treatment response.[5] The Met-variants of *COMT* gene Val-158Met were shown to be risk variants for depressed mood and low motivation in depressive Swedish men.[6]

Brain-derived neurotrophic factor (BDNF) is a molecular substrate of stress; data have demonstrated that BDNF expression is reduced by stress (an important risk factor for MDD and posttraumatic stress disorder)[7] and correlates to hippocampus volume in patients.[8] The levels of BDNF and its receptor, tropomyosin-related kinase B (TrkB) receptor, are decreased in regions of the hippocampus in postmortem tissue taken from suicide victims and patients with MDD, and in the serum of MDD patients.[9–11] Researchers have investigated the *BDNF* gene for a single nucleotide polymorphism (SNP) that might be linked to MDD. The most common *BDNF* SNP in humans is at codon 66, resulting in the Val66Met protein variant, which prevents the activity-dependent release of BDNF.[12] Men homozygous for the mutation might be at greater risk for MDD.[13] It has been hypothesized that monoamine and BDNF are associated with the pathogenesis of MDD.[14]

The white matter (WM) abnormalities constitute one element of the pathogenesis of MDD.[15–17] Various fiber tract alterations have been seen in MDD patients.[18–22] Magnetic resonance imaging (MRI) is a noninvasive method used to examine WM abnormalities. Diffusion tensor imaging (DTI) is an MRI technique that can study the orientation and integrity of WM fiber tracts in vivo.[23] DTI-based quantitative measures, such as fractional anisotropy (FA), represents intact myelin and axons, and has been shown to be a useful marker of the microstructural changes in WM.

Although several studies using a voxel-based DTI analysis demonstrated lower FA values in the frontal, temporal, and parietal lobes and the cerebellum of MDD patients,[24–28] such an analysis is not a mainstream of statistical parametric mapping and is not officially supported. Therefore, there has not been a consensus about the optimal method to spatially normalize FA images and the size of the smoothing kernel. A voxel-wise approach of tract-based spatial statistics (TBSS) has been introduced. The TBSS method projects all subjects' FA data onto an average FA tract skeleton before applying voxel-wise cross-subject statistics, and it minimizes the misalignment effects and is more robust and sensitive than voxel-based DTI analyses.[18]

The findings from individual reports of WM abnormalities in MDD patients indicate a widespread pattern of alterations, and the extent of WM abnormalities might be associated with clinical features. Indeed, the severity of illness and poorer treatment outcomes have been associated with increased WM pathology, indicating that patients with a greater illness burden are more likely to have microstructural damages.[29] The most pronounced WM FA reductions have been observed in the main body and genu of the corpus callosum, consistent with some, but not all, DTI reports in MDD. FA values of the WM in the right frontal lobe, right fusiform gyrus, left frontal lobe, and right occipital lobe were also demonstrated to be reduced. Fiber tracking has shown that the main fascicles involved were the right inferior longitudinal fasciculus, right inferior fronto-occipital fasciculus, right posterior thalamic radiation, and interhemispheric fibers running through the genu and body of the corpus callosum.[30]

Carballedo et al[31] recently reported that they observed a significant interaction, in the uncinated fasciculus, between a *BDNF* allele and diagnosis: patients carrying the *BDNF* Met allele had lower FA values in the uncinated fasciculus compared with healthy subjects carrying the Met allele. Kim et al[32] reported an association between altered WM connectivity and *COMT* gene Val158Met polymorphism in panic disorder patients. We hypothesize that the *COMT* gene and the *BDNF* gene are associated with WM connectivity in MDD patients.

In the present study, therefore, we compared the status of polymorphism of the *COMT* gene or *BDNF* gene and DTI findings between drug-naïve MDD patients and age- and sex-matched healthy controls.

Subjects and methods

Subjects

Thirty first-episode, right-handed, treatment-naïve outpatients were recruited. Major depressive episodes were diagnosed using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) according to the DSM-IV, text revision (TR) criteria. The severity of depression was evaluated using the 17-item Hamilton Rating Scale for Depression (HAMD17). Only those patients with a HAMD17 score $\geq$14 were eligible for the study. Exclusion criteria were: any history of neurological disease or other physical disease, and comorbidity with other mental disorders (no evidence of schizoaffective disorder, bipolar disorder, Axis II personality disorders, or mental retardation). In all, 17 subjects were male, and 13 were female. The age range was from 20 to 67 years, with a mean $\pm$ standard deviation (SD) age of 44$\pm$12 years. Similarly, 30 right-handed healthy subjects, 17 male and 13 female, with mean age 44$\pm$13 years were recruited from the community.

The DTI scans for all 60 subjects were performed on the day when each subject was enrolled. The 30 control subjects

were interviewed by the same psychiatrists that interviewed the MDD patients, using the Structured Clinical Interview for DSM-IV, nonpatient edition. None of the control subjects had a history of serious medical or neuropsychiatric illness or a family history of major psychiatric or neurological illness in their first-degree relatives, and all were well matched with the patients in terms of age, sex, and years of education. All subjects were given complete information about the procedures. Written informed consent was obtained from all subjects via forms approved by the local Ethics Committee of the University of Occupational and Environmental Health, Kitakyushu, Japan.

Methods

Diffusion tensor images: MRI scanning protocol

All MRI examinations were performed using a 3T MRI system (Signa® EXCITE™ 3T; GE Healthcare, Little Chalfont, UK) with an eight-channel brain phased-array coil. DTIs were acquired by a single-shot, spin-echo planar sequence, with the following parameters: TR/TE =12,000/83.3 msec; 4 mm slice thickness; no gap; field of view =26 cm; number of excitations =1, spatial resolution =1.02×1.02×4 mm. Diffusion gradients (b-value of 1,000 sec/mm^2) were always applied on two axes simultaneously around the 180-degree pulse. The diffusion properties were measured along 25 noncollinear directions. The spatial distortion of diffusion-weighted MRIs was corrected based on each T2-weighted echo-echo planar image (b=0 sec/mm^2)[33] using registration functional MRI of the brain (FMRIB) tools.

Image processing

Maps of FA were computed for all subjects from the DTIs, after eddy current correction and automatic brain extraction using the FMRIB Diffusion Toolbox, which is part of the FMRIB Software Library (The Oxford Centre for Functional MRI of the Brain, Oxford, UK).[34] We performed a voxel-wise statistical analysis of the DTI data using TBSS[35] (implemented in the FMRIB Software Library 4.1.6). The FA, radial diffusivity (RD), and axial diffusivity (AD) were created by fitting a tensor model to the raw diffusion data. Brain extraction was then performed using the Brain Extraction Tool 2.1.[36]

The FA data of all subjects were aligned into a common space by means of nonlinear registration.[37] Next, a mean FA image was created and thinned to create a mean FA skeleton representing the centers of all tracts common to the group. This skeleton was thresholded at FA >0.2. Each subject's aligned FA data were then projected onto this skeleton, and the resulting data were fed into a voxel-wise cross-subject statistical analysis. Subsequently, other relevant DTI output images (AD and RD) were projected onto the mean FA skeleton so that other diffusivity values could be compared between groups in the same spatial location.

We compared the DTI metrics between the MDD and control groups using a TBSS analysis.

Genotyping and serum catecholamine metabolites assay

Genomic DNA was extracted from peripheral leukocytes using a QIAamp® DNA Blood Kit (Qiagen, Venlo, the Netherlands) and was stored at −20°C until used for analysis. Genotyping for the presence of the *BDNF* Val66Met and *COMT* Val158Met polymorphisms was performed using direct sequencing in the region.

We analyzed the subjects' plasma concentrations of homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). The plasma HVA levels were analyzed by HPLC-ECD according to the method of Yeung et al,[38] with slight modification. In brief, each cyano-bonded solid-phase extraction cartridge was preconditioned with methanol, followed by glass-distilled water. To each cartridge we added 0.3 mL of plasma sample or standard, and 0.1 mL of working internal standard solution (5 ng of 5-hydroxyindolecarboxylic acid in 0.01 M KH_2PO_4, pH 7.2). The samples were deproteinized with 1 mL of acetonitrile. After mixing by vortex and centrifugation (1,760× *g*, 4°C for 10 minutes), an aliquot (5 µL) of supernatant was allowed to pass through the cartridge slowly, under a mild vacuum (15 mmHg). The cartridge was washed with 0.2 mL of distilled water and extracted containing 1 mL of ethylacetate, and then an aliquot was evaporated to dryness under nitrogen gas. After dissolution in mobile phase (200 µL), a 10 µL portion of this solution was injected into the HPLC system. The detection limit was 0.5 ng/mL, and the calibration curve was linear up to 40 ng/mL. The intra- and interassay coefficients of variation were 6% and 8%, respectively. The recovery rate was more than 80%.

The subjects' plasma MHPG levels were also analyzed by HPLC-ECD, according to the method of Minegishi and Ishizaki.[39] In brief, the plasma was separated by centrifugation at 600× *g* at 4°C. Extraction was performed under a vacuum using Bond-Elut columns (Varian Medical Systems, Inc., Palo Alto, CA, USA) prepacked with 100 mg of C18-bonded silica (40 µm) in a 1 mL capacity disposable syringe. The columns, which were inserted into a vacuum chamber connected to an

aspirator, were prepared by washing with 1 mL methanol followed by 1 mL of water. After the addition of 50 μL of a solution of vanillyl alcohol (internal standard equivalent to 5 ng/mL) to 1 mL of plasma, the samples were passed through the columns, followed by 0.75 mL of water to rinse off both residual samples and easily eluted hydrophilic compounds.

The adsorbed materials were eluted with 200 μL of methanol to a 0.1 M phosphate buffer (pH 4.8) mixture (40:60, v/v [volume/volume]). A 20 μL portion of this solution was injected into the HPLC system. The detection limit was 0.5 ng/mL, and the calibration curve was linear up to 40 ng/mL. The intra- and interassay coefficients of variation were 6% and 8%, respectively. The recovery rate was more than 80%.

Statistical analyses

The significance threshold for between-group differences was set at family-wise error (FWE)-corrected $P<0.05$; this was corrected for multiple comparisons across voxels by using the threshold-free cluster-enhancement option. The number of permutations was set to 20,000 in all voxel-wise analyses. The chi-square test was used to compare the number of patients with the *COMT* or *BDNF* genotype Val/Val, and the number of Met-carriers in both the MDD patient and the control groups. The unpaired *t*-test was used to compare the items of the HAMD17 scores, and the plasma levels of MHPG and HVA between the Val/Val group and Met-carriers in the MDD patient group. The unpaired *t*-test was also used to compare serum BDNF levels between the MDD patients and the healthy controls. A significance level of $P<0.05$ was used. Statistical procedures were performed using the Japanese version of SPSS, version 15 (SPSS Inc., Chicago, IL, USA).

