1. Introduction

Diffusion tensor imaging (DTI) (Basser et al., 1994), a newly developed method to estimate the white matter (WM) integrity, provides information about the diffusion of water in biological tissues. In the WM, water diffusion is highly anisotropic, with greater diffusion in the direction parallel to axonal tracts. Thus, diminished anisotropy of water diffusion has been proposed to reflect compromised WM integrity (Lim et al., 1999). Fractional anisotropy (FA) (Basser, 1995) is a quantitative measure of diffusion anisotropy acquired from DTI.

The normally aging brain exhibits an assortment of micro- and macroscopic changes in the WM as well as the cerebral cortex. Histological studies demonstrate a decrease in myelin density and in the number of myelinated fibers (Meier-Ruge et al., 1992). Postmortem brain (Meier-Ruge et al., 1992) and volumetric neuroimaging studies (Christiansen et al., 1994; Salat et al., 1999) have suggested that WM changes are more prominent than cortical changes with aging, at least during certain segments of the age span and in certain regions of the brain. For example, volume loss in prefrontal WM is disproportionately greater than that in prefrontal cortex with late aging {comparison of elderly adults aged 60–75 with those aged >85 years (Salat et al., 1999)}. Several DTI studies have demonstrated age-related reductions of WM anisotropy in the genu of the corpus callosum (Pfefferbaum et al., 2000b), anterior WM (Pfefferbaum et al., 2000a; O'Sullivan et al., 2001), periventricular WM (Nusbaum et al., 2001), and the prefrontal WM (Nusbaum et al., 2001; Pfefferbaum et al., 2005; Salat et al., 2005).

Regarding schizophrenia, impairments of the neural connectivity between certain cortical areas, such as frontal and temporal areas, have been implicated in the pathophysiology of the disease (Frith and Dolan, 1996; Andreasen et al., 1997; Bullmore et al., 1997). Indeed, volumetric magnetic resonance (MR) studies and pathological studies demonstrated abnormalities of the WM in schizophrenia (Miyakawa et al., 1972; Cannon et al., 1998; Davis et al., 2003; Ho et al., 2003; Uranova et al., 2004). Changes in WM integrity in schizophrenia has relevance to the neural disconnection model of the disorder and may provide a basis for focal abnormalities as well. Several previous DTI studies in chronic schizophrenia showed decrease of FA in schizophrenics mainly in the front-temporal white matter and corpus callosum (Buchsbaum et al., 1998; Lim et al., 1999; Agartz et al., 2001; Burns et al., 2003). Furthermore, FA decrease in patients with first episode schizophrenia might be less pronounced compared to chronic patients (Price et al., 2005; Szeszko et al., 2005), suggesting that the decreased FA in schizophrenics might be attributed, at least in part, to progressive and exaggerated age-dependent changes in schizophrenics rather than neurodevelopmental abnormalities in the WM. To date, there is only one cross-sectional study with a small sample size investigating age-related FA changes in schizophrenia that demonstrated an age-related FA increase in schizophrenics (Jones et al., 2006).

The present study was aimed to examine whether patients with chronic schizophrenia do have reduced FA values compared to controls and whether such changes in FA progress in an age-dependent manner.

2. Methods

2.1. Subjects

Table 1 shows the characteristics of participants of this study. Forty-two patients with chronic schizophrenia were recruited at the National Center of Neurology and Psychiatry, Tokyo, Japan. Consensus diagnosis was made for each patient by at least two trained psychiatrists according to the DSM-IV criteria (American Psychiatric Association, 1994), based on all available information, including clinical interviews, medical records and other research assessments. All patients were stable and/or partially remitted and had been taking antipsychotic medication at the time of MR measurement and neuropsychological tests. Forty-two healthy volunteers who had no current or past contact to any psychiatric services served as controls. All the subjects were biologically unrelated Japanese. After description of the study, written informed consent was obtained from every subject. The study protocol was approved by the ethics committee of the National Center of Neurology and Psychiatry, Tokyo, Japan. Exclusion criteria for all the participants included asymptomatic or symptomatic cerebral infarctions detected by T2 weighted MRI, serious neurological or endocrine disorder, any medical condition that could potentially affect the central nervous system, or mental retardation according to DSM-IV criteria.

2.2. Image acquisition

MR studies were performed on a 1.5 tesla Magnetom Vision Plus system (Siemens, Erlangen, Germany). Axial DTI scans aligned to the plane containing anterior and posterior commissures were acquired with a pulsed-

Table 1
Characteristics of participants

	Controls	Schizophrenics	Two sample *t*-test	(Two-tailed; df=82)
			t	*P*
Number of subjects	42	42		
Gender (male/female)	26/16	26/16		
Handedness (right/left)	41/1	41/1		
Age (years)	39.2 (9.0)	40.0 (9.3)	−0.42	0.68
Range of age (years)	22–59	22–59		
Education (years)	17.1 (3.5)	13.0 (2.9)	8.1	<0.001
Full-scale IQ (WAIS-R)	114.3 (11.6)	86.0 (21.3)	6.0	<0.001
Age of onset		23.3 (7.0)		
Duration of illness (years)		16.8 (9.0)		
Duration of hospitalization (months)		31.2 (61.3)		
Dose of total antipsychotic drugs (mg/day, chlorpromazine equivalent)		1005.1 (735.3)		
Dose of typical antipsychotic drugs (mg/day, chlorpromazine equivalent)		694.8 (748.3)		
Dose of atypical antipsychotic drugs (mg/day, chlorpromazine equivalent)		310.3 (464.2)		

Mean (S.D.).
WAIS-R: Wechsler Adult Intelligence Scale-Revised.

gradient, spin-echo, single-shot echo planar imaging (EPI) sequence (TR/TE=4000/100 ms; acquisition matrix, 256×256; NEX=4, FOV 240 mm; b=1000 s/mm^2; 20 slices, slice thickness 5 mm, gap 1.5 mm). Diffusion was measured along six non-collinear directions. For each of six gradient directions, four acquisitions were averaged. Four acquisitions without diffusion weighting (b=0) were also averaged. Additionally, a three dimensional volumetric acquisition of a T1-weighted gradient echo sequence with a gapless series of thin sagittal sections using an MPRage sequence (TR/TE=11.4/4.4 ms; flip angle, 15 degree; acquisition matrix, 256×256; NEX=1, FOV 315 mm; slice thickness 1.23 mm) was acquired for evaluating the volume of grey matter (GM), WM and cerebrospinal fluid (CSF) space.

2.3. Image processing

FA images for each subject were computed from seven diffusion images acquired as above by an in-house script on Matlab 6.5 software (Mathworks, Inc., MA, USA). Then, the FA images were spatially-normalized using high-dimensional-warping algorithm (Ashburner et al., 1999) and were matched to the FA template image. To make the FA template image, we warped FA images of 4 normal subjects (other than 42 control subjects) to the single-subject T1 template (skull stripped image) using spatial normalization function of SPM2 and averaged the 4 warped FA images. The transformed FA images were smoothed with a Gaussian kernel. The filter size (full-width at half-maximum: FWHM) was varied from zero to 16 mm in steps of 2 mm to validate the consistency of results of SPM analyses, because a previous study (Jones et al., 2005) reported that the statistical results of SPM analyses were differed depending on filter size. For measuring the volume of GM, WM and CSF space, an additional function of an optimized VBM script (http://dbm.neuro.uni-jena.de/vbm) was used (Good et al., 2001).

2.4. Statistical analysis

2.4.1. Voxel-by-voxel analysis

The resultant FA images were analyzed using statistical parametric mapping with the framework of the General Linear Model in SPM2 (Wellcome Department of Cognitive Neurology, London, UK) (Friston et al., 1995). We constituted following three

Table 2
The relationship between smoothing kernel sizes (FWHM) and number of resels in our sample

FWHM (mm)	Number of resels
None	12460.4
2×2×2	5131.1
4×4×4	1720.2
6×6×6	706.0
8×8×8	289.4
10×10×10	119.7
12×12×12	52.1
14×14×14	24.4
16×16×16	12.4

statistical analyses: 1) a two-sample *t*-test for estimating group differences (controls versus schizophrenics), 2) a correlational analysis between age and FA values in both controls and the schizophrenics and 3) a correlational analysis of FA values with duration of illness, age of onset, duration of hospitalization, and daily dose of

