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Multimodal Associations of *FKBP5* Methylation With Emotion-Regulatory Brain Circuits

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ABSTRACT

BACKGROUND: Understanding the biological processes that underlie individual differences in emotion regulation and stress responsivity is a key challenge for translational neuroscience. The gene *FKBP5* is a core regulator in molecular stress signaling that is implicated in the development of psychiatric disorders. However, it remains unclear how *FKBP5* DNA methylation in peripheral blood is related to individual differences in measures of neural structure and function and their relevance to daily-life stress responsivity.

METHODS: Here, we characterized multimodal correlates of *FKBP5* DNA methylation by combining epigenetic data with neuroimaging and ambulatory assessment in a sample of 395 healthy individuals.

RESULTS: First, we showed that *FKBP5* demethylation as a psychiatric risk factor was related to an anxiety-associated reduction of gray matter volume in the ventromedial prefrontal cortex, a brain area that is involved in emotion regulation and mental health risk and resilience. This effect of epigenetic upregulation of *FKBP5* on neuronal structure is more pronounced where *FKBP5* is epigenetically downregulated at baseline. Leveraging 208 functional magnetic resonance imaging scans during a well-established emotion-processing task, we found that *FKBP5* DNA methylation in peripheral blood was associated with functional differences in prefrontal-limbic circuits that modulate affective responsivity to daily stressors, which we measured using ecological momentary assessment in daily life.

CONCLUSIONS: Overall, we demonstrated how *FKBP5* contributes to interindividual differences in neural and real-life affect regulation via structural and functional changes in prefrontal-limbic brain circuits.

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Prefrontal-limbic brain circuits are crucial for human emotion processing and maintaining mental health (1). Emotion regulation requires prefrontal control over limbic structures, such as the amygdala and the hypothalamus, that feed into the endocrine, autonomic, metabolic, and immunological pathways that mediate coordinated responses to stressful stimuli (2). Within this circuitry, perigenual/ventral parts of the anterior cingulate cortex (vACC) and the ventromedial prefrontal cortex (vmPFC) are altered in stress-related disorders (3) and are central to emotion regulation and stress processing (4), which are major categories of transdiagnostic psychiatric risk prioritized here. Converging evidence suggests that gene-environment interactions, in particular stress exposure during neural development in childhood and early adolescence in genetically vulnerable individuals, influences vmPFC structure and function by exaggerated hypothalamic-pituitary-adrenal (HPA) axis activation and glucocorticoid exposure (2,5–8).

FKBP5 encodes for the Hsp90-associated cochaperone FK506 binding protein 51 (FKBP51) (9), which is a core regulator of molecular stress signaling and is strongly induced by glucocorticoid-dependent mechanisms during acute stress (10–12). This serves to downregulate and limit acute stress responses (10,12). Through an inhibitory feedback loop with the glucocorticoid receptor, FKBP51 modulates HPA axis activity by reducing ligand binding (13), reducing nuclear translocation (14), and decoupling glucocorticoid-responsive transcripts from glucocorticoid exposure (15,16). Early-life stress seems to influence this function of *FKBP5* via glucocorticoid-dependent DNA demethylation (15–17). Genetic and epigenetic variation in *FKBP5* have been repeatedly associated with the development of stress-related psychiatric disorders such as depression, anxiety disorders, and post-traumatic stress disorder (18–23). Translational research using animal models has associated *FKBP5* overexpression with elevated anxiety (24,25) and impaired stress responsivity (10,12,26). Conversely, pharmacological inhibition of *FKBP5* has anxiolytic properties and facilitates stress coping (25,27). Therefore, *FKBP5* seems to be a key regulator of endocrine,

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neural, and affective facets of stress processing, which are crucial for mental health (18).

We studied 2 specific CpG sites (cg00130530 and cg20813374) that are available on the Illumina Infinium MethylationEPIC bead chip and are promising epigenetic loci in the context of mental health risk. The CpG sites are located in the promoter region close to the transcription starting site (−462 bp for cg20813364 and −484 bp for cg00130530) of *FKBP5* (15) and are flanking a nuclear factor-κB (NF-κB) binding site and colocate with H3K4me1 and H3K27me3 signatures in a likely poised enhancer (15). Demethylation at these loci is associated with depressive symptomatology (15) and multiple psychiatric risk factors including early-life stress (15,28), aging (15), accelerated DNA methylation (DNAm) aging (28), chronic low-grade inflammation (29), and cardiometabolic risk (15,28). DNAm at cg00130530 and cg20813374 epigenetically regulates *FKBP5* expression in the canonical direction of upregulation with demethylation (15) and modifies glucocorticoid-dependent induction of *FKBP5* expression (15). A series of in vivo and in vitro studies has shown that cg00130530 and cg20813374 functionally interlink glucocorticoid-dependent and inflammatory pathways, indicating a particular relevance of this specific epigenetic locus in explaining *FKBP5*-dependent effects in stress-related psychiatric disorders (15).

Current knowledge of the effects of *FKBP5* DNAm on neural and real-life affective processing in humans is incomplete. Existing evidence suggests associations between *FKBP5* DNAm and prefrontal gray matter volume (30,31), but adequately powered studies of healthy individuals that exclude disease-typical confounding factors and have a broad multimodal data base are lacking.