Results

The genotype distributions of the *COMT* Val158Met polymorphism were determined in both the MDD patients and the control subjects, as shown in Table 1. The table provides the allele and genotype distributions as well as the chi-square and *P*-values of Hardy–Weinberg equilibria. As can be seen in Table 2, there were no significant differences among the MDD patients in each item of the HAMD17, with the exception that the responses to item 16 (weight loss) differed significantly between the *COMT* Val/Val group and the *COMT* Met-carriers.

The analysis of the plasma levels of catecholamine metabolites (MHPG and HVA) revealed no significant differences between the MDD patients and the controls (MHPG was 5.3±1.0 ng/mL for MDD and was 5.4±1.2 ng/mL for controls [$P=0.23$]; HVA was 5.5±1.4 ng/mL for MDD and was 5.1±0.8 ng/mL for the controls [$P=0.13$]). In addition, no significant differences between the Val/Val group and the Met-carriers were found in plasma MHPG, a major metabolite of norepinephrine (5.0±1.4 ng/mL [Val/Val group]; 4.9±1.5 [Met-carriers] [$P=0.85$]) or in plasma HVA, a major metabolite of dopamine (8.5±2.7 ng/mL [Val/Val group]; 8.7±2.7 ng/mL [Met-carriers] [$P=0.81$]). The serum BDNF levels were significantly lower in the MDD patients (4.8±0.4 ng/mL) compared with the controls (5.6±0.5 ng/mL) ($P=0.044$).

Table 1 Gene distribution of *COMT* and *BDNF* in patients with MDD and healthy controls

	Control	χ^2	*P*-value	Number of patients	χ^2	*P*-value
***COMT* Val158Met**						
GG	10			9		
GA	18	2.566	0.1099	16	0.222	0.5377
AA	2			5		
***BDNF* Val66Met**						
GG	18			15		
GA	10	0.14	0.7082	8	1.12	0.2900
AA	2			7		

Abbreviations: *BDNF, brain-derived neurotrophic factor; COMT, catechol-O-methyltransferase.*

Table 2 Between-group comparisons of scores on each item of the HAMD17, in MDD patients with the *BDNF* gene polymorphism

HAMD 17-item	Val/Val	Met-carrier	*P*-value
1	2.53±0.96	2.60±0.88	0.84
2	0.8±0.75	0.87±0.72	0.81
3	1.40±0.80	1.93±1.18	0.17
4	1.33±0.60	1.07±0.57	0.23
5	1.07±0.25	0.93±0.44	0.33
6	1.13±0.60	1.20±0.65	0.76
7	2.80±0.91	2.93±0.93	0.7
8	0.87±0.65	1.00±0.73	0.6
9	0.80±0.65	0.67±0.70	0.6
10	1.67±1.14	1.67±1.01	1
11	1.53±0.81	1.40±0.80	0.66
12	1.07±0.68	1.07±0.25	1
13	0.8±0.54	1.00±0.52	0.32
14	1.07±0.57	1.47±0.62	0.08
15	0.73±0.77	0.60±0.80	0.65
16	0.53±0.72	0.80±0.91	0.039
17	0.20±0.40	0.47±0.72	0.23
Total	19.50±6.35	21.70±4.91	0.31

Notes: 1, Depressed mood; 2, Feeling of guilt; 3, Suicide; 4, Insomnia early; 5, Insomnia middle; 6, Insomnia late; 7, Work and activity; 8, Retardation; 9, Agitation; 10, Anxiety (psychological); 11, Anxiety (somatic); 12, Somatic symptoms (gastrointestinal); 13, Somatic symptoms (general); 14, Genital symptoms; 15, Hypochondriasis; 16, Loss of weight; 17, Insight.
Abbreviations: *BDNF, brain-derived neurotrophic factor;* HAMD17, 17-item Hamilton Rating Scale for Depression; MDD, major depressive disorder.

Regarding *BDNF* Val66Met, no significant differences between the Val/Val group and the Met-carriers were observed in plasma MHPG (5.0±1.4 ng/mL [Val/Val group]; 4.9±1.5 [Met-carriers] [P=0.85]), plasma HVA (5.3±1.1 ng/mL [Val/Val group]; 5.0±1.3 [Met-carriers] [P=0.54]), or serum BDNF (5.4±1.2 ng/mL [Val/Val group]; 4.8±1.6 [Met-carriers] [P=0.39]). No differences were observed in any items of the HAMD17 between the Val/Val genotype and the Met-carriers (Table 3).

In the voxel-wise-based group comparison, no significant differences were observed regarding FA, AD, or RD, in all patients compared with the controls. We found a significant FA decrease ($P<0.05$) within the temporal lobe WM in the Met-carriers among the MDD patients compared with those of the healthy controls (Figure 1A–C), on the basis of the Johns Hopkins University (JHU) white-matter tractography atlas and the International Consortium for Brain Mapping DTI-81 WM labels (part of the FMRIB Software Library package). In the voxel-wise-based group comparison, there was no significant difference in FA, AD, or RD, between the MDD patients and the healthy controls.

After dividing the MDD patients into genotype subgroups, we found a significant FA decrease ($P<0.05$) in the temporal lobe among the MDD patients who were Met-carriers compared with the corresponding values among the healthy controls (Figure 1A–C). Significantly decreased AD in the temporal lobe ($P<0.05$) was also found in the Met-carrier MDD patients compared with the healthy controls (Figure 1D–F). Significantly decreased AD in the temporal lobe ($P<0.05$) was also found in the MDD patients compared with the controls (Figure 1D–F). Moreover, the genotype–diagnostic interaction effect on FA was seen in the same position (uncorrected $P<0.05$), although no voxels could survive after the correction for multiple comparisons (FWE <0.05). The results of the image analyses are shown in Table 4.

No significant differences were observed in FA or AD, at any brain regions, between the MDD patients with the *COMT*

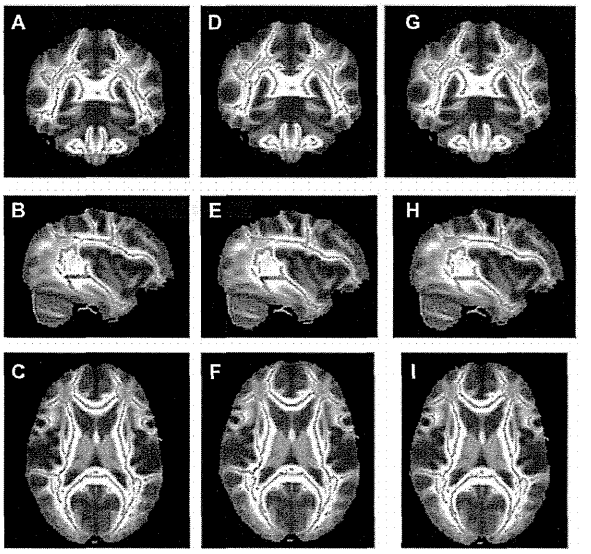

Figure 1 Corrected *P*-maps for the *COMT* gene polymorphism.
Notes: These maps show the regions where FA is reduced in the red voxels (**A–C**), where AD is reduced in the red voxels (**D–F**), and where RD shows no change (**G–I**). The FA, AD, and RD skeletons are projected in green on the MNI 152 template (Montreal Neurological Institute, Montreal, QC, Canada) average brain section.
Abbreviations: AD, axial diffusivity; *COMT*, *catechol-O-methyltransferase*; FA, fractional anisotropy; RD, radial diffusivity.

Table 3 Between-group comparisons of scores on each item of the HAMD17 for the two groups of patients with MDD

HAMD 17-item	Val/Val	Met-carrier	*P*-value
1	2.11±0.34	2.32±0.64	0.71
2	0.73±0.75	0.80±0.69	0.65
3	1.67±0.59	1.81±1.03	0.19
4	1.48±0.76	1.29±0.49	0.18
5	1.02±0.29	1.09±0.38	0.30
6	1.13±0.60	1.20±0.65	0.76
7	2.49±0.67	2.99±1.08	0.52
8	0.91±0.72	1.14±0.81	0.53
9	0.92±0.54	0.71±0.52	0.49
10	1.82±1.02	1.52±0.94	0.78
11	1.23±0.74	1.57±0.79	0.72
12	0.98±0.68	1.12±0.43	0.92
13	0.87±0.63	1.14±0.61	0.43
14	1.21±0.62	1.44±0.78	0.11
15	0.65±0.52	0.63±0.79	0.59
16	0.53±0.69	0.78±0.88	0.13
17	0.37±0.41	0.52±0.51	0.49
Total	20.33±7.29	20.97±5.84	0.51

Notes: 1, Depressed mood; 2, Feeling of guilt; 3, Suicide; 4, Insomnia early; 5, Insomnia middle; 6, Insomnia late; 7, Work and activity; 8, Retardation; 9, Agitation; 10, Anxiety (psychological); 11, Anxiety (somatic); 12, Somatic symptoms (gastrointestinal); 13, Somatic symptoms (general); 14, Genital symptoms; 15, Hypochondriasis; 16, Loss of weight; 17, Insight.
Abbreviations: HAMD17, 17-item Hamilton Rating Scale for Depression; MDD, major depressive disorder.

Table 4 The results of image analyses

Anatomical regions	Cluster size	*P*-value (FWE-corrected)	MNI coordinate		
			x	y	z
FA analysis (HS > MDD in Met carriers)					
Right temporal	81	0.046	54	87	84
lobe	10	0.049	53	91	80
AD analysis (HS > MDD in Met carriers)					
Right temporal	151	0.046	58	90	87
lobe	9	0.049	51	81	71

Abbreviations: AD, axial diffusivity; FA, fractional anisotropy; FWE, family-wise error; HS, healthy subjects; MD, mean diffusivity; MDD, major depressive disorder; MNI, Montreal Neurological Institute.

Val/Val genotype and the healthy controls with the *COMT* Val/Val genotype. No significant difference was observed in RD between the MDD patients and the healthy controls, both among the *COMT* Val/Val group and the *COMT* Met-carriers (Figure 1G–I). In addition, there were no significant differences regarding FA, AD, or RD between the Val/Val group and the Met-carriers among the MDD patients, and no correlations in FA, AD, or RD were observed, at any regions of the brain, between the MDD patients and the healthy controls, both those with the *BDNF* Val/Val genotype and the *BDNF* Met-carriers (Table 4).

