Effect of the Interaction Between Childhood Abuse and rs1360780 of the *FKBP5* Gene on Gray Matter Volume in a General Population Sample

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Abstract: *Objective:* The *FKBP5* gene codes for a co-chaperone that regulates glucocorticoid receptor sensitivity and thereby impacts the reactivity of the hypothalamic–pituitary–adrenal (HPA)-axis. Evidence suggested that subjects exposed to childhood abuse and carrying the TT genotype of the *FKBP5* gene single nucleotide polymorphism (SNP) rs1360780 have an increased susceptibility to stress-related disorders.

Method: The hypothesis that abused TT genotype carriers show changes in gray matter (GM) volumes in affect-processing brain areas was investigated. About 1,826 Caucasian subjects (age ≤ 65 years) from the general population [Study of Health in Pomerania (SHIP)] in Germany were investigated. The interaction between rs1360780 and child abuse (Childhood Trauma Questionnaire) and its effect on GM were analyzed. *Results:* Voxel-based whole-brain interaction analysis revealed three large clusters (FWE-corrected) of reduced GM volumes comprising the bilateral insula, the superior and middle temporal gyrus, the bilateral hippocampus, the right amygdala, and the bilateral anterior cingulate cortex in abused TT carriers. These results were not confounded by major depressive disorders. In region of interest analyses, highly significant volume reductions in the right hippocampus/parahippocampus, the bilateral anterior and middle cingulate cortex, the insula, and the amygdala were confirmed in abused TT carriers compared with abused CT/CC carriers. *Conclusion:* The results supported the hypothesis that the *FKBP5* rs1360780 TT genotype predisposes subjects who have experienced childhood abuse to widespread structural brain changes in the subcortical and cortical emotion-processing brain areas. Those brain changes might contribute to an increased vulnerability of stress-related disorders in TT genotype carriers. *Hum Brain Mapp* 37:1602–1613, 2016. © 2016 Wiley Periodicals, Inc.

Key words: major depression disorder; stress-related disorders; childhood abuse; general population; *FKBP5* gene; gene–environment interaction; CTQ; MRI; SPM8; VBM8

INTRODUCTION

A dysfunction of the hypothalamic–pituitary–adrenal (HPA)-axis has been suggested to represent an important pathogenic factor in depressive disorders [Heim and Nemeroff, 2001; Heim et al., 1997; Holsboer, 2000]. There is accumulating evidence that childhood trauma is associated with sensitization of the neuroendocrine stress response, glucocorticoid resistance, increased central corticotropin-releasing factor (CRF) activity and reduced hippocampal volume [Heim et al., 2008]. The biological predisposition toward a dysregulated HPA-axis has been addressed in recent studies focusing on the role of genes involved in the regulation of the HPA-axis as potentially underlying the HPA dysregulation in depression, for example, genes coding for corticotrophin-releasing hormone receptor 1 [Grabe et al., 2010; Tyrka et al., 2009] and for *FKBP5* [Binder, 2009; Binder et al., 2004, 2008].

The *FKBP5* gene is a key regulator of HPA-axis sensitivity and activity. This gene is located on chromosome 6p21 and codes for FK506 binding protein 51 (*FKBP5*), a co-chaperone of hsp90 that regulates glucocorticoid receptor (GR) sensitivity [Binder, 2009]. *FKBP5* is relevantly expressed in the brain [Gawlik et al., 2006]. Functionally, cortisol induces *FKBP5* gene expression by activation of glucocorticoid-response-elements [Vermeer et al., 2003]. In turn, *FKBP5* binding to the GR reduces GR affinity for cortisol and diminishes the amount of activated GRs being translocated to the cell nucleus [Wochnik et al., 2005].

Some common single nucleotide polymorphisms (SNPs) within the *FKBP5* gene have been found to increase *FKBP5* protein expression [Binder et al., 2004; Hubler and Scammell, 2004]. In line with a pathophysiological model, these high-expression-inducing alleles of the *FKBP5* gene (e.g., T allele of rs1360780) are associated with relative GR resistance [Binder et al., 2008].

In gene–environment interaction analyses, Binder et al. determined that rs1360780 interacts with childhood abuse as a predictor of adult posttraumatic stress disorder (PTSD) [Binder et al., 2008]. In a previous study, we found a prominent interaction between the high-induction TT genotype of rs1360780 and childhood physical abuse for adult depression [Appel et al., 2011]. A prospective study in adolescent participants, Zimmermann et al. [2011] confirmed the risk-increasing effect of *FKBP5* variants on depression in traumatized subjects.