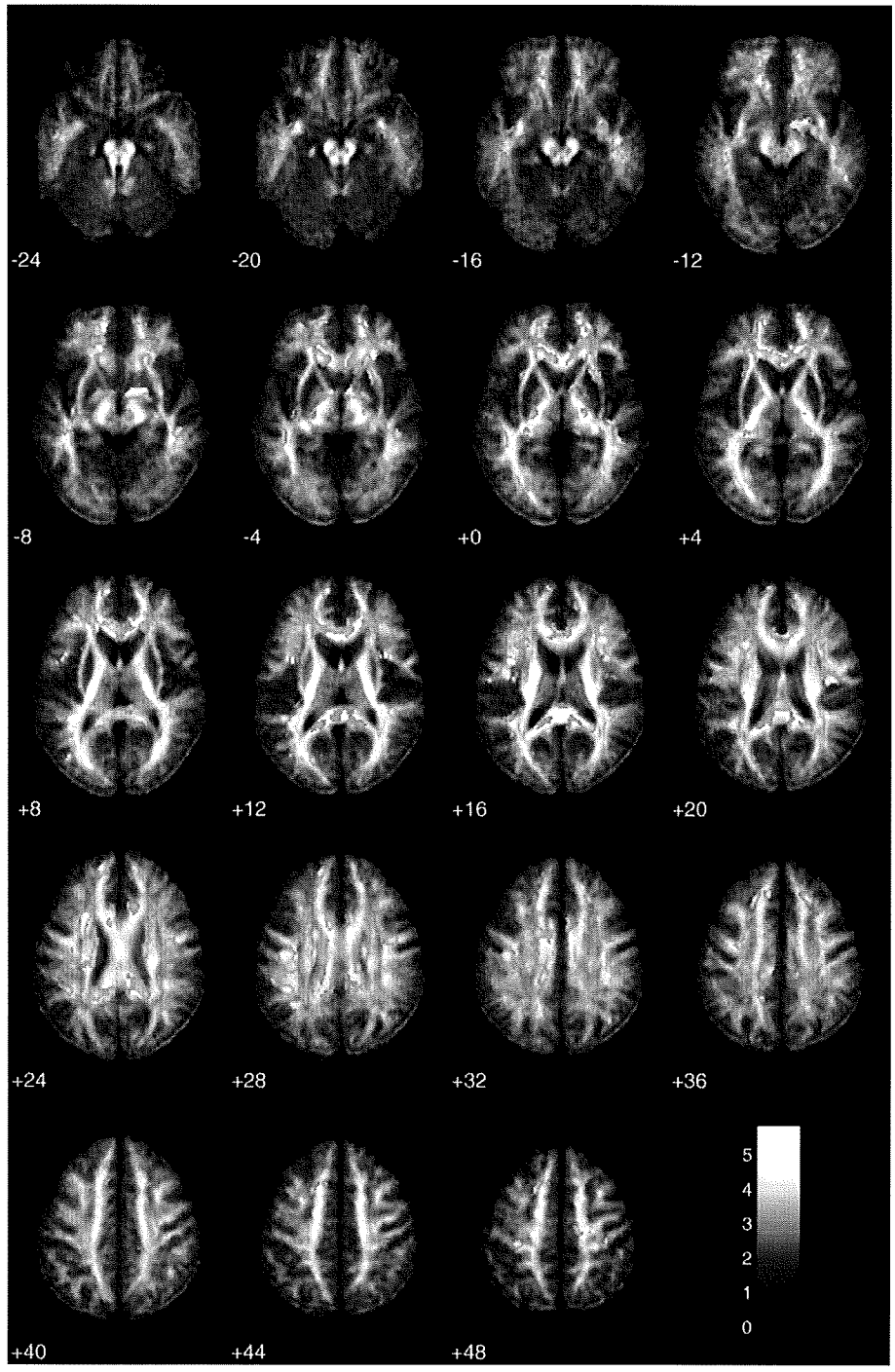

Fig. 1. Comparison in FA values between patients with schizophrenia and controls. The SPM {t} is displayed onto axial FA template images. The WM areas in which significantly lower FA values in patients compared with controls were observed, including the bilateral frontal and temporal WM, uncinate fasciculi, cingulum bundles, and genu and splenium of the corpus callosum ($P<0.001$, uncorrected).

antipsychotic drugs in the schizophrenics. In all the three analyses, relative WM volume (WM volume divided by the summation of GM, WM and CSF volumes) and WAIS-R (Wechsler Adult Intelligence Scale-Revised) full-scale IQ score were treated as nuisance variables. The former was included for eliminating the possible effect of WM volume change associated with aging on the FA values through partial voluming from non-WM voxels. The latter was included to allow for the effects of IQ, because there was some evidence which suggested DTI measures were correlated with cognitive decline in elderly (O'Sullivan et al., 2004). We additionally conducted the analyses without these two nuisance variables to check whether there were any differences in the results with or without nuisance variables in the statistical models. To estimate population effects (diagnostic effects), we used a single-subject, condition (controls or schizophrenics) and covariate (no covariate of interest) model for the SPM analysis. In the second analysis, we applied the single subject condition (controls or schizophrenics) and covariate (interaction with condition, covariate of interest; age) model. A single-subject, covariate only model was applied in the third analysis. For these three analyses, we set masking threshold for FA values of 0.2 for excluding voxels containing partial volume of WM and other tissues. Since the previous study demonstrating a positive correlation between FA values and age in schizophrenics reported mean FA values of around 0.4 (Jones et al., 2006), we additionally set masking threshold for FA values of 0.35 for examining correlation between age and FA values of more anisotropic WM structure in the second analysis. For the evaluation of the statistical models, we used Wake Forest University Pickatlas (Maldjian et al., 2003) to pick up cerebral WM in the Montreal Neurological Institute (MNI) space. We used uncorrected $P<0.001$ as a statistical threshold to search significant differences. As demonstrated in Table 2, the number of resels differed profoundly depending on smoothing kernel sizes (FWHM) and the statistical results with correction for multiple comparisons could change dramatically relying on number of resels. On the other hand, SPM results without correction for multiple comparisons were essentially unchanged regardless of smoothing kernel size (data not shown). Therefore, we did not perform correction for multiple comparisons. The resultant set of t values constituted statistical parametric map (SPM {t}). We employed the filter size of 6 mm for presentation of the results considering for the original voxel dimensions of acquired data {0.94 mm × 0.94 mm × (5.00+1.50) mm}.

2.4.2. ROI analysis

To ensure the robustness of the results of the voxel-by-voxel analyses, we additionally performed ROI analyses. We used MarsBar (http://marsbar.sourceforge.net/) for extracting region of interest (ROI) containing all the voxels classified as WM with Wake Forest University Pickatlas from spatially normalized and smoothed FA images and calculated mean FA values of the ROI. Then, we performed correlational analyses of mean FA values with the same variables in voxel-by-voxel analysis using Statistical Package for Social Science (SPSS), 1) in both controls and schizophrenics, 2) in controls and 3) in schizophrenics. We constituted a General Linear Model for the first analysis and entered diagnosis-by-age interaction effects into the statistical model to examine if there were any diagnosis-by-age interaction effects. For the second and third analyses, Pearson's correlation coefficients between mean WM FA values and covariates were calculated.

3. Results

3.1. Voxel-by-voxel analyses

3.1.1. Comparison between schizophrenics and controls

Schizophrenics demonstrated significantly lower FA values in widespread WM areas, compared with controls. These WM areas included bilateral frontal and temporal lobes, uncinate fasciculi (external capsules), cingulum bundles, and genu and splenium of corpus

Table 3
The summary of the WM areas in which significantly lower FA values in patients compared with controls were observed

Anatomical regions	t-value	MNI coordinates		
	(Voxel level)	x	y	z
Rt frontal lobe white matter	4.34	22.5	52.5	−4.5
Lt frontal lobe white matter	5.43	−13.5	49.5	−6
Rt temporal lobe white matter	4.25	48	−33	−7.5
Lt temporal lobe white matter	4.19	−45	−31.5	−10.5
Rt uncinate fasciculus (external capsule)	4.00	33	12	−1.5
Lt uncinate fasciculus (external capsule)	3.84	−33	12	−1.5
Rt cingulate bundle	4.23	6	6	33
Lt cingulate bundle	4.32	−7.5	6	30
genu of corpus callosum	3.79	6	24	10.5
splenium of corpus callosum	4.18	−3	−33	19.5

callosum (Fig. 1, Table 3). There would be increased possibility of alpha errors because we did not perform correction for multiple comparisons. However, our results were in well concordance with the results of the previous studies (Buchsbaum et al., 1998; Lim et al., 1999; Agartz et al., 2001; Burns et al., 2003; Kubicki

