disease, kidney disease, chronic hepatic disease, cancer, or diabetes mellitus. The patients were diagnosed on the basis of DSM-IV criteria, information from medical records and a clinical interview. All patients were stable and/or partially remitted at the time of MR measurement and neuropsychological tests.

According to genotypes, each group (control and schizophrenia) was categorized into three groups; the homozygous Val-COMT group (control: n = 38, two were left-handed, schizophrenia: n = 19, one was left-handed), the Val/Met-COMT group (control: n = 25, three were left-handed, schizophrenia: n = 22, all were right-handed) and the remaining homozygous Met-COMT group (control: n = 13, all were right-handed, schizophrenia: n = 6, all were right-handed). Because of the small number of subjects with homozygous Met-COMT, the Val/Met-COMT and homozygous Met-COMT groups were combined and treated as one group, the Met-COMT carriers. Table 1 shows the characteristics of each group. All groups were of comparable age, gender (χ^2 test, df = 3, P = 0.38) and handedness (χ^2-test, df = 3, P = 0.53). No genotype effects and genotype-diagnosis interaction effects were found in years of education, scores of full scale Intelligence Quotient (IQ) and scores of premorbid IQ [Japanese version of National Adult Reading Test (JART) score], however, patients who had fewer years of education ($P < 0.0001$), had lower scores of both full scale IQ and JART ($P < 0.001$). The duration of illness, medication and hospitalization, the age at disease onset and drug dose (chlorpromazine equivalent) of those homozygous for the Val-COMT did not differ from the Met-COMT carriers.

SNP genotyping

Venous blood was drawn from subjects and genomic DNA was extracted from whole blood according to the standard procedures. The Val158Met polymorphism of the COMT gene (dbSNP accession: rs4680) was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay, described previously (Hashimoto *et al.*, 2004, 2005). Briefly, primers and probes for detection of the SNP are: forward primer 5′-GACTGTGCCGCCATCAC-3′, reverse primer 5′-CAGGCATGCACACCTTGTC-3′, probe 1 5′-VIC-TTTCGCTG-GCGTGAAG-MGB-3′ and probe 2 5′-FAM-CGCTGGCATGAAG-MGB-3′. PCR cycling conditions were: at 95°C for 10 min, 50 cycles of 92°C for 15 s and 60°C for 1 min.

MRI procedures

All MR studies were performed on a 1.5 tesla Siemens Magnetom Vision plus system. A three dimensional (3D) volumetric acquisition of a T_1-weighted gradient echo sequence produced a gapless series of thin sagittal sections using an MPRage sequence (TE/TR, 4.4/11.4 ms; flip angle, 15°; acquisition matrix, 256 × 256; 1 NEX, field of view, 31.5 cm; slice thickness, 1.23 mm).

Image analysis (TBM)

