**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.

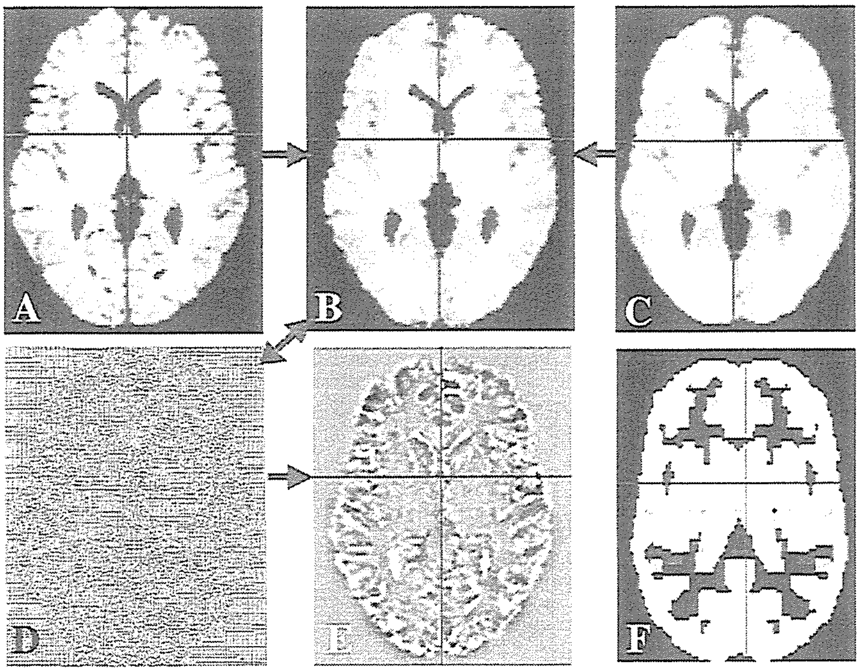


**Fig. 1** Steps of analysis for tensor-based morphometry. An example is shown for a single subject in one axial slice. The single object brain (**A**) has been corrected for orientation and overall size to the template brain (**C**). Non-linear spatial normalization removes most of the anatomical differences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

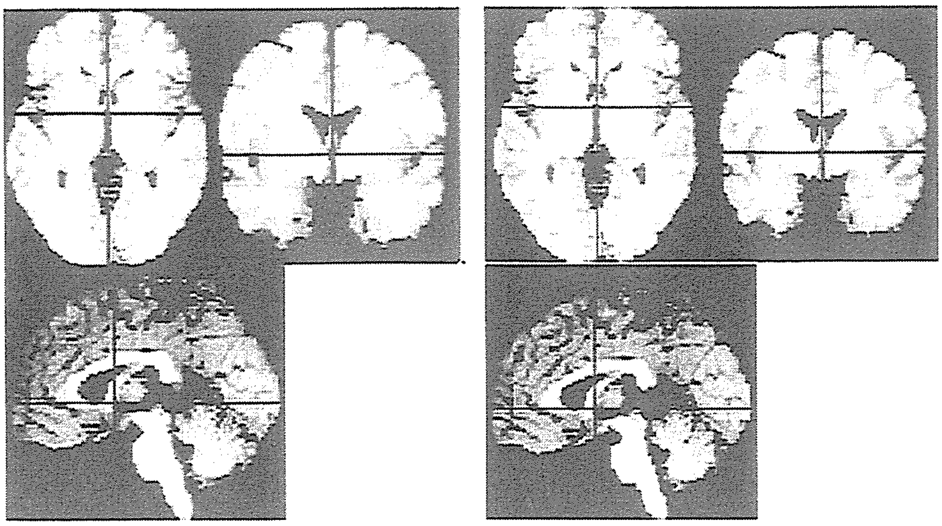

**Fig. 2** Mean images after high dimensional warping control subjects and schizophrenics. *Left*: The mean image of warped MR images obtained from 76 controls. Even after averaging, the mean image is not blurred. *Right*: The mean image of warped MR images obtained from 47 schizophrenics. The mean image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

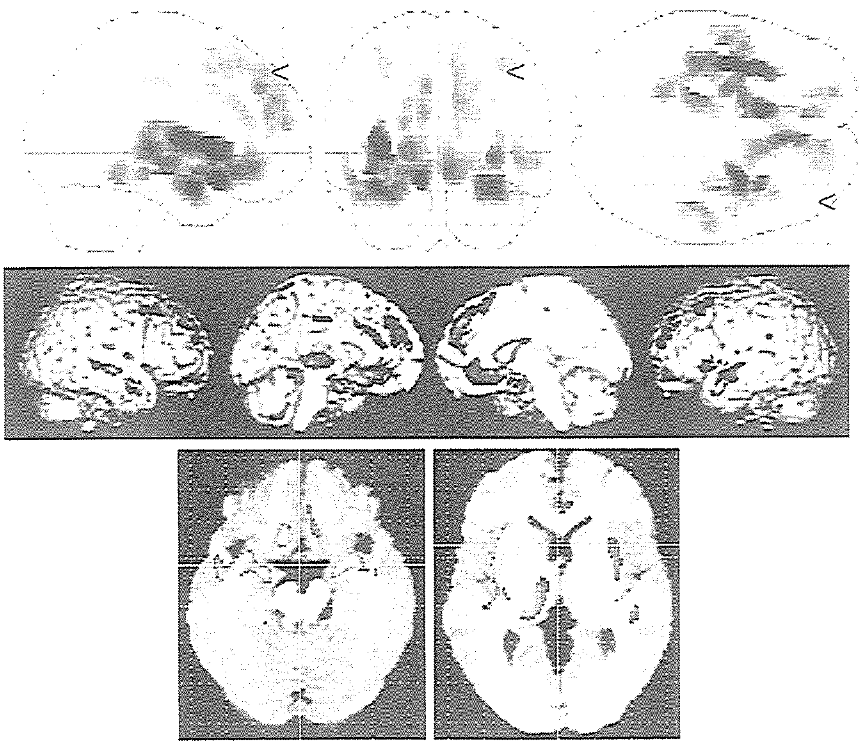

**Fig. 3** Decreased volumes in schizophrenics ($n = 47$) as compared to controls ($n = 76$). *Top*: The SPM {$t$} is displayed in a standard format as a maximum-intensity projection (MIP) viewed from the right, the back and the top of the brain. The anatomical space corresponds to the atlas of Talairach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

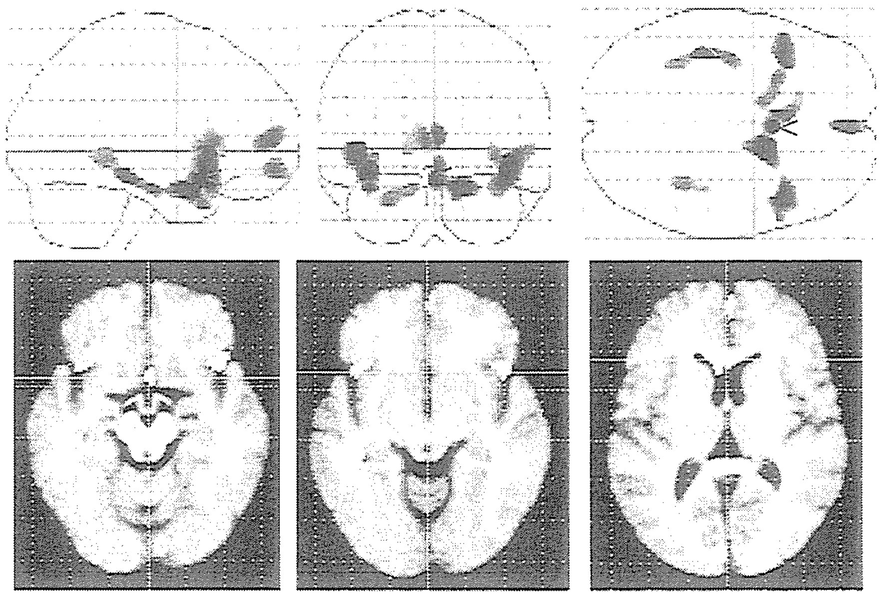

**Fig. 4** Increased volumes in schizophrenics as compared to controls. *Top*: The SPM {*t*} is displayed in a standard format as a MIP. Patients with schizophrenia showed a significantly increased volume of the CSF space. *Bottom*: The SPM {*t*} is displayed onto axial $T_1$-weighted MR images. A significantly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

