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A Functional Interleukin-18 Haplotype Predicts Depression and Anxiety through Increased Threat-Related Amygdala Reactivity in Women but Not Men

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Common functional polymorphisms in the gene encoding *interleukin-18* (*IL18*), a cytokine belonging to the IL-1 superfamily that can induce synthesis of several other cytokines, have been associated with major depressive episodes following the experience of stressful life events. The neural mechanisms underlying these associations remain unexamined. Here we use an imaging genetics strategy to examine the effects of risk-related *IL18* haplotypes comprising rs187238 and rs1946518 on threat-related amygdala reactivity and, through an indirect effect, stress-related symptoms of depression and anxiety in 448 non-Hispanic Caucasian university students. Analyses indicated that women but not men possessing an *IL18* haplotype comprising both risk-related alleles evidenced increased threat-related left centromedial amygdala reactivity relative to other haplotype groups. Moreover, in women only, increased threat-related left centromedial amygdala reactivity predicted increased symptoms of depression and anxiety in individuals also reporting higher levels of life stress. Path analyses revealed a significant indirect effect of *IL18* risk haplotype on symptoms of depression and anxiety through increased threat-related amygdala reactivity. These results suggest that a common functional *IL18* haplotype associated with heightened proinflammatory responses confers susceptibility to stress-related depression and anxiety through effects on threat-related amygdala function, a risk pathway specific to women. If replicated, these patterns can inform the search for personalized interventions targeting neurobiological pathways of risk associated with inflammation.
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INTRODUCTION

Individual differences in immune functioning may affect susceptibility to stress-precipitated mood and anxiety disorders. In fact, there is considerable evidence for a link between chronic inflammation and depression (Dowlati *et al*, 2010; Howren *et al*, 2009; Valkanova *et al*, 2013) as well as anxiety disorders (Gill *et al*, 2009, 2010; Passos *et al*, 2015). Recently, common polymorphisms in inflammation-related genes have been identified as a potential source of interindividual variability in risk for internalizing disorders (Bufalino *et al*, 2013). Among these candidate loci, common polymorphisms in the gene encoding *interleukin-18* (*IL18*) have specifically been linked to the experience of major depressive episodes following stressful life events.

Prior research has demonstrated a greater frequency of risk-related variants of *IL18* promoter region polymorphisms, rs187238 and rs1946518, which form a haplotype, in individuals with major depressive disorder (MDD) whose illness was preceded by a stressful life event in comparison with individuals with MDD having no such experience before developing disorder (Haastrup *et al*, 2012), although effects would not survive correction for multiple comparisons. Moreover, an *IL18* haplotype comprising the major C risk allele of rs187238 and the major G risk allele of rs1946518 has been associated with increased expression of IL-18 mRNA in peripheral blood mononuclear cells (Giedraitis *et al*, 2001) and plasma IL-18 (Haastrup *et al*, 2012). Although these prior functional and disease associations were modest and the functional associations did not reach statistical significance, they are nevertheless informative because IL-18 is a cytokine belonging to the IL-1 superfamily that can induce synthesis of several other cytokines, including tumor necrosis factor-α (TNF-α) and IL-1β (Dinarello, 1999). Increased levels of IL-18 have been noted in individuals with MDD and panic disorder (Al-Hakeim *et al*, 2015; Kokai *et al*, 2002; Merendino *et al*, 2002; Prossin *et al*, 2011), and peripheral IL-18 concentrations are positively correlated with the severity of symptoms in MDD (Alcocer-Gomez *et al*, 2014). Moreover, post-mortem data indicate that *IL18* gene expression is upregulated in the

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prefrontal cortex of individuals who had MDD compared with controls (Shelton *et al*, 2011).

There are several potential neural mechanisms through which functional *IL18* polymorphisms may influence susceptibility to stress-related disorders. One such mechanism is threat-related reactivity of the amygdala, which functions as the hub of an extended corticolimbic circuit that plays a central role in transducing signals of danger into coordinated sympathetic changes in behavior and physiology, including activation of the HPA axis (Herman and Cullinan, 1997). Importantly, IL-18 is expressed within the central nervous system and the IL-18 receptor is expressed throughout the corticolimbic circuit, including the amygdala (Alboni *et al*, 2009; Conti *et al*, 1999). Because increased expression of IL-18 can trigger the upregulation of other proinflammatory cytokines such as TNF-α, the impact of *IL18* polymorphisms on threat-related amygdala function may be mediated indirectly as well (Capuron and Miller, 2011; Miller *et al*, 2009).