Discussion

In the genotype comparison (significant genotype–diagnosis interactions), we found that the reduction of the FA values in the temporal lobe was significantly larger in the MDD patients compared with the healthy subjects. FA has been shown to have an increased sensitivity to WM damage, as its decrease has been reported in the normal-appearing WM of patients with MDD.[24] The use of other DTI parameters, such as AD, which is related to axonal loss, and RD, which is associated with demyelination,[37,40] may increase the specificity of DTI to particular microstructural abnormalities. The most noteworthy finding in the present study was that the FA and AD, but not the RD, in the temporal lobe in the Met-carriers with MDD were significantly decreased compared with those in the healthy controls. These results may indicate that neuronal degeneration (axonal loss) can occur in the temporal lobe of Met-carriers with MDD.

In contrast, Seok et al[41] recently reported that FA reduction in the temporal lobe was significant only in the MDD patients with the Val/Val group of *COMT* Val158Met polymorphism. This finding indicates that MDD patients with a homozygote Val gene might have further abnormalities and brain pathological changes. Taken together, the above-described findings show that it is controversial whether the *COMT* Val158Met polymorphism is associated with structural changes of WM in the temporal lobe.

However, no significant differences were found in the plasma levels of MHPG and HVA between the present MDD patients and the control subjects. Depression is a heterogeneous condition characterized by multiple symptoms and subtypes. The different symptoms and subtypes are likely mediated by different neurocircuitry, and neurotransmitters such as noradrenaline, dopamine, and serotonin, and they might or might not be present in any particular individual with MDD. MDD might be characterized by an increase or a decrease in certain symptoms.

We reported that MDD patients with high plasma MHPG demonstrated severe anxiety and agitation, whereas those with low MHPG and/or HVA demonstrated severe psychomotor retardation.[42,43] We suspect that this is one of the reasons that no significant between-group differences were found in the catecholamine metabolites, in the present study.

In addition, each component of depressive symptoms might be related to the some brain regions and neurocircuits. Anhedonia, for example, has been found to be positively correlated with the ventromedial prefrontal cortex activity and negatively correlated with amygdala/ventral striatal activity in response to "happy" stimuli, using functional MRI (fMRI).[44] Psychomotor symptoms have been associated with frontal and caudate abnormalities in depression.[45] A recent fMRI study has shown that vulnerability to MDD is associated with temporofrontolimbic decoupling that is selective for self-blaming feelings.[46]

According to the meta-analysis of Liao et al[30] using DTI, there are four consistent locations of decreased FA in patients with MDD: WM in the right frontal lobe, the right fusiform gyrus, the left frontal lobe, and the occipital lobe. Fiber tracking showed that the main fascicles involved were the right inferior fronto-occipital fasciculus, the right posterior thalamic radiation, and interhemispheric fibers running through the genu and the body of the corpus callosum.

Taken together, these results indicate that *COMT* gene Val158Met polymorphism did not reflect the plasma and cerebrospinal fluid levels of catecholamine metabolites. The weight loss item scores of the HAMD17 were significantly lower in the present *COMT* Val/Val group than in the Met-carriers. It might be possible that the higher activity of COMT leads to reduced physical activity by influencing the catecholaminergic pathways.

The specificity of WM hyperintensities to age-associated vascular depression[47] reinforces the notion that MDD is a heterogeneous disorder. Although the data suggest that T2-weighted WM is related to late-onset MDD, findings suggestive of microstructural WM changes, as evinced by DTI, in young adults with MDD were reported by Li et al.[48] The age-associated relationship between WM and MDD may preclude the use of this trait to identify young individuals at risk of developing MDD. Nevertheless, an understanding of the mechanisms by which microvascular lesions lead to depression may help elucidate important pathophysiological pathways and facilitate the development of new treatments. In the present study, however, no correlations were found with the *BDNF* Val/Met polymorphism in patients with MDD.

On the other hand, Carballedo et al[31] reported that they observed a significant interaction between *BDNF*

alleles in the uncinate fasciculus and diagnosis. In short, their patients with the *BDNF* Met allele had smaller FA in the uncinate fasciculus compared with the patients in the Val/Val group and compared with healthy controls with Met allele. In the present study, we did not examine the regions of the temporal lobe in detail. One of the reasons for the discrepancy between the results of Carballedo et al[31] and those of the present study was that our MDD patients were at the early stage of depressive state and were drug-naïve.

Our finding that the serum BDNF levels in the MDD patients were lower than those in the healthy controls is in agreement with previous reports.[11,49–51] We also found that the *BDNF* gene Val66Met polymorphism was not associated with serum BDNF levels in patients with MDD and that the *BDNF* Val66Met polymorphism is independent of the WM disturbance in MDD. Taken together, these results indicate that the *BDNF* gene Val66Met polymorphism is not critical for WM disturbances in patients with MDD.

The present study has several limitations. The sample size was too small to allow a second statistical analysis. The sample was heterogeneous, and the severity of illness was relatively moderate. A replication study that accounts for these limitations should be performed to confirm our preliminary results. Since the *COMT* gene Met-carriers showed more decreased body weight (Table 2), the possibility that the finding reflected the changed distribution in the brain could not be completely ruled out.

In conclusion, we observed an association between the *COMT* gene Val158Met polymorphism and the reduction of FA and AD, but not RD, in the temporal lobe of patients with MDD.

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Disclosure

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References

1. Yoshimura R, Nakamura J, Shinkai K, Ueda N. Clinical response to antidepressant treatment and 3-methoxy-4-hydroxyphenylglycol levels: mini review. *Prog Neuropsychopharmacol Biol Psychiatry*. 2004;28(4):611–616.
2. Karege F, Bovier P, Gaillard JM, Tissot R. The decrease of erythrocyte catechol-O-methyltransferase activity in depressed patients and its diagnostic significance. *Acta Psychiatr Scand*. 1987;76(3):303–308.
3. Massat I, Souery D, Del-Favero J, et al. Association between COMT (Val158Met) functional polymorphism and early onset in patients with major depressive disorder in a European multicenter genetic association study. *Mol Psychiatry*. 2005;10(6):598–605.
4. Lachman HM, Morrow B, Shprintzen R, et al. Association of codon 108/158 catechol-O-methyltransferase gene polymorphism with the psychiatric manifestations of velo-cardio-facial syndrome. *Am J Med Genet*. 1996;67(5):468–472.
5. Baune BT, Hohoff C, Berger K, et al. Association of the COMT val158met variant with antidepressant treatment response in major depression. *Neuropsychopharmacology*. 2008;33(4):924–932.
6. Åberg E, Fandiño-Losada A, Sjöholm LK, Forsell Y, Lavebratt C. The functional Val158Met polymorphism in catechol-O-methyltransferase (COMT) is associated with depression and motivation in men from a Swedish population-based study. *J Affect Disord*. 2011;129(1–3):158–166.
7. Martinowich K, Manji H, Lu B. New insights into BDNF function in depression and anxiety. *Nat Neurosci*. 2007;10(9):1089–1093.
8. Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS. Hippocampal volume reduction in major depression. *Am J Psychiatry*. 2000;157(1):115–118.
9. Castrén E, Rantamäki T. The role of BDNF and its receptors in depression and antidepressant drug action: Reactivation of developmental plasticity. *Dev Neurobiol*. 2010;70(5):289–297.
10. Thompson Ray M, Weickert CS, Wyatt E, Webster MJ. Decreased BDNF, trkB-TK+ and GAD67 mRNA expression in the hippocampus of individuals with schizophrenia and mood disorders. *J Psychiatry Neurosci*. 2011;36(3):195–203.
11. Molendijk ML, Spinhoven P, Polak M, Bus BA, Penninx BW, Elzinga BM. Serum BDNF concentrations as peripheral manifestations of depression: evidence from a systematic review and meta-analyses on 179 associations (N=9484). *Mol Psychiatry*. Epub August 20, 2013.
12. Egan MF, Kojima M, Callicott JH, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*. 2003;112(2):257–269.
13. Frielingsdorf H, Bath KG, Soliman F, Difede J, Casey BJ, Lee FS. Variant brain-derived neurotrophic factor Val66Met endophenotypes: implications for posttraumatic stress disorder. *Ann N Y Acad Sci*. 2010;1208:150–157.
14. aan het Rot M, Mathew SJ, Charney DS. Neurobiological mechanisms in major depressive disorder. *CMAJ*. 2009;180(3):305–313.
15. Fields RD. White matter in learning, cognition and psychiatric disorders. *Trends Neurosci*. 2008;31(7):361–370.
16. Herrmann LL, Le Masurier M, Ebmeier KP. White matter hyperintensities in late life depression: a systematic review. *J Neurol Neurosurg Psychiatry*. 2008;79(6):619–624.
17. Kumar A, Cook IA. White matter injury, neural connectivity and the pathophysiology of psychiatric disorders. *Dev Neurosci*. 2002;24(4):255–261.
18. Kieseppä T, Eerola M, Mäntylä R, et al. Major depressive disorder and white matter abnormalities: a diffusion tensor imaging study with tract-based spatial statistics. *J Affect Disord*. 2010;120(1–3):240–244.
19. Korgaonkar MS, Grieve SM, Koslow SH, Gabrieli JD, Gordon E, Williams LM. Loss of white matter integrity in major depressive disorder: evidence using tract-based spatial statistical analysis of diffusion tensor imaging. *Hum Brain Mapp*. 2011;32(12):2161–2171.
20. Zhu X, Wang X, Xiao J, Zhong M, Liao J, Yao S. Altered white matter integrity in first-episode, treatment-naive young adults with major depressive disorder: a tract-based spatial statistics study. *Brain Res*. 2011;1369:223–229.
21. Cullen KR, Klimes-Dougan B, Mueller BA, et al. Altered white matter microstructure in adolescents with major depression: A preliminary study. *J Am Acad Child Adolesc Psychiatry*. 2010;49(2):173–183.

