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Allele-specific DNA methylation level of *FKBP5* is associated with post-traumatic stress disorder

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ABSTRACT

Background: FK506-binding protein 5 (FKBP5) binds to glucocorticoid receptors and modulates glucocorticoid sensitivity. The *FKBP5* gene has been implicated in the dysregulation of human stress responses, contributing to the risk and treatment response of stress-related disorders. The present study examined whether epigenetic changes in *FKBP5* are associated with chronic post-traumatic stress disorder (PTSD) status in the context of *FKBP5* genetic variation (rs1360780 polymorphism) among male veterans exposed to combat trauma.

Methods: Korean male veterans who served on active duty during the Vietnam War were categorized into 2 groups: with PTSD (n = 123) and without PTSD (n = 116). The genotype of *FKBP5* rs1360780 and DNA methylation levels of two CpG sites at the *FKBP5* intron 7 region were assessed in peripheral blood. Analysis of covariance was performed to examine main and interaction effects of PTSD status and *FKBP5* genotype on *FKBP5* DNA methylation level, with age, trauma levels, and alcohol use as covariates.

Results: A significant main effect of *FKBP5* rs1360780 and PTSD and an interaction effect between genotype and PTSD status were found on mean *FKBP5* DNA methylation level. The T allele of rs1360780 was associated with lower *FKBP5* methylation level. In addition, the PTSD group showed significantly higher methylation than did the non-PTSD group among veterans carrying the risk T allele (n = 96), while no group difference was observed on methylation levels among veterans with the CC genotype (n = 143). Among veterans carrying the T allele, *FKBP5* methylation levels were positively correlated with the severity of PTSD symptoms.

Conclusions: The present study demonstrated different *FKBP5* methylation levels in PTSD depending on *FKBP5* genetic variation among veterans exposed to combat trauma. The present finding suggests that the genetic and epigenetic modulation of *FKBP5* is involved in the pathophysiology of PTSD. Further longitudinal research involving people exposed to trauma is required to understand causal relationships of *FKBP5* in the development and recovery of PTSD.

1. Introduction

Post-traumatic stress disorder (PTSD), a chronic and debilitating stress-related psychiatric disorder, has characteristic symptoms including re-experiencing traumatic events, avoidance of trauma-related stimuli, negative alterations in cognition and mood, and alterations in arousal after exposure to severe psychological trauma (APA, 2013). Dysfunction of the hypothalamic–pituitary–adrenal (HPA) axis, the central coordinator of the neuroendocrine stress response systems, has been considered a core biological feature of PTSD (Mehta and Binder, 2012; Pervanidou and Chrousos, 2010). Although the pathophysiology of PTSD remains unclear, it is thought to involve a complex interplay between genetic and environmental factors (Koenen et al., 2009; Sheerin et al., 2017). Epigenetic alterations, such as DNA methylation, representing key mechanisms by which environmental factors elicit enduring changes in gene expression, are proposed to be involved in PTSD pathophysiology (Malan-Muller et al., 2014; Voisey et al., 2014; Zannas et al., 2015; Zovkic and Sweatt, 2013).

Accumulating evidence suggests that genetic factors involving the HPA axis and glucocorticoid responsivity react with severe stressors, contributing to changes in gene expression and functional alterations in stress-related neurobiological systems and PTSD development (Mehta and Binder, 2012). FK506-binding protein 5 (FKBP5), a major regulatory protein of the HPA axis, is emerging as an important molecule in PTSD pathophysiology (Klengel et al., 2013; Watkins et al., 2016; Zannas et al., 2016). FKBP5 binds to glucocorticoid receptors (GRs) and

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modulates GR sensitivity in response to stressors, leading to the regulation of the HPA axis (Binder, 2009; Lekman et al., 2008; Matosin et al., 2018; Roy et al., 2010). An enhanced expression of *FKBP5*, located on the short arm of chromosome 6, has been reported to be significantly associated with reduced GR sensitivity, leading to impaired HPA negative feedback and chronic stress response (Scammell et al., 2001; Vermeer et al., 2003; Westberry et al., 2006). Moreover, genetic variants of *FKBP5* have been implicated in the dysregulation of human stress responses, contributing to the risk of psychiatric disorders including depression (Rao et al., 2016), alcohol use disorders (Huang et al., 2014; Qiu et al., 2016), and PTSD (Watkins et al., 2016) as well as treatment response (Binder et al., 2004; Wilker et al., 2014).