Prior human studies provide novel evidence supporting a positive reciprocal association between amygdala reactivity and inflammation (Inagaki *et al*, 2012; Muscatell *et al*, 2015; although see Harrison *et al*, 2009 for contrary evidence) and an association between other inflammation-related genotypes and amygdala reactivity (see, eg, Baune *et al*, 2010). Importantly, heightened amygdala reactivity to social threat also predicts greater risk for the future development of mood and anxiety symptoms in response to stress (Swartz *et al*, 2015), raising the possibility that inflammation may confer risk for mood and anxiety disorders through its effects on amygdala reactivity. Emerging evidence also suggests that effects of inflammation may differ by sex. In one recent paper, women who were administered a low-dose endotoxin reported greater increases in depressed mood and social disconnection compared with men, even though there were no differences in the degree to which peripheral IL-6 and TNF-α levels increased (Moieni *et al*, 2015). It is possible that similar moderation by sex may occur for the association between inflammation and its effects on brain function.

Here, we use an imaging genetics strategy to test the hypothesis that *IL18* haplotypes comprising rs187238 and rs1946518 risk alleles predict increased mood and anxiety symptoms through increased threat-related amygdala reactivity. We further hypothesized that these associations would be moderated by exposure to stressful life events because stress may moderate the association between the *IL18* risk haplotypes and threat-related amygdala reactivity (Figure 1, path A; Sekiyama *et al*, 2005; Sugama and Conti, 2008). Moreover, stress may further moderate the association between amygdala reactivity and behavioral symptoms (Figure 1, path B; Swartz *et al*, 2015). Thus, our goal was to test both of these paths and the indirect effect from *IL18* risk haplotypes on mood and anxiety symptoms. Finally, based on reported sex differences in these and related pathways, we examined moderating effects of sex (Derry *et al*, 2015; Moieni *et al*, 2015; Morris *et al*, 2011).

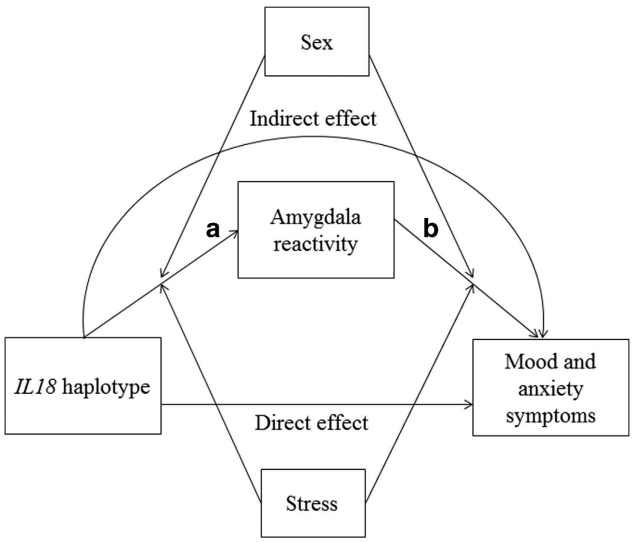

Figure 1 Theoretical model. Risk alleles in the *IL18* haplotype are hypothesized to have an indirect effect on susceptibility to mood and anxiety symptoms through increased threat-related amygdala reactivity. Moderation by sex and stressful life events was tested for both paths.

