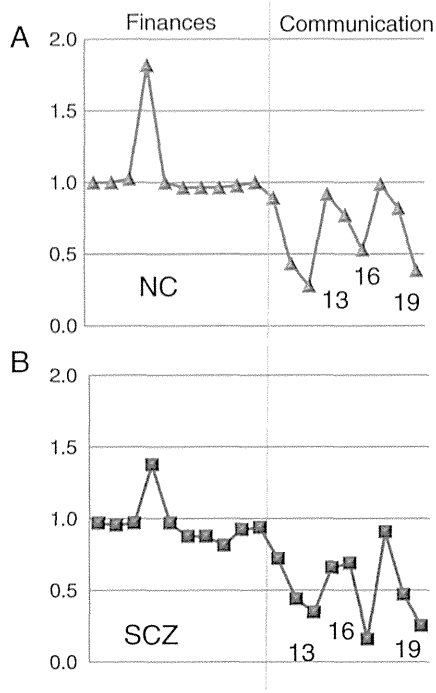

Fig. 2. UPSA-B profiles for normal controls (A) and patients with schizophrenia (B). Task 13: Speaking to the phone a name and an address given by a tester. Task 16: Leaving a voice-mail message to a reschedule of an appointment. Task 19: Remembering preparations designated by a medical appointment.

tester), Task 16 (leaving a voice-mail message to reschedule an appointment), and Task 19 (remembering preparations designated by a medical appointment) (Fig. 2 A, B).

3.4. Correlations between the UPSA-B and the MCCB

Correlations between the MCCB measures and the UPSA-B Total score scores were presented in Table 3. On the whole, the patient group yielded relatively larger *r*s than the normal control group (Table 3). The MCCB Overall composite, Working Memory composite, and Verbal Learning in the patient group showed moderate correlations (≥0.3) reaching a statistical significance. Attention/Vigilance was also significantly correlated with the UPSA-B Total score. in both groups, although the *r*s were modest (0.23–0.27). The MSCEIT had the weakest relationship to the UPSA-B Total score, not revealing substantial correlations (≤0.1) in either group (Table 3).

Table 3
Correlations between the MCCB and the UPSA-B.

MCCB domains and tests	NC	SCZ
Overall composite[a]	0.27*	0.35**
Speed of Processing Composite[b]	0.15	0.25
Trail Making Test		
BACS Symbol Coding		
Fluency		
Working Memory Composite[c]	0.21	0.36**
WMS-II Spatial Span		
Letter Number Sequencing		
Verbal Learning	0.06	0.36**
HVLT-R		
Reasoning and Problem solving	−0.01	0.20
NAB Maze		
Visual Learning	0.19	0.24
BVMT-R		
Social Cognition	0.16	−0.10
MSCEIT Managing Emotion		
Attention/Vigilance	0.23*	0.27*
CPT-IP		

NC: normal controls, SCZ: patients with schizophrenia.
* $p < 0.05$.
** $p < 0.01$.
[a] The sum of the T scores for seven domains.
[b] The sum of TMT, BACS SC, and Fluency.
[c] The sum of WMS III SS and LNS.

4. Discussion

The principal findings of the current study are summarized as follows: (1) the Japanese version of the UPSA-B has good discriminating power for diagnostically different groups yielding a large effect size ($d \geq 0.8$) and optimal cut-off point (around 80), (2) the Finance subscale of the UPSA-B was markedly better performed than the Communication subscale in both healthy adults and patients, showing poor performances on certain tasks in the Communication subscale, and (3) Performance on the UPSA-B Japanese version reflected neurocognitive functioning especially in patients with schizophrenia.

The average score of the UPSA-B Total score of the normal sample obtained in the current study was above 80, consistent with Leifker et al. (2010). Given that a majority of the studies from the US and Europe have reported that the averages of patients fell under 80 (Table 1), and that the optimal cut-off point in the current study was 78.8, it seems to be feasible to set the discriminative point at around 80 (less than 3–4 failures out of 20 tasks). In other words, the point around here is suggested to be an endpoint for patients with schizophrenia to live and function well in the community.

The disassociation between patients and normal controls found here was also in accord with a study in China (McIntosh et al., 2011), yielding relatively larger effect sizes ($d \geq 0.8$). Although the scores of Chinese samples were lower than other studies (Table 1), this is due to a wide range of educational attainment in the research participants who were selected, in order to show that the UPSA-B was sensitive to education and that patients with low levels of education would have poor performance because of both educational and illness related variables (McIntosh et al., 2011).

In addition to the diagnostically critical point noted above, identification of 'functional milestones' (e.g. goal lines for achievement in residential independence or employment) (Mausbach et al., 2011) would be informative for patients undergoing their rehabilitation. Previous studies exploring those cut-offs have reported that a score of 60 or above was able to be used to determine independent- vs. assisted-living status (Mausbach et al., 2007), and a score of 80 or above was able to be used to predict employment status (≥20 h/week) (Mausbach et al., 2011). It would be of importance to investigate whether a similar degree of achievement is required for those functional milestones in westernized Asian countries like Japan.

As was suggested by the average score of subscales (Table 2) and the profile analyses (Fig. 2), the performance on the UPSA-B may vary depending on the domains; ANOVA results indicated that the Finances subscale was considerably higher than that of the Communication subscale in both the normal and the patient groups. In fact, our normal controls nearly gained the maximum score of 50, suggesting that healthy adults are expected to be almost perfect in this domain. Previous studies have also reported that the average of the Finances domain exceeded 40 even in people with schizophrenia (Burton et al., 2013; Mausbach et al., 2007; Vesterager et al., 2012) (Table 1). Given these discrepant figures between the subscales, the Total score on the UPSA-B is preferable for the purposes of general classification of functioning on the part of people with schizophrenia. Also, our supplementary analyses showed that discriminative ability of single subscales alone (Finances: AUC = 0.68, $d' = 1.0$; Communication: AUC = 0.73, $d' = 1.3$) was poorer than that of theTotal score (AUC = 0.77, $d' = 1.3$).

The profile analysis showed similar trends between normal controls and patients for the Communication subscale. Both groups presented a relatively low score at memory-demanding tasks (Tasks 13, 16, and 19; Fig. 2). Our preliminary analysis including university students ($N = 30$, mean age = 20.6) performed well on those tasks, suggesting that age- and/or disease-based degradation of memory may account for poor performance.

The UPSA-B Total score showed a significant and moderately good correlation with the MCCB Composite score in patients with schizophrenia ($r \geq 30$), indicating sensitivity of the Japanese version of the UPSA-B to overall cognitive functioning in a clinical population. Moreover, in the patient group, Working memory and Verbal learning, in particular, showed relatively larger correlations compared to other domains, consistent with a previous report by Burton et al. (2013). Interestingly, the MSCEIT was excluded from the MCCB composite score in their study, assuming that social cognition would substantially differ from neurocognition in terms of the relationship to everyday functioning. Our result seems to support their assumption, as revealed by the weakest correlations between the MSCEIT and the UPSA-B measure in both normal controls and patients (Table 3).

Several issues are worth discussing in order to enhance the utility of the UPSA-B Japanese version. First, essential psychometric properties, including test–retest reliability, practice effect, and potential sensitivity in response to treatment need to be examined. Although previous studies (Leifker et al., 2010; Velligan et al. in press) have reported the UPSA-B has good qualities in these aspects, these issues should be replicated with the Japanese version of the UPSA-B.

Second, stratified cut-off points according to types of functional recovery (e.g. residential status, employment) (Mausbach et al., 2011) or demographic variables (e.g. age) would be worth investigating. Regarding the latter, as noted before, healthy adults have been shown to perform well (≥80) on the UPSA-B (Leifker et al., 2010). Similarly, a study with the full version of the UPSA (Harvey et al., 2010) has reported that basic daily-living activities were relatively unaffected by age, based on data from healthy elderly population. Despite those facts, our preliminary data indicate that younger subjects (university students) performed better than middle-aged workers for the Communication subscale (students = 38.5, workers = 33.4). This suggests that the execution of this battery might be age-dependent to a certain degree. Besides, the normal achievement might become less demanding as a person gets older; elderly people may not be expected to be as equally efficient as younger people who are generally more productive in work or social activities. Whether the performance on the UPSA-B is affected by age or socio-economical requirements warrants further investigations. Results from such studies may indicate a need for stratified standard scores or cut-offs.

Finally, as noted in a previous study (Vesterager et al., 2012), updated versions with or without supplemental tasks/props would facilitate the application to subjects with a wide range of demographic backgrounds.

To conclude, our study has presented data which confirmed the utility of the Japanese version of the UPSA-B. The battery has a good discriminative validity in differentiating patients with schizophrenia from a healthy middle-aged population, consistent with previous studies using elderly samples. Further, the responsiveness to cognitive functioning in patients with schizophrenia adds support to the utility of the UPSA-B as a co-primary measure in westernized countries like Japan.

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Contributors

CS designed the study, analyzed data, and wrote the draft. TS organized the international collaborative team for this study and supervised it. CS, MT, and OS collected data and supported the interpretation. TS, TP, and PH revised the draft critically for important intellectual content. All authors contributed to manuscript writing.

Conflict of Interest

The authors report no potential conflicts of interest.