In particular, single-nucleotide polymorphism (SNP) rs1360780 of *FKBP5*has been reported to have functional effects, such as HPA axis regulation (Binder et al., 2004; Fujii et al., 2014; Klengel and Binder, 2015; Klengel et al., 2013). The risk T allele of rs1360780 was found to be associated with peritraumatic dissociation (Koenen et al., 2005) and a risk of PTSD (Klengel et al., 2013; Watkins et al., 2016). Individuals with T allele of rs1360780 have an altered chromatin structure after childhood trauma exposure, leading to greater GR-induced FKBP5 upregulation and HPA axis dysregulation (Klengel et al., 2013). An imaging genetics study showed that subjects carrying the T allele of rs1360780 had attention bias for threat as well as increased hippocampal activation in response to threat (Fani et al., 2013). A recent study involving Gulf War veterans showed that the T allele of rs1360780 and child abuse were separately associated with PTSD status, and that the risk allele was also associated with lower cortisol levels (Young et al., 2018). Klengel et al. suggested that allele-specific epigenetic changes in *FKBP5* are related to genes by environment interactions, contributing to long-term adverse effects of stress on neurobiological functions and development of stress-related psychiatric disorders (Klengel and Binder, 2015). There is scarce information on the relationship of allele-specific epigenetic changes in *FKBP5* and PTSD.

The present study aimed to investigate whether epigenetic changes in *FKBP5* are associated with chronic PTSD status in the context of *FKBP5* genetic variation (rs1360780 polymorphism) among male veterans exposed to combat trauma after adjustment for possible confounders, including age, trauma exposure levels, and alcohol use. Moreover, the potential confounding effect of antidepressant use was considered with respect to the *FKBP5* methylation level. Our main hypothesis was that *FKBP5* methylation levels would be different in patients with PTSD depending on the *FKBP5* risk allelic status.

2. Materials and methods

2.1. Participants

In total, 256 male veterans were recruited from the Veterans Health Service (VHS) Medical Center (Seoul, South Korea). All subjects underwent a structured, face-to-face interview according to Diagnostic and Statistical Manual of Mental Disorders IV-Text Revision (APA, 2000) by a trained psychiatrist. Inclusion criteria were combat veterans who served on active duty in the Korean Armed Forces during the Vietnam War (1964–1973) and ethnically Korean men. Subjects with a history of head trauma and organic brain syndrome including cerebrovascular accidents or dementia, psychosis or bipolar disorder, or substance-related disorders other than alcohol and nicotine abuse were excluded. The study was approved by the Institutional Review Board of the VHS Medical Center. All subjects provided written informed consent before participation in the study.

2.2. Clinical assessments

Clinician-Administered PTSD Scale (CAPS), a structured clinical interview for PTSD, was used for assessing PTSD symptoms and determining PTSD status (Blake et al., 1995; Weathers et al., 2001, 1999). Current PTSD status was determined by current CAPS and DSM-IV criteria. Non-PTSD group was free from current and lifetime diagnosis of PTSD. Combat Exposure Scale (CES) was also used for assessing the subjective stress level of wartime stressors (Keane et al., 1989). The total CES score was calculated by using a sum of weighted score. In addition, Alcohol Use Disorders Identification Test (AUDIT) was applied to assess hazardous and harmful alcohol use (Babor et al., 2001). Harmful alcohol use was defined as a score of ≥ 12 using the validated Korean version of AUDIT (Lee et al., 2000).

2.3. Genotyping of FKBP5 variants

rs1360780 polymorphism on the *FKBP5* locus was selected, based on previous studies on *FKBP5* (Binder et al., 2008; Fujii et al., 2014; Kang et al., 2012; Klengel et al., 2013). The genotyping method and primer pairs for the assay were identical to those in our previous study on stress responses in cancer patients (Kang et al., 2012). The genotype frequencies of rs1360780 was in accordance with Hardy-Weinberg equilibrium.