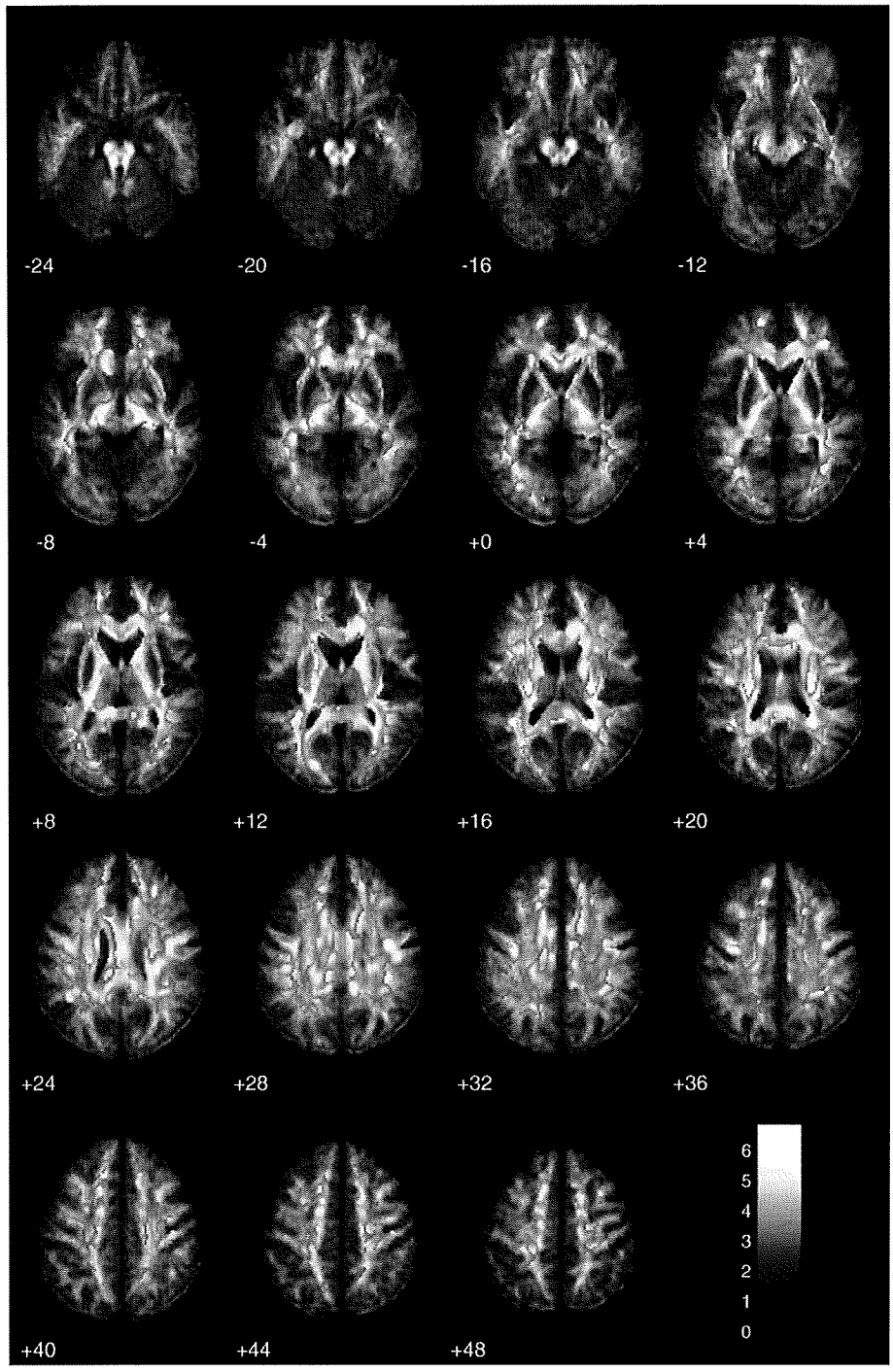

Fig. 2. Correlational analysis between FA values and age with 0.2 as a masking threshold in schizophrenics. The SPM {t} is displayed onto axial FA template images. The widespread WM areas showed a significant negative correlation between FA values and age in schizophrenics ($P<0.001$, uncorrected).

et al., 2003). Therefore, we might be able to regard the results of these previous studies as a priori hypotheses. There were no areas of significantly higher FA values in patients compared with controls even at a lenient threshold ($P<0.05$, uncorrected). In these results of the analysis without nuisance variables in the statistical

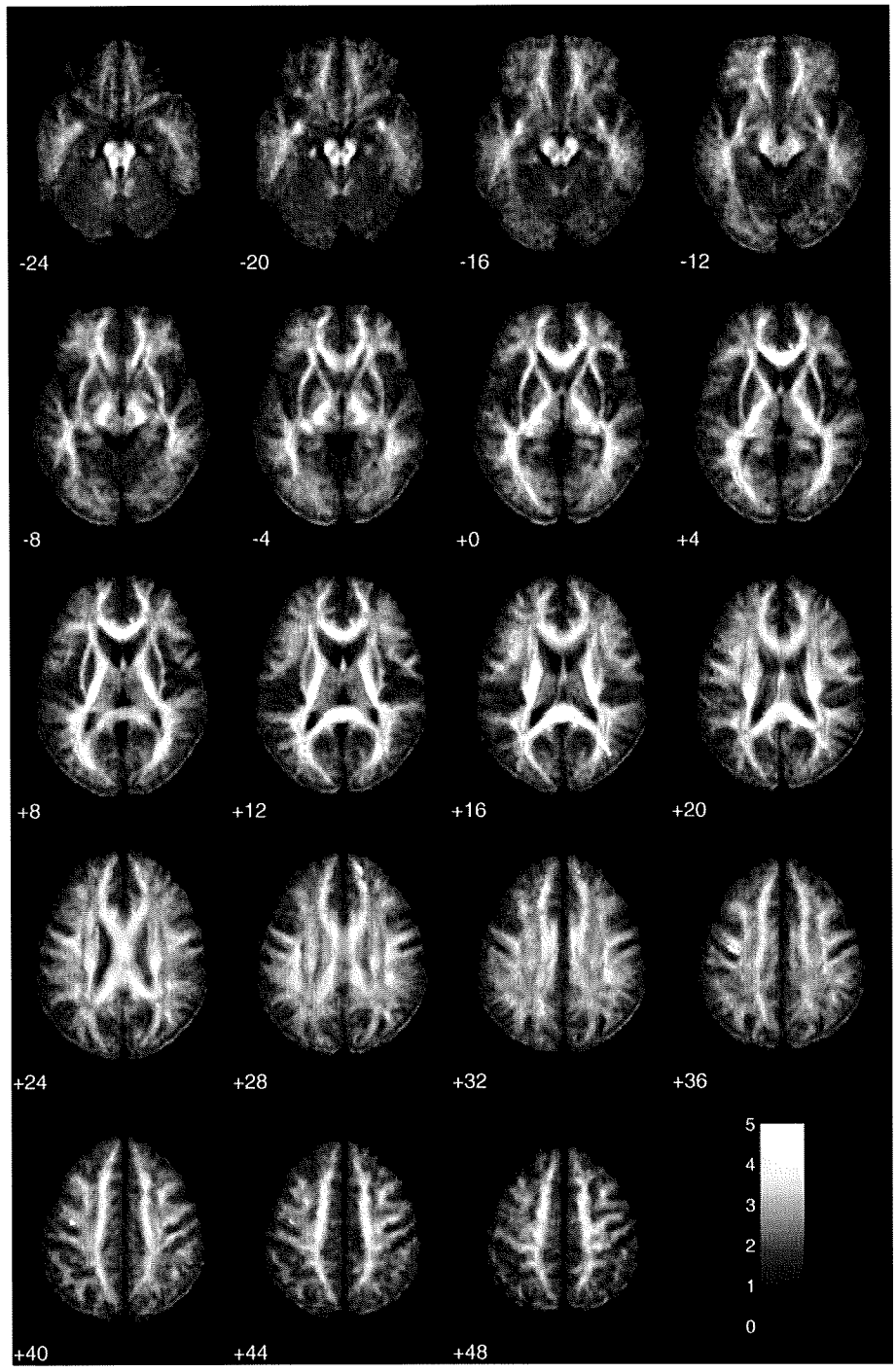

Fig. 3. Correlational analysis between FA values and age with 0.2 as a masking threshold in controls. The SPM {t} is displayed onto axial FA template images. The WM areas showed a significant negative correlation between FA values and age in controls ($P<0.001$, uncorrected), including right prefrontal {(15.0, 49.5, 30.0) in MNI coordinates, $t=5.03$}, left frontal {(−37.5, −15.0, 34.5), $t=4.51$} and bilateral temporo-occipital WM {(31.5, −60.0, 16.5), $t=4.75$; (−30.0, −60.0, 15.0), $t=4.47$}.

models, the distributions of the statistically significant areas were essentially unchanged compared to the results with nuisance variables although the spatial extents of the statistically significant areas were slightly larger (data not shown), which was the case with the results of other two analyses.