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RESEARCH ARTICLE

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Replication and Cross-Phenotype Study Based Upon Schizophrenia GWASs Data in the japanese Population: Support for Association of MHC Region with Psychosis

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Recent genome-wide association studies (GWASs) of schizophrenia (SCZ) identified several susceptibility genes and suggested shared genetic components between SCZ and bipolar disorder (BD). We conducted a genetic association study of single nucleotide polymorphisms (SNPs) selected according to previous SCZ GWAS targeting psychotic disorders (SCZ and BD) in the Japanese population. Fifty-one SNPs were analyzed in a two-stage design using first-set screening samples (all SNPs: 1,032 SCZ, 1,012 BD, and 993 controls) and second-set replication samples ("significant" SNPs in the first-set screening analysis: 1,808 SCZ, 821 BD, and 2,321 controls). We assessed allelic associations between the selected SNPs and the three phenotypes (SCZ, BD, and "psychosis" [SCZ + BD]). Nine SNPs revealed nominal association signals for all comparisons ($P_{uncorrected} < 0.05$), of which two SNPs located in the major histocompati-

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bility complex region (rs7759855 in *zinc finger and SCAN domain containing 31* [*ZSCAN31*] and rs1736913 in *HLA-F antisense RNA1* [*HLA-F-AS1*]) were further assessed in the second-set replication samples. The associations were confirmed for rs7759855 ($P_{corrected} = 0.026$ for psychosis; $P_{corrected} = 0.032$ for SCZ), although the direction of effect was opposite to that in the original GWAS of the Chinese population. Finally, a meta-analysis was conducted using our two samples and using our data and data from Psychiatric GWAS Consortium (PGC), which have shown the same direction of effect. SNP in *ZSCAN31* (rs7759855) had the strongest association with the phenotypes (best $P = 6.8 \times 10^{-5}$ for psychosis: present plus PGC results). These data support shared risk SNPs between SCZ and BD in the Japanese population and association between MHC and psychosis.

Key words: bipolar disorder; major histocompatibility complex; genome-wide association study; single nucleotide polymorphism

INTRODUCTION

The modern diagnosis criteria for non-organic psychoses, such as the Diagnostic and Statistical Manual of Mental Disorders and the International Statistical Classification of Diseases and Related Health Problems, are based on the Kraepelinian dichotomy: dementia praecox (schizophrenia [SCZ]) and manic-depressive illness (bipolar disorder [BD]). Although these manuals clearly describe the diagnostic criteria for SCZ and BD, psychiatrists occasionally encounter cases that are difficult to diagnose. These patients present similar but "unusual" symptoms (e.g., psychotic symptoms, depressive symptoms, and cognitive impairments) without the typical manifestations of each disorder. Moreover, the initial diagnosis of these patients may change during the treatment course because of shifts in their main symptoms.

Epidemiological surveys have identified the common features for SCZ and BD. These include (1) heritability (approximately 80%) for both disorders and (2) lifetime prevalence (approximately 1%) worldwide (although BD type II or bipolar spectrum disorder are more common) [O'Donovan et al., 2009]. More recently, a population-based cohort study also reported that the prevalence of either disease was higher in family members of an index proband regardless of his/her diagnosis [Lichtenstein et al., 2009], suggesting that the genetic risk for SCZ or BD partially overlapped. Recent genome-wide association studies (GWASs) support this shared genetic risk for SCZ and BD. For example, candidate susceptibility genes based on BD GWAS findings showed highly significant associations with SCZ for single nucleotide polymorphisms (SNPs) in *CACNA1C*, *ANK3*, and *ITIH3-ITIH4* loci, although the effect size in specific variants is small (odds ratio: approximately 1.2) [Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011; Schizophrenia Psychiatric Genome-Wide Association Study Consortium, 2011; Williams et al., 2011]. In addition, a large number of accumulated risks for SCZ or BD can weakly predict disease status based on very liberal "risk" alleles [Purcell et al., 2009; Schizophrenia Psychiatric Genome-Wide Association Study Consortium, 2011; Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013]. Therefore, combining SCZ and BD samples into a single "psychosis" group [Craddock and Owen, 2010] can increase the statistical power, especially for the detection of overlapping risk SNPs. Several studies used this concept to enhance the detection of risk SNPs for SCZ or BD [Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Hamshere et al., 2013].

The aim of the current study was to conduct replication and cross-phenotype analyses of SNPs selected on the basis of GWAS findings for SCZ and BD in the Japanese population. This study provided evidence for common risk associations between the two phenotypes, allowing us to assess the trans-population effects of the psychiatric disorders.

MATERIALS AND METHODS

Subjects

We used two independent sample sets in this study. The first-set screening analysis involved 1,032 patients with SCZ (48.3% female subjects; mean age ± standard deviation [SD] = 46.8 ± 14.8 years), 1,012 patients with BD (51.8% female subjects; mean age 50.7 ± 14.3 years; BD type I = 621, BD type II = 380, schizoaffective disorder [SA] = 7, BD type information not available = 4), and 993 healthy controls (51.1% female subjects; mean age 49.7 ± 14.0 years).

For the two SNPs that showed significant association in the first-set screening analysis, we used an independent second set for replication analysis. This sample consisted of 1,808 patients with SCZ (45.1% female subjects; mean age ± SD = 49.8 ± 14.8 years), 821 patients with BD (54.6% female subjects; 48.2 ± 14.4; BD type I = 387, BD type II = 344, SA = 89, unknown type of diagnosis = 1), and 2,321 healthy controls (57.2% female subjects; 42.3 ± 14.2 years).

The general characteristics and psychiatric profiles of all these subjects were reported previously [Matsunaga et al., 2012; Kondo et al., 2013]. Written informed consent was obtained from each subject. The ethics committees of Fujita Health University, RIKEN BSI, and the institutes participating in the Collaborative Study of Mood Disorder (COSMO) approved this study [Matsunaga et al., 2012].

SNP Selection and Quality Control

In total, 51 SNPs were selected from the SCZ GWAS data published prior to September 2011 [O'Donovan et al., 2008; Shi et al., 2009, 2011; Stefansson et al., 2009; Schizophrenia Psychiatric Genome-Wide Association Study Consortium, 2011; Yue et al., 2011; Rietschel et al., 2012; Ikeda et al., 2013]. The selection was based on the following inclusion criteria. The potential risk SNPs in autosomal chromosomes must have (1) a *P*-value of $< 1 \times 10^{-5}$ if the original GWAS was conducted on a Caucasian population or (2) a *P*-value of $< 1 \times 10^{-4}$ if the study was on an Asian population, because we assume that the sample size of the previous GWAS for Asian population did not have sufficient power [Shi et al., 2011; Yue

et al., 2011]. Moreover, the minor allele frequency (MAF) must not be zero based on the HapMap JPT panel.

All SNPs were genotyped using the Sequenom iPLEX Gold system (Sequenom, San Diego, CA). In the optimization step, we designed primers for all the SNPs and verified the genotype clustering. For the four SNPs that did not show acceptable genotyping calls, we designed new primers for proxy SNPs to replace these SNPs. Thus, we determined the genotype distribution for a total of 51 SNPs in the final genotyping stage (Table SI; Fig. S1).

The quality control (QC) procedures were based on the missing rate per person (<10%) and per SNP (<5%) and the existence of Hardy–Weinberg equilibrium with a *P*-value of >0.0001 with visual inspection (Figs. S2 and S3). In total, 46 SNPs were eligible for association analysis in the first-set screening samples.

Statistical Analysis

All statistical procedures were performed using PLINK version 1.07 [Purcell et al., 2007]. We assessed the allelic association (two-tailed) of the selected SNPs and the three phenotypes: (1) SCZ versus control (referred to as "SCZ association"), (2) BD versus control (referred to as "BD association"), and (3) "psychosis" (SCZ + BD) versus control (referred to as "psychosis association") in the first and second sets of samples. A comparison between multiple variables is a major concern to be addressed in a genetic study in which multiple SNPs and phenotypes are analyzed. However, thus far, no gold standard has been established. Therefore, we used a two-stage analysis in the first-set screening sample. In addition, to account for linkage disequilibrium (LD) between SNPs selected for the analysis, we used the Single Nucleotide Polymorphism Spectral Decomposition (SNPSpD) interface [Nyholt, 2004; Li and Ji, 2005] to identify a sufficient number of independent variables for the first-set screening analysis (N = 42). We used an adjusted significance level ($P = 0.05/42 = 0.0012$) on the basis of the number of independent variables.

A meta-analysis of the SNPs was conducted by combining the screening and replication datasets. Furthermore, we combined our data and the Psychiatric GWAS Consortium (PGC) mega-analysis of SCZ [Schizophrenia Psychiatric Genome-Wide Association Study Consortium, 2011]. A binary effects model, one of the novel random effects approaches, which is providing more statistical power under the existence of heterogeneity, was applied in each analysis using METASOFT program [Han and Eskin, 2011].

RESULTS

Following the QC procedure, a total of 46 SNPs and 2,819 samples from the first-set screening samples remained eligible for association analysis (965 SCZ, 930 BD, and 924 healthy controls). Table I shows the nominal association signals ($P_{uncorrected} < 0.05$) for the three phenotypes (SCZ, BD, and psychosis). The detailed results can be found in Table SII. We also listed the results of the subgroup analysis for our BD samples divided by the diagnostic subtype in Table SIII, as the proportion of BD type II in our sample was relatively high in comparison to the previous BD GWASs. No large difference was observed between BD type I (plus SA) and BD type II.