22. Zuo N, Fang J, Lv X, et al. White matter abnormalities in major depression: a tract-based spatial statistics and rumination study. *PLoS One*. 2012;7(5):e37561.
23. Sexton CE, Mackay CE, Ebmeier KP. A systematic review of diffusion tensor imaging studies in affective disorders. *Biol Psychiatry*. 2009;66(9):814–823.
24. Henderson CE, Higginson JS, Barrance PJ. Comparison of MRI-based estimates of articular cartilage contact area in the tibiofemoral joint. *J Biomech Eng*. 2011;133(1)014502.
25. Ma N, Li L, Shu N, et al. White matter abnormalities in first-episode, treatment-naive young adults with major depressive disorder. *Am J Psychiatry*. 2007;164(5):823–826.
26. Wu F, Tang Y, Xu K, et al. Whiter matter abnormalities in medication-naive subjects with a single short-duration episode of major depressive disorder. *Psychiatry Res*. 2011;191(1):80–83.
27. Ouyang X, Tao HJ, Liu HH, et al. White matter integrity deficit in treatment-naïve adult patients with major depressive disorder. *East Asian Arch Psychiatry*. 2011;21(1):5–9.
28. Jia Z, Huang X, Wu Q, et al. High-field magnetic resonance imaging of suicidality in patients with major depressive disorder. *Am J Psychiatry*. 2010;167(11):1381–1390.
29. de Diego-Adeliño J, Pires P, Gómez-Ansón B, et al. Microstructural white-matter abnormalities associated with treatment resistance, severity and duration of illness in major depression. *Psychol Med*. Epub August 21, 2013.
30. Liao Y, Huang X, Wu Q, et al. Is depression a disconnection syndrome? Meta-analysis of diffusion tensor imaging studies in patients with MDD. *J Psychiatry Neurosci*. 2012;38(1):49–56.
31. Carballedo A, Amico F, Ugwu I, et al. Reduced fractional anisotropy in the uncinate fasciculus in patients with major depression carrying the met-allele of the Val66Met brain-derived neurotrophic factor genotype. *Am J Med Genet B Neuropsychiatr Genet*. 2012;159B(5):537–548.
32. Kim B, Yoo E, Lee JY, et al. The effects of the catechol-O-methyltransferase val158met polymorphism on white matter connectivity in patients with panic disorder. *J Affect Disord*. 2013;147(1–3):64–71.
33. Haselgrove JC, Moore JR. Correction for distortion of echo-planar images used to calculate the apparent diffusion coefficient. *Magn Reson Med*. 1996;36(6):960–964.
34. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage*. 2004;23 Suppl 1:S208–S219.
35. Smith SM, Jenkinson M, Johansen-Berg H, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage*. 2006;31(4):1487–1505.
36. Smith SM. Fast robust automated brain extraction. *Hum Brain Mapp*. 2002;17(3):143–155.
37. Rueckert D, Sonoda LI, Hayes C, Hill DL, Leach MO, Hawkes DJ. Nonrigid registration using free-form deformations: application to breast MR images. *IEEE Trans Med Imaging*. 1999;18(8):712–721.
38. Yeung PK, Buckley SJ, Pedder SC, Dingemanse J. Determination of 3,4-dihydroxyphenylacetic acid and 5-hydroxyindoleacetic acid in human plasma by a simple and rapid high-performance liquid chromatography assay. *J Pharm Sci*. 1996;85(4):451–453.
39. Minegishi A, Ishizaki T. Determination of free 3-methoxy-4-hydroxyphenylglycol with several other monoamine metabolites in plasma by high-performance liquid chromatography with amperometric detection. *J Chromatogr*. 1984;311(1):51–57.
40. Brett M, Leff AP, Rorden C, Ashburner J. Spatial normalization of brain images with focal lesions using cost function masking. *Neuroimage*. 2001;14(2):486–500.
41. Seok JH, Choi S, Lim HK, Lee SH, Kim I, Ham BJ. Effect of the COMT val158met polymorphism on white matter connectivity in patients with major depressive disorder. *Neurosci Lett*. 2013;545:35–39.
42. Ueda N, Yoshimura R, Shinkai K, Nakamura J. Plasma levels of catecholamine metabolites predict the response to sulpiride or fluvoxamine in major depression. *Pharmacopsychiatry*. 2002;35(5):175–181.
43. Shinkai K, Yoshimura R, Ueda N, Okamoto K, Nakamura J. Associations between baseline plasma MHPG (3-methoxy-4-hydroxyphenylglycol) levels and clinical responses with respect to milnacipran versus paroxetine treatment. *J Clin Psychopharmacol*. 2004;24(1):11–17.
44. Stuhrmann A, Dohm K, Kugel H, et al. Mood-congruent amygdala responses to subliminally presented facial expressions in major depression: associations with anhedonia. *J Psychiatry Neurosci*. 2013;38(4):249–258.
45. Der-Avakian A, Markou A. The neurobiology of anhedonia and other reward-related deficits. *Trends Neurosci*. 2012;35(1):68–77.
46. Green S, Lambon Ralph MA, Moll J, Deakin JF, Zahn R. Guilt-selective functional disconnection of anterior temporal and subgenual cortices in major depressive disorder. *Arch Gen Psychiatry*. 2012;69(10):1014–1021.
47. Sneed JR, Rindskopf D, Steffens DC, Krishnan KR, Roose SP. The vascular depression subtype: evidence of internal validity. *Biol Psychiatry*. 2008;64(6):491–497.
48. Li L, Ma N, Li Z, et al. Prefrontal white matter abnormalities in young adult with major depressive disorder: a diffusion tensor imaging study. *Brain Res*. 2007;1168:124–128.
49. Bocchio-Chiavetto L, Bagnardi V, Zanardini R, et al. Serum and plasma BDNF levels in major depression: a replication study and meta-analyses. *World J Biol Psychiatry*. 2010;11(6):763–773.
50. Fernandes BS, Gama CS, Ceresér KM, et al. Brain-derived neurotrophic factor as a state-marker of mood episodes in bipolar disorders: a systematic review and meta-regression analysis. *J Psychiatr Res*. 2011;45(8):995–1004.
51. Sen S, Duman R, Sanacora G. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. *Biol Psychiatry*. 2008;64(6):527–532.

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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)

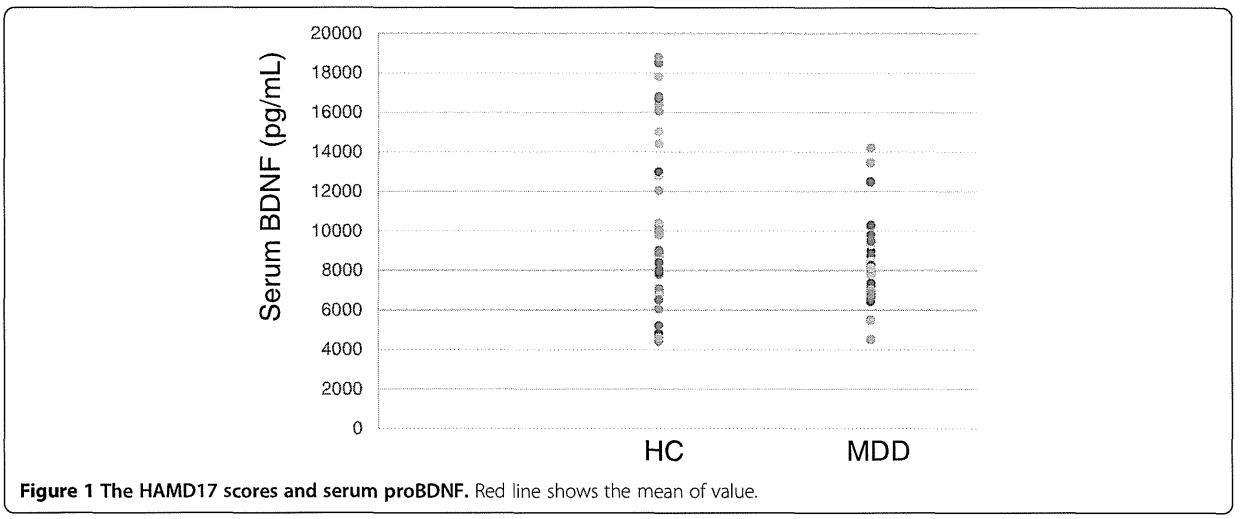

Figure 1 The HAMD17 scores and serum proBDNF. Red line shows the mean of value.

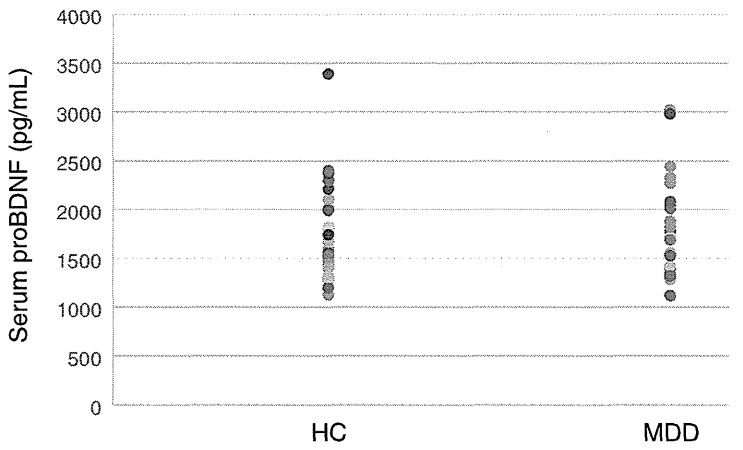

Figure 2 Serum proBDNF change during treatment with fluvoxamine. Red line shows the mean of value.

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.).

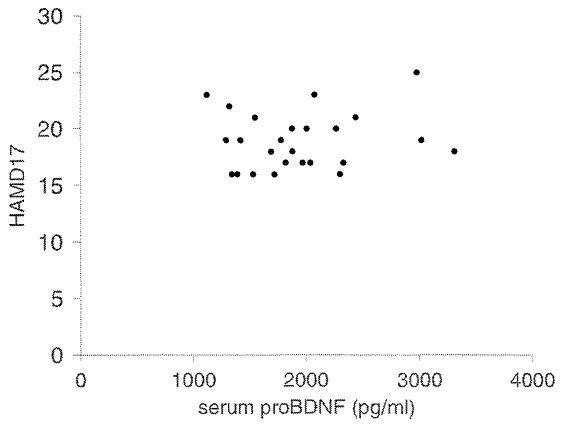

Figure 3 Serum proBDNF in the responders' and the nonresponders' treatment with fluvoxamine.

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

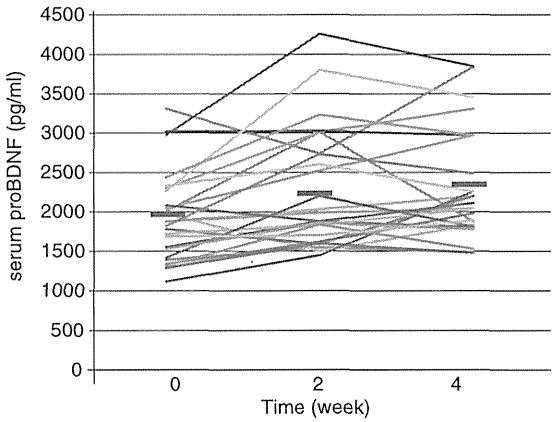

Figure 4 The HAMD17 scores and serum BDNF. Red line shows the mean of value.