In the current study, we followed the model that early-life stress leads to dysregulated HPA-axis function, especially in genetically predisposed subjects carrying the TT genotype rs1360780 [Heim and Nemeroff, 2001; Heim et al., 1997, 2009; Rice et al., 2008]. An exaggerated cortisol response may then impair neuroplasticity [Pittenger and Duman, 2008] and trigger structural brain changes, which have been shown in animal models [Conrad et al., 1999; Vyas et al., 2002, 2006]. In humans, there is accumulating evidence that these mechanisms and structural brain changes are linked to mental disorders [Dannlowski et al., 2012; Sacher et al., 2012].

However, this model of the interaction among early life stress, the *FKBP5* risk-SNP rs1369780 and brain structures has not been investigated comprehensively. To date, brain imaging studies of the *FKBP5* gene have either only investigated direct gene effects without integrating the important interactions with life stress [Fani et al., 2013; Fujii et al., 2014; Zobel et al., 2010] or if they investigated $G \times E$ interactions effects, the outcomes used predefined region of interests (ROIs) (hippocampus and amygdala) [Holz et al., 2015; Pagliaccio et al., 2014]. Moreover, the small sample sizes investigated did not allow for an analysis of homozygous TT carriers in interactions with life stress. Furthermore, the different developmental stages of study participants, ranging from children and young adults [Holz et al., 2015; Pagliaccio et al., 2014] to adults with ages up to 65 years [Fani et al., 2013; Fujii et al., 2014; Zobel et al., 2010], additionally introduced a relevant source of heterogeneity. Thus, the studies to date are difficult to compare and add limited evidence to the $G \times E$ interaction model of brain structure.

Thus, interaction analyses of the *FKBP5*-childhood trauma interplay that apply a whole-brain approach, which could discover more widespread interaction effects on the brain structure, are lacking.

In this study, we investigated $n = 1,826$ subjects from the general population. As the most robust evidence to date has been in favor of a recessive model of action for the T allele [Appel et al., 2011; Binder et al., 2004; Holz et al., 2015; Zimmermann et al., 2011], our leading hypothesis was that abused subjects carrying the TT genotype would show structural brain differences, particularly in affect-processing regions, compared with C allele carriers. We performed interaction analyses using voxel-based morphometry (VBM) to gain insight into the structural effects of the $G \times E$ interaction throughout the whole-brain gray matter. Secondly, we performed ROI analyses of brain areas known to be especially sensitive to cortisol (hippocampus and amygdala) and of emotion-processing areas, such as the insula and the cingulate cortex, to further elucidate the interaction effects on those target structures.

MATERIALS AND METHODS

General Population Sample

We analyzed data from the Study of Health in Pomerania (SHIP) [Völzke et al., 2011] comprising adult German residents of northeastern Germany. A two-stage stratified cluster sample of adults aged 20–79 years (baseline) was randomly drawn from local registries. At baseline (SHIP-0, 1997–2001), 4,308 Caucasian subjects participated. To date, two regular follow-ups have been carried out (SHIP-1 with $n = 3,300$ from 2002 to 2006 and SHIP-2 with $n = 2,333$ from 2008 to 2012). In parallel, detailed assessments of life events and mental disorders were conducted within the SHIP-LEGEND study (Life Events and Gene–Environment

Interaction in Depression) from 2007 to 2010, comprising $n = 2,400$ of the SHIP participants. Furthermore, a new independent sample called SHIP-TREND-0 ($n = 4,420$) in the same area was drawn in 2008, and similar examinations were undertaken.

Subjects from SHIP-2 and SHIP-TREND-0 were asked to participate in a whole-body magnetic resonance imaging (MRI) assessment [Hegenscheid et al., 2009; Stein et al., 2012]. In total, 1,163 subjects from SHIP-2 and 2,154 subjects from SHIP-TREND-0 underwent the MRI. Thus, approximately 50% of all subjects were eligible for the MRI study and volunteered to participate. The ethics committee of the University of Greifswald approved SHIP, SHIP-LEGEND and SHIP-TREND. A complete description of the study was provided to the subjects, and written informed consent was obtained. After exclusion of medical conditions (e.g., a history of cerebral tumor, stroke, Parkinson's Diseases, multiple sclerosis, epilepsy, hydrocephalus, enlarged ventricles, or pathological lesions) and technical reasons (e.g., severe movement artifacts or inhomogeneity of the magnetic field), complete data sets were available for 2,276 subjects. We only included subjects ≤ 65 years of age to rule out the major effects of brain aging and memory bias toward childhood experiences ($N = 1,827$). Based on the homogeneity check of the VBM 8 toolbox (developed by Christian Gaser, University of Jena, Germany, <http://www.neuro.uni-jena.de/>), we excluded one extreme outlier ($N = 1,826$).

