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Letter to the editor

# A polymorphism of the methylenetetrahydrofolate reductase gene confers susceptibility to schizophrenia and related brain changes

*Letter to the Editor*

Folate is important in the human nervous system was demonstrated in previous studies. The 5,10-methylenetetrahydrofolate reductase (MTHFR) plays a central role in folate metabolism. Two common single nucleotide polymorphisms (SNPs) in *MTHFR* are known: a C → T transition at nucleotide 677 (rs1801133) and an A → C transversion at position 1298 (rs1801131). Associations of *MTHFR* polymorphisms with schizophrenia have been reported (Gilbody et al., 2007). In the present study, we examined whether genetic variations of *MTHFR* are associated with the development of schizophrenia in our Japanese patient series. We also investigated the potential influence of the disease-associated genotype of *MTHFR* on the regional brain structure measured by voxel-based morphometry (VBM) analysis.

The subjects were 538 patients with schizophrenia (302 men and 236 women), and 1263 healthy controls (393 men and 870 women). The study protocol was approved by the Ethics Committee of the National Center of Neurology and Psychiatry, Japan, and written informed consent was obtained for participation in the study from all subjects. Among the subjects, 64 male schizophrenia patients (50 subjects carried the 677T allele) and 67 healthy male controls (42 subjects carried the 677T allele) underwent brain magnetic resonance imaging (MRI). Genomic DNA was prepared from venous blood according to standard procedures. Rs1801131 and rs1801133 were genotyped using the Taqman 5′-exonuclease allelic discrimination assay. The allele-specific fluorescence was measured with an ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). The MRI data acquisition was described previously (Ota et al., 2016). MR images were processed using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/). We assessed the deviation of genotype distributions from the Hardy-Weinberg equilibrium (HWE) with the $\chi^2$ test for goodness of fit. Genotype distributions were compared between patients and controls using the $\chi^2$ test for independence. These tests were performed with SPSS software ver. 22 (SPSS Japan, Tokyo). Statistical analyses for MR images were performed by Statistical Parametric Mapping 8 (http://www.fil.icon.ucl.ac.uk/spm), using age as a nuisance variance.

Among the subjects as a whole, the genotype distribution and the allele distribution did not differ significantly between the patients with schizophrenia and the controls for either SNP (rs1801131; A/A:A/C:C/C = 358:163:16, rs1801133; C/C:C/T:T/T = 181:255:102 in schizophrenia patients, rs1801131; A/A:A/C:C/C = 820:395:47, rs1801133; C/C:C/T:T/T = 458:604:201 in control subjects). However, when the men and women were examined separately, a nominally significant difference in the genotype distribution for rs1801133 (C677T) was observed in the men (C/C:C/T:T/T = 88:155:59 in schizophrenia patients, and C/C:C/T:T/T = 151:183:59 in control subjects, $p = 0.028$) but not in the women ($p = 0.259$). When we further analyzed the observed difference in the genotype distribution for rs1801133 based on the recessive and dominant models, we observed a significant difference in the dominant model ($\chi^2 = 6.52$, $df = 1$, $p = 0.011$) in the male subjects. There was no significant difference in the genotype distributions of rs1801131 even when the subjects were stratified by gender. Based on the above, we grouped the male subjects for whom MRI data was available into four groups based on the case-control status and whether or not the subject carried the 677T allele. We first evaluated the differences in gray matter volume between the healthy male controls and the male patients with schizophrenia. In the patient group, there were significant gray matter volume reductions such as in insula, medial frontal gyrus, and hippocampus (Fig. 1A) compared to the healthy male controls. However, when we estimated the main effect of 'genotype,' we did not detect a significant difference in any brain regions between the participants with and without the 677T allele. We next estimated the genotype-diagnosis interaction effects. There was a significant interaction between diagnosis and genotype in the left hippocampus (Fig. 1B). We then used follow-up simple effect tests to explore the exact nature of the interaction. Our analysis revealed that the male controls with the 677T allele showed a significant volume reduction in the left hippocampus compared to that of the male controls without the 677T allele (Fig. 1C). In the patient group however, there were no significant volume changes between the groups with and without the 677T allele. When we compared the gray matter volume between the male controls without the 677T allele and the male patients without the 677T allele, we observed a significant volume reduction in bilateral insulae and hippocampi among the schizophrenia patients (Fig. 1D).

