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FKBP5 and Emotional Neglect Interact to Predict Individual Differences in Amygdala Reactivity

Michael G. White[1,2], **Ryan Bogdan**[1], **Patrick M. Fisher**[3], **Karen Muñoz**[4], **Douglas E. Williamson**[5], and **Ahmad R. Hariri**[1,6]

[1]Laboratory of NeuroGenetics, Department of Psychology & Neuroscience, Duke University, Durham, NC USA

[2]Genes, Cognition and Psychosis Program, National Institute of Mental Health, National Institutes of Health, Bethesda, MD USA

[3]Center for Integrated Molecular Brain Imaging, Neurobiology Research Unit, Copenhagen, Denmark

[4]Department of Psychology, University of Pittsburgh, Pittsburgh, PA USA

[5]Genetic Epidemiology Program, Department of Psychiatry, University of Texas Health Sciences Center at San Antonio, San Antonio, TX USA

[6]Institute for Genome Sciences & Policy, Duke University, Durham, NC USA

Abstract

Individual variation in physiological responsiveness to stress mediates risk for mental illness and is influenced by both experiential and genetic factors. Common polymorphisms in the human gene for FK506 binding protein 5 (*FKBP5*), which is involved in transcriptional regulation of the hypothalamic-pituitary-adrenal (HPA) axis, have been shown to interact with childhood abuse and trauma to predict stress-related psychopathology. In the current study, we examined if such gene-environment interaction effects may be related to variability in the threat-related reactivity of the amygdala, which plays a critical role in mediating physiological and behavioral adaptions to stress including modulation of the HPA axis. To this end 139 healthy, Caucasian youth completed a BOLD fMRI probe of amygdala reactivity and self-report assessments of emotional neglect (EN) and other forms of maltreatment. These individuals were genotyped for six *FKBP5* polymorphisms (rs7748266, rs1360780, rs9296158, rs3800373, rs9470080, and rs9394309) previously associated with psychopathology and/or HPA axis function. Interactions between each SNP and EN emerged such that risk alleles predicted relatively increased dorsal amygdala reactivity in the context of higher EN, even after correcting for multiple testing. Two different haplotype analyses confirmed this relationship as haplotypes with risk alleles also exhibited increased amygdala reactivity in the context of higher EN. Our results suggest that increased threat-related amygdala reactivity may represent a mechanism linking psychopathology to interactions between common genetic variants affecting HPA axis function and childhood trauma.

Keywords

FKBP5; amygdala; emotional neglect; stress; PTSD; HPA

Correspondence: Douglas E. Williamson, Genetic Epidemiology Program, Department of Psychiatry, University of Texas Health Sciences Center at San Antonio, 7703 Floyd Curl Drive, Suite 772E, Mail Code 7792, San Antonio, TX, 78229-3900, Tel: (210) 567-5449, Fax: (210) 567-5507, williamsonde@uthscsa.edu.

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Introduction

Childhood adversity predicts over 32% of all psychiatric disorders (Green et al., 2010). Understanding the neurobiological mechanisms that underlie this association and the individual differences that moderate risk, will inform disease etiology and may lead to more effective treatment and prevention strategies. Research across species suggests that early life adversity may precipitate psychopathology by aggravating physiological reactivity, biasing cognitive attention to threatening and stressful stimuli, and disrupting the regulation of emotional responses (Lupien et al., 2009).

The amygdala is critical for recruiting physiological and behavioral resources to adaptively respond to environmental challenges (LeDoux, 2000), particularly threat, with the magnitude of reactivity predicting individual differences in anxiety and depression (Hariri, 2009, Siegle et al., 2002). Consistent with non-human animal research (Tottenham and Sheridan, 2010), emerging human research has linked extreme childhood adversity (e.g., institutional rearing) to heightened amygdala reactivity (Maheu et al., 2010, Tottenham et al., 2011, Gianaros et al., 2008), and volumetric enlargement (Mehta *et al.*, 2009, Tottenham *et al.*, 2010). Because disrupted hypothalamic-pituitary-adrenal (HPA) axis function is associated with dysfunctional stress responsiveness, heightened amygdala reactivity (Maheu et al., 2008), childhood maltreatment (McCrory et al., 2010, Lupien et al., 2009) and psychopathology (Yehuda, 2002), genetic variation affecting HPA axis function may moderate associations between emotional neglect and amygdala reactivity which may, in turn, precipitate stress-related psychopathology.

One intriguing HPA axis candidate gene is FK506 binding protein 5 (*FKBP5*), located on chromosome 6 (6p21.31). *FKBP5* is a co-chaperone that mediates nuclear translocation of the cortisol-glucocorticoid receptor (GR) complex and hence GR-mediated gene transcription. Elevated *FKBP5* levels confer reduced GR sensitivity to circulating cortisol, leading to decreased negative feedback regulation of the HPA axis and a slower resolution of the stress response (Binder, 2009). Consistent with these effects, rodent research has linked *FKBP5* expression to anxiety-like behavior (Attwood et al., 2011). Of primary importance to the current study, *FKBP5* polymorphisms are associated with differential HPA axis function as well as psychopathology in the context of stress (Table 1). However, to our knowledge, neither the effect of *FKBP5* nor *FKBP5*-by-stress interaction effects have been investigated in the context of threat-related brain circuitry. Because heightened threat-related amygdala reactivity has been associated with early adversity, elevated HPA axis function, and stress-related psychopathology, it is a promising candidate mechanism by which *FKBP5* genotypes may interact with stress to predict psychopathology.