The basic principle of TBM is to analyse the local deformations of an image and to infer local differences in brain structure. In TBM, MRI scans of individual subjects are mapped to a template image with three-dimensional (3D) non-linear normalization routines. Local deformations were estimated by a univariate Jacobian approach. The basic principle of TBM is the same as a method used in a previous report described as deformation-based morphometry (Gaser *et al.*, 2001). Firstly, inhomogeneities in MR images were corrected using a bias correction function in statistical parametric mapping (SPM2), then the corrected image was scalp-edited by masking with a probability image of brain tissue obtained from each image using a segmentation function in SPM2. Using a linear normalization algorithm in SPM2, all brains were resized to a voxel size of 1.5 mm and adjusted for orientation and overall width, length and height (Fig. 1A). Therefore, brains were transformed to the anatomical space of a template brain whose space is based on Talairach space (Talairach and Tournoux, 1988). Subsequent non-linear normalization introduced local deformations to each brain to match it to the same scalp-edited template brain (Fig. 1C). The non-linear transformation was done using the high-dimension-warping algorithm (Ashburner and Friston, 2004). After the high dimensional warping, each image (Fig. 1B) looks similar to the template (Fig. 1C). Figure 2 demonstrated a mean MR image of 76 controls (left) and a mean MR image of 47 schizophrenics after high dimensional warping (Fig. 2). We obtained 3D deformation fields for every brain (Fig. 1D). Each of these 3D deformation fields consists of displacement vectors for every voxel, which describe the 3D displacement needed to locally deform the brain to match it to the template. We calculated the Jacobian determinants to obtain voxel by voxel parametric maps of local volume change relative to the template brain (Fig. 1E). The local Jacobian determinant is a parameter commonly used in continuum mechanics (Gurtin, 1987), which characterizes volume changes, such as local shrinkage or enlargement caused by warping. The parametric maps of Jacobian determinants were analysed using SPM2, which implements a 'general linear model'. To test hypotheses about regional population effects and interaction, data were analysed by an analysis of covariance (ANCOVA) without global normalization. There was no significant difference in age among the four groups, however, patients with schizophrenia, particularly those homozygous for the Val-COMT allele, were older than controls. Therefore, we treated age and years of education and scores of JART as nuisance variables. Since TBM explores the entire brain (grey matter, CSF space and white matter) at once, the search volume of TBM has a large number of voxels and since our interest was in morphological changes in the grey matter and CSF space, we excluded white matter tissue from analyses by using an explicit mask (Fig. 1F). We used $P < 0.001$, corrected for multiple comparisons with false discovery rate (FDR) <0.05 as a statistical threshold. The resulting sets of t values constituted the statistical parametric maps {SPM (t)}. Firstly, we estimated the main effects, the genotype effect in total subjects (the Val/Val-COMT versus the Met-COMT carriers) and the diagnostic effect (schizophrenia versus controls) and then the genotype-diagnosis interaction effect was estimated. Furthermore, the effects of genotypes in each group (controls carrying the Val/Val-COMT gene versus controls carrying the Met-COMT gene and schizophrenics carrying the Val/Val-COMT gene versus schizophrenics carrying the Met-COMT gene) were estimated within the ANCOVA design matrix. Anatomical localization accorded both to MNI coordinates and Talairach coordinates obtained from M. Brett's transformations (www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html) and are presented as Talairach coordinates (Talairach and Tournoux, 1988). Since previous studies have demonstrated the association between the Val158Met polymorphism and the dorsolateral PFC (DLPFC), we applied an additional hypothesis-driven region of interest (ROI) method to test regional population effects in the DLPFC. For this ROI analysis, we used the Wake Forest University PickAtlas (Maldjian *et al.*, 2003) within the ANCOVA design matrix for SPM analysis. We set $P < 0.05$ (uncorrected) with a small volume correction ($P < 0.05$ within the ROI) to assess grey matter volume changes in the DLPFC (Brodmann area 46, 9 and 8).

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.