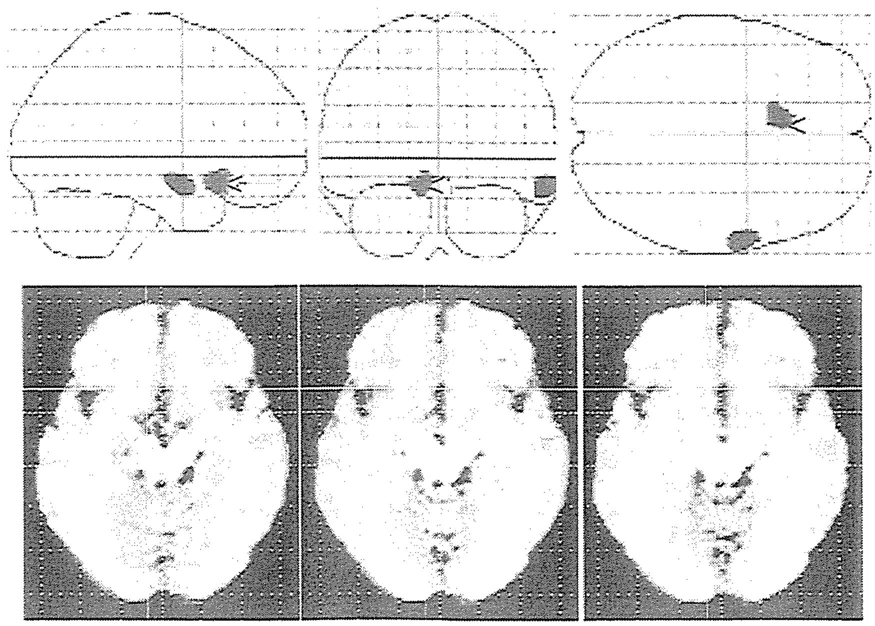

**Fig. 5** The result of comparison between individuals homozygous for the Val-COMT allele ($n = 57$) and Met-COMT carriers ($n = 66$) (genotype effects). *Top*: Representation in stereotaxic space of regions with significant reduction of volume in individuals homozygous for the Val-COMT allele demonstrated.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

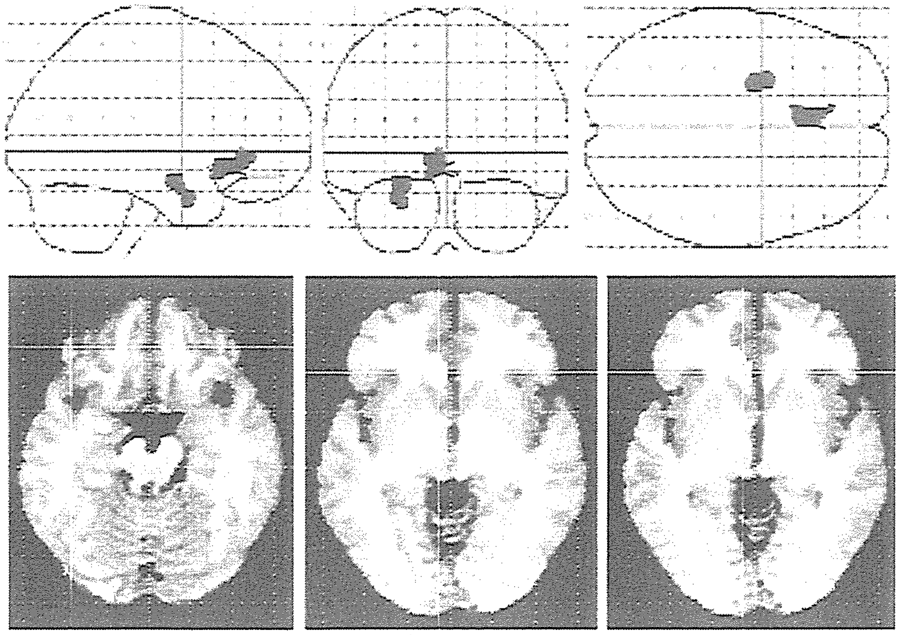

**Fig. 6** Results of genotype-diagnosis interaction effects on brain morphology. *Top*: The SPM {t} is displayed in a standard format as a MIP. The stronger effects of Val158Met polymorphism on brain morphology in schizophrenia than those in controls were noted in the left ACC, left parahippocampal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

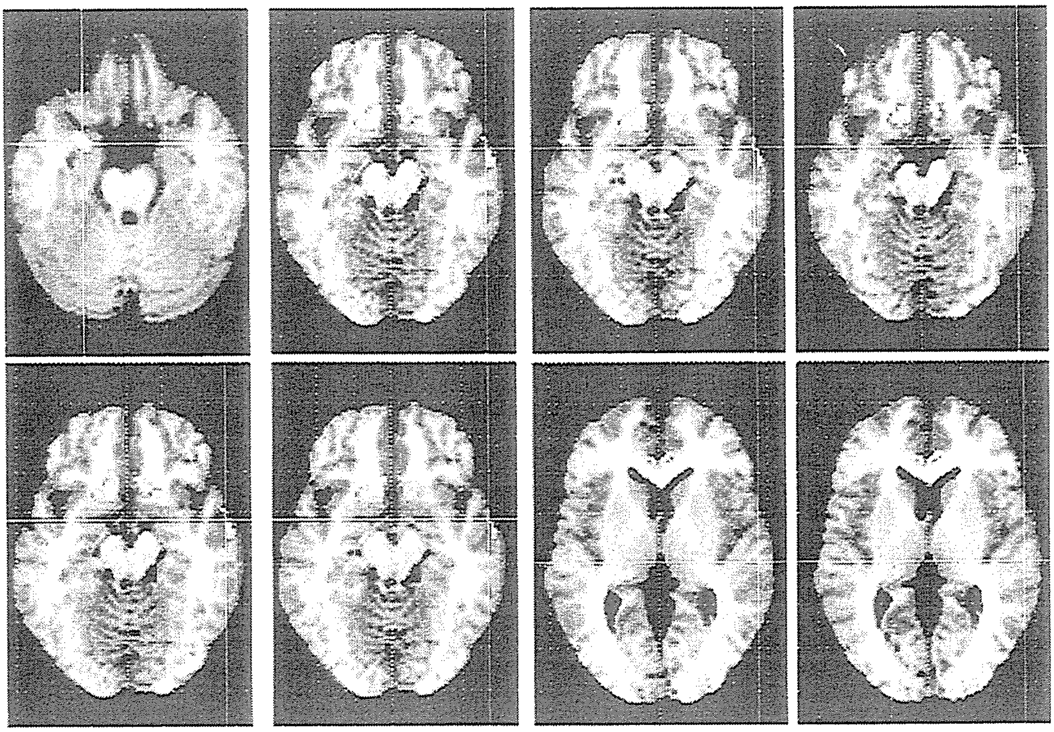

**Fig. 7** The effects of the Val158Met polymorphism of the COMT gene on brain morphology in schizophrenics. The SPM {*t*} is displayed onto axial $T_1$-weighted MR images. The schizophrenics homozygous for the Val-COMT allele (*n* = 19) showed a significant reduction of volumes in the left parahippocampal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

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### References

Arango C, Breier A, McMahon R, Carpenter WT Jr, Buchanan RW. The relationship of clozapine and haloperidol treatment response to prefrontal, hippocampal, and caudate brain volumes. Am J Psychiatry 2003; 160: 1421–7.

