levels, it can be speculated that increase in resistin levels is induced only during certain time period after dexamethasone administration. Further investigation is warranted to better understand the mechanism and time course of the resistin change induced by dexamethasone.

Consistent with a previous finding [9], *RETN* rs3219175 was a strong determinant of plasma resistin levels in our sample. We found no significant interaction between genotype and dexamethasone administration. Therefore, administration of dexamethasone increased the protein and mRNA levels of resistin regardless of the rs3219175 genotype.

The present study was limited by the small number of subjects. However, the results obtained by the microarray analysis were highly significant and the results of the protein analysis were consistent with that of the microarray analysis. Therefore, we believe that the possibility of type I error is low. Another limitation was that quantitative real-time PCR was not performed to confirm the results obtained from microarray analysis. However, the probe corresponding to the *RETN* gene in Agilent Human Genome 4 × 44 K arrays has been previously confirmed as a fully valid reporter [21]. Therefore, the expression levels of *RETN* obtained in the present study are likely to be a reliable data set. Another major limitation was that we measured gene expression and protein levels of resistin only once after dexamethasone administration and did not track the resistin levels over time. Further studies with larger number of subjects are necessary to better determine the influence of dexamethasone administration on resistin change.

In conclusion, oral administration of dexamethasone resulted in increased expression levels and plasma levels of resistin. Consistent with previous findings, *RETN* rs3219175 was a strong

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determinant of plasma resistin levels. The present study showed that oral administration of dexamethasone increases the protein and mRNA levels of resistin irrespective of the rs3219175 genotype.

Figure legend

Figure 1: Resistin levels at baseline and after dexamethasone administration

[A] Resistin expression levels in the whole blood increased significantly after the dexamethasone administration ($P = 9.54 \times 10^{-10}$, Wilcoxon signed-rank test). [B] Plasma protein levels of resistin also increased significantly after the dexamethasone administration ($P = 0.023$, Wilcoxon signed-rank test).

DEX: dexamethasone

Figure 2: Mean fold changes in gene expression levels of resistin after dexamethasone administration

Table legends

Table 1: Clinical characteristics of subjects

The clinical characteristics of the A carriers and non-A carriers of *RETN* rs3219175 are shown. Values are shown as mean ± standard deviation.

[a] Body mass index (BMI) and body weight data for the microarray study were available for 17 of the participants (i.e. 8 A carriers and 9 non-A carriers).

[b] Three patients with major depressive disorder and 3 with bipolar disorder

[c] Four patients with major depressive disorder and 2 with bipolar disorder

[d] Fisher's exact test

[e] Two patients with major depressive disorder and 2 with schizophrenia

[f] Three patients with major depressive disorder and 1 with bipolar disorder

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Table 2: Probes showing significant change in gene expression levels in whole blood after dexamethasone administration

The list of probes which showed a significant change in gene expression levels in whole blood after dexamethasone administration (Bonferroni-corrected $P < 0.05$) is shown. The highest significance was obtained for the probe of *RETN*.

DEX: dexamethasone, SD: standard deviation

Table 3: The alterations of resistin levels in human samples induced by glucocorticoid administration reported in present and previous studies

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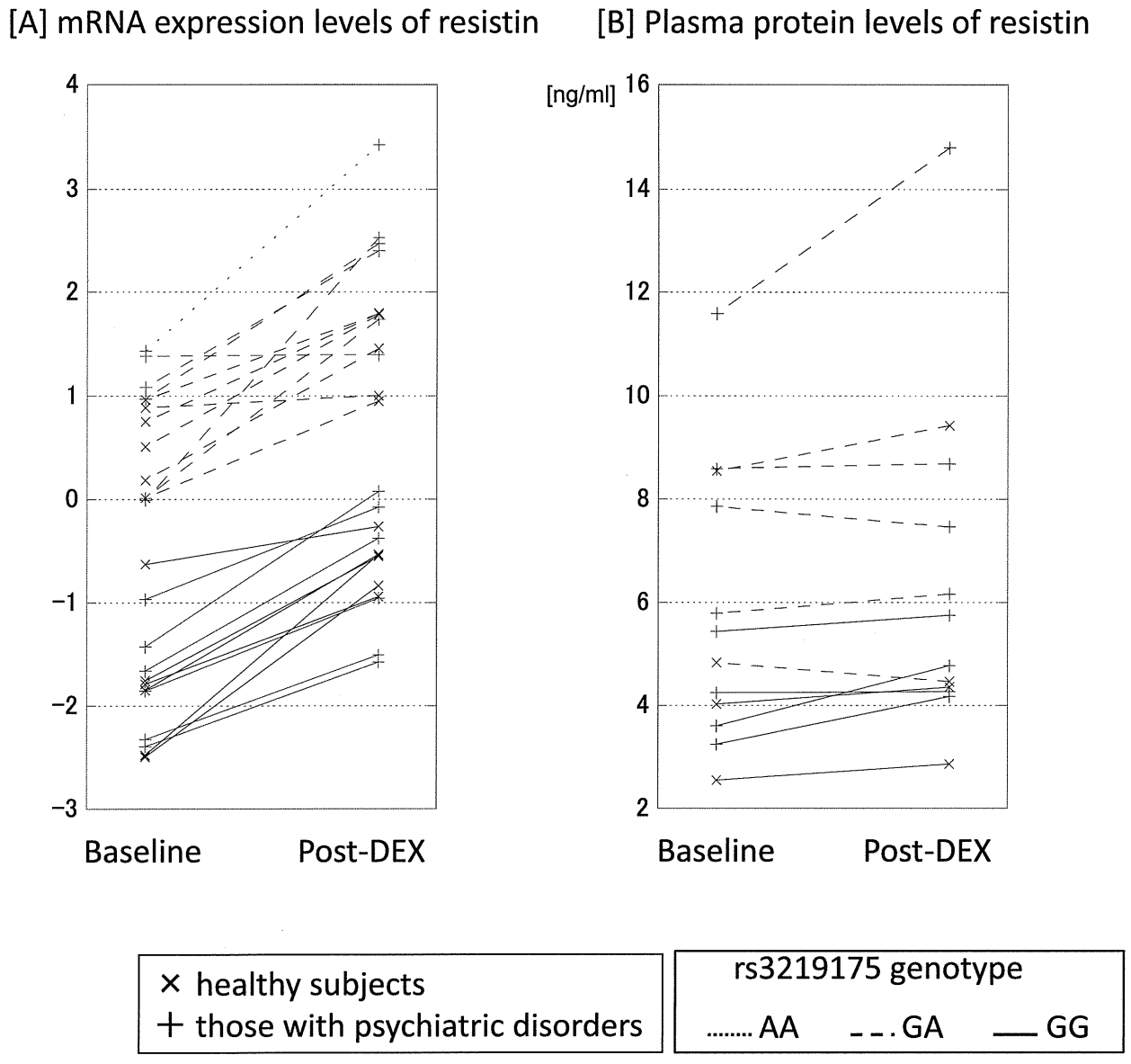

