoms on the Delusions-Symptoms-State Inventory (DSSI; Bedford & Foulds, 1978). Full details of the subsample selection can be found in Hammen and Brennan (2001). Of the 815 mother-child dyads recruited for the study, 705 were followed longitudinally until offspring were age 20. Between age 22 and 25, these

705 offspring were recontacted and asked to participate in DNA sample collection, of whom 512 consented and 416 were genotyped for the four loci of interest. From the original 815 subsample, there were no differences between those who did and did not participate in the genotyping, with the exception that genotyped participants were more likely to be female. In the present study, stress reactivity data was available for 399 of the genotyped offspring (156 males, 243 females) while depressive symptom data was available for a 383 of these individuals (149 males, 234 females). In order to maximize use of the available data, different sample sizes were used to investigate the two outcomes of interest.

Measures

Maternal Stress **–** The Reeder Stress Inventory (RSI) (Reeder, Schrama, & Dirken, 1973) is a four-question measure of self-perceived daily strain that probes individuals' physiological and psychological reactions to personal situations such as daily hassles, major events, and coping resources. During pregnancy and at offspring age 5 years, mothers indicated the extent to which four statements applied to them on a 5-point Likert scale: 1) "In general, I am usually tense or nervous"; 2) "There is a great amount of nervous strain connected with my daily activities"; 3) "At the end of the day I am completely exhausted mentally and physically"; and 4) "My daily activities are extremely trying and stressful." The measure demonstrated high internal consistency, with a Cronbach's alpha of 0.804 for the pregnancy measure and 0.836 for the 5-year measure.

Offspring Stress Reactivity **–** Stress reactivity was established in 20-year-old offspring by calculating appraisal bias scores from the UCLA Life Stress Interview (LSI) (Hammen and Brennan, 2001). Participants were asked to rate the stress severity of each

stressful event they reported on a 5-point scale. *Appraisal bias scores* were calculated by regressing mean subjective severity rating scores on mean objective severity rating scores (i.e., those determined by a trained team of raters who were blind to participants' subjective responses). These scores have been established as a reliable measure of stress reactivity in numerous studies utilizing the LSI (e.g., Krackow & Rudolph, 2008; Cole et al., 1998; De Los Reyes & Prinstein, 2004; Conway et al., 2012).

Offspring Depressive Symptomatology **–** Offspring depressive symptoms at age 20 were measured using the Beck Depression Inventory-II (BDI; Beck, Steer, & Brown, 1996), a widely used measure depressive symptoms in both clinical and community samples. It provides continuous symptom data, rather than a dichotomous diagnosis of depression, which provides greater statistical power for detecting interaction effects. In the present study, the BDI demonstrated high internal consistency with a Cronbach's alpha of 0.92.

Maternal Depressive Disorder **–** A dichotomous diagnosis of lifetime maternal depression/dysthymia was established using the Structured Clinical Interview for DSM-IV (SCID; First, Spitzer, Gibbon & Williams, 1995). The interview was administered by trained Master's-level clinical psychology students at the age 15 visit. A depressive disorder was coded as present if mothers reached criteria for depression and/or dysthymia. Given that the sample was enriched for mothers with depressive symptoms early in the offspring's life, maternal depressive disorder at offspring age 15 was included as a covariate when examining offspring depressive symptoms and stress reactivity at age 20.

***Genotyping* —**Between ages 22 and 25 (mean of 23.32 years), participants were mailed blood collection kits and had blood samples drawn at local health facilities. Samples were delivered to the Genetic Epidemiological Laboratory of the Queensland Institute of Medical Research, and aliquots were later shipped to the Social Genomics Core of the USC/UCLA Biodemography Center. Genotyping of the four polymorphisms was conducted using a commercial TaqMan Genotyping Assay (Applied Biosystems, Foster City, CA) performed on an iCycler real-time PCR instrument (BioRad, Hercules, CA). Test-retest reliability analyses based on duplicated samples yielded a total genotyping error rate of <1%. All SNPs were in Hardy-Weinberg Equilibrium (*p*s > 0.05) (CRHR1: $\chi^2 = 1.42$; FKBP5: $\chi^2 = 1.42$; NR3C1: $\chi^2 = 0.37$; BDNF: $\chi^2 = 0.027$).