MATERIALS AND METHODS

Participants

Our analyses focused on 549 young adult university students aged 18–22 years who completed the ongoing Duke Neurogenetics Study (DNS) as of December 2015 and self-reported their race and ethnicity as non-Hispanic Caucasian (Table 1). This focus was chosen to mitigate potential spurious effects due to population stratification. All procedures were approved by the Duke University Medical Center and participants provided informed consent before study initiation. Recruitment and exclusion criteria have been described in detail elsewhere (Nikolova *et al*, 2014; Prather *et al*, 2013; Swartz *et al*, 2015, 2016). Relatedness was assessed using pairwise identity by descent estimation in PLINK 1.07; pairs with a PI_Hat of >0.20 had one member excluded from analyses ($n=2$). Diagnosis of any past or current DSM-IV Axis I disorder or select Axis II disorders (antisocial personality disorder and borderline personality disorder), assessed with the electronic Mini International Neuropsychiatric Interview (Sheehan *et al*, 1998) and Structured Clinical Interview for the DSM-IV subtests (First *et al*, 1996), was not an exclusion. In the sample reported here, 22% ($n=122$) of the participants met criteria for at least one current or past psychiatric diagnosis (see Supplementary Table S1 for specific diagnoses). However, none of the participants in the DNS were using psychotropic medications. The most commonly used medications in the sample were oral contraceptives ($n=136$), antihistamines ($n=40$), antibiotics ($n=18$), bronchodilators ($n=15$), inhaled glucocorticoids ($n=8$), nonsteroidal anti-inflammatory drugs ($n=7$), stimulants ($n=6$), thyroid hormone ($n=6$), and nasal decongestants ($n=4$). Given that these medications may affect inflammatory pathways, we included a covariate for medication use in all analyses (further details below).

Table 1 Participant Characteristics

	CG/CG haplotype	Other haplotypes	Difference
Female	n = 83	n = 178	
Age (years)	19.8 (1.2)	19.7 (1.2)	$t(259) = -0.45$, $p = 0.65$
Diagnosis	24%	19%	$\chi^2(1) = 0.86$, $p = 0.35$
BMI	23.3 (3.8)	23.2 (3.1)	$t(242) = -0.02$, $p = 0.99$
Stressful life events	3.0 (1.3)	2.9 (1.3)	$t(259) = -0.51$, $p = 0.61$
MASQ depression	21.3 (7.8)	20.8 (7.8)	$t(259) = -0.43$, $p = 0.67$
MASQ anxiety	18.2 (5.2)	17.5 (5.7)	$t(259) = -1.02$, $p = 0.31$
MASQ anxious arousal	21.0 (4.0)	20.7 (5.4)	$t(259) = -0.41$, $p = 0.68$
MASQ anhedonia	49.8 (11.8)	48.7 (11.5)	$t(259) = -0.71$, $p = 0.48$
MASQ total	0.36 (2.8)	0.01 (3.4)	$t(259) = -0.82$, $p = 0.41$
Male	n = *79*	n = *138*	
Age (years)	20.0 (1.3)	19.7 (1.2)	$t(215) = -1.50$, $p = 0.14$
Diagnosis	35%	20%	$\chi^2(1) = 6.69$, $p = 0.01$
BMI	23.8 (3.2)	23.8 (2.9)	$t(206) = 0.19$, $p = 0.85$
Stressful life events	2.9 (1.2)	2.7 (1.3)	$t(215) = -1.32$, $p = 0.19$
MASQ depression	20.3 (8.3)	19.4 (7.1)	$t(215) = -0.83$, $p = 0.41$
MASQ anxiety	17.0 (5.1)	16.4 (4.5)	$t(215) = -0.88$, $p = 0.38$
MASQ anxious arousal	20.7 (5.9)	20.2 (5.1)	$t(215) = -0.57$, $p = 0.57$
MASQ anhedonia	53.5 (12.5)	49.7 (12.6)	$t(215) = -2.17$, $p = 0.03$
MASQ total	0.26 (3.5)	−0.38 (3.1)	$t(215) = -1.40$, $p = 0.17$

Abbreviations: BMI, body mass index; MASQ, Mood and Anxiety Symptom Questionnaire.
Diagnosis indicates the percentage of participants with any past or present psychiatric diagnosis. Stressful life events indicate the summed impact scores across all life events reported on the Life Event Scale for Students (square root transformed). MASQ Total was calculated by standardizing each subscale score and summing them; a score of 0 therefore indicates mean levels of symptoms.

Amygdala Reactivity Paradigm

Amygdala reactivity to threat was assessed using an emotional face matching challenge paradigm. The paradigm version used in the DNS consists of four blocks of a face-processing task (one block each for fearful, angry, surprised, and neutral faces) interleaved with five blocks of a sensorimotor control task. Details on the task paradigm have been reported in previous research (Swartz *et al*, 2015, 2016).

