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Association of *MTHFR* C677T polymorphism with schizophrenia and its effect on episodic memory and gray matter density in patients

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Abstract

Growing evidence suggests that the methylenetetrahydrofolate reductase (*MTHFR*) may play a role in the pathogenesis of schizophrenia. Recent studies suggested that the *MTHFR* 677T, as a risk allele, has an impact on brain activation and memory function in schizophrenia patients. To confirm further the association between this functional polymorphism and schizophrenia, we detected genotypes of *MTHFR* C677T polymorphism in 1,002 schizophrenic patients and 1,036 controls of Chinese Han population, by using direct DNA sequencing method. To explore further effects of *MTHFR* C677T polymorphism on memory and brain function in schizophrenia, 33 schizophrenia patients and 29 healthy participants were selected from above samples to be assessed with MRI scanning and episodic memory (EM) examination. The case - control association study results showed that the *MTHFR* C677T was associated with schizophrenia ($\chi^2 = 14.11$, $P = 1.74 \times 10^{-4}$, OR = 0.79; 95% CI = 0.70 – 0.89). We also found that the *MTHFR* 677T allele had a load-dependent effect on EM in schizophrenic patients, but not in healthy control participants. Further analysis on gray matter density (GMD) revealed significant diagnostic effects in bilateral frontal cortices, bilateral insula, left medial temporal cortex and bilateral occipital cortices, effects of *MTHFR* genotype in the right insula, right inferior frontal gyrus, right rolandic opercula, right parahippocampal gyrus and right medial temporal pole, and effects of genotype-diagnosis interaction in the right temporal gyrus. Our findings suggested that the *MTHFR* 677T allele might have effect on risk of schizophrenia, memory impairment and GMD changes in patients.

Keywords

schizophrenia; methylenetetrahydrofolate reductase (*MTHFR*); association study; gray matter density (GMD); episodic memory (EM)

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1. Introduction

Schizophrenia is a severe neuropsychiatric disorder with a lifetime risk of approximately 1%, characterized by profound disturbances of cognition, emotion, and social functioning. Family and twin studies have provided cumulative evidence for a genetic basis of schizophrenia [1–4]. The methylenetetrahydrofolate reductase (*MTHFR*) gene has been reported as one of the susceptibility genes of schizophrenia [5]. *MTHFR* is a key enzyme in the process of folate metabolism [6]. One common functional polymorphism in coding region of the *MTHFR* gene, C677T (rs1801133), may cause 35% of a reduction in enzyme activity in TT carriers [7]. Several studies previously published have focused on the relationship between the *MTHFR* C677T polymorphism and schizophrenia, however, the results of these studies are controversial. Various studies in subjects with different ethnic backgrounds have found that the *MTHFR* C677T polymorphism is a risk factor for schizophrenia [8–14]. However, other reports indicated a lack of association between *MTHFR* C677T polymorphism and schizophrenia [15–21]. Interestingly, meta-analyses hitherto published have provided evidence of the T-allele or the TT genotype as risk factors for schizophrenia [3,7,22–26]. Furthermore, we recently used a two-staged genome-wide association study (GWAS) and found that the *MTHFR* C677T polymorphism might be associated with schizophrenia in Chinese Han population [27]. The potential causes of the controversial findings of association between *MTHFR* C677T and schizophrenia might be explained by genetic heterogeneity across various populations and diversities of clinical phenotypes, especially for different endophenotypes, such as cognitive impairments.

Schizophrenia is characterized by heritable deficits in cognitive function, such as episodic memory (EM) impairments [28]. The contribution of genetic factors to the memory has been widely acknowledged. Deficits of EM together with attention and executive function are putative endophenotypes for schizophrenia [29]. The potential underlying mechanisms might involve the genes involved in the dopaminergic pathway, as well as *MTHFR* and catechol - O- methyltransferase (*COMT*). The *MTHFR* C677T has been associated with risk of schizophrenia and cognition impairments in patients, which may exert an effect on dopamine signaling pathway through changing upstream methylation of *COMT* [30–31]. Deficits of methylation of the *COMT* promoter region, which is strongly heritable, have been widely reported to play roles in the pathogenesis of schizophrenia [32–33]. The *MTHFR* 677T may contribute to diminished promoter methylation of *COMT* and then increased *COMT* expression, and further reduced function level of dopamine signaling pathway. Genetic risk for schizophrenia could affect functional activity in the brain; such changes have been shown to mediate disturbed memory function [34]. Understanding how risk genes cumulatively impair brain function in schizophrenia could provide critical insights into its pathophysiology. Collective data have suggested that the pathogenesis of schizophrenia was the results of interplay between genetic and environmental risk factors operating on brain or neural network maturational processes [35]. Neuroimaging studies have found significant gray matter density (GMD) reduction in certain frontal, temporal and subcortical regions, which somewhat explained the memory impairments in schizophrenic patients [36–38]. Moreover, recent twin studies provided evidence that cortical gray matter distribution was under significant genetic control in many brain regions [39–41].