2.4. Pyrosequencing of the targeted FKBP5 region

Genomic DNA was isolated from whole blood cells using standard techniques for DNA methylation analysis of *FKBP5* intron 7. Two CpG sites in the CpG-rich region of *FKBP5* intron 7 were targeted based on

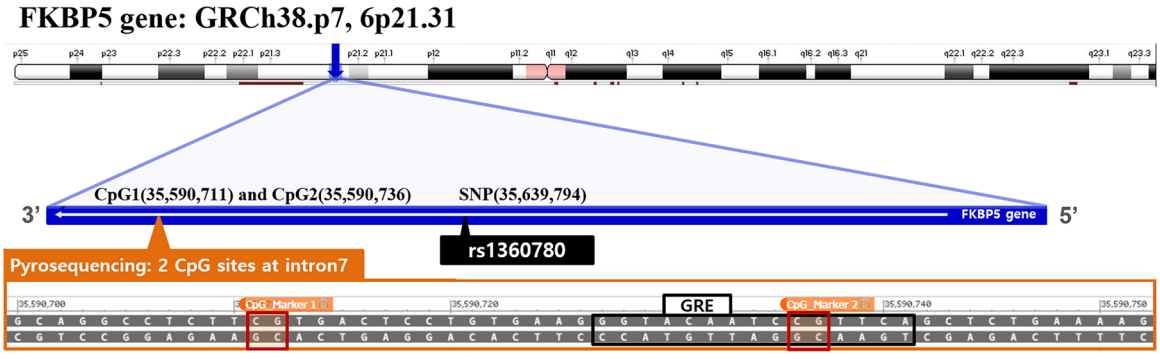

Fig. 1. Schematic representation of targeted single-nucleotide polymorphism (SNP) and two cytosine-phosphate-guanine (CpG) sites selected for DNA methylation analysis of the *FKBP5* structure of chromosome 6, based on the Genome Reference Consortium Human Build 38 patch release 7 primary assembly in the NCBI Variation Viewer. The two CpG sites analyzed based on the proximity to glucocorticoid response element (GRE, black box) at intron 7 are shown in red boxes (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 1
Sociodemographic and clinical characteristics of subjects with and without current PTSD.

	PTSD (N = 123)	Non-PTSD (N = 116)	T or χ^2	*p*
Age	63.11 ± 3.54	62.66 ± 4.19	0.89	0.37
Education (years)	10.49 ± 2.75	10.72 ± 2.75	−0.66	0.51
Marital status Married/Other, n	105/18	107/9	2.82	0.093
Socioeconomic status High/Medium/Low, n	25/55/43	19/56/41	0.67	0.72
CAPS				
Total score	62.70 ± 22.24	9.31 ± 11.72	23.40	< 0.001
Re-experiencing	20.72 ± 8.58	4.33 ± 7.63	15.62	< 0.001
Avoidance	21.28 ± 10.14	1.68 ± 3.47	20.23	< 0.001
Hyperarousal	20.69 ± 8.19	3.38 ± 4.29	20.63	< 0.001
CES score	20.48 ± 7.16	14.14 ± 7.61	6.64	< 0.001
AUDIT score	11.93 ± 11.03	6.73 ± 7.47	4.29	< 0.001
Harmful alcohol use Yes/No, n	60/63	32/84	11.33	0.001
Use of antidepressants Yes/No, n	76/47	18/98	56.57	< 0.001
Genotypes of the rs1360780, n				
CC	72	71	0.230	0.892
CT	41	37		
TT	10	8		
HWE	0.239	0.305		

CAPS, Clinician-Administered PTSD Scale; CES, Combat Exposure Scale; AUDIT, Alcohol Use Disorders Identification Test; HWE, *p*-value for Hardy-Weinberg equilibrium.

