Table 1 Subject characteristics

	Control Val/Val	Met carriers	Schizophrenia Val/Val	Met carriers	Diagnosis F (P)	Genotype F (P)*	Genotype by diagnosis F (P)
Number of subjects	38	38	19	28			
Gender (M/F)	16 out of 22	14 out of 24	11 out of 8	13 out of 15			
Handedness (R/L)	36 out of 2	35 out of 3	18 out of 1	28 out of 0			
Age (years)	41.47 (13.42)	39.26 (10.6)	45.98 (15.29)	43.05 (10.57)	3.633 (0.059)	1.7 (0.195)	0.21 (0.647)
Education (years)	17 (3.16)	16.06 (2.57)	12.67 (2.43)	13.33 (3.31)	30.855 (<0.0001)	0.047 (0.828)	1.61 (0.208)
Full scale IQ (WAIS-R)	113.42 (12.05)	108.93 (13.58)	80.69 (17.68)	88.958 (22.08)	57.9 (<0.001)	0.29 (0.59)	3.41 (0.068)
JART	78.8 (10.45)	75.42 (13.65)	54.69 (20.74)	62.25 (27.06)	23.366 (<0.001)	0.292 (0.59)	2.014 (0.159)
Wechsler Memory Scale—Revised							
Verbal memory	111.78 (15.001)	111.061 (12.89)	78.0 (21.623)	81.33 (18.57)	86.93 (<0.001)	0.147 (0.702)	0.354 (0.553)
Visual memory	112.1 (8.51)	106.55 (11.99)	74.78 (24.32)	83.29 (20.613)	85.51 (<0.001)	0.204 (0.65)	4.605 (0.03)
General memory	113.31 (13.92)	110.85 (12.22)	74.43 (21.3)	79.33 (19.14)	111.93 (<0.001)	0.135 (0.715)	1.226 (0.27)
Attention/concentration	104.47 (13.25)	102.94 (16.51)	87.79 (19.09)	92.54 (17.38)	16.08 (0.001)	0.228 (0.634)	0.866 (0.14)
Delayed recall	111.88 (15.46)	112.48 (10.08)	77.07 (20.92)	81.21 (19.19)	99.74 (<0.001)	0.52 (0.475)	0.284 (0.59)
WCST (preservative error)	2.5 (3.89)	3.14 (3.90)	12.08 (11.54)	8.52 (10.63)	24.5 (<0.0001)	0.93 (0.34)	1.93 (0.17)
Digit span	11.12 (3.25)	10.77 (3.34)	7.83 (3.93)	9.09 (2.74)	12.165 (0.0007)	0.415 (0.52)	1.28 (0.261)
Onset age			25.38 (10.34)	23.74 (7.992)		0.52	
Duration of illness (years)			19.86 (14.93)	18.84 (9.8)		0.77	
Duration of hospitalization (months)			66 (153.41)	59.59 (91.18)		0.86	
Duration of medication (years)			12.86 (14.21)	16.4 (9.89)		0.29	
Drug dose of typical antipyschotic drugs (mg/day, chlorpromazine equivalent)			617.9 (720.18)	700.38 (752.67)		0.69	
Drug dose of atypical antipyschotic drugs (mg/day, chlorpromazine equivalent)			282.3 (428.29)	340.23 (482.19)		0.66	

Mean (standard deviation); WAIS-R = Wechsler Adult Intelligence Scale—Revised; JART = Japanese version of National Adult Reading Test; WCST = Wisconsin Card Sorting Test.

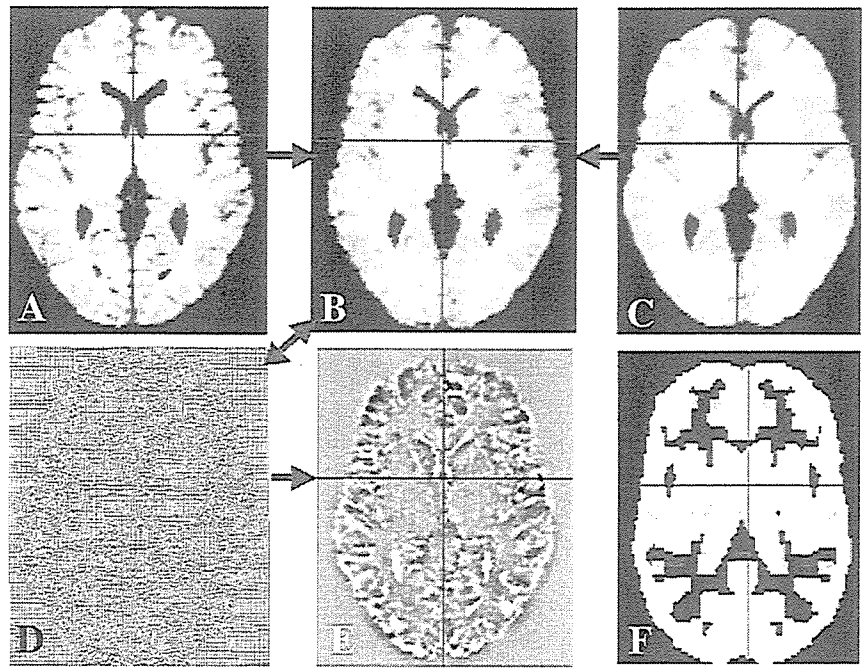

Fig. 1 Steps of analysis for tensor-based morphometry. An example is shown for a single subject in one axial slice. The single object brain (**A**) has been corrected for orientation and overall size to the template brain (**C**). Non-linear spatial normalization removes most of the anatomical differences between the two brains by introducing local deformations to the object brain, which then (**B**) looks as similar as possible to the template. Image (**D**) shows the deformations applied to the object brain by a deformed grid. Statistical analysis can be done univariate using the local Jacobian determinant as a derivative of the field (**E**). An explicit mask image (**F**) was used to explore morphology in the grey matter and CSF space.

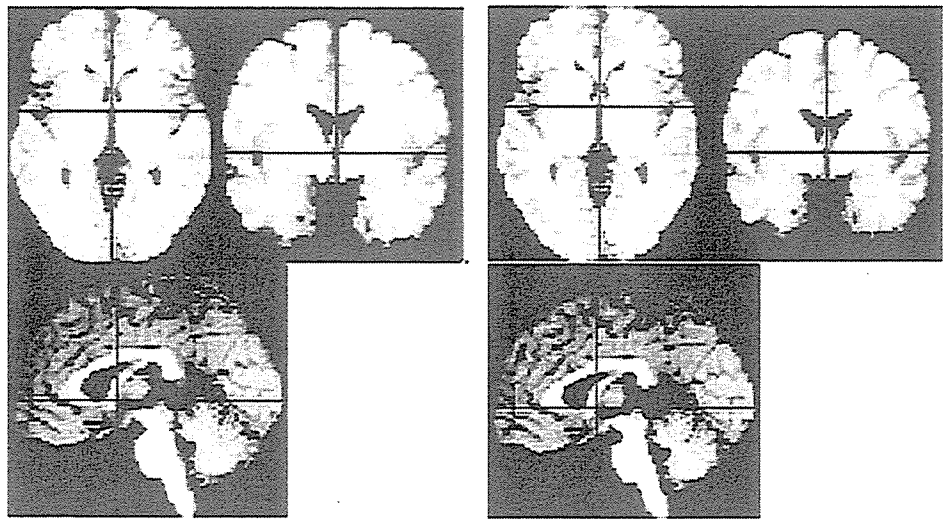

Fig. 2 Mean images after high dimensional warping control subjects and schizophrenics. *Left*: The mean image of warped MR images obtained from 76 controls. Even after averaging, the mean image is not blurred. *Right*: The mean image of warped MR images obtained from 47 schizophrenics. The mean image of schizophrenic looks similar to that of controls.

