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***FKBP5* and Attention Bias for Threat:**

Associations With Hippocampal Function and Shape

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Abstract

Importance—The *FKBP5* gene product regulates glucocorticoid receptor (GR) sensitivity and hypothalamic-pituitary-adrenal axis functioning and has been associated with many stress-related psychiatric disorders. The study of intermediate phenotypes, such as emotion-processing biases and their neural substrates, provides a way to clarify the mechanisms by which *FKBP5* dysregulation mediates risk for psychiatric disorders.

Objective—To examine whether allelic variations for a putatively functional single-nucleotide polymorphism associated with *FKBP5* gene regulation (rs1360780) would relate differentially to attention bias for threat. this was measured through behavioral response on a dot probe task and hippocampal activation during task performance. Morphologic substrates of differential hippocampal response were also measured.

Design—Cross-sectional study conducted from 2010 to 2012 examining associations between genotype, behavioral response, and neural response (using functional magnetic resonance imaging [fMRI]) on the dot probe; voxel-based morphometry and global and local shape analyses were used to measure structural differences in hippocampi between genotype groups.

Setting—Participants were recruited from primary care clinics of a publicly funded hospital in Atlanta, Georgia.

Participants—An African American cohort of adults (N=103) was separated into 2 groups by genotype: one genotype group included carriers of the rs1360780 T allele, which has been

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Online-Only Material: The eTables, eMethods, and eResults are available at <http://www.jamapsych.com>.

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associated with increased risk for posttraumatic stress disorder and affective disorders; the other group did not carry this allele. Behavioral data included both sexes (N=103); the MRI cohort (n=36) included only women.

Main Outcome Measures—Behavioral and fMRI (blood oxygen level–dependent) response, voxel-based morphometry, and shape analyses.

Results—Carriers of the rs1360780 T allele showed an attention bias toward threat compared with individuals with-out this allele ($F_{1,90}=5.19$, $P=.02$). Carriers of this allele demonstrated corresponding increases in hippocampal activation and differences in morphology; global and local shape analyses revealed alterations in hippocampal shape for TT/TC compared with CC genotype groups.

Conclusion—Genetic variants of *FKBP5* may be associated with risk for stress-related psychiatric disorders via differential effects on hippocampal structure and function, resulting in altered attention response to perceived threat.

IN THE PAST DECADE, *FKBP5* (OMIM 602623) has emerged as a promising genetic candidate for investigations of vulnerability to mood and anxiety disorders.¹ The *FKBP5* gene encodes for the protein FK506 binding protein 5–51, which regulates sensitivity of the glucocorticoid receptor (GR). The GR is a critical component of the body's stress response system and a primary binding site for cortisol, a corticosteroid released during acute and chronic stress. The FKBP5 protein interacts with heat shock protein 90 to reduce GR affinity, modulating cortisol activity and preventing translocation of the GR complex to the nucleus.² Through this process, FKBP5 appears to decrease GR sensitivity (or increase GR resistance) to cortisol following stress. Dysregulated GR signaling is common in affective disorders^{1,3} and may be mediated by *FKBP5* gene expression. Polymorphisms in *FKBP5* have been associated with posttraumatic stress disorder (PTSD),^{4–10} depression,¹¹ depressive episode recurrence,¹² and bipolar disorder.¹³ Notably, associations between *FKBP5* polymorphisms and psychiatric disorders have been somewhat variable between white and African American populations.^{6,14}

Although these studies have outlined clear associations between *FKBP5* and various affective disorders, mechanisms by which *FKBP5* dysregulation may mediate symptom risk are still unknown. This relationship may be clarified through the study of intermediate phenotypes: discrete, heritable traits associated with genetic variance and disease phenotype.¹⁵ Given the role of *FKBP5* in endocrine response to stress, this gene is likely to influence maladaptive behaviors that comprise intermediate phenotypes that underlie stress-related psychiatric disorders.¹⁶ One candidate intermediate phenotype is biased attention to emotionally valenced cues, which has been widely observed in anxiety¹⁷ and depression¹⁸ and linked to allelic variations in the serotonin transporter gene.^{19,20} There is some evidence to suggest the involvement of *FKBP5* in this phenomenon. Multiple studies indicate that attention biases are closely associated with circulating levels of cortisol^{21–25} and that these biases may be influenced by cortisol administration.²⁴ Given the role of the GR in modulating the body's response to cortisol, attention biases for emotional stimuli could be regulated through GR signaling and thus moderated by polymorphisms in genes that influence the GR receptor complex, such as *FKBP5*.