previous studies (Han et al., 2017; Klengel et al., 2013), which were located between 35,590,711 and 35,590,741 of Chr 6 based on Genome Build 38 of NCBI human reference assembly (Fig. 1). CpG1 (Chr 6: 35,590,711) and CpG2 (Chr 6: 35,590,736) corresponded to +52,080 and +52,105 relative to the transcriptional start of exon 6 (CpG1 = +52,080 and CpG2 = +52,105), respectively. Polymerase chain reaction (PCR) was performed in a volume of 20 μL with 20 ng or more converted DNA, 2.5 μL of 10 × Taq buffer, 5 U Hot/Start Taq polymerase (Enzynomics, South Korea), 2 μL of each 2.5 mM dNTP mixture, 1 μL of 10 pmol/μl Primer-S, and 1 μL of 10 pmol/μL biotinylated-Primer-As. The amplification was performed according to the general guidelines of the pyrosequencing method using primers (forward primer, 5′- TGGGATAATAATTTGGAGTTATAGTGTA-3′ and biotinylated reverse primer, 5′-AAGAACAAGTCTAGGAACAAATAAGGGAAC-3′) and the following cycling conditions: denaturation at 95 °C for 10 min, followed by 45 cycles at 95 °C, 53 °C, and 72 °C, each for 30 s, followed by a final extension cycle at 72 °C for 5 min. The PCR product (2 μL) was electrophoresed on 2% agarose gel (SeaKem LE Agarose) and visualized by ethidium bromide staining. Single-stranded DNA templates were prepared from 16 to 18 μL of biotinylated PCR products using streptavidin-coated Sepharose® HP beads (Amersham Biosciences, Sweden) and following the PSQ 96 sample preparation guide for multichannel pipettes. Fifteen picomoles of the sequencing primer (5′- ACTTGGAGCCACAGTGCAGGCCTC-3′) was used for analyses. The percentages of individual methylation at two CpG sites were calculated. For internal quality control, the analysis of a non-CpG cytosine during pyrosequencing was included. Sequencing was performed using a PyroMark ID system with the Pyro Gold reagents kit (Qiagen). The pyrosequencing procedures were performed using the service of Genomictree, Inc. (Daejeon, South Korea).

2.5. Statistical analyses

All statistical analyses were performed using Statistical Package for the Social Sciences Version 23.0 (SPSS Inc., Chicago, IL, USA). Sociodemographic and clinical characteristics between the groups were compared using the chi-squared or Student's *t*-test. For the genotype groups of rs1360780, we compared the low-risk allele group with homozygous C allele and the high-risk allele group carrying the risk T allele (CT + TT) based on a risk allele carrier model. A 2-way analysis of covariance (ANCOVA) was performed to determine the main and interaction effects of PTSD status (PTSD or non-PTSD) and genotype of rs1360780 (CC or CT/TT) on mean *FKBP5* DNA methylation levels in the intron 7 region. Assuming that the methylation of CpGs around a transcription factor-binding site is a functional unit (Klengel et al., 2013), combined effect of the two CpG sites was considered by using the mean value in analysis. Covariates for the basic ANCOVA model included age, the CES score and harmful alcohol use. Partial eta squared (η_p^2) values were calculated as estimates of effect size, considering that a partial eta-squared of 0.01 was small, 0.04 moderate, and 0.1 large (Huberty, 2002). In another model, we conducted ANCOVA with antidepressant use as a further covariate in addition to age, the CES, and alcohol use. In case of a significant interaction between the factors, *post-hoc* ANCOVA were performed to determine the association of PTSD status with *FKBP5* DNA methylation levels according to *FKBP* genotypes. Moreover, partial correlation after controlling for same potential confounders (age, CES, and alcohol use) were performed between mean *FKBP5* methylation levels and clinical variables (CAPS total score and three symptom clusters of re-experiencing, avoidance, and hyperarousal). All statistics were two-tailed, and statistical significance was set at $p < 0.05$. For correlation analyses, we adjusted *p* values using Bonferroni approach for the four tests, while considering an alpha level of 0.05/4 ($p < 0.0125$) as statistically significant.

3. Results

Among 256 participants, 239 with complete clinical and genetic data sets were included in the final analysis. Using the CAPS interview, the subjects were categorized into 2 groups: with PTSD (n = 123) and without PTSD (n = 116). Sociodemographic and clinical characteristics of the subjects are presented in Table 1. The mean age in the final sample was 63.11 years (standard deviation; SD = 3.54 years) for the PTSD group and 62.66 years (SD = 4.19 years) for the non-PTSD group. No statistically significant differences were observed between the two groups in sociodemographic characteristics including age, education, marital status, and socioeconomic status.