Results

Behavioural data

Patients had a lower full scale IQ, measured by the Wechsler Adult Intelligence Scale—Revised, than controls. They also had a lower expected premorbid IQ measured by a JART, lower scores of Wechsler Memory Scale—Revised and demonstrated poorer performance of working memory measures such as the number of preservative errors in the WCST and digit span (Table 1). No genotype or genotype-diagnosis interaction effects were found in working memory measures

Table 2 Results of image analyses

Anatomical regions	Brodmann area	Cluster size	Corrected P FDR	*T*-value (voxel level)	Talairach coordinates		
					x	*y*	*z*
Main effects							
Diagnosis effects (control > schizophrenia) (Fig. 3)							
Limbic system							
R insula	BA13	4682	0.000	6.41	33	11	−2
L insula	BA13	4017	0.000	8.81	−33	11	4
R parahippocampal gyrus, amygdala-uncus	BA36	4682	0.000	7.32	30	1	−17
R parahippocampal gyrus	BA36	186	0.000	5.04	30	−41	−8
L parahippocampal gyrus, hippocampus-amygdala	BA34/36	637	0.000	5.46	−20	−41	−8
R anterior cingulate cortex	BA32	147	0.000	4.9	9	33	20
L anterior cingulate cortex	BA32	200	0.000	4.63	−11	32	20
L cingulate gyrus	BA32	275	0.001	4.2	−12	−16	39
Prefrontal cortex							
R inferior frontal gyrus	BA47,11	145	0.000	4.99	27	28	−11
R superior frontal gyrus	BA8/9	1889	0.000	6.08	12	43	39
L medial frontal gyrus	BA9	1333	0.000	5.13	−8	47	19
L inferior frontal gyrus	BA45	141	0.000	4.55	−44	23	15
L middle frontal gyrus	BA8	482	0.000	4.44	−30	24	43
L superior frontal gyrus	BA8	482	0.000	4.39	−35	17	51
Premotor area							
R dorsal premotor area	BA6	429	0.000	4.37	41	13	45
Temporal cortex							
R superior temporal gyrus	BA22	806	0.000	5.04	47	−23	−1
R middle temporal gyrus	BA21	806	0.000	4.87	56	−15	−3
L superior temporal gyrus	BA38	4017	0.000	7	−36	1	−17
Central grey matter							
L thalamus		4017	0.000	7.26	−15	−17	2
Diagnosis effects (control < schizophrenia) (Fig. 4)							
L sylvian fissure		621	0.000	6.7	−45	17	−3
R sylvian fissure		774	0.000	6.59	44	17	−8
Lateral ventricle (anterior horn)		279	0.000	5.27	−5	21	4
Lateral ventricle (L inferior horn)		248	0.000	6.18	−41	−30	−10
Lateral ventricle (R inferior horn)		137	0.000	5.02	36	−40	−1
Interhemisphrenic fissure		154	0.000	5.28	3	55	−12
Genotype effects (Val/Val-COMT < Met-COMT carriers) (Fig. 5)							
Limbic system							
L anterior cingulate cortex	BA24/25	334	0.033	4.29	−8	17	−13
Temporal cortex							
R middle temporal gyrus	BA21	285	0.016	5.10	59	−3	−14
Genotype-diagnosis interaction effects (Fig. 6)							
Limbic system							
L anterior cingulate gyrus	BA24/25/32	264	0.044	3.77	−6	25	−6
L parahippocampal gyrus, amygdala-uncus	BA34	219	0.048	3.74	−24	−6	−14
The effects of polymorphism in control group (no significant difference)							
The effects of polymorphism in schizophrenia							
Val/Val-COMT < Val/Met, Met/Met-COMT (Fig. 7)							
Limbic system							
L parahippocampal gyrus, amygdala-uncus	BA28	81	0.010	4.17	−26	2	−22
L anterior cingulate cortex	BA24/25/32	263	0.007	4.38	−7	20	−8
Central grey matter							
L thalamus		91	0.014	3.94	−21	−28	6

and IQ, however, a significant genotype-by-diagnosis interaction effect was found in a visual memory measure (F = 4.605, df = 1, P = 0.03) (Table 1). However, a *post hoc t*-test (Bonferroni test) demonstrated no genotype effect in each diagnostic category (control: P = 0.15, schizophrenia: P = 0.11).

Morphological changes in schizophrenia (diagnosis effects)

In comparison with controls, patients with schizophrenia demonstrated a significant reduction of volumes in multiple brain areas, such as the limbic and paralimbic systems, neocortical areas and the subcortical regions (Table 2 and Fig. 3).

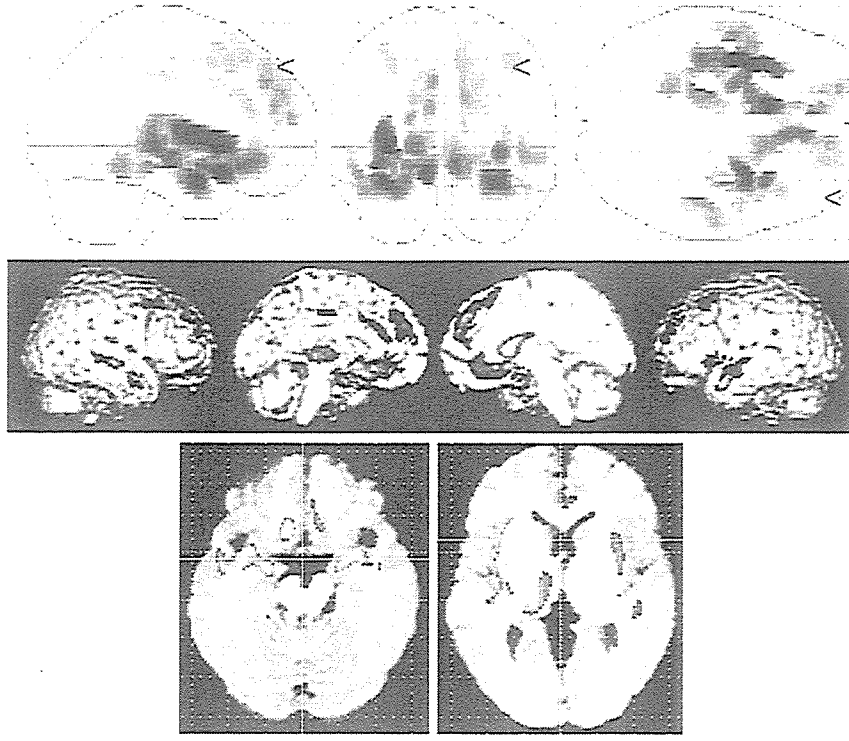

Fig. 3 Decreased volumes in schizophrenics ($n = 47$) as compared to controls ($n = 76$). *Top*: The SPM {*t*} is displayed in a standard format as a maximum-intensity projection (MIP) viewed from the right, the back and the top of the brain. The anatomical space corresponds to the atlas of Talairach and Tournoux. Representation in stereotaxic space of regions with significant reduction of volume in schizophrenia was demonstrated. Schizophrenics demonstrated a significant reduction of volumes in the multiple brain areas, such as the limbic and paralimbic systems, neocortical areas and the subcortical regions. *Middle*: The SPM {*t*} is rendered onto T_1-weighted MR images. *Bottom*: The SPM {*t*} is displayed onto axial T_1-weighted MR images. A significantly decreased volume of the amygdala-uncus, bilateral insular cortices, ACC, temporal cortex and the left thalamus in schizophrenics was noted.

In the limbic and paralimbic systems, patients with schizophrenia showed reduction of volumes in the parahippocampal gyri, amygdala-uncus, insular cortices and the anterior cingulate cortices (ACC). They also demonstrated reduced volumes in the frontal and temporal association areas, dorsal premotor areas and the left thalamus. In comparison with controls, patients with schizophrenia showed significantly increased volume in the CSF space such as lateral ventricle, sylvian and the interhemispheric fissures but not in the grey matter (Table 2 and Fig. 4).

Morphological changes associated with the Val158Met polymorphism (genotype effects)

In comparison with Met-COMT carriers, individuals homozygous for the Val-COMT allele demonstrated a significant reduction of volumes in the left ACC and the right middle temporal gyrus (MTG) (Table 2 and Fig. 5). The hypothesis-driven analysis demonstrated a genotype effect on volumes in the bilateral DLPFC (right BA9, left BA8) at a lenient threshold (uncorrected $P = 0.05$) (data are not shown), however, no voxels could survive after the correction for multiple comparisons (FDR < 0.05) within the ROI. There were no areas that individuals homozygous for the Val-COMT allele demonstrated a significant increment of volume compared to Met-COMT carriers.

Genotype—diagnosis interaction effects

We found significant genotype-diagnosis interaction effects on brain morphology. The stronger effects of Val158Met polymorphism on brain morphology in schizophrenia than those in controls were noted in the left ACC and the left amygdala-uncus (Table 2 and Fig. 6). The hypothesis-driven analysis demonstrated a genotype-diagnosis interaction effect on the volume of the right DLPFC (BA9/46) at a lenient threshold (uncorrected $P = 0.05$) (data not shown), however, no voxels could survive after the correction of multiple comparisons (FDR < 0.05) within the ROI.