Furthermore, emerging evidence suggests that attention biases may be associated with alterations in brain function among individuals who hold increased genetic risk for psychiatric disorders. For example, Thomason and colleagues²⁶ found that children who carried specific allelic variants of the serotonin transporter gene (related to depressive vulnerability) demonstrated an attention bias toward fearful facial expressions and increased

blood oxygen level–dependent (BOLD) signal in parietal and paralimbic regions compared with participants without this allele.

For *FKBP5* in particular, the hippocampus is an attractive target for brain-behavior phenotype investigations given that this region has been associated with high GR density²⁷ and *FKBP5* expression; furthermore, psychiatric disorders linked to *FKBP5* genetic status, particularly PTSD, have been associated with abnormal hippocampal volume^{28–30} and function.^{31,32} The hippocampus also has been implicated in the processing of context during acquisition of conditioned fear responses and, in concert with prefrontal regions,³³ participates in the appraisal of potential threat in new situations.^{34–36} These hippocampal functions may be mediated partly by *FKBP5* given the potential role of this gene in the stress response.

Thus, the goal of the present study was to investigate the relationship between an *FKBP5* polymorphism and a candidate cognitive phenotype, attention bias for threat cues, in highly traumatized individuals, some of whom met criteria for Axis I psychiatric disorders. We selected a putatively functional³⁷ *FKBP5* single-nucleotide polymorphism (SNP) that has been associated with anxious and depressive psychiatric disorders: rs1360780. Recent experiments³⁷ have shown that the rs1360780 risk allele is associated with increased transcription of *FKBP5* following stimulation by glucocorticoids, possibly by increasing binding of the risk-allele containing DNA sequence to TATA-box binding protein, a transcriptional enhancer. Increased *FKBP5* messenger RNA transcription in peripheral blood cells immediately after trauma exposure has been associated with a higher risk for subsequent PTSD.³⁸

Attention biases were characterized through behavioral and neural responses during functional magnetic resonance imaging (fMRI) in a subset of these individuals. We tested the hypothesis that risk allele carriers would demonstrate significantly greater attention bias for threat and increased neural response in the hippocampus compared with individuals without this allele. In addition, we conducted structural analyses within this region of interest (ROI) to characterize the neural substrates of differential task response between individuals with and without the risk allele.

METHODS

PARTICIPANTS

A total of 110 adult (aged 20–62 years) African American men and women (sex and race self-identified) were enrolled as part of a larger study investigating genetic risk for stress-related disorders; study procedures were approved by the institutional review boards of Emory University School of Medicine and Georgia State University. Participants were recruited from the general medical clinics of a publicly funded hospital, as detailed previously.^{4,39} Individuals were deemed eligible for participation if they could give informed consent and understand English, as determined by a study researcher. Participants were administered the Traumatic Events Inventory (TEI) to detail the frequency and type of traumatic experiences, the PTSD Symptom Scale (PSS)⁴⁰ to measure the presence and frequency of current PTSD symptoms, and the Structured Clinical Interview for *DSM-IV* disorders⁴¹ to detect comorbid psychiatric disorders such as major depressive disorder (MDD) or bipolar disorder. Demographic and clinical characteristics of this sample are detailed in eTable 1 (<http://www.jamapsych.com>).

A subset of these participants (47 women) completed magnetic resonance imaging (MRI); data from these (all traumatized) individuals were included in a prior study of PTSD⁴² and were selected from the larger sample on the basis of inclusion/exclusion criteria for the

present study. Exclusion criteria for this study included current psychotropic medication use; medical or physical conditions that preclude MRI scanning; a diagnosis of bipolar disorder, schizophrenia, or other psychotic disorder; medical conditions that contribute significantly to psychiatric symptoms; history of head injury or loss of consciousness for more than 5 minutes; and a history of neurologic illness.

DNA EXTRACTION, GENOTYPING, AND QUALITY CONTROL

Samples for DNA were extracted from saliva (DNAAdvance extraction kit; Beckman Coulter Genomics) collected in vials (Oragene; DNA Genotek Inc) or from whole blood (E.Z.N.A. Blood DNA Midi Kit; Omega Bio-tek) collected in EDTA tubes. All DNA for genotyping was quantified (PicoGreen; Invitrogen Corp, or NanoDrop; Thermo Fisher Scientific Inc) and normalized to a concentration of 5 ng/ μ L. The DNA was plated into 384 plates at 10 ng and dried prior to performing the genotyping reactions.