With respect to combat trauma exposure, the PTSD group was more likely to be associated with exposure to severe trauma based on the CES score ($p < 0.001$). In addition, 48.8% of patients with PTSD were classified as harmful drinkers, while 27.6% of people without PTSD were classified in this category ($p < 0.001$). In the present sample, 61.8% and 15.5% of subjects with and without PTSD, respectively ($p < 0.001$), were taking antidepressants.

With respect to rs1360780 polymorphism, the genotype distribution was CC (58.5%), CT (33.3%), and TT (8.1%) in the PTSD group and CC (61.2%), CT (31.9%), and TT (6.9%) in the non-PTSD group. The genotype frequencies of rs1360780 were in Hardy-Weinberg equilibrium in both groups. Subjects with high-risk alleles (CT + TT) accounted for 41.5% in the PTSD group and 38.8% in the non-PTSD group. The genotype and allele frequencies of rs1360780 were not significantly different between the PTSD and non-PTSD groups (Table 1).

ANCOVA with age, the CES, and harmful alcohol use as covariates revealed significant main effects of the PTSD status ($F = 6.322$, $p = 0.013$) and rs1360780 genotype ($F = 7.280$, $p = 0.007$) as well as a significant interaction effect between the PTSD status and genotype on the *FKBP5* DNA methylation level ($F = 4.424$, $p = 0.037$; Table 2 and Fig. 2). Controlling additionally for antidepressant use did not change the results of the interaction effect ($F = 4.488$, $p = 0.035$) and main effect of the genotype ($F = 6.950$, $p = 0.009$), but weakened the main effect of the PTSD status ($F = 2.779$, $p = 0.097$). *Post-hoc* comparisons revealed that subjects with PTSD showed significantly higher

Table 2
Main and interaction effects on *FKBP5* DNA methylation levels by ANCOVA (N = 239).

Variable	ANCOVA				
	df	Mean Square	F	*p*-value	η_p^2
Main effect					
PTSD status	1	19.02	6.322	0.013	0.027
rs1360780 genotype (CC or CT/TT)	1	21.90	7.280	0.007	0.030
Covariates					
Age	1	10.51	3.493	0.063	0.015
CES	1	6.29	3.704	0.149	0.009
Harmful alcohol use	1	2.31	0.767	0.382	0.003
Interaction effect					
PTSD status x Genotype	1	13.31	4.424	0.037	0.019
Error	232	3.01			

Abbreviations: ANCOVA Analysis of covariance; df degrees of freedom; CES Combat Exposure Scale Age, CES, and harmful alcohol use were used as covariates in ANCOVA. Effect sizes were presented using partial eta squared (η_p^2); 0.01, 0.04, and 0.1 are considered, respectively, small, moderate, and large effect sizes.

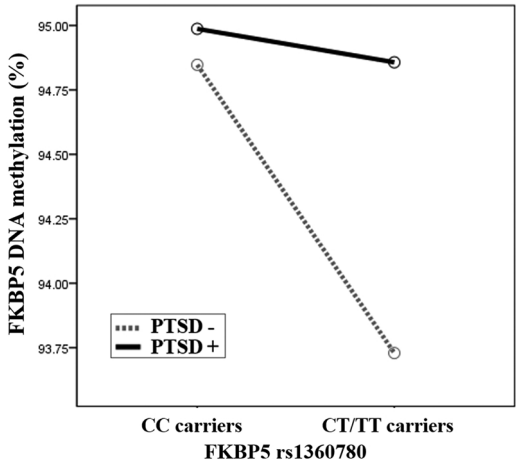

Fig. 2. DNA methylation levels at *FKBP5* intron 7 between PTSD status and genotype of FKBP5 rs1360780. A significant interaction effect between PTSD status and genotype (p = 0.037) was found for the mean FKBP5 methylation level. *Post-hoc* comparisons showed that the PTSD group showed significantly higher methylation than did the non-PTSD group among veterans with the CT/TT genotype (n = 96), while there was no group difference with respect to methylation levels among those with the CC genotype (n = 143).

methylation than those without PTSD among veterans carrying the risk T allele (F = 9.452, $p = 0.003$), while there was no group difference with respect to methylation levels among those with the CC genotype (Fig. 2).

