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Research Article

RESEARCH ARTICLE

FKBP5 Genotype-Dependent DNA Methylation and mRNA Regulation After Psychosocial Stress in Remitted Depression and Healthy Controls

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Abstract

Background: Polymorphisms in the FK506 binding protein 5 (*FKBP5*) gene have been shown to influence glucocorticoid receptor sensitivity, stress response regulation, and depression risk in traumatized subjects, with most consistent findings reported for the functional variant rs1360780. In the present study, we investigated whether the *FKBP5* polymorphism rs1360780 and lifetime history of major depression are associated with DNA methylation and *FKBP5* gene expression after psychosocial stress.
Methods: A total of 116 individuals with a positive (n = 61) and negative (n = 55) lifetime history of major depression participated in the Trier Social Stress Test. We assessed plasma cortisol concentrations, *FKBP5* mRNA expression, and CpG methylation of *FKBP5* intron 7 in peripheral blood cells.
Results: Genotype-dependent plasma cortisol response to psychosocial stress exposure was observed in healthy controls, with the highest and longest-lasting cortisol increase in subjects with the TT genotype of the *FKBP5* polymorphism rs1360780, and healthy controls carrying the T risk allele responded with a blunted *FKBP5* mRNA expression after psychosocial stress. No genotype effects could be found in remitted depression.
Conclusions: The *FKBP5* rs1360780 polymorphism is associated with plasma cortisol and *FKBP5* mRNA expression after psychosocial stress in healthy controls but not in remitted depression. Preliminary results of the DNA methylation analysis suggest that epigenetic modifications could be involved.

Keywords: major depression, HPA axis, *FKBP5*, gene expression, DNA methylation

Introduction

The hypothalamus-pituitary-adrenocortical (HPA) axis is the major neuroendocrine response system that regulates the somatic stress reaction. Dysregulation of the HPA axis can be a consequence of an impaired function of the glucocorticoid receptors (GRs) that mediate the effects of corticosteroids (Holsboer, 2000; Pariante, 2004). Proper GR function is essential for a negative feedback regulation of the HPA axis. The HSP90 co-chaperone FK506 binding protein 5 (*FKBP5*) is an important functional regulator in the GR complex. *FKBP5* is part of the ultra-short intracellular negative feedback loop with GR where

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FKBP5 reduces glucocorticoid binding affinity (Denny et al., 2000) and nuclear translocation of the GR (Wochnik et al., 2005) and thus acts as a negative regulator of the GR transcriptional activity. GR activation, in turn, leads to an increase in *FKBP5* mRNA and protein expression, as glucocorticoids such as cortisol induce the expression of *FKBP5* (Vermeer et al., 2003; Binder, 2009; Jääskeläinen et al., 2011).

Single nucleotide polymorphisms (SNPs) within the *FKBP5* gene are known to influence GR sensitivity and thus HPA axis regulation, which has been discussed as a key endocrine marker for stress-related disorders such as major depression (MD) (Lekman et al., 2008; Lavebratt et al., 2010; Zobel et al., 2010; Appel et al., 2011; Zimmermann et al., 2011) or posttraumatic stress disorder (PTSD) (Binder et al., 2008; Xie et al., 2010; Mehta et al., 2011). The most consistent findings have been reported for rs1360780, a SNP located in the second intron of the *FKBP5* gene; the T allele of this SNP forms a putative transcription start site (Klengel et al., 2013). Healthy subjects carrying the T risk allele of this SNP show relative GR resistance as indicated by an escape from the dexamethasone (dex)-induced suppression of plasma cortisol (Binder et al., 2008; Touma et al., 2011). GR resistance was also demonstrated after activation of the HPA axis during a psychosocial stress test (Ising et al., 2008; Buchmann et al., 2014). Healthy individuals with the rs1360780 TT genotype responded with prolonged plasma cortisol concentrations and more anxiety symptoms during the recovery period after the stress test (Ising et al., 2008).

Gene expression studies further elucidate the association between *FKBP5* and MD. It could be shown in mice that chronic corticosterone treatment increases *FKBP5* mRNA to a similar extent in hippocampus, hypothalamus, and peripheral blood cells, while a corresponding reduction of *FKBP5* DNA methylation could be observed in the hypothalamus and blood cells (Lee et al., 2010). *FKBP5* mRNA levels are also associated with depression outcome, as successful antidepressant treatment response was related to reduced *FKBP5* gene expression (Cattaneo et al., 2013). GR resistance, which is a common phenomenon in MD, can be detected by measuring *FKBP5* mRNA levels in peripheral blood. Menke et al. (2012b) investigated the GR-stimulated gene expression in peripheral blood cells in 2 cohorts of patients and healthy controls. Stimulated mRNA levels of *FKBP5* and other candidate genes could discriminate better between cases and controls than baseline gene expression or suppression of plasma cortisol by dex. The same research group found different *FKBP5* mRNA levels between acutely depressed patients and healthy controls, which were associated with rs1360780 (Menke et al., 2013).