For genotyping, 1 mL of whole blood or 500 μ L of Oragene saliva was used for extraction. Genotyping of *FKBP5* SNP rs1360780 was conducted (TaqMan SNP Genotyping Assay with TaqMan Genotyping Master Mix; Applied Biosystems Inc) and alleles were discerned (7900HT Fast Real-Time PCR System; Applied Biosystems Inc). Negative controls and within- and across-plate duplicates were used for quality control. All negative controls passed quality control. Forty percent of the samples were genotyped in duplicate with no genotype discordants. The TaqMan genotype call rate was 97%. Alleles were in Hardy-Weinberg equilibrium (African Americans only, $P=.19$).

The *FKBP5* SNP rs1360780 was selected on the basis of prior studies^{37,43} with *FKBP5* polymorphisms within this population. We tested for associations under a risk=T allele carrier model. Participants were categorized into 2 groups on the basis of genotype: individuals with 1 or 2 copies of the T allele, previously associated with risk of psychiatric disorders, were categorized in the risk group (TC/TT), whereas individuals without this allele (CC) were categorized in the resilient group.

ATTENTION BIAS (DOT PROBE) TASK DESCRIPTION

A dot probe task was used to measure attention biases for threat; this measure has reliably detected biases in prior studies¹⁷ of individuals with emotional disorders. Task procedures have been described⁴⁴ (also detailed in eMethods), and the trial structure is illustrated in Figure 1A. Briefly, in each of 80 trials a central fixation cross appears for 500 ms, followed by a pair of face photographs (both of the same model) presented for 500 ms. Each pair comprises either 1 emotionally expressive (threatening/happy) and 1 neutral face or 2 neutral faces. After the offset of the faces, an asterisk replaces 1 of the faces for 1100 ms. Participants quickly indicate, with a forced-choice button press, whether the asterisk appeared on the left or right side of the screen. A modified version of this task, detailed elsewhere,⁴² was presented during neuroimaging.

Emotion bias scores were first calculated by subtracting response time to emotion-congruent stimuli (probes that replace happy or angry/threatening pictures) from response time to emotion-incongruent stimuli (probes that replace neutral pictures); these scores were decomposed into threat bias and happy bias scores for all stimuli combined and separately for African American and white faces. Positive scores indicated attention capture by the threat or happy cue, and negative scores reflected attention avoidance of this cue.

STATISTICAL ANALYSIS OF BEHAVIORAL DATA

Univariate analyses of covariance were conducted to examine whether genotype status was related to attention bias for threatening faces in general (threat bias) or for threatening white

(threat bias white) or African American (threat bias African American) faces after covarying for the presence of bipolar disorder, MDD, total trauma exposure (TEI total score), and PTSD symptoms (PSS total score). A similar analysis was conducted to examine potential associations with happy bias score. In addition, 3 separate factorial analyses of covariance were conducted to examine potential interactions of genotype, PTSD diagnosis, and trauma exposure (in childhood or adulthood) on threat bias score after covarying for depressive psychiatric disorders. A final analysis was conducted to compare bias scores for the CC, TC, and TT groups after covarying for psychiatric disorders. A threshold of $P < .05$ was used to determine statistical significance.

MRI ACQUISITION AND PREPROCESSING, fMRI DATA ANALYSIS

Scanning took place in a research-dedicated scanner (Siemens 3-Tesla; Siemens AG). A high-resolution T1-weighted structural scan was acquired using an MPRAGE sequence (176 slices, field of view [FOV]=256 mm cubic voxels; $1 \times 1 \times 1$ mm slice; repetition time [TR]=2600 ms; echo time [TE]=3.02 ms; inversion time=900 ms; flip angle=8°). During task administration, a total of 26 contiguous echo-planar, T2-weighted images parallel to the anterior-posterior commissure line were acquired (TR=2530 ms; TE=30 ms; FOV=240 mm; 64×64 matrix; $3.75 \times 3.75 \times 4.0$ mm voxel). Statistical Parametric Mapping, version 5 (SPM5, Wellcome Department of Neurology, London, England; <http://www.fil.ion.ucl.ac.uk/spm/>) was used for file conversion, image preprocessing, and fMRI statistical analyses. Functional images were slice-time corrected (with a high-pass filter applied) and realigned to the first image to correct for motion. The mean of the realigned undistorted images was then coregistered with the structural T1 volume, spatially normalized to standardized Montreal Neurological Institute (MNI) space⁴⁵ based on the position of the anterior and posterior commissure and smoothed with an 8-mm full-width half-maximum gaussian kernel.