In subjects carrying the risk T allele ($n = 96$), mean DNA methylation levels at *FKBP5* intron 7 were positively correlated with the total score ($r = 0.285$, $p = 0.006$), avoidance ($r = 0.299$, $p = 0.004$), and hyperarousal symptom scores ($r = 0.282$, $p = 0.006$) of CAPS after controlling for age, CES, and alcohol use (Fig. 3). For re-experiencing cluster, the correlation did not reach statistical significance ($r = 0.203$, $p = 0.051$).

4. Discussion

The present study showed that the DNA methylation level at *FKBP5* intron 7 is associated with the current PTSD status in the presence of the risk allele of *FKBP5* rs1360780 in a relatively homogenous group of male veterans exposed to combat trauma. Significant main and interaction effects were observed between the genotype and PTSD status on the *FKBP5* DNA methylation level. The present finding suggests that allele-specific DNA methylation level of *FKBP5* is involved in PTSD pathophysiology.

We found a significant independent main effect of *FKBP5* rs1360780 on the *FKBP5* methylation level, even after adjustment for antidepressant use. This finding indicates that the mean methylation level at *FKBP5* intron 7 is reduced in subjects carrying the risk T allele of *FKBP5* rs1360780 compared those carrying the CC allele. The T allele of rs1360780 has been previously reported to be associated with enhanced *FKBP5* expression, reduced GR sensitivity, and an insufficient recovery of cortisol activation in response to stress (Matosin et al., 2018). Because lower DNA methylation levels at *FKBP5* intron 7 can be assumed to reflect higher *FKBP5* expression (Klengel et al., 2013), our finding regarding the association between the T allele of rs1360780 and lower *FKBP5* methylation levels is relevant to previous findings that showed associations of the T allele with enhanced *FKBP5* mRNA and protein levels (Binder et al., 2004). Binder and colleagues reported that 1360780, the closest variant to a functional GRE, was associated with differential induction of *FKBP5* following GR activation and that individuals carrying the risk T allele were associated with a more exaggerated *FKBP5* mRNA response in context of early life trauma exposure across multiple ethnicities using the 1000 Genomes project data (Klengel et al., 2013). They proposed that allele-specific, early life trauma-dependent DNA demethylation and upregulation in *FKBP5*, creating reduced GR sensitivity, confers a risk of stress-related conditions such as depression and PTSD in adulthood.

In addition, we found a significant main effect of PTSD on the *FKBP5* methylation level after adjustment for age, trauma exposure, and alcohol use, showing that subjects with PTSD have higher methylation levels than those without PTSD. When considering an inverse association between the *FKBP5* methylation level at intron 7 and *FKBP5* gene expression (Klengel et al., 2013) and the negative effects of enhanced FKBP5 expression on GR sensitivity (Scammell et al., 2001), although we did not assess gene expression and GR sensitivity, our observation of higher methylation levels in PTSD may reflect reduced *FKBP5* expression and enhanced GR sensitivity in PTSD subjects. Because chronic stress can increase *FKBP5* expression and the T allele of *FKBP5* has been reported to be associated with *FKBP5* demethylation/GR insensitivity and contribute to psychiatric risk (Scammell et al., 2001), our result regarding increased *FKBP5* methylation in PTSD seems to be apparently contrasting and might represent compensatory changes in PTSD patients. However, the present finding is in accordance with accumulating evidence of enhanced GR sensitivity in PTSD, in contrast to reduced GR sensitivity in depression (Rohleder et al., 2010; Yehuda, 2009). A recent postmortem study showed 3.5-fold downregulation in *FKBP5* expression in PTSD (Holmes et al., 2017), which also supports enhanced GR sensitivity in PTSD.