The aim of this study was to assess whether *FKBP5* polymorphisms associated with HPA axis function and/or psychopathology (Table 1) predict variation in threat-related amygdala reactivity in the context of childhood adversity. To this end, 139 youth without history of mental illness completed a widely used and well-characterized functional magnetic resonance imaging (fMRI) probe of threat-related amygdala reactivity (Brown et al., 2005, Neumann et al., 2006, Brown et al., 2006, Manuck et al., 2007) and provided self-reports of experienced childhood trauma. This population allowed us to preclude any differences in brain function that may be attributable to psychopathology. Because of the prospective nature of this study, it allows continued assessment of this sample so that the value of our measures in predicting the development of psychopathology may be examined. Because *FKBP5* gentoypes only predict psychopathology in the context of adversity and resulting changes in HPA axis regulation are more salient in the context of stress reactivity, we

hypothesized that *FKBP5* "risk" alleles would be associated with increased threat-related amygdala reactivity, but only in the context of prior childhood emotional neglect.

Materials & Methods

Sample

A total of 139 Caucasian, non-Hispanic, psychiatrically healthy adolescents aged 12 to 15 (Table 2) with genotype information for six *FKBP5* polymorphisms (rs9394309, rs7748266, rs1360780, rs9296158, rs3800373, and rs9470080) and functional neuroimaging data were selected from the ongoing Teen Alcohol Outcomes Study (TAOS), which is designed to investigate the contributions of genes, environments, and neural systems to the onset of alcohol use disorders during adolescence. Subjects in the current analyses did not meet threshold or sub-threshold criteria for lifetime/present mood disorder, as assessed by the Kiddies Schedule for Affective Disorders and Schizophrenia for School-Age Children, Present and Lifetime (6–18 Years) Present and Lifetime Episode version (K-SADS-PL) (Ambrosini, 2000, Kaufman et al., 2000).

TAOS subjects were recruited from the San Antonio, Texas, metro area via commercially available phone lists containing families living within a 30-mile radius of University of Texas Health Science Center at San Antonio (UTHSCA) and likely to have adolescents between the ages of 12 and 15 years. Families with medically healthy adolescents who did not have braces and were in the desired age range completed in-person interviews, which included a comprehensive battery of self-report behavioral assessments. Eligible participants who completed the MRI protocol and blood draw were compensated $140.00, while those only completing the in-person interview received $40.00. All subjects participated after parents provided informed consent per a UTHSCA Institutional Review Board approved protocol.

Childhood Trauma Questionnaire (CTQ)

The CTQ assesses 5 different types of childhood trauma: emotional, physical, and sexual abuse, as well as emotional and physical neglect (Bernstein et al., 2003). Each CTQ subscale has excellent internal consistency and convergent validity (Bernstein et al., 1994). We focused on the emotional neglect subscale because of previous research associating severe forms of emotional neglect with heightened amygdala reactivity (Maheu et al., 2010, Tottenham et al., 2011), and a previous report from our group on a larger TAOS sample documenting associations between emotional neglect, but not other CTQ subscales, and amygdala reactivity (Bogdan et al., in press). Moreover, there was a substantial amount of variability on this scale relative to the other subscales in the present sample.

fMRI Challenge Paradigm

A widely used and validated challenge paradigm was used to elicit robust amygdala reactivity (Hariri et al., 2002, Kleinhans et al., 2010, Carré et al., 2010, Wang et al., 2004). In this task, participants complete 4 blocks of a perceptual face processing task in which they view a trio of faces (expressing either anger or fear) and select 1 of 2 faces (displayed on the bottom) identical to the target stimulus (displayed on top). Each block consists of 6 images derived from a standard set of facial affect pictures (Ekman and Friesen, 1976). Faces are balanced for gender and target affect. Each of the 6 face trios is presented for 4 seconds with a variable inter-stimulus interval of 2-6 seconds, for a total block length of 48 seconds. Interleaved between these blocks, participants complete 5 blocks of a sensorimotor control task during which they view a trio of geometric shapes (circles, horizontal ellipses, vertical ellipses) and select 1 of 2 shapes (displayed on the bottom) identical to the target shape (displayed on top). Each sensorimotor task consists of 6 different shape trios. Each of

the 6 different shape trios is presented for 4 seconds with a fixed inter-stimulus interval of 2 seconds, for a total block length of 36 seconds. The total paradigm length is 390 seconds. Reaction times and accuracy were recorded through an MR-compatible button-box. The stimuli used in this task are socially derived threats that are indirect and/or ambiguous (e.g., Should I be afraid of what this person is afraid of? Is this angry person a threat to me?). These types of stimuli engage brain circuitry, particularly the amygdala, responsible for adjusting vigilance to changing contingencies (for review see Davis and Whalen, 2001, Hariri and Whalen, 2011).

Genetic Analyses

Genomic DNA from all participants was isolated from mouthwash samples using the Oragene DNA self-collection kit following the manufacturer's instructions (DNA Genotek, Inc., 2006). *FKBP5* SNPs rs1360780, rs9296158, rs3800373 and rs9470080 were genotyped using TaqMan allelic discrimination assay (Livak, 1999). Two additional *FKBP5* SNPs (rs7748266 and rs9394309) were derived from an Illumina Human 610-Quad BeadChip assay (Illumina, San Diego, CA, USA). No other polymorphisms from this assay were tested in the current study. All SNPs were in accordance with Hardy-Weinberg Equilibrium (all $\chi^2 < 0.5$, all $p > 0.4$) and genotyping concordance of rs1360780 and rs9470080 across TaqMan genotyping and Illumina BeadChip assays were 100%.