For each participant's data, a first-level, fixed-effects model was created with vectors for onset time of each condition, including threat/neutral, happy/neutral, and neutral/neutral trials; each trial included face-pair presentation and probe. Box-car functions using 1, -1 contrast conventions were used to indicate voxels that had a higher activation level for the primary contrast condition, which was activation to threat probe-incongruent trials (ie, the probe replaced the neutral face of a threat-neutral pair) minus threat probe-congruent trials (the probe replaced the threat face of a threat-neutral pair), the same events that were used to calculate threat bias score. Analysis using 2-tailed unpaired t tests was then conducted to examine between-group differences in *FKBP5* genotype and BOLD signal change to threat within ROIs. Primary analyses were conducted with ROIs, and brainwide analyses were conducted for completeness. First, an uncorrected statistical threshold of $P < .005$ and an extent threshold of 5 or more voxels per cluster were applied to brainwide analyses. Region-of-interest analyses were conducted with masks of the parahippocampal gyrus created with the WFU PickAtlas Toolbox (Wake University School of Medicine)⁴⁶; a $P < .05$ threshold, extent threshold of 5 or more voxels per cluster was used to determine statistical significance. A nonlinear transformation (<http://bioimagesuite.yale.edu/mni2tal/index.aspx>) was used to convert coordinates from MNI to Talairach space,⁴⁷ and a Talairach daemon⁴⁸ was used to localize anatomic coordinates of voxels associated with statistically significant patterns of BOLD activation.

VOXEL-BASED MORPHOMETRY ANALYSIS

With the brain extraction toolkit in the Oxford Centre for Functional MRI of the Brain (FMRIB) Software Library,⁴⁹ all structural images were (1) skull-stripped (evaluated individually for errors), (2) segmented with FMRIB's automated segmentation tool,⁵⁰ (3) aligned to MNI152 standard space using affine registration, and (4) underwent nonlinear registration using FMRIB's nonlinear image registration tool. Following alignment, a study-

specific template was generated. The segmented images were then smoothed using an isotropic gaussian kernel with a sigma of 3 mm, and voxelwise general linear model was carried out using permutation-based, nonparametric testing⁵¹ to control for type I error. Statistical thresholding was set at uncorrected $P < .001$ for ROIs.

SHAPE ANALYSIS

Global shape analysis was performed using methods fully detailed in eTable 2. Following skull extraction, images were fed through a global shape analysis workflow that automatically extracted each brain into 56 distinct regions. Inhomogeneity correction of images was followed by affine registration, nonlinear registration to the Laboratory of Neuroimaging Probabilistic Brain Atlas (LPB40),⁵² and subsequent automatic volume parcellation, during which each voxel in the brain was labeled.⁵³ The regional boundaries of the resulting labeled image were then converted into 56 distinct shapes,⁵⁴ which underwent subsequent preprocessing, modeling, and analysis. A total of 5 shape metrics were used to perform group comparisons between the TC/TT and CC genotypes: volume, surface area, mean curvature, shape index, and curvedness. The resultant output of this analysis included sets of raw P values and false-discovery rate (FDR)-corrected P values.⁵⁵ eTable 2 shows the standard intrinsic shape measures computed for each region.

Local shape analyses of both hippocampi⁵⁴ were conducted to identify the specific vertices/subregions that demonstrated significant radial distance and/or displacement vector field variations from the study-defined mean hippocampal shape template.⁵⁶ In the local shape analysis protocol, the structural attributes and cortical measures are calculated per vertex on specific shape regions that are first coregistered across participants to establish homologous anatomic features before statistically analyzing them against various participant demographic, clinical, or phenotypic data.⁵⁷ To control for multiple comparisons testing, FDR correction was applied. An atlas (Figure 2B) was used to identify anatomic locations of between-group differences.⁵⁶

RESULTS

GROUP CHARACTERISTICS

Genotype data were not available for 5 participants because of insufficient quality of the DNA samples, leaving a total participant sample of 105. In this sample, participants reported experiencing a range of 0 to 10 traumatic experiences, with 7.7% of participants reporting no trauma exposure. Approximately 6.7% of participants met criteria for a lifetime history of bipolar disorder, 13.3% met the criteria for current MDD and, based on PSS items (in keeping with *DSM-IV* PTSD criteria), 41.2% met the criteria for PTSD. Within this sample, no significant differences ($P > .05$) emerged in demographic or clinical characteristics between CC and TC/TT genotype groups (eTable 1). Although significant differences were observed between genotype groups for psychotropic medication use ($P = .006$, $\chi^2 = 7.51$), no significant differences in threat or happy bias score were observed between participants who were or were not taking psychotropic medication ($P > .05$). Within the imaging cohort, age and level of current PTSD symptoms were similar for risk and resilient genotypes. However, because the incidence of trauma exposure differed significantly between groups ($P < .05$), this served as a covariate in subsequent analyses in addition to PTSD symptoms. A comparison of clinical and demographic characteristics between the entire sample and the MRI cohort revealed no significant differences in age, PTSD symptoms, trauma exposure, household monthly income, and educational level (all $P > .05$).