To fill this gap, here we studied the neural and real-life behavioral correlates of *FKBP5* DNAm in a sample of 395 healthy individuals. First, using structural magnetic resonance imaging (MRI), we identified brain structural correlates of *FKBP5* DNAm in peripheral blood and related them to trait anxiety. Second, using public data from the Allen Human Brain Atlas, we explored how these identified brain regions were related to *FKBP5* expression. Third, we linked *FKBP5* DNAm in peripheral blood to functional changes in the prefrontal-limbic circuitry during affective processing. Finally, using ambulatory assessment methods, we showed how *FKBP5*-associated brain responses were related to stress responsivity in daily life. Taken together, our results suggest that the vmPFC contributes to the association between *FKBP5* methylation and mental health risk and protection.

METHODS AND MATERIALS

Sample

Our sample of 395 participants (194 women, mean age ± SD = 28.12 ± 10.39 years) consists of 2 cohorts (for details, see the Supplement). Exclusion criteria were a history of psychiatric or neurological illness including alcohol or substance use disorder, intake of psychopharmacological medication, contraindications for MRI, and non-Caucasian ethnicity to avoid population-stratification artifacts.

Preprocessing of DNAm Data

Using Illumina Infinium MethylationEPIC bead chips, we obtained DNAm profiles from whole-blood samples. As described in detail by Chen *et al.* (32), the data were preprocessed using the *minfi* Bioconductor package (33) in R and corrected for potential confounders, which comprised age (34), sex, cigarette smoking (35,36), and cellular composition (34,37), and the autosomal methylome derived the first 10 principal components (38) by residualizing each given DNAm probe using the specified covariates in a general linear model. The resulting residuals were used for downstream analyses. Here, we chose to study 2 specific CpG sites (cg20813374 and cg00130530) that are located in the promoter region and in close proximity to the transcription starting site (−462 bp for cg20813364 and −484 bp for cg00130530) of *FKBP5* (chr.6p21.31) (15). The use of the Illumina Infinium MethylationEPIC bead chip at these 2 CpG sites has previously been validated by Zannas *et al.* using targeted bisulfite sequencing with the Illumina MiSeq System (15). Following Zannas *et al.* (15), we averaged the residualized DNAm values of these 2 highly intercorrelated CpG sites of interest (cg00130530 and cg20813374; Pearson's $R = 0.47$, $p < .001$) for our analyses (hereafter *FKBP5* DNAm).

Structural MRI

Data Acquisition and Processing. All participants underwent T1–magnetization-prepared rapid acquisition gradient-echo structural MRI scans on one of the 2 identical 3T Siemens Trio scanners (for details, see the Supplement). The structural data were preprocessed using CAT12 in SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/), including segmentation, spatial normalization using default parameters, and smoothing with an 8-mm full width at half maximum Gaussian kernel (39).

Definition of Regions of Interest. We defined regions of interest (ROIs) using the HCP-MMP 1.0 (Human Connectome Project's multimodal parcellation 1.0) atlas (40) for cortical and the Harvard-Oxford Atlas (41) for subcortical structures. Our selection of ROIs was guided by 3 key criteria. Firstly, we considered functional relevance in emotion regulation (1,4), emphasizing its pivotal role in the major transdiagnostic category of psychiatric risk under examination. Secondly, we prioritized a strong association with stress-related psychiatric disorders, including depression and posttraumatic stress disorder (3,42). Lastly, we focused on responsiveness to environmental adversities (5,43), consistent with the theoretical foundation of our study. These criteria collectively led us to target specific brain regions, namely the amygdala, hippocampus, and the vmPFC and vACC. Thus, we specified an ROI mask comprising the amygdala and hippocampus (40,41), which are subcortical structures involved in emotion processing, and the vmPFC and vACC, which are cortical areas involved in emotion regulation.

Voxel-Based Morphometry. Using voxel-based morphometry (VBM) in SPM12 (44), second-level regression analysis on gray matter volume was performed defining *FKBP5* DNAm as the predictor of interest and age, sex, total intracranial volume, and cohort as covariates of no interest and corrected for multiple comparisons using the familywise error rate within each ROI. Additionally, we tested for a change in this effect over age by defining the interaction term of *FKBP5*

DNAm and age as the predictor of interest. For subsequent analyses, we extracted the first eigenvariate of the *FKBP5*-related vmPFC voxel cluster (2503 voxel) including the peak voxel (at x = 6, y = 62, z = −15 in Montreal Neurological Institute [MNI] space) thresholded at $p = .01$ (hereafter vmPFC gray matter volume).

Allen Human Brain Atlas Data

Preprocessing. Whole-brain gene expression data were downloaded from https://human.brain-map.org/ and comprise brainwide transcriptomic microarray data of 6 neurotypical postmortem brains. Preprocessing of gene expression data followed Arnatkevičiūtė *et al.* (45) using HCP-MMP 1.0 (40) (for details, see the Supplement). Parcelwise expression values were mapped onto the HCP-MMP 1.0 (40) and retrieved for downstream analyses.