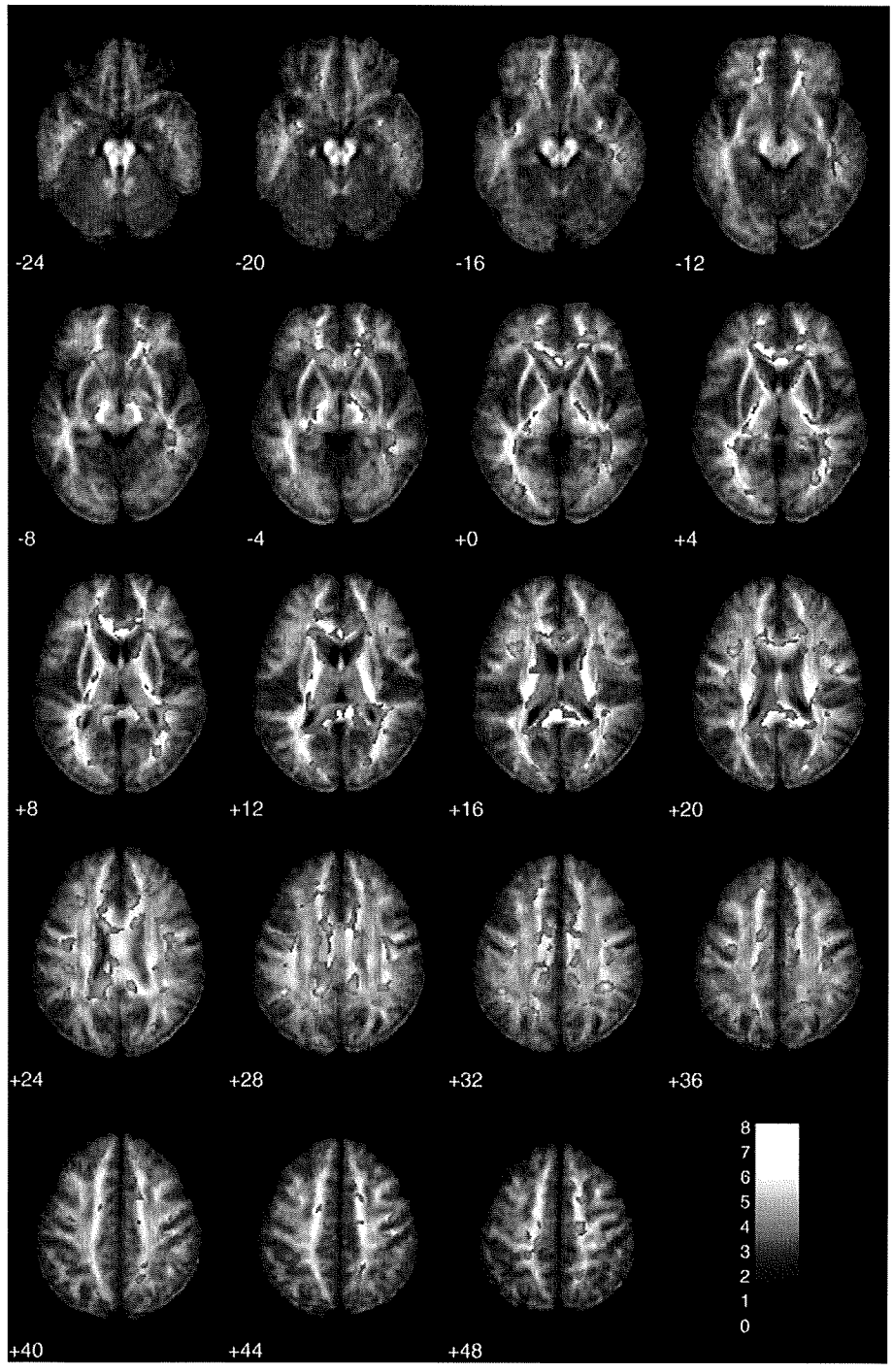

Fig. 4. Correlational analysis between FA values and duration of illness with 0.2 as a masking threshold in schizophrenics. The SPM {t} is displayed onto axial FA template images. The widespread WM areas showed a significant negative correlation between FA values and duration of illness in schizophrenics ($P<0.001$, uncorrected).

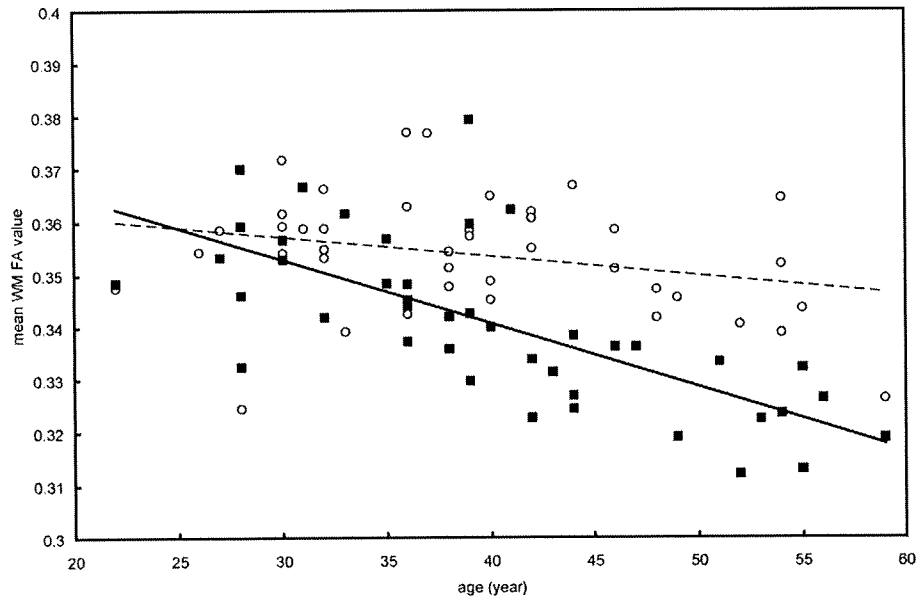

Fig. 5. A scatter plot between age and mean WM FA value when masking threshold for FA values was set to 0.2. Filled squares represent schizophrenics and open circles represent controls. The solid line indicates a regression line for schizophrenics ($y=-0.0012\times+0.3888$, $R^2=0.49$, test for regression slope: df=40; $t=-6.24$; $P<0.0001$). The dashed line indicates a regression line for controls ($y=-0.0004\times+0.3679$, $R^2=0.083$, test f or regression slope: df=40; $t=-1.90$; $P=0.065$). A significant diagnosis-by-age interaction effect (general linear model: $P=0.009$) was noted.

3.1.2. *Correlational analysis in schizophrenic and control groups*

As the results of the second analysis considering aging effects, a significant negative correlation with age was observed in the FA values of widespread, almost diffuse WM areas in the schizophrenic group (Fig. 2), while in the control group, only FA values in right prefrontal {(15.0, 49.5, 30.0) in MNI coordinates, $t=5.03$}, left frontal {(−37.5, −15.0, 34.5), $t=4.51$} and bilateral temporo-occipital WM {(31.5, −60.0, 16.5), $t=4.75$;

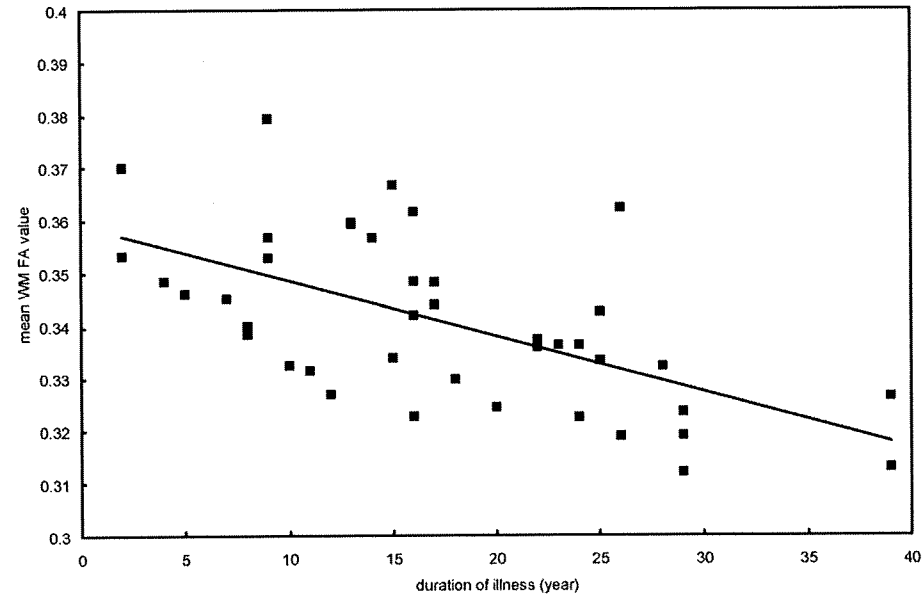

Fig. 6. A scatter plot between duration of illness and mean WM FA value when masking threshold for FA values was set to 0.2. Filled squares represent schizophrenics. The solid line indicates a regression line for schizophrenics ($y=-0.0011\times+0.3590$, $R^2=0.36$, test for regression slope: df=40; $t=-4.78$; $P<0.0001$).

(−30.0, −60.0, 15.0), t=4.47} demonstrated a significant negative correlation with age (Fig. 3). Even if the analysis was done on voxels with FA values higher than 0.35, to examine more anisotropic WM areas, the results were essentially unchanged (data not shown).

3.1.3. Correlational analysis between FA values and clinical factors in schizophrenics

There was a significant negative correlation between FA values and duration of illness in widespread WM areas (Fig. 4), while there was no significant correlation of FA values with age of onset, duration of hospitalization or daily dose of antipsychotic drugs (data not shown).

3.2. ROI analyses

3.2.1. ROI-based correlational analysis in both schizophrenics and controls

First, we constituted a General Linear Model putting diagnosis as a fixed factor and age, IQ and relative WM volume as covariates. F values (significance probabilities) were as follows; diagnosis: 10.8 (P=0.001), age: 26.1 (P<0.001), IQ: 0.029 (P=0.865) and relative WM volume: 16.6 (P<0.001). Then, we added diagnosis-by-age interaction into the model. F values (significance probabilities) changed as follows; diagnosis: 2.34 (P=0.130), age: 27.8 (P<0.001), IQ: 0.059 (P=0.809), relative WM volume: 14.1(P<0.001) and diagnosis-by-age interaction: 7.08 (P=0.009). Effect of IQ was not significant in both models. There was a significant diagnosis-by-age interaction effect.