Interview and Psychometric Data

Sociodemographic factors and medical history were assessed by a computer-assisted face-to-face interview. The diagnoses of any depressive disorders were assessed using the Munich-Composite International Diagnostic Interview (M-CIDI; [Wittchen HPa, 1997]) in SHIP-LEGEND and SHIP-TREND-0 [Völzke et al., 2011]. The M-CIDI is a standardized fully structured instrument for assessing psychiatric disorders over the lifespan according to DSM-IV criteria. Test-retest reliability analyses of the diagnosis of major depressive disorder (MDD) revealed kappas between 0.62 and 0.77 [Wittchen and Pfister, 1997].

Current depressive symptoms were assessed using the BDI-II, which is a 21-item self-report questionnaire with high reliability and validity [Beck and Steer, 1987] (see Supporting Information). The Childhood Trauma Questionnaire (CTQ) [Bernstein et al., 2003] has a total of 28 items that are rated on a five-point Likert scale, with higher scores indicating a higher exposure to traumatic experiences. The CTQ manual provides threshold scores to determine the severity of abuse (none = 0, mild = 1, moderate = 2 and severe to extreme = 3). We used the three-abuse dimensions of emotional, sexual, and physical abuse. In addition to the sum score of the abuse dimensions (CTQ abuse score), we generated one dichotomized variable of overall abuse as follows: a subject was rated as

positive for overall abuse when at least one of the abuse dimensions at a severity score of ≥ 1 (at least mild) was reported. In independent studies, the abuse dimensions have particularly been found to have good reliability and validity [Bernstein et al., 2003; Wingenfeld et al., 2010].

Genetic Methods

Genotyping and imputation

The SHIP sample was genotyped using the Affymetrix Human SNP Array 6.0. The overall genotyping efficiency of the GWA was 98.55%. As the *FKBP5* gene SNP rs1360780 is not genotyped by the Affymetrix Human SNP Array 6.0, genotype data for rs1360780 were derived from standard imputation procedures using the software IMPUTE v0.5.0 based on HapMap II. The observed to expected variance ratio of 0.99 indicates very high imputation quality for rs1360780.

Genotyping a subset of the SHIP-TREND-0 subjects ($n = 986$) was performed using the Illumina HumanOmni 2.5-Quad. The final sample call rate was 99.51%. Imputation of genotypes in the SHIP-TREND-0 cohort was performed with the software IMPUTE v2.1.2.3 against the HapMap II (CEU v22, Build 36) reference panel. The imputation quality for rs1360780 was very high ($0.995576 = 1$).

In the other subjects from SHIP-TREND-0, genotyping of rs1360780 was performed using the pre-developed TaqMan[®] SNP Genotyping Assay C__8852038_10 (Life Technologies, Applied Biosystems, Darmstadt, Germany). All failing samples were repeated at least twice (for further information on genotyping see Supporting Information). Information on the Hardy Weinberg equilibrium and genotype distribution for each of the three platforms is given in Supporting Information Table 1 (all P -values > 0.05). As we had an overlap of $N = 981$ subjects between the Illumina Imputation and TaqMan genotyping, we could compare the performance of both methods. We observed $n = 5$ (0.5%) subjects with different genotypes across both platforms. In these five cases, we decided to use the values from the SHIP-TREND-0 imputation. However, none of these five subjects was a member of the abused TT carriers group.

Image acquisition. Having completed the interview, patients underwent a routine medical examination. All images were obtained using a 1.5 T Siemens MRI scanner (Magnetom Avanto, Siemens Medical Systems, Erlangen, Germany) with a T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence and the following parameters: axial plane, TR = 1,900 ms, TE = 3.4 ms, Flip angle = 15° , and an original resolution of $1.0 \times 1.0 \times 1.0 \text{ mm}^3$ image processing.

Preprocessing. We preprocessed the images with SPM 8 (Wellcome Trust Centre for Neuroimaging, University College London) and the VBM 8 toolbox. We applied the “set the origin using center-of-mass” procedure within the

VBM 8 toolbox to automatically align the images spatially and to avoid errors caused by misaligned images. Following this step, the images were bias-corrected, spatially normalized by using the high-dimensional DARTEL normalization, segmented into the different tissue classes, modulated for non-linear warping only and smoothed by a Gaussian kernel of 8 mm FWHM. Different brain sizes were already taken into account in the modulation step. Therefore, no further correction for total brain volume was required in the VBM analyses.

The homogeneity of GM images was checked using the covariance structure of each image with all other images (outlier ≥ 3 standard deviations from the mean), as implemented in the check data quality function in the VBM 8 toolbox.

To access the absolute local GM volume within predefined masks, we prepared a second set of GM segmentations that additionally took the affine transformations into account (affine and non-linear modulated warped GM images).

Statistical Analyses for MRI Data

We used SPM8 to analyze the preprocessed GM segments. First, the direct effects of the *FKBP5* genotypes and abuse (yes/no) on whole-brain GM volume (VBM) were analyzed separately using an ANOVA model. Covariates in both models were age and sex, and in the analysis of abuse, we additionally adjusted for lifetime diagnosis of MDD.