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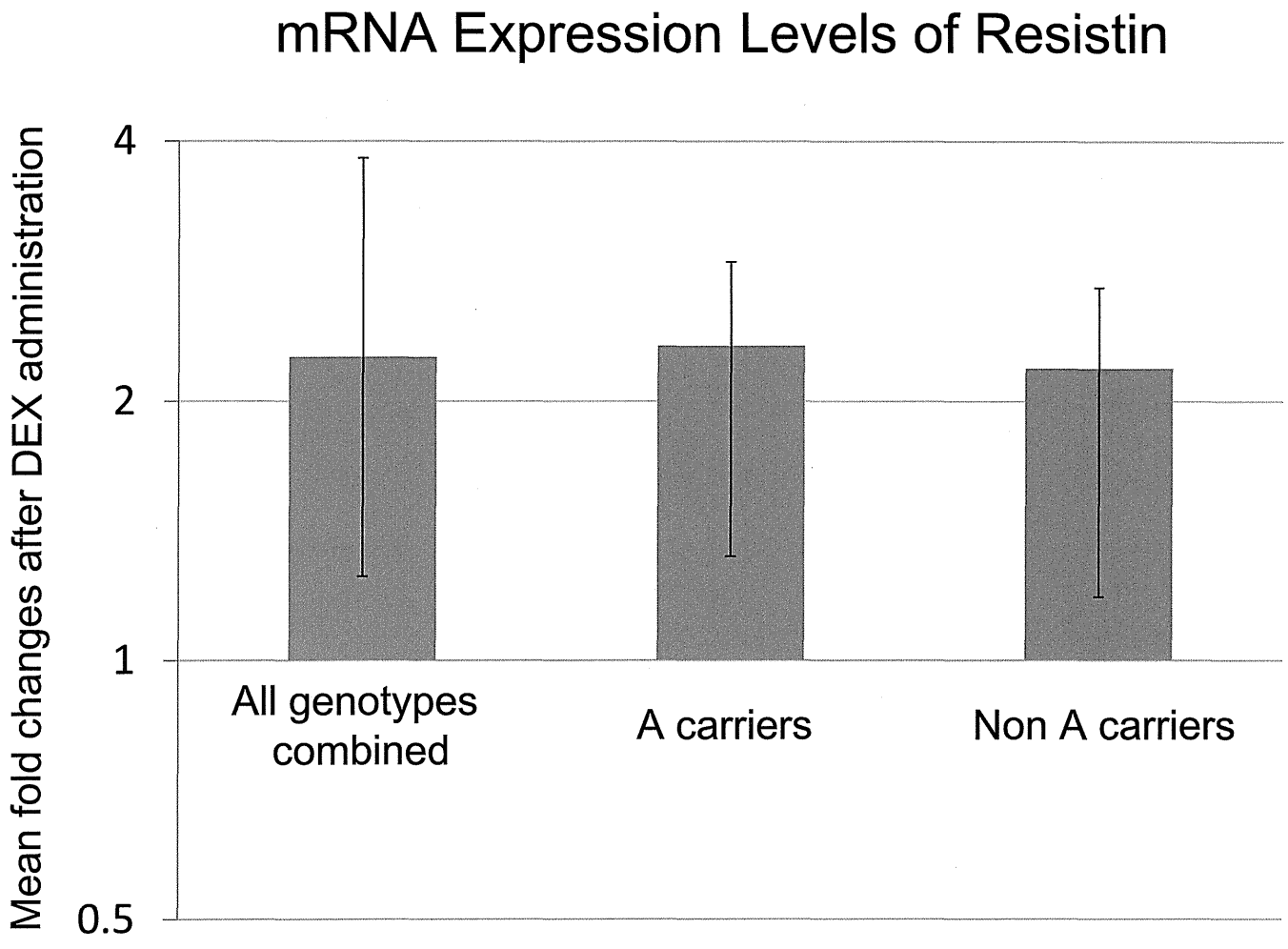
mRNA Expression Levels of Resistin
Mean fold changes after DEX administration
4
2
1
0.5
All genotypes combined
A carriers
Non A carriers

÷	*RETN* rs2535629		Statistics
	A carriers	non-A carriers	
Microarray study (*N* = 24)	*N* = 12	*N* = 12	
Sex (Male/Female)	8/4	6/6	$\chi^2 = 0.69$, $df = 1$, $P = 0.68$
Age	40.8 ± 6.8	38.9 ± 8.8	$t = 0.59$, $df = 22$, $P = 0.56$
Body weight (kg)	73.3 ± 22.8	60.8 ± 13.8	$t = 1.38$, $df = 15$, $P = 0.19$
BMI (kg/m^2) [a]	25.5 ± 6.8	23.4 ± 4.7	$t = 0.76$, $df = 15$, $P = 0.46$
Percentage with psychiatric diagnosis	50% [b]	50% [c]	$\chi^2 = 0.00$, $df = 1$, $P = 1.00$
Plasma level measurement of resistin (*N* = 12)	*N* = 6	*N* = 6	
Sex (Male/Female)	3/3	3/3	$P = 1.00$ [d]
Age	42.8 ± 8.8	36.3 ± 11.8	$t = 1.08$, $df = 10$, $P = 0.30$
Body weight (kg)	58.4 ± 8.2	72.1 ± 14.2	$t = 2.04$, $df = 10$, $P = 0.07$
BMI (kg/m^2)	21.6 ± 2.4	24.6 ± 3.9	$t = 1.62$, $df = 10$, $P = 0.14$
Percentage with psychiatric diagnosis	66.7% [e]	66.7% [f]	$P = 1.00$ [d]