Epigenetic modifications such as DNA methylation are important determinants of mRNA gene expression, as recently demonstrated for *FKBP5*. *FKBP5* mRNA can be upregulated by glucocorticoids (Vermeer et al., 2003; Jääskeläinen et al., 2011) but also by demethylation of specific regions in the *FKBP5* gene (Lee et al., 2010; Klengel et al., 2013; Yehuda et al., 2013). Interestingly, genetic variations, in particular *FKBP5* SNP rs1360780, also seem to influence how environmental exposure impacts epigenetic marks. Klengel et al. (2013) discovered in a sample of patients with PTSD that CpG methylation patterns around glucocorticoid response elements (GREs) in intron 7 of the *FKBP5* gene are associated with the T risk allele of rs1360780 and early childhood trauma, presumably due to a genotype-dependent interaction between the distal GREs and the transcription start site in intron 2, which is formed by the T allele of rs1360780. This interpretation is in agreement with in vitro findings suggesting that *FKBP5* activation is majorly regulated by distal rather than proximal GREs (Paakinaho et al., 2010).

Thus, combining measures of HPA axis regulation with mRNA expression and DNA methylation will be important to elucidate the role of *FKBP5* in stress regulation. In the present study, we exposed participants with a lifetime history of MD who were in full remission for at least 6 months, as well as healthy control subjects, to a psychosocial stress test to examine the effect of a previous MD and the *FKBP5* SNP rs1360780 on: (1) plasma cortisol concentrations, (2) *FKBP5* mRNA levels in peripheral blood cells after psychosocial stress, and (3) DNA methylation of intron 7 in the *FKBP5* gene.

Methods

Subjects

A total of 116 subjects aged 30 to 42 years (mean(M) = 34.35, standard deviation (SD) = 3.43) were recruited from participants of the Early Developmental Stages of Psychopathology (EDSP) study. The EDSP study is a longitudinal epidemiological study designed to investigate the prevalence, incidence, risk factors, comorbidity, and course of mental disorders in a community sample of adolescents and young adults. The original EDSP sample was randomly drawn from the 1994 government registries of all residents aged 14 to 24 years in Munich (Germany). After the baseline investigation, 3 follow-up investigations were conducted covering an overall time period of 10 years (Wittchen et al., 1998; Lieb et al., 2000; Zimmermann et al., 2011). The present study sample was selected on the basis of the aggregated diagnostic information of all EDSP assessments.

The remitted MD group included participants with a lifetime DSM-IV diagnosis of MD who were in full remission for at least 6 months. Exclusion criteria were DSM-IV lifetime diagnoses of dysthymia, schizophrenia, substance use disorder, social phobia, or the specific phobia of a blood-injection-injury type. As a control sample, we recruited from the same epidemiological cohort subjects with a negative history of any affective disorder, general anxiety disorder, or any other mental disorder mentioned within the exclusion criteria of the MD sample. Medical conditions known to influence HPA axis activity were also excluded. We further excluded subjects on current antidepressant medication for the analysis (Wagner et al., 2012; Cattaneo et al., 2013).

For comparison reasons, mRNA data are shown from N = 14 unstressed control subjects without a history of mental disorders and aged 27 to 39 years (M = 32.93, SD = 3.77) who underwent repeated afternoon blood collections without participation in a stress test.

All subjects were Caucasians. Participants gave oral and written consent after being informed about the study procedure. The study was approved by the local Ethics Committee of the Ludwig Maximilians University, Munich, Germany.

Diagnostic Assessment

Eligible EDSP participants were invited by letter and contacted by telephone. If respondents gave their consent to participate and matched inclusion criteria cross-checked by a brief telephone interview, they were invited for a diagnostic appointment. Diagnostic assessment was based on the computerized Munich version of the Composite International Diagnostic

Interview (M-CIDI), which allows for the standardized assessment of symptoms, syndromes, and diagnoses of DSM-IV disorders as well as their onset, duration, and severity (Wittchen and Pfister, 1997). Moreover, childhood adverse events were assessed with the M-CIDI. A childhood adverse event was defined as separation from a parent (through death or parental divorce/separation) or exposure to a PTSD-qualifying trauma prior to the age of 13 years. Information on childhood adverse events was derived from the PTSD and family history section of the M-CIDI. The M-CIDI interview was used in the EDSP study in all assessments.

Trained interviewers conducted the interval version of the M-CIDI covering the time period between the previous assessment and the current evaluation. Presence of acute depression symptoms within the last 14 days was evaluated using the Beck Depression Inventory II (BDI) (Hautzinger et al., 2009). Participants were invited for the experimental part of the study if they did not exceed a BDI score of 14.