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 ($t = 3.046$, $p = 0.0018$, $1\text{-}\beta = 82.3\%$) (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

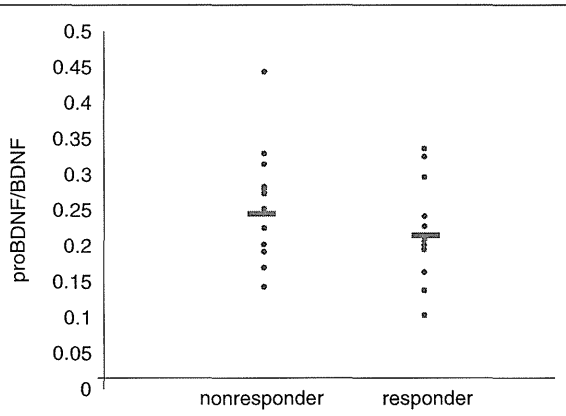

Figure 5 Serum BDNF change during treatment with fluvoxamine.

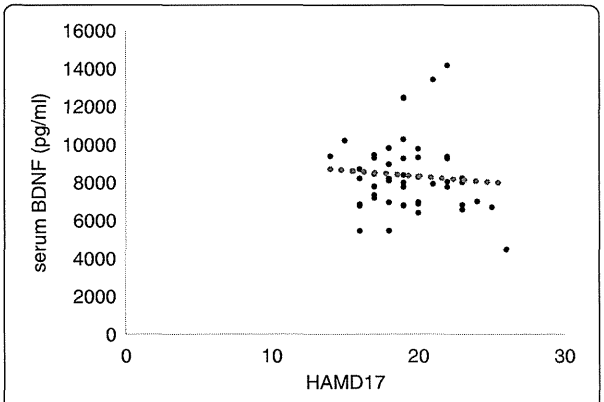

Figure 6 Serum BDNF in the responders' and the nonresponders' treatment with fluvoxamine. Red dot line shows regression line.

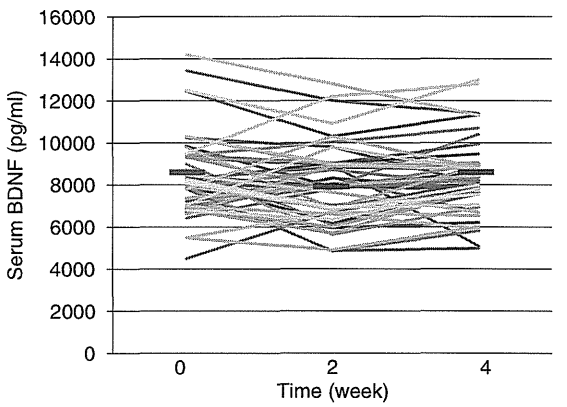

Figure 7 Serum BDNF change during treatment with fluvoxamine. Red line shows the mean of value.

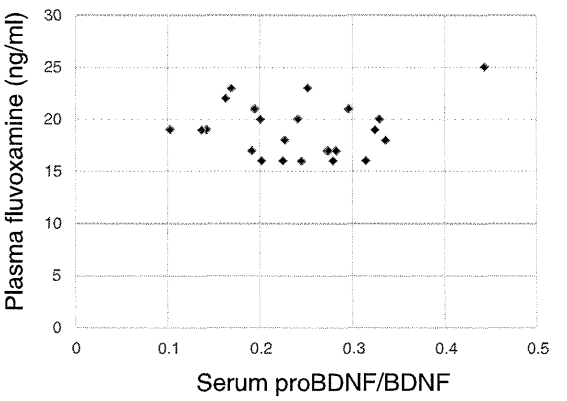

Figure 9 The proBDNF/BDNF ratio and plasma fluvoxamine concentration.

between plasma fluvoxamine levels at 4 weeks and the changes in serum BDNF levels (from 0 to 4 weeks) (r = 0.117, p = 0.691) (Figure 11).

Discussion

Recent meta-analyses demonstrated that mature BDNF levels in serum in patients with MDD were decreased compared to those in healthy controls. The result in the present study regarding serum BDNF confirms the recent meta-analyses [10,14,15]. Low serum mature BDNF levels increased over the course of antidepressant treatment [10,14,15]. We have previously reported that a significant correlation was found between the HAMD17 score and serum BDNF levels before pharmacotherapy [16]. In the present study, we reconfirmed our previous finding. A correlation was not however observed between serum proBDNF levels and the HAMD17 scores before starting fluvoxamine. In addition, there was no relationship between serum levels of proBDNF/BDNF and HAMD17. Taking these findings into account, the BDNF level, but not proBDNF and proBDNF/BDNF, reflects the severity of MDD. Moreover, no correlation was observed between serum fluvoxamine levels and serum levels of proBDNF/BDNF. The result in the present study suggests that the plasma fluvoxamine level was not independent of proBDNF/BDNF in MDD patients after fluvoxamine treatment. In addition, serum levels of BDNF and proBDNF/BDNF did not change at least 4 weeks after fluvoxamine administration. However, serum proBDNF increased during fluvoxamine treatment but did not reach the statistically significant level. Taking these findings into account, our hypothesis was not confirmed. In other words, the influence of fluvoxamine on serum levels of proBDNF, BDNF, and proBDNF/BDNF is complicated. Another interpretation is that 4 weeks is not enough to alter the dynamics of proBDNF and BDNF. Zhou et al. [17] reported that protein and serum levels of proBDNF were higher in MDD than in

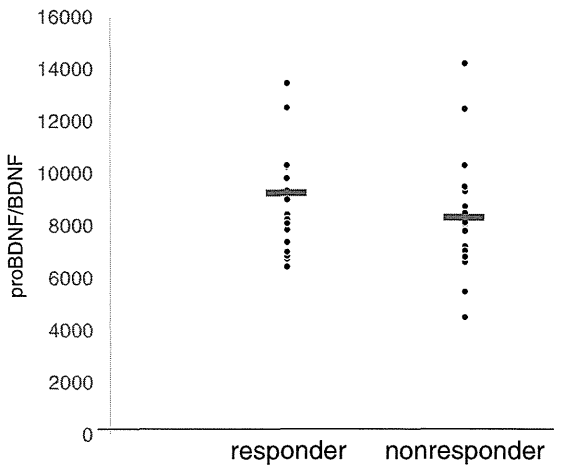

Figure 8 The proBDNF/BDNF ratio between the responders and the nonresponders. Red line shows the mean of value.

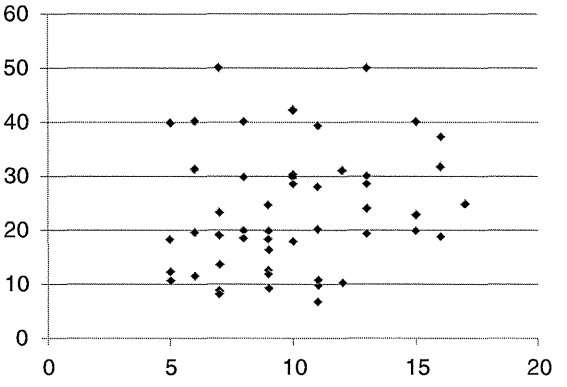

Figure 10 The changes in the HAMD17 (0–4 weeks) and plasma fluvoxamine concentration at week 4.

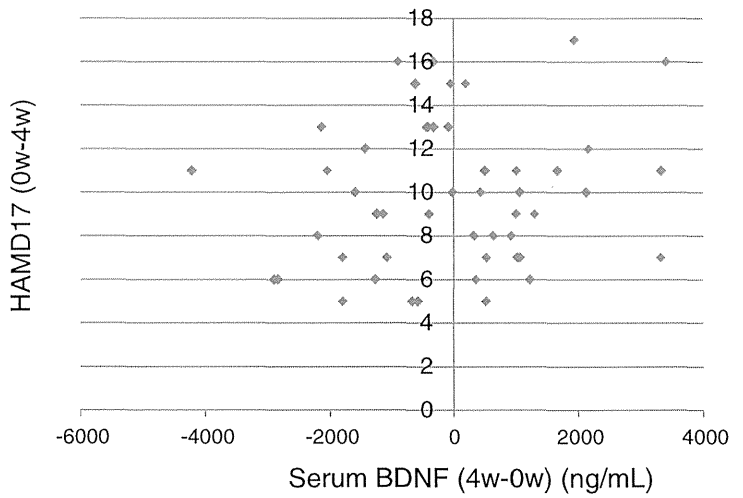

Figure 11 The changes in the HAMD17 (0–4 weeks) and plasma concentration at week 4.

healthy control subjects while BDNF levels were lower. The authors also demonstrated that the levels of BDNF and proBDNF negatively and positively correlated with major depression severity, respectively. These results suggest that the balance between proBDNF and BDNF is disturbed in MDD. Sodersten et al. [18] recently reported a very interesting finding using two independent cohort studies (Sahlgrenska cohort and Karolinska cohort). The authors found that serum MDNF, proBDNF, the ratio of BDNF/proBDNF, and interaction with MMP-9 were different between patients with bipolar disorders and healthy controls. The function of proBDNF however remains precisely elucidated.

Furthermore, there is little information about the role of serum proBDNF. We know that a controversy exists about the relationship between brain BDNF and peripheral BDNF. A recent study reported that circulating BDNF revealed a positive correlation with hippocampal BDNF, which reinforces the relevance to identify a potentially useful therapeutic biomarker [19].

No correlation was found between the changes in the HAMD17 scores and plasma fluvoxamine levels, which indicates that the effect of plasma fluvoxamine levels is independent of an individual's depressive clinical efficacy in fluvoxamine. In other words, the pharmacodynamic factors of each patient might also be involved in the effects of fluvoxamine. We should consider various factors for predicting the treatment response, and it could be more complicated in the fluvoxamine response. The present study had several limitations: (i) small samples, (ii) assaying serum proBDNF was tricky and the detection rate of serum proBDNF was very low using the ELISA kit, and (iii) we did not measure MMP-9 levels. Thus, we are undergoing reconfirmation of these preliminary results using another ELISA kit or Western blotting method.

Conclusions

We reconfirmed that serum levels of BDNF, but not proBDNF or proBDNF/BDNF ratio, in MDD were lower than those in healthy controls. Fluvoxamine however did not change serum levels of BDNF, proBDNF, and proBDNF/BDNF ratio at least within 4 weeks. Finally, no correlations exist between plasma levels of fluvoxamine and the changes in the HAMD17 scores or serum BDNF levels. In short, there is no association between serum levels of proBDNF, BDNF, or proBDNF/BDNF ratio and fluvoxamine response in MDD patients at least within 4 weeks of treatment. Using a different antidepressant medication on proBDNF/BDNF could be useful to determine the specificity of the effect of fluvoxamine.

Competing interests

Professor Nakamura has received grant support from Dainippon-Sumitomo Pharma Co., Tanabe-Mitsubishi Pharma Co., and Astellas Pharma Co., Ltd in 2013. The other authors declare that they have no competing interests.