The results of our present analyses demonstrated a significant relationship between susceptibility to schizophrenia and the 677T allele only in the male participants. A meta-analysis showed that being a carrier of the 677T allele is a genetic risk factor for autism spectrum disorders as well (Shaik et al., 2016). Autism spectrum disorder is more common in males than in females (CDC, 2014), and individuals diagnosed with autism spectrum disorder in childhood have a substantial burden of co-occurring psychiatric disorders, such as schizophrenia spectrum disorder (Mouridsen et al., 2008). In light of these reports, it would not be entirely unexpected that an association exists between the *MTHFR* polymorphisms and susceptibility to schizophrenia only in males. When we estimated the genotype-diagnosis interaction effects, we observed significant interaction effects in the left hippocampus. This effect was observed between the healthy male controls with the 677T allele and the healthy male controls without the allele. Additionally, the schizophrenia patients without the 677T allele showed significant volume reductions of the hippocampi compared to the healthy subjects without the 677T allele. It is possible that the genotype effects

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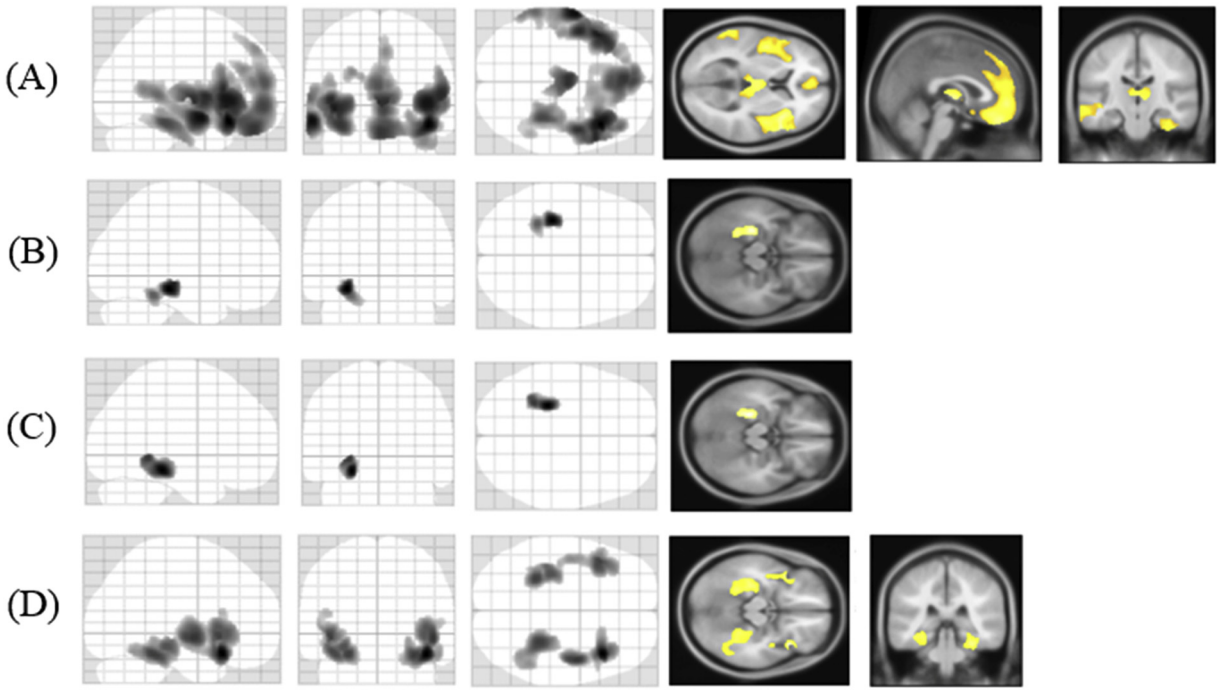