Probe Name	Gene Symbol	Gene Name	Baseline levels		÷	Post-DEX levels		P value	Bonferroni-corrected P
			Mean	SD		Mean	SD	(Paired t-test)	value
A_23_P119222	*RETN*	resistin	-0.56	1.38		0.61	1.45	0.000000000954	0.0000298
A_23_P258912	*MYOM2*	myomesin 2	-0.75	2.04		-0.12	1.95	0.0000000105	0.000329
A_32_P170879			1.16	2.11		-0.51	1.55	0.000000628	0.0197
A_32_P216715	÷	÷	-1.72	3.56	÷	0.78	2.41	0.00000110	0.0345

Table 3: Alterations of resistin levels in human samples induced by glucocorticoid administration reported in present and previous studies

Study	Samples		Type and dose of glucocorticoid administered	Time after glucocorticoid administration								
				3 h	11 h	16 h	24 h	48 h	1 w	2 w	3 w	4 w
Menke et al [7]	whole blood	mRNA	DEX 1.5 mg	n.s.								
Present study	whole blood	mRNA	DEX 1.5 mg		↑							
	plasma	Protein	DEX 0.5 mg			↑						
Tsiotra et al [15]	monocyte-enriched mononuclear cells	mRNA	DEX 10^{-7} M				n.s.					
		Protein					↑					
Lewandowski et al [16]	serum	Protein	DEX 0.5 mg every 6 hours				n.s.[a]	n.s.				
Tanaka et al [11]	LPS-stimulated peripheral blood mononuclear cells	Protein	DEX (10^{-6}, 10^{-7}, 10^{-8} M)			↓[b]						
		mRNA				↓[b]						
	serum[c]	mRNA	Prednisolone 46.7 ± 9.4 mg/day						n.s.	n.s.	↓	↓

↑: significantly increased; ↓: significantly decreased; n.s.: not significant; LPS: lipopolysaccharide; DEX: dexamethasone

[a] Nearly significant (P = 0.051) increase was observed.

[b] Dexamethasone was added overnight to LPS-stimulated peripheral blood mononuclear cells.

[c] Samples were from patients with systemic autoimmune diseases who received glucocorticoid therapy.

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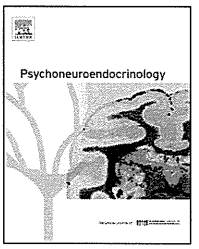

Effect of the common functional *FKBP5* variant (rs1360780) on the hypothalamic-pituitary-adrenal axis and peripheral blood gene expression

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FK506 binding protein 5;
Polymorphism;
Post-traumatic stress disorder;
Dexamethasone/corticotropin-releasing hormone test;
Glucocorticoid receptor;
Age-dependent

Summary Regulation of hypothalamic-pituitary-adrenal (HPA) axis reactivity plays an important role in the development of stress-related psychiatric disorders. FK506 binding protein 5 (*FKBP5*) modulates HPA axis reactivity via glucocorticoid receptor (GR; *NR3C1*) sensitivity and signaling. The T allele of the single nucleotide polymorphism, *FKBP5* rs1360780 (C/T), is associated with higher FKBP5 induction by glucocorticoids. In the present study, we performed the dexamethasone/corticotropin releasing hormone (DEX/CRH) test and quantitative real-time PCR analysis of peripheral blood mononuclear cell (PBMC) cDNA samples in 174 and 278 non-clinical individuals, respectively. We found increased suppression of the stress hormone (cortisol) response to the DEX/CRH test ($P = 0.0016$) in aged (>50 years) individuals carrying the T allele compared with aged non-T allele carriers. T carriers showed significant age-related changes in *GR* and *FKBP5* mRNA expression levels in PBMCs ($P = 0.0013$ and $P = 0.00048$, respectively). Our results indicate that *FKBP5* rs1360780 regulates HPA axis reactivity and expression levels of *GR* and *FKBP5* in PBMCs in an age-dependent manner. Because these phenotypes of aged T carriers are similar to endophenotypes of people with post-traumatic stress disorder, our findings may be useful for determining the molecular mechanisms, treatment, and preventive strategies for this disease.

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1. Introduction

The hypothalamic-pituitary-adrenal (HPA) axis plays an essential role in stress responses and stress-related psychiatric disorders (Holsboer, 2000; Yehuda, 2001; Kunugi et al., 2010). Glucocorticoids (GCs) are released into blood as end products of the HPA axis, penetrate into the brain, bind to glucocorticoid receptors (GRs), and exert negative feedback effects to maintain homeostasis. Accumulating evidence suggests that FK506 binding protein 5 (FKBP5), a GR-regulating co-chaperone molecule of HSP90, plays an important role in HPA axis regulation (Binder, 2009). FKBP5 binds to GRs in the cytosol, decreasing GR ligand affinity and nuclear translocation (Binder, 2009). Moreover, GCs have been shown to induce *FKBP5* mRNA expression in native lymphocytes and the human B-lymphoblastoid cell line, IM-9 (Vermeer et al., 2003).

Among *FKBP5* polymorphisms, rs1360780 is the only common single nucleotide polymorphism (SNP) that has functional effects. This SNP is located less than 500 bp from the highly conserved, functional hormone response element within intron 2 of *FKBP5* (Hubler and Scammell, 2004; Billing et al., 2007). This SNP is associated with FKBP5 protein expression levels in lymphocytes. Therefore, the T allele is associated with higher FKBP5 induction by cortisol compared with the C allele (Binder et al., 2004). The sequence containing the T allele of rs1360780 forms a putative TATA box, and compared with the C allele, exhibits stronger binding to the TATA box binding protein. This enhances *FKBP5* mRNA transcription by altering the chromatin interaction between the *FKBP5* transcription start site and long-range enhancer (Klengel et al., 2013).

FKBP5 is considered as a promising genetic candidate for vulnerability to stress-related disorders (Koenen et al., 2005; Binder et al., 2008; Koenen and Uddin, 2010; Xie et al., 2010; Zimmermann et al., 2011; Hauger et al., 2012; Collip et al., 2013; Klengel et al., 2013; Perez-Ortiz et al., 2013). Accumulating evidence has supported an interaction of *FKBP5* rs1360780 with early/childhood trauma to predict post-traumatic stress disorder (PTSD) (Koenen et al., 2005; Binder et al., 2008), suicide attempts (Roy et al., 2010), and major depression (Appel et al., 2011; Zimmermann et al., 2011). The T allele of rs1360780 is associated with a higher risk for current PTSD symptoms in individuals with a severe traumatic history (Klengel et al., 2013).