Authors' contributions

RY designed the study, measured the serum BDNF, proBDNF, and plasma fluvoxamine, wrote the first draft, and managed the literature searches. TK performed the statistical analyses. HH, KA, AK, WU-N, and AI-S collected the clinical data. NI and JN wrote the final manuscript. All of the authors took part in either drafting the article or revising it critically for important intellectual content and approved the final manuscript.

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References

1. Smith DJ, Nicholl BI, Cullen B, Martin D, Ul-Haq Z, Evans J, Gill JM, Roberts B, Gallacher J, Mackay D, Hotopf M, Deary I, Craddock N, Pell JP: **Prevalence and characteristics of probable major depression and bipolar disorder within UK Biobank: cross-sectional study of 172,751 participants.** *PLoS One* 2013, **25**:e75362.
2. Lima NN, do Nasciment VB, de Carvalho SM, de Abreu LC, Neto ML, Brasil AQ, Junior FT, de Oliveira GF, Reis AO: **Childhood depression: a systematic review.** *Neuropsychiatr Dis Treat* 2013, **9**:1317–1425.
3. Judd LL, Schettler PJ, Coryell W, Akiskal HS, Fliedorowicz JG: **Overt irritability/anger in unipolar major depressive episode: past and characteristics and implications and for long-term course.** *JAMA Psychiatry* 2013, **70**:1171–1180.
4. Katoh Y, Uchida S, Kawai M, Takei N, Mori N, Kawakami J, Kagawa Y, Yamada S, Namiki N, Hashimoto H: **Onset of clinical and plasma concentration of fluvoxamine in Japanese patients.** *Biol Pharm Bull* 2010, **33**:1999–2002.
5. Iron J: **Fluvoxamine in the treatment of anxiety disorders.** *Neuropsychiatr Dis Treat* 2005, **1**:289–299.
6. Omori IM, Watanabe N, Nakagawa A, Akechi T, Cipriani A, Barbui C, McGuire H, Churchill R, Furukawa TA, Meta-Analysis of New Generation Antidepressants (MANGA) Study Group: **Efficacy, tolerability and side-effect profile on fluvoxamine for major depression: meta-analysis.** *J Psychopharmacol* 2009, **23**:539–550.
7. Dwivedi Y: **Brain-derived neurotrophic factor: role in depression and suicide.** *Neuropsychiatr Dis Treat* 2009, **5**:433–449.
8. Hashimoto K: **Brain-derived neurotrophic factor as a biomarker for mood disorders: an historical overview and future directions.** *Psychiatry Clin Neurosci* 2010, **64**:341–357.
9. Jiang C, Salton SR: **The role of neurotrophins in major depressive disorder.** *Transl Neurosci* 2013, **4**:46–58.
10. Sen S, Duman R, Sanacora G: **Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications.** *Biol Psychiatry* 2008, **64**:527–532.
11. Green MJ, Matheson SL, Shepherd A, Weickert CS, Carr VJ: **Brain-derived neurotrophic factor levels in schizophrenia: a systematic review with meta-analysis.** *Mol Psychiatry* 2011, **16**:960–972.
12. Yoshida T, Ishikawa M, Niitsu T, Nakazato M, Watanabe H, Shiraishi T, Shiina A, Hashimoto T, Kanahara N, Hasegawa T, Enohara M, Kimura A, Iyo M, Hashimoto K: **Decreased serum levels of mature brain-derived neurotrophic factor (BDNF), but not its precursor proBDNF, in patients with major depressive disorder.** *PLoS One* 2012, **7**:e42676.
13. Hori H, Yoshimura R, Ueda N, Ikenouchi-Sugita A, Umene-Nakano W, Nakamura J: **A case with occurring adverse effects when cross-over titration from fluvoxamine to paroxetine associated with increasing the plasma fluvoxamine level in major depressive disorder.** *World J Biol Psychiatry* 2009, **10**:620–622.
14. Bocchio-Chiavetto L, Bagnardi V, Zanardini R, Molteni R, Nielsen MG, Placentino A, Giovannini C, Rillosi L, Ventriglia M, Riva MA, Gennarelli M: **Serum and plasma BDNF levels in major depression: a replication study and meta-analyses.** *World J Biol Psychiatry* 2010, **11**:763–773.
15. Fernandes BS, Beck M, Turck CW: **Decreased peripheral brain-derived neurotrophic factor levels are a biomarker of disease activity in major psychiatric disorders: a comparative meta-analysis.** *Mol Psychiatry* 2013, [Epub ahead of print].
16. Yoshimura R, Kishi T, Suzuki A, Umene-Nakano W, Ikenouchi-Sugita A, Hori H, Otani K, Iwata N, Nakamura J: **The brain-derived neurotrophic factor (BDNF) polymorphism Val66Met is associated with neither serum BDNF level nor response to selective serotonin reuptake inhibitors in depressed Japanese patients.** *Prog Neuropsychopharmacol Biol Psychiatry* 2011, **35**:1022–1025.
17. Zhou L, Xiong J, Lim Y, Ruan Y, Huang C, Zhu Y, Zhong JH, Xiao Z, Zhou XF: **Upregulation of blood proBDNF and its receptors in major depression.** *J Affect Disord* 2013, **150**:776–784.
18. Sodersten K, Palsson E, Ishima T, Funa K, Landen M, Hashimoto K, Agren H: **Abnormality in serum levels of mature brain-derived neurotrophic factor (BDNF) and its precursor proBDNF in mood-stabilized patients with bipolar disorder: a study of two independent cohorts.** *J Affect Disord* 2014, **160**:1–9.
19. Eker C, Kitis O, Taneli F, Eker OD, Ozan E, Yucel K, Coburn K, Gonul AS: **Correlation of serum BDNF levels with hippocampal volumes in first episode, medication-free depressed patients.** *Eur Arch Psychiatry Clin Neurosci* 2010, **260**(7):527–533.

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ORIGINAL RESEARCH

Plasma levels of 3-methoxy-4-hydroxyphenylglycol are associated with microstructural changes within the cerebellum in the early stage of first-episode schizophrenia: a longitudinal VBM study

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Abstract: The aims of this study are to determine how the interval changes of the brain structures in the early stage of first-episode schizophrenia relate to the interval changes in the clinical data, including the clinical symptoms of schizophrenia and catecholaminergic measures (plasma homovanillic acid [HVA] and 3-methoxy-4-hydroxyphenylglycol [MHPG]). Regional brain volumes and fractional anisotropy (FA)/mean diffusivity (MD) with diffusion tensor imaging (DTI) were measured at baseline and 6-month follow-up in a 3T magnetic resonance imaging (MRI) system in a cohort of 16 schizophrenic patients, who were in their first episode at the time of baseline MRI. At the time of baseline and follow-up MRI, all 16 patients underwent evaluations that included a psychopathological assessment (Positive and Negative Syndrome Scale [PANSS]) and peripheral catecholaminergic measures (plasma MHPG or HVA). For interval changes between baseline and follow-up MRI data (morphological change, MD, and FA), the correlation/regression analysis was performed as a series of single regression correlations in Statistical Parametric Mapping 5, with the interval changes in PANSS or plasma HVA and MHPG as the covariates of interest. Positive and inverse correlations contrasts were created, and in this preliminary analysis, a family-wise error-corrected threshold of $P<0.05$ was considered significant. In the correlation/regression analysis, a positive correlation between the FA in the right cerebellar vermis and the MHPG was observed. No significant correlations between the brain volume or MD and any laboratory data (plasma HVA and MHPG) were found. During the 6-month follow-up in the early stage of first-episode schizophrenia, the MHPG changes were correlated with the microstructural FA changes in the cerebellum, which may reflect the functional connections of the noradrenergic system in the cerebellum.

Keywords: 3T MR imaging, apparent diffusion coefficient, fractional anisotropy, mean diffusivity, voxel-based morphometry, homovanillic acid

Introduction

Schizophrenia is a neuropsychiatric disorder that shows detectable evidence of brain structural abnormalities in the majority of afflicted individuals during the chronic stages.[1] The identification of the structural brain abnormalities in patients with schizophrenia, using magnetic resonance imaging (MRI), has become an important area of neuroimaging research in recent years. Several previous studies using a voxel-wise analysis have reported that the morphological changes are detectable on 3D MRI and/or the reduced fractional anisotropy (FA) on diffusion tensor imaging (DTI) in several regions at the first manifestation of schizophrenia (first-episode schizophrenia).[2–5] Use of voxel-wise analysis has also allowed correlation/regression analysis to be performed between the

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patient's MRI data and their clinical data. Recently, many studies have looked at the relationship between structural data (including DTI) and clinical data (including the Positive and Negative Syndrome Scale [PANSS]).[6,7] One study reported inverse correlations of FA values with PANSS positive symptom scores in the left uncinate fasciculus, right sagittal stratum, and the left superior longitudinal fasciculus.[7] In these studies, however, it seems unclear whether the relationship between the structural brain abnormalities and the clinical data really existed, because no study has evaluated the longitudinal changes in patients with first-episode schizophrenia.

The actions of antipsychotic drugs on the dopamine system have led to many examinations of dopaminergic metabolites as possible markers for psychosis and antipsychotic response. However, it has also become clear that the noradrenergic system has extensive interactions with the dopamine system, and may play a role in schizophrenia,[8] and may also have a key role in psychotic relapse.[9] Interactions between the two systems have been well-studied in the cerebrospinal fluid and plasma of schizophrenic patients through their respective metabolites; plasma homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) are the major degradation products of the monoamines dopamine and noradrenaline, respectively. Plasma HVA and MHPG are good predictors of response to antipsychotic treatment;[10–12] the effects of risperidone (a benzisoxazole derivative belonging to a family of atypical antipsychotic drugs) on plasma levels of HVA and MHPG have been related to its clinical efficacy in ameliorating the positive and negative symptoms of schizophrenia, respectively.[11,13] Moreover, recent studies reported that plasma levels of MHPG are decreased in patients in the early stage of first-episode schizophrenia,[10,14] which suggests that the MHPG may be a useful biomarker in the early stages of schizophrenia. Although it is of interest to explore whether and how anatomic deficits relate to clinical manifestations and alterations in brain physiology, we know of no voxel-wise correlation/regression analysis in which correlation between MRI data and peripheral catecholaminergic measures have been shown.

Herein, we report the results of a longitudinal MRI study of first-episode schizophrenia conducted to date, to our knowledge, using well-validated and highly reliable state-of-the-art neuroimaging tools. We measured regional brain volumes, FA, and mean diffusivity (MD) during an average of 6 months using high-resolution MRI with a 3T MRI system in a cohort of schizophrenic patients, who were in their first episode at the time of baseline MRI. The aims of this study were to determine how the interval changes of the brain structures in the early stage of first-episode schizophrenia relate to interval changes in the clinical data, including clinical symptoms of schizophrenia and peripheral catecholaminergic measures (plasma HVA and MHPG).