Second, combining the *FKBP5* genotype and childhood trauma, we set up an interaction model (VBM) to evaluate the putative interaction effect between the *FKBP5* genotype and abuse. In addition to using the dichotomized abuse variable, we exploratively applied a dimensional CTQ abuse score. For descriptive reasons, we further conducted three whole-brain VBM analyses to study the putative interaction effect between the *FKBP5* genotype and each of the three dimensional abuse subscales of the CTQ (emotional, physical, and sexual abuse). In all the VBM interaction models, the following covariates were used: age, sex, and lifetime diagnosis of MDD. Additional analyses were performed omitting the lifetime diagnosis of MDD (see Supporting Information Table 4).

The statistical threshold for voxels in the whole-brain analysis was set at $P_{\text{uncorrected}} < 0.001$, with subsequent Family Wise Error (FWE) correction for peak level and cluster level significance using SPM8.

Furthermore, a ROI approach was performed, focusing our analyses on the bilateral hippocampus (including the parahippocampal gyrus), amygdala, anterior/middle/posterior cingulate cortex and insula as defined by the AAL-atlas [Tzourio-Mazoyer et al., 2002].

To determine statistical significance of putative clusters in each of the six bilateral ROIs, we used Monte Carlo simulation. Based on the uncorrected voxel-wise P -value threshold, the inherent smoothness of the data within the

TABLE I. Descriptive characteristics for the combined SHIP-2 and SHIP-TREND-0 sample

	Total sample	rs1360780 = CC	rs1360780 = CT	rs1360780 = TT	<i>P</i> -value ^a
N	1,826	905	775	146	
Sex (male/female)	853/973	424/481	361/414	68/78	0.993
Age (in years)	47.88 (10.8)	48.17 (11.0)	47.69 (10.6)	47.10 (10.8)	0.435
Childhood abuse ^b (yes/no)	319/1,507	150/755	149/626	20/126	0.165
CTQ abuse score	17.36 (4.8)	17.26 (4.9)	17.51 (4.9)	17.23 (4.1)	0.539
MDD lifetime (yes/no)	342/1,484	165/740	145/630	32/114	0.571
BDI-II ^c	7.23 (6.4)	7.47 (6.6)	6.90 (6.3)	7.44 (6.1)	0.168
Education (<10, =10, >10 years in school)	126/1,151/549	66/570/269	49/495/231	11/86/49	0.774
Smoking (never/ex-/current smoker)	703/674/449	347/332/226	307/283/185	49/59/38	0.727
Alcohol (g/day)	9.56 (13.6)	9.76 (14.9)	9.56 (12.2)	9.84 (12.4)	0.550

^aAccording to one-way ANOVA or χ^2 -tests to check for possible differences in the genotype groups.

^bChildhood abuse derived from the Childhood Trauma Questionnaire (none versus mild/moderate/severe).

^cBDI-II: in SHIP-TREND-0 BDI-II generated from PHQ-9 scores (37).

ROI, and the predefined α -level significance, this simulation provides a minimum cluster size threshold (k). All clusters within the ROI exceeding k in size are significant on this α -level. We used SPM 8 to estimate the smoothness of the residual images within each ROI and AlphaSim (implemented in the toolbox for Data Processing & Analysis of Brain Imaging (DPABI V1.2_141101) <http://rfmri.org/dpabi>) to perform the simulations. We chose the uncorrected voxel-wise $P < 0.01$, $\alpha = 0.05$ and ran 10,000 iterations to estimate the cluster size threshold in every ROI.

To study the interaction effect between rs1360780 and childhood abuse on local GM volume reduction, we extracted the volumes of the ROIs mentioned above. We applied the bilateral ROI masks of the modulated (affine + non-linear) and warped GM images and summed up the GM content of each voxel within the masks.

We adjusted those ROI volumes for total brain volume (TBV), age, gender, and MDD. Then, the ROI volume residuals were further analyzed in interaction models (linear regression) with the CTQ abuse score and the dichotomized SNP (C carriers vs. TT).

To rule out the confounding effects of smoking status (never, ex-, or current smoker) and alcohol intake (milligrams per day), we additionally performed VBM, ROI analysis, and ROI volume interactions with adjustment for age, gender, MDD, smoking and alcohol (for details see Supporting Information Tables 5 and 7–9).

Statistical Analyses for $G \times E$ Interaction with Depression

To investigate the $G \times E$ effects of childhood abuse and rs1360780 on depressive symptoms in our MRI sample according to our previous article Appel et al. [2011], we used Tobit regression, a technique designed to address

censored distribution [Persons and Perloff, 1989]. In addition to a continuous measure of current depressive symptoms (BDI-II), we investigated the $G \times E$ effect on categorical MDD as sensitivity analysis.