Effects of the Val58Met polymorphism on brain morphology

Since genotype–disease interaction effects were found, we estimated the effects of genotypes on brain morphology in the control groups and the schizophrenic groups separately.

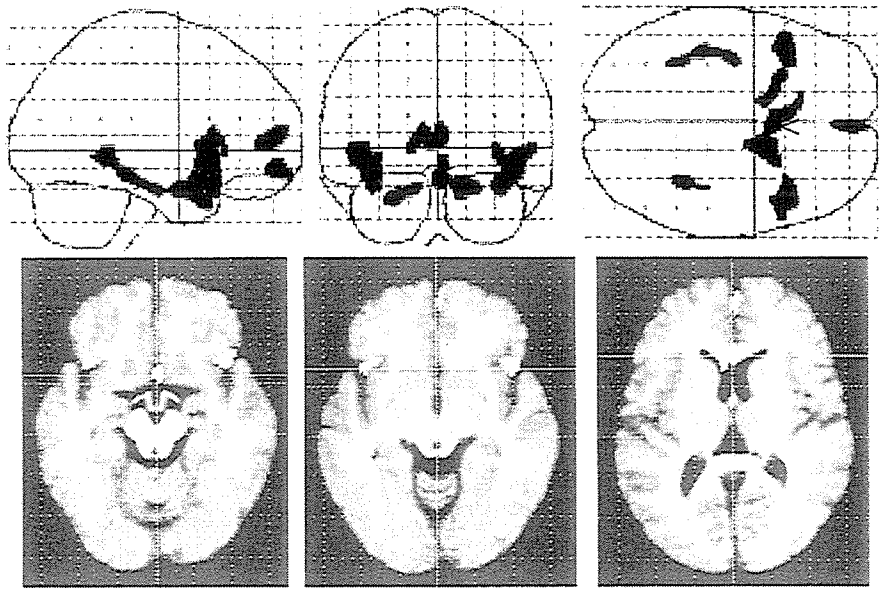

Fig. 4 Increased volumes in schizophrenics as compared to controls. *Top*: The SPM {*t*} is displayed in a standard format as a MIP. Patients with schizophrenia showed a significantly increased volume of the CSF space. *Bottom*: The SPM {*t*} is displayed onto axial T_1-weighted MR images. A significantly increased volume of the CSF space such as the lateral ventricle, sylvian fissures and the interhemisphrenic fissure was noted.

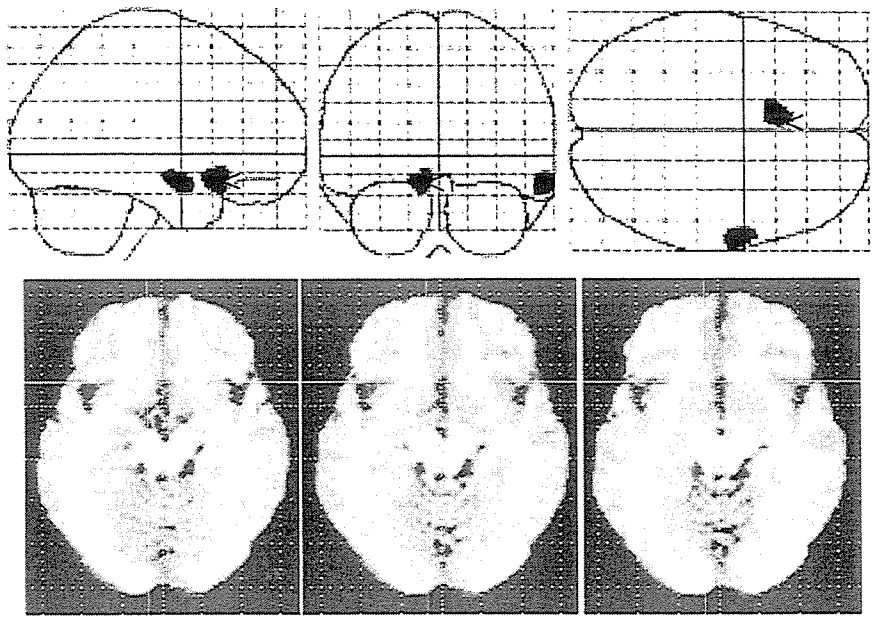

Fig. 5 The result of comparison between individuals homozygous for the Val-COMT allele (n = 57) and Met-COMT carriers (n = 66) (genotype effects). *Top*: Representation in stereotaxic space of regions with significant reduction of volume in individuals homozygous for the Val-COMT allele demonstrated. *Bottom*: The SPM {*t*} is displayed onto axial T_1-weighted MR images. Individuals homozygous for the Val-COMT allele demonstrated a significant reduction of volumes in the left ACC and right MTG as compared to Met-COMT carriers.

In the control group, we found no significant morphological differences between individuals homozygous for the Val-COMT allele and Met-COMT carriers. Even the hypothesis driven analysis with a lenient statistical threshold ($P < 0.05$) could not detect any significant morphological changes in the DLPFC between the two groups. Contrary to the control group, schizophrenics homozygous for the Val-COMT allele showed a significant reduction of volumes in the left amygdala-uncus, bilateral ACC, right MTG and the left thalamus when compared to the patients carrying the Met-COMT

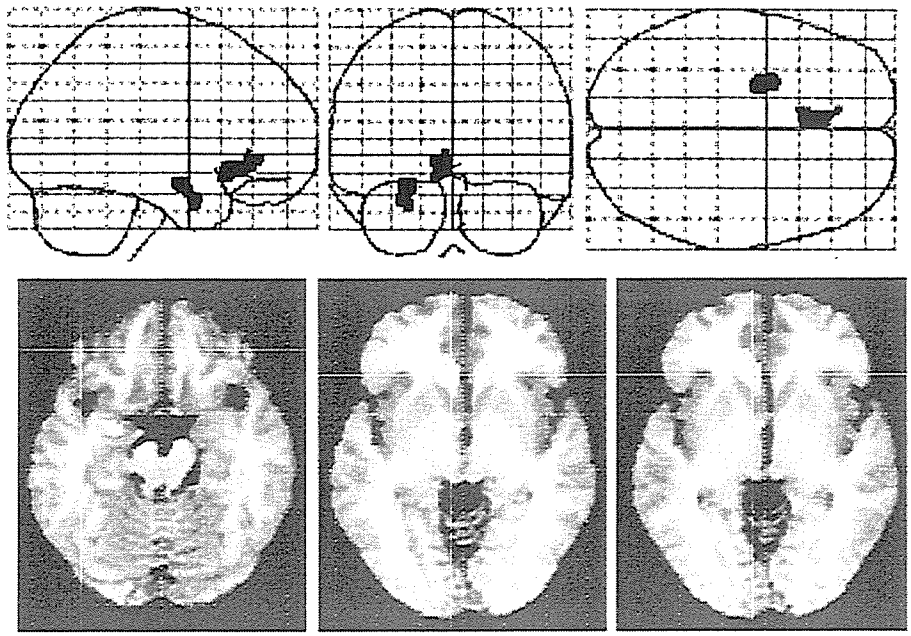

Fig. 6 Results of genotype-diagnosis interaction effects on brain morphology. *Top*: The SPM {*t*} is displayed in a standard format as a MIP. The stronger effects of Val158Met polymorphism on brain morphology in schizophrenia than those in controls were noted in the left ACC, left parahippocampal gyrus and the amygdala-uncus. *Bottom*: The SPM {*t*} is displayed onto axial T_1-weighted MR images.

allele (Table 2, Fig. 7). The hypothesis-driven analysis demonstrated a significantly decreased volume of the bilateral DLPFC in schizophrenics homozygous for the Val-COMT allele when compared to the Met-COMT schizophrenics at a lenient threshold (uncorrected $P = 0.05$) (data not shown). However, no voxels could survive after the correction for multiple comparisons (FDR < 0.05) within the ROI. There are no significantly increased volumes in the schizophrenics homozygous for the Val-COMT allele. All the results were essentially unchanged even if all the left-handed subjects were excluded in all analyses (data not shown).