Statistical Analysis. An association between brainwide *FKBP5* expression and a parcellated *T* map from the VBM analysis between *FBKP5* DNAm and gray matter volume was tested using Spearman correlation. As an index of the correlational structure between gray matter volume and *FKBP5* DNAm, we derived the unthresholded *T* map from the VBM analysis described above and mapped the *T* values onto the HCP-MMP 1.0 (40). Following established recommendations for brain map comparisons, we accounted for spatial autocorrelation and gene nonspecificity (46) (for details, see the Supplement).

Functional MRI

Data Acquisition and Processing. Blood oxygen level–dependent functional MRI was performed on a 3T Siemens Trio scanner (for details, see the Supplement). Fourteen participants were not included in our functional analyses due to excessive head motion (for details, see the Supplement). As described previously (47), functional MRI data were processed and analyzed in SPM12 (http://www.fil.ion.ucl.ac.uk/spm) using standard procedures (for details, see the Supplement).

Psychophysiological Interaction. Voxelwise estimates of task-specific vmPFC connectivity for each participant were quantified by calculating a psychophysiological interaction (48) during an emotional face-matching task (for details, see the Supplement) (49). Because the functional connectivity analysis was informed by the outcome of the initial VBM analysis, we extracted the vmPFC time series, defined as a 6-mm sphere around the peak voxel of our previous analysis (at x = 6, y = 62, z = −15 in MNI space) (50). By including the interaction term of extracted vmPFC time series and task conditions in a general linear model, we computed voxelwise task-dependent vmPFC connectivity maps, corrected for artifacts by including 24 head motion parameters (51), white matter signal, and cerebrospinal fluid signal as regressors of no interest. For group inference, the contrast images (faces > forms) were entered into a linear regression analysis in which we defined *FKBP5* DNAm as predictor of interest and included age, sex, total intracranial volume, and vmPFC gray matter volume as covariates of no interest. For subsequent analyses, we extracted the first eigenvariate of the *FKBP5*-related vmPFC-amygdala coupling voxel cluster (17 voxel) including the peak voxel (at x = 15, y = −7, z = −16 in MNI space) thresholded at $p = .01$ (hereafter vmPFC-amygdala coupling).

Ambulatory Assessment

E-Diary Sampling. As has been done in previously published work by our group (47,52,53), 64 participants carried a smartphone (Motorola Moto G, Motorola Mobility) for 7 consecutive days in daily life before completing the emotion-processing functional MRI paradigm. On each study day, e-diary assessments were prompted between 7:30 AM and 10:30 PM with a minimum interval of 40 minutes and a maximum interval of 100 minutes, resulting in an average of 12.02 at 9 to 23 possible e-diary prompts per day. For the implementation of the e-diaries and sampling strategy, we used the ambulatory assessment software movisensXS, version 0.6.3658 (movisens GmbH, https://xs.movisens.com).

Multilevel Analysis. To investigate whether *FKBP5*-related neural connectivity was associated with daily-life stress responsivity, we conducted a multilevel analysis using SAS software (SAS 9.4; SAS Institute). Our model included our predictor of interest (that is, the interaction term of vmPFC-amygdala coupling with negative event intensity), the corresponding main effects, as well as time of day, time of day squared, and age and sex as covariates of no interest (for details, see the Supplement).

RESULTS

FKBP5 DNAm Is Associated With Anxiety-Related vmPFC Structure

Both *FKBP5* DNAm (16) and morphometry in emotion-regulatory brain circuits seem to be responsive to environmental influences such as early-life stress (18,43) and are plausible intermediates in the association of environmental influence and human well-being. Hypothesizing an association between *FKBP5* DNAm and gray matter volume in these brain regions, we showed that *FKBP5* DNAm was related to brain structural variance in the vmPFC (peak voxel at x = 6, y = 62, z = −15 in MNI space; $T = 3.99$, familywise error–corrected p [p_{FWE}] = .039, corrected in ROI) (Figure 1A, B), i.e., *FKBP5* hypermethylation was associated with higher gray matter volume. No voxel outside the predefined mask was significantly correlated with *FKBP5* DNAm. This effect did not change over age when the interaction term of age and *FKBP5* DNAm was defined as predictor of interest, both in imaging space (all $p_{FWE} > .8$ in ROI) and when testing the vmPFC's extracted eigenvariate directly ($p > .9$). Post hoc, we analyzed both cohorts separately using the same multiple regression model described above and found that this effect of peripheral *FKBP5* DNAm on vmPFC structure replicated in both cohorts (cohort A: peak voxel at x = 8, y = 56, z = 20 in MNI space; $T = 4.52$, $p_{FWE} = .046$, whole-brain corrected, or $p_{FWE} = .004$, corrected in ROI; cohort B: peak voxel at x = 4, y = 62, z = −15 in MNI space; $T = 3.95$, $p_{FWE} = .049$, corrected in ROI) (for details, see the Supplement). In the next step, we extracted the eigenvariate of the *FKBP5*-related vmPFC voxel cluster (2503 voxel) including the peak voxel (at x = 6, y = 62, z = −15 in MNI space) thresholded at $p = .01$ (hereafter vmPFC gray matter