Based on these findings, in the current study, we focused on the common functional polymorphism, rs1360780, in the *FKBP5* gene. We hypothesized that *FKBP5* rs1360780 is associated with regulation of HPA reactivity, even in a non-clinical population. We performed the dexamethasone/corticotropin-releasing hormone (DEX/CRH) test to evaluate HPA axis reactivity. Furthermore, to estimate differences in *GR* and *FKBP5* expression levels between the genotype groups of *FKBP5* rs1360780, we conducted quantitative real-time polymerase chain reaction (qPCR) analysis using peripheral blood monocytes (PBMCs).

2. Methods

2.1. Participants

Subjects were volunteers who had no current or past history of psychiatric disorders. The numbers of subjects for the DEX/CRH test and qPCR analysis were 174 and 278, respectively (Table 1). Participants were screened using the Japanese version of the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998; Otsubo et al., 2005) and unstructured interviews by a research psychiatrist. All subjects were biologically unrelated Japanese without current/past history of psychiatric disorders. Individuals who had a prior medical history of central nervous system disease, substance abuse/dependence, severe head injury, dementia, or intellectual disability were not enrolled in the study. The study protocol was approved by the ethics committee at the National Center of Neurology and Psychiatry, Japan. After description of the study, written informed consent was obtained from every subject.

2.2. Genotyping

Venous blood was drawn from subjects and genomic DNA was extracted from whole blood according to standard procedures. The genotyping protocol was performed similar to our previous study (Fujii et al., 2012). Briefly, the SNP rs1360780 was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (assay ID C_8852038_10; Applied Biosystems, Foster City, CA). PCR thermal cycling conditions were as follows: one cycle at 95 °C for 10 min followed by 50 cycles

Table 1 Age and gender distribution of subjects for the DEX/CRH test and qPCR analysis of GR expression level in PBMCs.

DEX/CRH test	Genotype groups		Statistics	*P* value
	CC (*N* = 103)	CT/TT (*N* = 71)		
Mean age, years (standard deviation; SD)	46.4 (13.4)	45.1 (15.0)	$F = 0.36$, $df = 1$	0.55[a]
Gender, female: *n* (%)	77 (74.8)	48 (67.6)	$\chi^2 = 1.06$, $df = 1$	0.30

qPCR analysis	Genotype groups		Statistics	*P* value
	CC (*N* = 155)	CT/TT (*N* = 123)		
Mean age, years (SD)	43.0 (14.8)	41.0 (15.8)	$F = 1.19$, $df = 1$	0.28[a]
Gender, female: *n* (%)	113 (72.9)	87 (70.7)	$\chi^2 = 0.16$ $df = 1$	0.69

SD; standard deviation.
[a] Determined using one-way ANOVA.

at 92 °C for 15 s, and 60 °C for 1 min. Genotype data were assessed blind to case–control status.

2.3. DEX/CRH test

The DEX/CRH test, originally developed by Heuser et al. (1994a), was performed according to our previously reported protocol (Kunugi et al., 2006; Hori et al., 2010). Briefly, subjects took 1.5 mg of DEX orally at 2300 h. On the next day, a vein was cannulated at 1430 h to collect blood at 1500 and 1600 h. Human CRH (100 μg) was administered intravenously at 1500 h, immediately after the first blood collection.

Cortisol concentrations obtained from the DEX suppression test (DST) (defined as DST-cortisol) were obtained from blood specimens taken at 1500 h (i.e., plasma cortisol concentrations 16 h after oral DEX intake but immediately before CRH infusion). Cortisol concentrations obtained at 1600 h were defined as DEX/CRH-cortisol. The difference between DEX/CRH-cortisol and DST-cortisol values was reported as delta values. These definitions are similar to those defined by Heuser et al. (1994b). In addition, we calculated the area under the time course curve (AUC, given in arbitrary units), which was determined by the trapezoidal rule. Proportions of categorical data (e.g., sex) were compared using the χ^2 test for independence. Intergroup cortisol comparisons were performed using the Mann–Whitney *U* test. Nonparametric tests were used because endocrine values do not always show a normal distribution, and some subjects showed values below detectable limits. All reported *P* values are two-tailed. A value of $P < 0.05$ was considered statistically significant.

2.4. Gene expression analysis

Venous blood was collected from each subject into PAXgene tubes (Qiagen, Valencia, CA) between 1100 h and 1200 h, and incubated at room temperature for 24 h for RNA stabilization. RNA was extracted from whole blood using the PAXgene Blood RNA System Kit (PreAnalytix GmbH, Hombrechtikon, Switzerland), according to the manufacturer's guidelines. RNA was quantified by optical density readings at 260 nm using NanoDrop ND-1000 (Thermo Fisher Scientific, Rockford, IL). PCR amplifications were performed in triplicate (in a 5-μl volume) in 384-well plates using an ABI prism 7900HT (Applied Biosystems). Each reaction contained cDNA (0.28 μl), qPCR QuickGoldStar Mastermix Plus (Eurogentec, Seraing, Belgium) and human GR primer (*NR3C1*, Hs00353740_m1), human *FKBP5* primer (Hs01561006_m1), or primers for the housekeeping genes (human beta-actin [*ACTB*, 4326315E] or human glyceraldehyde-3phosphate dehydrogenase [*GAPDH*, 4326317E)])(these primers were purchased from Applied Biosystems). Negative control reactions were performed using no RNA. Real-time PCR reactions were performed at 50 °C for 2 min, 95 °C for 10 min, and then 50 cycles of 95 °C for 15 s and 60 °C for 1 min. Standard amplification curves were made by serial dilution of a standard, with a pooled cDNA sample included in each plate. Mean values (from triplicates of each sample) were normalized to the standard curve. *NR3C1* and *FKBP5* values from each sample were normalized to *ACTB* and *GAPDH* values.