Discussion

In this study, we found reduction of volumes in the limbic and paralimbic systems, neocortical areas (prefrontal and temporal cortices) and thalamus in patients with schizophrenia when compared to control subjects. The schizophrenia patients demonstrated a significant enlargement of CSF spaces including the lateral and sylvian fissure, which could be interpreted as a result of impaired neurodevelopment and/or global brain atrophy. These findings are concordant with previous studies of MR morphometry of schizophrenia. According to a recent review and meta-analyses of the morphometry of schizophrenia, the consistent abnormalities in schizophrenia are as follows; (i) ventricular enlargement (lateral and third ventricles); (ii) medial temporal lobe involvement; (iii) superior temporal gyrus involvement (iv) parietal lobe involvement; and (v) subcortical brain region involvement including the thalamus (Okubo *et al.*, 2001; Shenton *et al.*, 2001; Davidson and Heinrichs, 2003). The other regions observed in this study, such as the insula, DLPFC and the ACC have also often been demonstrated as abnormal areas in schizophrenia (Shenton *et al.*, 2001; Takahashi *et al.*, 2004; Yamasue *et al.*, 2004). Using the TBM technique, we replicated the morphological abnormalities observed in previous MR studies on schizophrenia, suggesting that TBM was able to detect morphological changes associated with this disease. As well as neuroimaging studies, post-mortem studies have also reported morphological abnormalities in schizophrenia, but not necessarily as common neuropathological features. Regions including the hippocampus, ACC, thalamus and the DLPFC are regularly associated with abnormalities of cell size, cell number and neuronal organization (Bogerts, 1993; Arnold and Trojanowski, 1996; Selemon, 2001; Selemon and Lynn, 2002, 2003). Selemon *et al.* reported that schizophrenics demonstrated abnormalities in overall and laminar neuronal density in the DLPFC (Brodmann area 9) and suggested that the DLPFC should be a particularly vulnerable target in the disease process (Selemon 2001; Selemon and Lynn, 2002, 2003).

Importantly, our results suggest that some of the morphological changes in schizophrenia mentioned above are associated with the Val158Met polymorphism of the COMT gene. In the schizophrenic group, the polymorphism was associated with the volumes in the limbic and paralimbic systems, temporal cortices and the left thalamus, whereas no morphological changes related to the polymorphism were found in

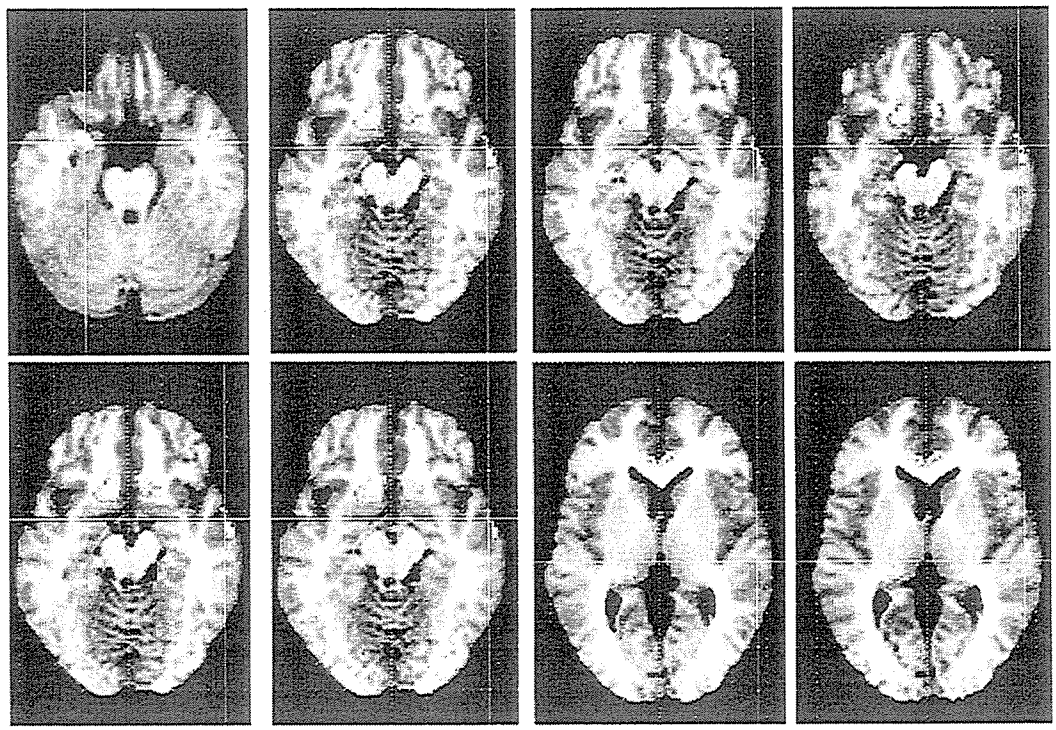

Fig. 7 The effects of the Val158Met polymorphism of the COMT gene on brain morphology in schizophrenics. The SPM {*t*} is displayed onto axial T_1-weighted MR images. The schizophrenics homozygous for the Val-COMT allele (n = 19) showed a significant reduction of volumes in the left parahippocampal gyrus, amygdala-uncus, ACC, left thalamus and the right MTG when compared to patients who carried the Met-COMT allele (n = 28).

normal individuals. As a consequence, significant genotype-diagnosis interaction effects were found in the left ACC and the amygdala-uncus. These results indicate that the Val158-Met polymorphism of the COMT gene is strongly associated with morphological changes in schizophrenia, particularly those in the limbic and paralimbic systems. Longitudinal MRI studies of schizophrenia strongly suggest that progressive changes should occur after onset of the illness (Okubo *et al.*, 2001; Ho *et al.*, 2003). Recent studies have demonstrated that antipsychotic drugs, particularly haloperidol, have considerable effects on brain morphology (Arango *et al.*, 2003; Lieberman, 2005; Dorph *et al.*, 2005). Because of the long duration of illness and medication taken by our subjects, the effects of antipsychotics may be a possible confounding factor for our findings. However, the duration of medication and the dose of antipsychotics taken by the Val/Val-COMT schizophrenics did not differ from those of the Met-COMT schizophrenics. Although the effects of antipsychotics on brain morphology may contribute to the observed morphological changes in patients with schizophrenia in this study, it is unlikely that the effects of antipsychotics contributed to morphological differences between the two schizophrenic groups.

When we were preparing this manuscript, another study demonstrated no genotype and genotype-diagnosis interaction effects of the Val158Met polymorphism on morphology of the frontal lobe in controls and schizophrenia (Ho *et al.*, 2005). Although there are differences between the two studies, such as mean ages of subjects, duration of illness, methods for image analysis and a racial factor (Caucasians versus Japanese), that study also demonstrated no genotype and genotype-diagnosis interaction effects on morphology of the DLPFC. However, we found these effects on DLPFC morphology at a very lenient statistical threshold. Further studies with a larger sample will clarify whether Val158Met polymorphism does affect DLPFC morphology. As well as prefrontal morphology, we found no significant genotype or genotype-diagnosis interaction effects on working memory, however, schizophrenics homozygous for the Val-COMT allele tended to have poorer performances on working memory measures, compared to Met-COMT carriers with schizophrenia. Although there were no significant effects of Val158Met polymorphism on working memory and other neuropsychological measures, a significant effect of the polymorphism was noted in brain morphology. The brain morphology has been considered to be useful as an intermediate phenotype in genetic research in neuropsychiatric disorders (Baare *et al.*, 2001; Durston *et al.*, 2005). Therefore, morphological changes might be more sensitive to the effects of genotype than behavioural measures such as the performance of working memory measures. In a previous study (Ho *et al.*, 2005) a similar phenomenon—no significant effect of Val158Met polymorphism on working memory performance but significant

effects on brain activities during a working memory task—was found. Further studies with a larger sample size are needed to clarify whether morphological changes are a more sensitive marker of genotype effects than behavioural measures.

Unexpectedly, we found effects of the polymorphism on the ACC volume rather than the DLPFC which is crucial for working memory. Since the ACC is associated with a variety of cognitive tasks involving mental efforts, and also plays important roles in working memory (Paus *et al.*, 2001; Kondo *et al.*, 2004), it is feasible that the Val158Met polymorphism may be associated with the ACC morphology. In fact, a previous study demonstrated that the Val-COMT allele was associated with abnormal ACC function as well as abnormal prefrontal cortical function, relative to the Met-COMT allele, as measured by cognitive tests and fMRI activation in normal subjects (Egan *et al.*, 2001).