Experimental Procedure

The Trier Social Stress Test (TSST) was applied during afternoon sessions at the Max Planck Institute of Psychiatry in Munich, Germany. In fact, the TSST was administered twice during the experimental sessions; however, this analysis will focus on acute stress effects obtained from the first TSST. Study investigators and participants were not aware of the group assignment as an MD or healthy control according to the M-CIDI diagnoses.

A venous catheter was placed in the forearm vein at least 30 minutes prior to the collection of the baseline blood sample. After baseline evaluations, the TSST protocol was started (Kirschbaum et al., 1993; Ising et al., 2008). The TSST is a public speaking task involving a mock job interview and a mental arithmetic task. The participants were informed about the stress procedure and were given 10 minutes of preparation time for a presentation about their professional education. After that, the subjects were escorted to another room to give their presentation in front of a mixed-gender panel of 2 judges who are trained to withhold verbal and nonverbal feedback. The TSST itself consists of a 5-minute mock job interview and an unexpected 5-minute mental arithmetic task. Both tasks were audio- and videotaped so as to increase task engagement. Blood samples were drawn before and subsequently after the stress procedure in 15-minute intervals following the TSST. During the recovery period, participants could choose between watching movies and reading newspapers.

Hormone Assessment

Plasma adrenocorticotropic hormone (ACTH) was measured using an immunoradiometric assay (cobas ECLIA, Roche Diagnostics, Rotkreuz, Switzerland). The detection limit for plasma ACTH was 1.0 pg/mL. Plasma cortisol was determined using a radioimmunoassay kit (CT Cortisol RIA, DRG Diagnostics, Marburg, Germany). The detection limit was 1.7 ng/mL.

DNA Extraction and Genotyping

Blood samples (7.5 mL) were collected in ethylenediaminetetraacetic acid containing tubes at baseline and DNA was extracted using the Puregene whole blood DNA-extraction kit (Gentra Systems Inc.). The *FKBP5* SNP rs1360780 was genotyped by using real-time PCR and subsequent melting curve analysis performed with a Lightcycler 480 Genotyping Master (Roche Applied Science, Mannheim, Germany). The minor allele frequency was 28%. Genotype distribution did not deviate from the Hardy-Weinberg equilibrium as indicated by a nonsignificant *P* value of the exact test ($P = .110$). The SNP could be successfully genotyped in all samples (call rate = 100%).

Assessment of *FKBP5* Gene Expression

Whole blood samples using PAXgene Blood RNA tubes (QIAGEN GmbH, Hilden, Germany) were collected at baseline as well as +45 and +70 minutes after the TSST. *FKBP5* gene expression analysis was performed using fast real-time PCR with a TaqMan gene expression assay (Applied Biosystems Deutschland GmbH, Darmstadt, Germany). Gene expression was quantified in relation to the average activity of 4 housekeeping genes (glucose-6-phosphate dehydrogenase, ß-glucuronidase, TATA box binding protein, phospholipase A) showing excellent concordance for all time points (average intraclass correlation: $r = .922$). The call rate was 100%.

DNA Methylation Analysis

DNA methylation analysis was performed by Varionostic GmbH (http://www.varionostic.de) using an EpiTYPER assay, which is based on bisulfite conversion and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Sequenom Inc, San Diego, CA). The region of interest was defined as the genomic region located in intron 7 of the *FKBP5* gene (chr6:35,666,288-35,666,763, hg18) corresponding to the P1 region as defined by Klengel et al. (2013). The amplicon was designed with the Qiagen Assay Design Software 2.0. Bisulfite treatment, PCR, and post-PCR steps of the EpiTYPER assay were performed according to the manufacturer's protocol. Seven CpGs are contained within the target region (units 1 to 7); units 5 (chr6:35,666,544), 6 (chr6:35,666,688), and 7 (chr6:35,666,699) could not be assessed due to technical reasons. We averaged for the statistical analysis the methylation of units 1 to 4 (chr6:35,666,364; chr6:35,666,416; chr6:35,666,466; chr6:35,666,491) covering the area of the GRE, which has been described to be associated with altered stress responsiveness (Klengel et al., 2013). Complete methylation data of units 1 to 4 were available for N = 96 subjects (call rate = 82%).

Statistical Analysis

Demographic data were compared between groups (remitted MD/controls) using an independent sample *t* test for continuous data and Pearson's chi-square test or Fisher's exact test for categorical data.