To explore further association between the *MTHFR* C677T polymorphism and schizophrenia, we analyzed the *MTHFR* C677T polymorphism in another case-control sample independent from our previously published GWAS sample of Chinese Han population [27]. Furthermore, to explore whether the *MTHFR* C677T impacts on gross morphological changes in the brain cortex and examine the effect of this variant in the *MTHFR* gene on memory functions in patients with schizophrenia, we analyzed high resolution anatomical magnetic resonance images (MRI) with voxel-based morphometry

(VBM) technique and verbal EM in 33 schizophrenia patients and 29 normal healthy volunteers [42–43].

2. Methods

2.1. Participants

The study consisted of 1,002 patients with schizophrenia (540 males and 462 females; mean age 31.2 ± 9.9 years) and 1,036 healthy controls (602 males and 434 females; mean age 32.5 ± 8.3 years). The consensus diagnoses were made by at least two experienced psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, 4th ed (DSM-IV). Healthy controls were recruited from local communities with simple non-structured interview performed by psychiatrists, who excluded those individuals with history of mental health and neurological diseases. The healthy controls were drawn from the same geographical areas with patients, and well matched to patients group on gender, age and ethnicity. All participants were unrelated Chinese Han nationality born residing in northern China. Among these participants, 33 schizophrenia patients and 29 normal healthy volunteers were processed with MRI scanning and EM assessment. For the 33 patients, exclusion criteria were treatment with electroconvulsive therapy within the last 6 months, a history of seizure disorder or a serious medical illness. These patients were on anti-psychotic medication, and the medication dosage was converted to chlorpromazine equivalent. The symptom severity was assessed by a trained and experienced psychiatrist using the Positive and Negative Syndrome Scale (PANSS) within one week of MR scanning. All the 33 patients and 29 controls were right-handed, and without a history of head injury, neurological disorder, alcohol or substance abuse. There was no significant difference in sex proportion, mean age and years of education between the two groups (Table 1). The study was approved by the Medical Research Ethics Committee of the Institute of Mental Health, Peking University. All participants were given detailed verbal and written information regarding the purpose and procedures of the study. Written consents were obtained from the patients and/or their parents, and all healthy participants enrolled in this study.

2.2. Genotyping

Genomic DNA was extracted from venous blood using a commercially available QIAamp[®] DNA Blood Mini Kit (Qiagen Inc., Hilden, Germany). The SNP rs1801133 (C677T) of *MTHFR* gene was genotyped with the direct DNA sequencing method. The primers sequences were as follows: 5' AGCCCAGCCACTCACTGTTTT 3' and 5' CAGCGAACTCAGCACTCCA 3'. The PCR amplification was performed in a 25 μ l volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μ M of each dNTP, 0.25 μ M of each primer, 1U of Taq DNA polymerase and 40 ng genomic DNA. The conditions used for PCR amplification included an initial denaturation at 94 $^{\circ}$ C for 5 min, followed by 35 cycles at 94 $^{\circ}$ C for 30s, 64 $^{\circ}$ C for 30s, 72 $^{\circ}$ C for 30s, and a final elongation at 72 $^{\circ}$ C for 7 min. The PCR products were sequenced by DNA sequencing after cleaning the PCR product using BigDye Terminator Cycle Sequencing Ready Reaction Kit with Ampli-Taq DNA polymerase (PE Biosystem). The inner primers were used for the cycle-sequencing reaction, and fragments were separated by electrophoresis on an ABI PRISM 377-96 DNA sequencer (Applied Biosystem). According to the ABI standardized protocol, we have used the positive control DNA sequence in the sequencing process. Moreover, 51 samples (2.5%) have been randomly genotyped twice and no genotyping errors have been found.

2.3. Genotyping data analysis

Deviation of the genotype counts from the Hardy-Weinberg equilibrium was tested using a Chi-square goodness-of-fit test. Comparisons of gender and age were performed by using Chi-square test and Student's *t*-test respectively with the SPSS version 16.0 (Inc., Chicago,

IL, USA). Statistical differences in genotypic and allelic between schizophrenia and control participants were evaluated by the Chi-square test, by using the Haploview version 4.0 [44]. Statistical power estimate for case-control association analysis was obtained with the Genetic Power Calculator [45]. Results were considered significant at two-tailed $p < 0.05$.