BOLD fMRI Data Acquisition, Preprocessing, and Quality Assurance

Participants were scanned using a research-dedicated GE MR750 3T scanner at the Duke-UNC Brain Imaging and Analysis Center. A series of 34 interleaved axial functional slices aligned with the anterior commissure–posterior commissure (AC-PC) plane were acquired for full-brain coverage using an inverse-spiral pulse sequence to reduce susceptibility artifact (TR = 2000 ms; TE = 30 ms; flip angle = 60; FOV = 240 mm; $3.75 \times 3.75 \times 4$ mm voxels; interslice skip = 0).

Functional MRI data were processed in SPM8 using the standard preprocessing stream used in previously published research from the DNS (Nikolova *et al*, 2014; Prather *et al*, 2013; Swartz *et al*, 2015, 2016). Further details on preprocessing and quality control procedures are reported in the Supplementary Materials and Methods. The current sample includes 502 participants with fMRI data meeting all quality control criteria.

BOLD fMRI Data Analysis

The general linear model of SPM8 was used to conduct fMRI data analyses. Following preprocessing, a first-level model was created for each participant that included separate regressors for each block type (fearful, angry, surprised, and neutral faces and shape matching control blocks), as well as motion regressors created by artifact detection software (see Supplementary Information for further details). Next, individual contrast images for effects of each expression were generated at the first level for each participant and then entered into second-level random effects models to determine mean condition-specific regional responses. We extracted parameter estimates from functional clusters within amygdala regions of interest activated to the condition at $p < 0.05$ family-wise error (FWE) corrected across the search volumes, for the contrast of Angry and Fearful blocks > Control blocks. Amygdala regions of interest were derived from probabilistic cytoarchitectonic mapping of the basolateral and centromedial amygdala subregions (Amunts *et al*, 2005). Analyses were first performed for the contrast of Angry and Fearful blocks > Control blocks; when significant effects were observed, *post hoc* analyses were conducted to determine whether effects were driven by either the Angry or Fearful blocks specifically.

Genotyping

Genotyping was conducted by 23andMe. Genomic DNA from all participants was isolated from buccal cells derived from Oragene DNA self-collection kits (DNA Genotek, Kanata, Canada) customized for 23andMe. DNA extraction and genotyping were performed at the National Genetics Institute, a CLIA-certified clinical laboratory and subsidiary of Laboratory Corporation of America. One of two different Illumina arrays with custom content was used to provide genome-wide SNP data, the HumanOmniExpress or HumanOmniExpress-24 (Do *et al*, 2011; Eriksson *et al*, 2010; Tung *et al*, 2011). Both rs187238 and rs1946518 were genotyped on these arrays. Genotype distribution did not deviate from Hardy–Weinberg equilibrium for rs187238 ($\chi^2(1) = 1.58$, $p = 0.21$) or for rs1946518 ($\chi^2(1) = 0.004$, $p = 0.95$). PHASE was used to generate *IL18* haplotypes (Stephens *et al*, 2001); in all cases posterior probability was >99%. Based on prior research (Giedraitis *et al*, 2001; Haastrup *et al*, 2012), we coded individuals homozygous for the CG haplotype (ie, homozygous for the major C allele of rs187238 and for the major G allele of rs1946518) as the group of interest ($n = 162$) and all other individuals as the reference group ($n = 316$). See the Supplementary Materials and Methods for additional analyses supporting this grouping. Genotype data were available in 478 participants (448 of whom had valid fMRI data).

Stress

Concurrent to the collection of genotype and fMRI data, recent life stress was assessed with the Life Events Scale for Students (LESS; Clements and Turpin, 1996). The LESS is a checklist of life events; for each event, participants reported whether or not that event had occurred in the past year and rated the severity of the impact of that event on a 1 to 4 scale (4 = severe impact). Similar to our prior work (Swartz *et al*, 2015), we summed the severity ratings for each event present to create a total impact score, reflecting both a greater number and severity of life events. Because this variable was skewed, a square root transformation was applied to yield a more normal distribution.

Mood and Anxiety Symptoms

Concurrent to the collection of genotype, fMRI, and stress data, participants reported on their mood and anxiety symptoms with the Mood and Anxiety Symptoms Questionnaire (MASQ) Short Form (Watson *et al*, 1995). The MASQ consists of four subscales including general depression, general anxiety, anxious arousal, and anhedonia. Participants were asked to report on symptoms they experienced in the past week (eg, 'Felt worthless') on a scale from 1 (not at all) to 5 (extremely). Total scores on the MASQ were calculated by standardizing scores on the four subscales and then summing scores. When significant effects were detected with MASQ total scores, *post hoc* analyses were conducted to examine whether they were driven by specific subscales of the MASQ.