Arnold SE, Trojanowski JQ. Recent advances in defining the neuropathology of schizophrenia. Acta Neuropathol (Berl) 1996; 92: 217–31.

Ashburner J, Friston KJ. High-dimensional image warping. In: Frackowiak R, editor. Human brain function. 2nd edn. Academic Press; 2004. p. 673–94.

Baare WF, Hulshoff Pol HE, Boomsma DI, Posthuma D, de Geus EJ, Schnack HG, et al. Quantitative genetic modeling of variation in human brain morphology. Cereb Cortex 2001; 11: 816–24.

Bogerts B. Recent advances in the neuropathology of schizophrenia. Schizophr Bull 1993; 19: 431–45.

Cannon TD, Mednick SA, Parnas J, Schulsinger F, Praestholm J, Vestergaard A. Developmental brain abnormalities in the offspring of schizophrenic mothers. I. Contributions of genetic and perinatal factors. Arch Gen Psychiatry 1993; 50: 551–64.

Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 2004; 75: 807–21.

Daniels JK, Williams NM, Williams J, Jones LA, Cardno AG, Murphy KC, et al. No evidence for allelic association between schizophrenia and a polymorphism determining high or low catechol O-methyltransferase activity. Am J Psychiatry 1996; 153: 268–70.

Davidson LL, Heinrichs RW. Quantification of frontal and temporal lobe brain-imaging findings in schizophrenia: a meta-analysis. Psychiatry Res 2003; 122: 69–87.

Dorph Petersen KA, Pierri JN, Perel JM, Sun Z, Sampson AR, Lewis DA. The influence of chronic exposure to antipsychotic medications on brain size before and after tissue fixation: a comparison of haloperidol and olanzapine in macaque monkeys. Neuropsychopharmacology 2005; 30: 1649–61.

Durston S, Fossella JA, Casey BJ, Hulshoff Pol HE, Galvan A, Schnack HG, et al. Differential effects of DRD4 and DAT1 genotype on fronto-striatal gray matter volumes in a sample of subjects with attention deficit hyperactivity disorder, their unaffected siblings, and controls. Mol Psychiatry 2005; 10: 678–85.

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, et al. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci USA 2001; 98: 6917–22.

Fan JB, Zhang CS, Gu NF, Li XW, Sun WW, Wang HY, et al. Catechol-O-methyltransferase gene Val/Met functional polymorphism and risk of schizophrenia: a large-scale association study plus meta-analysis. Biol Psychiatry 2005; 57: 139–44.

Galderisi S, Maj M, Kirkpatrick B, Piccardi P, Mucci A, Invernizzi G, et al. COMT Val(158)Met and BDNF C(270) T polymorphisms in schizophrenia: a case-control study. Schizophr Res 2005; 73: 27–30.

Gaser C, Nenadic I, Buchsbaum BR, Hazlett EA, Buchsbaum MS. Deformation-based morphometry and its relation to conventional volumetry of brain lateral ventricles in MRI. Neuroimage 2001; 13: 1140–5.

Gogtay N, Sporn A, Clasen LS, Greenstein D, Giedd JN, Lenane M, et al. Structural brain MRI abnormalities in healthy siblings of patients with childhood-onset schizophrenia. Am J Psychiatry 2003; 160: 569–571.

Goldberg TE, Egan MF, Gscheidle T, Coppola R, Weickert T, Kolachana BS, et al. Executive subprocesses in working memory: relationship to catechol-O-methyltransferase Val158Met genotype and schizophrenia. Arch Gen Psychiatry 2003; 60: 889–96.

Gurtin, ME. An introduction to continuum mechanics. Boston: Academic Press; 1987.

Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry 2005; 10: 40–68.

Hashimoto R, Yoshida M, Ozaki N, Yamanouchi Y, Iwata N, Suzuki T, et al. Association analysis of the -308G>A promoter polymorphism of the tumor necrosis factor alpha (TNF-alpha) gene in Japanese patients with schizophrenia. J Neural Transm 2004; 111: 217–21.

Hashimoto R, Yoshida M, Kunugi H, Ozaki N, Yamanouchi Y, Iwata N, et al. A missense polymorphism (H204R) of a Rho GTPase-activating protein, the chimerin 2 gene, is associated with schizophrenia in men. Schizophr Res 2005; 73: 383–5.

Ho BC, Andreasen NC, Nopoulos P, Arndt S, Magnotta V, Flaum M. Progressive structural brain abnormalities and their relationship to clinical outcome: a longitudinal magnetic resonance imaging study early in schizophrenia. Arch Gen Psychiatry 2003; 60: 585–94.

Ho BC, Wassink TH, O'leary DS, Sheffield VC, Andreasen NC. Catechol-O-methyl transferase Val(158)Met gene polymorphism in schizophrenia: working memory, frontal lobe MRI morphology and frontal cerebral blood flow. Mol Psychiatry 2005; 10: 287–98.

Kendler KS. Overview: a current perspective on twin studies of schizophrenia. Am J Psychiatry 1983; 140: 1413–25.

Kondo H, Osaka N, Osaka M. Cooperation of the anterior cingulate cortex and dorsolateral prefrontal cortex for attention shifting. Neuroimage 2004; 23: 670–9.

Kunugi H, Vallada HP, Sham PC, Hoda F, Arranz MJ, Li T, et al. Catechol-O-methyltransferase polymorphisms and schizophrenia: a transmission disequilibrium study in multiply affected families. Psychiatr Genet 1997; 7: 97–101.

Lieberman JA, Tollefson GD, Charles C, Zipursky R, Sharma T, Kahn RS, et al. Antipsychotic drug effects on brain morphology in first-episode psychosis. Arch Gen Psychiatry 2005; 62: 361–70.

Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage 2003; 19: 1233–9.

McGue M, Gottesman II, Rao DC. The transmission of schizophrenia under a multifactorial threshold model. Am J Hum Genet 1983; 35: 1161–78.

Nelson KB, Lynch JK. Stroke in newborn infants. Lancet Neurol 2004; 3: 150–8.

Norton N, Kirov G, Zammit S, Jones G, Jones S, Owen R, et al. Schizophrenia and functional polymorphisms in the MAOA and COMT genes: no evidence for association or epistasis. Am J Med Genet 2002; 114: 491–6.

Ohmori O, Shinkai T, Kojima H, Terao T, Suzuki T, Mita T, et al. Association study of a functional catechol-O-methyltransferase gene polymorphism in Japanese schizophrenics. Neurosci Lett 1998; 243: 109–12.

Okubo Y, Saijo T, Oda K. A review of MRI studies of progressive brain changes in schizophrenia. J Med Dent Sci 2001; 48: 61–7.

Palmatier MA, Kang AM, Kidd KK. Global variation in the frequencies of functionally different catechol-O-methyltransferase alleles. Biol Psychiatry 1999; 46: 557–67.

Paus T. Primate anterior cingulate cortex: where motor control, drive and cognition interface. Nat Rev Neurosci 2001; 2: 417–24.

Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS, Straub RE, et al. The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. J Neurosci 2004; 24: 10099–102.

Selemon LD. Regionally diverse cortical pathology in schizophrenia: clues to the etiology of the disease. Schizophr Bull 2001; 27: 349–77.

Shenton ME, Dickey CC, Frumin M, McCarley RW. A review of MRI findings in schizophrenia. Schizophr Res 2001; 49: 1–52.

Steel RM, Whalley HC, Miller P, Best JJ, Johnstone EC, Lawrie SM. Structural MRI of the brain in presumed carriers of genes for schizophrenia, their affected and unaffected siblings. J Neurol Neurosurg Psychiatry 2002; 72: 455–8.

Stefanis NC, Van Os J, Avramopoulos D, Smyrnis N, Evdokimidis I, Hantoumi I, et al. Variation in catechol-O-methyltransferase val158 met genotype associated with schizotypy but not cognition: a population study in 543 young men. Biol Psychiatry 2004; 56: 510–5.

Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 2003; 60: 1187–92.

Takahashi T, Suzuki M, Hagino H, Zhou SY, Kawasaki Y, Nohara S, et al. Bilateral volume reduction of the insular cortex in patients with schizophrenia: a volumetric MRI study. Psychiatry Res 2004; 132: 187–96.

Talairach J, Tournoux P. A coplanar stereotaxic atlas of a human brain. Three-dimensional proportional system: an approach to cerebral imaging. Stuttgart: Thieme; 1988.

Tunbridge EM, Bannerman DM, Sharp T, Harrison PJ. Catechol-O-methyltransferase inhibition improves set-shifting performance and elevates stimulated dopamine release in the rat prefrontal cortex. J Neurosci 2004; 24: 5331–5.

Weinberger DR, Egan MF, Bertolino A, Callicott JH, Mattay VS, Lipska BK, et al. Prefrontal neurons and the genetics of schizophrenia. Biol Psychiatry 2001; 50: 825–44.

Wright IC, McGuire PK, Poline JB, Travere JM, Murray RM, Frith CD, et al. A voxel-based method for the statistical analysis of gray and white matter density applied to schizophrenia. Neuroimage 1995; 2: 244–52.

Yamasue H, Iwanami A, Hirayasu Y, Yamada H, Abe O, Kuroki N, et al. Localized volume reduction in prefrontal, temporolimbic, and paralimbic regions in schizophrenia: an MRI parcellation study. Psychiatry Res 2004; 131: 195–207.

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# Possible association between nonsynonymous polymorphisms of the anaplastic lymphoma kinase (ALK) gene and schizophrenia in a Japanese population

*Short Communication*

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**Summary.** We examined, for the first time, the possible association between schizophrenia and the anaplastic lymphoma kinase (ALK) gene which plays an important role in neurodevelopment. When two nonsynonymous polymorphisms (Arg1491Lys and Glu1529Asp) were examined, there were significant differences in genotype and allele distributions between patients and controls. Individuals homozygous for the minor allele (1491Lys–1529Asp) were more common in patients than in controls ($p = 0.0064$, odds ratio 2.4, 95% CI 1.3–4.6). These results suggest that genetic variations of the ALK gene might confer susceptibility to schizophrenia.

**Keywords:** Schizophrenia, anaplastic lymphoma kinase (ALK), single nucleotide polymorphism (SNP), association, susceptibility.

## Introduction

Growing evidence has suggested that alterations of neurotrophic factors may be involved in the morphological, cytoarchitectural and neurobiochemical abnormalities in the brain of schizophrenic patients (Thome et al., 1998; Durany and Thome, 2004). Anaplastic lymphoma kinase (ALK) was originally identified as an oncogene activated in anaplastic large cell lymphomas with chromosomal translocation t (2;5) (Morris et al., 1994; Shiota et al., 1994). Subsequent cloning of the ALK gene revealed that it encodes a receptor-type protein tyrosine kinase (RTK) of the insulin receptor family (Iwahara et al., 1997; Morris et al., 1997). Neurotrophic factors exert their effects through binding to RTKs and play an important role in neurodevelopment such as differentiation, proliferation, survival, and synaptic formation. Indeed, ALK was found to be a receptor for heparin-binding growth factors, midkine (Stoica et al., 2002) and pleiotrophin (Stoica et al., 2001). Midkine and pleiotrophin show approximately 50% identity in amino acid sequence and share the same genomic organization. These proteins play an important role in early neurogenesis, neurite outgrowth, nerve cell migration, and neuroprotection (reviewed by Kadomatsu and Muramatsu, 2004). Of note, a recent study reported alterations in serum midkine levels in patients with schizophrenia (Shimizu et al., 2003).

ALK is expressed almost exclusively in perinatal neural cells. In the central nervous system, it is highly expressed in diencephalons, midbrain, and the ventral half of the spinal cord. After birth, its expression decreases; however, it persists to be expressed in some regions such as the thalamus, olfactory bulb, and midbrain (Iwahara et al., 1997). These brain regions have been implicated in the pathophysiology of schizophrenia (e.g., Moberg and Turetsky, 2003; Clinton and Meador-Woodruff, 2004). The ALK gene is, therefore, a good candidate gene for association analysis with schizophrenia. To our knowledge, however, there is no study examining the possible association between the ALK gene and schizophrenia. The ALK gene maps to chromosome 2p23 (Morris et al., 1994). We searched for nonsynonymous single nucleotide polymorphisms (SNPs) in the ALK gene *in silico* and found only 2 common SNPs which have been well validated: a nucleotide substitution (G>A: NCBI SNP ID rs1881420) resulting in an amino acid change of Arg1491Lys (amino acid numbering is according to NCBI protein data base accession NP_004295) and G>C (rs1881421) resulting in Glu1529Asp. Since these polymorphisms may alter functions of ALK protein, we performed an association study between these polymorphisms and schizophrenia.

## Materials and methods

### *Subjects*

Subjects were 300 patients with schizophrenia (154 males, mean age of 45.3 years [SD 14.3]) and 308 healthy controls (140 males, 39.8 years [SD 11.5]). All subjects were biologically unrelated Japanese and recruited from the same geographical area (Western part of Tokyo Metropolitan). Consensus diagnosis by at least two psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4$^{th}$ edition (DSM-IV) criteria (American Psychiatric Association, 1994) on the basis of unstructured interviews and information from medical records. The controls were healthy volunteers recruited from hospital staffs and their associates. They were interviewed and those individuals who had current or past history of psychiatric treatment were not enrolled in the study.

The study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). After description of the study, written informed consent was obtained from every subject. The study protocol was approved by the ethics committees at the Showa University School of Medicine and the National Center of Neurology and Psychiatry, Japan.

### *Genotyping*

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to the standard procedures. The index SNPs (rs1881420 and rs1881421) were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay, as described previously (Hashimoto et al., 2004, 2005). Primers and probes for detection of the SNPs were as follows: 5′-TTCTCTCAGTCCAACCCTCCTT-3′ (forward primer), 5′-CTGGTGGGCTTGTTTCTGGAT-3′ (reverse primer), 5′-VIC-TTGCACAAGGTCCAC-MGB-3′ (probe 1), and 5′-FAM-TGCACAGGGTCCAC-MGB-3′ (probe 2) for rs1881420; 5′-AGAGAAACCC ACCAAAAAGAATAATCCT-3′ (forward primer), 5′-GTTAGGTGGGACAGTACAGCTT-3′ (reverse primer), 5′-VIC-CAGGTTACCCCTGTCGTGT-MGB-3′ (probe 1), and 5′-FAM-CAGGTTACCCCTCTCGTGT-MGB-3′ (probe 2) for rs1881421. Thermal cycling for polymerase chain reaction (PCR) were 1 cycle at 95°C for 10 minutes followed by 50 cycles of 92°C for 15 seconds and 60°C for 1 minute. Genotype data were read blind to the case-control status.

### *Statistical analysis*

The presence of Hardy-Weinberg equilibrium was examined by using the $\chi^2$ test for goodness of fit.

**Table 1.** Genotype distributions and allele frequencies of the Glu1529Asp polymorphism of the ALK gene (rs1881421) in patients with schizophrenia and controls

| | Genotype distribution | | | | Allele frequency | | |
|---|---|---|---|---|---|---|---|
| | N | Glu/Glu | Glu/Asp | Asp/Asp | N | Glu | Asp |
| Patients | 300 | 141 (47%) | 128 (43%) | 31 (10%) | 600 | 410 (68%) | 190 (32%) |
| Controls | 308 | 171 (55%) | 123 (40%) | 14 (5%) | 616 | 465 (75%) | 151 (25%) |

Genotype and allele distributions were compared between patients and controls by using the $\chi^2$ test for independence. All p-values reported are two-tailed.