2.4. Episodic memory (EM) examination

Verbal EM was assessed with subtest of Logical Memory from the Wechsler Memory Scale-Chinese Revised (WMS-CR). Participants were presented auditorily two story passages and were asked to recall each story immediately after hearing it using as many of the same words of the original passage as they could remember. There are 50 gists (important story ideas units) in the two story passages. The gist recall was evaluated in each participant. The full score was 25 points (0.5 for each gist), then the raw score was converted to scale score according to converting table in the manual of WMS-CR, which was defined on the basis of the common model of Chinese adults. One patient did not finish the EM assessment. The scale score of EM was evaluated using the univariate analysis of variance, with “diagnosis” and “genotype” (the genotype of rs1801133 of *MTHFR*) as fixed factors, by using SPSS for windows (SPSS 13.0, SPSS Inc, Chicago, IL, USA). In order to further control the confounding effects, we also did another univariate analysis of variance, with “diagnosis” and “genotype” as fixed factors, with age, sex, years of education and medication as covariates.

2.5. MRI examination

2.5.1. MRI data acquisition—Structural MRI scans were obtained at the Department of the Radiology, the Third Hospital, Peking University, with a 3.0-Tesla Magnetom Trio MR system (Siemens Medical System, Erlangen, Germany). High-resolution T1-weighted images were acquired in a sagittal orientation employing a 3D-MPRAGE sequence with the following parameters: time repetition = 2350 ms, time echo = 3.44 ms, field of view = $256 \times 256 \text{ mm}^2$, flip angle = 7° , 192 sagittal slices, slice thickness = 1 mm, matrix size = 256×256 , total acquisition time = 363 seconds.

2.5.2. MRI data preprocessing—The MRI data were processed with the Statistical Parametric Mapping Software (SPM5, Wellcome Department of Imaging Neuroscience, London; <http://www.fil.ion.ucl.ac.uk/spm>). The voxel-based morphometry (VBM) analysis was performed using the VBM5 toolbox (<http://dbm.neuro.uni-jena.de>), which implements segmentation algorithm from SPM5 and extends the core segmentation algorithm by using the Hidden Markov Random Field (HMRF) approach [42]. This procedure yielded modulated and unmodulated types of tissues images. Unmodulated gray matter images preserve the original MR signal intensity of each voxel, which allow detection of relatively subtle changes in tissue contrast (i.e., gray matter density [GMD]). In present study, according to our previous experience [46–47], only unmodulated images were used for GMD analysis. Resulting gray matter images were smoothed with a 12mm full width-half maximum (FWHM) Gaussian kernel.

2.5.3. MRI data statistical analysis—The images were analyzed within the framework of the general linear model implemented in SPM5. To determine the main effects and interaction effect of “diagnosis” by “genotype (the genotype of rs1801133 of *MTHFR*)”, we used a full factorial model with two factors (diagnosis and genotype) using age, gender as covariates. Cumulative evidence from neuroimaging studies has suggested that GMD was reduced in schizophrenia, but few studies have reported the effect of *MTHFR* C677T polymorphism on brain morphological changes. Therefore, in this exploratory analysis, the P value of voxel level was set at 0.01 for “diagnosis” effect, while for the main effect of “genotype” and the interaction effect of “diagnosis” by “genotype”, the P value of voxel

level was relaxed to 0.05, as we expected the effect of “genotype” to be more subtle. Results were considered significant after correction for multiple comparisons across the whole brain and correction for non-stationary smoothness at the cluster level ($P < 0.05$) [48].

To investigate the correlation of EM with GMD in schizophrenia, the brain regions showing significant effects of “diagnosis”, “genotype” and “diagnosis” by “genotype” were combined as a brain mask. Within the combined mask, a correlation of EM with GMD was conducted in the patient group, with the age, sex and medication as covariates. A height threshold of $P_{\text{voxel level}} < 0.05$ and $P_{\text{cluster level}} < 0.05$ was used for multiple comparisons correction.

The brain imaging results were labeled with the Automated Anatomical Labeling (AAL) software in combination with the Brodmann templates implemented in MRIcroN software (www.mricro.com) [49]. The GMD value of the peak voxels in the group analysis was extracted for the subsequent analysis by using the MarsBar toolbox (<http://marsbar.sourceforge.net/>).

3. Results

3.1. Case - control association of *MTHFR* C677T polymorphism with schizophrenia

The sample size was sufficient to detect a difference for rs1801133 with a genetic power of about 80% assuming an odds ratio (OR) of 1.27 with a risk allele frequency of 0.609. The genotype proportions did not deviate significantly from those expected under conditions of Hardy-Weinberg equilibrium among healthy controls ($\chi^2 = 0.23$, $df = 1$, $P = 0.62$) or patients ($\chi^2 = 2.81$, $df = 1$, $P = 0.09$).