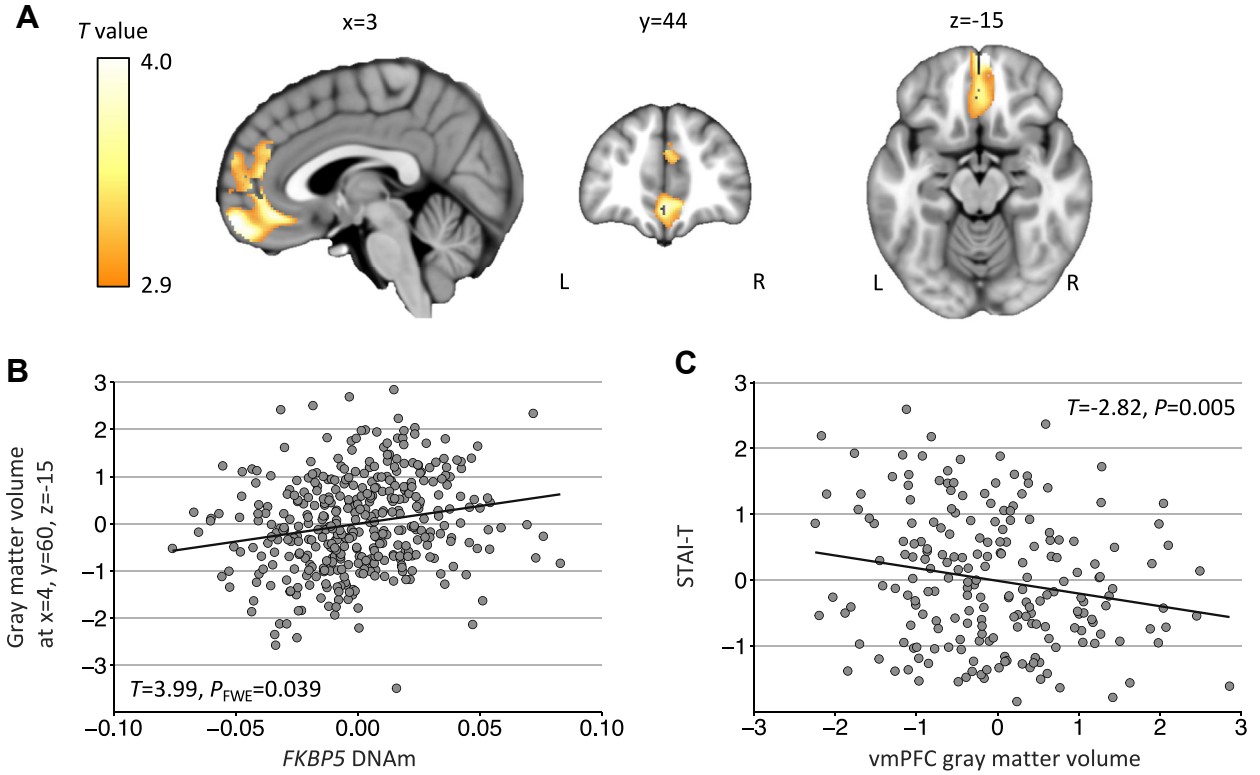

Figure 1. *FKBP5* DNA methylation (DNAm) is associated with anxiety-related ventromedial prefrontal cortex (vmPFC) gray matter volume. **(A)** *T* map showing gray matter volume associated with *FKBP5* DNAm (cg00130530 and cg20813374) thresholded at $p < .01$, uncorrected, for presentation purposes (peak voxel at x = 6, y = 62, z = −15 in Montreal Neurological Institute space; $T = 3.99$, familywise error–corrected p [p_{FWE}] = .039, corrected in region of interest). **(B)** Scatterplot showing the association between residualized *FKBP5* DNAm and peak-voxel vmPFC gray matter volume. Statistical analysis details are the same as for **(A)**. **(C)** Scatterplot showing the association between residualized vmPFC gray matter volume and State-Trait Anxiety Inventory–Trait (STAI-T) sum scores ($T = -2.82$, $p = .005$). L, left; R, right.

volume). In 204 of the 395 participants who completed the State-Trait Anxiety Inventory–Trait (for details, see the Supplement) (54), we tested whether the extracted vmPFC gray matter volume was related to trait anxiety, which is highly prevalent in patients with stress-related psychiatric disorders and in individuals at risk for developing an affective disorder (55,56). Using linear regression on State-Trait Anxiety Inventory–Trait scores, we defined vmPFC gray matter volume