A general genotypic model with exploratory posthoc comparisons between genotypes was used for all genetic analyses. For the analysis of plasma cortisol, we applied a repeated-measures analysis of covariance (ANCOVA) with the between-subject factors depression (remitted MD/controls) and *FKBP5* genotype of the SNP rs1360780 (CC/CT/TT genotype) and with the within-subject factor time (baseline, prestress, poststress, +15 minutes, +30 minutes, +45 minutes, +70 minutes). Baseline cortisol was added as a covariate. *FKBP5* mRNA expression after psychosocial stress was also evaluated with a repeated-measures ANCOVA including the factors depression, *FKBP5* genotype, and time (baseline, +45 minutes, +70 minutes). In a preliminary analysis, a 2-way ANCOVA was used to test the effects of the

factors depression and *FKBP5* genotype on the DNA methylation rate in intron 7 of the *FKBP5* gene. As covariates in the mRNA and DNA methylation analysis, we included gender, age, and the relative concentration of blood cell types (erythrocytes, lymphocytes, granulocytes, monocytes) of the baseline blood sample. Correction effects of the blood cell types on mRNA and DNA methylation were determined using Cohen's f (Cohen, 1988). Correction effects on mRNA concentrations showed small effects for lymphocytes, monocytes, and granulocytes (f = .10 – .25), or were below the threshold of a small effect for erythrocytes (f < .10). Correction effects on DNA methylation were below the threshold of a small effect (f < .10) for all cell types.

Huynh-Feldt–corrected *P*-values are reported when appropriate. The level of significance was set to *P* = .05. Analyses were performed with PASW Statistics for Windows (Version 18.0., SPSS Inc. released 2009, Chicago, IL).

Results

Sociodemographic variables and study characteristics are listed in Table 1. Subjects with remitted depression and healthy controls did not differ in age, gender, body mass index, and nicotine or caffeine consumption. A total of 38 participants suffered previously from a single episode of MD, and 23 subjects had recurrent MD with on average 5.61 (SD = 4.70) previous episodes. The mean age at onset was 20.48 years (SD = 5.51) and the last episode was on average 10.73 years (SD = 5.79) ago, which did not differ between single episode and recurrent depression. The mean BDI score at the test day was significantly higher in the depression group (M = 2.23, SD = 3.00) than in controls (M = 1.17, SD = 1.66, *P* = .019) but remained within the range of "no depression" according to the BDI manual.

Plasma Cortisol and ACTH Concentrations

The repeated-measures ANCOVA of plasma cortisol concentrations, corrected for baseline cortisol concentrations, showed a significant effect of time ($F_{5,545}$ = 23.39, *P* < .001), indicating a successful stress induction by the TSST. There was a nonsignificant trend toward an interaction of depression × *FKBP5* genotype ($F_{2,109}$ = 2.98, *P* = .055). Furthermore, we found a significant interaction of time × depression ($F_{5,545}$ = 3.16, *P* = .028) and an interaction of time × depression × *FKBP5* genotype ($F_{10,545}$ = 3.66, *P* = .002). Healthy control subjects with the TT genotype at rs1360780 showed the highest plasma cortisol concentrations in the recovery period following psychosocial stress (Figure 1). To disentangle these interactions, we conducted pointwise comparisons separately for each group and found a significant *FKBP5* genotype effect in healthy controls (+15 minutes poststress, *P* = .017; +30 minutes, *P* = .006; +45 minutes, *P* = .011; +70 minutes, *P* = .015). Healthy controls with the TT genotype showed increased plasma cortisol concentrations after the TSST (Figure 1a). No genotype-dependent differences in the plasma cortisol response to the TSST were observed in the remitted MD group (Figure 1b). When comparing cortisol differences separately for each genotype, we found in the TT genotype significant group effects at +15 minutes (*P* = .016), +30 minutes (*P* = .015), and +45minutes poststress (*P* = .007) with higher cortisol plasma levels in controls. Regarding plasma ACTH, there was a significant effect of time, indicating the efficacy of the stress induction ($F_{5,545}$ = 26.19, *P* < .001). However, we did not observe significant depression or genotype effects for ACTH.

FKBP5 mRNA Gene Expression

Repeated-measures ANCOVA of *FKBP5* mRNA revealed a significant interaction effect of time × depression × *FKBP5* genotype ($F_{4,208}$ = 3.43, *P* = .010). Healthy subjects with the rs1360780 CC genotype showed an increase of *FKBP5* mRNA after stress exposure, whereas healthy subjects with the CT or TT genotype showed blunted *FKBP5* mRNA levels. Again, no genotype effect on mRNA expression was found within the remitted MD group (Figure 2). Posthoc analyses separately for each group revealed a significant genotype effect in healthy controls +70 minutes (*P* = .015) and a nonsignificant trend +45 minutes poststress

Table 1. Demographic and Clinical Characteristics

	Total	Remitted MD	Healthy Controls	
	n = 116	n = 61	n = 55	*P*
Gender, n (%)				n.s.[a]
Female	56 (48)	29 (47)	27 (49)	
Male	60 (52)	32 (53)	28 (51)	
Age (range 30–42 y), M (±SD)	34.35 (3.46)	34.65 (3.32)	34.02 (3.61)	n.s.[b]
BMI, M (±SD)	24.35 (3.90)	23.95 (3.29)	24.79 (4.47)	n.s.[b]
Relationship status, n (%)				n.s.[a]
Single	59 (51)	28 (46)	31 (56)	
Married	52 (45)	29 (48)	23 (42)	
Divorced	5 (4)	4 (6)	1 (2)	
Depression history, n (%)				
Single episode MD		38 (62)		
Recurrent MD		23 (38)		
Age at onset, M (±SD)		20.48 (5.51)		
Previous episodes, M (±SD)		5.61 (4.70)		
BDI TSST, M (±SD)	1.73 (2.50)	2.23 (3.00)	1.17 (1.66)	.019[b]

Abbreviations: BMI, body mass index; BDI TSST, Beck Depression Inventory score on the day of the stress experiment; M, mean; MD, major depression; n.s., not significant; SD, standard deviation.