3.2.2. ROI-based correlational analysis in controls

Pearson's correlation coefficients (significance probabilities of the test of significance of the correlation: two-tailed) of mean WM FA value with age, IQ and relative WM volume in controls were as follows; FA vs. age: −0.287 (P=0.065), FA vs. IQ: −0.108 (P=0.496) and FA vs. mean WM volume: 0.481 (P=0.001). Only positive correlation between mean WM FA value and relative WM volume was statistically significant.

3.2.3. ROI-based correlational analysis in schizophrenics

Pearson's correlation coefficients (significance probabilities of the test of significance of the correlation: two-tailed) of mean WM FA value with clinical factors in schizophrenics were as follows; FA vs. age: −0.702 (P< 0.001), FA vs. duration of illness: −0.603 (P<0.001), FA vs. age of onset: −0.305 (P=0.049), FA vs. total daily dose of antipsychotics: 0.110 (P=0.489), FA vs. duration of hospitalization: −0.172 (P=0.277), FA vs. IQ: −0.064 (P=0.686), FA vs. relative WM volume: 0.421 (P=0.006). Significant positive correlation was observed between mean WM FA value and relative WM volume. Fig. 5 shows a scatter plot between age and mean WM FA value in controls and schizophrenics. Fig. 6 shows a scatter plot between duration of illness and mean WM FA value in schizophrenics. Significant negative correlations were observed between mean WM FA value and age (or duration of illness).

4. Discussion

In this study, we obtained three main findings; 1) lower FA values in schizophrenic patients compared with controls in WM areas including frontal and temporal WM, bilateral uncinate fasciculi (external capsules) and cingulum bundles and genu and splenium of corpus callosum, 2) age-related reductions of FA value in the widespread WM were more prominent in schizophrenics than in controls, and 3) a negative correlation between FA value in the widespread WM and duration of illness in schizophrenics.

Recent studies demonstrated age-related FA decline in normal individuals occurred in the prefrontal WM, while temporal WM were relatively preserved (Pfefferbaum et al., 2005; Salat et al., 2005). However, in this study, negative age-dependent effects were observed only in the lenient statistical threshold in the FA values of restricted areas of the WM in controls. This could be explained by the fact that all our subjects were under the age of 60, relatively less old compared to the participants of normal aging studies.

We replicated the results of the most of the previous studies, decreased FA values in the WM of schizophrenics. In the earlier studies concerning FA values in WM of patients with schizophrenia, an inherent abnormality in WM was expected to be detected since the decrease of FA values in the WM of the schizophrenic brain was assumed to occur as neurodevelopmental impairments before onset of the illness. Several studies demonstrated that schizophrenics had reduced FA value in the prefrontal WM (Buchsbaum et al., 1998), prefrontal and parieto-occipital WM (Lim et al., 1999), splenium of the corpus callosum (Agartz et al., 2001) and adjacent occipital WM (forceps major) (Agartz et al., 2001), left uncinate fasciculus and bilateral arcuate fasciculus (Burns et al., 2003), bilateral cingulum bundles (Kubicki et al., 2003). Some of them indicated that the reduction of FA values in schizophrenics might occur independently of reduction of the white matter volume. Although some studies reported no significant FA changes in schizophrenics (Steel et al., 2001; Foong et al., 2002), most studies with chronic

schizophrenia demonstrated lower FA values in schizophrenia (Kanaan et al., 2005). A few DTI studies have examined first episode patients (Price et al., 2005; Szeszko et al., 2005). Szeszko et al. found FA decrease in the left internal capsule and left-hemisphere WM of the middle frontal gyrus and posterior superior temporal gyrus of first-episode schizophrenics and schizoaffective disorder patients, however, the decrease was less pronounced compared with results of the majority of the studies in chronic schizophrenics. On the other hand, Price et al. reported that there was no FA decrease in the corpus callosum of patients with first-episode schizophrenia. They suggested that FA reduction in schizophrenia might reflect neuropathological abnormalities, which may occur after the onset of the disease and could be progressive. Our results, 1) age related FA reduction was more prominent in schizophrenics than controls, and 2) duration of illness was related to FA reduction in schizophrenics, suggest that changes of FA value in schizophrenia are attributable, at least in part, to progressive neuropathological changes after onset of the illness.

Contrary to our results, a previous DTI study demonstrated 'positive' correlation between age and FA in schizophrenics (Jones et al., 2006). They measured FA values of WM tracts captured from tractography, and they set seedpoints of the tracts manually from one slice of FA images. Such methods might overlook general decline of FA values in the WM. Their mean FA values (average of 8 WM tracts in each subjects) were around 0.4, which was relatively higher than those of our study {our mean FA value of entire WM was 0.35 + 0.01 (mean + S.D.)}. To simulate the analysis of the previous study, we additionally performed an analysis setting masking threshold for FA values of 0.35. As a result, the significant negative correlation remained to be present even in more anisotropic WM areas.

Previous pathological studies demonstrated microscopic abnormalities of the WM in schizophrenia such as decreased expression of myelin and oligodendrocyte-related genes, the decrease in density of oligodendrocytes (Hof et al., 2002), damage of myelin sheath lamellae (Uranova et al., 2001) and maldistribution of interstitial neurons (Akbarian et al., 1996) in prefrontal WM of the brains of schizophrenic patients. Further, a previous longitudinal MR study demonstrated progressive atrophy of the white matter in schizophrenics (Ho et al., 2003). Given these previous findings and ours, it seems likely that age-dependent FA decrease, but not increase, occurs in schizophrenic brains.

As well as a negative correlation with age, FA values of schizophrenics showed negative correlation with duration of illness but not with age of onset or daily dose of antipsychotics. The facts seem to support the hypothesis that FA reduction in schizophrenia might be associated with neuropathological abnormalities which may emerge, at least in part, after the onset of the disease and could be progressive. Further, the spatial distribution of age-related FA reduction in schizophrenics was different from those of normal individuals in previous studies that demonstrated preserved temporal white matter (Pfefferbaum et al., 2005; Salat et al., 2005). Such different distributions suggest that FA changes in schizophrenics might be associated with disease progression rather than merely exaggerated aging effects. However, it is difficult for neuroimaging studies, even for longitudinal studies, to discriminate disease progression from aging effects. The correlational study between DTI findings and pathological findings should be conducted to clarify whether reduction of FA values in schizophrenics reflect pure disease progression or merely exaggerated aging effects.

Several limitations should be considered in our study. First, our study is a cross-sectional study. To confirm progressive pathological process in the WM of the patients of schizophrenia, longitudinal studies should be conducted. Second, IQ score was not matched between groups, i.e., mean IQ score was significantly lower in schizophrenics in our samples. O'Sullivan et al. (2004) reported DTI measures were correlated strongly with cognitive decline in elderly. Thus, it could be problematic whether age-related FA decrease in our study was reflected by cognitive decline. However, no significant correlation was observed between mean WM FA values and IQ in our sample. Also, regarding schizophrenia, it has been hypothesized that most cognitive change takes place early in their psychotic episodes and it remains relatively stable through long term in the illness (Hoff et al., 2005). Hence, at least from our data, we cannot attribute age-related FA decline in schizophrenia to IQ changes. Third, the issue of partial volume effect should be addressed. In schizophrenia, progressive WM atrophy has been reported in the previous studies (Ho et al., 2003). Due to the atrophy, it is possible that the voxels located in the border of the WM and other tissues in schizophrenics were estimated as having lower FA values. However, we minimized the problem by using the high dimensional warping algorithm, threshold masking for FA values and adopting relative WM volume as a nuisance variable. Another issue is the possible effect of long-term medication with antipsychotics. Although daily dose of antipsychotics was not correlated with FA values in schizophrenics, we could not estimate accurate cumulative doses of antipsychotics

throughout the duration of illness. Several morphological MR studies and animal studies suggested that the administration of antipsychotics could affect brain morphology (Wang et al., 2004; Lieberman et al., 2005). It is possible that long-term medication with antipsychotics also affects microstructure of the WM in schizophrenics. The longitudinal animal studies may clarify this issue.

In conclusion, we confirmed decreased FA in schizophrenics, compared to controls in the widespread WM areas in a Japanese sample. We found that age-dependent FA decline was more pronounced in chronic schizophrenics compared to controls, and that such FA decline was significantly correlated with duration of illness in patients. These observations suggest that decreased FA values in schizophrenia might be attributable, at least in part, to progressive changes in the WM after the onset of the illness.