One would argue that the effects of one polymorphism of the gene could not explain the morphological changes in schizophrenia. As well as the effects of the Val158Met polymorphism, we agree that other polymorphisms of schizophrenia susceptibility genes and genotype–genotype interaction may relate to individual brain morphology. Such interactions might contribute to the different effects of the Val158Met polymorphism on brain morphology observed in this study. Further studies of each effect and interaction of several schizophrenia susceptibility genes on brain morphology, brain functions and performances of neuropsychological tests should be conducted to clarify how polymorphisms of these genes affect intermediate phenotypes of schizophrenia.

In conclusion, we found an association between the Val158Met polymorphism and morphological abnormalities in schizophrenia. Although the underlying mechanisms of our observation remain to be clarified, our data indicate that brain morphology as an intermediate phenotype should be useful for investigating how genotypes affect endophenotypes of schizophrenia.

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BRIEF REPORT

A possible association between the −116C/G single nucleotide polymorphism of the *XBP1* gene and lithium prophylaxis in bipolar disorder

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Abstract

Bipolar disorder (BPD) is a severe, chronic, and life-threatening illness, and its pathogenesis remains unclear. Recently, a functional polymorphism (−116C/G) of the X-box binding protein 1 (*XBP1*) gene was reported to be a genetic risk factor for BPD. Moreover, the endoplasmic reticulum stress responses were impaired in cultured lymphocytes from BPD patients with the −116G allele and only valproate rescued such impairment among three major mood stabilizers. In this context, we hypothesized that BPD patients with different genotypes respond differently to mood stabilizers. We investigated the association between the −116C/G polymorphism of the *XBP1* gene and lithium response in Japanese patients with BPD. We found that lithium treatment is more effective among BPD patients with the −116C allele carrier than in patients homozygous for the −116G allele. The association between the −116C/G polymorphism and clinical efficacy of mood stabilizers should be further investigated in a prospective study with a larger sample.

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Key words: Bipolar disorder, lithium, SNP (single nucleotide polymorphism), *XBP1*.

Introduction

Bipolar disorder (BPD) is a severe, chronic, and life-threatening illness characterized by recurrent episodes of mania and depression. Despite extensive research, its pathogenesis is still unclear. Lithium is listed as a first-line agent for the treatment of BPD by American Psychiatric Association guidelines (APA, 2002). However, a significant percentage of patients with BPD show partial or no response to lithium treatment (Abou-Saleh, 1987). Psychopathological and biological markers that predict lithium response in BPD are not yet elucidated. Therefore, many researchers explored psychopathological and biological markers for lithium response in BPD, and several genetic markers are considered to be good candidates for lithium response (for reviews, see Gelenberg and Pies, 2003; Ikeda and Kato, 2003).

Recently, a functional polymorphism (−116C/G) of the X-box binding protein 1 (*XBP1*) gene that plays a pivotal role in endoplasmic reticulum (ER) stress response was shown to confer susceptibility to BPD (Kakiuchi et al., 2003). The single nucleotide polymorphism (SNP) in the promoter region of the *XBP1* gene was significantly more common in Japanese patients with BPD [odds ratio (OR) 4.6] and over-transmitted to affected offspring in trio samples of the NIMH Bipolar Disorder Genetic Initiative. The *XBP1*-dependent transcription activity of the −116G allele was lower than that of the −116C allele, and induction of *XBP1* expression after ER stress was markedly reduced in the cell with the G allele. Moreover, valproate rescued the impaired response of the cell with the G allele by inducing *ATF6*, the gene upstream of *XBP1*, although lithium and carbamazepine did not. Based on the observations, we hypothesized that BPD

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patients with different genotypes respond differently to treatment with mood stabilizers such as lithium and valproate.

The aim of the study was to examine the possible association between lithium response and the XBP1 −116C/G polymorphism in patients with BPD.

Methods

Subjects

A total of 66 patients with BPD (20 BP I disorders and 46 BP II disorders) were recruited at Hokkaido University Hospital. They were composed of 38 males and 28 females with a mean age of 50.6 yr (S.D. = 11.9 yr) and a mean age at onset of 34.4 yr (S.D. = 11.4 yr). All subjects were biologically unrelated Japanese. Consensus diagnosis was made for each patient by at least two psychiatrists according to DSM-IV criteria (APA, 1994). The presence of concomitant diagnoses of mental retardation, drug dependence, or other Axis I disorder, together with somatic or neurological illnesses that impaired psychiatric evaluation, represented exclusion criteria. Patients had been treated with lithium carbonate and its serum concentration was maintained between 0.4–1.2 mequiv/l at least for 1 yr. Treatment response to lithium was determined for each patient from all available information including clinical interview and medical records, by at least two psychiatrists according to criteria described by Kato et al. (2000). Briefly, lithium responders were defined as those patients who had less frequent and/or severe relapse, including no relapse, during lithium treatment than prior to lithium treatment. Among 66 patients, 43 patients were determined as responders and 23 patients as non-responders. In the 23 non-responders, 15 patients had been treated with valproate at least for 1 yr. We secondarily evaluated the treatment response to valproate using the same criteria as for response to lithium. After complete description of the study, written informed consent was obtained from every subject. The study protocol was approved by the ethics committees of Hokkaido University Graduate School of Medicine and the National Center of Neurology and Psychiatry.

Genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to the standard procedures. Genotypes for the −116C/G SNP were determined using the TaqMan 5′-exonuclease allelic discrimination assay, described previously (Hashimoto et al., 2004). Briefly, probes and primers for detection of the polymorphism were: forward primer 5′-CTGTCACTCCGGATGGAAATA-AGTC-3′, reverse primer 5′-ATCCCTGGCCAAAGG-TACTTG-3′, probe 1 5′-VIC-CTCCCGCACGTAAC-MGB-3′, and probe 2 5′-FAM-TCCCGCAGGTAAC-MGB-3′. PCR cycling conditions were: 95 °C for 10 min, 45 cycles of 92 °C for 15 s and 60 °C for 1 min.

Statistical analysis

Difference in clinical features between responders and non-responders to lithium treatment was analysed using the χ^2 tests for categorical variables and the t tests for continuous variables. The presence of Hardy–Weinberg equilibrium was examined by using the χ^2 test for goodness of fit. Genotype and allele distributions between responders and non-responders to lithium treatment were analysed by the χ^2 test for independence. Association between genotype and serum lithium levels was analysed by analysis of variance (ANOVA). All p values reported are two-tailed. Statistical significance was defined at $p < 0.05$.

Results

The clinical characteristics of patients with BPD are shown in Table 1. Significant differences were not found in clinical features between patients who were defined as responders and non-responders to lithium treatment. Allele frequencies and genotype distributions of the −116C/G polymorphism of the *XBP1* gene among responders and non-responders to lithium treatment are shown in Table 2. The genotype distributions for the total patients, responders, and non-responders were both in Hardy–Weinberg equilibrium (total patients: $\chi^2 = 1.19$, d.f. = 1, $p = 0.28$; responders: $\chi^2 = 1.8$, d.f. = 1, $p = 0.18$; non-responders: $\chi^2 = 0.13$, d.f. = 1, $p = 0.72$). Serum lithium levels in responders did not differ among *XBP1* genotypes [C/C 0.64 (S.D. = 0.10) mequiv/l; C/G 0.66 (S.D. = 0.24) mequiv/l; G/G 0.53 (S.D. = 0.18) mequiv/l; $F = 1.83$, $p = 0.17$, ANOVA]. On the other hand, there was a trend towards increased serum lithium levels in non-responders homozygous for the −116G allele [C/C 0.48 mequiv/l ($n = 1$); C/G 0.53 (S.D. = 0.16) mequiv/l; G/G 0.69 (S.D. = 0.19) mequiv/l], but it did not reach statistical significance ($t = 2.0$, d.f. = 20, $p = 0.059$, t test comparing patients with C/G and G/G).