## Results

Nearly all the subjects except for three (99.5%) had the same genotype for the two SNPs of rs1881420 and rs1881421, i.e., genotypes of G/G, G/A, and A/A in the former corresponded to those of G/G, G/C, and C/C in the latter. Thus, we show results of statistical analyses for the SNP rs1881421 (Glu1529Asp) only. Genotype distributions and allele frequencies in patients and controls are shown in Table 1. The genotype distribution was not significantly deviated from Hardy-Weinberg equilibrium for patients and controls (patients: $\chi^2 = 0.1$, df = 1, p = 0.81; controls: $\chi^2 = 1.9$, df = 1, p = 0.16). There was a significant difference in the overall genotype distribution between patients and controls ($\chi^2 = 9.3$, df = 2, p = 0.0095). Individuals homozygous for the minor allele (1529Asp) was significantly more common in patients than in controls ($\chi^2 = 7.4$, df = 1, p = 0.0064, odds ratio 2.4, 95% CI 1.3–4.6). When allele frequencies were compared, the 1529Asp allele was significantly more frequent in patients than in controls ($\chi^2 = 7.7$, df = 1, p = 0.0055, odds ratio 1.4, 95% CI 1.1–1.8).

## Discussion

We examined, for the first time, the possible association between schizophrenia and the anaplastic lymphoma kinase (ALK) gene which plays an important role in neurodevelopment such as early neurogenesis, neurite outgrowth, nerve cell migration, and neuroprotection. We found that the minor allele (1529Asp) of the Glu1529Asp polymorphism (rs1881421) and homozygosity for this allele were significantly more common in patients with schizophrenia than in controls. Since nearly all the subjects had the same genotype for the other SNP, Arg1491Lys (rs1881420), the risk alleles constitute a haplotype 1491Lys–1529Asp. Thus, our results suggest that the 1491Lys–1529Asp haplotype or its homozygosity may confer susceptibility to schizophrenia. However, we do not know whether these nonsynonymous polymorphisms do alter functions of the ALK protein to give susceptibility to schizophrenia. Accordingly, there remains a possibility that other polymorphisms, which are in linkage disequilibrium to these polymorphisms, are truly responsible for giving susceptibility.

The ALK gene encodes a 1620 amino acid protein containing a putative 26 amino acid signal peptide, an extracellular domain of 1004 amino acid after signal peptide cleavage, a transmembrane domain of 28 hydrophobic amino acids, a juxtamembrane segment of 64 amino acids, a catalytic domain (protein tyrosine kinase domain) of 254 amino acids, followed by the carboxyl-terminal tail of 244 amino acids (Morris et al., 1997). The Arg1491Lys and Glu1529Asp residues lie close to a NPTY motif (residue 1504–1507) in the carboxyl-terminal tail (Morris et al., 1997). Such motifs mediate the interaction of RTKs with signaling substrates such as the insulin receptor substrate-1 and Src homology and collagen proteins through the substrates's phosphotyrosine binding (PTB) domain (van der Geer and Pawson, 1995). It is possible that amino acid changes of Arg1491Lys and Glu1529Asp may alter protein structure and affect functions (e.g., binding to these substrates).

ALK is a receptor-type protein kinase (RTK) that is expressed preferentially in neurons of the central and peripheral nervous systems at late embryonic stages (Iwahara et al., 1997; Morris et al., 1997). Neurotrophic factors exert their effects through binding to RTKs, and ALK is a receptor for heparin-binding growth factors, midkine and pleiotrophin (Stoica et al., 2001, 2002). Thus it is likely that ALK play an important role in neurodevelopment such as differentiation, proliferation, survival, neurite outgrowth and synaptic formation, and alterations of ALK functions may result in vulnerability to developing schizophrenia, which accords with the neurotrophic factor theory of schizophrenia (Thome et al., 1998; Durany and Thome, 2004). Indeed, alterations in other neurotrophic factors such as brain-derived neurotrophic factors (BDNF) and neurotrophin-3 have been implicated in schizophrenia (e.g., Durany et al., 2001; Nanko et al., 2003; Hattori et al., 2002).

A limitation in the present study might be that the obtained evidence for association was not very strong (p-values of $<0.01$ level in a single sample). Replication studies in independent samples are required. If our results are replicated, experiments elucidating the possible effects of the amino acid substitutions (Arg1491Lys and Glu1529Asp) on the ALK protein functions may serve to advance our understanding of the molecular mechanisms of schizophrenia and may provide clues to production of new treatment of the illness.

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## References

American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders, 4th edn. American Psychiatric Association, Washington DC

Clinton SM, Meador-Woodruff JH (2004) Thalamic dysfunction in schizophrenia: neurochemical, neuropathological, and in vivo imaging abnormalities. Schizophr Res 69: 237–253

Durany N, Michel T, Zochling R, Boissl KW, Cruz-Sanchez FF, Riederer P, Thome J (2001) Brain-derived neurotrophic factor and neurotrophin 3 in schizophrenic psychoses. Schizophr Res 52: 79–86

Durany N, Thome J (2004) Neurotrophic factors and the pathophysiology of schizophrenic psychoses. Eur Psychiatry 19: 326–337

Hashimoto R, Yoshida M, Ozaki N, Yamanouchi Y, Iwata N, Suzuki T, Kitajima T, Tatsumi M, Kamijima K, Kunugi H (2004) Association analysis of the −308G>A promoter polymorphism of the tumor necrosis factor alpha (TNF-alpha) gene in Japanese patients with schizophrenia. J Neural Transm 111: 217–221

Hashimoto R, Okada T, Kato T, Kosuga A, Tatsumi M, Kamijima K, Kunugi H (2005) The breakpoint cluster region (BCR) gene on chromosome 22q11 is associated with bipolar disorder. Biol Psychiatry 57: 1097–1102

Hattori M, Kunugi H, Akahane A, Tanaka H, Ishida S, Hirose T, Morita R, Yamakawa K, Nanko S (2002) Novel polymorphisms in the promoter region of the neurotrophin-3 gene and their associations with schizophrenia. Am J Med Genet 114: 304–309

Iwahara T, Fujimoto J, Wen D, Cupples R, Bucay N, Arakawa T, Mori S, Ratzkin B, Yamamoto T (1997) Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. Oncogene 14: 439–449

Kadomatsu K, Muramatsu T (2004) Midkine and pleiotrophin in neural development and cancer. Cancer Lett 204: 127–143

Moberg PJ, Turetsky BI (2003) Scent of a disorder: olfactory functioning in schizophrenia. Curr Psychiatry Rep 5: 311–319

Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Saltman DL, Look AT (1994) Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 263: 1281–1284

Morris SW, Naeve C, Mathew P, James PL, Kirstein MN, Cui X, Witte DP (1997) ALK, the chromosome 2 gene locus altered by the t(2;5) in non-Hodgkin's lymphoma, encodes a novel neural receptor tyrosine kinase that is highly related to leukocyte tyrosine kinase (LTK). Oncogene 14: 2175–2188

Nanko S, Kunugi H, Hirasawa H, Kato N, Nabika T, Kobayashi S (2003) Brain-derived neurotrophic factor gene and schizophrenia: polymorphism screening and association analysis. Schizophr Res 62: 281–283

Shimizu E, Hashimoto K, Salama RH, Watanabe H, Komatsu N, Okamura N, Koike K, Shinoda N, Nakazato M, Kumakiri C, Okada S, Muramatsu H, Muramatsu T, Iyo M (2003) Two clusters of serum midkine levels in drug-naive patients with schizophrenia. Neurosci Lett 344: 95–98

Shiota M, Fujimoto J, Semba T, Satoh H, Yamamoto T, Mori S (1994) Hyperphosphorylation of a novel 80 kDa protein-tyrosine kinase similar to Ltk in a human Ki-1 lymphoma cell line, AMS3. Oncogene 9: 1567–1574

Stoica GE, Kuo A, Aigner A, Sunitha I, Souttou B, Malerczyk C, Caughey DJ, Wen D, Karavanov A, Riegel AT, Wellstein A (2001) Identification of anaplastic lymphoma kinase as a receptor for the growth factor pleiotrophin. J Biol Chem 276: 16772–16779