In the phenotype-wise analyses conducted in this study, nine SNPs showed nominal significant association ($P_{uncorrected} < 0.05$), half of which (five SNPs) were located in the major histocompatibility complex (MHC) region of chromosome 6, a known risk locus for SCZ (Table I). Interestingly, our study revealed a direction of effect that was opposite to that in the original GWAS [Shi et al., 2011]. The most significant SNP association was found for psychosis at rs7759855 in *zinc finger and SCAN domain containing 31* (*ZSCAN31*) ($P_{uncorrected} = 2.2 \times 10^{-4}$, $P_{corrected} = 0.0093$, Table I). Significant "SCZ association" was found for SNPs in chromosome 1 (rs10489202 and rs1060041: LD with $r^2 = 0.99$ and $D' = 1.0$), chromosome 8 (rs7004633), and chromosome 15 (rs4775413). However, they did not retain significance after correction for multiple testing. It is also of interest that "BD association" was more significant than "SCZ association" in chromosome 6 but not in the other chromosomes.

Only three SNPs retained significant associations with BD and psychosis after SNPSpD correction, and they were all located in the MHC region of chromosome 6 (rs7759855, rs6927023, and rs1736913). They were further tested using the second-set replication samples. Of note, because rs7759855 and rs6927023 were in LD ($r^2 = 0.91$, $D' = 0.96$ in our screening data), rs6927023 was excluded. This analysis revealed an association of rs7779855 in *ZSCAN31* with SCZ ($P_{corrected} = 0.032$) and psychosis ($P_{corrected} = 0.026$), but not with BD ($P_{corrected} = 0.25$), despite the same direction of effect (Table I).

Finally, a meta-analysis was conducted using the screening and replication samples and the two SNPs selected for replication: rs7759855 and rs1736913 (Table II). Both SNPs showed stronger evidence of associations with all three phenotypes, however, we did not detect associations of genome-wide significance (5×10^{-8}). We did not perform a meta-analysis using our results and results of the original GWAS [Shi et al., 2011] because of the opposite direction of effect. Instead, we conducted a merging analysis with PGC SCZ data of the two SNPs (Table II), and found significant association of rs7759855 with SCZ ($P = 5.0 \times 10^{-4}$) and psychosis ($P = 6.8 \times 10^{-5}$). Another SNP (rs1736913) in *HLA-F antisense RNA1* (*HLA-F-FAS1*) also increased their significance ($P = 0.0045$ for SCZ and 3.4×10^{-4} for psychosis) by this meta-analysis, because the non-significant trend for association was observed with same direction of effect in the second-set replication analysis (SCZ: $P_{uncorrected} = 0.043$, $P_{corrected} = 0.087$, psychosis: $P_{uncorrected} = 0.025$, $P_{corrected} = 0.051$).

DISCUSSION

In this study, a two-stage association analysis was conducted on candidate SNPs identified from SCZ GWAS targeting SCZ, BD, and psychosis (SCZ + BD) in a Japanese population. The two SNPs showing significant associations with the first-set screening samples revealed significant replication of rs7759855 in *ZSCAN31* in the second set of samples.

The top two SNPs in the screening analysis are located in the MHC region (28.3 and 29.7 Mb on chromosome 6, for rs7759855 and rs1736913, respectively). Although the precise mechanism of

TABLE I. Association Analysis in the First-Set Screening Samples (SNPs With Nominal Significant Association)

							First-set screening						Second-set replication					
Chr	SNP	Closest gene	BP	A1	A2	Phenotype	F_A	F_U	$P_{corrected}$	*P*	OR (95% CI)	Direction	F_A	F_U	$P_{corrected}$	*P*	OR (95% CI)	Direction
1	rs10489202	MPC2	167903079	T	G	SCZ	0.150	0.125	1	0.0267	1.24 (1.02–1.49)	+						
						BD	0.126		1	0.901	1.01 (0.83–1.23)	+						
						Psychosis	0.138		1	0.169	1.12 (0.95–1.33)	+						
1	rs1060041	DCAF6	167973976	T	C	SCZ	0.147	0.125	1	0.0481	1.21 (1.00–1.46)	+						
						BD	0.129		1	0.749	1.03 (0.85–1.25)	+						
						Psychosis	0.138		1	0.180	1.12 (0.95–1.32)	+						
6	rs7759855	ZSCAN31	28283113	G	A	SCZ	0.0803	0.0559	0.12	0.00292	1.48 (1.14–1.91)	−	0.0791	0.0653	**0.0322**	0.0161	1.23 (1.04–1.46)	−
						BD	0.0866		**0.012**	$\mathbf{2.82 \times 10^{-4}}$	1.60 (1.24–2.07)	−	0.0765		0.249	0.125	1.19 (0.95–1.48)	−
						Psychosis	0.0834		**0.0093**	$\mathbf{2.21 \times 10^{-4}}$	1.54 (1.22–1.94)	−	0.0783		**0.0264**	0.0132	1.22 (1.04–1.42)	−
6	rs1233710	ZKSCAN4	28323425	T	C	SCZ	0.373	0.363	1	0.534	1.04 (0.91–1.19)	−						
						BD	0.395		1	0.0459	1.15 (1.00–1.31)	−						
						Psychosis	0.383		1	0.135	1.09 (0.97–1.23)	−						
6	rs6927023	GPX6	28454221	A	G	SCZ	0.0809	0.0570	0.16	0.00384	1.46 (1.13–1.88)	−						
						BD	0.0841		0.056	0.00132	1.52 (1.18–1.96)	−						
						Psychosis	0.082		**0.026**	$\mathbf{6.28 \times 10^{-4}}$	1.49 (1.18–1.87)	−						
6	rs1736913	HLA-F-AS1	29704400	A	G	SCZ	0.253	0.221	0.89	0.0212	1.19 (1.03–1.39)	−	0.249	0.230	0.0866	0.0433	1.11 (1.00–1.23)	−
						BD	0.270		**0.019**	$\mathbf{4.63 \times 10^{-4}}$	1.31 (1.13–1.52)	−	0.250		0.214	0.107	1.12 (0.98–1.27)	−
						Psychosis	0.261		**0.040**	$\mathbf{9.43 \times 10^{-4}}$	1.25 (1.10–1.43)	−	0.250		0.0505	0.0253	1.11 (1.01–1.22)	−
6	rs2021722	TRIM26	30174131	A	G	SCZ	0.102	0.130	0.32	0.00759	0.76 (0.62–0.93)	+						
						BD	0.101		0.28	0.00678	0.76 (0.62–0.93)	+						
						Psychosis	0.102		0.068	0.00161	0.76 (0.64–0.90)	+						
8	rs7004633	MMP16	89829427	G	A	SCZ	0.205	0.238	0.65	0.0155	0.83 (0.71–0.96)	−						
						BD	0.226		1	0.384	0.93 (0.80–1.09)	−						
						Psychosis	0.215		1	0.0559	0.88 (0.77–1.00)	−						
15	rs4775413	CYCSP38	59627395	T	C	SCZ	0.416	0.377	0.65	0.0156	1.18 (1.03–1.34)	+						
						BD	0.406		1	0.0731	1.13 (0.99–1.29)	+						
						Psychosis	0.411		0.64	0.0152	1.15 (1.03–1.29)	+						

BP, base position based upon hg19; A1, minor allele based upon whole sample; A2, major allele; F_A, frequency of A1 in affected subjects; F_U, frequency of A1 in unaffected subjects; OR, odds ratio for A1 (i.e., A2 is reference); CI, confidence interval; Direction, direction of the effect size based upon the original studies; +, same direction; −, opposite direction. SCZ, schizophrenia; BD, bipolar disorder; psychosis (SCZ + BD).
P-values based upon two-tailed test.
Bold numbers represents significant *P*-values after correction of multiple testings.

TABLE II. Meta-Analysis of the Two SNPs Detected in the First-Set Screening Analysis

				Shi et al. [2011]		PGC_SCZ		Current study (first-set screening_second-set replication)			PGC + current study	
Chr	SNP	Gene	RA	RA	P	RA	P	Phenotype	P	I^2	P	I^2
6	rs7759855	ZSCAN31	G	A	5.0×10^{-5}	G	0.54	SCZ	6.65×10^{-4}	25.4	5.02×10^{-4}	0.0
								BD	2.70×10^{-4}	67.7		
								Psychosis	7.32×10^{-5}	63.8	6.75×10^{-5}	27.8
6	rs1736913	HLA-F-AS1	A	G	4.6×10^{-5}	A	0.22	SCZ	0.00800	0.0	0.00445	59.7
								BD	4.29×10^{-4}	59.0		
								Psychosis	3.89×10^{-4}	48.8	3.39×10^{-4}	77.8

RA, risk allele reported in each study (our study, Shi et al., PGC group); SCZ, schizophrenia; BD, bipolar disorder; psychosis (SCZ + BD).