Notably, as shown in Table 2 and Fig. 2, a significant interaction effect between *FKBP5* rs1360780 genotype and PTSD status was found on *FKBP5* methylation levels, which indicated an association of allele-specific *FKBP5* methylation with PTSD. No difference was shown in *FKBP5* methylation levels between PTSD and non-PTSD groups without the risk T allele of *FKBP5* rs1360780, while, among veterans carrying the risk allele, those with PTSD showed a significantly higher methylation level than those without PTSD, and the *FKBP5* methylation levels were positively correlated with the severity of PTSD symptoms. These findings suggest that lower *FKBP5* expression and enhanced GR sensitivity may play an important role in the pathophysiology or course of PTSD, especially in the context of the presence of the risk allele of *FKBP5* rs1360780. In line with our findings, Yehuda et al., in their genetic studies involving survivors of the World Trade Center attacks, demonstrated lower *FKBP5* expression in PTSD group than in non-PTSD group (Yehuda et al., 2009), and lower *FKBP5* expression was associated with the *FKBP5* risk allele, lower plasma cortisol, and greater PTSD symptom severity (Sarapas et al., 2011). In addition, a genetic study of the effects of PTSD on GR sensitivity revealed genotype-

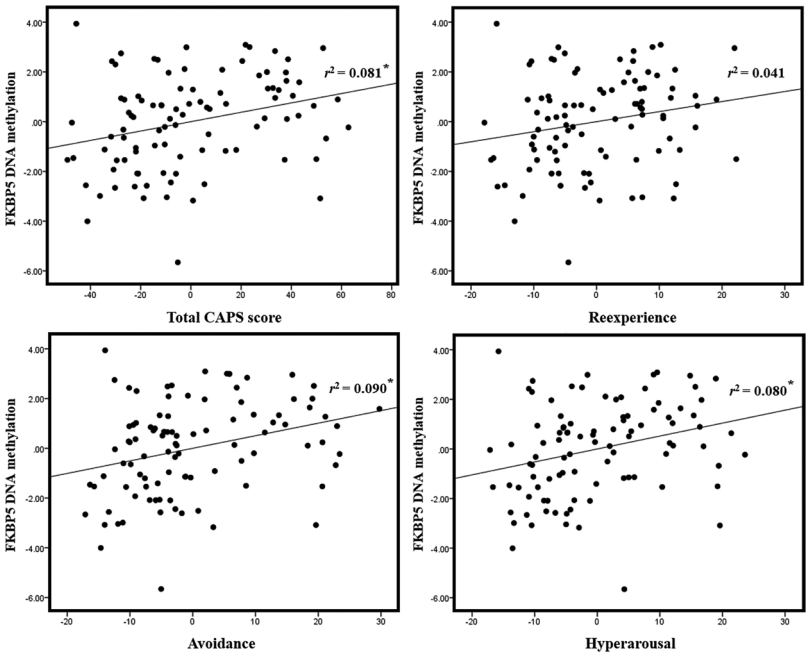

Fig. 3. Scatter plots showing correlations between PTSD symptom scores of CAPS and *FKBP5* DNA methylation levels in veterans carrying the risk T allele of rs1360780 ($n = 96$). Data are partial correlation after controlling for age, combat exposure level, and harmful alcohol use. Asterisk (*) indicates significance at $p < 0.0125$.

dependent differences in endocrine and GR with PTSD symptoms, in which enhanced GR sensitivity was observed only in PTSD subjects carrying the risk allele of FKBP5 SNP rs9296158 (Mehta et al., 2011). The authors suggested that a network of genes associated with PTSD symptom severity are differentially modulated depending on the FKBP5 genotype and GR sensitivity (Mehta et al., 2011). These findings suggest that the risk allele of *FKBP5* in PTSD affects increased *FKBP5* methylation, reduced *FKBP5* expression, and enhanced GR sensitivity by interacting with trauma exposure and other stress-responsive molecules, contributing to the mechanisms underlying subsets of PTSD.