3. Results

3.1. T carriers did not show an age-associated increase in the cortisol response to the DEX/CRH test

We examined the association between the rs1360780 genotype and cortisol response to the DEX/CRH test in 174 healthy individuals. Because we identified only seven subjects homozygous for the minor T allele, homozygous and heterozygous T allele subjects were combined in the analysis. There were no significant differences in mean age and sex distribution between the genotype groups (CC vs CT/TT; Table 1). We determined cortisol responses in the subjects (Table 2), and then examined the correlation of the cortisol response with age, after stratification by genotype (Fig. 1). After calculating the AUC value, we found that the total subjects showed a significant correlation between age and cortisol response ($r = 0.22$, $P = 0.0037$). Such a significant positive correlation was observed in non-T carriers ($r = 0.33$, $P = 0.00061$; Fig. 1A), but not in T carriers (Fig. 1B). In T carriers, there seems to be no change in the AUC value over ages.

The mean AUC value was significantly greater in non-T carriers than in T carriers ($P_{AUC} = 0.0083$; Fig. 1C and Table 2). Similar results were obtained with other cortisol response values (Table 2). For considering the effects of age on hormonal responses, subjects were split into aged (>50 years) and young (≤50 years) groups, according to our previous study (Kunugi et al., 2006). There was no significant difference in mean age or sex distribution between the genotype groups (CC vs CT/TT) in aged or young group (supplemental Table S1). In the aged group, the mean AUC value was significantly greater in non-T carriers than in T carriers ($P_{AUC} = 0.0016$; Fig. 1D and Table 2). The other cortisol response values were consistently greater in non-T carriers than in T carriers (Table 2). In contrast, in the young group, there was no significant difference in any cortisol response value between the two genotype groups (Fig. 1E and Table 2). Aged non-T carriers have enhanced responses to the DEX/CRH test when compared with young subjects, while the T carrier group appeared to be unchanged over ages (Fig. 1 and Table 2).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2014.01.007.

To examine the relationship between hypocortisolism and rs1360780, we divided the subjects into enhanced-suppressors (i.e., DEX/CRH-cortisol < 1 mg/dl) and the remaining non-enhanced suppressors, according to our previous study (Hori et al., 2010). There were 13 enhanced-suppressors in our sample (Table 3). Among them, nine (69%) individuals were T carriers and four (31%) were non-T carriers. Among the remaining 161 non-enhanced suppressors, 62 (39%) individuals were T carriers and 99 (61%) were non-T carriers. Enhanced-suppressors were significantly more common in T carriers than in non-T carriers (Fisher's exact test, $P = 0.040$; odds ratio: 3.59; 95% confidence interval: 1.06–12.17). In previous studies of depressed patients (Heuser et al., 1994a; Schule et al., 2009), the DEX suppression status within the DEX/CRH test was defined by a cut-off criterion of 27.5 ng/ml (75.3 nmol/l) applied to DST-cortisol. When this criterion was

Table 2 Comparison of the cortisol response to the DEX/CRH test between *FKBP5* rs1360780 genotype groups.

Total	Mean cortisol concentration (SD) (μg/dl)		Mann–Whitney *U*	*P* value[a]
	CC (*n* = 103)	CT/TT (*n* = 71)		
DST-cortisol[b]	0.89 (1.16)	0.51 (0.90)	2862.5	**0.0074**
DEX/CRH-cortisol[c]	6.37 (5.49)	4.36 (4.22)	2875	**0.017**
Delta[d]	5.48 (5.04)	3.85 (3.98)	2984	**0.039**
AUC[e]	3.63 (3.07)	2.44 (2.31)	2794.5	**0.0083**
Aged (>50 years)	Mean cortisol concentration (SD) (μg/dl)		Mann–Whitney *U*	*P* value[a]
	CC (*n* = 42)	CC (*n* = 27)		
DST-cortisol[b]	1.01 (1.13)	0.47 (0.65)	387.5	**0.019**
DEX/CRH-cortisol[c]	8.38 (6.15)	4.09 (3.69)	321.5	**0.0025**
Delta[d]	7.37 (5.55)	3.61 (3.64)	337.5	**0.0048**
AUC[e]	4.70 (3.44)	2.28 (1.92)	311	**0.0016**
Young (≤50 years)	Mean cortisol concentration (SD) (μg/dl)		Mann–Whitney *U*	*P* value[a]
	CC (*n* = 61)	CC (*n* = 44)		
DST-cortisol[b]	0.80 (1.18)	0.54 (1.02)	1133.5	0.13
DEX/CRH-cortisol[c]	4.98 (4.55)	4.53 (4.55)	1238.5	0.50
Delta[d]	4.18 (4.23)	3.99 (4.21)	1306.5	0.82
AUC[e]	2.89 (2.56)	2.53 (2.54)	1187	0.31

[a] Determined using the Mann–Whitney *U* test. Statistically significant *P* values are labeled with bold text.
[b] DST-cortisol: cortisol concentration obtained at 1500 h (i.e., plasma cortisol concentration 16 h after oral DEX intake but immediately before CRH infusion).
[c] DEX/CRH-cortisol: cortisol concentration at 1600 h (i.e., plasma cortisol concentration 17 h after oral DEX intake and 1 h after CRH infusion).
[d] Delta: difference between DEX/CRH-cortisol and DST-cortisol values.
[e] AUC: area under the time course curve, determined by the trapezoidal rule and reported in arbitrary units.

applied, there were only 5 non-suppressors for their criterion in our non-clinical subjects. Therefore, we selected the median of AUC values as the cut-off. Among the 87 suppressors, 43 (49.4%) individuals were non-T carriers and 44 (50.6%) were T carriers. Among the remaining 87 non-suppressors, 60 (69.0%) individuals were non-T carriers and 27 (31.0%) were T carriers. Suppressors were significantly more common in T carriers than in non-T carriers ($\chi^2 = 6.88$, $df = 1$, $P = 0.0087$; odds ratio: 2.27; 95% confidence interval: 1.22–4.22).

3.2. T carriers show age-related changes in *GR* and *FKBP5* mRNA expression levels in PBMCs

GR and *FKBP5* expression levels in PBMCs were determined in 278 subjects. There were no significant differences in mean age and sex distribution between the two genotype groups (Table 1). Even when subjects were split into aged or young group, there was no significant difference in mean age or sex distribution between the genotype groups (CC vs CT/TT) for either group (supplemental Table S1).

We normalized *GR* and *FKBP5* expression levels to *ACTB* expression levels in each sample (Fig. 2). We examined the correlation between age and *GR* expression levels after stratification by the rs1360780 genotype (Fig. 2A–C), and found no significant correlation in non-T carriers (Fig. 2A). However, we detected a significant age-related increase in *GR* expression in T carriers ($r = 0.29$, $P = 0.0013$; Fig. 2B). The mean *GR* expression level was significantly higher in T carriers than in non-T carriers in the aged group (Mann–Whitney $U = 740$, $P = 0.0093$), but there was no significant difference in the young group (Mann–Whitney $U = 4190$, $P = 1.00$) (Fig. 2C).