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Gene expression in the peripheral leukocytes and association analysis of PDLIM5 gene in schizophrenia

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Abstract

PDLIM5 modulates neuronal calcium signaling, co-localizes with synaptic vesicles of neurotransmitters and positive association between its gene and schizophrenia was reported but its relation is still ambiguous. The differential expression of the PDLIM5 gene both in the brain and in the lymphoblasts has been found in schizophrenia compared to control subjects. In this study, we measured the expression level of the PDLIM5 gene transcripts in the peripheral leukocytes from 19 medication-free and 21 chronically medicated schizophrenic patients as well as age- and sex-matched control subjects using a quantitative real-time PCR method. The mRNA levels of the PDLIM5 gene in the leukocytes of medication-free schizophrenic patients were significantly higher than those of control subjects. On the other hand, our group has previously shown that its mRNA expression in the leukocytes of medication-free major depressive patients was significantly lower compared with controls. There was no difference in the PDLIM5 mRNA levels between chronic schizophrenic patients with antipsychotic medication and their controls. Further, we failed to find any genetic association between the PDLIM5 gene and schizophrenia with six single nucleotide polymorphics (SNPs) of the PDLIM5 gene in Japanese subjects (279 subjects each) and there was no significant relation between PDLIM5 gene and schizophrenia with the haplotype analysis ($P = 0.48$), either. We suggest that the higher expression levels of the PDLIM5 mRNA in the peripheral leukocytes may be a candidate marker for medication-free schizophrenic patients.

Keywords: PDLIM5; Gene expression; Leukocytes; Association analysis; Schizophrenia

PDLIM5 is an intermediate protein that has been shown to regulate intracellular calcium levels by linking calcium channel and protein kinase C (PKC) [2,3,16]. PDLIM5 is ubiquitously expressed and its cellular localization in the brain is identical to Synapsin which is known to be involved in the neurotransmitter release [16]. The PDLIM5 gene lies on chromosome 4q22, a locus previously reported to be linked with schizophrenia [13,19]. While Kato et al. failed to find any association between the PDLIM5 gene and schizophrenia [15], Horiuchi et al. found a significant association between them [6]. It was reported that the expression level of PDLIM5 mRNA was significantly increased in the postmortem brain tissues of patients with schizophrenia, bipolar disorder and major depression, but was decreased in the immortalized lymphoblastoid cell lines derived from patients with schizophrenia and bipolar disorder [10,11]. Our group has recently shown that levels of mRNA expression in the peripheral leukocytes of the PDLIM5 gene were significantly lower in medication-free major depressive patients compared with controls [8].

The expressional alterations of genes in the peripheral blood lymphocytes and leukocytes have been reported to indicate the changes of the central nervous systems in schizophrenia and

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Table 1a
Demographic data for medication-free schizophrenic patients studied in PDLIM5 mRNA expression analysis ($N = 19$)

	Age (y.o)	Gender	Age at onset (years)	BPRS score	Family history of schizophrenia in first-degree relative
S1	25	M	22	64	+
S2	24	M	24	42	−
S3	24	M	24	31	−
S4	27	M	24	37	−
S5	36	M	36	34	−
S6	39	M	38	59	−
S7	27	M	26	58	−
S8	20	F	19	46	−
S9	23	F	23	48	−
S10	34	F	31	36	−
S11	47	F	47	30	−
S12	15	F	13	30	+
S13	26	F	21	100	−
S14	23	M	23	31	−
S15	28	M	25	63	−
S16	47	F	47	37	−
S17	37	F	21	36	−
S18	30	F	25	41	−
S19	45	F	43	36	+

The age (years old: y.o) represents the age of the subject when the leukocytes were drawn. M: male, F: female, '+' indicates that at least one of the first-degree relatives has schizophrenia.

major depressive disorder [7,8,9,17,21]. In this study, we measured the PDLIM5 mRNA expression levels in the peripheral leukocytes in unmedicated and medicated schizophrenic patients as well as in control subjects, using a quantitative real-time PCR method. In addition, we examined the genetic case-control study of the PDLIM5 gene with schizophrenia in Japanese subjects comprising of 279 patients with schizophrenia and 279 controls.

All patients and controls were biologically unrelated Japanese. The diagnosis of schizophrenia was made by at least two experienced psychiatrists according to DSM-IV criteria [1]. Clinical symptoms were evaluated by the Brief Psychiatric Rating Scale scores (BPRS) [20] when blood samples were taken. Age- and sex-matched controls were in good physical health without a history of any psychiatric or serious somatic diseases and taking any medication during the sample collection period. Probands who had first-degree relatives with psychiatric disorders were excluded from the control subjects.

For the measurement of expression levels of the PDLIM5 mRNA, the subjects consisted of 19 medication-free patients with schizophrenia (subject number S1–S19, Tables 1a and 1b) (14 first-episode and drug-naïve schizophrenic patients, 5 schizophrenic patients without antipsychotic treatment for at least 2 months; 9 males and 10 females, mean age: 30.4 ± 9.3), 19 age- and sex-matched controls (9 males and 10 females, mean age: 30.6 ± 8.6), 21 chronically treated patients with schizophrenia who were stably controlled under the same amount dosage of antipsychotics for at least 3 months (subject number S20–S40, Tables 2a and 2b) (13 males and 8 females, mean age: 47.7 ± 11.3) and 21 age- and sex-matched controls (mean age: 47.7 ± 11.1).

For the genetic association study, we used DNA samples from 279 in patients (189 male and 90 female; mean age: 51.3 ± 13.7 years) with schizophrenia from 13 psychiatric hospitals in the neighboring area of Tokushima Prefecture in Japan (population: about 820,000). Age- and sex-matched controls were selected from volunteers after assessing the psychiatric problems (189 male and 90 female; mean age: 51.4 ± 12.0) for the association and haplotype-based case-control study.

All subjects signed written informed consent to participate in the expression and genetic association studies approved by the institutional ethics committees.

Table 1b
PDLIM5 mRNA expression in medication-free schizophrenic ($N = 19$) and control subjects ($N = 19$)

	Male ($N = 9$)	Female ($N = 10$)	Total ($N = 19$)
Schizophrenia (S1–S19)			
Age	28.1 ± 5.6	32.4 ± 11.5	30.4 ± 9.3
The PDLIM5 mRNA expression before treatment	1.13 ± 0.3	1.29 ± 0.3	$1.21 \pm 0.3^*$
Control			
Age	27.6 ± 4.8	33.4 ± 10.4	30.6 ± 8.6
The PD1IM5 mRNA expression	0.95 ± 0.2	1.03 ± 0.4	1.00 ± 0.3

The mean PDLIM5 mRNA levels in the peripheral leukocytes from medication-free schizophrenia patients were significantly higher than those of age- and sex-matched controls (Mann–Whitney U test: $P = 0.023$); $^*P < 0.05$. No correlation between PDLIM5 mRNA levels and baseline BPRS scores were observed (Spearman's correlation efficient: $P = 0.38$).

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Table 2a
Demographic data for chronic schizophrenic patients studied in PDLIM5 mRNA expression analysis ($N = 21$)

	Age (y.o)	Gender	Medication	BPRS Score
S20	57	M	QTP 75 mg, LP 150 mg, CP 300 mg	55
S21	56	M	Ris 6 mg	29
S22	56	M	Ris 5 mg, QTP 200 mg, sulpiride 150 mg	44
S23	60	M	Ris 8 mg, LP 20 mg	67
S24	57	M	HPD 9 mg, BPD 9 mg propericyazine 60 mg	52
S25	40	M	Ris 12 mg	33
S26	46	M	Ris 6 mg, HPD 9 mg, sultopride 900 mg	49
S27	45	M	BPD 9 mg, clocaprarmine 75 mg	59
S28	31	M	BPD 2 mg, HPD 1 mg, LP 15 mg Perospirone 24 mg	49
S29	49	F	Ris 6 mg, HPD 6 mg, CP 20 mg, HPD decanoate 150 mg	33
S30	53	F	HPD 2.25 mg, sulpride 150 mg	33
S31	65	F	HPD 4.5 mg, CP 37.5 mg	47
S32	51	F	Olz 10 mg	23
S33	43	F	Ris 6 mg, zotepine 50 mg	45
S34	54	F	Olz 20 mg, LP 50 mg	38
S35	54	M	Ris 12 mg, zotepine 150 mg timiperone 6 mg	42
Sc36	25	M	Ris 9 mg, perospirone 16 mg	39
Sc37	49	M	Ris 12 mg, LP 150 mg	54
Sc38	23	M	Ris 12 mg, LP 150 mg	38
Sc39	35	F	Olz 20 mg	33
Sc40	53	F	QTP 400 mg	27

The age (years old: y.o) represent the age of the subject when the leukocytes were drawn. M: male, F: female, Olz: olanzapine, Ris: risperidone, HPD: hapoperidol, BPD: bromperidol, LP; levom epromazine.