Haploview was used to determine linkage disequilibrium (LD; Figure 1E) and establish haplotype blocks for the candidate *FKBP5* SNPs (Barrett et al., 2005). The underlying haplotype phase for each subject was determined using PHASE software (Stephens et al., 2001). Because the genomic region of *FKBP5* has a low degree of haplotype complexity (i.e., high LD with common haplotypes) all subjects had posterior pairwise probabilities greater than 95% and were thus included in haplotype analyses. One participant had a posterior probability of 70% and 30% for two different haplotypes within the haplotype block generated from Haploview. However, because this subject would be grouped in the same fashion regardless of block assignment, we included this subject in analyses.

A haplotype block was formed using the confidence interval (CI) method (Gabriel et al., 2002). This block was composed of 5 of the 6 SNPs included in this study (i.e., rs9296158, rs7748266, rs1360780, rs9394309 and rs9470080) and resulted in the following haplotype frequencies: GCCAC (67.3%), ATTGT (15.5%), ACTGT (9.4%), GCCGT (3.6%), ACTAT (3.2%), ACCAT (0.7%), GCCAT (0.4%). Subjects were categorized based on the presence of the GCCAC haplotype (i.e., 0, 1 or 2 copies), which was comprised of “non-risk” alleles as identified in previous studies (Table 1). A second post-hoc haplotype block was also formed by including the four SNPs (i.e., rs3800373, rs9296158, rs1360780, and rs7748266) with interaction effects surviving both SNPSpD correction and FWE correction for multiple brain regions of interest comparisons ($p < 0.0048$, see below). This resulted in the following haplotype frequencies: TGCC (71.2%), GATT (13.3%), GACT (12.6%), TATT (2.2%), GACC (0.4%), TACC (0.4%). In a similar fashion to the Haploview generated haplotype, subjects were categorized based on the presence of the TGCC haplotype (i.e., 0, 1 or 2 copies), which was comprised of “non-risk” alleles (Table 1).

fMRI Acquisition Parameters

Blood oxygen level-dependent (BOLD) fMRI data were acquired with a gradient-echo EPI sequence (TR/TE = 2000/25 milliseconds, FOV = 20 cm, matrix = 64 × 64) covering 34 interleaved 3 mm thick axial slices on a Siemens 3T Trio Scanner. Prior to collecting fMRI data for each participant, a reference echoplanar imaging scan was collected; this scan was visually inspected for artifacts and good signal.

Neuroimaging Data Analyses

BOLD fMRI data were processed with SPM8 (Wellcome Department of Imaging Neuroscience, London, England). Images for each participant were realigned to the first volume in the time series to correct for head motion, spatially normalized into a standard stereotactic space (Montreal Neurological Institute template) using a 12- parameter affine model, and smoothed to minimize noise and residual difference in gyral anatomy with a gaussian filter set at 6 mm full-width at half-maximum. Next, the ARtifact detection Tool (ART) (Whitfield-Gabrieli, 2009) was used to generate regressors to account for images with large motion (i.e., >0.6 mm relative to the previous time frame) or spiking artifacts (i.e., global mean intensity 2.5 standard deviations from the entire time series).

After preprocessing, linear contrasts using canonical hemodynamic response functions estimated condition specific (i.e., faces>shapes) BOLD activation for each individual. As we were not interested in neural networks associated with face-specific processing per se, but rather in eliciting a maximal amygdala response across all participants, we chose not to use neutral faces as control stimuli because neutral faces can be subjectively experienced as affectively laden or ambiguous and thus engage the amygdala (Wright et al., 2003, Schwartz et al., 2003). Individual contrast images (i.e., weighted sum of the beta images) were used in a second-level random effects model to determine mean condition-specific responses using a 1-sample *t* test, corrected for family-wise error (FWE) at a voxel level of $p < 0.05$ with a voxel extant of 10 contiguous voxels across amygdala regions of interest.

Regions of Interest

Mean BOLD contrast estimates were extracted from functional clusters exhibiting a main effect of task (FWE $p < .05$; ≥ 10 contiguous voxels) within anatomically defined amygdala regions of interest (ROIs). Extracting parameter estimates from functional clusters activated by our fMRI paradigm, rather than clusters specifically correlated with our independent variables of interest, precludes the possibility of any correlation coefficient inflation that may result when an explanatory covariates is used to select a region of interest (Viviani, 2010). We have used this more conservative and rigorous analytic strategy in recent studies (Hyde et al., 2011, Carré et al., 2010). To account for distinct functional subregions within the amygdala, we constructed separate ventral (i.e., basolateral complex; 1024 mm^3/42 voxels) and dorsal (i.e., central nucleus and substantia inominata; 1920 mm^3/93.33 voxels) amygdala ROIs as previously described (Manuck et al., 2010, Carré et al., 2010). The ventral and dorsal distinctions allowed for independent examination of regions primarily involved with receiving sensory input and those critical for the expression of responses to threatening stimuli, respectively (Davis and Whalen, 2001, LeDoux, 2007). In humans, fMRI has revealed differences in function between these subregions, such as the conscious versus unconscious processing of fearful faces (Lerner et al., 2011, Etkin et al., 2004), as well as structural and functional connectivity (Lerner et al., 2011). All participants had >98% BOLD signal coverage in these amygdala ROIs.