Stoica GE, Kuo A, Powers C, Bowden ET, Sale EB, Riegel AT, Wellstein A (2002) Midkine binds to anaplastic lymphoma kinase (ALK) and acts as a growth factor for different cell types. J Biol Chem 277: 35990–35998

Thome J, Foley P, Riederer P (1998) Neurotrophic factors and the maldevelopmental hypothesis of schizophrenic psychoses. J Neural Transm 105: 85–100

van der Geer P, Pawson T (1995) The PTB domain: a new protein module implicated in signal transduction. Trends Biochem Sci 20: 277–280

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# Antipsychotic medication and cognitive function in schizophrenia

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**Abstract**

Antipsychotic polypharmacy and excessive dosing still prevail worldwide in the treatment of schizophrenia, while their possible association with cognitive function has not well been examined. We examined whether the "non-standard" use of antipsychotics (defined as antipsychotic polypharmacy or dosage >1000 mg/day of chlorpromazine equivalents) is associated with cognitive function. Furthermore, we compared cognitive function between patients taking only atypical antipsychotics and those taking only conventionals. Neurocognitive functions were assessed in 67 patients with chronic schizophrenia and 92 controls using the Wechsler Memory Scale-Revised (WMS-R), the Wechsler Adult Intelligence Scale-Revised (WAIS-R), the Wisconsin Card Sorting Test (WCST), and the Advanced Trail Making Test (ATMT). Patients showed markedly poorer performance than controls on all these tests. Patients on non-standard antipsychotic medication demonstrated poorer performance than those on standard medication on visual memory, delayed recall, performance IQ, and executive function. Patients taking atypical antipsychotics showed better performance than those taking conventionals on visual memory, delayed recall, and executive function. Clinical characteristics such as duration of medication, number of hospitalizations, and concomitant antiparkinsonian drugs were different between the treatment groups (both dichotomies of standard/non-standard and conventional/atypical). These results provide evidence for an association between antipsychotic medication and cognitive function. This association between antipsychotic medication and cognitive function may be due to differential illness severity (e.g., non-standard treatment for severely ill patients who have severe cognitive impairment). Alternatively, poorer cognitive function may be due in part to polypharmacy or excessive dosing. Further investigations are required to draw any conclusions.


*Keywords:* Schizophrenia; Cognitive function; Atypical antipsychotics; Conventional antipsychotics; Polypharmacy

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## 1. Introduction

Schizophrenia is associated with wide-ranging deficits in neurocognitive function, including memory, attention, executive function, and working memory (Bozikas et al., 2006; Fioravanti et al., 2005; Gold et al., 1992; Keefe et al., 2005; Reed et al., 2002; Silver et al., 2003; Suwa et al., 2004), and these deficits are considered to be core to the pathophysiology of the illness. Reports of cognitive impairments in schizophrenia date back to the pioneering efforts of Kraepelin (1919) and Bleuler (1950) and, more recently, the characteristics of these deficits have been clarified with increasing sophistication and precision. School children who will later develop schizophrenia are more likely than their classmates to under-perform in school (Erlenmeyer-Kimling et al., 2000; Fuller et al., 2002; Kremen et al., 1998; Reichenberg et al., 2005), and cognitive deficits become widely present at the onset of psychosis (Bilder et al., 1992; Hoff et al., 1992). Growing evidence has suggested that cognitive deficits in schizophrenia are not byproducts of positive symptoms (Addington et al., 1991; Davidson et al., 1995) or negative symptoms (Bell and Mishara, 2006; Harvey et al., 1996; Harvey et al., 2005).

As impaired performance on measures of neurocognition is more closely linked to functional outcome than symptoms (Green, 1996; Green et al., 2000), enhancement of cognitive functioning is considered an important component of treatment for schizophrenia (Green et al., 2005; Hofer et al., 2005). Investigators have focused on the cognitive pathology of schizophrenia and have sought to assess the effects of treatment on this dimension. A large number of treatment studies have demonstrated that therapeutic effects of conventional drugs are limited to the positive symptoms of the illness and they have substantially less impact on cognitive impairments (Medalia et al., 1988; Spohn and Strauss, 1989), whereas atypical antipsychotics may ameliorate cognitive deficits (Bender et al., 2006; Bilder et al., 2002; Harvey et al., 2006; Keefe et al., 1999, 2006; Kern et al., 1999; Meltzer and McGurk, 1999; Muller et al., 2005; Purdon et al., 2000; Rossi et al., 1997; Sumiyoshi et al., 2005; Thornton et al., 2006).

In spite of extensive research and recommendations as to the optimal prescription of antipsychotics, antipsychotic polypharmacy and excessive dosing are still highly prevalent worldwide, especially in Japan (Bitter et al., 2003; Chong et al., 2004; Faries et al., 2005; Ganguly et al., 2004; Procyshyn et al., 2001; Sim et al., 2004a; Weissman, 2002). This may be due in part to the scarcity of evidence for the possible association between antipsychotic medication in terms of dosage or type and cognitive function in these countries. In this context, the present study was aimed (1) to examine whether the "non-standard" use of antipsychotics (defined as antipsychotic polypharmacy or dosage >1000 mg/day of chlorpromazine equivalents) is associated with cognitive functions and (2) to compare cognitive deficits between patients treated with atypical antipsychotics and those with conventional drugs, using a comprehensive set of neurocognitive tests and by examining extensive clinical characteristics of patients.

## 2. Methods

### 2.1. Subjects

Patients with schizophrenia ($n=67$) who were under treatment at the National Center of Neurology and Psychiatry Musashi Hospital, Tokyo, Japan were recruited. All patients met the DSM-IV criteria (American Psychiatric Association, 1994) for schizophrenia. Consensus diagnosis was made by treating and research clinicians who were all senior psychiatrists, based on clinical interviews, observations, and case notes. Patients were chronic schizophrenia and were prescribed a stable dose of antipsychotic medication for at least 3 months prior to neuropsychological test sessions. Schizophrenic symptoms were rated by using the Positive and Negative Syndrome Scale (PANSS, Kay et al., 1987). Healthy volunteers ($n=92$) who had no history of current or past contact to psychiatric services were recruited from the hospital staffs and their associates through fliers and by word of mouth. Those individuals who had a history of regular use of psychotropic agents were not enrolled in the control group. Participants were excluded from both the patient and control groups if they had prior medical histories of central nervous system disease or severe head injury, or if they met criteria for alcohol/drug dependence or mental retardation. All subjects were biologically unrelated Japanese who resided in the same geographical area (Western part of Tokyo Metropolitan). Written informed consent was obtained from all subjects and the study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

### 2.2. Neuropsychological test measures

A comprehensive battery of neurocognitive tests was administered to all subjects in a random order that took at least 4 h to complete. The battery included the Wechsler Memory Scale-Revised (WMS-R, Sugishita, 2001; Wechsler, 1987), the Wechsler Adult Intelligence Scale-Revised (WAIS-R, Shinagawa et al., 1990; Wechsler, 1981), the Wisconsin Card Sorting Test (WCST, Heaton, 1981; Kashima et al., 1987), and the Advanced Trail Making Test (ATMT, Nakahachi et al., 2006; Takahashi et al., 2005).

#### 2.2.1. Wechsler Memory Scale-Revised

A full version of the WMS-R (Wechsler, 1987) was administered. The average score and standard deviation (S.D.) of WMS-R in the general population are 100 and 15, respectively. This test mainly measures memory functions, while it can also assess attention. Its four main outcome measures were verbal memory, visual memory, attention, and delayed recall.

#### 2.2.2. Wechsler Adult Intelligence Scale-Revised

A full version of the WAIS-R (Wechsler, 1981) was administered, yielding scores of verbal IQ, performance IQ, and full-scale IQ.