MHC (or HLA) to SCZ, BD, and psychosis susceptibility is largely unknown [Corvin and Morris, 2014], our results, based on Japanese population, support the genetic association between psychotic disorders and the MHC region where the recent SCZ GWAS or mega-analysis identified as risk region [Purcell et al., 2009; Shi et al., 2009; Stefansson et al., 2009; Schizophrenia Psychiatric Genome-Wide Association Study Consortium, 2011; Yue et al., 2011]. Interestingly, the association signals of all SNPs (including rs7759855 and rs1736913) in the MHC region selected from GWAS of the Chinese population [Shi et al., 2011] had an opposite direction of effect with Chinese but same with Caucasian population. In general, genetic differences between Japanese and Chinese populations are considered smaller than those between Asian and Caucasian populations. However, such discrepancy may not apply specifically to the MHC region because it contains complex and population-specific LDs. For instance, pharmacogenomic studies of carbamazepine-induced Stevens–Johnson syndrome/toxic epidermal necrolysis identified HLA-B*15:02 as a risk factor for the Chinese (MAF = 8.6%), but not for the Japanese (MAF = 0.1%) or Caucasian (MAF = 0.1%), individuals. Furthermore, HLA-A*31:01 is a risk factor for the Japanese (MAF = 9.1%) and Caucasian (MAF = 2–5%), but not for the Chinese (MAF = 1.8%), individuals [Chung et al., 2004; Aihara, 2011; McCormack et al., 2011; Ozeki et al., 2011]. Therefore, it is difficult to pinpoint the risk genes in MHC region by comparing trans-population effect. Considering the tight LD around this region, the association signal we detected by replication of rs7759855 around *ZSCAN31* (located around 28 Mb of the MHC region) may tag specific HLA alleles or haplotypes, such as HLA Class I (approximately 29 Mb), although this gene play a role as transcription factor involved in human embryo development [Pi et al., 2002].

It is also peculiar that the SNP in *ZSCAN31* was associated with BD in the screening samples but with SCZ in the replication samples, despite the fact that a nominal signal was found for SCZ and BD in the screening and replication samples. Nonetheless, this SNP may share genetic risks between the two psychotic disorders, because we assume the failure to detect significant association at individual level of phenotypes (SCZ or BD) is due to less statistical power (see below) under small effect size, reported previously [Schizophrenia Psychiatric Genome-Wide Association Study Consortium, 2011].

The other SNP (rs1736913 in *HLA-F-AS1*), which we detected in the screening samples, is located closer to HLA Class I, suggesting that this SNP may also tag HLA alleles. However, this SNP failed to replicate if we corrected for multiple testing, again probably due to statistical power issues related to sample size, which is the main limitation of our study. Power calculation shows that our sample size may not be sufficient to detect the associations under the estimated effect size (odds ratio = approximately 1.2): 19% power for SCZ/BD and 29% power for psychosis to detect significance of risk with 23% MAF (average MAF in our SNPs in the control subjects) under an additive model (type I error rate = 0.001) [Purcell et al., 2003]. Therefore, these results need to be verified with a larger sample size in future.

In conclusion, we detected significant association between SNPs in the MHC region and psychosis in the Japanese population. These data support common risk factors between SCZ and BD in the Japanese population.

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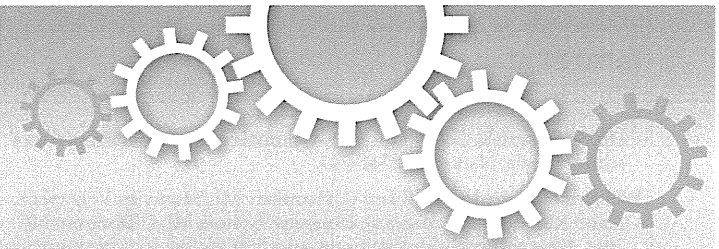

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The common functional *FKBP5* variant rs1360780 is associated with altered cognitive function in aged individuals

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The common single nucleotide polymorphism (SNP) rs1360780 (C/T) of the FK506 Binding Protein 5 (*FKBP5*) gene has been reported to be associated with an altered response of the hypothalamic-pituitary-adrenal (HPA) axis and the development of stress-related psychiatric disorders such as posttraumatic stress disorder (PTSD). In the present study, we examined whether this SNP is associated with cognitive function in a non-clinical population. The full versions of the Wechsler Memory Scale-Revised and Wechsler Adult Intelligence Scale-Revised were administered to 742 and 627 Japanese individuals, respectively, followed by genotyping of rs1360780 by the TaqMan 5′-exonuclease allelic discrimination assay. For both cognitive tests, we found significantly poorer attention/concentration (working memory) in aged (>50 years old) individuals carrying the T allele compared with their counterparts. This finding accords with an altered HPA axis and vulnerability to stress-related psychiatric disorders.

FK506 Binding Protein 5 (FKBP5) is a key molecule in the stress response and the pathophysiology of stress-related disorders including post-traumatic stress disorder (PTSD) and major depressive disorder (MDD)[1,2]. An altered stress response in hypothalamic- pituitary- adrenal (HPA) axis reactivity has been implicated in these disorders[3–5]. In the HPA axis, FKBP5 plays a role as a glucocorticoid receptor (GR)-regulating co-chaperone molecule of heat shock protein 90 by binding to GRs in the cytosol and decreasing GR nuclear translocation[1]. FKBP5 thereby inhibits the function of GRs which regulate adrenocortical secretion of glucocorticoids (cortisol in humans and corticosterone in rodents) during stress-induced HPA axis activity.

To our knowledge, *FKBP5* rs1360780 (C/T) is the only common single nucleotide polymorphism (SNP) among *FKBP5* polymorphisms that has been demonstrated to have functional effects, despite being located within intron 2. The association between this SNP and FKBP5 protein expression levels has been well established. The T allele has been considered a high induction allele for *FKBP5* by cortisol when compared with the C allele[6]. The sequence containing the T allele of this SNP forms a putative TATA box, and exhibits stronger binding activity to the TATA box binding protein when compared with the C allele. These molecular changes lead to alteration of the chromatin interaction between the *FKBP5* transcription start site and long-range enhancer, and results in the enhancement of *FKBP5* mRNA transcription[7]. Accumulating evidence suggests that the T allele is a risk factor in early/childhood trauma, and predicts PTSD[8,9], suicide attempts[10], MDD[11,12], and current PTSD symptoms[7]. In the recent study[13], FKBP5 levels in the human brain were reported to be associated with Alzheimer's disease (AD) progression although there is no genetic association study between *FKBP5* rs1360780 and AD.

Recently, we found that, in the aged (>50 years old) non-clinical population, individuals carrying the T allele of rs1360780 showed lower cortisol reactivity to the dexamethasone/corticotropin-releasing hormone (DEX/CRH) test than non-T carriers[2]. Furthermore, aged T carriers showed significantly higher and lower mRNA expression levels of *GR* and *FKBP5* in peripheral blood mononuclear cells, respectively, when compared with aged non-T carriers[2]. Interestingly, these biochemical phenotypes of the aged non-clinical subjects carrying the risk T allele accords with the endophenotypes reported in patients with PTSD[4,14].

Patients with PTSD are known to have difficulty in concentrating[15] and exhibit mild cognitive deficits[16]. HPA axis reactivity contributes to normal cognitive function[17]. To our knowledge, however, there is no information in the literature on the genetic effects of *FKBP5* on neurocognitive functions.

In the present study, we examined the possible association between *FKBP5* rs1360780 and neurocognitive function. We hypothesized that even non-clinical individuals carrying the T allele would show impaired cognitive function. Because the above-mentioned association of rs1360780 with HPA axis reactivity was observed in an age-dependent manner in our previous study[2], we took age into account in this present study.

Results

Demographic characteristics of non-clinical subjects. Neurocognitive performance was assessed using the Wechsler Memory Scale-Revised (WMS-R) and the Wechsler Adult Intelligence Scale-Revised (WAIS-R) in 743 and 627 subjects, respectively. Since the genotype frequency of homozygotes for the T allele (T/T) was small (0.044), those individuals homozygous and heterozygous for the T allele were combined in the analysis. There was no significant difference in mean age, education years, or gender distribution between the genotype groups (CC vs. CT/TT; Table 1). The genotype distribution for subjects administered either the WMS-R or WAIS-R did not significantly deviate from the Hardy-Weinberg equilibrium (HWE) ($P > 0.05$).

Aged T carriers showed poorer attention/concentration on the WMS-R. Across all subjects, there was no significant difference in the WMS-R between the two genotype groups (Figure 1a). When young (≤50 years old) and aged groups were examined separately, as in our previous study[2], the mean score of attention/concentration was significantly lower in T than in non-T carriers in the aged group ($F = 14.9$, $df = 1$, $P = 0.00014$; Figure 1c), but not in the young group (Figure 1b). The result remains significance even after the Bonferroni correction (critical $P = 0.05/8 = 0.00625$ due to 4 cognitive domains for 2 age groups). There was no significant difference in the other categories (verbal memory, visual memory, or delayed recall) between the genotype groups, even in the aged subjects (Figure 1a–c). Relationships between the genotype and WMS-R attention/concentration subtests are shown in Figure 1d–f. In the aged group, compared to non-T carriers, T carriers showed statistically lower mean scores on all subtests (mental control, $F = 7.0$, $df = 1$, $P = 0.0087$; digit span, $F = 8.4$, $df = 1$, $P = 0.0041$; visual span, $F = 4.6$, $df = 1$, $P = 0.033$). Although 3 subtests comprising WMS-R attention/concentration index were examined in young and aged groups, the main effect for digit span was significant in the aged group even after the Bonferroni correction (critical $P < 0.05/6 = 0.0083$).