Statistical Analyses

A general linear model (GLM) was used in Statistica version 7.0 to test emotional neglect-by-*FKBP5* SNP genotype interaction (EN × *FKBP5*) effects for each SNP/haplotype on each of the four amygdala ROIs (left/right dorsal amygdala and left/right ventral amygdala). EN scores, *FKBP5* genotype/haplotype (number of minor alleles/haplotypes), EN × *FKBP5*, age, and gender were used as covariates in the GLM. The interaction term was calculated for each subject by multiplying the EN score by the number of minor alleles. Using a GLM in this manner ensures that significant EN × *FKBP5* effects exist even after accounting for independent main effects of EN and *FKBP5* (see Hayes and Matthes, 2009). It is important to note that we hypothesized that *FKBP5* would not exhibit main effects on amygdala

reactivity but would only affect amygdala reactivity in the context of childhood emotional neglect. For one SNP, rs7748266, minor allele homozygotes were combined with heterozygotes for GLM analyses because the minor allele frequency (MAF) was less than 20%.

The program SNPSpD, which uses LD and the number of SNPs to determine a corrected significance threshold, identified $\alpha = 0.019$ to correct for the six *FKBP5* SNPs tested (Nyholt, 2004). An additional bonferroni correction was applied to this threshold to correct for multiple ROI comparisons ($\alpha = 0.019/4 = 0.0048$). SNPs with individual interaction effects that survived this threshold were used as part of haplotype analyses (see above). One-way ANOVAs and chi-square tests assessed differences in self-report and demographic variables across genotype groups.

Results

Genotype groups did not differ significantly on any self-reported variables including EN (all p's > 0.07), CTQ total (all p's > 0.07), gender ratio (all p's > 0.25), or age (all p's > 0.5; Table 2). Consistent with previous research the task reliably recruited threat-related reactivity of the dorsal and ventral amygdala (Figure 1a,b) (Brown et al., 2005, Neumann et al., 2006, Brown et al., 2006, Manuck et al., 2007). Additionally, consistent with our previous report in a larger sample from TAOS, both right ventral and dorsal ROIs exhibited a positive main effect of EN score ($F_{[1, 137]} = 5.87$, $p = 0.017$; $F_{[1, 137]} = 4.55$, $p = 0.035$; respectively) (Bogdan et al., in press).

GLM revealed significant EN-by-genotype interaction effects on both the right and left dorsal amygdala ROIs (see Table 2), which survived SNPspD correction for multiple testing ($p < 0.019$). However, no significant interaction effects were observed on ventral amygdala ROIs after accounting for SNPspD correction (All $F_{[5, 133]}$'s < 4.57, all p's > 0.034). *FKBP5* SNPs rs7748266 ($F_{[5, 133]} = 6.58$, $p = 0.011$), rs1360780 ($F_{[5, 133]} = 10.31$, $p = 0.002$), rs9296158 ($F_{[5, 133]} = 9.99$, $p = 0.002$), rs3800373 ($F_{[5, 133]} = 9.16$, $p = 0.003$), and rs9470080 ($F_{[5, 133]} = 6.59$, $p = 0.011$) had significant interaction effects on right dorsal amygdala reactivity (see Figure 2a-e); while SNPs rs9394309 ($F_{[5, 133]} = 6.39$, $p = 0.013$) and rs7748266 ($F_{[5, 133]} = 8.51$, $p = 0.004$) had significant interaction effects on left dorsal reactivity (see Figure 3a,b). These effects survived a multiple SNP comparison generated by SNPspD ($p < 0.019$), and four SNPs (rs7748266, rs1360780, rs9296158, and rs3800373) survived an additional FWE correction for multiple ROI comparisons ($p < 0.0048$). However, there was no significant main effect of genotype for any SNP on dorsal (see Table 2) or ventral amygdala ROIs (all p's > 0.06). Exploratory haplotype analyses revealed that both the five-SNP haplotype block generated from Haploview (rs9296158, rs7748266, rs1360780, rs9394309 and rs9470080) and the four-SNP haplotype block created post-hoc with SNPs showing the most significant interaction effects (rs3800373, rs9296158, rs1360780 and rs7748266), interacted with EN to predict right dorsal amygdala reactivity, (Haploview: $F_{[5, 133]} = 6.59$, $p = 0.011$; Figure 4a; post-hoc composition: $F_{[5, 133]} = 9.99$, $p = 0.002$;Figure 4b). Both haplotype analyses are significant after Bonferroni correction for multiple haplotype comparisons ($p = 0.025$). Results using a whole amygdala ROI (Figure S1) were similar to those seen in the dorsal subregion (Table S2). Additionally, similar results in the dorsal amygdala were obtained when using the total CTQ score (Table S2). We also tested the interactions including subjects (n = 17) with a current or past diagnosis of a mood or anxiety disorder. The results were essentially unchanged; EN × *FKBP5* interactions emerged for the same SNPs predicting right (rs7748266, rs9296158, rs1360780, rs3800373, and rs9470080) and left (rs7748266 and rs9394309) dorsal amygdala reactivity (all $F_{[5, 150]}$'s > 6.4, all p's < 0.012).

Discussion

This study examined how common *FKBP5* polymorphisms previously linked to HPA axis dysfunction and/or stress-related psychopathology impact threat-related amygdala reactivity in the context of childhood adversity. Consistent with predictions, *FKBP5* genotypes previously associated with impaired negative feedback of the HPA axis and/or stress-related psychopathology (i.e., rs1360780 T allele, rs9296158 A allele, rs9470080 T allele, rs3800373 G allele, rs7748266 T allele, rs9394309 G allele) interacted with childhood emotional neglect to predict heightened threat-related dorsal amygdala reactivity. While *FKBP5* genotype alone may have a modest effect on amygdala reactivity, the further unmasking of this regulatory bias by environmental stressors results in a significant bias in reactivity. This reactivity bias in the context of emotional neglect may represent a mechanism through which individuals carrying these *FKBP5* alleles are at increased risk for stress-related psychopathology.