Total RNA was extracted from the peripheral leukocytes using the PAX gene Blood RNA kit (Qiagen, Tokyo, Japan) according to the manufacturer's recommendations. One microgram of total RNA was used for cDNA synthesis by QuantiTect Reverse Transcription Kit (Qiagen) after assessing RNA quality and quantity with NanoDrop (NanoDrop Technologies, DE, USA). Expression of the PDLIM5 gene transcript was quantified by real-time PCR with the TaqMan Gene Expression Assay (Applied Biosystems, CA, USA). Primers and probes (Hs00179051_m1) were purchased from Applied Biosystems as well as Horiuchi's group [6]. GAPDH gene expression was used as an internal control and measurement of threshold cycle (Ct) was performed in triplicate. Data were collected and analyzed with Sequence Detector Software version 2.1 (Applied Biosystems) and the standard curve method. Relative gene expression was calculated as the ratio of PDLIM5 to GAPDH gene and the mean of the three replicate measures was assigned to each individual. Almost all of blood samples were taken in the morning before lunch. The expression of the PDLIM5 mRNA was not changed among blood samples collected at several points during the day time or over several weeks in the same control subjects.

Genotyping was performed using commercially available TaqMan probes (C_2095059_10, C_16015055_20, C_3226622_10, C_16015313_10, C_1569781_10, C_11567561_10) with Applied Biosystems 7500 Fast Real Time PCR System according to the protocol recommended by the manufacturer (Applied Biosystems). We selected six single nucleotide polymorphic (SNP) markers for genotyping according to linkage disequilibrium (LD) and haplotype blocks in the PDLIM5 gene region [6]. Two SNPs (rs10008257, rs2433320) in the 5′-flanking region and four SNPs left in the genomic region are covered about 169-kb in the whole 214-kb of the PDLIM5 gene. The heterozygocities of four of these six SNPs, rs10008257, rs2433320, rs2433327 and rs2452600 in Japanese population are reported as 0.39, 0.18, 0.26 and

Table 2b
PDLIM5 mRNA expression in chronic treated schizophrenic ($N = 21$) and control subjects ($N = 21$)

	Male ($N = 13$)	Female ($N = 8$)	Total ($N = 21$)
Schizophirenia (S20–S40)			
Age	46.1 ± 12.7	50.4 ± 8.7	47.7 ± 11.3
The PDLIM5 mRNA expression	0.78 ± 0.2	0.93 ± 0.2	0.83 ± 0.2
Control			
Age	46.2 ± 12.3	50.1 ± 9.0	47.7 ± 11.1
The PDlIM5 mRNA expression	0.90 ± 0.3	1.14 ± 0.4	1.00 ± 0.3

The mean PDLIM5 mRNA levels in the peripheral leukocytes from schizophrenia patients who has been treated with antipsychotic drugs for many years were not different from controls' (Mann–Whitney U test: $P = 0.16$). No correlation between PDLIM5 mRNA levels and baseline BPRS scores were observed (Spearman's correlation efficient: $P = 0.82$).

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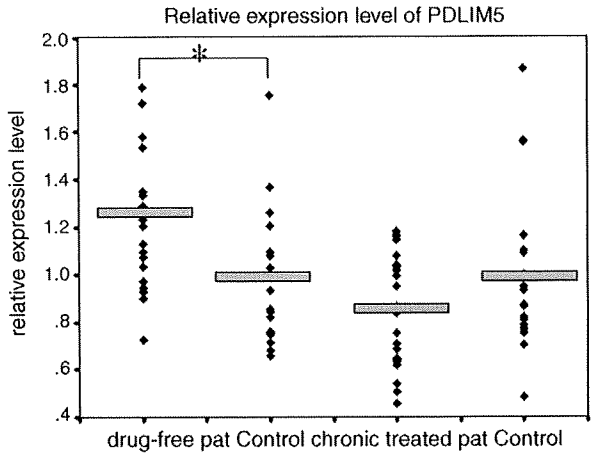

Fig. 1. Relative expression levels of PDLIM5 in the peripheral leukocytes in schizophrenic patients and control subjects. Compared with the normal control group, the mean PDLIM5 mRNA level in the leukocytes of medication-free schizophrenic patients ($N = 19$) was significantly higher (patients: 1.21 ± 0.29, controls: 1.00 ± 0.29, Mann–Whitney U test: $P = 0.023$). The mean PDLIM5 mRNA level in the leukocytes of chronic schizophrenic patients ($N = 21$) showed no significant difference compared with controls (patients: 0.83 ± 0.23, controls: 1.00 ± 0.32, Mann–Whitney U test: $P = 0.16$).

0.34, respectively. The heterozygocities of the other two SNPs, rs12641023 and rs14082, are not reported.

Statistical calculations were carried out using the SPSS Statistical Software Package 11.5 (SPSS, Tokyo, Japan). Expressional differences between patients and control subjects were calculated using the Mann–Whitney U test. Spearman correlation coefficients were used to evaluate the correlations between PDLIM5 mRNA levels and BPRS scores. Two-way ANOVA was performed to determine the independent and combined effects of age and the expression of PDLIM5 between groups. Allele and genotype frequencies of patients and control subjects were compared using Fisher's exact test. The SNPAlyze 3.2 Pro software (DYNACOM, Japan) was used to estimate haplotype frequencies, LD, and permutation P-values. Pair-wise linkage disequilibrium indices, D' and r2, were calculated in the control subjects. The criterion for significance was set at $P < 0.05$ for all tests. Data are presented as mean ± standard deviation.

Relative expression levels of PDLIM5 mRNA in 19 medication-free patients (S1–S19) were 1.21 ± 0.29 in the range of 0.73–1.79, while 1.00 ± 0.29 (range: 0.66–1.75) in healthy volunteers, showing a statistical difference (Mann–Whitney U test: $P = 0.023$, Fig. 1). Mean BPRS scores was 45.2 ± 17.4. No correlation between PDLIM5 mRNA levels and baseline BPRS scores were observed (Spearman's correlation efficient: $P = 0.38$). There was no significant expressional difference of PDLIM5 mRNA levels either between males and females or between genotypes of the single nucleotide polymorphism (rs2433320) both in patients with schizophrenia and in control subjects.

Relative PDLIM5 mRNA level was 0.83 ± 0.23 (0.46–1.18) in 21 chronically treated patients (S20–S40), while 1.00 ± 0.32 (0.49–1.87) in healthy volunteers, showing no significant statistical difference (Mann–Whitney U test: $P = 0.16$; Fig. 1). Mean chlorpromazine-equivalent doses were 932.1 ± 510.5 mg/day and mean duration of treatment was 23.5 ± 10.7 years and mean BPRS scores was 43.1 ± 10.8. No significant relationship between PDLIM5 mRNA levels and BPRS scores was observed (Spearman correlation efficient: $P = 0.71$). There was no significant expressional difference of PDLIM5 mRNA levels either between males and females or between genotypes of the single nucleotide polymorphism (rs2433320) both in patients with schizophrenia and in control subjects.

There were no significant deviations in all six SNPs from Hardy-Weinberg equilibrium in either patients or control subjects. Allele and genotype frequencies of the six SNPs are shown in Table 3. There were no associations between these SNPs and schizophrenia neither in the allelic frequency nor in the genotypic distributions. Although both rs2433320-rs2443327 and rs12641023-rs14082 were in a tight LD ($D' = 0.936$, 0.968, each), permutation test showed no significant difference in estimated frequencies of these haplotypes between the controls and patients (global permutation $P = 0.58$, 0.45, each, Table 4). Haplotypes of six SNPs were evaluated, but no significant difference was observed in frequencies of any estimated haplotype or in distributions of all estimated haplotypes between the controls and patients (global permutation $P = 0.48$).

The present study is the first report on the PDLIM5 gene expression in the peripheral leukocytes in schizophrenia. The mean PDLIM5 mRNA levels in the peripheral leukocytes from medication-free schizophrenia patients were significantly higher than those of age- and sex-matched controls. Altered mRNA expression in the peripheral lymphocytes could reflect the altered metabolism of brain cells [4]. Our result is consistent with the result of higher expression in the postmortem brains from schizophrenic patient but not with the result of lower expression in the lymphoblastoid cells derived from schizophrenic patients [10,11]. The differences of the mRNA expression between studies may be partly attributed to the difference in the materials. When using lymphoblastoid cells, the effect of virus infection or chromosomal alterations during culture must be taken into account [12]. On the other hand, the mRNA expression level of PDLIM5 gene was not significantly higher in chronically treated schizophrenics compared with that of controls. This finding in the chronic patients may be a consequence of pharmacological effects of antipsychotics or clinical improvement. This result suggests that expression of PDLIM5 mRNA may not be trait-oriented but state-related change. To confirm whether the expression of this gene is a state marker, a follow-up investigation is needed in the same patients before and after treatment.