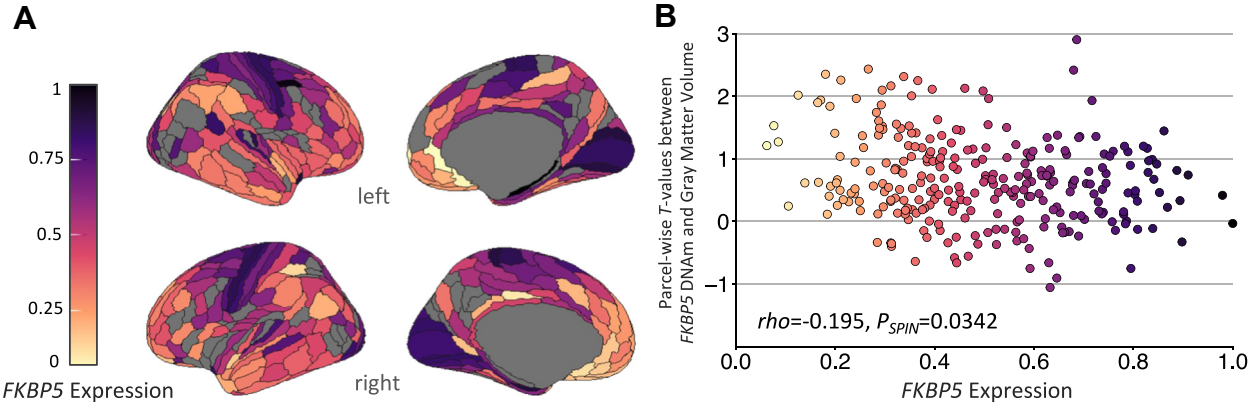

Figure 2. Brainwide distribution of *FKBP5* expression co-locates with *FKBP5*-related brain structural variance. **(A)** *FKBP5* expression from the Allen Human Brain Atlas, averaged within each cortical parcel, mapped onto the HCP-MMP 1.0 (Human Connectome Project's multimodal parcellation 1.0) (40). **(B)** Scatterplot of the association between parcelwise *FKBP5* expression and parcelwise *T* values reflecting the relationship between *FKBP5* DNA methylation (DNAm) (cg00130530 and cg20813374) and brain structure (rho = −0.195, p_{SPIN} = .0342 after spatial permutation testing).

as the predictor of interest and age, sex, and total intracranial volume as covariates of no interest. We found a negative association showing that lower *FKBP5*-related vmPFC structure was related to elevated trait anxiety ($T = -2.82$, $p = .005$) (Figure 1C). Trait anxiety was not significantly correlated with *FKBP5* DNAm in peripheral blood ($T = -0.32$, $p = .75$). Overall, these findings suggest that *FKBP5* demethylation as a psychiatric risk factor is related to an anxiety-associated reduction of gray matter volume in the vmPFC, a brain area that is crucial in emotion regulation and mental health risk and resilience (1,4).

Brainwide *FKBP5* Expression Coincides With *FKBP5*-Related Morphometric Variance

Above, we demonstrated an association between peripheral *FKBP5* DNAm and the vmPFC, a brain region strongly involved in neuroendocrine signaling (1). A plausible explanation for this highly localized effect of peripheral *FKBP5* DNAm on neural structure in the vmPFC could be a distinct pattern of *FKBP5* expression that renders this brain area prone to these epigenetic changes. In mice, regions with low *FKBP5* expression are more sensitive to dynamic changes in the epigenetic regulation of *FKBP5* under stress (11,57). Accordingly, we hypothesized that the transcriptional pattern of *FKBP5* (Figure 2A) contributes to the region-dependent sensitivity of this brain structure to changes in peripheral *FKBP5* DNAm (Figure 1A). We found a negative Spearman correlation between parcelwise *T* values and *FKBP5* expression (rho = −0.195, $p = .001$) (Figure 2B), with the vmPFC (e.g., parcel r10v that includes the peak voxel at x = 6, y = 62, z = −15 in MNI space) showing a particularly high *T* value of 2.43 (ranking at 359 of 360 parcels, which is the 100th percentile) and low *FKBP5* expression of 0.26 (ranking at 248 of 281 parcels, which is the 88th percentile). In accordance with recent recommendations (46), we performed spatial permutation testing with 10,000 spherical rotations of both brain maps, i.e., 1) peripheral *FKBP5* DNAm correlated brain volume and 2) *FKBP5* expression to mitigate the potential influence of spatial autocorrelation (58). The result remained significant after performing spatial permutation testing ($p_{SPIN} = .034$) (for details, see the Supplement) and turned out to be relatively specific to *FKBP5* because the resulting statistic (rho = −0.195) exceeds 98.15% of comparable analyses with over 10,000 other gene maps (9842 of 10,072) (for details, see the Supplement). This suggests that epigenetic upregulation of *FKBP5* may have a more pronounced effect on neuronal structures where *FKBP5* is epigenetically downregulated at baseline, particularly in the vmPFC.

FKBP5 DNAm in Peripheral Blood Is Related to Emotion-Regulatory Brain Function

Previous findings suggest that higher-order vmPFC and vACC facilitate amygdala reactivity and thereby affective responsivity to environmental stimuli, particularly under stress (4,59,60). Subsequent to early-life stress, this prefrontal-limbic interplay seems to be enhanced in individuals suffering from stress-related psychiatric disorders (61,62). Investigating effects of *FKBP5* on this emotion-regulatory prefrontal-limbic brain circuitry (59,60,63), we found that *FKBP5* DNAm in peripheral blood was negatively correlated with the connectivity between the vmPFC and the right amygdala (peak voxel at x = 15, y = −7, z = −16 in MNI space; $T = -3.49$, $p_{FWE} = .015$, corrected for bilateral amygdala) (Figure 3A, B). Lower *FKBP5* DNAm was associated with stronger vmPFC-amygdala coupling, while weaker coupling was associated with higher *FKBP5* DNAm in peripheral blood. This effect was statistically independent of vmPFC brain structure because when we included vmPFC gray matter volume as the covariate of no interest, there was no correlation between vmPFC connectivity and structure ($T = -0.01$, $p = .99$), suggesting an independent modulation of vmPFC connectivity by FKBP5 DNAm. Taken together, these findings indicate that *FKBP5* demethylation is related to enhanced prefrontal-limbic coupling during affective processing.