Such heightened threat-related amygdala reactivity is consistent with collective evidence that these *FKBP5* risk genotypes are associated with elevated and prolonged cortisol response to stress. First, elevated endogenous cortisol is associated with potentiated amygdala reactivity (Maheu et al., 2008, van Stegeren et al., 2007). Second, extreme forms of childhood emotional neglect (e.g, institutionalization) are associated with relative hyper-responsiveness of the HPA axis (McCrory et al., 2010, Lupien et al., 2009), as well as potentiated amygdala reactivity (Maheu et al., 2010, Tottenham et al., 2011, Gianaros et al., 2008). Third, increased *FKBP5* expression (Binder, 2009, Binder et al., 2008, Wochnik et al., 2005) and adverse experiences (Heim et al., 2008, Meaney, 2001) impair negative feedback regulation of the stress response, and *FKBP5* is upregulated in the amygdala following stress (Scharf et al., 2011) as well as associated with anxious behavior (Attwood et al., 2011). Thus, HPA axis dysregulation, associated with both *FKBP5* risk genotypes and prior emotional neglect, may be further exaggerated by their interaction (i.e., GxE) leading to heightened amygdala reactivity and sensitivity to environmental threat, which may subsequently mediate increased risk for stress-related psychopathology.

Although the amygdala is traditionally analyzed as a unitary structure in neuroimaging studies, this assumption is not consistent with non-human animal research, which suggests there are different roles for the different nuclei within the amygdala (for review see Davis and Whalen, 2001, LeDoux, 2007). Recent human neuroimaging studies (Bzdok et al., 2012, Lerner et al., 2012, Roy et al., 2009, Amunts et al., 2005) support this observed heterogeneity by highlighting differences between the connectivity of the ventral and dorsal subregions, the same *a priori* ROIs used in this study. Also, differences between ventral and dorsal subregions have been found in the conscious versus unconscious processing of fearful faces (Lerner et al., 2012, Etkin et al., 2004). However, more studies are needed to definitively establish these observed differences. This caveat notwithstanding, our findings were specific to reactivity within the dorsal amygdala, which contains the central nucleus as well as dorsal extended regions including the nucleus basalis of Meynert. Unlike the ventral amygdala, which generally receives sensory, hippocampal and prefrontal afferents, the dorsal amygdala sends efferents to the brainstem and hypothalamus, which drive autonomic and motor functions, as well as prefrontal cortex, which modulates attention and vigilance (LeDoux, 2007). Because all of the EN-by-*FKBP5* genotype interactions reported here predicted differences in dorsal, but not ventral amygdala reactivity, the risk associated with these variants may reflect sensitized responses to, as opposed to sensory perceptions, of threat. It is also important to note that we observed nominal, but not corrected, significance levels within whole amygdala ROIs (see supplemental material). This is likely due to differences in the spatial distribution of *FKBP5* expression, since animal models show

stress-related increases in expression of *FKBP5* occurring in the central nucleus, which is part of the dorsal amygdala, and not other regions of the amygdala (Scharf et al. 2011).