There was a trend towards an increased frequency of the −116C allele in the responders rather than non-responders ($\chi^2 = 3.72$, d.f. = 1, $p = 0.054$; OR 2.18, 95% CI 0.98–4.87). Subsequent Mantel–Haenszel tests showed a differential genotype distributions between

Table 1. Background and clinical characteristics of bipolar (BP) patients

	Lithium-treated patient			
	Total (66)	Responder (43)	Non-responder (23)	Responders vs. non-responders
Sex				
Males	38 (57.6%)	28 (65.1%)	10 (43.5%)	$\chi^2=2.87$, d.f.=1,
Females	28 (42.4%)	15 (34.9%)	13 (56.5%)	$p=0.09$
Diagnosis				
BP I	20 (30.3%)	14 (32.6%)	6 (26.1%)	$\chi^2=0.30$, d.f.=1,
BP II	46 (69.7%)	29 (67.4%)	17 (73.9%)	$p=0.59$
Psychotic features				
Present	7 (10.6%)	6 (14.6%)	1 (4.3%)	$\chi^2=1.46$, d.f.=1,
Absent	59 (89.4%)	37 (85.4%)	22 (95.7%)	$p=0.23$
History of rapid cycling				
Present	10 (15.2%)	4 (9.3%)	6 (26.1%)	$\chi^2=3.28$, d.f.=1,
Absent	56 (84.8%)	39 (90.7%)	17 (73.9%)	$p=0.07$
Medication				
Lithium monotherapy	14 (21.2%)	11 (25.6%)	3 (13.0%)	$\chi^2=1.41$, d.f.=1,
Presence of co-administration[a]	52 (78.8%)	32 (74.4%)	20 (87.0%)	$p=0.24$
				t test
Age (yr)[b]	50.6±11.9	51.1±11.3	49.7±13.1	$t=0.44$, d.f.=64, $p=0.66$
Age at onset (yr)[b]	34.4±11.4	34.0±11.7	35.1±11.0	$t=0.38$, d.f.=64, $p=0.70$
Serum lithium concentration[b] (mequiv/l)	0.62±0.20	0.62±0.21	0.62±0.19	$t=0.03$ d.f.=64, $p=0.98$

[a] Aditional administration of valproate, carbamazepine, antidepressants, antipsychotics are included.
[b] Continuous variables are shown as mean±S.D.

responders and non-responders ($\chi^2=4.30$, d.f.=1, $p=0.038$). Thus, we examined the C allele carriers and non-carriers separately, and found that the C allele carriers were significantly more common in the responder group than the non-carriers ($\chi^2=4.34$, d.f.=1, $p=0.037$; OR 3.00, 95% CI 1.05–8.58).

The genotype distributions among responders and non-responders to valproate treatment are shown in Table 3. There was no association between the −116C/G polymorphism of the *XBP1* gene and response to valproate ($\chi^2=1.25$, d.f.=2, $p=0.54$).

Discussion

We investigated the possible association between the *XBP1* gene and the response to lithium treatment in BPD for the first time. Our results suggest that lithium treatment is more effective in BPD patients with the −116C allele of the *XBP1* gene than in patients homozygous for the G allele.

Kakiuchi et al. (2003) proposed that impaired response against ER stress in BPD patients with the G allele might be one of the possible cellular and molecular pathophysiology of BPD. Among three representative mood stabilizers, only valproate rescued this impairment of ER stress response in cultured lymphocytes, although lithium or carbamazepine did not. These findings suggested that the effectiveness of lithium on BPD patients with the G allele might be weaker than those with the C allele. Our clinical observations were consistent with the proposed mechanisms. A possible explanation for the mechanisms of the better efficacy of lithium treatment in −116C carriers is that −116C carrier patients might have other cellular and molecular impairments, which lithium could influence in the nervous system, e.g. inhibition of glycogen synthase kinase-3, inositol monophosphatase and *N*-methyl-D-aspartate receptor activity, activation of the BDNF/Trk pathway, or enhancement of neurogenesis and neuronal progenitor

Table 2. Genotype and allele frequencies of the C −116G polymorphism of the X box-binding protein 1 (*XBP1*) gene and response for lithium treatment

Response for lithium treatment	Allele frequency		χ^2		Genotype distribution			MH		χ^2	
	C	G	*p* value	OR (95% CI)	C/C	C/G	G/G	*p* value	C/C, C/G	G/G	*p* value	OR (95% CI)
Responders (43)	35	51	0.054	2.18 (0.96–3.03)	5	25	13	0.038	30	13	0.037	3.00 (1.05–8.58)
	(40.7%)	(59.3%)			(11.6%)	(58.1%)	(30.2%)		(69.8%)	(30.2%)		
Non-responders (23)	11	35			1	9	13		10	13		
	(23.9%)	(76.1%)			(4.3%)	(39.1%)	(56.5%)		(43.5%)	(56.5%)		
Total patients (66)	46	86			6	34	26		40	26		
	(34.8%)	(65.2%)			(9.1%)	(51.5%)	(39.4%)		(60.6%)	(39.4%)		

OR, Odds ratio; CI, confidence interval; MH, Mantel–Haenszel.

Table 3. Genotype of the −116C/G polymorphism of the *XBP1* gene and response for valproate treatment in lithium non-responders

Response for valproate treatment	Genotype distribution			χ^2
	C/C	C/G	G/G	*p* value
Responders (7)	1 (14.3%)	2 (28.6%)	4 (57.1%)	0.53
Non-responders (8)	0 (0%)	3 (37.5%)	5 (62.5%)	
Total patients (15)	1 (6.7%)	5 (33.3%)	9 (60.0%)	

proliferation (Chen et al., 2000; Hallcher and Sherman, 1980; Hashimoto et al., 2002a,b; 2003; Klein and Melton, 1996). Recently, it has been reported that chronic lithium treatment increased 78-kDa glucose-regulated protein (GRP78), a molecular chaperone of the heat shock protein 70 family, and showed cyto-protective effects in rat PC12 cells (Hiroi et al., 2005). In this regard, one of therapeutic actions of lithium might be associated with reducing ER stress, including signal transduction by *XBP1*. Although there was no direct evidence suggesting that *XBP1* is involved in the pathway of action of lithium, the −116C allele of the *XBP1* gene may contribute to reduce ER stress more effectively by lithium treatment.

Considering the action of valproate in cells with the −116G allele, it is possible that BPD patients with the −116G allele respond to valproate treatment better than those with the −116C allele. Therefore, we investigated the association between valproate response and the −116C/G polymorphism in non-responders to lithium treatment using the same criteria as for lithium response. However, we did not find any association in our small sample. It has been reported that lithium is effective for classical mania, while valproate is effective for both classical and irritable mania (Swann et al., 2002). In this context, valproate is likely to have a wider treatment spectrum than lithium, which may explain our finding. To clarify the association between the −116C/G polymorphism and treatment response to valproate, an independent and larger sample should be investigated.

After Kakiuchi et al. (2003) showed that the −116G allele was a risk factor of BPD in a Japanese sample, there have been two negative studies investigating American and European samples (Cichon et al., 2004), and a Chinese sample (Hou et al., 2004). Among our sample, the allele frequency of the −116G allele in

patients (0.65) was closer to that in controls (0.64) than that in BPD patients (0.71) in Kakiuchi et al.'s report (2003), although both subjects were of the same ethnicity (Japanese). To conclude whether the −116C/G contributes to the genetic risk factor for BPD in the Japanese population, larger number of BPD patients of Japanese origin should be examined.

On the other hand, two positive association studies between the −116G allele and schizophrenia have been reported (Chen et al., 2004; Kakiuchi et al., 2004). It has been reported that schizophrenia and BPD share several susceptibility loci such as 22q12 where the *XBP1* gene is located (Badner and Gershon, 2002). Therefore, these studies concerning schizophrenia might help to identify a shared pathogenesis of these two mental disorders.

To our knowledge, this is the first report indicating that long-term lithium treatment was more effective in BPD patients with the −116C allele on the promoter region of the *XBP1* gene than in those without the −116C allele. The mechanism of lithium response in the C allele-carrier patients is still unknown, however, it may be related to other mechanisms than dysregulation of ER stress response caused by the −116G allele. The limitations of the current study are retrospective design and small sample size. The association between the −116C/G polymorphism and clinical efficacy of mood stabilizers should be further investigated in a prospective study with a larger sample.

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Statement of Interest

None.

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Gene expression and association analysis of LIM (PDLIM5) in major depression

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Abstract

LIM (PDLIM5) is a small protein that interacts with protein kinase C-epsilon and the N-type calcium channel alpha-1B subunit and modulates neuronal calcium signaling. Recently, the *LIM* mRNA expression in postmortem brains and immortalized lymphoblastoid cells from mood disorder patients was reported to be changed and seems to be involved in its pathophysiology. We hypothesized that the expression of the *LIM* mRNA in the native peripheral leukocytes may be a good candidate for the biological marker for mood disorders. Twenty patients with major depression and age- and sex-matched control subjects were included in this expression study. The *LIM* mRNA levels in the peripheral leukocytes from drug-naive depressive patients were significantly lower than those from control subjects and increased significantly after 4-week paroxetine treatments, to almost the same level as controls'. Hamilton depressive scores (HAM-D) were improved about 50% after 4-week treatment but neither paroxetine concentrations nor the changes of HAM-D scores showed significant correlation with the change of the mRNA levels. Then, we genotyped three single nucleotide polymorphic markers of *LIM* gene, which were reported to be associated with bipolar disorder in patients with major depression and control subjects ($n = 130$, each), but there were no associations between these SNPs and major depression. Our investigation indicates that the lower expression levels of *LIM* mRNA in the peripheral leukocytes are associated with the depressive state and that its recovery after treatment may be an adaptive change induced by the antidepressant.