On the other hand, while the PTSD status was associated with an altered methylation level of *FKBP5* depending on rs1360780, the genotype distribution of rs1360780 did not significantly vary between the PTSD and non-PTSD groups. This suggests that the risk allele of FKBP5 alone is not likely enough to confer a risk for PTSD. Previously, the T allele of rs1360780, interactively with childhood trauma as well as directly, was found to be associated with a risk factor for PTSD (Binder et al., 2008; Klengel et al., 2013; Watkins et al., 2016) as well as depression (Appel et al., 2011; Rao et al., 2016). A few studies have reported a main effect of the *FKBP5* risk allele on stress-related psychiatric disorders, but stronger evidence suggests that combined effects of environmental factors such as adverse life events on the risk allele influence individual stress vulnerability and resilience over lifetime, as well as contribute to the development and recovery of psychiatric disorders (Matosin et al., 2018; Zannas et al., 2016). Klengel et al. showed that allele-specific *FKBP5* demethylation mediates gene-childhood trauma interactions on the risk of stress-related psychiatric disorders such as PTSD and depression, leading to a differential FKBP5 transcriptional activation and HPA axis dysregulation in response to childhood trauma (Klengel et al., 2013). In addition, *FKBP5* expression is considered to be modulated by interaction with various molecules involved in stress response (Matosin et al., 2018). Altogether, the risk allele of FKBP5 could be associated with altered GR sensitivity and dysregulated HPA axis responses by interacting environmental factors, but the directions may be subject to trauma exposure, developmental periods, other genetic factors such as stress-responsive molecules, and disease context.

The study has several potential limitations. First, because it is difficult to directly assess DNA methylation patterns in the brain tissue in human studies, we measured *FKBP5* methylation levels only in peripheral blood, which was assumed to reflect DNA methylation patterns in the brain. Although changes in DNA methylation are tissue-specific, significant relationships were reported between DNA methylation levels of *FKBP5* in peripheral blood and its DNA methylation and expression levels in the hippocampus (Ewald et al., 2014). The finding suggests that DNA methylation levels in blood are a useful surrogate for those in the brain. Second, approximately 60% of patients with PTSD in the present study were taking antidepressants. Although our results still remained significant after controlling for antidepressant use, the results of *FKBP5* DNA methylation may have been confounded by the possible HPA axis modulating effect of antidepressant treatment (Cattaneo et al., 2013). Therefore, replication of our findings in drug-naïve traumatized people is warranted. Third, the cross-sectional nature of the present study does not allow for causality and directions about their relationship between PTSD status and *FKBP5* DNA methylation levels. Although *FKBP5* methylation levels were applied as an outcome variable in the present study of veterans who were exposed to combat trauma approximately 45 years before, it cannot be concluded whether the observed DNA methylation reflects a consequence of chronic PTSD and cumulating stress responses or a moderating/mediating factor for developing PTSD. Therefore, it is required to longitudinally assess changes in *FKBP5* DNA methylation levels over time, including before and after trauma exposure, to better understand the epigenetic mechanism of *FKBP5* in PTSD pathophysiology. Fourth, since our main purpose was to determine the associations of PTSD status with *FKBP5* after controlling for the effect of trauma exposure itself, we employed combat-exposed subjects without PTSD as the present control group, and we did not recruit trauma-unexposed healthy controls. Without comparing with the results from trauma-unexposed healthy individuals, we could not tell whether the finding of reduced *FKBP5* methylation in T allele-carrying non-PTSD group reflected a healthy state without PTSD or an effect related to trauma exposure of having served in the military or some artifact caused by confounders. Therefore, it is necessary to determine whether there are allele-specific differences in *FKBP5* DNA methylation in trauma-unexposed healthy individuals. In addition, given that DNA methylation differences can be influenced by cell type composition effects (Houseman et al., 2015), it remains unclear whether the epigenetic changes observed are due to the changes in cellular composition of blood between groups. Finally, although bisulfite pyrosequencing is known as a highly sensitive and specific screening technique for DNA methylation analysis, it may often limit to determine small between-group differences of 1% to 2% in a methylation level

accurately, which may make a false positive result or lead to miss detecting a subtle difference. Therefore, results of the present study should be considered as a preliminary finding of *FKBP5* methylation in the Korean male veterans, and replication of independent studies using larger sample sizes are needed.

In summary, we demonstrated differences in *FKBP5* DNA methylation levels in PTSD depending on *FKBP5* genetic variation among veterans exposed to combat trauma. The present findings suggest that genetic and epigenetic factors for *FKBP5* and altered HPA axis are involved in the molecular mechanisms underlying PTSD. Further longitudinal research in individuals exposed to trauma is needed to understand causal relationships of the genetic and epigenetic modulation of *FKBP5* in the development and recovery of PTSD.

Conflict of interest

The authors declare that they have no conflicts of interest.

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