#### 2.2.3. Wisconsin Card Sorting Test

The WCST (Heaton, 1981) mainly assesses executive function including cognitive flexibility in response to feedback. We used a modified and computerized version of the test (Kashima et al., 1987; Kobayashi, 1999). Outcome measures were numbers of categories achieved, total errors, and perseverative errors of Milner and Nelson types.

#### 2.2.4. Advanced Trail Making Test

The ATMT (Takahashi et al., 2005) is a computerized task modified from the original Trail Making Test (Reitan and Wolfson, 1993), and is considered to measure subjects' abilities of spatial working memory and psychomotor speed. In the present study, only spatial working memory was rated in all subjects.

### 2.3. Grouping procedures

Daily doses of antipsychotics, including depot antipsychotics, were converted to approximate chlorpromazine equivalents (CPZeq) using published guidelines (American Psychiatric Association, 1997; Inagaki et al., 1999). The patient group was subdivided into two different types of subgroups by medication patterns. One grouping criterion was a "standard" or "non-standard" use of antipsychotics. The "standard" was defined as receiving antipsychotic monotherapy with a CPZeq dose of 1000 mg/day or less, and "non-standard" as polypharmacy (the use of more than one antipsychotic) or a CPZeq dose of more than 1000 mg/day. This classification was according to several precedent studies (Diaz and De Leon, 2002; Edlinger et al., 2005; Ito et al., 2005; Lehman and Steinwachs, 1998; Sim et al., 2004b; Waddington et al., 1998; Weissman, 2002). The other grouping criterion was whether patients were treated only with conventional or only with atypical antipsychotics, and those who were treated with both types of antipsychotics were excluded from this grouping criterion.

### 2.4. Statistical analyses

Averages are reported as means ± S.D. Demographic characteristics and test results were compared between groups. We used the *t*-test to compare mean scores. Categorical variables were compared with $\chi^2$ test or Fisher's exact test where appropriate. The analysis of covariance (ANCOVA) was used to compare neuropsychological test results of patients and those of controls, controlling for a confounding variable. All comparisons were made between two groups, namely between patients and controls, the "standard" and "non-standard" groups, or conventional and atypical groups. Statistical significance was set at two-tailed $p < 0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Tokyo).

## 3. Results

### 3.1. Sample characteristics

Demographic and clinical characteristics are presented in Table 1. There were no differences between patients and controls in sex, age, or handedness. Patients with schizophrenia demonstrated significantly shorter education years and a higher rate of cigarette smoking compared to controls. The "non-standard" group, as expected, showed significantly greater CPZeq and more frequent use of conventional antipsychotics and antiparkinsonian drugs than the "standard" group. The number of hospitalizations was significantly larger in "non-standard" than in "standard" group. The conventional group showed significantly longer duration of medication, more frequent use of antiparkinsonian drugs, and larger number of hospitalizations than the atypical group.

### 3.2. Neuropsychological test scores in patients vs. controls

Patients with schizophrenia showed significantly poorer performance than healthy controls on all the neuropsychological tests (Table 2). Although control

Table 1
Demographic and clinical characteristics of schizophrenia patients and controls (including comparisons of "standard" vs. "non-standard" and conventional vs. atypical group)

| Variable | Schizophrenia patients ($N$=67) | Healthy controls ($N$=92) | $p$ value (patients vs. controls) | Standard group ($N$=26) | Non-standard group ($N$=41) | $p$ value (standard vs. non-standard) | Conventional group ($N$=23) | Atypical group ($N$=22) | $p$ value (conventional vs. atypical) |
|---|---|---|---|---|---|---|---|---|---|
| Sex (male/female) | 40/27 | 54/38 | 0.90 | 14/12 | 26/15 | 0.44 | 16/7 | 11/11 | 0.18 |
| Age (years) | 42.7±11.9 | 43.0±14.5 | 0.90 | 42.4±14.5 | 42.9±10.1 | 0.87 | 46.2±11.7 | 39.8±13.6 | 0.095 |
| Education (years) | 13.4±2.7 | 16.4±3.1 | <0.001 | 13.8±2.1 | 13.0±3.0 | 0.24 | 13.5±3.3 | 13.6±2.2 | 0.93 |
| Handedness (right/left) | 64/3 | 84/8 | 0.50 | 26/0 | 38/3 | 0.28 | 22/1 | 22/0 | 1.0 |
| Smoking (yes/no) | 32/35 | 23/69 | 0.0029 | 12/14 | 20/21 | 0.83 | 12/11 | 10/12 | 0.65 |
| Family history of schizophrenia (yes/no) | 15/52 | | | 7/19 | 8/33 | 0.48 | 17/6 | 17/5 | 0.79 |
| Age at onset (years) | 25.0±8.3 | | | 26.2±9.4 | 24.2±7.5 | 0.33 | 24.2±7.2 | 26.7±10.1 | 0.34 |
| Duration of medication (years) | 13.3±10.4 | | | 10.6±12.8 | 15.1±8.3 | 0.090 | 17.9±11.5 | 8.2±9.9 | 0.0045 |
| CPZeq of total antipsychotic drugs (mg/day) | 877±749 | | | 473±268 | 1134±840 | <0.001 | 804±634 | 521±322 | 0.065 |
| Antipsychotics (conventional/both/atypical) | 23/22/22 | | | 5/0/21 | 18/22/1 | <0.001 | – | – | |
| Antiparkinsonian drug use (yes/no) | 48/19 | | | 14/12 | 34/7 | 0.010 | 2/21 | 12/10 | 0.0012 |
| Age at first hospitalization (years) | 29.1±12.2 | | | 29.0±15.2 | 29.1±10.7 | 0.98 | 30.3±10.7 | 27.5±14.9 | 0.52 |
| Number of hospitalizations | 2.1±2.1 | | | 1.2±1.7 | 2.7±2.1 | 0.0043 | 2.4±2.1 | 1.2±1.7 | 0.042 |
| Duration of total hospitalizations (months) | 35.0±78.4 | | | 27.0±68.8 | 40.1±84.4 | 0.51 | 46.3±107.2 | 20.0±53.1 | 0.31 |
| Outpatients/inpatients | 45/22 | | | 19/7 | 26/15 | 0.41 | 17/6 | 16/6 | 0.93 |
| PANSS total score | 63.1±17.4 | | | 59.7±17.9 | 65.1±17.1 | 0.36 | 63.3±17.0 | 57.6±16.4 | 0.39 |

Underlined figures represent significant differences.

Table 2
Test results of patients with schizophrenia and control subjects (including comparisons of standard vs. non-standard and conventional vs. atypical group)