**Fig. 1.** Comparisons of gray matter volume across the patients with schizophrenia and the healthy controls with and without the 677T allele of *MTHFR*. A: There were significant gray matter volume reductions of the patients with schizophrenia in bilateral insulae, thalami, medial frontal gyri, anterior cingulate gyri, left temporal gyrus, and right hippocampus compared to the healthy male subjects. B: There was a significant interaction effect of genotype × diagnosis on the left hippocampus among the four groups. C: The healthy male subjects with the 677T allele showed a significant volume reduction in the left hippocampus compared to the healthy subjects without the 677T allele. D: There were significant volume reductions in the schizophrenic male patients without the 677T allele compared to the healthy male subjects without the 677T allele. Only differences that achieved a seed level of $p < 0.001$ (uncorrected) and a cluster level of $p < 0.05$ (uncorrected) were deemed significant.

in our schizophrenia patient group might be masked by the schizophrenia-related hippocampal shrinkage. It is known that the MTHFR 677T polymorphism causes a reduction of enzyme activity and increases the plasma homocysteine levels (Gilbody et al., 2007). Homocysteine is known to have neuronal toxicity by increasing the vulnerability to NMDA-mediated excitotoxicity (Kim and Pae, 1996) and oxidative injury (Kruman et al., 2000). Previous study showed that high plasma homocysteine levels were associated with hippocampus atrophy in healthy subjects (den Heijer et al., 2003).These are possible mechanisms underlying our present observation of an association between the C677T polymorphism and gray matter volume reduction in the hippocampus.

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### Conflict of interest

All authors declare that they have no conflicts of interest.

### Contributors

Miho Ota designed the study and wrote the first draft of the manuscript. Noriko Sato managed the analyses. Fuyuko Yoshida collected data. Kotaro Hattori collected data. Shinsuke Hidese collected data. Toshiya Teraishi collected data. Hiroshi Kunugi managed the analyses. All authors contributed to and have approved the final manuscript.

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## References

den Heijer, T., Vermeer, S.E., Clarke, R., Oudkerk, M., Koudstaal, P.J., Hofman, A., Breteler, M.M., 2003. Homocysteine and brain atrophy on MRI of non-demented elderly. Brain. 126, 170–175.

Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators; Centers for Disease Control and Prevention (CDC), 2014. Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. MMWR Surveill. Summ. 63. 1–21.

Gilbody, S., Lewis, S., Lightfoot, T., 2007. Methylenetetrahydrofolate reductase (MTHFR) genetic polymorphisms and psychiatric disorders: a HuGE review. Am. J. Epidemiol. 165, 1–13.

Kim, W.K., Pae, Y.S., 1996. Involvement of N-methyl-d-aspartate receptor and free radical in homocysteine-mediated toxicity on rat cerebellar granule cells in culture. Neurosci. Lett. 216, 117–120.

Kruman, I.I., Culmsee, C., Chan, S.L., Kruman, Y., Guo, Z., Penix, L., Mattson, M.P., 2000. Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. J. Neurosci. 20, 6920–6926.

Mouridsen, S.E., Rich, B., Isager, T., Nedergaard, N.J., 2008. Psychiatric disorders in individuals diagnosed with infantile autism as children: a case control study. J. Psychiatr. Pract. 14, 5–12.

Ota, M., Hori, H., Sato, N., Yoshida, F., Hattori, K., Teraishi, T., Kunugi, H., 2016. Effects of ankyrin 3 gene risk variants on brain structures in patients with bipolar disorder and healthy subjects. Psychiatry Clin. Neurosci. 70, 498–506.

Shaik, Mohammad N., Sai, Shruti P., Bharathi, V., Krishna, Prasad C., Hussain, T., Alrokayan, S.A., Naik, U., Radha, Rama Devi A., 2016. Clinical utility of folate pathway genetic polymorphisms in the diagnosis of autism spectrum disorders. Psychiatr. Genet. 26, 281–286.

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