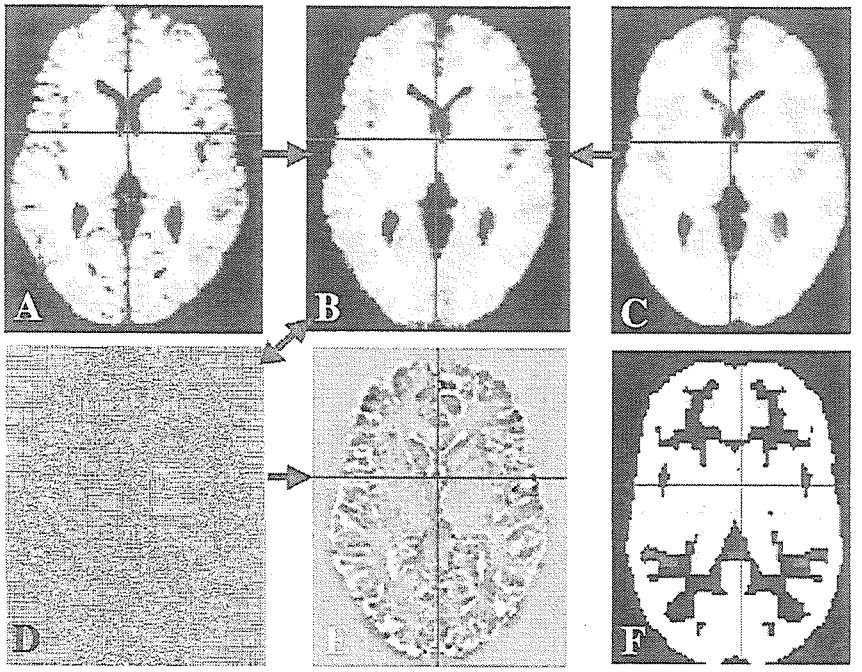

Fig. 1 Steps of analysis for tensor-based morphometry. An example is shown for a single subject in one axial slice. The single object brain (**A**) has been corrected for orientation and overall size to the template brain (**C**). Non-linear spatial normalization removes most of the anatomical differences 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.

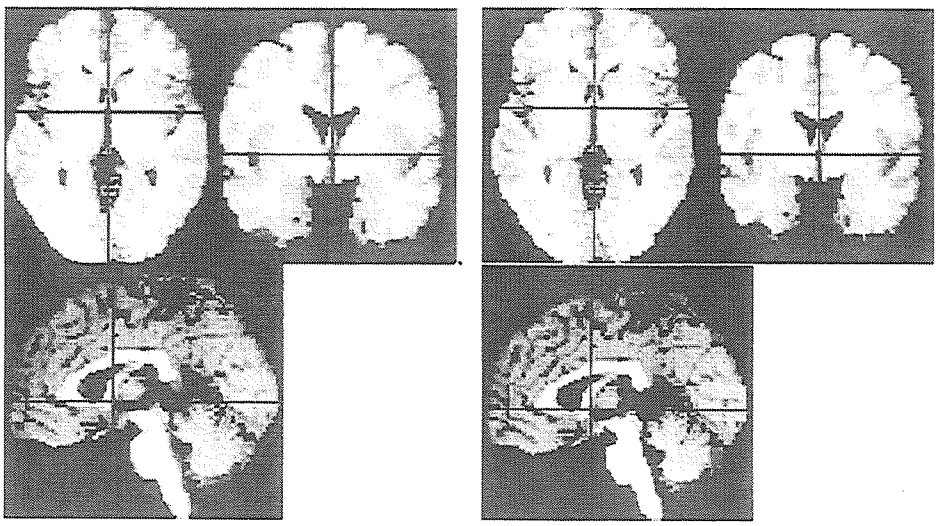

Fig. 2 Mean images after high dimensional warping control subjects and schizophrenics. *Left*: The mean image of warped MR images obtained from 76 controls. Even after averaging, the mean image is not blurred. *Right*: The mean image of warped MR images obtained from 47 schizophrenics. The mean image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