BEHAVIORAL DIFFERENCES IN ATTENTION BIAS TO THREAT BETWEEN *FKBP5* (rs1360780) GENOTYPES

Two other participants were excluded from behavioral data analyses because of many skipped trials or inaccurate responses on trials (>20%), leaving a total of 103 participants with valid behavioral data. Univariate tests including trauma exposure and presence of PTSD, MDD, and bipolar disorder as covariates and 3 threat bias indices (threat bias score for the combined race types threat bias score for African American, or threat bias score for whitefaces) as dependent variables were conducted. After covarying for trauma exposure and the presence of PTSD, MDD, and bipolar disorder, risk allele (TC/TT) carriers demonstrated a statistically significant ($F_{1,90}=5.19$, $P=.02$) bias toward threat (African American and white races combined: mean [SD] threat bias score, 13.1 [37.2]) compared with the CC genotype group (-6.8 [46.2]) (Figure 1B). These findings were verified with permutation testing, using PLINK software, version 1.07,⁵⁸ to ensure that results did not depend on distributional assumptions; a total of 100 000 permutations were performed.

Separate univariate analyses revealed no significant genotype associations with happy bias (composite bias score or separated by face race; $P>.05$). Further univariate analyses (including the same covariates) revealed no significant genotype effects for threat bias for white faces ($P=.10$), although threat bias findings for African American faces approached statistical significance ($F_{1,90}=3.48$, $P=.06$). Results from separate factorial analyses of covariance including psychiatric disorder covariates and threat bias score as the dependent variable indicated no significant interactions of genotype status and (1) childhood sexual or physical abuse ($P=.85$) or (2) adult trauma exposure (presence or absence, as reported in the TEI) ($P=.44$). A univariate analysis including genotype and PTSD status as factors did not reveal significant interactions of these variables on threat bias score ($P=.69$). Genotype contributed significantly to this model ($P=.04$), whereas PTSD status did not, although findings approached statistical significance ($P=.17$). A final factorial analysis of covariance revealed that, when CC, TC, and TT genotype groups were examined separately, no significant main effects were observed after covarying for psychiatric disorders (possibly because of limited sample size), though findings approached statistical significance ($F_{1,90}=2.47$; $P=.09$); individuals in the CC genotype group demonstrated a slight bias away from threat (mean bias score, -6.78 [46.21]), whereas both the TC (13.25 [39.26]) and TT (12.71 [41.40]) groups demonstrated a bias toward threat.

fMRI DIFFERENCES IN HIPPOCAMPAL ACTIVATION TO THREAT BETWEEN *FKBP5* GENOTYPES

Because of excessive motion (7) and/or brain parenchyma abnormalities (4), 11 participants were excluded from fMRI and MRI data analyses, leaving 36 participants (10 CC, 26 TC/TT); another (TC/TT) participant was excluded from fMRI (but not structural MRI) analyses, resulting from many skipped trials (>20%) on the task. Behavioral data from the cohort of participants who completed the fMRI version of the task ($n=35$) yielded no significant genotype effects on threat bias regardless of face race ($P>.05$). Correlations among attention bias scores from these 2 data sets are provided in the eResults.

In a whole-brain analysis for the threat incongruent vs threat congruent trials contrast condition, participants with the TC/TT genotype demonstrated significantly greater BOLD signal in the right hippocampus and left parahippocampal gyrus compared with those having the CC genotype (uncorrected $P<.005$) (eTable 3 and Figure 3); these findings did not change significantly when hippocampal volume was entered as a covariate (eTable 4). The CC genotype, relative to TC/TT genotype, did not demonstrate greater BOLD signal in hippocampal regions (eTable 3).

Subsequent ROI analyses confirmed these findings, with the TC/TT genotype group demonstrating increased activation in the right and left hippocampus relative to the CC group ($P < .05$) (eTable 3). The BOLD signal values were extracted from 6-mm spheres within these regions and entered into 2 separate, 3-level hierarchical regressions, with trauma incidence (TEI total; level 1), PTSD symptoms (PSS total; level 2), and genotype (level 3) serving as predictors of BOLD signal in these regions. Trauma exposure did not predict a significant amount of variance in BOLD signal within the right ($\beta = -0.15$, $R^2 = 0.02$, $P > .05$) or left ($\beta = -0.11$, $R^2 = 0.01$, $P > .05$) hippocampus; when added to this model, PTSD symptoms likewise did not predict a significant proportion of variance in BOLD signal within the right ($\beta = 0.24$, $R^2 = 0.08$, $P > .05$) or left hippocampus ($\beta = 0.03$, $R^2 = 0.02$, $P > .05$). However, when added in the third step of these models, *FKBP5* genotype accounted for a significant amount of variance in BOLD signal for the right ($\beta = -0.44$, $R^2 = 0.28$, $P < .05$) and left hippocampus ($\beta = -0.46$, $R^2 = 0.21$, $P < .05$). There was no direct association between threat bias score and neural response within ROIs.