Covariates

Higher body mass index (BMI) is associated with greater proinflammatory response to social stress (McInnis *et al*, 2014). Therefore, BMI was calculated using participants' height and weight and was entered as a covariate in all analyses. BMI was missing for 44 participants. To control for potential circadian variation in amygdala reactivity and inflammation, we also entered time of day for the scan as a covariate in all analyses (range: 0800 to 1900 h). Psychiatric diagnoses (both past and current) were dummy-coded as mood and anxiety disorders ($n = 61$) or other psychopathology, generally alcohol and substance use disorders ($n = 61$); in the case of comorbid diagnoses, they were included in the mood and anxiety disorder group. These two dummy-coded variables were entered as covariates in all analyses. A covariate was also created for medication use (0 = no medication, 1 = medication), including all classes of medications other than oral contraceptives. In addition, to control for ancestral genetic heterogeneity within the self-reported non-Hispanic Caucasian participants, we computed multidimensional scaling (MDS) components using identity-by-state (IBS) analyses in PLINK of the whole genome data within this subgroup. We included the top two MDS components as covariates in all of our statistical models. Additional supplementary analyses were conducted in women only controlling for oral contraceptive use and phase of menstrual cycle (see Supplementary Information).

Statistical Analysis

All analyses were conducted in Mplus v7 (Muthen & Muthen) using full information maximum likelihood estimation (FIML). For all analyses, 1000 bootstrapped samples were requested to generate standard errors robust to non-normality. Our goal was to build an indirect effects model linking *IL18* haplotype to mood and anxiety symptoms via threat-related amygdala reactivity (Figure 1). To do so, we tested each path separately first, and then used results from these analyses to construct the full model. Full details on the analytical approach are reported in the Supplementary Materials and Methods.

RESULTS

Effects of IL18 Haplotype on Amygdala Reactivity

The omnibus test was significant ($\Delta\chi^2(7) = 20.60$, $p = 0.004$) indicating that the effects of *IL18* haplotype on amygdala reactivity differed by sex and/or amygdala subregion. Follow-up analyses to test for interactions between haplotype and sex across the four subregions indicated that sex significantly moderated the effect of *IL18* haplotype on left centromedial amygdala reactivity ($\Delta\chi^2(1) = 6.73$, $p = 0.01$, false discovery rate (FDR)-corrected $p = 0.04$). This was because of a significant effect of the haplotype on amygdala reactivity in women (B = 0.07, SE = 0.03, $\beta = 0.17$, $p = 0.01$, $\Delta R^2 = 0.03$) but not in men (B = −0.04, SE = 0.04, $\beta = -0.09$, $p = 0.24$, $\Delta R^2 = 0.01$; Figure 2). The effect also remained significant in women when winsorizing continuous variables to limit the influence of outliers. Further *post hoc* testing indicated that the effect in women was specific to the left centromedial

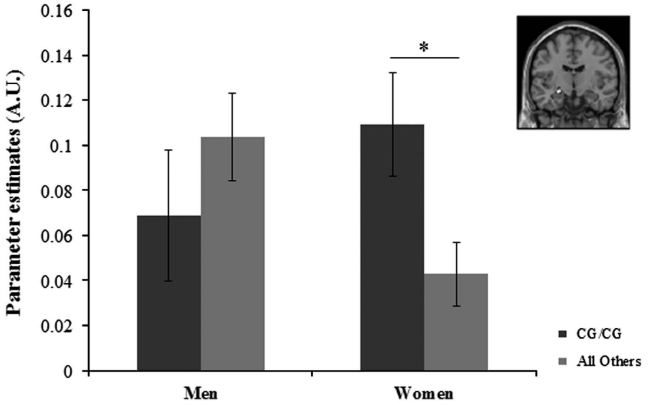

Figure 2 The association between *IL18* haplotype and amygdala reactivity is moderated by sex. Parameter estimates were extracted for the contrast of Angry and Fearful Faces > Control blocks within a functional cluster significantly activated at $p < 0.05$ family-wise error-corrected within the left centromedial amygdala (inset). *IL18* haplotype predicted significant differences in left centromedial amygdala reactivity in women but not men. CG/CG haplotype group indicates participants homozygous for the rs187238 major C allele and for the rs1946518 major G allele. Error bars represent 1 SEM; $*p < 0.05$.