These conclusions should be tempered, however, as there were several limitations to this study. First, we did not collect neuroendocrine measures (e.g., cortisol), which would have allowed us to test our speculations regarding HPA axis mechanisms that may drive the observed interaction. Second, we did not collect contemporaneous measures of psychopathology symptomatology making it impossible for us to link amygdala reactivity to individual differences in clinical presentations. Notably, however, TAOS is a prospective study, which will allow us to assess whether genetically-driven variability in brain function interacts with environmental stressors to predict future development of psychopathology. Third, we relied on retrospective self-report of emotional neglect, which could be biased by current mood (Teasdale and Russell, 1983) and personality traits, such as trait anxiety (Reidy and Richards, 1997). It will be important for future studies to use additional measures (e.g., public records, in person interviews) to derive more accurate estimates of adversity. Fourth, while all SNPs showed significant EN-by-genotype interaction effects on right or left dorsal amygdala reactivity, only effects from four SNPs survived both SNPspD correction and correction for multiple ROI comparisons (rs3800373, rs9296158, rs7748266, rs1360780). However, correcting for the number of SNPs tested in this case is quite conservative because of the *a priori* rationale we had for testing each SNP (Table 1). Additionally, correcting for the number of ROIs tested does not account for any correlation between the regions and is likely overly conservative, as well. Therefore, the SNPspD correction for multiple comparisons is likely more than sufficient to protect against Type 1 error, and for this reason, we include all resulting significant associations. In addition, the observed interaction effects predicting amygdala reactivity were present in a sample of psychiatrically healthy children and adolescents with modest levels of childhood stress (notably, the findings were entirely consistent when including individuals with current or past diagnosis of a mood or anxiety disorder [n =17]). Thus, it will be important for future research to assess whether differential amygdala reactivity conferred by our observed interactions actually predicts meaningful differences in psychopathology and behavior. Indeed, following these samples into peak periods of risk for psychopathology, as TAOS is designed to do, will be particularly valuable (Somerville et al., 2011). Lastly, the SNPs selected do not represent full coverage of the *FKBP5* gene as they were carefully selected a priori based on associations with HPA function and/or psychopathology (see Table 1). Thus, there may be *FKBP5* variants exhibiting yet to be discovered interaction effects with EN on amygdala function. These limitations notwithstanding, if our results can be replicated, they provide a plausible biological mechanism (i.e., heightened threat-related amygdala reactivity) by which common *FKBP5* polymorphisms may confer risk for psychopathology in the context of childhood adversity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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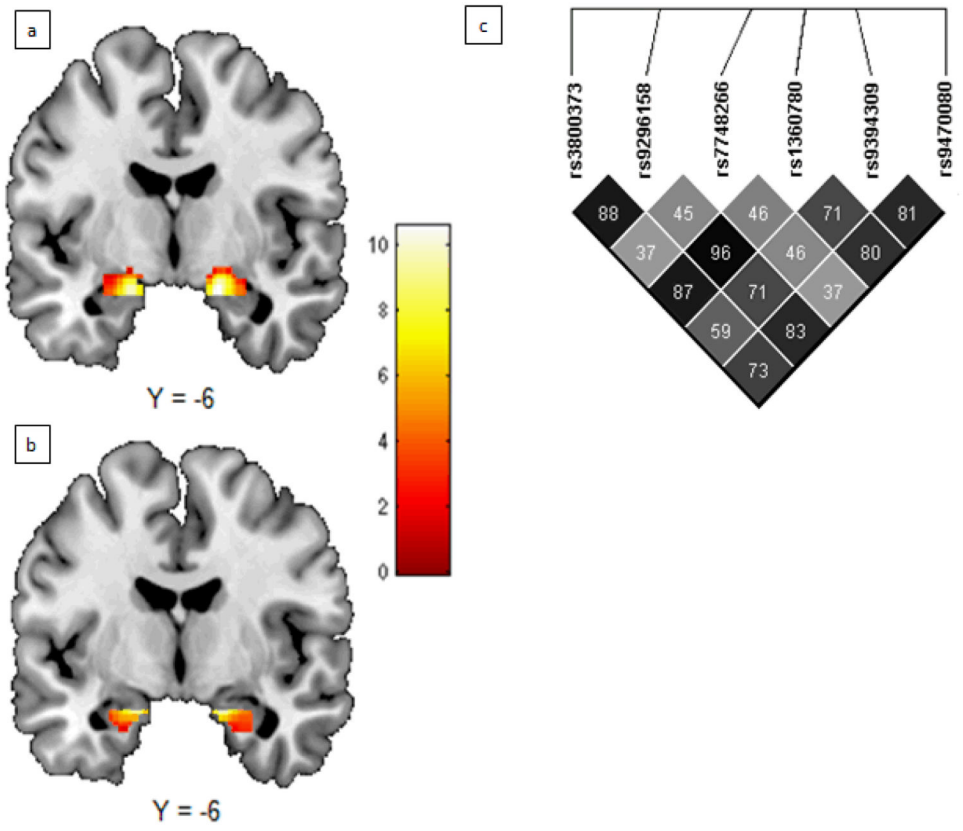

Figure 1. Main and Interaction Effects

Significant group-level activation in **(a)** dorsal (left: [-18 -4 -18], k = 152 voxels, t = 10.58; right: [18 -8 -16], k = 164 voxels, t = 10.52) and **(b)** ventral (left: [-18 -4 -20], k = 67 voxels, t = 9.53; right: [18 -4 -20], k = 89 voxels, t = 9.20) amygdala ROIs ($p_{FWE} < 0.05$). LD map for *FKBP5* SNPs shown in **(e)**. Darker squares denote higher LD and numbers shown are r^2 values.

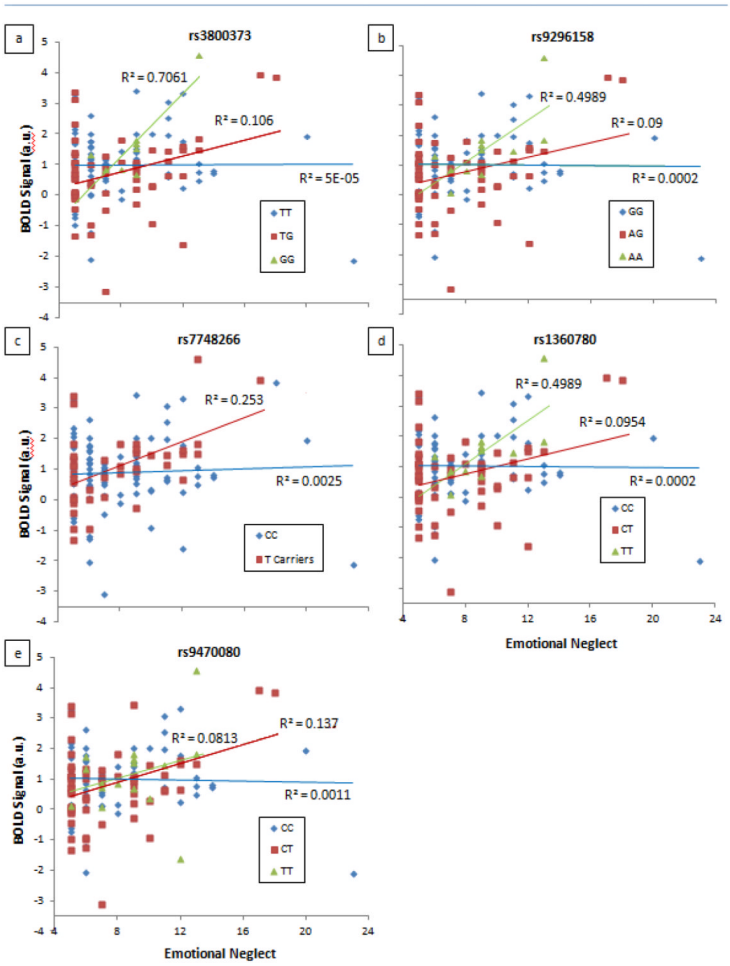

Figure 2. *FKBP5* SNPs showing significant interaction effects with emotional neglect on right dorsal amygdala reactivity