FKBP5-Related vmPFC-Amygdala Coupling Alters Real-Life Stress Responsivity

To examine effects of *FKBP5*-related neural function on stress responsivity in daily life, we conducted smartphone-based ecological momentary assessments. Within participants, higher intensities of negative events were robustly related to negative affect reflecting stress responsivity as observed in previous studies (64). *FKBP5*-related emotion-regulatory vmPFC-amygdala coupling significantly moderated the influence of negative events on negative affect ($T = -2.21$, $p = .027$) (Figure 3C) in the absence of a significant association between vmPFC-amygdala coupling and negative affect ($T = -0.69$, $p = .49$). Individuals with lower vmPFC-amygdala coupling were more sensitive to stress than participants with high vmPFC-amygdala coupling (Figure 3C). Residualizing vmPFC-amygdala coupling for *FKBP5* DNAm attenuated this result ($T = -0.85$, $p = .40$), suggesting that the *FKBP5*-related coupling dimension is essential for this effect of brain connectivity on stress responsivity. Prefrontal gray matter volume did not significantly moderate the effect of negative events on affect in our sample ($T = 0.24$, $p = .81$). Therefore, our findings suggest that *FKBP5*-related vmPFC-amygdala coupling is associated with stress responsivity in daily life, a key component of transdiagnostic psychiatric risk.

DISCUSSION

We examined multimodal associations of *FKBP5*, a central regulator of molecular stress signaling that is associated with stress-related psychiatric disorders (18,65). By combining epigenetic data on *FKBP5* DNAm (at cg00130530 and cg20813374) with structural and functional neuroimaging and ambulatory assessment in a large sample of 395 healthy individuals, we demonstrated that *FKBP5* was related to structural, functional, and transcriptional patterns in a prefrontal-limbic circuitry that was associated with trait anxiety and real-life stress responsivity. Our findings suggest the critical importance of *FKBP5*-related neural mechanisms for real-life stress processing as well as mental health risk and resilience.

First, we demonstrated that *FKBP5* DNAm in peripheral blood was associated with anxiety-related morphometric variance in the vmPFC, a pivotal brain region in neural stress processing. *FKBP5* upregulation, lower vmPFC volume, and elevated trait anxiety are transdiagnostic correlates of stress-related mental disorders (3,18,43) that can be found in