The pathophysiological mechanism remains unknown, but we speculate that the higher expression of PDLIM5 is related with putatively elevated Ca^{2+} signaling in schizophrenia. It has been suggested that abnormalities in Ca^{2+} signaling was associated with molecular etiology of schizophrenia. Regulator of G protein signaling-4 (RGS4) and B-cell limphoma/leukaemia-2 gene (Bcl-2) which reduce free Ca^{2+} in a cell have been found to be down regulated in the temporal cortex of schizophrenic patients [14,18]. It was reported that there was high levels of free intracellular Ca^{2+} in platelets of schizophrenic patients

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Table 3
Genetic studies of PDLIM5 gene with schizophrenia in case-control samples

Group	Genotype			*n*	Hardy-Weinberg equilibrium	*P*-value	Allele		*P*-value
ra1 0008257	A/A	A/G	G/G				A	G	
sch	42	127	105	274	0.823	0.471	211	337	0.804
cont	34	140	102	276	0.229		208	344	
rs2433320									
sch	7	75	197	279	0.858	0.601	89	469	0.871
cont	11	70	198	279	0.205		92	466	
rs2433327	T/T	T/C	C/C				T	C	0.833
sch	169	88	16	273	0.414	0.917	426	120	
cont	164	92	15	271	0.788		420	122	
rs2452600	T/T	T/C	C/C				T	C	
sch	54	125	96	275	0.306	0.232	233	317	0.080
cont	68	130	81	279	0.325		266	292	
rsl2641023	A/A	A/G	G/G				A	G	
sch	51	126	93	270	0.555	0.497	228	312	0.295
cont	42	131	103	276	0.924		215	337	
rs14082	A/A	A/G	G/G				A	G	
sch	58	124	91	273	0.243	0.302	240	306	0.141
cont	45	125	103	273	0.582		215	331	

sch: Schizophrenia, cont: control subjects. *P*-values are calculated by Fisher s exact test.

[22]. PDLIM5 regulates intracellular calcium levels by linking calcium channel and protein kinase C [2,3,16]. The levels of PDLIM5 might be up-regulated both in the brain and in the peripheral leukocytes in patients with schizophrenia in response to increased intracellular calcium levels. It has been demonstrated that antipsychotic drugs block IP3-induced release of Ca^{2+} [23] and Ca^{2+} dependence of PKC is well known [5]. So antipsychotic medication might normalize the up-regulation of PDLIM5 expression in schizophrenia by reducing Ca^{2+} signaling.

PDLIM5 may be involved in other mental disorders. Iwamoto et al. reported that expression level of PDLIM5 was significantly and commonly increased in the postmortem brain tissues of patients with schizophrenia, major depression and bipolar disorder [11]. However, we have already shown that mean PDLIM5 mRNA level in the peripheral leukocytes of medication-free patients with major depression was significantly lower than in control subjects [8]. Therefore, the higher expression of this gene in the peripheral leukocytes of medication-free patients with schizophrenia may be disease-specific and not due to non-specific stress of psychiatric condition. Further investigations of other psychiatric diseases including bipolar disorder are needed.

Table 4
Linkage disequilibrium (LD) indices (lower left are r2, upper right are *D'*)

	rs10008257	rs2433320	rs2443327	rs2452600	rs12641023	rs14082
rs10008257		0.37227	0.44147	0.28294	0.12734	0.15919
rs2433320	0.01632		0.9364	0.50709	0.37209	0.40839
rs2443327	0.03427	0.57719		0.54423	0.43945	0.45693
rs2452600	0.0447	0.05573	0.09541		0.19114	0.18089
rs12641023	0.00626	0.04284	0.08854	0.02152		0.96845
rs14082	0.01002	0.05068	0.09508	0.01918	0.93062	

Horiuchi et al. reported that there were significant association between polymorphisms (rs2433320 and rs2433322) of PDLIM5 gene and schizophrenia. Their group also showed that the different alleles of the rs2433320 showed different DNA–protein complexes on electrophoreic mobility shift assay and GA heterozygotic genotype might have higher transcriptional activity in schizophrenia [6]. However, our result showed that there was not significant association between schizophrenia and six polymorphisms of PDLIM5 gene, including rs2433320, and this result is consistent with a previous study with a large number of subjects ($n = 562$) [15]. In addition, neither patients nor controls showed a significant difference of the PDLIM5 mRNA expression in the peripheral leukocytes between GG and GA genotypes of this SNP in our subjects although the typeII error was not denied.

In conclusion, our investigation revealed that the mean PDLIM5 mRNA levels in medication-free schizophrenic patients were significantly higher compared to those in controls and the chronic schizophrenic patients with antipsychotic treatment for many years showed almost the same expression levels as healthy control levels. There were no associations between schizophrenia and PDLIM5 gene. These results suggest that the higher expression levels of PDLIM5 mRNA in the leukocytes may be a candidate marker for medication-free schizophrenic patients. Further studies are necessary to confirm the present results.

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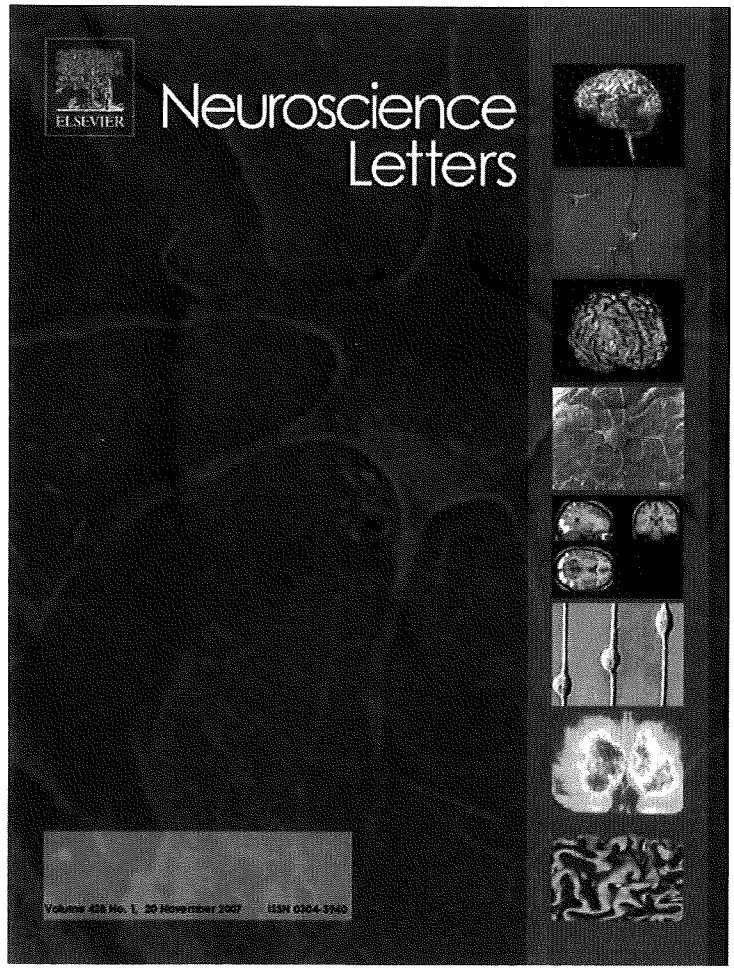

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A possible association between the Val158Met polymorphism of the catechol-*O*-methyl transferase gene and the personality trait of harm avoidance in Japanese healthy subjects

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Abstract

Catechol-*O*-methyltransferase (COMT) is an enzyme that degrades various biogenic amines, which have been hypothesized to be associated with personality traits. We investigated a possible relationship between the COMT Val158Met polymorphism and personality traits assessed by the Temperament and Character Inventory (TCI) in 139 healthy subjects in a Japanese population. The number of Met alleles of the COMT Val/Met genotype tended to relate to harm avoidance (HA) scores parametrically, while no significant difference was observed between genotype groups in either novelty seeking, reward dependence, persistence, self-directedness, cooperativeness or self-transcendence. These results suggest that the Val/Met polymorphism of the COMT gene may play a role in HA in Japanese population.

Keywords: Catechol-*O*-methyltransferase (COMT); Temperament and Character Inventory (TCI); Harm avoidance; Polymorphism

Genetic factors significantly contribute to the determination of human personality traits, although environmental influence is also important. Personality traits assessed by self-report questionnaires show moderate heritability [6]. Such inheritance is ultimately attributable to functional variants of the genes programming brain development and function [5]. Catechol-*O*-methyl transferase (COMT) is an enzyme involved in monoamine metabolism, and a common single nucleotide polymorphism (SNP) in the COMT gene, producing an amino acid substitution of methionine (Met) to valine (Val) at position 108/158 (Val158Met), affects dopamine regulation in the prefrontal cortex [19]. This polymorphism impacts on the stability of the enzyme, such that the Val allele is associated with significantly higher enzyme activity than the Met allele [4]. Several studies have revealed that the Val allele is associated with poorer performances, compared with the Met allele, in cognitive tasks of frontal function such as the Wisconsin Card Sorting Test (WCST) and N-back task [7,8]. The underlying mechanism of such behavioral differences may be related to lower prefrontal dopamine levels arising from the higher dopamine catabolism mediated by the Val allele [4,23]. Thus, it is likely that the Val/Met polymorphism of the COMT gene could be associated with a personality trait; however, the relationship between the Val/Met polymorphism of the COMT gene and the personality traits using the Temperament and Character Inventory (TCI) has not been studied in Japanese population [1,2,9,14–16,21,22]. In this study, we examined the relationship between the Val158Met of the COMT gene and the personality traits measured by TCI in Japanese healthy subjects.

One hundred and thirty-nine healthy subjects participated in the study. A Japanese version of TCI, a full version of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) [20,24]

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