MORPHOLOGIC ANALYSIS OF DIFFERENCES IN HIPPOCAMPAL VOLUME AND SHAPE BETWEEN *FKBP5* GENOTYPES

At the uncorrected $P < .001$ statistical threshold, voxel-based morphometry findings indicated no between-group differences in gray matter density of the hippocampus. The global shape analysis revealed some between-group differences in shape index of the left hippocampus (uncorrected $P < .05$). Additionally, local shape analysis results (Figure 2B) revealed significant between-group differences in displacement vector field measure in the left and right hippocampus (FDR corrected $P < .001$). Relative to the right hippocampus, a larger area of the left hippocampus displayed between-group spatial displacement vector metric differences, predominantly in the cornu ammonis 1 (CA1) region. The genotypic effect on hippocampal surface area was reduced ($P > .05$) when TEI score and/or hippocampal volume were included as covariates.

To ensure that these genotypic effects were not present in similar cortical and subcortical regions, these local shape analyses were conducted on the left and right insula and caudate. None of these regions showed statistically significant genotypic effects ($P > .05$).

COMMENT

The present study investigated associations among genotypic variants of a putatively functional *FKBP5* SNP, rs1360780, and response to threat cues in an attention bias paradigm. In addition to investigating behavioral response, we examined corresponding differences in function and structure within the hippocampus, a brain region that appears to be affected by *FKBP5* gene expression. Our findings from these parallel behavioral, functional, and structural neuroimaging data sets revealed that, relative to individuals without this allele, carriers of the T allele (which has been linked to incidence of psychiatric disorders^{37,43}) demonstrated (1) an attention bias toward threat, (2) increased hippocampal activation to threat, and (3) differences in hippocampal shape.

Preferential attention processing of threat cues has purported relevance to the development and maintenance of pathologic anxiety.⁵⁹ The findings from the present study document, for what we believe to be the first time, an association between *FKBP5* polymorphism and threat bias. An increasing number of studies indicate that *FKBP5* genetic variability is linked to behaviors that reflect atypical or dysregulated emotion processing, including aggression⁶⁰ and suicidality.⁶¹ Furthermore, earlier findings⁴ reflected interactive effects of genetics and environmental stress (ie, childhood abuse), that is, the genetic effects were most pronounced in the presence of high levels of prior stress or trauma. Notably, we found that the relationship between *FKBP5* allelic variance and attention bias for threat remained

significant after controlling for variance associated with psychiatric disorders, as well as trauma exposure, and no interactive effects were observed among *FKBP5* genotype, trauma exposure, and posttraumatic psychiatric disorders. Thus, the associations observed between *FKBP5* genotypic variance and attention bias were not better accounted for by variance linked to environmental stress or psychiatric disorders. It is also possible that, when considering the relationship between *FKBP5* genotype and attention bias for threat, the effects of maltreatment, exposure to traumatic events in adulthood, and a relatively high number of life stressors (eg, occupational stress, racial discrimination, bereavement, and divorce) may be additive and not interactive. Although the cross-sectional design of this study precludes definitive statements regarding risk, our findings suggest that *FKBP5* risk allele carriers, compared with individuals without this allele, are more likely to demonstrate a heightened vigilance for ostensibly mild threat cues. An attentional preference for these mild threat cues, particularly among individuals with trait anxious characteristics, indicates increased susceptibility to psychiatric disorders under stressful conditions.

In this study, risk allele carriers (who are likely to demonstrate increased transcription of *FKBP5* following stress exposure) also demonstrated heightened hippocampal activation in response to threat, specifically, to a condition that approximates threat bias. To our knowledge, no previous studies have examined associations between *FKBP5* polymorphisms and hippocampal function, particularly response to threat. However, prior data¹⁶ identified *FKBP5* as a putative modulator of physiologic stress response systems, suggesting that these associations merit attention. Our findings indicate that increased hippocampal responsiveness to threat cues was selectively associated with risk allele carrier status, implicating this gene in neural mechanisms of threat evaluation. Given the density of GRs in the hippocampus and the role of *FKBP5* in regulating GR function, the increased hippocampal activation may represent, in part, *FKBP5*-mediated sensitization of neural systems to external threat signals. Risk allele carriers may have a lower threshold for perceiving threat in the environment compared with individuals without this allele, and thus may demonstrate heightened responsiveness in neural circuits mediating threat evaluation. Notably, earlier studies of pathologically anxious individuals have revealed enhanced activation in hippocampal regions,⁶² as well as frontal regions with limbic connections, during expression of attention biases.^{33,63} Thus, the heightened hippocampal response to mild threat cues observed in risk allele carriers could represent higher baseline responsiveness in neural networks engaged during threat evaluation in these individuals.