| Variable | Patients with schizophrenia (N=67) | Control subjects (N=92) | p value (patients vs. controls) | Standard group (N=26) | Non-standard group (N=41) | p value (standard vs. non-standard) | Conventional group (N=23) | Atypical group (N=22) | p value (conventional vs. atypical) |
|---|---|---|---|---|---|---|---|---|---|
| WMS-R | | | | | | | | | |
| Verbal memory | 81.9±18.3 | 112.2±14.2 | <0.001 | 86.0±19.9 | 79.2±16.8 | 0.14 | 85.3±16.0 | 83.6±20.1 | 0.75 |
| Visual memory | 83.8±21.0 | 110.1±10.4 | <0.001 | 92.2±20.1 | 78.4±20.0 | 0.008 | 79.6±21.0 | 94.7±19.1 | 0.016 |
| Attention | 90.8±14.0 | 107.9±14.5 | <0.001 | 92.3±14.6 | 89.8±13.7 | 0.46 | 92.0±12.5 | 92.5±14.6 | 0.89 |
| Delayed recall | 80.4±19.8 | 113.2±12.8 | <0.001 | 88.8±20.0 | 75.1±17.8 | 0.005 | 78.2±14.7 | 90.8±20.6 | 0.023 |
| WAIS-R | | | | | | | | | |
| Information | 8.7±3.7 | 11.3±3.0 | <0.001 | 9.4±3.2 | 8.2±3.9 | 0.16 | 9.5±3.5 | 8.6±3.5 | 0.38 |
| Digit span | 8.5±2.8 | 11.4±3.1 | <0.001 | 8.7±2.5 | 8.4±3.0 | 0.74 | 8.5±3.3 | 8.8±2.0 | 0.72 |
| Vocabulary | 8.3±3.3 | 12.1±2.9 | <0.001 | 9.5±2.9 | 7.6±3.3 | 0.015 | 8.4±3.4 | 9.2±3.2 | 0.40 |
| Arithmetic | 8.0±3.2 | 12.6±3.0 | <0.001 | 8.3±2.6 | 7.8±3.5 | 0.47 | 8.7±3.4 | 8.3±3.0 | 0.66 |
| Comprehension | 7.6±3.3 | 11.7±2.8 | <0.001 | 8.4±3.3 | 7.2±3.3 | 0.15 | 7.1±3.0 | 8.3±3.4 | 0.22 |
| Similarities | 9.6±3.3 | 12.7±2.0 | <0.001 | 10.2±3.4 | 9.2±3.2 | 0.22 | 9.5±3.3 | 10.2±3.5 | 0.49 |
| Picture completion | 8.5±3.0 | 10.6±2.4 | <0.001 | 9.3±2.3 | 8.0±3.3 | 0.056 | 8.8±3.1 | 9.1±2.2 | 0.70 |
| Picture arrangement | 8.1±3.3 | 11.7±2.4 | <0.001 | 8.3±3.0 | 7.9±3.4 | 0.59 | 7.9±2.8 | 8.5±3.2 | 0.48 |
| Block design | 9.3±3.5 | 13.1±2.6 | <0.001 | 10.6±3.3 | 8.4±3.4 | 0.012 | 9.1±3.4 | 10.6±3.0 | 0.12 |
| Object assembly | 8.3±3.4 | 11.5±3.0 | <0.001 | 9.4±3.0 | 7.7±3.5 | 0.036 | 7.6±3.3 | 9.4±3.0 | 0.062 |
| Digit symbol | 7.0±2.8 | 12.6±2.7 | <0.001 | 7.2±2.6 | 6.8±3.0 | 0.58 | 7.6±2.1 | 7.1±2.8 | 0.52 |
| Verbal IQ | 90.2±16.7 | 112.7±13.7 | <0.001 | 94.4±14.2 | 87.6±17.8 | 0.090 | 91.3±16.2 | 93.0±15.3 | 0.72 |
| Performance IQ | 86.8±16.6 | 112.1±11.5 | <0.001 | 92.1±13.9 | 83.4±17.4 | 0.036 | 86.4±14.0 | 91.7±14.2 | 0.21 |
| Full-scale IQ | 87.7±17.0 | 113.5±12.4 | <0.001 | 92.7±13.9 | 84.4±18.0 | 0.0502 | 88.3±15.0 | 91.7±14.6 | 0.44 |
| WCST | | | | | | | | | |
| Categories achieved | 2.0±2.0 | 3.7±2.0 | <0.001 | 2.6±2.1 | 1.7±1.8 | 0.080 | 1.6±1.9 | 2.4±2.2 | 0.18 |
| Total errors | 26.1±10.3 | 18.4±8.5 | <0.001 | 22.1±9.8 | 28.7±9.9 | 0.010 | 30.6±11.3 | 21.9±10.0 | 0.0089 |
| Perseverative errors of Milner | 7.5±9.2 | 2.7±3.7 | <0.001 | 5.8±8.2 | 8.5±9.7 | 0.26 | 11.3±12.5 | 5.3±7.1 | 0.051 |
| Perseverative errors of Nelson | 9.9±8.9 | 4.9±5.2 | <0.001 | 7.2±8.1 | 11.5±9.1 | 0.055 | 13.5±11.3 | 6.0±7.0 | 0.0098 |
| ATMT (spatial working memory) | 27.8±8.9 | 35.8±8.6 | <0.001 | 30.3±8.7 | 26.3±8.8 | 0.068 | 27.3±8.4 | 30.4±9.1 | 0.25 |

Underlined figures represent significant differences.

subjects in the present study performed rather better than general population on the standardized WMS-R and WAIS-R, performance on all indices of the two tests in patients were poorer than that in general population. Since the difference in education years between the two diagnostic groups had a possibility of confounding the difference in the test results, we performed ANCOVA, controlling for education years. It revealed that all the performance on the tests were significantly poorer in patients than in controls (all $p<0.01$).

### 3.3. Test scores in standard vs. non-standard group

As presented in Table 2, mean scores on all indices of the cognitive tests were better in patients treated with the standard use of antipsychotics than in those treated with the non-standard use; seven measures reached statistical significance, i.e., visual memory, delayed recall (WMS-R), vocabulary, block design, object assembly, performance IQ (WAIS-R), and number of total errors (WCST).

### 3.4. Test scores in conventional vs. atypical antipsychotics group

Test results in patients treated with conventional drugs and those with atypical drugs are presented in Table 2. All mean scores except verbal memory (WMS-R), information, arithmetic, and digit symbol (WAIS-R) were favorable to the atypical antipsychotic group. The atypical group performed significantly better than the conventional group on visual memory, delayed recall, WCST total errors, and perseverative errors of Nelson.

## 4. Discussion

Our results confirmed that a wide range of cognitive functions including memory, attention, working memory, executive function, and general intellectual function are substantially impaired in patients with chronic schizophrenia, which is consistent with an abundance of studies (Bozikas et al., 2006; Fioravanti et al., 2005; Gold et al., 1992; Joyce and Huddy, 2004; Keefe et al., 2005; Reed et al., 2002; Silver et al., 2003; Suwa et al., 2004).

### 4.1. Standard vs. non-standard medication

Congruent with recent reports (Bitter et al., 2003; Chong et al., 2004; Sim et al., 2004a), non-standard use of antipsychotics (i.e., excessive use of antipsychotics or polypharmacy) was frequent in our Japanese patients with schizophrenia (standard 39% vs. non-standard 61%). Patients in non-standard group showed significantly poorer performance than those in standard group on visual memory, delayed recall, performance IQ, and executive function. Since the symptom severity (assessed with PANSS) of the two groups was similar, the difference in cognitive performance cannot be ascribed to difference in symptom severity at the time of neurocognitive tests. However, other clinical characteristics such as number of hospitalizations (with statistical significance) and duration of medication (with statistical trend), from which the original illness severity would be presumed, were different between the two treatment groups. In this situation, the illness of the non-standard group might be severer than that of the standard group at the outset, thus requiring the additional medication to reach the same level of improvement. Moreover, Joyce et al. (2005) reported that cognitive heterogeneity was present in patients with schizophrenia at illness onset. In this context, primary explanation for the association between differences of antipsychotic medication (standard/non-standard) and of cognitive function could be that both of them are due to the same cause, namely the difference of original illness severity. Alternatively, the other plausible explanation for the difference of cognitive performance between the two medication groups might be that polypharmacy and excessive dosing of antipsychotics have detrimental effects on brain and cause poorer cognitive function. This raises the possibility that cognitive deficits could be reduced by changing non-standard to standard prescription if symptoms of patients permit. Since the non-standard treatment group was more likely to be on concomitant antiparkinsonian medication, such drugs could also play a causal role in the poorer cognitive function, which was in line with prior reports (McGurk et al., 2004; Minzenberg et al., 2004; Strauss et al., 1990). To draw any conclusion, longitudinal studies that investigate from illness onset to chronic phase are necessary.

### 4.2. Conventional vs. atypical

When patients were divided into conventional and atypical antipsychotic groups, the latter demonstrated significantly better performance than the former on visual memory, delayed recall, and executive function. In our subjects, most patients in the atypical group were medicated with either risperidone or olanzapine. Indeed, these drugs have been reported to be superior to conventional drugs or even to other atypical antipsychotics (Bilder et al., 2002; Cuesta et al., 2001; Kern et al., 1999; McGurk et al., 2005; Mori et al., 2004;