[a] Chi-square test/Fisher's exact test.

[b] Student's *t* test.

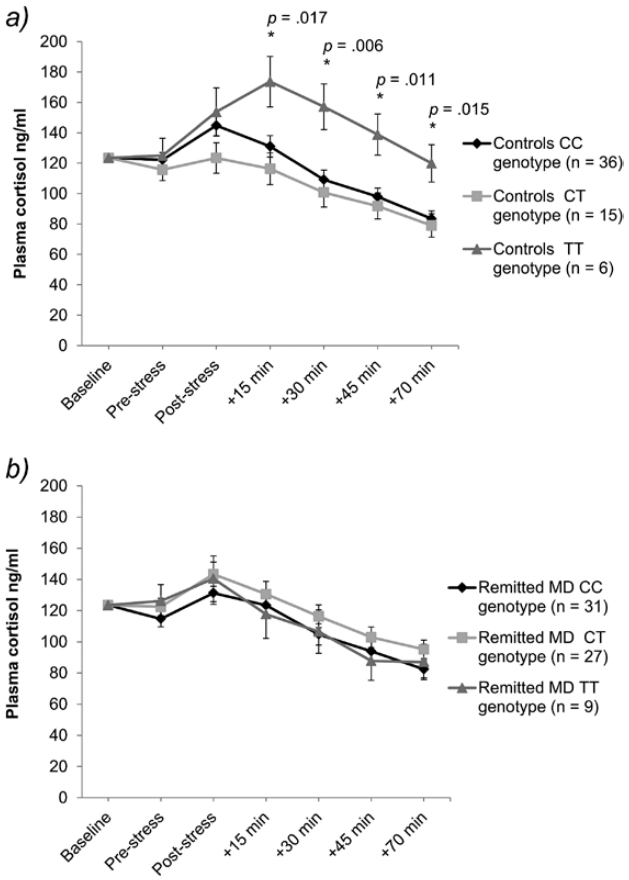

Figure 1. Genotype-dependent (rs1360780) change of plasma cortisol levels corrected for baseline cortisol concentration in (a) healthy controls (n = 55) and (b) subjects with remitted lifetime history of major depression (MD) (n = 61). The figure shows estimated means with standard error bars and *P*-values from pointwise comparisons of the *FKBP5* genotype effect within the control group.

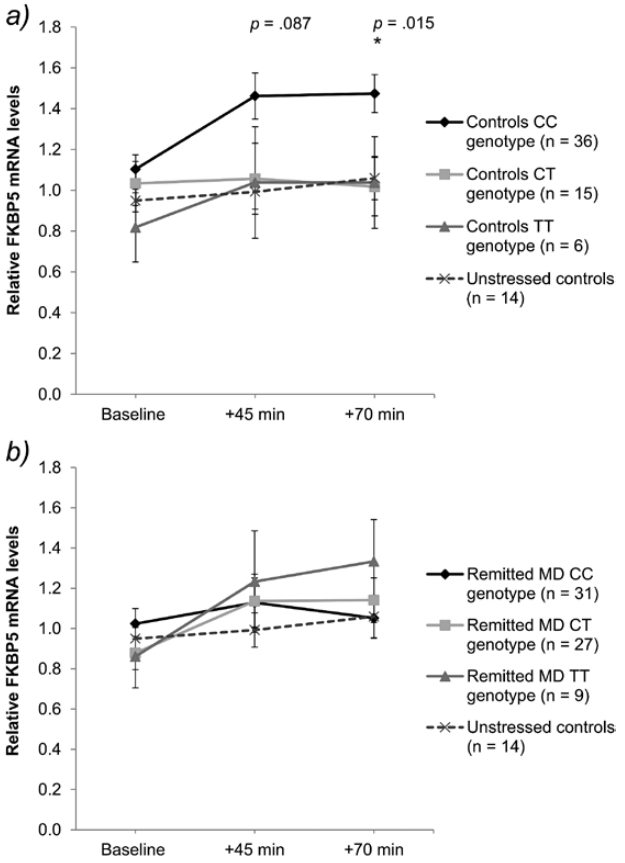

Figure 2. Genotype-dependent (rs1360780) change of *FKBP5* mRNA levels corrected for baseline cortisol concentration in (a) healthy controls (n = 55) and (b) subjects with remitted lifetime history of major depression (MD) (n = 61). *FKBP5* mRNA in unstressed control subjects (dotted lines) is additionally included as a reference. The figure shows estimated means with standard error bars and *P*-values from pointwise comparisons of the *FKBP5* genotype effect within the control group.