All analyses were performed with STATA/MP software, version 10.1 (StataCorp LP, College Station, Texas) and free-ware R, version 2.11.1. [Team RDC, 2010].

RESULTS

The sample characteristics are shown in Table I. We did not find any direct effect of the TT genotype rs1360780 versus CT/CC on the BDI-II ($P = 0.96$) or MDD ($P = 0.27$) after adjustment for sex and age. In interaction analyses between rs1360780 and childhood abuse, no statistically significant interaction with BDI-II scores emerged in our MRI sample. Neither the broader scale definition of abuse (none vs. mild/moderate/severe) (Supporting Information Table 2) nor the narrow definition of overall childhood abuse (none/mild vs. moderate/severe) (Supporting Information Table 3) or the CTQ- abuse dimensions demonstrated interaction with rs1360780 (all P -values ≥ 0.20). $G \times E$ analyses of MDD were restricted to the broad abuse phenotype due to small cell counts in the interaction term. Again, this revealed no significant interaction (all P -values ≥ 0.28).

Effects of rs1360780 on Gray Matter Volume in the Total sample—Whole-Brain Analysis

Group comparisons between the $N = 146$ TT genotype carriers versus $N = 1,680$ CT/CC carriers showed no significant results in the whole-brain analyses or ROI analyses ($P_{\text{FWEcorrected}} > 0.05$ for all comparisons).

Figure 1.

Inverse interaction effect of *FKBP5* (CC/CT vs. TT) and childhood abuse (none vs. mild/moderate/severe) on gray matter. Voxel based morphometry (VBM) interaction analysis in $N = 1,826$ subjects revealed three clusters after the adjustment for age, gender, and lifetime diagnosis of MDD.

Effects of Abuse on Gray Matter Volume in the Total Sample—Whole-Brain Analysis

There were no statistically significant results for comparisons of the $N = 1,507$ non-abused versus $N = 319$ abused subjects in the whole-brain analyses. No significant GM volume difference was found in the abused subjects compared with non-abused subjects in the ROI analyses ($P_{\text{FWEcorrected}} > 0.05$ for all comparisons).

Interaction Effect of rs1360780 and Abuse on Gray Matter Volume

This analysis is based on 299 abused subjects with the CC/CT genotype, 20 abused subjects with the TT genotype, 1,381 non-abused with the CC/CT genotype, and 126 non-abused subjects with the TT genotype. Three large clusters showed a significant negative interaction among *FKBP5* genotype (0 = CC/CT, 1 = TT) and childhood abuse (0 = none, 1 = mild/moderate/severe) and GM with adjustment for age, gender, and lifetime MDD (Fig. 1 and Table II). After applying peak-level FWE corrections ($P < 0.05$), peak voxels in three clusters within the left hemisphere survived (77 voxels: superior temporal gyrus, insula; 62 voxels: insula, putamen; and 28 voxels: temporal pole, superior temporal gyrus).

Omitting lifetime MDD as covariate did not change the results (see Supporting Information Table 4). Additionally, adjusting for smoking status and alcohol intake had no noticeable impact, except that the cluster within the anterior cingulate cortex and frontal gyrus slightly missed sig-

nificance (see Supporting Information Table 5). The three clusters in the left hemisphere that survived peak-level FWE correction were robust under these various adjustments.

When performing the VBM interaction analyses using the dimensional CTQ abuse score instead of the dichotomized CTQ abuse score, none of the clusters reached the level of FWE-corrected significance, clearly indicating non-linear interaction effects.

Effects of the CTQ Subscales

In VBM interaction analyses, no significant interaction effects were revealed for the dimensional physical and emotional CTQ subscales.

A significant cluster of 2,106 voxels ($P_{\text{cluster, FWE}} = 3.4 \times 10^{-4}$) with a negative interaction effect of the *FKBP5* genotype and the dimensional sexual abuse CTQ subscale ($n = 9$ with TT genotype and sexual abuse CTQ subscale > 5) on GM was found in the left hemisphere comprising regions within the inferior orbital frontal gyrus, hippocampus, temporal gyrus, amygdala, putamen, and insula (Supporting Information Fig. 1 and Supporting Information Table 6). After applying peak-level FWE correction ($P < 0.05$), a cluster of 80 voxels in the left temporal pole, superior gyrus and superior temporal gyrus survived.

Interaction Effect of rs1360780 and Abuse on Gray Matter Volume—ROI Analyses

ROI analyses revealed significant clusters of decreased GM volume in abused TT genotype carriers in the left and right amygdala, left and right hippocampus/parahippocampus, bilateral ACC, bilateral middle cingulate cortex, and the left and right insula (Table III). Additional adjustment for smoking status and alcohol intake did not change the results substantially, and only the cluster within the left hippocampus/parahippocampus missed significance (see Supporting Information Table 7).