Keywords: Major depression; *LIM (PDLIM5)*; Gene expression; Leukocytes; Association analysis; Paroxetine

Recent findings from molecular biology suggest that LIM (PDLIM5) may be involved in the pathophysiology of major depression. LIM is expressed at various region of brain such as hippocampus, thalamus, hypothalamus, cortex and amygdala and its cellular localization is identical to Synapsin I, which is known to be involved in neurotransmitter release [17]. LIM is known to interact specifically with N-type calcium channel alpha-1B subunit and protein kinase C-epsilon and is critical for rapid and efficient potentiation of the calcium channel activation by PKC in neurons [4]. Because an extensive clinical literature suggests a role for calcium homeostasis in the pathophysiology of major depression and the actions of antidepressants (reviewed in [10]), the abnormalities of calcium signaling cascade induced by the altered expression of LIM may be involved in the pathophysiology of major depression.

Clinical genetic studies also suggest that LIM may be a potential candidate for one of the etiological factors in major depression. It was reported that the expression level of *LIM* mRNA was significantly increased in the postmortem brain tissues of patients with bipolar disorder, schizophrenia and major depression, and was decreased in the immortalized lymphoblastoid cell lines of patients with bipolar disorder [13,14]. The association of single nucleotide polymorphisms in the upstream region of *LIM* gene with bipolar disorder was confirmed [15]. *LIM* gene

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is located at 4q22 [25] and there are several linkage studies between this region and mental disorders, bipolar disorder [5], schizophrenia [19] and major depression [3].

Recently, several studies including our own have shown altered mRNA expressions in the native peripheral leukocytes of patients with mental disorders [11,21]. We reported that the expression of serotonin transporter (*5HTT*) mRNA in leukocytes was higher in depressive patients compared with that of control subjects and normalized after antidepressant treatment [11]. Not only neurochemical transmitters, such as serotonin and noradrenaline, but also peptides and proteins including hormones, cytokines and even structural proteins in the whole body may play a part in the depressive states (reviews: [6,20]). Thus, the activity of the circulating blood leukocytes in depressive patients may reflect the brain dysfunction occurring in the depressive state. Since *LIM* mRNA is expressed both in the brain and in the peripheral leukocytes, we hypothesized that the expression level of *LIM* mRNA in the leukocytes would also be a good candidate marker of major depression like that of *5HTT* mRNA. Thus, we compared the *LIM* mRNA levels between major depressive patients and controls. Then, we conducted a genetic association analysis of *LIM* gene polymorphisms.

The subjects consisted of 20 patients with major depression (6 males, 14 females and mean age 41.4 ± 12.7) and 20 age- and sex-matched controls (6 males, 14 females and mean age 42.6 ± 12.8). Before study participation, all subjects signed an informed consent form approved by the Ethical Committee of The University of Tokushima Graduate School. All patients were diagnosed as Major Depressive Disorder according to DSM-IV [1] by at least two trained psychiatrists. All subjects underwent extensive medical, neurological, psychological and laboratory evaluations before participating in the study. The persons who had axis II disorders were removed from the study. The diagnosis and the eligibility of the patients were reconfirmed during follow-up periods. Seventeen patients were in the first and other three were in the recurrent depressive episode. All patients did not receive any antidepressants for the current episode before blood sampling. All patients were treated with paroxetine for 4 weeks. The dose of paroxetine was started with 10 or 20 mg for the first two weeks and gradually increased up to 40 mg based on judgment of the trained clinician. At baseline and 4 weeks, subjects were rated with Structured Interview Guide for the 17-item Hamilton Depression Rating Scale (SIGH-D 17, [27]; Japanese version, Y. Nakane, 2000) before blood collection. Peripheral blood was also collected from 20 sex- and age-matched volunteers who were in good physical health with a history of neither psychiatric nor serious somatic disease and were not taking any medication. Probands who had first-degree relatives with psychiatric disorders were excluded from the control subjects.

Samples were collected in the Tokushima University Hospital and the Ehime University Hospital. These included 130 patients with major depression (61 males, 69 females and mean age 46.1 ± 14.6) and 130 age–sex-matched control subjects (61 males, 69 females and mean age 45.8 ± 14.4). Patients were diagnosed with the consensus of at least two trained psychiatrists using DSM-IV [1]. Controls were selected by the same criteria as the expression study. All these subjects were Japanese, unrelated to each other, and living in the same area (Shikoku Island in Japan). All participants signed an informed consent form approved by the Ethical Committee of The University of Tokushima Graduate School or Ehime University.

The paroxetine quantification was performed using high performance liquid chromatography with 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole (NBDF)-derivatization, according to the method of Irie et al. [12] with slight modification in that the separation was performed on a Phenomenex C18 column (4.6 mm × 250 mm).

Total RNA was extracted from peripheral leukocytes of whole blood samples using the PAXgene Blood RNA kit (Qiagen, Tokyo, Japan) according to the manufacturer's recommendations. Residual genomic DNA was digested with RNase-free DNase I (Qiagen). One to five micrograms of total RNA was used for cDNA synthesis by oligo (dT) primers and Powerscript Reverse Transcriptase (BD Biosciences, Japan) after assessing RNA quality and quantity with NanoDrop (NanoDrop Technologies, DE, USA). Primers and hybridization probes were selected and optimized at exon–intron boundary of *LIM* gene (Nihon Gene Research Lab's Inc., Sendai, Japan). Primers were as followed: forward primer: 5′-GATGGTGAACCCTACTGTGA-3′; reverse primer: 5′-GTCTGACCTTCCAAACTTTC-3′. Hybridization probes were as followed: 5′-TCCCATAGAAGCTGGTGACATGTTCCTGG-3′-fluorescein, 5′-LCRed640-AGCTCTGGGCTACACCTGGCATGACACTT-3′-phosphorylation. Quantitative real-time PCR was performed with LightCycler (Roche Diagnostics, Tokyo, Japan). The *G6PD* gene (glucose-6-phosphate dehydrogenase, Qiagen) was used as a housekeeping gene for normalization. Measurements of each gene expression were conducted in duplicate. Proper amplification of the quantitative PCR products of *LIM* and *G6PD* genes was confirmed by agarose gel electrophoresis in all samples.

Genotyping was performed using commercially available TaqMan probes and Applied Biosystems 7500 Fast Real Time PCR System according to the protocol recommended by the manufacturer (Applied Biosystems, CA, USA).

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. Change before and after treatment was calculated with the Wilcoxon rank sum test. Spearman correlation coefficients were used to evaluate the correlations between LIM mRNA levels and either paroxetine concentration or HAM-D score. All significance levels were two-sided. The distribution of genotypes and alleles in the two study groups was compared using the Fisher's exact test. Haplotypic associations were examined with PHASE software [23,24]. The criterion for significance was set at $P < 0.05$ for all tests. Data are presented as mean ± standard deviation.

The relative amount of *LIM* mRNA in the peripheral leukocytes was standardized with G6PD mRNA as an internal standard. The coefficient of variance was less than 20%. There was no significant difference of *LIM* mRNA expression between males and females. *LIM* mRNA levels (*LIM* mRNA/*G6PD* mRNA × 10^3) was in the range of 0.2–19.0 (mean ± S.D.,

Table 1
Association of SNPs in *LIM* gene with major depression

SNP		HWE	*n*	Allele		*P*-value	Genotype			*P*-value
				A	G		A/A	A/G	G/G	
rs10008257	MD	0.91	123	128	118	0.79	33	62	28	0.38
	CT	0.07	125	134	116		41	52	32	
rs2433320	MD	0.73	116	33	199	0.80	2	29	85	0.96
	CT	0.96	123	38	208		3	32	88	
SNP		HWE	*n*	Allele		*P*-value	Genotype			*P*-value
				T	C		T/T	T/C	C/C	
rs2452600	MD	0.88	130	108	152	0.21	22	64	44	0.20
	CT	0.09	130	123	137		24	75	31	

HWE, Hardy–Weinberg equilibrium; MD, major depression; CT, control. *P*-values were calculated by Fisher's exact test.