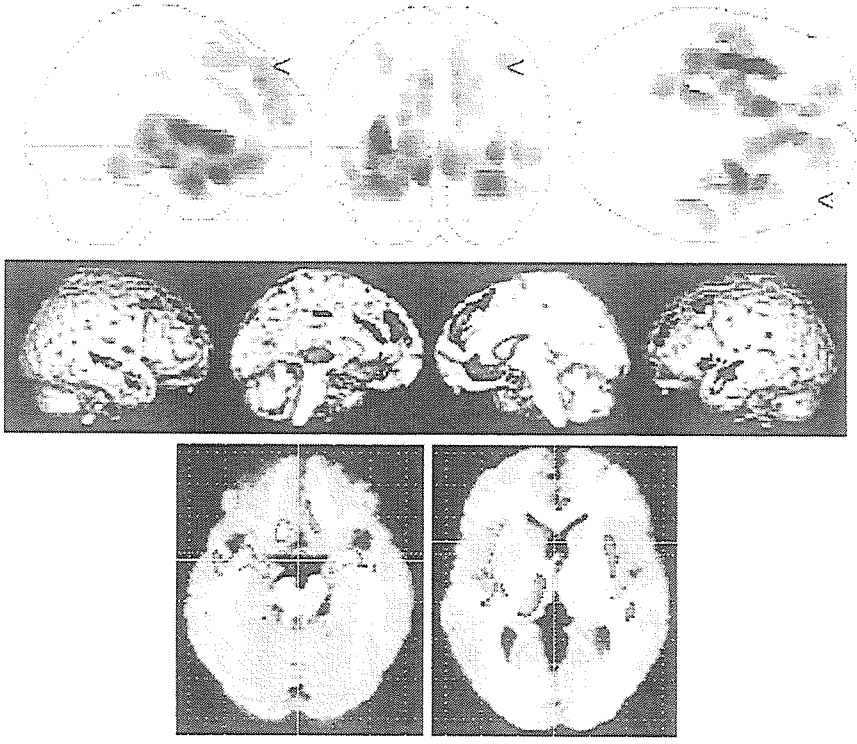

Fig. 3 Decreased volumes in schizophrenics ($n = 47$) as compared to controls ($n = 76$). *Top*: The SPM {*t*} is displayed in a standard format as a maximum-intensity projection (MIP) viewed from the right, the back and the top of the brain. The anatomical space corresponds to the atlas of Talairach 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.

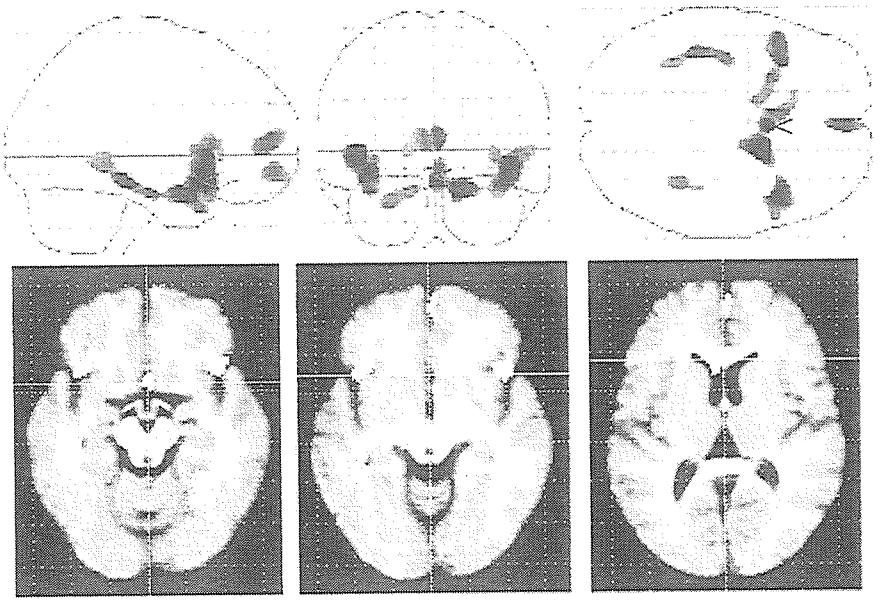

Fig. 4 Increased volumes in schizophrenics as compared to controls. *Top*: The SPM {*t*} is displayed in a standard format as a MIP. Patients with schizophrenia showed a significantly increased volume of the CSF space. *Bottom*: The SPM {*t*} is displayed onto axial T_1-weighted MR images. A significantly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