(*P* = .087). Healthy controls with the CC genotype showed higher *FKBP5* mRNA levels after stress exposure than subjects carrying the TT or CT genotype (Figure 2a). There were no genotype effects in the remitted MD group (Figure 2b). Pointwise comparison by *FKBP5* genotype showed a significant group effect for the CC genotype +45 minutes (*P* = .049) and +70 minutes poststress (*P* = .003). Healthy subjects with the CC genotype showed higher *FKBP5* mRNA levels than subjects with a lifetime history of MD and this genotype.

DNA Methylation of Intron 7 in the FKBP5 Gene

Following Klengel et al. (2013), we analyzed DNA methylation in intron 7 of the *FKBP5* gene and evaluated differences between groups and *FKBP5* genotype in n = 96 subjects. In this preliminary analysis, we found a significant group × genotype interaction ($F_{2,84} = 3.12$, *P* = .049) for the mean *FKBP5* DNA methylation in this region in intron 7. Posthoc comparisons did not reach significance but suggested a nonsignificant trend toward genotype-dependent CpG methylation differences among subjects with a lifetime history of MD (*P* = .096). Furthermore, there was a nonsignificant trend for a group effect in subjects carrying the TT genotype (*P* = .096). Subjects with the TT genotype and a lifetime history of MD had a 10% higher DNA methylation rate than healthy controls with the same *FKBP5* genotype (Figure 3).

Discussion

To investigate the role of genetic and genomic markers of the stress regulator *FKBP5*, we performed the TSST in 116 participants from an epidemiological sample with a positive (n = 61) and a negative (n = 55) lifetime history of MD. Given the converging findings from previous studies, we focused on the effects of the intronic *FKBP5* variant rs1360780, on *FKBP5* mRNA expression in peripheral blood cells, and on *FKBP5* intron 7 CpG methylation. Three major findings could be observed. First, we found a genotype-dependent plasma cortisol response to psychosocial stress exposure in healthy subjects with the highest and longest-lasting cortisol increase in subjects with the TT genotype of rs1360780, whereas no genotype-dependent effects were detected in participants remitted from MD. Second, healthy controls with the CC genotype showed an increase of *FKBP5* mRNA following stress, whereas controls with the CT genotype or the TT genotype responded with a blunted *FKBP5* mRNA expression after psychosocial stress. Again, we could not observe genotype effects in subjects with a lifetime history of MD. Third, a preliminary analysis of the CpG methylation around GREs in intron 7 of the *FKBP5* gene suggested depression and genotype-specific differences in *FKBP5* DNA methylation. Our results point to an important role of the *FKBP5* genotype and *FKBP5* gene activity for the HPA axis response to psychosocial stress. Expanding upon

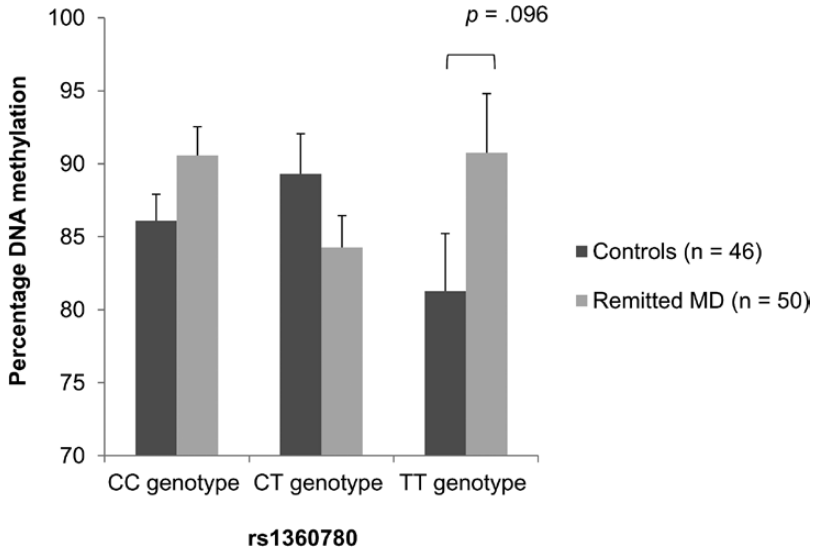

Figure 3. CpG methylation rate of *FKBP5* intron 7 between groups and *FKBP5* genotype. The figure shows estimated means with standard error bars and *P*-values from pairwise comparison of group effects for the TT genotype. MD, lifetime history of major depression. Rs1360780 genotype frequencies: Healthy controls: CC genotype n = 27, CT genotype n = 13, TT genotype n = 6; Remitted depression: CC genotype n = 24, CT genotype n = 20, TT genotype n = 6.

previous research, we could demonstrate that these effects distinctly differ between subjects with a lifetime history of MD and healthy controls.