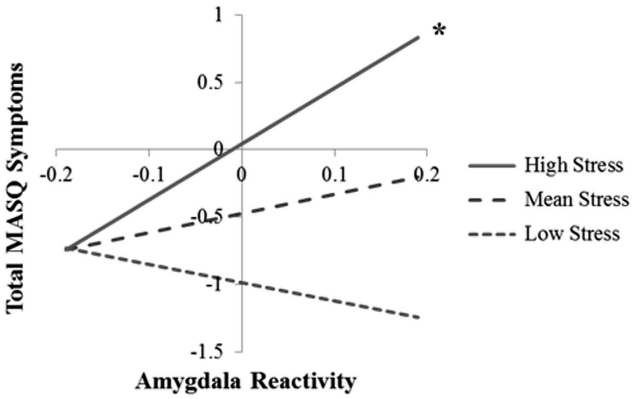

Figure 3 Interaction between left centromedial amygdala reactivity and recent stress predicts concurrent mood and anxiety symptoms in women. Effects are shown for women only. Simple slopes analysis was conducted using parameter estimates obtained in Mplus. Effects were estimated at mean levels of stress, 1 SD below the mean (low stress) and 1 SD above the mean (high stress). The symbol '*' indicates that a simple slope is significant at $p < 0.05$. Amygdala reactivity was mean centered for this analysis. Total MASQ symptoms were calculated by summing the four standardized subscale scores; 0 indicates mean symptom levels across the subscales.

amygdala, as parameter estimates differed significantly for this region compared with the left basolateral ($\Delta\chi^2(1) = 8.09$, $p = 0.004$, FDR-corrected $p = 0.012$), the right centromedial ($\Delta\chi^2(1) = 5.26$, $p = 0.02$, FDR-corrected $p = 0.02$), and the right basolateral amygdala ($\Delta\chi^2(1) = 5.20$, $p = 0.02$, FDR-corrected $p = 0.02$). The *post hoc* testing also indicated this effect did not differ by facial expression ($\Delta\chi^2(1) = 0.07$, $p = 0.79$); in other words, in women the effect of *IL18* haplotype was equivalent for left centromedial amygdala reactivity to angry and fearful facial expressions. Moderating effects of sex and main effects of haplotype were not significant for the other subregions tested (all p's > 0.05). See Supplementary Materials and Methods for further analyses supporting these results.

Moderation of the Effect of IL18 Haplotype by Recent Life Stress

The omnibus test indicated that there were no significant *IL18* haplotype × stress interaction effects for the four amygdala subregions in either men or women ($\Delta\chi^2(8) = 4.65$, $p = 0.79$). Looking just at the left centromedial amygdala in women, when the *IL18* haplotype × stress interaction was entered into the regression, the main effect of *IL18* haplotype remained significant ($p = 0.012$), but the interaction of haplotype × stress was not significant ($B = -0.03$, $SE = 0.02$, $\beta = -0.10$, $p = 0.16$).

Effect of Amygdala Reactivity on Concurrent Mood and Anxiety Symptoms

Because the effects of the *IL18* haplotype were only significant for left centromedial amygdala reactivity, we examined the interaction of this subregion and recent stress as a predictor of concurrent mood and anxiety symptoms. Multigroup analyses indicated that the main effect of left centromedial amygdala reactivity on MASQ total symptoms was not significantly moderated by sex ($p = 0.71$) and was not significant ($B = 1.07$, $SE = 0.76$, $p = 0.16$). However, multigroup analyses indicated the interaction between amygdala reactivity and stress in predicting symptoms was significantly moderated by sex ($\Delta\chi^2(1) = 5.84$, $p = 0.02$). In women, there was a significant interaction between left centromedial amygdala reactivity and stress in predicting total MASQ symptoms ($B = 2.08$, $SE = 0.79$, $\beta = 0.16$, $p = 0.01$, $\Delta R^2 = 0.02$). As demonstrated in Figure 3, greater amygdala reactivity predicted greater mood and anxiety symptoms, but only under conditions of higher stress. This interaction was not significant in men ($B = -0.45$, $SE = 1.11$, $\beta = -0.04$, $p = 0.69$, $\Delta R^2 = 0.001$). The effect also remained significant in women when winsorizing continuous variables to limit the influence of outliers. The *post hoc* analyses indicated that this interaction effect was equivalent for amygdala reactivity to fearful and angry faces ($\Delta\chi^2(1) = 0.02$, $p = 0.89$). The *post hoc* analyses also indicated this interaction was significant in women for the subscales of depression ($B = 5.00$, $SE = 1.82$, $\beta = 0.16$, $p = 0.006$, $\Delta R^2 = 0.02$), and marginally anxiety ($B = 2.85$, $SE = 1.57$, $\beta = 0.13$, $p = 0.07$, $\Delta R^2 = 0.02$), anxious arousal ($B = 2.99$, $SE = 1.51$, $\beta = 0.15$, $p = 0.05$, $\Delta R^2 = 0.02$), and anhedonia ($B = 3.13$, $SE = 2.58$, $\beta = 0.07$, $p = 0.23$, $\Delta R^2 = 0.004$). Constraining parameter estimates to be equal across the four subscales did not result in a significant reduction in model fit ($\Delta\chi^2(3) = 2.86$, $p = 0.41$), indicating that effects were generally equivalent across the four subscales.