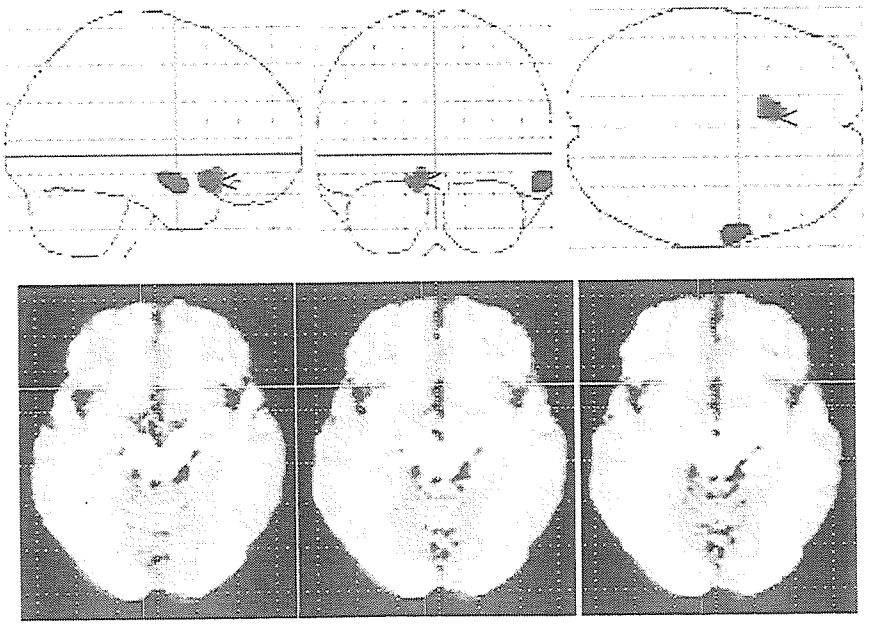

Fig. 5 The result of comparison between individuals homozygous for the Val-COMT allele (n = 57) and Met-COMT carriers (n = 66) (genotype effects). *Top*: Representation in stereotaxic space of regions with significant reduction of volume in individuals homozygous for the Val-COMT allele demonstrated. *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

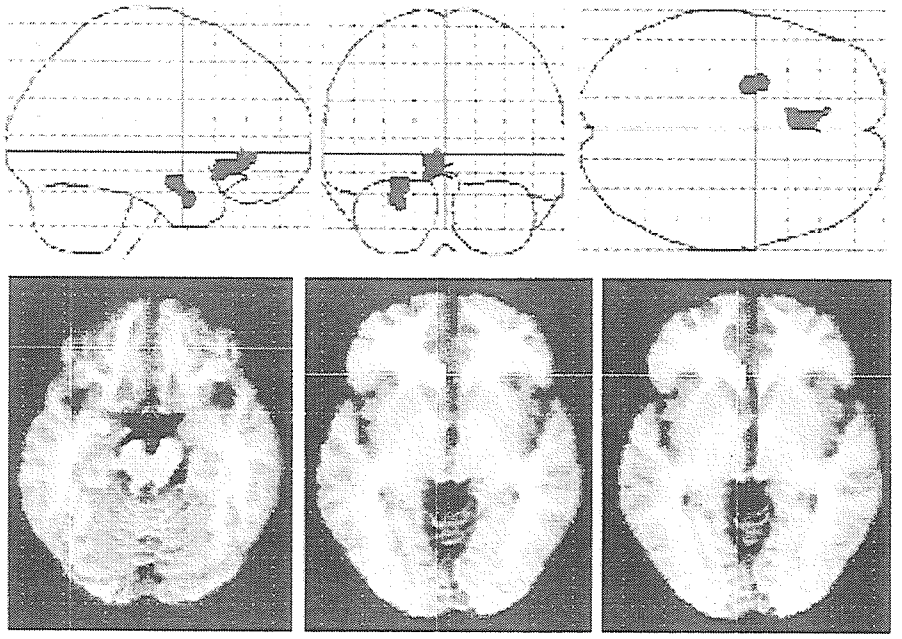

Fig. 6 Results of genotype-diagnosis interaction effects on brain morphology. *Top*: The SPM {*t*} is displayed in a standard format as a MIP. The stronger effects of Val158Met polymorphism on brain morphology in schizophrenia than those in controls were noted in the left ACC, left parahippocampal gyrus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