***Multilocus Genetic Profile Score* –** Each polymorphism was coded such that increasing values corresponded to increasing risk according to the literature. To model an unweighted additive effect, polymorphisms were coded as 0, 1, or 2 such that individuals homozygous for a protective allele received a 0 and individuals homozygous for a risk allele received a 2 (see *Table 1*). We then summed all four polymorphism scores to calculate each participant's total risk score, the HPA-axis MGPS.

Results

Descriptive Statistics and Main Effects

Table 2 displays descriptive statistics for study variables for the present sample. *Table 3* lists bivariate correlations for study variables. Preliminary analyses identified a significant association between HPA-axis MGPS and offspring ethnicity, with white offspring exhibiting lower scores than non-white offspring. Age 20 BDI and stress reactivity both showed a significant bivariate correlation with offspring sex and maternal

depressive disorder. As such, offspring sex, ethnicity (dummy coded as white versus non-white), and maternal depressive disorder were included as covariates in all regression analyses. Bivariate correlations indicated that HPA-axis MGPS was not significantly associated with age 20 BDI or stress reactivity. Maternal prenatal stress showed a significant bivariate correlation with age 20 BDI but was not correlated with age 20 stress reactivity. Similarly, maternal stress at offspring age 5 was significantly correlated with age 20 BDI but not with stress reactivity.

After entering covariates in the first step (i.e., sex, ethnicity, maternal depressive disorder), maternal prenatal stress and HPA-axis MGPS were entered simultaneously into a regression predicting age 20 BDI. Results indicated that maternal prenatal stress was no longer significantly associated with BDI at age 20 (ΔR^2=0.007, t=1.65, p=0.1) and HPA-axis MGPS remained nonsignificant (ΔR^2=0.007, t=-0.31, p=0.76).

Next, after entering covariates in the first step (i.e., sex, ethnicity, maternal depressive disorder), maternal prenatal stress and HPA-axis MGPS were entered simultaneously into a regression predicting age 20 stress reactivity. Both predictors' association with age 20 stress reactivity remained nonsignificant (ΔR^2=0.001, t=0.36, p=0.72; ΔR^2=0.001, t=0.56, p=0.58).

Interaction of Maternal Prenatal Stress and MGPS in Predicting Age 20 Stress Reactivity

We next examined whether greater genetic vulnerability (i.e., higher HPA-axis MGPS) increased susceptibility to maternal prenatal stress, using age 20 stress reactivity as the outcome. Both predictor variables were mean-centered. We entered covariates in the first step (i.e., sex, ethnicity, maternal depressive disorder), main effects for maternal

prenatal stress and MGPS in the second step, and their interaction in the third step. The interaction term was nonsignificant (see *Table 4*).

Interaction of Maternal Prenatal Stress and MGPS in Predicting Age 20 BDI

We then examined whether greater genetic vulnerability (i.e., higher HPA-axis MGPS) increased susceptibility to maternal prenatal stress, using age 20 BDI as the outcome. We entered covariates in the first step (i.e., sex, ethnicity, maternal depressive disorder), main effects for maternal prenatal stress and MGPS in the second step, and their interaction in the third step. The interaction term was significant (see *Table 4*). To probe the interaction further, we estimated the *regions of significance* (Roisman et al., 2012) for the crossover interaction and determined that individuals with greater genetic vulnerability reported significantly higher depressive symptoms in the context of greater maternal prenatal stress (t=2.57, p=0.01) and significantly fewer depressive symptoms in the context of lower maternal prenatal stress (t=2.18, p=0.03). *Figure 1* illustrates this interaction.

Interaction of Maternal Stress at Age 5 and MGPS in Predicting Age 20 BDI

In order to differentiate genetic vulnerability to prenatal stress (i.e., fetal programming) from general vulnerability to life stress, we next examined whether higher HPA-axis MGPS increased susceptibility to maternal stress at age 5, again using age 20 BDI as the outcome. Both predictor variables were mean-centered. We entered covariates in the first step, main effects for age 5 maternal stress and MGPS in the second step, and their interaction in the third step. The interaction term was not significant (see *Table 4*), indicating that MGPS does not moderate the influence of maternal stress during

childhood on early adult depressive symptoms. In other words, the moderating effect of MGPS was specific to prenatal stress.