All significance levels survive SNPspD correction for multiple testing of p = 0.019: **(a)** rs3800373 (p = 0.003), **(b)** rs9296158 (p = 0.002), **(c)** rs7748266 (p = 0.011), **(d)** rs1360780 (p = 0.002), and **(e)** rs9470080 (p = 0.011). Plots show relationship between EN and amygdala BOLD separated by genotype group.

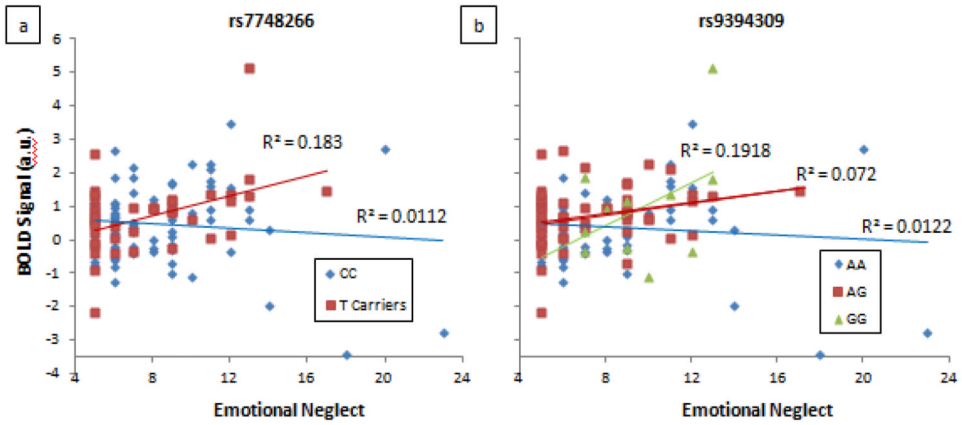

Figure 3. ***FKBP5*** **SNPs showing significant interaction effects with emotional neglect on left dorsal amygdala reactivity**

All significance levels survive SNPspD correction for multiple testing of p = 0.019: **(a)** rs7748266 (p = 0.004) and **(b)** rs9394309 (p = 0.013). Plots show relationship between EN and amygdala BOLD separated by genotype group.

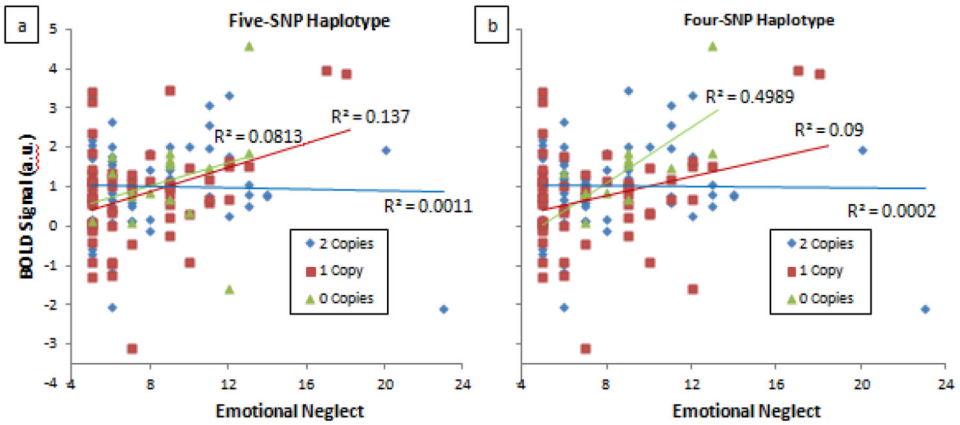

Figure 4. *FKBP5* haplotypes showing significant interaction effects with emotional neglect on right dorsal amygdala reactivity

(a) Five-SNP haplotype (rs9296158, rs7748266, rs1360780, rs9394309 and rs9470080) generated from Haploview (p = 0.011). Subjects were categorized based on the presence of the GCCAC haplotype (i.e. 0, 1 or 2 copies), which is comprised of "non-risk" alleles. **(b)** Four-SNP haplotype (rs3800373, rs9296158, rs1360780 and rs7748266) constructed from SNPs showing most significant interaction effects (p = 0.002). Subjects were categorized based on the presence of the TGCC haplotype (i.e., 0, 1 or 2 copies), which is comprised of "non-risk" alleles. Plots show relationship between EN and amygdala BOLD separated by haplotype group.

Table 1

Associations Between *FKBP5* Polymorphisms, Stress Responsiveness and Psychopathology.