These functional differences also may be understood in the context of structural differences. Although no significant differences in hippocampal density were noted, risk allele carriers demonstrated differences in hippocampal shape compared with individuals without this allele, specifically in the CA1 region. Abnormalities in hippocampal structure, particularly the CA1 region,^{64,65} have been noted frequently in individuals with stress-related disorders.^{66,67} This region may be particularly susceptible to the effects of stress,⁶⁸ and changes in CA1 plasticity could be partially mediated by *FKBP5* mechanisms given previous observations⁶⁹ of *FKBP5* messenger RNA upregulation following stress exposure. Individuals within our study population had experienced a relatively high incidence of trauma exposure and psychosocial stress, which has been linked to suppressed neuronal proliferation in the hippocampus.⁷⁰ These conditions are likely to interact with genetic vulnerability, and structural differences in the hippocampus may be particularly apparent among individuals who carry a risk genotype, one that has been associated^{37,43} with adverse behaviors and diagnostic outcomes.

In addition, there were no significant genotypic effects in other regions of the brain examined, providing evidence to support the specificity of *FKBP5* genetic effects on the hippocampus. Thus, the structural differences observed here indicate a putative morphologic

substrate for the differential response to threat observed between genotype groups. However, given that genotypic effects were no longer apparent after including trauma exposure in the statistical model, replication in a larger sample is needed to ascertain the robustness of these findings.

Other study limitations must be noted. First, because only female face stimuli were used in this version of the dot probe, it was impossible to investigate potential interactive effects of sex and attention biases. Similarly, the lack of white participants prohibited examination of stimulus×participant race interactions and their effects on attention biases. Also, our imaging cohort included only women, thus precluding investigation of sex-specific differences in hippocampal response or structure. In addition, some research⁷¹ suggests that *FKBP5* may be a target of progesterone; unfortunately, endocrine and menstrual cycle data were unavailable in the present study to examine potential relationships with behavioral and neural response. These factors merit consideration in future investigations. Although hypothesis testing was limited to a particular ROI, the relatively small size of our imaging cohort (particularly the size of 1 genotype group) increases the likelihood of a type II error. Furthermore, mediational analyses would provide an ideal statistical approach for determining the indirect effects of hippocampal function and size on attention bias for threat; however, our limited sample size did not confer sufficient statistical power to detect these effects. Thus, these neuroimaging findings should be viewed as preliminary and warrant confirmation in genetic neuroimaging studies conducted with larger samples.

In summary, we observed that genotype status for a putatively functional *FKBP5* SNP was associated with differential behavioral and hippocampal response during performance of an attention bias task. Structural differences in hippocampal formation served to further distinguish these genotype groups. Taken together, these data may represent behavioral and neural markers of *FKBP5* function and merit further investigation in further studies of genetic risk for affective psychiatric disorders.

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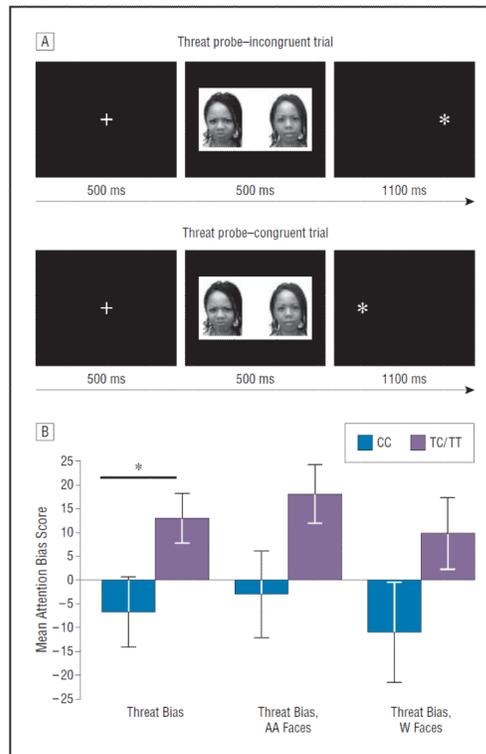


Figure 1. Attention bias (dot probe) task. A, Trial structure. Rows illustrate trials that were used to calculate threat bias and as functional magnetic resonance imaging contrast conditions. The top row displays trials in which the probe appears on the opposite side of the threatening expression (threat probe incongruent); the bottom row displays trials in which the probe appears on the same side of the threatening expression (threat probe congruent). B, Attention bias to threat as a function of *FKBP5* genotype: TC/TT genotypes demonstrate attention bias toward threat, compared with CC genotype. Chart illustrates mean attention bias score (error bars indicate standard error of the mean), for threat faces (both races, combined) and separated by African American (AA) and white (W) race type, as a function of genotype group. * $P < .03$.