Sensitivity Analyses

To assess the contribution of each SNP to the interaction between MGPS and maternal prenatal stress, we first conducted four GxE tests using the individual SNPs from the MGPS. Results indicated that no single SNP significantly interacted with maternal prenatal stress to predict age 20 BDI (see *Table 4*). Next, four additional GxE tests were conducted with 3-SNP MGPS variables created by removing each SNP, one at a time, from the original 4-SNP MGPS. The GxE interaction remained significant for all of the four tests (see *Table 4*).

Discussion

The present study demonstrated that an HPA-axis multilocus genetic profile score (MGPS) significantly interacts with maternal prenatal stress to predict offspring depressive symptoms in early adulthood. We show that the accumulation of genetic risk, quantified through the MGPS, interacts with maternal prenatal stress more robustly than any single polymorphism included in the profile score. These findings provide preliminary evidence to suggest that particular genetic variants associated with the HPA-axis may act in a polygenic, additive fashion to interact with maternal prenatal stress to predict depression. Further, we demonstrate that this interaction is specific to maternal stress that occurs during pregnancy, as maternal stress during early childhood does not exhibit the same interactive effects. These findings support the fetal programming hypothesis, which purports that stress exposure during pregnancy can reprogram the HPA-axis and have long-term effects on offspring outcomes. To our knowledge, this is

the first study demonstrating that the duration of programming effects on offspring depressive symptoms extends into early adulthood, while also corroborating findings from animal studies that have identified a moderating role of genetic risk on these programming effects (Glover, O'Connor, & O'Donnell, 2010).

Depressive disorders have been associated with a variety of factors including genetic predisposition (Silberg, Maes & Eaves, 2010), prenatal exposures (Graignic-Philippe et al., 2014), and postnatal environment (e.g., parenting, stressors) (Hammen, Brennan & Le Brocque, 2011). Although these factors have been individually studied for decades, and the interaction of genetic risk and *post*natal environment has been widely researched (see review by Nugent et al., 2011), there is a dearth of studies exploring the interaction of genetic risk with *pre*natal exposures in humans. In this study, we examined this interaction in order to interrogate a potential mechanism by which maternal prenatal stress might confer risk for offspring stress reactivity and depression. Given the role of each considered gene (i.e., BDNF, CRHR1, NR3C1, and FKBP5) in the development and functioning of the HPA-axis, the body's primary stress response system (Glover, O'Connor, & O'Donnell, 2010), it was hypothesized that variants within these genes would have a particularly strong impact on offspring outcomes when compounded with prenatal stress. Our results indicated that this was true for offspring depressive symptoms but not for stress reactivity. Given that previous studies identifying an association between maternal prenatal stress and offspring stress reactivity (Davis, Glynn, Waffarn & Sandman, 2011; Gutteling, deWeerth & Buitelaar, 2005; O'Connor, Bergman, Sarkar & Glover, 2013) have not followed offspring past childhood, it is possible that impact of fetal stress exposure on stress reactivity does not persist long-term. However, it is also of

note that our measure of stress reactivity differed from many of those used in the literature, as we did not have a measure of cortisol reactivity in the present sample. Considering that previous studies have identified discrepancies between cognitive appraisals of stress and physiological measures of stress reactivity (e.g., Voegtline et al., 2013), future studies may choose to utilize cortisol reactivity or another physiological measure of stress reactivity to further investigate these prenatal programming GxE effects in young adulthood.

With regard to depressive symptomatology, our analyses not only supported the hypothesis that genetic risk would confer greater risk for depression when compounded with stress but also indicated that a high HPA-axis MGPS was *beneficial* in the context of low maternal prenatal stress. These findings align with the *differential susceptibility model*, which contends that individuals differ in their sensitivity to environmental exposures based on their biological predispositions (Belsky, 1997). In other words, individuals at high genetic risk are more susceptible to environmental influences, both positive and negative. This model differs from the traditional *diathesis-stress model*, which views genetic risk as harmful in the presence of stress exposure but neutral in the absence of stress (Heim & Nemeroff, 1999). A recent meta-analysis (Leighton et al., 2017) comparing these two models indicated greater empirical support for differential susceptibility, and numerous studies of maternal prenatal stress have suggested the same (see review by Abbott et al., 2018). The present study adds to the growing body of literature suggesting that individuals at high genetic risk for depression or other psychopathology may also be the most responsive to positive exposures, while those at

low genetic risk show reduced variability in outcomes in the context of positive versus negative influences.