Indirect Effect of IL18 Haplotype on Symptoms via Amygdala Reactivity

Based on the results of the analyses above, we report an indirect effects model in women only, as neither path was significant in men. On the first path (Figure 1, path A) we modeled the main effect of *IL18* haplotype on left centromedial amygdala reactivity, given no evidence for

moderation by recent life stress. On the second path (Figure 1, path B), we modeled an interaction between recent life stress and left centromedial amygdala reactivity as a predictor of total MASQ symptoms. As shown in Figure 4, there was a significant indirect effect of *IL18* haplotype on mood and anxiety symptoms through its effect on threat-related amygdala reactivity. As expected, this indirect effect was significant when stress was 1 SD above the mean ($\alpha\beta = 0.29$, SE = 0.14, $p = 0.04$, 95% confidence intervals (0.08 to 0.67)), but not when stress was at mean levels ($\alpha\beta = 0.10$, SE = 0.08, $p = 0.22$ (−0.03 to 0.30)) or 1 SD below the mean ($\alpha\beta = -0.09$, SE = 0.13, $p = 0.50$, (−0.43 to 0.10)).

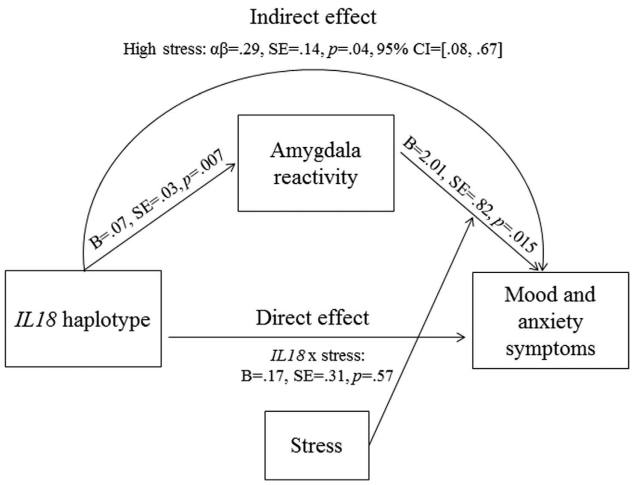

Figure 4 Indirect effect of *IL18* haplotype on mood and anxiety symptoms via amygdala reactivity in women. Indirect effect model is shown for women only; model is not shown for men because neither path was significant. The CG/CG *IL18* haplotype group (individuals homozygous for the rs187238 major C allele and for the rs1946518 major G allele) evidenced increased left centromedial threat-related amygdala reactivity. Increased threat-related amygdala reactivity in turn predicted higher mood and anxiety symptoms under higher levels of recent life stress. The indirect effect of *IL18* haplotype on mood and anxiety symptoms through amygdala reactivity was significant, but only in individuals reporting higher levels of life stress.

DISCUSSION

The goal of the present study was to examine whether an *IL18* risk-related functional haplotype predicted individual differences in threat-related amygdala reactivity and, indirectly, mood and anxiety symptoms as a function of stress. We found that in women *IL18* haplotype predicted relatively increased threat-related reactivity of the left centromedial amygdala. We also found an indirect effect of *IL18* haplotype on mood and anxiety symptoms under conditions of higher stress via its effect on amygdala reactivity.