Subjects remitted from depression and healthy controls showed different genotype effects of *FKBP5* on the plasma cortisol response to psychosocial stress. Healthy individuals with the rs1360780 TT genotype responded with increased plasma cortisol concentrations and a delayed cortisol peak in the recovery period after exposure to psychosocial stress. This genotype is known to be related to an impaired sensitivity of the GR as indicated by an escape from the suppressive effects of dex (Binder et al., 2008; Touma et al., 2011) and to a higher vulnerability to stress-related disorders in traumatized subjects including depression (Appel et al., 2011; Zimmermann et al., 2011) and PTSD (Binder et al., 2008; Klengel et al., 2013). This finding replicates our previous study using the same psychosocial stress test in a different sample of healthy controls (Ising et al., 2008). Healthy subjects with the rs1360780 TT genotype show a markedly increased and prolonged cortisol response to psychosocial stress, presumably as a result of a reduced sensitivity of the GR, and thus an impaired negative feedback inhibition of the HPA axis, which, in turn, contributes to the reported higher vulnerability to stress-related disorders. No genotype effects on ACTH could be observed, which is in agreement with previous reports (eg, Ising et al., 2008), suggesting cortisol as the most sensitive stress hormone regarding *FKBP5* effects.

FKBP5 affects the GR function by influencing the nuclear translocation (Wochnik et al., 2005) and binding affinity (Denny et al., 2000), and it is also part of an ultra-short feedback loop with the GR, which is an important mechanism in the restoration of the HPA axis after stress exposure (Binder, 2009). *FKBP5* mRNA can be upregulated by glucocorticoids (Jääskeläinen et al., 2011); thus, one would expect an increase of *FKBP5* mRNA following cortisol release after exposure to laboratory psychosocial stress. Indeed, oral administration of 1.5 mg of dex, a synthetic glucocorticoid with high affinity to the GR, resulted in a more than 5- to 10-fold stimulation of *FKBP5* expression in peripheral blood cells after 3 hours (Menke et al., 2013). However, in our study we did not observe a general induction of *FKBP5* mRNA after psychosocial stress. The reasons for the absence of a general *FKBP5* mRNA induction might be 2-fold: (1) oral administration of 1.5 mg of dex results in a nonphysiologically fast and intensive GR stimulation, whereas GR stimulation in a psychosocial stress situation is gradual and moderate; (2) dex directly stimulates peripheral GR, whereas the stress situation in the laboratory activates GR indirectly in response to the stress-induced cortisol surge. Furthermore, GR effects are restricted to peripheral GRs as dex hardly passes the blood-brain barrier (Meijer et al., 1998), while psychosocial stress by nature is perceived and processed in the central nervous system. Even though we did not find a general *FKBP5* mRNA response to psychosocial stress, we found an interaction between the *FKBP5* genotype and depression history on *FKBP5* expression. Healthy subjects with the CC genotype, which is associated with normal GR function (Binder et al., 2008; Ising et al., 2008), responded with a significant induction of *FKBP5* mRNA after psychosocial stress. Whereas the TT genotype seems to predispose healthy subjects to a resistant and nonadaptive stress response regulation resulting in an increased and extended cortisol response, the CC genotype is associated with a more adaptive response pattern: the cortisol response seems to be situationally adequate and is accompanied by an adaptive upregulation of *FKBP5*.

To our surprise, *FKBP5* genotype-related effects on plasma cortisol and *FKBP5* mRNA response to psychosocial stress were observed only in healthy controls. Previous studies reported *FKBP5* genotype-related differences on HPA axis responsivity in acutely depressed patients (Binder et al., 2004; Menke et al., 2013) and healthy controls (Binder et al., 2008; Ising et al., 2008), but, to the best of our knowledge, there are no functional studies on subjects with remitted depression. In our study, participants remitted from depression did not show a significant upregulation of *FKBP5* mRNA but presented with a regular cortisol response after psychosocial stress; both effects were independent of the *FKBP5* genotype. As rs1360780 is related to antidepressant action and course of disease (Binder et al., 2004; Kirchheiner et al., 2008; Lekman et al., 2008), it is possible that the remission from the disease might have resulted in changes of the *FKBP5*-dependent stress regulation in these patients. In particular, patients carrying the rs1360780 T risk allele are known to have a faster response to antidepressant medication and a stronger cortisol-dependent regulation of *FKBP5* mRNA (Binder et al., 2004), further supporting this assumption.

However, it remains unclear as to whether the history of a depressive episode or previous antidepressant medication might be responsible for the altered effects of *FKBP5* on the stress regulation. Recent discussions point to an interpretation of the T allele being the environment-sensitive haplotype compared with the CC genotype and therefore, it could be influenced more by positive and negative environments (Klengel and Binder, 2013). In our study, the increased cortisol response of healthy subjects with the TT genotype, which was previously shown in other samples (Binder et al., 2008; Ising et al., 2008), vanished in subjects with a lifetime history of MD. It seems that episodes of MD or overcoming depression lead to a long-lasting change of *FKBP5*-mediated vulnerability that is displayed by a normalized cortisol regulation after psychosocial stress in these subjects.