Aged T carriers had lower digit span scores on the WAIS-R. With respect to the WAIS-R, there was no significant difference between the two genotype groups in full-scale Intelligence Quotient (IQ), verbal IQ, or performance IQ in the total (Figure 2a), aged (Figure 2b), or young (Figure 2c) groups. Relationships between the genotype of *FKBP5* rs1360780 and WAIS-R subscales in the total, young, and aged groups are shown in Figure 2d–f, respectively. In the total group, the mean score on the digit span subtest of the WAIS-R was significantly lower in T carriers than in non-T carriers ($F = 7.6$, $df = 1$, $P = 0.0060$; Figure 2d). In the young group, there was no significant difference in any subscale scores between the two genotype groups (Figure 2e). In contrast, the mean score on the digit span subtest in the WAIS-R was significantly lower in the aged T carriers than in the aged non-T carriers ($F = 14.2$, $df = 1$, $P = 0.00020$; Figure 2f), as expected from the WMS-R results. Although 11 subtests in the WAIS were examined in young and aged groups, the main effect for digit span was significant in the aged group even after the Bonferroni correction (critical $P < 0.05/22 = 0.0023$).

Discussion

We found that the *FKBP5* functional polymorphism rs1360780 was associated with novel phenotypes in cognitive function, even in a non-clinical population. In accordance with our previous study[2] on the relationship between this SNP and HPA axis reactivity, an age-dependent effect was observed. Aged subjects with the T allele had a significantly lower mean score on the attention/concentration index in the WMS-R compared with their, non-T, aged counterparts. In particular, they showed poor performance on the digit span subtest of the WMS-R. Similar results were obtained when cognitive functions were assessed by the WAIS-R.

FKBP5 is responsive to stressor exposure and modulates GR sensitivity[1]. *FKBP5* expression was reported to be upregulated in many brain regions after exposure to stress[18]. However, there are differences in GR sensitivity among brain regions. For example, the baseline level of *FKBP5* expression is higher in the hippocampus than in other brain regions[19], and thereby extreme stressor exposure is needed to increase *FKBP5* levels in the hippocampus[18]. Such differences in *FKBP5* function among brain regions may create the *FKBP5* genotype-dependent vulnerability in some specific brain regions, thereby likely leading to the difference in cognitive function between the genotypes of rs1360780.

Attention/concentration impairment is considered one of the symptoms of PTSD[16]. PTSD is characterized by 4 primary symptoms, namely intrusion, numbing, avoidance, and impairment of arousal. Impaired attention/concentration is included within the arousal symptom[15]. According to a previous review[16], 16 out of 19 studies on neurocognitive functioning in PTSD provided evidence of attention/immediate memory deficits. In a more recent study using the WMS-R, patients with PTSD were significantly impaired in the attention/concentration index when compared with trauma-exposed non-PTSD volunteers[19]. Overall, accumulating evidence supports the mild impairment of attention and immediate memory in PTSD patients. Given the results of the present study in a non-clinical population, poor attention/concentration may be an endophenotype of individuals who have a genetic risk (i.e., the T allele of rs1360780) for PTSD rather than the result of the development of the illness.

Attention/concentration impairment is common in MDD[20]. In homozygotes for the T allele of rs1360780, trauma exposure during childhood and adolescence increased the risk of developing depression[12]. Patients with AD also show global cognitive impairment including decreased attention and concentration[21]. Thus, the pheno-

Table 1 | Age, education years and gender distribution of subjects administered either the WMS-R or WAIS-R

	Genotype Groups		Statistics	*P* value
WMS-R	CC (n = 423)	CT/TT (n = 320)		
Mean age, years (SD)	45.1 (14.7)	43.3 (15.3)	$t = 1.5$, $df = 741$	0.13
Mean education years (SD)	15.1 (2.6)	15.0 (2.6)	$t = 0.36$, $df = 741$	0.72
Gender, female: n (%)	325 (76.8)	229 (71.6)	$\chi^2 = 2.7$, $df = 1$	0.10
WAIS-R	CC (n = 360)	CT/TT (n = 267)		
Mean age, years (SD)	46.2 (14.4)	44.4 (16.0)	$t = 1.4$, $df = 625$	0.16
Mean education years (SD)	14.9 (2.7)	15.0 (2.6)	$t = -0.073$, $df = 625$	0.94
Gender, female: n (%)	276 (76.7)	192 (71.9)	$\chi^2 = 1.8$, $df = 1$	0.18

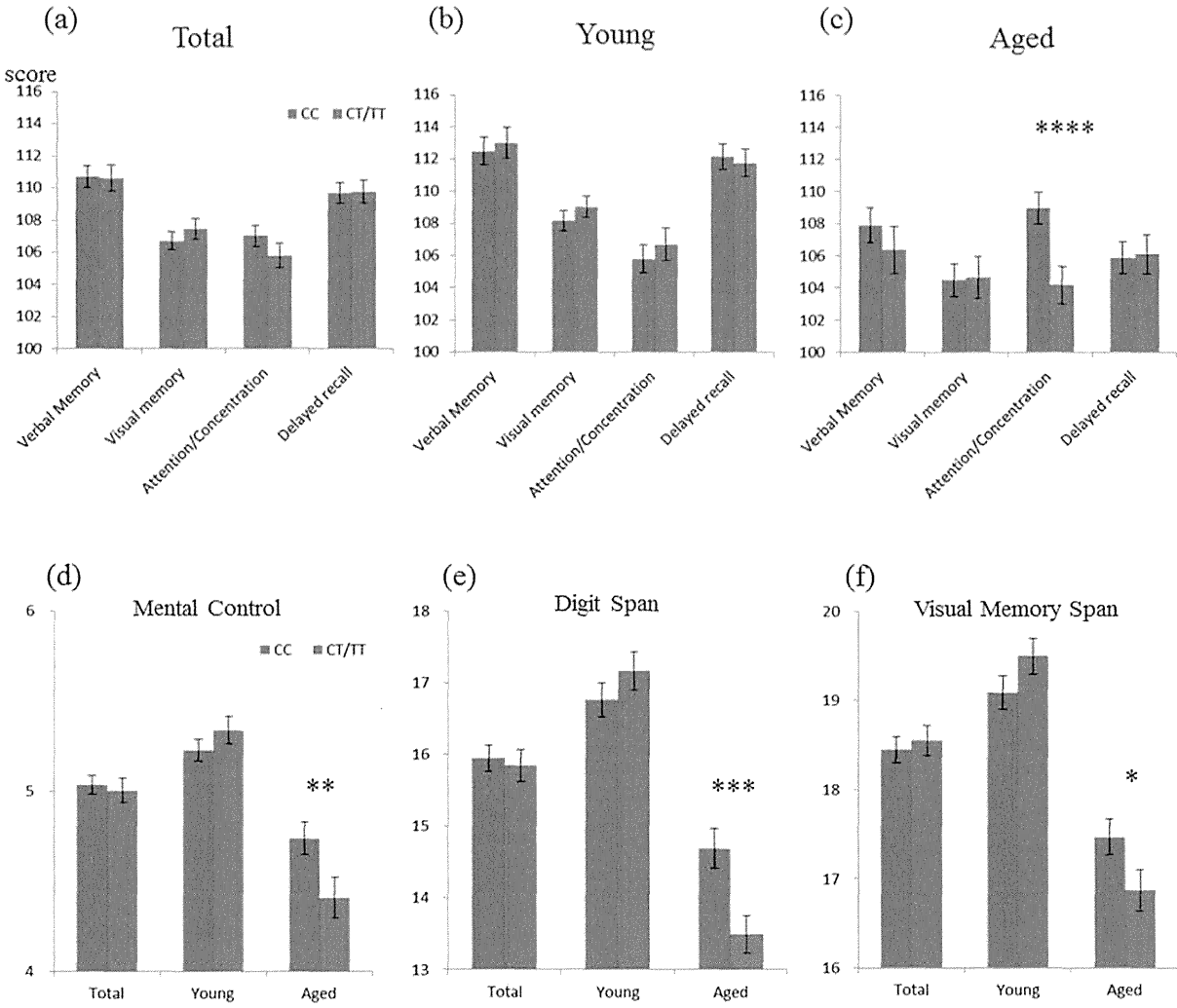

Figure 1 | Mean scores on the WMS-R tests for both genotypes (CC and CT/TT) in the total, aged, and young groups. No significant differences by genotype were observed in the total (a: CC [$n = 423$], CT/TT [$n = 320$]) or young (b: CC [$n = 256$], CT/TT [$n = 205$]) groups. We found a significant genotype difference in mean attention/concentration scores on the WMS-R in the aged group (c; $F = 14.94$, $df = 1$, **** $P = 0.00014$: CC [$n = 167$], CT/TT [$n = 115$]). No significant difference in any WMS-R attention/concentration subtests ([d] mental control, [e] digit span, [f] visual memory span) between the two groups (CC vs. CT/TT) was observed in the total or young groups. In the aged group, T carriers show statistically lower mean scores in all subtests ([d] mental control, $F = 6.98$, $df = 1$, $^{**}P = 0.0087$; [e] digit span, $F = 8.36$, $df = 1$, $^{***}P = 0.0041$; [f] visual memory span, $F = 4.60$, $df = 1$, $^{*}P = 0.033$), than non-T carriers. Error bars indicate standard error of the mean (S.E.M).

types of aged T carriers in the present study may be similar to the endophenotypes of not only patients with PTSD but also patients with MDD or AD.