Interaction Effects of rs1360780 on Gray Matter Volume in Abused Versus Non-Abused

We calculated the interaction analyses based on the residuals of the absolute GM volume (adjusted for total brain volume, age, sex, and lifetime diagnosis of MDD) of the respective ROI.

We observed significant interactions between dichotomized abuse (none vs. mild/moderate/severe) and rs1360780 (C carriers vs. TT) for the residuals of the volumes of the amygdala ($\beta = -90.48$, robust SE = 38.65, $P = 0.0194$), ACC ($\beta = -669.23$, robust SE = 183.00, $P = 0.0003$), middle cingulate gyrus ($\beta = -670.47$, robust SE = 240.08, $P = 0.0053$), hippocampus/parahippocampus ($\beta = -493.10$, robust SE = 189.26, $P = 0.0093$), and insula ($\beta = -494.84$, robust SE = 198.15, $P = 0.0126$) (see

TABLE II. VBM analysis of the whole brain in $N = 1,826$ subjects revealed a significant negative correlation of the interaction of FKBP5 (CC/CT vs. TT) and childhood abuse (none vs. mild/moderate/severe) in three clusters under the adjustment for age, gender, and lifetime diagnosis of MDD

k	Regions	Brodmann areas	Stereotaxic coordinates (mm)			t score
			x	y	z	
2,754 ^a	L insula	13, 38, 22, 21, 47	−44	−6	−8	4.81
	L hippocampus		−33	−13	−2	4.64
	L temporal pole, superior gyrus		−47	8	−12	4.61
	L middle temporal gyrus					
	L Heschl gyrus					
	L putamen					
1,408 ^a	L superior temporal gyrus	38, 13, 21, 22, 28				
	R middle temporal gyrus		36	−10	−15	4.39
	R insula		48	−1	−12	4.17
	R hippocampus		38	6	−27	3.78
	R temporal pole, superior gyrus					
	R parahippocampal gyrus					
	R superior temporal gyrus					
	R putamen					
	R temporal pole, middle gyrus					
	R amygdala					
775 ^a	L anterior cingulate cortex	32, 24, 10	5	44	4	4.31
	R anterior cingulate cortex		−3	48	9	3.29
	R medial orbital frontal gyrus					
	R medial superior frontal cortex					
155	R lingual gyrus	18	0	−76	−5	3.86
	L calcarine fissure		0	−69	4	3.15
	L cerebellum					
	L lingual gyrus					
261	Vermis	34, 28, 47				
	R superior frontal gyrus, orbital part		18	−1	−12	3.73
	R putamen		24	12	−14	3.53
	R gyrus rectus					
	R parahippocampal gyrus					
	R amygdala					
	R insula					
	R hippocampus					
	R olfactory cortex					
	R olfactory cortex					
358	L olfactory cortex	25	2	2	−9	3.55
	R putamen		6	11	−9	3.49
	R caudate nucleus		15	12	−5	3.10
	R olfactory cortex					
129	R middle cingulate cortex	24	5	−18	40	3.53
	L middle cingulate cortex					

k denotes the cluster size in voxels, all clusters with $k \geq 100$ are listed in the table, $P < 0.001$ (uncorrected).

^aClusters that reach cluster-level significance ($P < 0.05$, FWE corrected).

Supporting Information Table 9 for results using the residuals that were additionally adjusted for alcohol intake and smoking status). Only the posterior cingulate cortex did not yield a significant interaction ($\beta = -20.63$, robust SE = 48.27, $P > 0.6$). These findings were supported by the interaction analyses using the dimensional CTQ scores (Fig. 2; for additional adjustment for alcohol intake and smoking status see Supporting Information Table 8). Supporting Information Figure 2 depicts the results for CC, CT, and TT.

DISCUSSION

It is important to consider that investigations into direct genetic risk markers of depressive disorders to date have not led to independently replicated genetic associations that elucidate the pathophysiology of MDD [CONVERGE, 2015; PGC, 2013]. In this context, the *FKBP5*-gene is an outstanding marker that supports the model of stress-induced HPA-axis dysregulation in genetically predisposed subjects as a longstanding biological risk of

TABLE III. ROI analyses of FKBP5 rs1360780 in $N = 1,826$ subjects revealed clusters with a significant negative correlation of the interaction of FKBP5 (CC/CT vs. TT) and childhood abuse (none vs. mild/moderate/severe) in the left and right amygdala, the left and right hippocampus/parahippocampus, the bilateral anterior cingulate cortex, the bilateral middle cingulate cortex and left and right insula under the adjustment for age, gender, and lifetime diagnosis of MDD

Region of interest	Cluster sizes in voxels ($P < 0.01$, $k > 20$)	AlphaSim cluster size threshold ($P < 0.05$)
Amygdala	L: 301 ^a R: 481 ^a	64
Hippocampus/parahippocampus	L: 353 ^a , 35 R: 777 ^a , 60	324
Anterior cingulum	L+R: 2,532 ^a	287
Middle cingulum	L+R: 1,556 ^a R: 35	409
Posterior cingulum	—	101
Insula	L: 1,266 ^a R: 1,116 ^a	332

Masks are all bilateral and based on the AAL atlas.