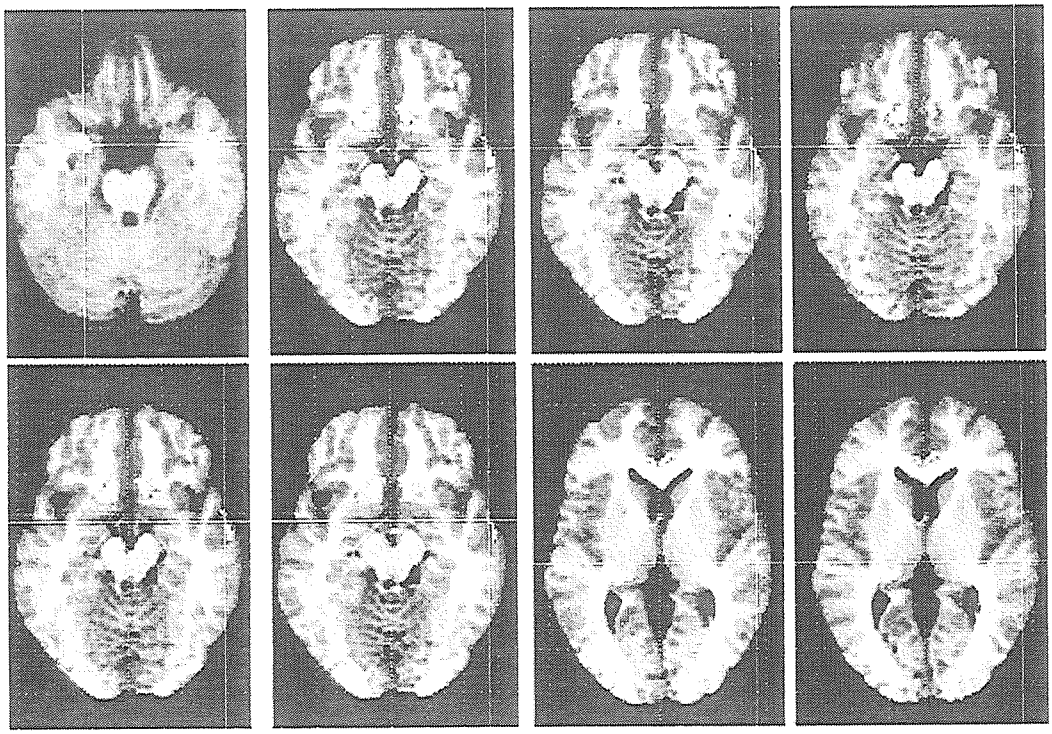

Fig. 7 The effects of the Val158Met polymorphism of the COMT gene on brain morphology in schizophrenics. The SPM {*t*} is displayed onto axial T_1-weighted MR images. The schizophrenics homozygous for the Val-COMT allele (n = 19) showed a significant reduction of volumes in the left parahippocampal gyrus, 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.

Acknowledgements

This study was supported by the Promotion of Fundamental Studies in Health Science of Organization for Pharmaceuticals and Medical Devices Agency. This work was also supported in part by Grants-in-Aid from the Japanese Ministry of Health, Labor and Welfare (H17-kokoro-007 and H16-kokoro-002), the Japanese Ministry of Education, Culture, Sports, Science and Technology and Core research for Evolutional Science and Technology of Japan Science and Technology Agency, Japan Foundation for Neuroscience and Mental Health.

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International Journal of Neuropsychopharmacology (2005), 8, 1–6. Copyright © 2005 CINP
doi:10.1017/S1461145705005523

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.

Received 13 October 2004; Reviewed 28 December 2004; Revised 7 February 2005; Accepted 20 February 2005

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 (40.7%)	51 (59.3%)	0.054	2.18 (0.96–3.03)	5 (11.6%)	25 (58.1%)	13 (30.2%)	0.038	30 (69.8%)	13 (30.2%)	0.037	3.00 (1.05–8.58)
Non-responders (23)	11 (23.9%)	35 (76.1%)			1 (4.3%)	9 (39.1%)	13 (56.5%)		10 (43.5%)	13 (56.5%)		
Total patients (66)	46 (34.8%)	86 (65.2%)			6 (9.1%)	34 (51.5%)	26 (39.4%)		40 (60.6%)	26 (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.

Acknowledgements

This work was supported by Grants-in-Aid from the Japanese Ministry of Health, Labor and Welfare, the Japanese Ministry of Education, Culture, Sports, Science and Technology, the Uehara Memorial Foundation, and Japan Foundation for Neuroscience and Mental Health. The authors thank Tomoko Shizuno and Reiko Fujita for technical assistance.

Statement of Interest

None.

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