We found that the effects of the *IL18* haplotype were strongest for the left centromedial amygdala subregion. This is notable as the centromedial subregion encompasses the central nucleus of the amygdala (CeA), which facilitates activation of the HPA axis in response to threat via projections to the paraventricular nucleus. The specificity of this effect to women aligns with the finding that acute activation of the inflammatory response through administration of low-dose endotoxin results in greater reductions in mood in women compared with men (Moieni *et al*, 2015). Future research will be necessary to confirm these sex differences in genetic influences on amygdala reactivity and to examine whether the *IL18* haplotype may affect functioning of regions other than the amygdala in men. Indeed, prior research has indicated that the structure, function, and connectivity of other regions implicated in internalizing disorders, including the hippocampus, ventral striatum, and prefrontal cortex, are associated with individual differences in proinflammatory markers and inflammation-related genotypes (Baune *et al*, 2012; Inagaki *et al*, 2015; Muscatell *et al*, 2015), suggesting that these regions should also be investigated as potential mediators of *IL18* haplotype effects on stress-related internalizing symptoms.

The molecular and cellular mechanisms driving the association between *IL18* haplotype and threat-related amygdala reactivity remain to be determined. Because IL-18 is expressed in the brain (Conti *et al*, 1999) and IL-18 receptors are present in the amygdala (Alboni *et al*, 2009), direct modulatory effects on threat-related reactivity are possible. As discussed earlier, IL-18 can also drive the production of other proinflammatory cytokines including IL-6 and IL-1β (Dinarello, 1999) that may affect brain function through a number of parallel pathways (Capuron and Miller, 2011). In another line of work in MDD, sadness-induced increases in IL-18 have been associated with sadness-induced endogenous opioid release in the left amygdala that was associated with changes in negative affective state (Prossin *et al*, 2016). Animal models also indicate that IL-18 may affect expression of a wide range of genes in the amygdala, with *IL18* knockout mice evidencing altered expression of over 1000 genes in the amygdala, including those encoding neuropeptides known to regulate threat-related amygdala reactivity such as oxytocin and arginine vasopressin (Yamamoto *et al*, 2010). Thus, there are many potential routes through which IL-18 may affect amygdala reactivity and broader corticolimbic circuit function. Counter to expectations, we did not observe that the association between *IL18* haplotype and threat-related amygdala reactivity was moderated by stressful life events. We might have observed stronger effects with a more acute measure of life stress (ie, life stress within the past week or the past month) than our measure of life stress within the past year, or it may be that our measure of stressful life events was too broad and that moderation may have been observed by focusing on specific types of stressors. Moreover, stronger moderating effects may be observed for more chronic and severe forms of stress, such as childhood maltreatment (Redlich *et al*, 2015).

Several limitations of the present study warrant discussion. First, we did not obtain measures of peripheral IL-18 in participants, and hence we were unable to test the effect of *IL18* haplotype on peripheral inflammation, or whether effects of the haplotype on amygdala reactivity were mediated by higher peripheral IL-18 levels. This is an important direction for future research. Second, we did not have a more acute measure of recent life stress (ie, within the past week, which may have reduced our ability to detect a moderating effect of life stress on the association between *IL18* haplotype and amygdala reactivity. Third, because a relatively small proportion of participants reported a current

mood or anxiety disorder ($n = 29$), we did not test whether threat-related amygdala reactivity predicted clinical diagnoses. Thus, it remains to be determined whether the associations observed with a continuous distribution of symptoms also apply when examining clinically significant categorical disorder. Fourth, because this study is cross-sectional, temporal order of the relationship between the amygdala by stressful life event interaction and mood and anxiety symptoms cannot be established. However, our prior work showing that this interaction precedes the development of prospectively assessed mood symptoms is consistent with our model (Swartz *et al*, 2015).

These limitations notwithstanding, we provide initial evidence that a functional *IL18* haplotype indirectly influences stress-related susceptibility to mood and anxiety symptoms through heightened threat-related amygdala reactivity, an effect observed only in women. As such, our results inform ongoing efforts to devise genetic risk profiles to identify specific pathophysiological mechanisms contributing to stress susceptibility in individuals and, ultimately, using personalized approaches to target preventions or interventions toward these pathways of risk.

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