FKBP5 expression levels were also examined (Fig. 2D–F). There was no significant correlation between age and *FKBP5* expression levels in non-T carriers (Fig. 2D). However, we found a significant age-related decrease in *FKBP5* expression in T carriers ($r = -0.31$, $P = 0.00048$; Fig. 2E). The mean *FKBP5* expression level was lower in T carriers than in non-T carriers in the aged group (Mann–Whitney $U = 794$, $P = 0.029$; Fig. 2F). However, *FKBP5* expression levels were higher in T carriers than in non-T carriers in the young group (Mann–Whitney $U = 3451$, $P = 0.039$; Fig. 2F). In T carriers, the aged group showed significantly lower *FKBP5* expression level than the young group (Mann–Whitney $U = 1203$, $P = 0.014$; Fig. 2F). Contrary to T carriers, in non-T carriers, the aged group showed higher *FKBP5* expression level than the young group at a trend level (Mann–Whitney $U = 2263$, $P = 0.081$; Fig. 2F), although the *P* value did not reach statistical significance. These results indicated an interaction between age and genotype.

We compared relative gene expression levels between each genotype group using another endogenous control, *GAPDH*, and obtained similar results (supplemental Fig. S1).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2014.01.007.

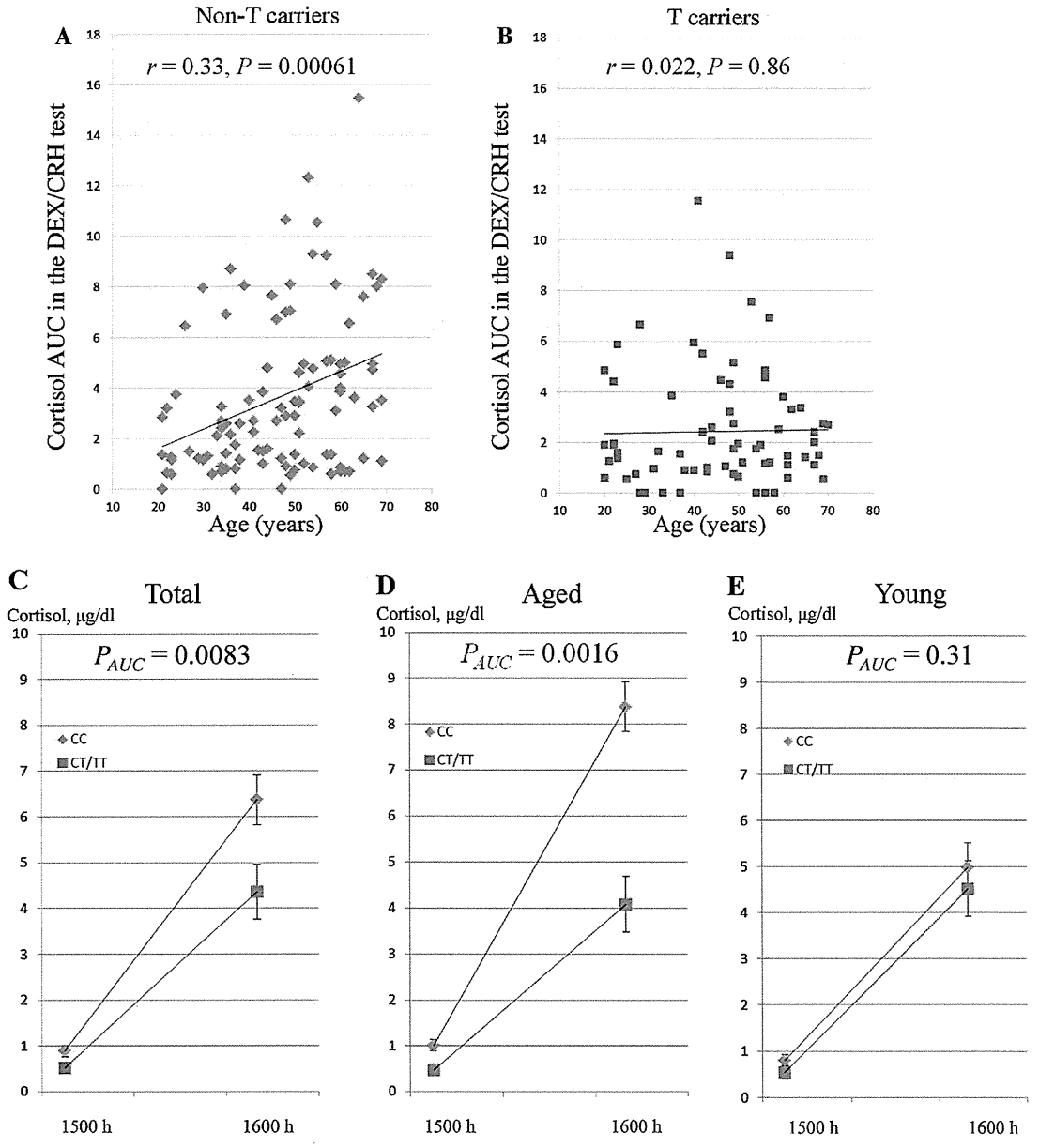

Figure 1 Age-related effects of rs1360780 on HPA axis reactivity. (A and B) Scatter plots of cortisol AUC values and age in each genotype group. Cortisol AUC values were obtained using the DEX/CRH test. Non-T carriers showed a significant correlation between cortisol AUC and age (A; CC, $n = 103$, $r = 0.33$, $P = 0.00061$), whereas T carriers did not show a correlation (B; CT/TT, $n = 71$, $r = 0.022$, $P = 0.86$). (C–E) Time course curves of the cortisol response to the DEX/CRH test in total (C), and in the aged (D) and young (E) groups. Error bars indicate SEM.

4. Discussion

We showed that the *FKBP5* functional polymorphism, rs1360780, was associated with novel phenotypes in HPA axis reactivity and gene expression changes in PBMCs, even in the non-clinical population. Subjects carrying the minor T allele had a suppressed cortisol response in the HPA axis and showed significant age-related changes of *GR* and *FKBP5* mRNA

Table 3 Genotype distribution of *FKBP5* rs1360780 in non-enhanced and enhanced suppressors.