5.1 ± 4.1) in healthy volunteers, while 0.3–8.2 (mean ± S.D., 2.4 ± 2.0) in 20 medication free depressed patients, showing a statistical difference (Mann–Whitney *U*-test: $P = 0.009$; Fig. 1). *LIM* mRNA levels at baseline was 2.6 ± 2.0 in 17 first episode patients, while 1.0 ± 0.6 in recurrent episode patients, showing no statistical difference. No significant relationship between *LIM* mRNA levels and baseline HAM-D score was observed (Spearman correlation efficient: $P = 0.899$).

Mean paroxetine doses were 29.0 ± 9.7 mg/day (10 mg/day, $n = 1$; 20 mg/day, $n = 7$; 30 mg/day, $n = 5$; 40 mg/day, $n = 7$) at 4-week treatments. Mean paroxetine concentration was 64.8 ± 47.6 ng/ml at that point. Depressive symptoms were improved after 4-week paroxetine treatments (HAM-D scores at baseline and 4 weeks, 22.2 ± 6.5 and 11.3 ± 7.2, respectively; Wilcoxon rank sum test: $P < 0.001$). The *LIM* mRNA level in the leukocytes was significantly increased at 4-week from baseline (2.4 ± 2.0 at baseline; 4.1 ± 4.4 at 4 weeks; Wilcoxon rank sum test: $P = 0.011$; Fig. 1). Neither paroxetine concentrations nor the changes of HAM-D scores showed significant correlation with the change of the mRNA levels.

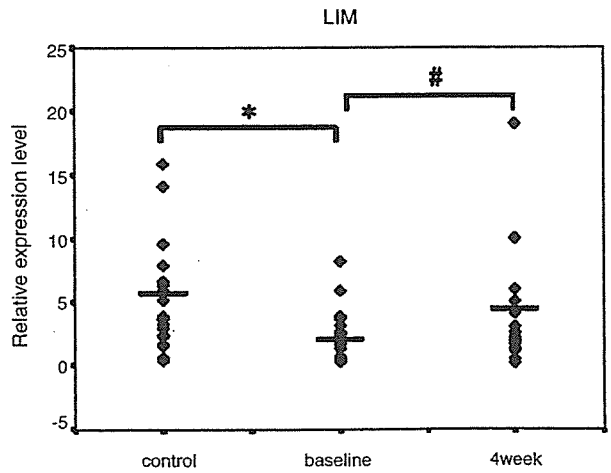

Fig. 1. The measurement of *LIM* mRNA/*G6PD* mRNA × E3 in peripheral leukocytes in depressive patients ($n = 20$) and control subjects ($n = 20$). The mean *LIM* mRNA level was significantly lower in patients (patients: 5.1 ± 4.1, controls: 2.4 ± 2.0, Mann–Whitney *U*-test: $^{*}P = 0.009$). The *LIM* mRNA levels in leukocytes of patients with major depression were significantly increased at 4 weeks compared with those at baseline (baseline: 2.4 ± 2.0, 4-week treatment: 4.1 ± 4.4, Wilcoxon rank sum test: $^{\#}P = 0.011$). The means for controls, baseline and 4-week treatment samples of patients are indicated by horizontal line.

We genotyped three single nucleotide polymorphic (SNP) markers, the two of them (rs10008257 and rs2433320) were reported to be associated with bipolar disorder [15], and the other (rs2452600) was a non-synonymous SNP that results in c to t (Ser136Phe) substitution at codon 136 in LIM protein. The heterozygocity of those three SNPs (rs10008257, rs2433320 and rs2452600) in Japanese population is reported as 0.39, 0.18 and 0.34, respectively and the ratio in the present study was the same as reported. There were no significant deviations in all three SNPs from the Hardy–Weinberg equilibrium in either patients or control subjects. Allele and genotype frequencies of the three SNPs are shown in Table 1. Haplotype analysis of the three SNPs is shown in Table 2. There were no associations between these SNPs and major depression.

The present study is the first report on gene expression in the peripheral leukocytes and association analysis of *LIM* gene in major depression. There are three major findings in our investigation.

First, the mean *LIM* mRNA levels in the peripheral leukocytes of depressive patients before treatment (baseline) were significantly lower than those of age- and sex-matched controls. Our result may be related with previous reports showing that the expression level of *LIM* mRNA was commonly decreased in the immortalized lymphoblastoid cell lines derived from patients with bipolar disorder and schizophrenia [13,14].

Table 2
Haplotypic association of SNPs in LIM with major depression

rs10008257	rs2433320	rs2452600	Frequencies (MD, CT)
A	G	C	0.243, 0.224
A	G	T	0.173, 0.196
A	A	C	0.026, 0.022
A	A	T	0.033, 0.023
G	G	C	0.259, 0.233
G	G	T	0.192, 0.200
G	A	C	0.041, 0.051
G	A	T	0.033, 0.051

P-values were calculated by PHASE software; $P = 0.77$.

However, Iwamoto et al. [13,14] reported that the *LIM* mRNA in the postmortem brains of patients with major depression, bipolar disorder and schizophrenia was commonly high. The cause of these changes in opposite directions is unclear but may be explained by the state-dependent factors such as the regulation of circulating fluid (hormones, cytokines and psychotropic drugs) and clinical symptoms. Further studies with the peripheral leukocytes of bipolar disorder and schizophrenia should be performed. Several findings suggest that N-type calcium channel and PKC epsilon and their adaptor protein LIM may be involved in the pathophysiology of major depression. Decreased anxiety-related behavior was reported in both mice lacking N-type calcium channel [22] and PKC-epsilon [8]. Activity-dependent release of BDNF from hippocampal neuron was triggered by calcium influx specifically through N-type calcium channel [2], which is known to be involved in the pathophysiology of major depression (reviewed in [18]). Because LIM protein is critical for rapid and efficient potentiation of the N-type calcium channel activation by PKC epsilon in neurons [4], down-regulation of *LIM* mRNA in the peripheral leukocytes may reflect the impairments of calcium signaling and neuronal plasticity in neurons.

Second, the *LIM* mRNA levels after 4 weeks of paroxetine treatment were significantly increased from the baseline levels and almost the same levels as those of healthy controls. Although our results fail to reveal the relationship among LIM expression, clinical symptoms and medication, the increase of LIM expression after treatment may be a consequence of state-dependent factors such as clinical improvement and pharmacological effects of antidepressant. Because administration of serotonin selective reuptake inhibitor was reported to decrease the plasma serotonin in patients with major depression [26], the action of serotonin may be involved in this change. Serotonin inhibits N-type calcium channels via 5-HT1A receptors in lamprey spinal neurons [7] and activates PKC epsilon via 5-HT7 receptors in human U373 MG astrocytoma cells [16]. These findings suggest that the alteration of serotonergic signaling induced by the pharmacological effects of antidepressant may influence the *LIM* mRNA expression via N-type calcium channel and PKC epsilon.

Third, there were no associations between major depression and *LIM* gene polymorphisms which were associated with bipolar disorder [15]. Recently, Horiuchi et al. [9] reported that the same SNP (rs2433320) was associated with schizophrenia as well as bipolar disorder. They also reported the different allele of this SNP showed different DNA-protein complexes on electrophoreic mobility shift assay and GA genotype might have higher transcriptional activity. In our study, however, neither patients (GG: 2.3 ± 2.0, $n = 16$; GA: 2.9 ± 2.0, $n = 4$ Mann–Whitney U-test: $P = 0.68$) nor controls (GG: 5.0 ± 4.4, $n = 18$; GA: 6.2 ± 0.5, $n = 2$: $P = 0.38$) showed significant difference of *LIM* mRNA expression between GG and GA genotype of this SNP. These results suggest that the altered expression level of *LIM* mRNA in major depression may be caused by the state-dependent factors. The major limitation of our association study may be the relatively small sample size and we cannot exclude the type II error.

In conclusion, our investigation revealed that the mean *LIM* mRNA levels in depressive patients were significantly decreased at baseline and were significantly increased up to healthy control levels after paroxetine treatment. There were no associations between major depression and *LIM* gene polymorphisms which were reported to be associated with bipolar disorder and schizophrenia. These results suggest that the levels of *LIM* mRNA in native leukocytes may be a useful biological marker of major depression. Further studies are necessary to confirm and extend the present results.

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