^aClusters that exceed the simulated AlphaSim thresholds for significant cluster sizes ($P < 0.05$).

depressive disorders. Consistent with this model, we identified three large clusters of reduced GM volumes based on whole-brain interaction between childhood abuse status and the FKBP5 rs1360780 TT genotype. Three smaller clusters within the left hemisphere (temporal gyrus, insula, and putamen) even survived peak-level FWE correction ($P < 0.05$), which strengthens the observed interaction effect.

Interestingly, these strong interaction effects were only observed when applying the dichotomized abuse variable (none vs. mild, moderate, severe abuse) but not when using the dimensional CTQ abuse score. This clearly points to the non-linear nature of this interaction. In fact, it is quite reasonable to conceive of a vulnerability-stress model that proposes an individual psychobiological resilience that outweighs the deleterious effects of stress and trauma until the psychobiological system decompensates. Evidence has even been reported that suggests a U-shaped relationship between life stress and mental well-being [Seery et al., 2010]. Again, this argues against merely linear effects of gene–environment interactions. However, linear statistical effects may also be detectable in non-linear relationships at a lower level of statistical power.

Our whole-brain results demonstrate reduced GM volumes in the bilateral insula, superior and middle temporal gyrus, bilateral hippocampus, right amygdala, and bilateral anterior cingulate cortex in abused subjects carrying the TT risk genotype. The VBM results were further elaborated and supported in subsequent ROI analyses using

Figure 2.

Interaction effect on residuals of ROI volumes between CTQ abuse score and rs1360780 (TT vs. CC/CT), $N = 1,826$ (1,680 C allele carriers, 146 TT genotype carriers). The CTQ abuse score is plotted on the x-axis against the residuals of the corresponding ROI volume (adjusted for total brain volume [TBV], age, sex, and lifetime depression) in mm³ on the y-axis. Subjects with genotype CC or CT are displayed in blue and TT carriers in yellow with a matching regression line. The genotype-CTQ abuse interaction effect sizes (beta), robust standard errors (SE), and P-values are shown in the plots.

GM volumes of the corresponding brain structures. It is remarkable that these results were virtually independent of any superimposing effects of lifetime MDD.

The GM volumes of the ventral and dorsal parts of the ACC were strongly affected by the abuse-genotype interaction in our study. The ventral (genual and subgenual) part of the anterior cingulate cortex (ACC) plays an important role within the emotion-processing network of the brain. The ventral ACC is connected to other brain regions that process emotional, motivational, and interoceptive

information (amygdala, nucleus accumbens, and anterior insula). Furthermore, the ventral ACC impacts the autonomous nervous system through connections to the hypothalamus [Allman et al., 2001]. The dorsal ACC (dACC) or middle cingulum has been implicated in cognitive processing of emotional content rather than pure emotion processing [Mohanty et al., 2007]. The GM volume of the dACC has been associated with attention to emotions and the ability to regulate negative feeling states [Koven et al., 2011]. Moreover, in emotion-processing studies in alexithymia, the dACC was prominently involved in the processing of emotional and social stimuli [van der Velde et al., 2013]. A direct effect of the T allele on the GM volume of dACC was found by Fujii et al. [2014]. Although GM changes in the same structure were supported by our $G \times E$ analyses, we could not confirm any direct effect of the T allele or the TT genotype on the brain structure in our sample.

Furthermore, the GM volume of the posterior cingulum was not affected by the abuse-genotype interaction in our study. In contrast to the ACC, the posterior cingulate cortex forms a central node in the default mode network of the brain and has been implicated as a neural substrate for human awareness [Leech et al., 2012]. Thus, the cingulate cortex provides an interesting example that the emotion-processing regions in particular (ventral and middle part) were affected by the abuse-genotype interaction.

We generally assume that trauma-induced up-regulation of the HPA-axis in childhood may negatively affect brain areas with a high density of glucocorticoid receptors and high sensitivity to cortisol (e.g., amygdala, prefrontal cortex and hippocampus; [Su et al., 2004; Wang et al., 2014; Webster et al., 2002]). Long-lasting changes in functional integrity (e.g., hypomethylation of T alleles because of excess cortisol) may add to structural changes [Klengel et al., 2013].

In contrast to the findings of Fujii et al. [2014], we observed no GM volume reduction in non-abused TT carriers. Additionally, abuse itself did not yield any effects on GM volume in the whole-brain VBM or ROI analyses.