Cortisol suppression pattern	*n*	Genotype count (frequency)		Fisher's exact test, two-sided
		CC	CT/TT	
Enhanced-suppressors	13	4 (0.31)	9 (0.69)	**0.040**
Others	161	99 (0.61)	62 (0.39)	

Bold text indicates a statistically significant difference.

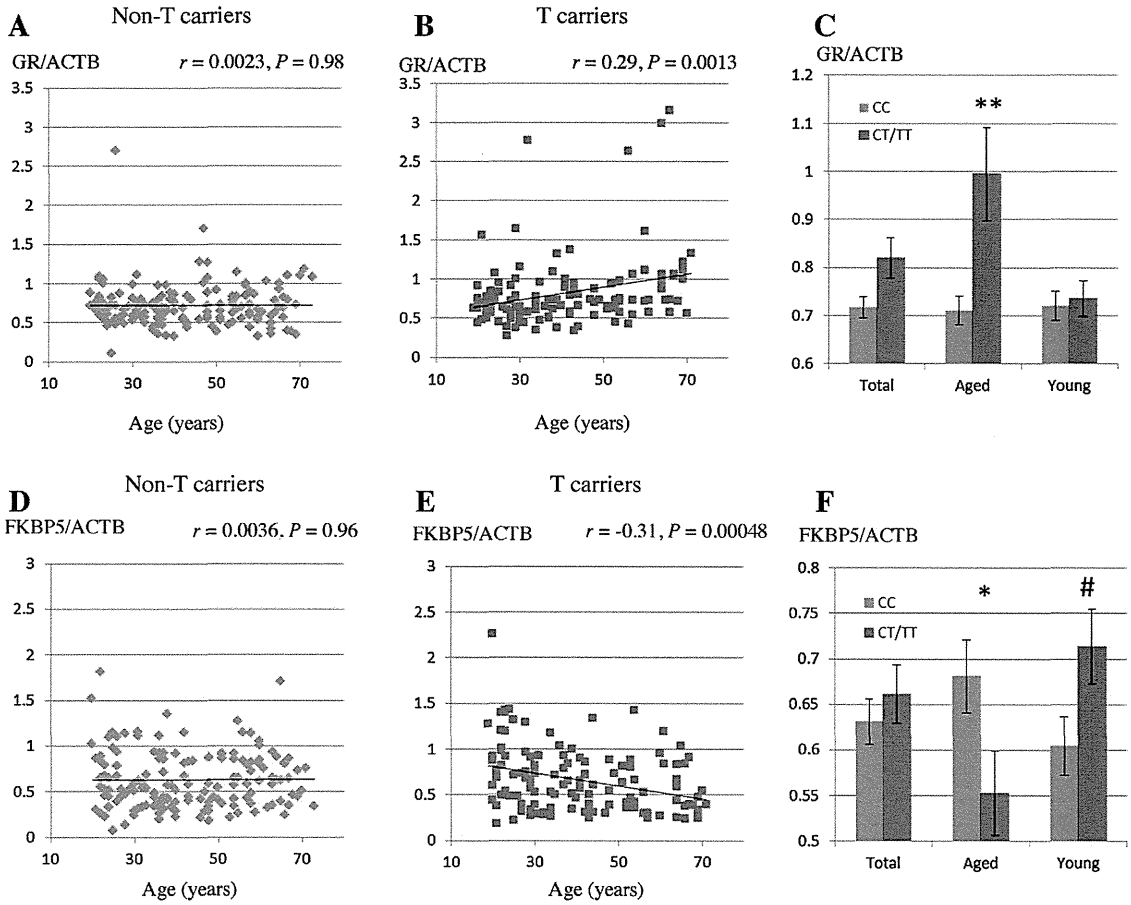

Figure 2 Age-related effects of rs1360780 on *GR* and *FKBP5* expression levels in PBMCs. (A and B) Scatter plots of *GR* mRNA levels in PBMCs by age in each genotype group. Non-T carriers did not show a significant correlation between *GR/ACTB* and age (A; CC, $n = 155$, $r = 0.0023$, $P = 0.98$), whereas T carriers showed a significant correlation (B; CT/TT, $n = 123$, $r = 0.29$, $P = 0.0013$). (C) Mean *GR/ACTB* mRNA ratios in PBMCs in total, and in the aged and young groups. (D and E) Scatter plots of *FKBP5* mRNA levels in PBMCs and age in each genotype group. Non-T carriers did not show a significant correlation between *FKBP5/ACTB* and age (D; CC, $n = 155$, $r = 0.0036$, $P = 0.96$), whereas T carriers showed a significant correlation (E; CT/TT, $n = 123$, $r = -0.31$, $P = 0.00048$). (F) Mean *FKBP5/ACTB* mRNA ratios in PBMCs in total, and in the aged and young groups. $^{**}P = 0.0093$ (Mann–Whitney $U = 740$), $^{*}P = 0.029$ (Mann–Whitney $U = 794$), $\#P = 0.039$ (Mann–Whitney $U = 3451$). Error bars indicate SEM.

expression levels. As a result, significant differences in HPA axis reactivity and gene expression were found in PBMCs in aged individuals (>50 years). These findings suggest an age-dependent effect of rs1360780.