Our previous study showed that aged non-clinical individuals carrying the T allele of rs1360780 had a suppressed cortisol response in the HPA axis[2]. However, previous studies in aged populations reported conflicting results regarding the association between cortisol levels and cognitive performance[22–27]. Although, compared with the C allele, the T allele is associated with higher FKBP5 induction by cortisol[6,7], aged T carriers exhibit low cortisol levels likely due to compensatory mechanisms that occur during aging[2,28]. The relationship between *FKBP5* genotype, cortisol, and cognitive performance is complex and further investigations are required to understand its underpinning molecular mechanisms. However, we could provide a possible mechanism. We previously found lower HPA axis reactivity in T carriers compared with non-T carriers in the aged population[2]. This lower reactivity was supported by the increased *GR* and reduced *FKBP5* expression levels in aged T carriers' peripheral blood mononuclear cells (PBMCs)[2]. Such changes could extend beyond PBMCs to the brain and be involved in the structural changes in specific brain regions. In our previous study, T carriers exhibited the smaller dorsal anterior cingulate cortex (dACC) than non-T carriers[29]. In T carriers, the smaller dACC may lead to the lower performance on the attention/concentration index. These differences between the genotypes of *FKBP5* rs1360780 are likely to contribute to the differences in vulnerability to PTSD.

In consideration of the importance of the education years in the neurocognitive tests[30], we confirmed that there was no significant difference in education years between the genotype groups not only among total individuals (Table 1), but also among young and aged individuals (data not shown).

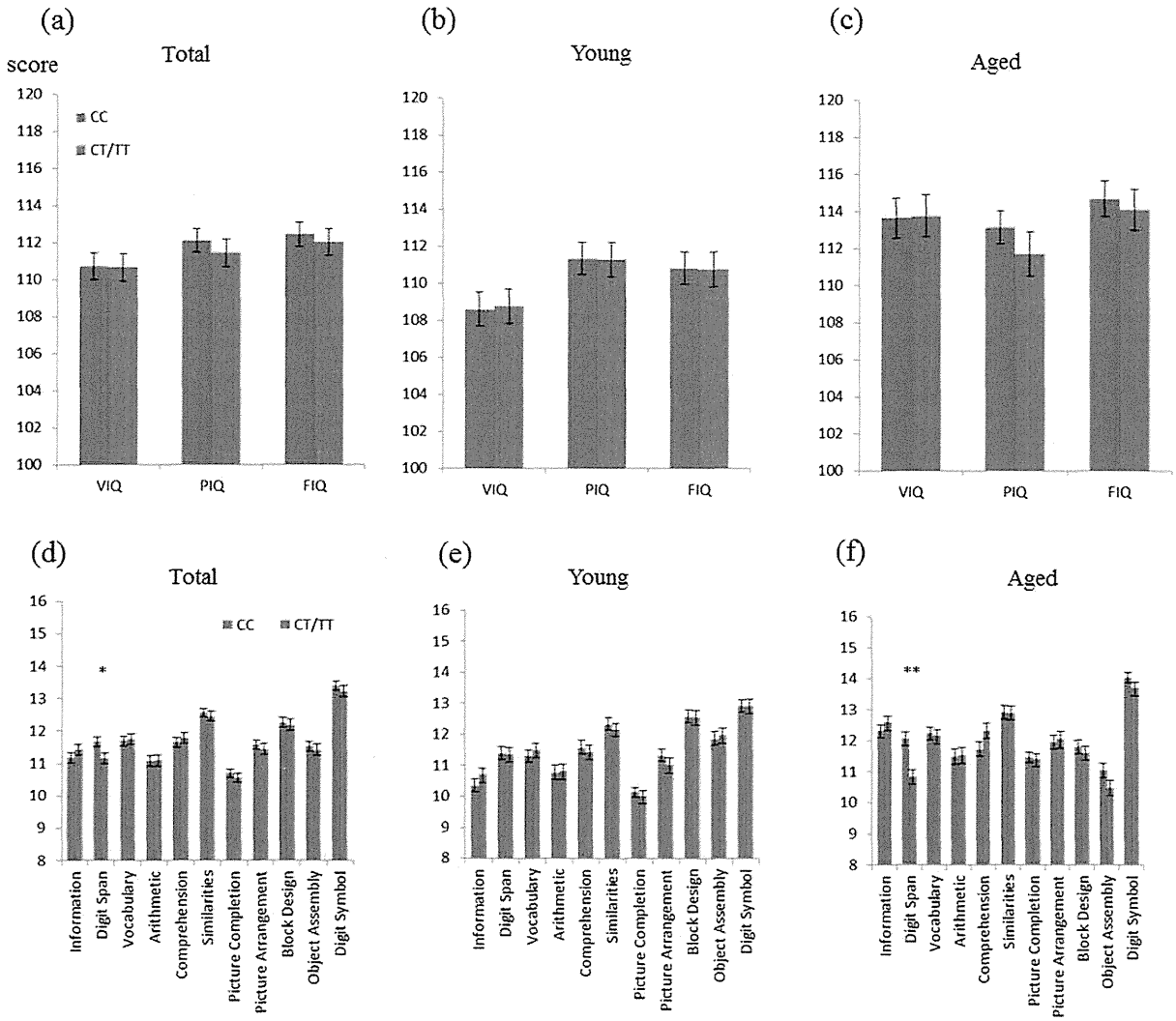

Figure 2 | Mean scores from IQ and each subset in the verbal and performance sections of the WAIS-R in the total, aged, and young groups. Mean IQ scores assessed using the WAIS-R in T and non-T carriers are shown for the total (a: CC [$n = 360$], CT/TT [$n = 267$]), young (b: CC [$n = 210$], CT/TT [$n = 165$]), and aged (c: CC [$n = 150$], CT/TT [$n = 102$]) groups. There was no significant difference in verbal IQ (VIQ), performance IQ (PIQ), or full-scale IQ (FIQ) between genotypes (CC vs. CT/TT) from any group (total, aged, or young). Error bars indicate S.E.M. A significant difference in digit span by genotype in the total (d) and aged (f) groups was observed using the WAIS-R. The young group (e) showed no significant differences in any of the subsets. Error bars indicate S.E.M. * $P = 0.0060$, ** $P = 0.00020$ (ANCOVA).

Several limitations to this study need to be mentioned. First, its cross-sectional nature did not allow us to draw any definitive conclusions regarding age-dependent effects. Second, although random sampling would be desirable for collecting an unbiased representative sample, we recruited non-clinical volunteers from the community through local magazine advertisements and an announcement on our website. Third, although all participants were healthy subjects without a history of psychiatric disorders, they were not assessed for childhood trauma. Fourth, we performed ANCOVA without controlling for education years in the present study. However, essentially similar results were obtained in ANCOVA even when we controlled for education years, age, and gender (data not shown). Fifth, mood may be a confounder for attention/concentration, although we did not control for mood status. Further studies controlling for mood status are required to address this issue. Sixth, we assessed only Japanese mostly female subjects in the present study. Further studies in other ethnic groups are required to confirm our findings. We controlled for gender in ANCOVA. These limitations should be resolved in future studies.

In conclusion, we found that aged non-clinical individuals with the T allele of *FKBP5* rs1360780 had significantly poorer attention/concentration than those without. Since such cognitive dysfunctions are involved in PTSD symptoms, the results in our non-clinical population suggest that poor attention/concentration may be an endophenotype of individuals with this particular genetic vulnerability to PTSD. Our findings are of potential importance to provide new insights into the pathogenesis of stress-related psychiatric disorders, including PTSD, and may lead to the development of effective preventive strategies. Further studies are warranted to elucidate the mechanisms underlying this observed association.

Methods

Participants. Subjects were volunteers with no current or past history of psychiatric disorders. The number of subjects for the WMS-R and the WAIS-R were 742 and 627, respectively (Table 1). All subjects were biologically unrelated and Japanese. Participants were screened using the Japanese version of the Mini-International Neuropsychiatric Interview (M.I.N.I.)[31,32] and unstructured interviews by a research psychiatrist. Individuals who had a prior medical history of central nervous system disease, substance abuse/dependence, severe head injury, dementia, or intellectual disability were not permitted to enroll in the study.

The present experiments on our participants were conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan. After hearing a comprehensive description of the study, written informed consent was obtained from every subject.

Genotyping. Venous blood was drawn from subjects and genomic DNA was extracted from whole blood according to standard procedures. The SNP rs1360780 was genotyped using the TaqMan 5′- exonuclease allelic discrimination assay (Applied Biosystems, Foster City, CA; assay ID C___8852038_10) as described previously[2]. PCR thermal cycling conditions were as follows: one cycle at 95°C for 10 min, followed by 50 cycles at 92°C for 15 s and 60°C for 1 min. Genotype data were assessed blinded to the case-control status. The genotyping protocol was performed according to our previous study[33].

Neurocognitive testing. To examine neurocognitive performance, the full Japanese versions of the WMS-R[34,35] and WAIS-R[36,37] were administered by research psychologists. The WMS-R measures me mory functions, namely verbal memory, visual memory, and delayed recall. In addition, it includes the attention/concentration index, which consists of the forward and backward digit and visual span subtests, thus measuring not only attention and concentration, but also verbal and spatial working memory. Auditory attention and verbal working memory were measured from the forward and backward digit span tests, respectively. Visual attention and visual working memory were measured from the forward and backward visual span tests, respectively. Of the 763 subjects, the WMS-R and WAIS-R were completed by 743 and 627 participants, respectively (Table 1). Among them, 607 ones overlapped in the WMS-R and WAIS-R test study groups.