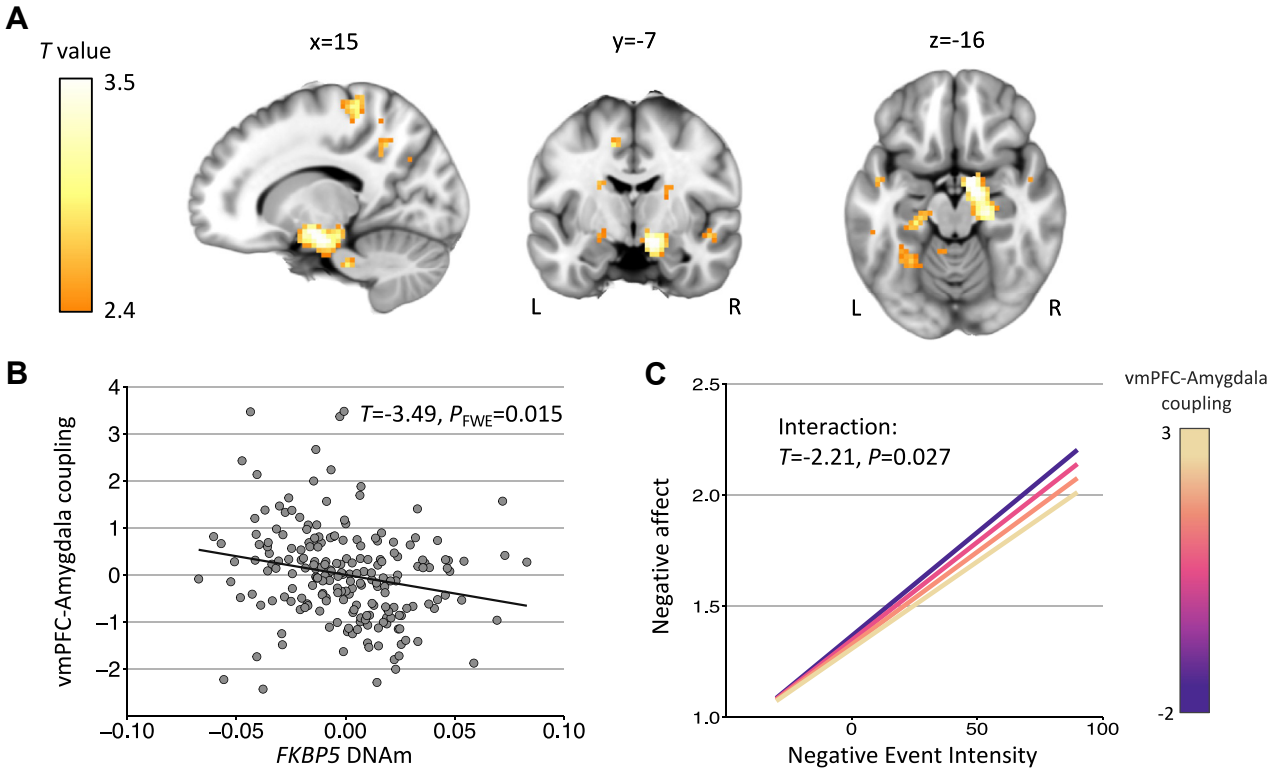

Figure 3. Emotion-regulatory ventromedial prefrontal cortex (vmPFC)–amygdala coupling is related to *FKBP5* DNA methylation (DNAm). **(A)** *T* map showing vmPFC-amygdala coupling associated with *FKBP5* DNAm (cg00130530 and cg20813374) thresholded at $p < .01$, uncorrected, for presentation purposes (peak voxel at x = 15, y = −7, z = −16 in Montreal Neurological Institute space; $T = -3.49$, familywise error–corrected p [p_{FWE}] = .015, corrected for bilateral amygdala). **(B)** Scatterplot showing the association between residualized *FKBP5* DNAm and peak-voxel vmPFC-amygdala coupling. Statistical analysis details are the same as for **(A)**. **(C)** vmPFC-amygdala coupling moderates the association between negative event intensity (person mean centered) and negative affect (interaction: $T = -2.21$, $p = .027$). A sample split into 4 equally sized groups based on their respective vmPFC-amygdala coupling was done for presentation purposes. L, left; R, right.

individuals who experienced excessive stress during their childhood (6,55,56). Via inhibition of intracellular glucocorticoid receptors, *FKBP5* prolongs HPA axis activity (16) and (dys) regulates glucocorticoid signaling, which is of importance in stress-induced dendritic remodeling of prefrontal neural structure (66,67). Considering this, *FKBP5*-associated morphometric changes in the vmPFC may result from aggravated neurostructural effects of glucocorticoids on the dendritic architecture of pyramidal neurons in the vmPFC. In vitro pharmacological experiments have shown that *FKBP5* inhibition leads to elongated neurite growth in neuronal tissue cultures (68), which could be a microscopic and reversible correlate of the macroscopic structural changes that we observed here. Furthermore, *FKBP5* mediates a bidirectional link between glucocorticoid-dependent and nuclear factor-κB (NF-κB)-dependent pathways (15). This provides a second mechanism contributing to *FKBP5*-related neurostructural variance because NF-κB regulates neural process growth and mediates stress-related effects on neuronal plasticity (69,70). In addition, NF-κB serves as an immunological master regulator in inflammatory pathways (69,70) that are frequently seen to be dysregulated in psychiatric patients with a history of adverse childhood experiences (71,72). It is tempting to speculate that *FKBP5*-related immune processes contribute to the *FKBP5*-related morphometric variability that we observed in the current study. Together, neurostructural effects of *FKBP5* in the vmPFC may explain why *FKBP5* inhibition in animal experiments has anxiolytic properties and supports stress coping (27,73), while *FKBP5* overexpression mediates the association between stress and anxiety-like behavior (25).

Second, we report the co-location of neurostructural correlates of peripheral *FKBP5* DNAm with the spatial distribution of *FKBP5* expression. Brain regions where gray matter volume is sensitive to changes in peripheral *FKBP5* DNAm are characterized by low *FKBP5* expression. This is consistent with previous animal studies that have shown that low expression of *FKBP5* renders surrounding neural structures more sensitive to changes in the epigenetic regulation of *FKBP5* (11,57). By modulating HPA axis signaling, low *FKBP5* expression enables tight neuroendocrine coupling of glucocorticoid-dependent pathways in brain regions that are pivotal to neural stress processing (11,57). This strong neuroendocrine coupling also implies a more pronounced glucocorticoid-dependent structural sensitivity to epigenetic upregulation of *FKBP5*, which may plausibly be related to the region-specific structural changes that we identified in the vmPFC.

Third, we showed that *FKBP5* DNAm in peripheral blood was related to functional changes in emotion-regulatory brain

circuits (4,59) that are implicated in the etiology of stress-related psychiatric disorders (1,61,62). During emotion processing, individuals with low *FKBP5* DNAm show increased emotion-regulatory vmPFC-amygdala coupling. *FKBP5* upregulation shifts the excitation-inhibition balance through elevated expression of excitatory glutamatergic receptors and reduced levels of inhibitory GABA (gamma-aminobutyric acid) (74,75) in electrophysiological experiments, particularly in prefrontal pyramidal neurons where stress-related upregulation of *FKBP5* is most pronounced (65). Low inhibition and overexcitation of prefrontal pyramidal neurons could explain increased engagement of prefrontal-amygdala projections (62), which we observed here as elevated vmPFC-amygdala coupling. This effect of *FKBP5* on prefrontal-limbic brain function, which is crucial for orchestrating neural affective regulation (4,59), may contribute to *FKBP5*-related individual differences in affective processing and responsivity to stress. The observed functional connectivity effect was not explained by the brain structural correlates of *FKBP5* DNAm in the vmPFC. While structure-function relationships likely exist in the vmPFC (76), this statistical independence suggests that the reported brain functional correlation of peripheral *FKBP5* DNAm is not merely a reflection of the reported structural association.