Interestingly, our analyses revealed that the interaction of MGPS and stress exposure was specific to stress that occurs prenatally, as the HPA-axis MGPS did not interact with maternal stress at offspring age 5. These results align with the *fetal programming hypothesis*, which posits that programming of the HPA-axis is altered in response to stress (i.e., cortisol) exposure during fetal development (Amiel-Tison et al., 2004; Barker, 2007; Gluckman & Hanson, 2005; Gluckman et al., 2009). Evidence indicates that the HPA-axis is most vulnerable to environmental influences during pregnancy and the first year postpartum (Gunnar, Brodersen, Krueger, & Rigatuso, 1996; Lewis & Ramsay, 1995), suggesting that stress exposure after infancy would be less impactful on long-term HPA-axis functioning. Findings from the present study support this theory, as maternal stress at age 5 did confer risk for offspring depression at age 20 but did not interact with HPA-axis-related genes. As such, the mechanism underlying the impact of early life stress on adult depression may differ from that of perinatal stress. Although previous studies have identified a significant influence of early postnatal stress on childhood cortisol reactivity (e.g., Gunnar et al., 2009), it is unclear whether these stressors have a lasting impact on the HPA-axis into adulthood. Future research is needed to delineate the biological mechanisms impacted by stress exposure at different periods in development.

Similarly, it remains unclear how different stress exposures may interact with one another to predict adult psychopathology. Preliminary studies have suggested that the effects of maternal prenatal stress may be moderated by later exposures to environmental

stress, as posited by the *three-hit hypothesis*. This model theorizes that offspring risk for depression increases as prenatal stress, postnatal stress, and genetic risk are compounded, as demonstrated by Daskalakis and colleagues (2013). Unfortunately, examining a three-way interaction of maternal prenatal stress, offspring postnatal stress, and HPA-axis MGPS was beyond the scope of this study. However, future studies with larger samples may utilize this life-span developmental approach to corroborate these findings and examine the accumulating impact of prenatal and postnatal stress exposure on a background of genetic risk.

A recent review of animal studies suggests that the impact of prenatal stress may also vary with respect to timing (Glover, O'Connor, & O'Donnell, 2010). For example, one study comprising 247 mother-infant pairs found that gestational timing of stress exposure affected outcomes of infant temperament and negative reactivity at 2-months of age, such that only third-trimester stress had a significant influence (Davis et al., 2007). Some have proposed that these timing effects may be due to *critical periods* of brain development during gestation, as different brain systems develop at different stages (Andersen et al., 2008; Rice & Barone, 2000). In other words, the effect of fetal exposures is contingent on the period of fetal brain development that coincides with the environmental stressor (Barker, 2002; Kajantie, 2006). We were not able to examine these timing effects in the present study, as there was little variability in the timing of the prenatal visit. Further, the majority of prenatal visits occurred during the first trimester of pregnancy. As such, the identified relationship between maternal prenatal stress and offspring depressive symptoms may have shown an even stronger effect had we collected measures of prenatal stress during the second or third trimesters. Similarly, the

association between maternal prenatal stress and offspring stress reactivity may be specific to later gestational periods. Future studies may benefit from considering both gestational timing and genetic risk as moderators of the prenatal stress-offspring depression relationship.

Finally, previous studies have also identified potential sex differences in fetal susceptibility to prenatal exposures. A number of studies have determined that higher levels of maternal cortisol or self-reported stress during pregnancy influences cognitive, psychological and physical development more strongly in male offspring than in female (Ellman et al., 2008; Sandman et al., 2013). Other studies have found that maternal prenatal stress is associated with cortisol reactivity in female offspring only (de Bruijn et al., 2009). Although the literature is mixed on how male and female offspring may differ in their vulnerability to prenatal exposures, it is apparent that sex may play an important moderating role in the influence on later offspring development. Again, our ability to investigate a three-way interaction of maternal prenatal stress, offspring sex, and HPA-axis MGPS was beyond the scope of the study. However, future research may consider teasing apart differences in male and female responsivity to fetal exposures in order to further clarify the complex interplay of biological and environmental factors contributing to developmental psychopathology.