Changes in the FKBP5 methylation pattern seem to play an important role in this context. Klengel et al. (2013) recently demonstrated a molecular mechanism of a gene environment interaction between childhood trauma and PTSD risk resulting from a rs1360780 allele specific demethylation of FKBP5 intron 7. Demethylation was associated with increased stress-dependent gene transcription followed by a long-term dysregulation of the HPA axis, which resulted in a higher risk of developing PTSD. In our study, we observed a significant interaction effect between the lifetime history of depression and the rs1360780 genotype with respect to the methylation rate of intron 7 in the FKBP5 gene. However, posthoc tests failed to show significant group differences, even though a nonsignificant trend toward an increased methylation in subjects with the TT genotype who remitted from depression was found. A posthoc power analysis based on the observed interaction effect revealed a power of .64 for the current analysis, which falls short of the recommended power threshold of .80/.90 (data not shown). Therefore, the failure to achieve a significant result in the posthoc tests can be related to a limited power of the analysis. Nevertheless, the suggestive trend corresponds to the observed group effect on the plasma cortisol response to psychosocial stress in subjects with the TT genotype as well as to a recent report by Yehuda et al. (2013), which found that low FKBP5 methylation in the promoter region of the gene is associated with higher cortisol levels in a sample of patients with PTSD. Given the limited power of the current DNA methylation analysis, these results should be considered as preliminary.

A strength of our study is that participants on current antidepressant medication were excluded from our analysis. Therefore, the gene expression results could not be confounded by current antidepressant medication, which is known to be associated with *FKBP5* gene expression (Wagner et al., 2012; Cattaneo et al., 2013). However, we do not have reliable information about prior antidepressant treatment that could have contributed to the diminished genotype-dependent effects in remitted depression. Also, childhood trauma could have modulated the outcome of the study. 13 controls and 14 subjects with remitted MD reported childhood adverse events; however, only one of them had the TT genotype (data not shown). Thus, a separate analysis by genotype × trauma was not suitable for our sample. Excluding the case with childhood trauma and TT genotype did not influence the significant interaction effects of cortisol and FKBP5 mRNA; however, the preliminary analysis of DNA methylation failed to show a significant effect ($P = .054$). Therefore, the history of childhood adverse events could have influenced in particular the DNA methylation analysis and should be included as a factor in future studies. Another limitation is the small sample size, especially of those participants with the rs1360780 TT genotype. In particular, results on mRNA and DNA methylation should be regarded as exploratory and require replication in independent samples. Finally, our study followed a strictly hypothesis-driven approach focusing on the role of *FKBP5* genetics, mRNA expression, and DNA methylation of *FKBP5* intron 7 as specific functional elements of the HPA axis regulation on the psychosocial stress. The genetic analysis was restricted to the rs1360780 polymorphism, as a large number of studies have described substantial effects of this specific *FKBP5* variant on HPA axis regulation and depression risk. However, regarding the absence of *FKBP5* effects in remitted depression, we cannot exclude that other *FKBP5* variants or methylation of different regions within the *FKBP5* gene might also contribute to stress response regulation in these subjects.

In summary, we found evidence of an adaptive negative feedback mechanism in healthy controls with the *FKBP5* rs1360780 CC genotype, reflected by a normal cortisol surge after psychosocial stress accompanied by an *FKBP5* mRNA upregulation in the expected direction. Healthy controls with the TT genotype, however, showed signs of GR resistance as indicated by a steeper stress-induced plasma cortisol increase and a slower recovery, corresponding to a low methylation rate of the intron 7 region within the *FKBP5* gene potentially contributing to the observed GR resistance. In remitted depressed individuals, we did not find genotype-dependent effects of rs1360780 with regard to plasma cortisol and *FKBP5* mRNA levels after psychosocial stress. It appears that previous depression or antidepressant treatment alters the role of *FKBP5* in stress response regulation, specifically in patients with the rs1360780 TT genotype. One could hypothesize that antidepressant treatment initiates epigenetic modifications of *FKBP5* and other stress-related genes (Menke et al., 2012a), which in turn contributes to the well-documented observation of an improved stress response regulation after successful depression therapy (Raison and Miller, 2003; Ising et al., 2005; Pariante, 2009). Longitudinal studies are warranted to elucidate the dynamics of *FKBP5*-related alterations before, during, and after depression therapy.

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Statement of Interest

Florian Holsboer and Manfred Uhr are co-inventors of the following patent: "FKBP51: a novel target for antidepressant therapy" (International publication number: WO2005054500). All other authors declare no conflict of interest.

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