Finally, we demonstrated the relevance of *FKBP5*-related neural connectivity for real-life affective processing because emotion-regulatory vmPFC-amygdala coupling is related to daily-life stress responsivity in our sample. Specifically, elevated vmPFC-amygdala coupling blunts the effect of negative events on negative affect without being associated with baseline measures of affect itself. At first, it may seem contradictory that elevated vmPFC-amygdala coupling, which we have shown to be associated with epigenetic risk (*FKBP5* demethylation), is related to a certain robustness to minor daily-life stressors in our sample of healthy individuals. However, this result is consistent with previous findings showing that prefrontal control over amygdala activity reduces stress responsivity in animals and humans (59,61). In principle, biological traces of early-life stress may serve a protective function against stress in healthy adults (77). This adaptive mechanism for environmental adversity has been suggested before regarding *FKBP5* demethylation and elevated vmPFC-amygdala coupling (18,61). In summary, increased prefrontal control over amygdala activity in individuals with stress-induced *FKBP5* demethylation may serve as an adaptive mechanism in response to childhood stress that conditions an altered stress response in adult life (61) and contributes to the effect of *FKBP5* on stress responsivity (18).

The content of this study is subject to several limitations. First, we studied 2 specific CpG sites located in the promoter of *FKBP5* (15) that do not represent the full epigenetic regulation or methylation of *FKBP5*. Second, in this study we did not assess data on epigenetic modifications of *FKBP5* other than DNAm, such as hydroxymethylation or histone modifications, that show rich interactive mechanisms and potentially entail crucial and distinct epigenetic information relevant to psychiatric research. One recent study of prefrontal cortical tissue of teenage suicide completers assessed both DNAm and hydroxymethylation in the *FKBP5* promoter region and found significant changes in both—decreased DNAm and increased hydroxymethylation (78). In many cell types, the specific CpG sites that were studied here colocalize with H3K4me1 and H3K27me3 signatures (15) that may also have impacted the findings of our study. Future studies need to harvest the tools of modern computational biology to do justice to the rich complexity of epigenetics. Third, the main findings of the current study are based on correlational analyses of *FKBP5* methylation without experimental manipulation of *FKBP5*. Therefore, any causal inferences should be treated with caution. Fourth, we did not include any patients in our study, and it is unclear to what extent findings in healthy individuals can be extrapolated to patient populations. However, we did implicitly apply a dimensional framework examining major categories of transdiagnostic psychiatric risk—emotion regulation and stress responsivity.

Conclusions

In summary, we characterized multimodal correlates of *FKBP5* DNAm in this article by combining epigenetic data on *FKBP5* DNAm in peripheral blood with neuroimaging and ecological momentary assessment. Our findings demonstrate how *FKBP5* is related to structural and functional changes in prefrontal-limbic brain circuits and real-life stress responsivity and how *FKBP5* thereby contributes to interindividual differences in neural and real-life affect regulation.

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TLK, UB, and HT had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. TLK, AM-L, UB, and HT were responsible for concept and design. All authors were responsible for acquisition, analysis, and interpretation of data. TLK, UB, and HT were responsible for drafting the manuscript. All authors were responsible for critical revision of the manuscript for important intellectual content. TLK was responsible for statistical analysis. AM-L and HT obtained funding. JC, AB, RM, JIS, LSG, ZZ, FS, SHW, MR, IR, MMN, MR, UWE-P, ES, and AM-L provided administrative, technical, or material support. AM-L, UB, and HT were responsible for supervision.

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Data supporting the findings of this study are available upon reasonable request from the corresponding author. Data sharing is subject to General Data Protection Regulation restrictions. Raw data containing sensible information that can be used to identify individuals, such a whole-genome data, cannot be shared under current data protection laws.

Citation Diversity Statement: Recent work in several fields of science has identified a bias in citation practices such that papers from women and other minorities are undercited relative to the number of such papers in the field (79). Here we sought to proactively consider choosing references that reflect

the diversity of the field in thought, form of contribution, gender, and other factors. We obtained predicted gender of the first and last author of each reference by using databases that store the probability of a name being carried by a woman (79). By this measure (and excluding self-citations to the first and last authors of our current paper), our references contain 9.4% woman(first)/woman(last), 15.1% man/woman, 20.8% woman/man, 50.9% man/man, and 0.4% unknown categorization. This method is limited in that 1) names, pronouns, and social media profiles used to construct the databases may not be indicative of gender identity in every case, and 2) it cannot account for intersex, nonbinary, or transgender people. We look forward to future work that could help us better understand how to support equitable practices in science.

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