It is of note that the effect sizes for the relationships identified in the present study were small in magnitude, despite their statistical significance. In addition to the aforementioned variables that contribute to depressive symptomatology, there are additional genetic factors that may explain these small effects. Primarily, although the size of our MGPS was moderate and consistent with that of other MGPSs (e.g., Nikolova

et al., 2011, Vrshek-Schallhorn et al., 2015), the number of SNPs that contribute to depression likely exceeds the four SNPs we included. Findings from genome-wide association studies (GWAS) of depression (e.g., Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2013) suggest that the confluence of hundreds, or even thousands, of common genetic variants likely confers risk for depression. As such, risk SNPs isolated by candidate gene studies – including those involved in this study – have not been identified with large-scale GWAS approaches due to the marginal effects of individuals SNPs (Ripke et al., 2013). Our findings align with the results of these GWAS studies, as well as the candidate-gene studies that have failed to identify significant main effects of the individual SNPs (i.e., CRHR1 [rs242924], FKBP5 [rs9296158], and NR3C1 [rs6198]) on depression (Heim & Binder, 2012). However, multilocus genetic profile scores provide a unique opportunity to examine polygenic risk in a hypothesis-driven manner (i.e., with a focus on a specific physiological system that underlies depression: the HPA-axis), which is not possible using a GWAS approach. Although each individual SNP has a marginal effect on depressive outcomes and even the additive burden of these SNPs remains marginal in isolation, the collective influence of these four HPA-axis-related SNPs emerges in the presence of environmental risk (i.e., maternal prenatal stress) that is known to act on the HPA-axis. These findings illustrate the importance of using a biologically informed multilocus approach to studying physiological systems impacted by environmental factors.

This study expands upon prior work that has identified an influence of prenatal stress on child and adolescent outcomes, providing evidence for the persistence of fetal

programming effects into early adulthood. The results highlight the importance of reducing maternal stress during pregnancy, and further suggest that providing clinical interventions during pregnancy may help prevent adult depressive outcomes in genetically high-risk offspring. Overall, this illustrates the utility of using an MGPS approach not only to clarify biological mechanisms of risk, but also to identify mothers who may benefit most strongly from intervention practices.

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Table 1. Allele classifications for the HPA-axis Multilocus Genetic Profile Score (MGPS). Allele frequencies are provided for the offspring with depressive symptom data (n=383).

Locus of Interest		**Polymorphism Score**		
Gene	**Polymorphism**	**0**	**1**	**2**
CRHR1	*rs242924*	TT *(n=127)*	GT *(n=177)*	GG *(n=79)*
FKBP5	*rs9296158*	GG *(n = 189)*	AG *(n=153)*	AA *(n=41)*
NR3C1	*rs6198*	TT *(n=279)*	CT *(n=94)*	CC *(n=10)*
BDNF	*Val66Met*	Val/Val *(n=245)*	Met/Val *(n=122)*	Met/Met *(n=16)*

Table 2. Descriptive Statistics

	M (SD)
Predictors	
Offspring MGPS	2.19 (1.2)
Maternal Prenatal Stress	9.42 (3.7)
Maternal Postnatal Stress at age 5	12.38 (4.2)
Outcomes	
Offspring Stress Reactivity at age 20 [a]	-0.01 (0.93)
Offspring BDI at age 20	7.49 (8.4)
Demographic Characteristics	
Sex	
Male	38.9%
Female	61.1%
Race [b]	
White	91.4%
Asian	5.0%
Maori/Islander	1.8%
Australian Aborigine	1.8%
Maternal Depressive Disorder at age 20	40.2%

[a] Stress reactivity scores were the residuals of objective team stress regressed on subjective participant stress. Residuals ranged from 2.28 (positive appraisals) to -3.24 (negative appraisals)

[b] Asian, Maori/Islander, and Australian Aborigine participants were grouped as 'non-White' to control for racial differences in MGPS

Table 3. Bivariate correlations between major study variables. ***Bold/italics*** indicate significance at the 0.05 level.

	Offspring MGPS	Maternal Prenatal Stress	Maternal Stress at age 5	Offspring BDI at age 20	Offspring Stress Reactivity at age 20
Offspring MGPS					
Maternal Prenatal Stress	0.007				
Maternal Stress at age 5	0.011	***0.413***			
Offspring BDI at age 20	-0.005	***0.117***	***0.170***		
Offspring Stress Reactivity at age 20	0.033	0.025	0.018	***0.182***	
Offspring Sex	-0.023	0.001	-0.035	***0.111***	***0.200***
Offspring Ethnicity	***-0.193***	0.033	0.006	0.034	-0.021
Maternal Depressive Disorder	***0.141***	***0.206***	***0.226***	***0.214***	0.047

Table 4. Multiple regression interaction results for primary and sensitivity analyses. ***Bold/italics*** indicate significance at the 0.05 level.