Statistical analysis. Deviation of genotype distributions from the HWE was assessed with a χ^2 test for goodness of fit. Demographic characteristics between genotype groups were compared by using either ANOVA or χ^2 test, as appropriate. Differences in WMS-R and WAIS-R scores between genotype groups were tested using an ANCOVA, controlling for age and gender. These tests were performed with SPSS ver.11 (SPSS Japan, Tokyo, Japan). Statistical tests were two-tailed and P values < 0.05 were considered significant.

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Author contributions

T.F. designed the study, performed the genotyping, undertook the statistical analyses, and wrote the draft of the manuscript. J.M., Y.K., I.I. and A.N. administered the neuropsychological tests. H.H., M.O., K.H. and T.T., contributed to the data collection. H.K. organized recruitment of non-clinical volunteers, supervised the entire project, and gave critical comments on the manuscript. All authors contributed to and have approved the final manuscript.

Additional information

Competing financial interests: The authors declare no competing financial interests.

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ANNALS OF GENERAL PSYCHIATRY

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Serum proBDNF/BDNF and response to fluvoxamine in drug-naïve first-episode major depressive disorder patients

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Abstract

Background: We investigated the association between serum proBDNF, a precursor of brain-derived neurotrophic factor (BDNF), and response to fluvoxamine in patients with major depressive disorder (MDD) using the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR): physically healthy and free of current alcohol or drug abuse, comorbid anxiety, or personality disorders.

Methods: Fifty-one patients with MDD (M/F, 19:32; age, 38 ± 19 years) and 51 healthy controls (M/F, 22:29; age, 34 ± 17 years) were studied using DSM-IV-TR: physically healthy and free of current alcohol or drug abuse, comorbid anxiety, or personality disorders. Serum levels of proBDNF and MDNF were measured by sandwich enzyme-linked immunosorbent assay (ELISA).

Results: Serum mature BDNF levels in the MDD patients were significantly lower than those in the healthy controls ($t = 3.046$, $p = 0.0018$). On the other hand, no difference was found in serum proBDNF between the MDD patients and the healthy controls ($t = -0.979$, $p = 0.833$). A trend of negative correlation was found between baseline serum BDNF and baseline scores of the 17 items of the Hamilton Rating Scale for Depression (HAMD17) ($r = -0.183$, $p = 0.071$). No correlation was however found between HAMD17 scores and proBDNF at baseline ($r = 0.092$, $p = 0.421$). Furthermore, no correlation was observed between baseline HAMD17 scores and baseline proBDNF/BDNF ($r = -0.130$, $p = 0.190$). No changes were observed in serum levels of proBDNF and BDNF during the treatment periods.

Conclusions: These results suggest that there is no association between serum proBDNF/BDNF and fluvoxamine response in MDD patients at least within 4 weeks of the treatment.

Keywords: BDNF, proBDNF, Major depressive disorder, Serum, Fluvoxamine

Background

Major depressive disorder (MDD) is a common and major psychiatric disorder that affects as many as about 20% of individuals within their lifetime [1-3]. A wide variety of pharmaceuticals are available for treating depression, including tricyclic antidepressants, monoamine oxidase inhibitors, and selective serotonin reuptake inhibitors (SSRIs). Fluvoxamine is an SSRI that is widely used for treatment of depression and other psychiatric disorders and has been suggested to have early effects when used as an antidepressant drug [4,5]. In addition, the results of a meta-analysis have shown that significant improvements in Hamilton Rating Scale for Depression (HAMD) scores achieved in the first few weeks were maintained after 6 weeks of treatment [6]. Results of a recent meta-analysis also suggest that treatment with fluvoxamine leads to symptomatic improvements in patients with MDD by the end of the first week of use [6].

Mature brain-derived neurotrophic factor (BDNF) is initially synthesized as a precursor protein. ProBDNF is converted to BDNF by extracellular proteases such as matrix metalloproteinase-9 (MMP-9). BDNF is biologically active. In contrast, proBDNF, which localizes intracellularly, serves as an inactive precursor. In short, new evidence shows that

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Table 1 Demographics of participants

	Values
Age (years)	38 (19)
Female (%)	62
Daily dose at week 4 (max) (mg)	103 (38)
DUP (months)	2.1 (0.9)
HAMD17 (baseline)	19.3 (2.8)

proBDNF and BDNF elicit opposing effects via the neurotrophin receptor p75 (p75NTR) and tropomyosin-related kinase B (TrkB) receptors, respectively, and that both proBDNF and mature BDNF play important roles in several physiological functions for neurons, which might be related to the pathology of psychiatric disorders such as mood disorders and schizophrenia [7-9]. Sen et al. [10] first performed a meta-analysis and demonstrated that serum BDNF levels are abnormally low in patients suffering from major depressive disorder and that the BDNF levels are elevated following a course of antidepressant treatment. Although the relationship of our findings to the pathophysiology of depression and the mechanism of drug action remains to be determined, the measure may have potential use as a biomarker for psychiatric disorders or as a predictor of antidepressant efficacy [10,11]. Recently, Yoshida et al. [12] reported that it was initially thought that only secreted mature BDNF was biologically active and that proBDNF, which localizes intracellularly, served as an inactive precursor. However, new evidence shows that proBDNF and BDNF elicit opposing effects via the p75NTR and TrkB receptors, respectively, and that both proBDNF and BDNF play important roles in several physiological functions [8,12]. In recent decades, the role of BDNF in first-episode major depressive disorder MDD patients has received intensive attention. However, the relationship between proBDNF and MDD has not been clearly elucidated. We hypothesized that (1) serum levels of BDNF, proBDNF, and proBDNF/BDNF are different between MDD patients and healthy controls, (2) fluvoxamine decreases serum proBDNF level and proBDNF/BDNF ratio and increases serum BDNF level, and (3) the plasma level of fluvoxamine is related to serum levels of BDNF and the HAMD17 scores.

This study aimed to determine whether serum levels of proBDNF/BDNF were different between MDD patients and the healthy controls. We also examined serum levels of proBDNF/BDNF between the responders and the nonresponders to fluvoxamine in patients with first-episode MDD. In addition, we also investigated longitudinal changes in proBDNF and BDNF in MDD patients treated with fluvoxamine before and after the treatment.

To the best of our knowledge, this is the first study investigating serum proBDNF/BDNF and response to fluvoxamine.

Materials and methods

Participants

Fifty-one drug-naïve and first-episode patients with MDD were studied. In the MDD group, 19 were males and 32 were females, ranging in age from 29 to 71 (mean ± standard deviation (SD), 38 ± 19) years. In 51 cases of the healthy control (HC) group, 22 males and 29 females, ranging in age from 24 to 68 (mean ± SD, 35 ± 16) years, were enrolled in the present study. Prior to the commencement of the study, all subjects provided written informed consent, after receiving a full explanation of the study as well as any potential risks and benefits of study participation. The study was approved by the Ethics Committee of the University of Occupational and Environmental Health and performed in accordance with the Declaration of Helsinki II. The demographics of the participants are shown in Table 1, ranging in age from 29 to 71 (mean ± SD, 38 ± 19)

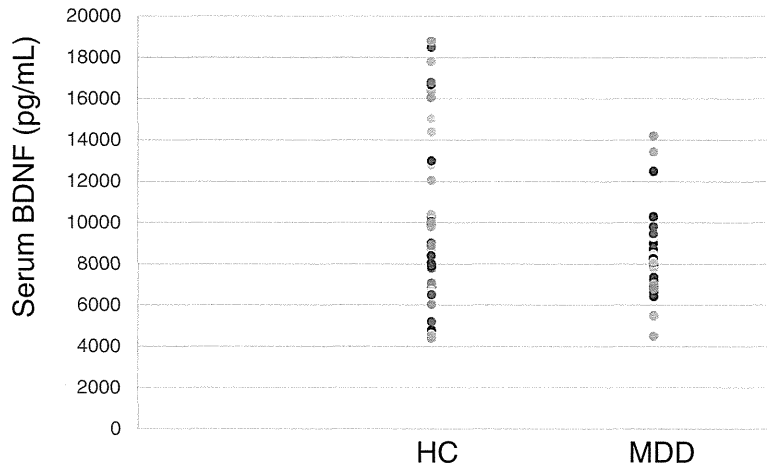

Figure 1 The HAMD17 scores and serum proBDNF. Red line shows the mean of value.

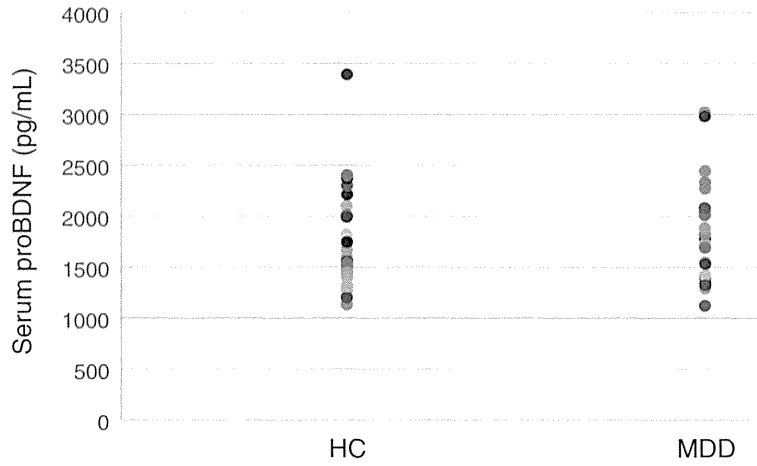

Figure 2 Serum proBDNF change during treatment with fluvoxamine. Red line shows the mean of value.

years. All patients fulfilled the MDD criteria using the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR): physically healthy and free of current alcohol or drug abuse, comorbid anxiety, or personality disorders. We defined the responders as those whose scores of the 17 items of the Hamilton Rating Scale for Depression (HAMD17) decreased 50% or more. All patients consented to participate after having been informed of the study's purpose. Benzodiazepines were the only hypnotics permitted, and their dosages were kept constant throughout the study period. The dosages of fluvoxamine varied among patients and were not fixed for ethical reasons.