Materials and methods

Subjects

The diagnosis of schizophrenia, according to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV-TR), requires at least a 6-month duration of signs of the disturbance. In this study, a total of 26 patients who fulfilled the DSM-IV-TR criteria A, B, D, E, and F, were recruited for the study, except for the criterion regarding the disease duration. All subjects underwent baseline magnetic resonance (MR) examinations within 6 months from the date from which the patients fulfilled these criteria. Therefore, all baseline MR examinations were performed during the early stage of first-episode schizophrenia, before a diagnosis of schizophrenia was established.[6] After a 6-month follow-up, a diagnosis of schizophrenia was established in 23 of the 26 patients. The symptoms of the remaining patients had completely remitted spontaneously without any medication use preceding the 6-month follow-up. One of the 23 patients was subsequently excluded from the study, because the image quality was impaired by severe artifacts from dental materials. After a 6-month follow-up, the remaining 22 patients were invited for a repeat MR examination (follow-up MRI). This MRI was unavailable for six patients. Therefore, 16 patients (eight males, eight females; mean age: 29.0±11.6 years; age range: 13–52 years) were finally enrolled in the longitudinal study. All were considered to have been in the early stage of first-episode schizophrenia at the time of the baseline MR examinations. All patients were screened by using the Structured Clinical Interview for DSM-IV-TR Disorders (SCID; New York State Psychiatric Institute 1995), and exclusion criteria for all groups were: 1) current or past serious medical illness such as cancer, diabetes mellitus, epilepsy, and cerebral infarction; 2) dependence on alcohol; and 3) illicit substance use such as methamphetamine, narcotics, and marijuana.

The mean duration from the date from which the patients fulfilled the DSM-IV-TR criteria A, B, D, E, and F to the date of baseline MR examination for the patients was 2.9±2.5 months. The duration of psychosis (DUP) was defined as the day of emergence of several symptoms of psychosis to the day when baseline MR examination was performed. The mean DUP was 6.9±6.4 months; therefore, the mean duration from the date of onset of psychotic symptoms to the date on which the patients fulfilled DSM-IV-TR criteria A, B, D, E, and F was 4.1±6.1 months. These two items were reconfirmed by the

patients themselves and their family members. At baseline MR examination, the total cumulative exposure to a chlorpromazine equivalent dose of antipsychotic drugs was 214±117 mg (mean ± standard deviation [SD]). One of the 16 patients had not received any medications, and the rest of the 15 patients were treated with atypical antipsychotic drugs (risperidone, n=2; aripiprazole, n=6; olanzapine, n=5; quetiapine, n=2). At follow-up MR examination, the total cumulative exposure to a chlorpromazine equivalent dose of antipsychotic drugs was 390±218 mg. All 16 patients were treated with atypical antipsychotic drugs (risperidone, n=2; aripiprazole, n=6; olanzapine, n=5; quetiapine, n=2; perospirone, n=1).

At the time of baseline and follow-up MR examinations, all 16 patients underwent an evaluation, which included a psychopathological assessment (PANSS) and peripheral catecholaminergic measures (plasma HVA and MHPG).

Magnetic resonance imaging acquisition and image processing for voxel-based morphometry

For baseline and follow-up MR examinations, the MRI data were obtained using a 3.0-Tesla scanner with a three-dimensional fast spoiled gradient recalled acquisition with steady state (3D-FSPGR), which was acquired with parameters of 10/4.1/700 (repetition time [ms]/echo time [ms]/inversion time), a flip angle of 10°, a 24 cm field of view (FOV), and 1.2 mm thick sections 0.47×0.47×1.2 mm resolution. All images were corrected for image distortion due to gradient nonlinearity using 'GradWarp'[15] and for intensity inhomogeneity using 'N3'.[16] Image processing for voxel-based morphometry (VBM),[17] a fully automatic technique for computational analysis of differences in regional brain volume throughout the entire brain, was conducted using SPM5 (Statistical Parametric Mapping; v5; Institute of Neurology, London, UK). The 3D-FSPGR images in native space were bias-corrected; spatially normalized; segmented into gray matter, white matter, and cerebrospinal fluid images; and intensity-modulated using SPM5.[18] The DARTEL (Diffeomorphic Anatomical Registration Through Exponential Lie Algebra) toolbox was used in a high-dimensional normalization protocol. DARTEL was proposed by Ashburner as an alternative method of normalization in the SPM package.[19] In an intensity modulation step, voxel values of the segmented images were multiplied by the measure of warped and unwarped structures derived from the nonlinear step of the spatial normalization. This step converted the relative regional gray matter density into absolute gray matter density, expressed as the amount of gray matter per unit volume of brain tissue before spatial normalization. The resulting modulated gray and white matter images were smoothed with an 8 mm Gaussian kernel.

Diffusion tensor images: MRI scanning protocol

All subjects also underwent DTI examinations with the same scanner and at the same time as 3D-FSPGR. The methods of DTI acquisition and data analysis were similar to those in a previous study.[20] In brief, a single-shot, spin-echo planar sequence was used (repetition time/echo time [TR/TE] =12,000/83.3 ms; 4 mm slice thickness; no gap; FOV 26 cm; number of excitations =1, spatial resolution 1.02×1.02×4 mm). The diffusion properties were measured at a b-value of 1,000 s/mm^2 along 25 noncollinear directions. Eddy current correction and patient motion correction were performed on the diffusion-weighted basis images using Functional MRI of the Brain (FMRIB)'s Linear Image Registration Tool (FLIRT) from the FMRIB Software Library (FSL).[21] The images were corrected for image distortion due to gradient nonlinearity using 'GradWarp'. Individual FA and MD maps were calculated using the DTIFIT tool implemented in FSL.

Spatial normalization of DTI for SPM analysis

The echo planar sequence used for the acquisition of the diffusion tensor dataset suffers from inherent geometric distortion from magnetic field inhomogeneities. Moreover, the contrast of the FA map is quite different from that of T1-, T2-, or proton density–weighted template images provided with SPM5. Therefore, an FA template specific to this study was created using the data from all participants. Each T2-weighted echo-planar image was co-registered into the 3D-FSPGR image, and the co-registration parameter was applied to the corresponding FA map. The parameters of the normalization used in the spatial normalization step of the 3D-FSPGR images in native space onto the T1 template were also applied to the co-registered FA map. The normalized FA maps were smoothed with an 8 mm isotropic Gaussian kernel, and a mean image (FA template) was created. Thereafter, all FA maps in native space were transformed onto the stereotactic space by registering each of the images to the customized FA template. The normalized FA map was smoothed with an 8 mm isotropic Gaussian kernel.

Image processing for tract-based spatial statistics

The structural distortion of the diffusion-weighted MR images was corrected based on each T2-weighted echo

planar image (b=0 s/mm^2) by using eddy current correction in the FMRIB Diffusion Toolbox software program (v5.0.4; parts of the FSL). Non-brain tissue of each MR image was deleted using the brain extraction tool. Voxel-wise statistical analysis of the DTI data was performed by using Tract-Based spatial analysis (TBSS; v1.1) software program. The FA volumes were aligned to a target image as follows: 1) apply nonlinear registration of each subject's FA into the FMRIB58_FA_1 mm standard-space image as the target image; and 2) the target image was affine transformed to 1×1×1 mm MNI 152 (Montreal Neurologic Institute, Montreal, QC) space. A mean FA image was created by averaging the aligned individual FA images, and was then thinned to create an FA skeleton representing white matter tracts common to all subjects. For the FA skeleton, a threshold was set at 0.2 to exclude voxels with low FA values, which are likely to include grey matter or cerebrospinal fluid. Individual FA data and voxel-wise statistical results were projected onto this FA skeleton. Subsequently, the MD were projected onto the mean FA skeleton and also compared between groups at the same spatial location.

Statistical analyses of voxel-based morphometry and DTI

In order to examine the interval changes of brain volume, a new set of images (temporal subtracting image [TSI]) was generated by calculating an interval change on MR images for each patient by subtracting the follow-up images from the baseline images.[22] The TSI were created using the following formula: TSI = (baseline image – follow-up image)/0.5 (baseline image + follow-up image). Therefore, the TSI represents the brain volume differences between the follow-up and baseline images for each voxel. Positive voxel values on the image indicate that the baseline MR images had higher intensity values than the follow-up images; negative voxel values on the image indicate that the follow-up images had higher intensity values than the baseline MR images. The same method was applied for creating the TSI on either the FA or MD maps for SPM analysis. We used the FA or MD skeleton to calculate TSI for TBSS.

Measurement of plasma levels of HVA and MHPG

Plasma concentrations of HVA and MHPG were analyzed by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). The plasma HVA levels were analyzed by HPLC-ECD according to the method of Yung et al with a slight modification.[23] The plasma MHPG levels were also analyzed by HPLC-ECD according to the method of Ohnishi et al.[24]

Correlational analysis

The interval changes in clinical or laboratory data were calculated for each subject by subtracting the follow-up data from the baseline data. For each interval change (TSI) between baseline and follow-up MR data (morphological changes, MD, and FA), the correlational analysis was performed as a series of single regression correlations in SPM5, with the interval changes of clinical data (positive and negative PANSS) or laboratory data (plasma HVA and plasma MHPG) as the covariates of interest. Positive and inverse correlations contrasts were created. Family-wise error (FWE) correction was applied. The significance level was set at false discovery rate (FDR)-corrected $P<0.05$. Significant clusters were identified by specific white matter tract through meticulous comparison to the MRI Atlas of Human White Matter.

Results

There was no significant difference in the global brain volume (total gray matter, total white matter, and intracranial volume) between baseline and follow-up MRI (Table 1). Both the positive and negative PANSS at follow-up MRI were significantly lower than those at baseline MRI. The plasma HVA and MHPG at follow-up MRI were significantly lower and higher, respectively, than that at baseline MRI. The mean duration between the baseline and follow-up MRI was 6 months. The laboratory data (plasma HVA and MHPG) were not associated with the dose of antipsychotic drugs both at baseline and after follow-up interval.