		ΔR^2	t	*p*-value
Stress Reactivity				
HPA-Axis MGPS	Maternal Prenatal Stress x MGPS	0.003	1.12	0.26
Depression Symptoms				
HPA-Axis MGPS	Maternal Prenatal Stress x MGPS	***0.02***	***2.66***	***0.008***
	Maternal Stress at age 5 x MGPS	0.004	1.23	0.22
Sensitivity Analyses	Maternal Prenatal Stress x BDNF [rs6265]	0.008	1.82	0.07
	Maternal Prenatal Stress x CRHR1 [rs242924]	0.001	0.53	0.60
	Maternal Prenatal Stress x NR3C1 [6198]	0.006	1.56	0.12
	Maternal Prenatal Stress x FKBP5 [rs9296158]	0.006	1.51	0.13
	Maternal Prenatal Stress x MGPS [w/o BDNF]	***0.01***	***1.97***	***0.05***
	Maternal Prenatal Stress x MGPS [w/o CRHR1]	***0.02***	***2.77***	***0.01***
	Maternal Prenatal Stress x MGPS [w/o NR3C1]	***0.01***	***2.17***	***0.03***
	Maternal Prenatal Stress x MGPS [w/o FKBP5]	***0.01***	***2.22***	***0.03***

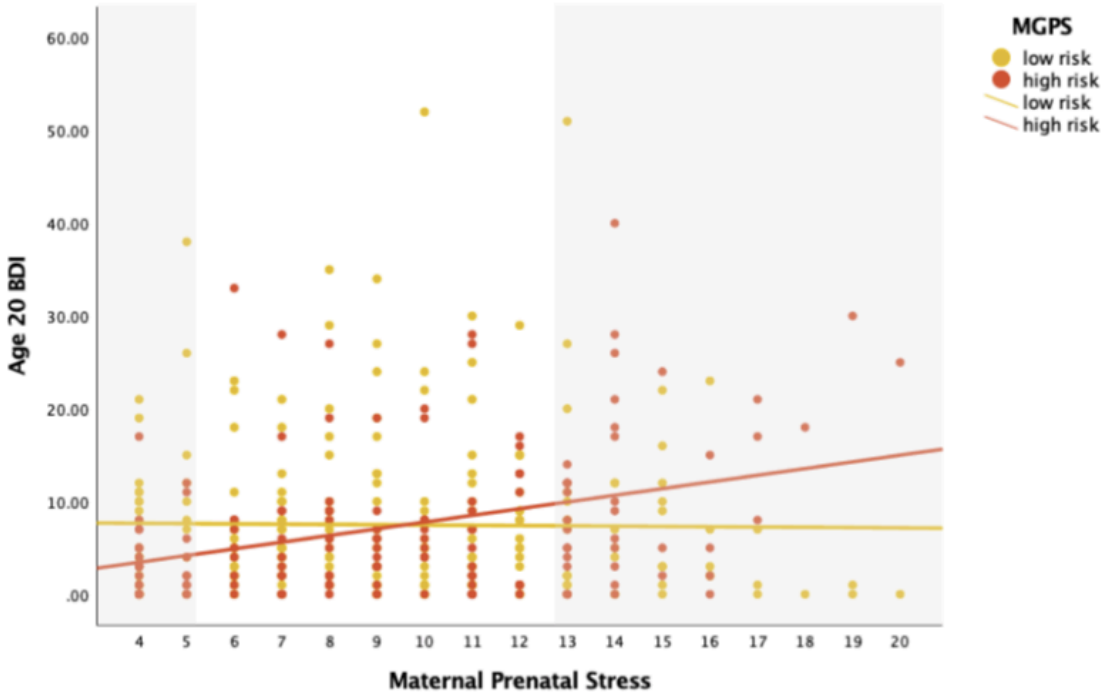

Figure 1. Crossover interaction of HPA-axis MGPS and maternal prenatal stress to predict offspring depressive symptoms at age 20. Grey regions indicate the regions of significance (i.e., levels of maternal prenatal stress at which offspring at high genetic risk significantly differ in depressive symptom severity from offspring at low genetic risk).