Assessment of clinical variables

Depression was assessed using the 17 items of the Structured Interview Guide for the Hamilton Depression Rating Scale (SIGH-D) by an experienced psychiatrist (R.Y.).

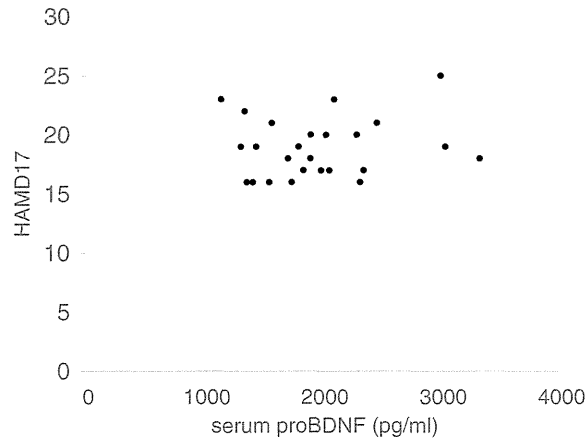

Figure 3 Serum proBDNF in the responders' and the nonresponders' treatment with fluvoxamine.

Serum proBDNF and BDNF assay

Blood was drawn at 9:00 a.m. Serum levels of BDNF and proBDNF were measured in duplicate using the human proBDNF enzyme-linked immunosorbent assay (ELISA) kit SK00572-06 (Adipo Bioscience, Santa Clara, CA, USA) and the human matureBDNF ELISA kit SK00572-01 (Adipo Bioscience, Santa Clara, CA, USA). All experiments were performed in duplicate. Protocols were performed according to the manufacturer's instructions. In short, 96-well microplates were coated with anti-BDNF monoclonal antibody and incubated at 4°C for 18 h. The plates were incubated in a blocking buffer for 1 h at room temperature. The samples were diluted 100-fold with an assay buffer, and BDNF standards were kept at room temperature with horizontal shaking for 2 h and then washed with the appropriate washing buffer. The plates were incubated with antihuman BDNF polyclonal antibody at room temperature for 2 h and washed with the washing buffer. Then, they were incubated with anti-IgY antibody conjugated to horseradish peroxidase for 1 h at room temperature and incubated again in peroxidase substrate and tetramethylbenzidine solution to induce a color reaction. The reaction was stopped with 1 mol/L hydrochloric acid. The absorbance at 450 nm was measured with an Emax automated microplate reader (Molecular Devices, Chuo-ku, Japan). Measurements were performed in duplicate.

Plasma fluvoxamine assay

The plasma fluvoxamine level was measured using high-performance liquid chromatography according to the method we previously described [13]. In brief, 1 mL of plasma alkalized with 500 μL of 2 M sodium hydrogen carbonate was extracted by hexane (10 mL) after the addition of the internal standard (clomipramine). Shaken horizontally for 20 min and then centrifuged at 2,000 *g* for 10 min, the upper organic layer was removed and dried

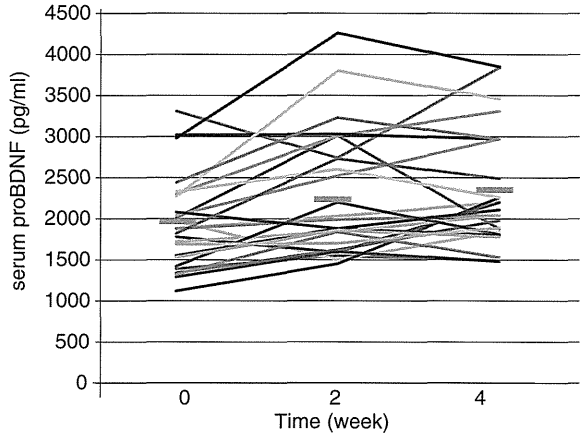

Figure 4 The HAMD17 scores and serum BDNF. Red line shows the mean of value.

under N_2. After being dissolved in 200 μL of mobile phase, a 50-μL aliquot of the final preparation was subjected to HPLC. All experiments were performed in duplicate.

Statistical analyses

Student's *t* test was used to compare two groups (serum levels of proBDNF and BDNF; healthy control vs. MDD). Serum levels of proBDNF and BDNF and plasma fluvoxamine concentrations were measured, and correlations with clinical variables were performed using Peason's correlation. One-way ANOVA was used regarding the time course of proBDNF and BDNF. Power analysis was performed in BDNF (0 week) × HAMD17 (0 week) and BDNF (healthy control, 0 week) × BDNF (MDD, 0 week). A significant value of $p < 0.05$ was judged as statistically significant. All analyses were carried out using SPSS version 19.0 (SPSS Inc, Chicago, IL, USA).

Results

The demographics of the participants are shown in Table 1. Twenty-five of 51 (49%) MDD patients responded to fluvoxamine at least within 4 weeks. Nine of 51 (18%) MDD patients had remission. Serum BDNF of all subjects could be measured. Serum proBDNF of 32 of 51 HC (63%) and 25 of 51 MDD patients (49%) could be assayed. Serum BDNF levels in MDD were significantly lower than those in HC ($t = 3.046$, $p = 0.0018$, 1-β = 82.3%) (Figure 1). On the other hand, no difference was found in serum proBDNF between the MDD patients and the HC ($t = -0.979$, $p = 0.833$) (Figure 2). Twenty-four of 51 MDD patients (47%) responded to fluvoxamine treatment at least within 4 weeks. No difference was found in baseline proBDNF between responders and nonresponders ($t = 1.837$, $p = 0.073$). No difference was also found in baseline BDNF between responders and nonresponders ($t = 1.19$, $p = 0.23$). A trend for negative correlation was found between baseline serum BDNF and baseline HAMD17 scores ($r = -0.183$, $p = 0.071$) (Figure 3). No correlation was however found between the HAMD17 scores and proBDNF at baseline ($r = 0.092$, $p = 0.421$) (Figure 4). No difference was found between serum levels of proBDNF ($r = 0.090$, $p = 0.336$) (Figure 5) and BDNF ($r = -0.084$, $p = 0.730$) (Figure 6) at week 0 in MDD patients. No changes were observed in serum levels of proBDNF at baseline, 2 weeks, and 4 weeks after administrating fluvoxamine ($F = 2.580$, $p = 0.080$) (Figure 7). No changes were also observed in serum levels of BDNF at baseline, 2 weeks, and 4 weeks after administrating fluvoxamine ($F = 0.579$, $p = 0.561$) (Figure 8). Furthermore, no correlation was observed between baseline HAMD17 scores and baseline proBDNF/BDNF in MDD patients ($r = -0.130$, $p = 0.190$) (Figure 9). No correlation was also found between plasma fluvoxamine levels at week 4 and the changes in HAMD17 scores ($r = 0.211$, $p = 1.514$) (Figure 10). No correlation was found

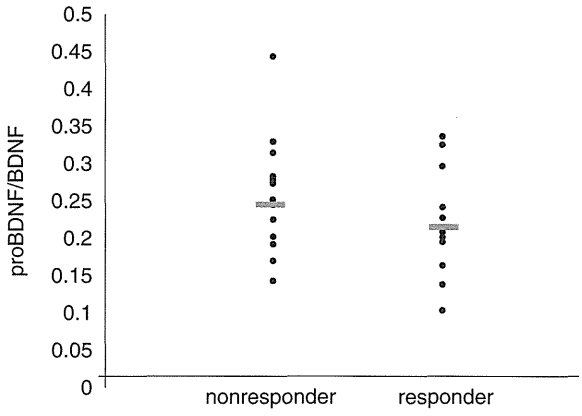

Figure 5 Serum BDNF change during treatment with fluvoxamine.

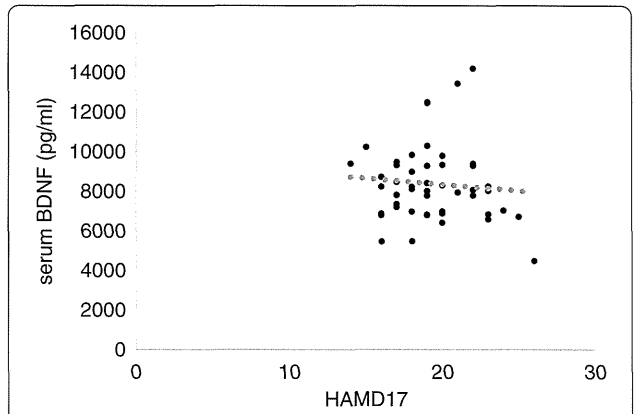

Figure 6 Serum BDNF in the responders' and the nonresponders' treatment with fluvoxamine. Red dot line shows regression line.