We observed a decreased cortisol response to the DEX/CRH test in aged subjects and a significantly increased proportion of enhanced-suppressors of cortisol in T carriers compared with non-T carriers. Moreover, we showed that rs1360780 affected the age-dependent increase in cortisol reactivity. Therefore, a significant age-dependent increase in HPA axis reactivity was observed in non-T carriers, but not in T carriers. Previous studies, including our study, showed an increase in cortisol reactivity with normal aging (Heuser et al., 1994b; Kunugi et al., 2006). There is also evidence showing enhanced hormonal responses to the DEX/CRH test in aged rats compared with young animals (Hatzinger et al., 1996, 2000). Consistent with these previous findings, we found a significant positive correlation between age and cortisol response to the DEX/CRH test (AUC: $r = 0.22$, $P = 0.0037$). Surprisingly, we found that this correlation was absent in T carriers, suggesting that FKBP5 plays an important role in the increase of cortisol reactivity with normal aging. We observed a lower cortisol response to the DEX/CRH test in aged T carriers compared with aged non-T carriers. Notably, recent studies (Touma et al., 2011; Velders et al., 2011) on the same polymorphism reported conflicting results regarding cortisol values. Velders et al. showed significantly lower total cortisol secretion during the day in T carriers compared with non-T carriers (total $n = 1711$; mean age: 74.9 ± 5.7 years), while Touma et al. reported no significant difference in the DEX/CRH test between the two genotype groups (total $n = 65$; mean age: 27.4 ± 6.5 years). This inconsistency between studies can be reconciled by our findings of age-dependent effects of rs1360780. In addition, Ising et al. (2008) examined the Trier social stress test (TSST) for 64 healthy subjects and showed that the homozygous subjects ($n = 7$) for the T allele were associated with insufficient cortisol recovery in psychosocial stress response. However, there was no significant difference between the genotype groups (CC [$n = 34$] vs CT [$n = 23$]). Therefore, when homozygous and heterozygous subjects for the T allele were combined in their analysis, there was likely no significant

difference between the genotype groups (CC vs CT/TT). Furthermore, they analyzed only young subjects (20–44 years). Because there was no significant difference between the genotype groups (CC vs CT/TT) in our young (≤50 years) group, our results were in line with theirs. Binder et al. (2008) reported no significant difference in DST-cortisol tests between T carriers and non-T carriers in 55 subjects without PTSD. Since their subjects were relatively young (mean: 43.3 ± 11.1 years), their results are consistent with ours in the young group. Mahon et al. (2013) found no significant difference in cortisol response to TSST across genotypes of rs1360780 in 368 healthy young adults (18–30 years), which is consistent with our results in young subjects. Menke et al. (2013) found that depressed patients showed a significantly greater GR resistance, as assessed by DEX-induced *FKBP5* mRNA expression in PBMCs and suppression of plasma cortisol levels, compared with healthy controls in T carriers, but not in non-T carriers. However, they seemed to have found no significant genotype effects on GR resistance. Klengel et al. (2013) reported that the T allele promotes greater transcriptional activity of *FKBP5* than the C allele in ex vivo experiments. In the present study, *FKBP5* expression levels in the young group were higher in T carriers than in non-T carriers, which is in line with Klengel et al. (2013). However, this relationship was flipped in the aged group due to an age-dependent decrease in *FKBP5* mRNA in T carriers. It is likely that this age-dependent effect in T carriers could be explained as a part of compensatory mechanisms that occur during aging.

We found an age-dependent increase in *GR* mRNA in PBMCs, specifically in T carriers, and mRNA expression was significantly higher in aged T carriers compared with aged non-T carriers. In contrast, we found an age-dependent decrease in *FKBP5* mRNA in PBMCs, specifically in T carriers, and mRNA expression was lower in aged T carriers compared with aged non-T carriers. Using the DEX/CRH test, we observed lower cortisol reactivity in aged T carriers. The increased *GR* and decreased *FKBP5* expression levels in aged T carriers are likely to act synergistically to increase GR signaling output, which is in accordance with the suppressed cortisol response and enhanced negative feedback system in the present study.

In aged T carriers, we observed lower cortisol reactivity using the DEX/CRH test. GR expression levels have been reported to decrease with aging in the hippocampus in rodents, which may contribute to HPA hyperactivity in aged animals (van Eekelen et al., 1992; Mizoguchi et al., 2009). Because FKBP5 plays an inhibitory role in GR function in the negative feedback of the HPA axis and the T allele of rs1360780 is associated with higher FKBP5 induction (Binder, 2009), it is possible that GR expression may not decrease with aging in T carriers due to compensatory mechanism. This possibility is in line with our observation of higher GR expression levels in aged T carriers than in aged non-T carriers.

Our findings would have important implications for the treatment of psychiatric disorders in the future. The cortisol response to the DEX/CRH test was reported to be affected by the type of drug used (Schule et al., 2006). Thus, rs1360780 genotype might be useful information in the choice of drugs especially in aged patients.

The phenotypes of aged T carriers in the present study were similar to PTSD endophenotypes/symptoms. PTSD patients tend to show hypocortisolism in the HPA axis (Yehuda, 2001; Grossman et al., 2003). Increased *GR* expression in PBMCs was reported to be a predictor of PTSD (van Zuiden et al., 2011, 2012). Lower *FKBP5* mRNA expression levels were reported in survivors of the 9/11 World Trade Center attacks with subsequent PTSD than in controls (Yehuda et al., 2009). This result was supported by their follow-up study (Sarapas et al., 2011). Low *FKBP5* mRNA expression in PBMCs prior to trauma is associated with an increased risk for the development of a high level of PTSD symptoms (van Zuiden et al., 2012). Some studies have reported a higher prevalence of PTSD in aged people than in young people (Liu et al., 2006; Jia et al., 2010; Viswanath et al., 2012), although conflicting results have also been reported (O'Donnell et al., 2004; Kessler et al., 2005; Frueh et al., 2007). Our findings suggest that the T allele of rs1360780 confers susceptibility to PTSD endophenotypes in the aged population.

Several limitations need to be mentioned. First, in general, the levels of both testosterone in men and estrogen in women are known to decrease with age. Moreover, these sex hormones are suggested to be associated with stress status, the HPA axis and *FKBP5* expression (Handa and Weiser, 2013). However, we did not collect data on testosterone/estrogen status or control for their compound effect. Second, we did not obtain data on menstrual cycle or menopausal status, which may have influenced the Dex/CRH test results as well as *FKBP5* mRNA levels (Kinouchi et al., 2008). Since our subjects were split into aged (>50 years) and young (≤50 years) groups, the majority of aged females had reached menopause. Third, although all participants were healthy subjects without current/past history of psychiatric disorders, they were not assessed on their childhood trauma. These limitations should be resolved in future studies.

In conclusion, we found that the *FKBP5* genetic variant rs1360780 affects HPA axis reactivity and expression levels of *GR* and *FKBP5* in PBMCs. These phenotypes of aged non-clinical subjects carrying the risk T allele are similar to PTSD endophenotypes. Our findings support the role of *FKBP5* in pathophysiology of stress-related psychiatric disorders. In addition, our finding should be valuable for determining the molecular mechanisms of the stress response, and for establishing more effective treatment and prevention strategies.

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Conflict of interest

All authors declare no conflict of interest that could influence their work.