Our findings suggest a GM decrease in the amygdala in abused TT carriers, which was a statistically robust finding in the categorical group comparison (Table III). However, the interaction analysis using the dimensional CTQ score failed to yield significant effects (Fig. 2). Additionally, Holz et al. [2015], who applied the dimensional CTQ score, did not observe any interaction effect on amygdala volume. This may point to the non-linear nature of the interaction.

In a sample of 120 school children, Pagliaccio et al. [2014] found that increased volume of the left amygdala was associated with an increased genetic risk score of stress-related genes but did not report on the *FKBP5* interaction effects on amygdala volume. Thus, their results are hardly comparable to our study.

We did not find any relevant structural GM volume reductions in abused CT carriers versus abused CC car-

riers that would point to some effect of the single T allele (data not shown in detail). Given the much higher number of heterozygotes compared with TT carriers, we should have been able to detect minor GM differences between CT and CC carriers. Thus, we conclude that CT status is not associated with any particular risk of structural brain changes in light of childhood abuse, at least in our Caucasian general population sample. Generally, this is in line with previous studies on $G \times E$ interactions that have identified homozygote TT status, but not heterozygotes, as a risk factor for depressive disorders in abused subjects, but not in non-abused subjects [Appel et al., 2011; Zimmermann et al., 2011].

For the interpretation of our results, it is important to keep in mind that no independent direct effects of the TT genotype or childhood abuse on brain structure were discovered. Moreover, the abused TT carriers in our sample had no higher symptom load or higher rate of lifetime diagnosis of MDD compared with the non-TT carriers. Thus, we conclude that the alterations to the brain structure in abused TT carriers most likely reflect the long-term consequences of a functional change in the HPA-axis but were not primarily driven by associated depressive disorders.

We can only speculate as to why the abused TT carriers had no signs of an increased burden of depressive symptoms. One explanation might be that 50% of all subjects of our general population study were excluded or refused to participate in the whole-body MRI scanning. Thus, there was relevant selection in the participation in the MRI scanning. Putatively, subjects who felt more severely distressed by symptoms of depression and anxiety might have refused participation to a larger extent. Thus, the structural brain differences observed in our study might represent a structural risk of depression derived by the trauma-genotype interaction but which, in our study sample, is counterbalanced by unmeasured protective factors. Alternatively, the structural alterations themselves could contribute to the obvious resilience to depressive symptoms and disorders in the risk subjects in our MRI study.

There were no significant differences between the carriers of the different genotypes in abused or non-abused subjects with regard to alcohol intake or cigarette smoking. However, descriptively, there were fewer non-smokers in the group of abused subjects, and on average, alcohol intake was higher in the non-abused, which was caused by the lower abstinence rate in non-abused. Comparing the average alcohol intake of the non-abstinence subjects of both groups, the abused subjects drank more. To minimize the likelihood that these lifestyle factors relevantly confounded the genotype-related findings, the additional adjustment in all analyses for smoking status and alcohol intake was applied [Fritz et al., 2014]. We only included subjects ≤ 65 years of age to rule out major effects of brain aging and memory bias toward childhood experiences. As the interaction between traumatic events and rs1360780

has also been implicated in the risk for PTSD, we aimed to consider confounding effects by PTSD. In the SHIP-LEGEND sample, the interview-based lifetime diagnosis of PTSD was available. However, only five subjects with childhood abuse were diagnosed with PTSD, and none of them carried the TT genotype. Thus, we do not assume that PTSD confounded our results in the SHIP-LEGEND subsample. However, as PTSD information was not available for SHIP-TREND-0, we cannot fully exclude an underlying role of PTSD in the reported interaction.

Although our overall sample was large, the risk group of abused TT carriers was relatively small ($n = 20$). Given a frequency of approximately 10% of the TT genotype, the prevalence of childhood abuse is critically important in determining the number of subjects at risk. However, it is important to consider that we performed whole-brain (VBM) interaction analyses. This indicates that statistically the interaction effect is carried by subjects without the risk SNP and without abuse, by subjects with the risk SNP and without abuse, by subjects with the risk SNP but without abuse and by subjects with the risk SNP and abuse. Thus, the effect of the “risk SNP and abuse” is contrasted to large groups of individuals included in the other three conditions, giving a high statistical credibility (significance) to the overall results. Moreover, even FWE-corrected peak voxel significances emerged in three clusters of the VBM interaction analysis, indicating high statistical power. Furthermore, it has to be acknowledged that our general population sample was not biased toward any treatment-seeking behavior.

In summary, this is the largest study to date on the structural effects of the gene–abuse interaction with *FKBP5* SNP rs1360780. We consider the GM volume reductions in affect processing and the regulation of brain regions to be long-lasting structural correlates of a dysregulated HPA-axis after child abuse in TT carriers that may contribute to increased risk of stress-related disorders. Our results add important evidence to the gene–environment interaction model for depressive disorders and may contribute to individual risk assessments after trauma.

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