In the correlation/regression analysis, a positive correlation between the MHPG and the FA in the right cerebellar vermis was observed in the SPM analysis ([MNI coordinate x, y, z] on DTI map = [−6, −50, −26], FWE-corrected P=0.012, Z=5.05, T=9.80; Figure 1), whereas an inverse correlation between the FA and MHPG was not found in any brain region. The TBSS analysis also showed a positive correlation between the MHPG and FA in the white matter of the right cerebellar vermis ([MNI coordinate x, y, z] on DTI map = [−6, −51, −27], uncorrected $P<0.001$, cluster size =390; Figure 2), although no voxels could survive after correction for multiple comparisons. No significant correlations between the brain volume or MD and any laboratory data (plasma HVA and MHPG) were evident. There were no significant correlations between any MR data (brain volumes, FA, and MD) and PANSS.

Table 1 Demographic and clinical characteristic of the patients at baseline and follow-up MRI

	Baseline MRI	Follow-up MRI	Paired *t*-test
	Mean ± SD	Mean ± SD	*P*-value
Age	29.0±11.6		
Male/female	8/8		
Total intracranial volume (mL)	1,476.6±123.5	1,484.6±131.0	0.21
Total GM volume (mL)	665.2±61.1	665.2±74.4	0.99
Total WM volume (mL)	477.6±57.9	482.3±62.2	0.28
PANSS-P	16.8±5.5	12.4±4.9	0.02
PANSS-N	17.8±5.7	14.3±4.6	0.02
PANSS-G	27.2±3.5	21.0±3.4	<0.01
PANSS-T	61.7±11.8	47.6±10.8	<0.01
MHPG	4.3±2.0	5.2±1.4	0.01
HVA	7.2±2.1	6.3±1.3	<0.01
CPZ (mg)	214±117	390±218	0.01
GAF	36.1±8.4	51.9±11.9	<0.01

Abbreviations: CPZ, chlorpromazine; GAF, Global Assessment of Functioning; GM, gray matter; HVA, homovanillic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol; MRI, magnetic resonance imaging; ; PANSS-P, PANSS positive symptoms subscale; PANSS-N, PANSS negative symptoms subscale; PANSS-G, PANSS general psychopathology subscale; PANSS-T, PANSS total score; SD, standard deviation; WM, white matter.

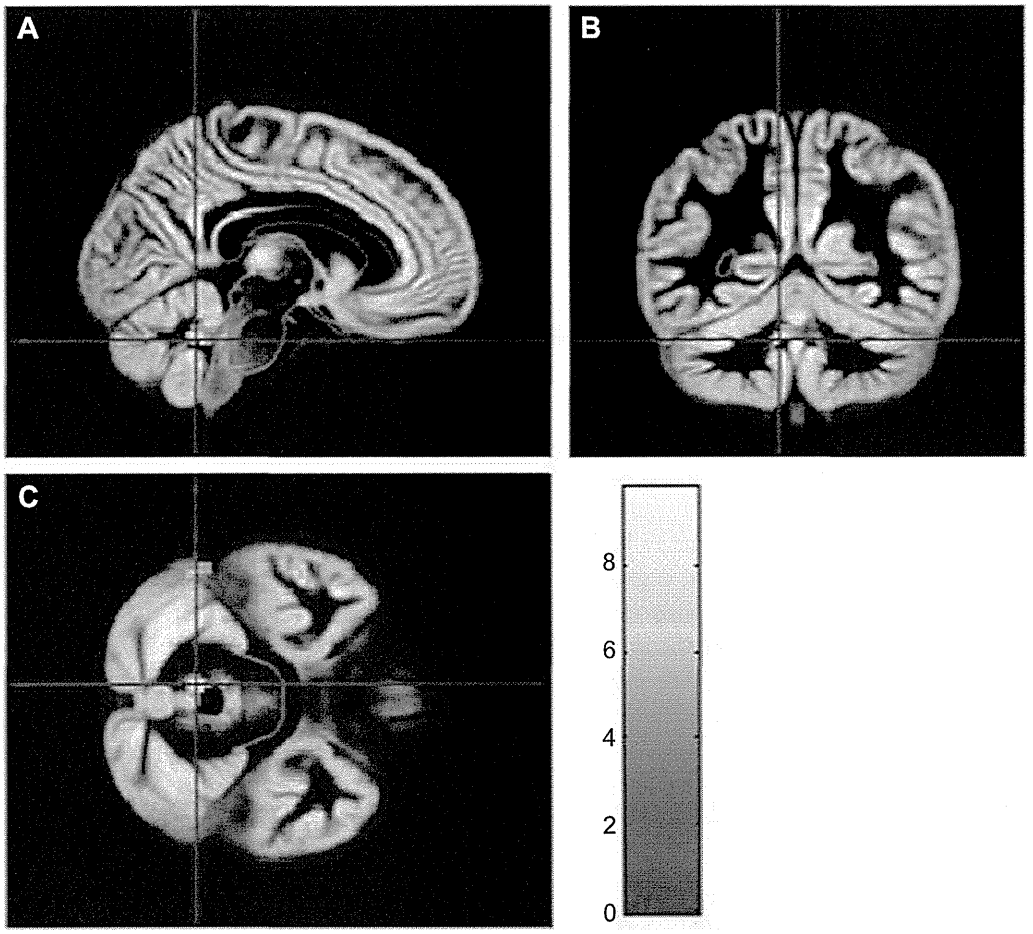

Figure 1 Correlation/regression analysis for 16 patients.
Notes: (**A**) Sagittal image; (**B**) Coronal image; (**C**) Axial image. Positive correlation of FA with plasma MHPG was seen in the cerebellar vermis. Blue lines indicate the most representative position and the color scale shows the T value. FWE-corrected threshold of $P<0.05$.
Abbreviations: FA, fractional anisotropy; FWE, family-wise error; MHPG, 3-methoxy-4-hydroxyphenylglycol.

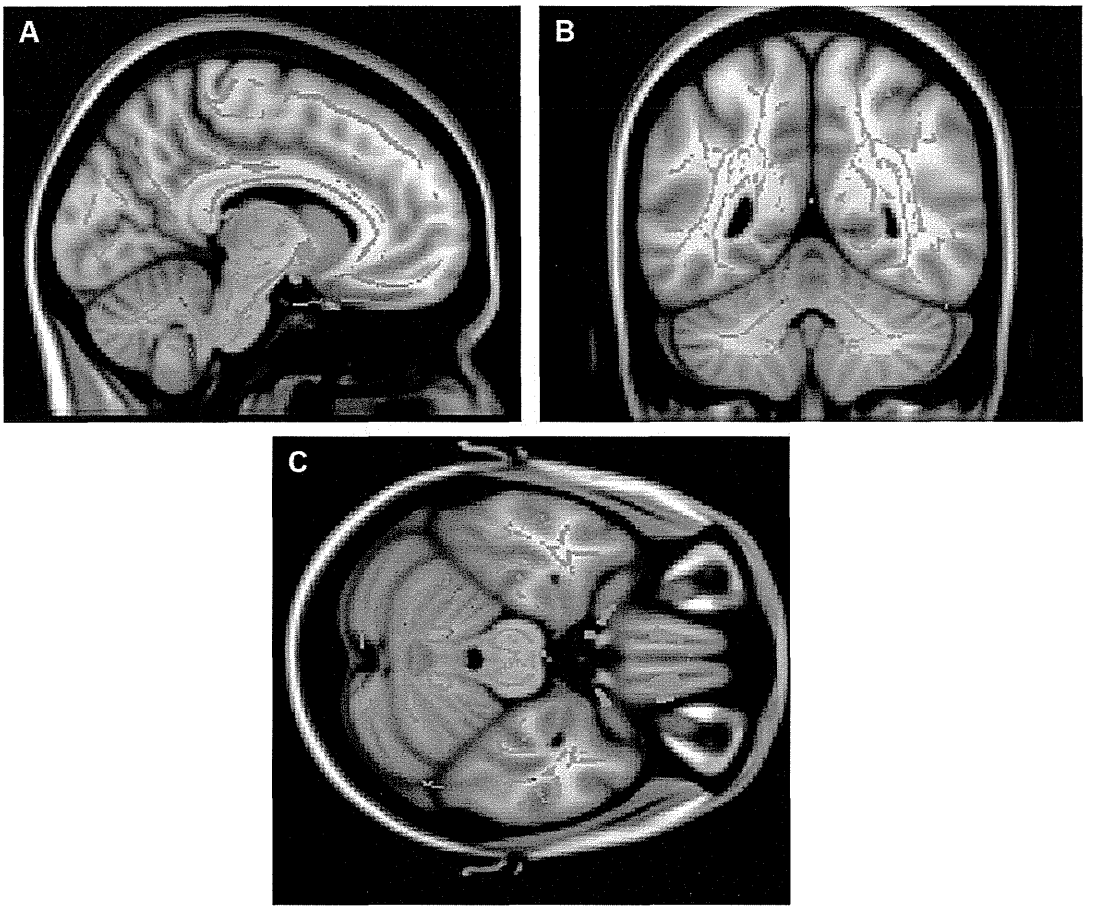

Figure 2 Correlation/regression analysis for 16 patients.
Notes: (**A**) Sagittal image; (**B**) Coronal image; (**C**) Axial image. Positive correlation of FA with plasma MHPG was seen in the white matter of the cerebellar vermis (red voxels). Green lines show an alignment-invariant tract representation (the "mean FA skeleton"). Uncorrected threshold of $P<0.001$.
Abbreviations: FA, fractional anisotropy; MHPG, 3-methoxy-4-hydroxyphenylglycol.

At the time of baseline and follow-up, no correlations were found in any brain region between any of the MR data (brain volumes, FA, and MD) and the laboratory data (plasma HVA and MHPG).

Discussion

Schizophrenia usually shows a prepsychotic phase of illness in which a change from premorbid functioning occurs.[23] This period is characterized by various mental state features, including nonspecific symptoms such as depressed mood and anxiety as well as subthreshold or attenuated psychotic symptoms. There is increasing interest in the potential for early detection and intervention during the early phase of a psychotic disorder.[25] Previous studies have reported that early detection and intervention in schizophrenia may offer a promising opportunity to redirect the illness' negative course.[26,27] In this study, all patients underwent the baseline MR examinations within 6 months from the time they fulfilled DSM-IV-TR criteria A, B, D, E, and F. Therefore, all baseline MR examinations were performed during the early stage of first-episode schizophrenia, before a diagnosis of schizophrenia was established. This is the first longitudinal MRI study to perform VBM analysis and voxel-based analysis of MD and FA maps computed from DTI obtained during the early stage of first-episode schizophrenia.

In the present study, we assessed the interval changes of MRI data and peripheral catecholaminergic measures during an average of 6 months using voxel-wise correlation/regression analysis, because there are individual variations in response to antipsychotic treatment; a positive correlation was observed between the FA in the right cerebellar vermis and MHPG. The reduced FA could be associated with microstructural alteration or damage involving the myelin sheath and/or directional coherence of fiber tracts. Some studies