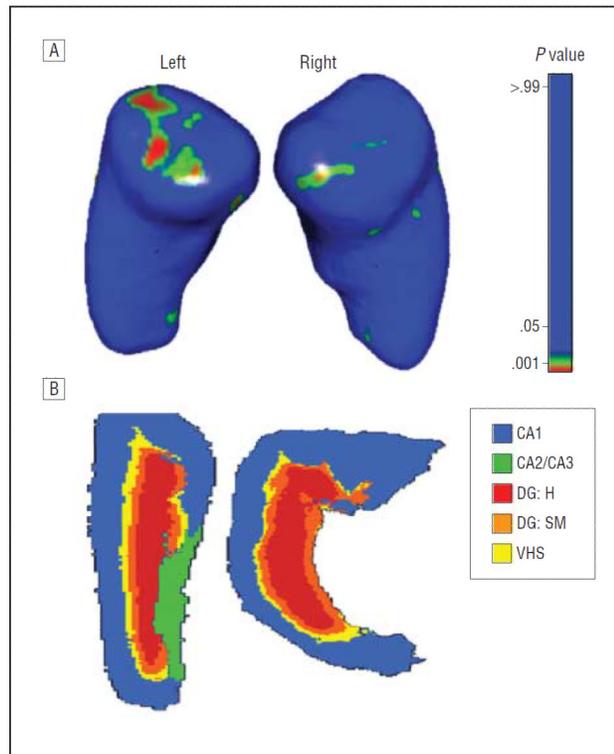


Figure 2.

FKBP5 polymorphism is associated with differences in hippocampal shape. A, Local shape analyses of the left and right hippocampus. Smaller *P* values (false-discovery rate corrected) reflect greater spatial displacement for TC/TT vs CC genotypes. B, Cross-sectional views of the 3-dimensional hippocampal atlas⁵⁶ used for reference. CA indicates cornu ammonis; DG, dentate gyrus; H, hilum; SM, stratum moleculare; and VHS, vestigial hippocampal sulcus.

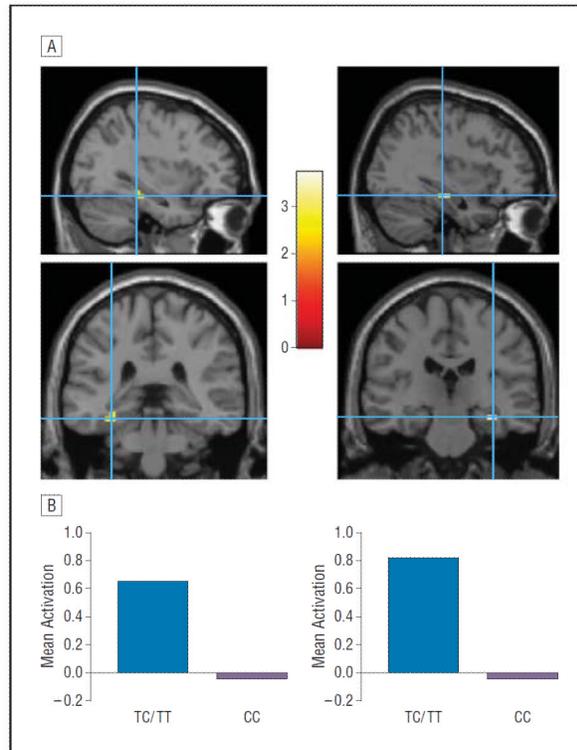


Figure 3. *FKBP5* polymorphism is associated with differential hippocampal activation during attention to threat. A, Statistical parametric maps of left and right hippocampus activation during the processing of threat probe-incongruent vs threat probe-congruent faces in TC/TT>CC genotype. Activations are shown overlaid onto a canonical T1 magnetic resonance image. The colored bar represents t scores for activations. Maximally activated voxels from the left parahippocampal gyrus ($x, y, z: -36, -35, -8$) and right hippocampus ($x, y, z: 36, -24, -12$), $P < .05$ (small-volume correction, family-wise error). Data are reported using the coordinate system of Talairach and Tournoux. B, Genotype differences in averaged blood oxygen level–dependent signal (contrast time series extracted from 6-mm spherical regions of interest) to this contrast condition.