SNP (Location)	Association
rs1360780 (Intron)	T allele: Increased *FKBP5* protein levels; reduced basal levels of cortisol; impaired HPA negative feedback following dexamethasone (DEX) and psychosocial stress; PTSD symptoms, incidence of depression, depressive symptoms and suicide in the context of childhood maltreatment; increased depression recurrence and more rapid response to antidepressant treatment; increased harm avoidance and reduced cooperativeness (Binder et al., 2004, Binder et al., 2008, Ising et al., 2008, Shibuya et al., 2010, Xie et al., 2010, Brent et al., 2010, Velders et al., 2011, Appel et al., 2011, Zimmermann et al., 2011)
rs9296158 (Intron)	A allele: Impaired HPA negative feedback of following DEX; reduced *FKBP5* downregulation with increasing PTSD severity; PTSD symptoms and incidence of depression in the context of childhood maltreatment (Binder et al., 2008, Xie et al., 2010, Mehta et al., 2011, Zimmermann et al., 2011)
rs9470080 (Intron)	T allele: Depressive symptoms; reduced basal cortisol levels; PTSD in the context of adverse environmental exposure; incidence of depression in the context of childhood maltreatment (Boscarino et al., 2011, Velders et al., 2011, Xie et al., 2010, Zimmermann et al., 2011)
rs3800373 (3′ UTR)	G allele: Increased rate of suicide among depressed individuals; more rapid antidepressant response; increased peri-traumatic dissociation following trauma; increased PTSD and incidence of depression in the context of childhood maltreatment; impaired negative feedback of cortisol following psychosocial stress (Binder et al., 2004, Brent et al., 2010, Koenen et al., 2005, Ising et al., 2008, Zimmermann et al., 2011)
rs7748266 (Intron)	T allele: Reduced basal cortisol levels (Velders et al., 2011)
rs9394309 (Intron)	G allele: Reduced basal cortisol levels (Velders et al., 2011)

Table 2

Demographics, effects of genotype and genotype-by-EN interaction effects on dorsal amygdala ROIs for *FKBP5* SNPs.

SNP/Haplotype	Genotype/Haplotype copies	N	Age, mean (SD)	CTQ, mean (SD)	EN, mean (SD)	Gender, female (%)	R Dorsal		L Dorsal	
							Main effect (p)	EN-by-genotype (p)	Main effect (p)	EN-by-genotype (p)
rs3800373	AA	74	13.5 (1.0)	32.3 (7.4)	7.8 (3.5)	39 (52.7)	0.091	0.003 **	0.082	0.212
MAF = 0.26	AC	57	13.5 (0.9)	31.9 (7.2)	7.8 (3.2)	25 (43.9)				
	CC	8	13.2 (0.9)	33.3 (4.9)	8.8 (2.1)	3 (37.5)				
rs9296158	GG	70	13.5 (1.1)	32.6 (7.5)	7.9 (3.5)	37 (52.9)	0.069	0.002 **	0.080	0.181
MAF = 0.29	AG	58	13.5 (0.9)	31.4 (7.2)	7.5 (3.1)	25 (43.1)				
	AA	11	13.3 (0.9)	33.7 (4.5)	9.2 (2.3)	5 (45.5)				
rs7748266	CC	98	13.5 (1.0)	32.6 (7.6)	7.8 (3.3)	50 (51.0)	0.316	0.011 *	0.280	0.004 **
MAF = 0.16	CT/TT	41	13.5 (0.9)	31.3 (6.0)	7.8 (3.1)	17 (41.5)				
rs1360780	CC	72	13.5 (1.0)	32.5 (7.4)	7.9 (3.5)	39 (54.2)	0.062	0.002 **	0.082	0.207
MAF = 0.28	CT	56	13.5 (0.9)	31.5 (7.3)	7.5 (3.1)	23 (41.1)				
	TT	11	13.3 (0.9)	33.7 (4.5)	9.2 (2.3)	5 (45.5)				
rs9394309	AA	71	13.4 (1.1)	33.1 (8.3)	8.0 (3.7)	37 (52.1)	0.537	0.097	0.119	0.013 *
MAF = 0.28	AG	57	13.6 (0.9)	30.6 (5.6)	7.3 (2.7)	24 (42.1)				
	GG	11	13.3 (0.9)	34.6 (4.6)	9.6 (2.3)	6 (54.5)				
rs9470080	CC	64	13.5 (1.1)	32.7 (7.7)	8.0 (3.6)	35 (54.7)	0.432	0.011 *	0.415	0.266
MAF = 0.33	CT	59	13.5 (0.9)	31.3 (7.1)	7.4 (3.0)	25 (42.4)				
	TT	16	13.4 (0.8)	33.4 (4.8)	8.8 (2.5)	7 (43.8)				
GCCAC	2 copies	64	13.5 (1.1)	32.7 (7.7)	8.0 (3.6)	35 (54.7)	0.432	0.011 *	0.415	0.266
Freq. = 0.67	1 copy	59	13.5 (0.9)	31.3 (7.1)	7.4 (3.0)	25 (42.4)				
	0 copies	16	13.4 (0.8)	33.4 (4.8)	8.8 (2.5)	7 (43.8)				
TGCC	2 copies	72	13.5 (1.0)	32.5 (7.4)	7.9 (3.5)	39 (54.2)	0.062	0.002 **	0.082	0.207
Freq. = 0.72	1 copy	56	13.5 (0.9)	31.5 (7.3)	7.5 (3.1)	23 (41.1)				
	0 copies	11	13.3 (0.9)	33.7 (4.5)	9.2 (2.3)	5 (45.5)				
All Groups		**139**	**13.5 (1.0)**	**32.2 (7.2)**	**7.8 (3.3)**	**68 (48.9)**				

* Significant effect after SNPspD correction

** Significant effect after SNPspD correction and correction for number of ROIs

Note: MAF = minor allele frequency. CTQ = childhood trauma questionnaire. EN = Emotional Neglect subscale of the CTQ.
No differences between genotype groups were observed for age, CTQ, EN, or gender