Genotype and allele distributions are shown in Table 2. There was a significant difference both in genotype frequencies ($\chi^2 = 14.41$, $df = 2$, $P = 7.44 \times 10^{-4}$) and in allele frequencies ($\chi^2 = 14.11$, $df = 1$, $P = 1.74 \times 10^{-4}$, OR = 0.79; 95% CI = 0.70 – 0.89) between patients and controls. The *MTHFR* 677T allele shown associated with schizophrenia as a risk allele (OR = 1.27, 95% CI = 1.12–1.43) for schizophrenia in our Chinese Han sample.

3.2. Association of the EM with *MTHFR* C677T polymorphism

The result revealed significant main effect of “diagnosis” ($F[1, 61] = 6.482$, $P = 0.014$) and interaction effect of “diagnosis” by “genotype” ($F[2, 61] = 4.348$, $P = 0.018$), but no significant main effect of “genotype” ($F[2, 61] = 0.792$, $P = 0.458$). With the age, sex, years of education and medication as the covariates, the results remained unchanged. The EM score showed significant main effect of “diagnosis” ($F[1, 61] = 4.808$, $P = 0.033$) and interaction effect of “diagnosis” by “genotype” ($F[2, 61] = 3.510$, $P = 0.037$), but no significant main effect of “genotype” ($F[2, 61] = 0.400$, $P = 0.672$). The scale score of EM was significantly reduced in the patients group (9.3 ± 2.0), compared with that in the control group (11.0 ± 1.8), which demonstrated that the episodic memory was impaired in patients with schizophrenia. Follow-up tests for simple effects showed that the “genotype” effect was significant in the patient group but not in the control group. The risk T allele of *MTHFR* had an allele-load-dependent decrease effect on EM in schizophrenic patients (Table 3 and Figure 1). There was no significant difference in sex proportion, age, years of education and clinical data between different *MTHFR* genotype carriers in controls and patients groups, respectively (Table S1).

3.3. Association of the brain GMD with *MTHFR* C677T polymorphism

3.3.1. Morphological changes in schizophrenia (diagnosis effects)—The covariate analysis revealed a significant main effect of “diagnosis” in bilateral temporal

cortices, right frontal cortex, left parietal cortex, left cuneus, right calcarine sulcus, left occipital cortex, etc. (Figure S1, Table S2). The GMD in those brain areas with significant “diagnosis” effect was reduced in patients with schizophrenia. There were no significant GMD increases in schizophrenic patients compared to healthy controls.

3.3.2. Morphological changes associated with *MTHFR* C677T polymorphism (genotype effects)

—The covariate analysis revealed significant main effect of “genotype” in parahippocampal gyrus, temporal pole, inferior frontal cortex and frontal operculum. The post hoc analysis of the peak-voxel-GMD values for the 6 survived clusters showed that the individuals with CT genotype had significantly higher GMD compared with the individuals with risk homozygotes TT genotype. For the right insula (Brodmann area [BA] 48) and the right inferior frontal cortex (BA 44), the individuals with CT genotype had significantly higher GMD than homozygotes. There were no significant differences of GMD between TT and CC carriers. (Figure S2, Table S3).

3.3.3. Genotype-diagnosis interaction effects

—Our results revealed that a significant interaction effects of “diagnosis” by “genotype” on GMD was in the right temporal cortex (Figure 2 and Table 4).

Since disease-genotype interaction effects were found, follow-up simple effect tests were used to explore the exact nature of the interaction, i.e., the effect of *MTHFR* genotypes within patient and control group separately. We found that, in healthy control group, the CT carriers had significantly higher GMD than homozygotes. In the patient group however, the CT carriers had non-significantly lower GMD than homozygotes. Moreover, the GMD significantly reduced in CT carriers in patients compared with those in controls.

3.3.4. Correlation of EM with GMD

—In the above brain regions showing significant effect of “diagnosis” and “genotype” and interaction effect of “diagnosis” by “genotype”, we found that, in no region, EM showed significant correlation with GMD in patient group.

4. Discussion

In the present case-control association study, we investigated the *MTHFR* C677T polymorphism to explore its role in susceptibility to schizophrenia in a Chinese Han sample, which was independent from our previously GWAS samples [27]. Our study further verified a significant association between the *MTHFR* C677T polymorphism and schizophrenia in the Chinese Han population. When comparing with healthy controls, the T allele was over-expressed in patients with schizophrenia (frequencies of T allele were 0.609 in cases vs. 0.551 in controls, $P = 1.74 \times 10^{-4}$, Table 2). There were also significant differences in the frequency distributions of genotypes between the schizophrenic patient and control groups ($P < 0.05$).

Our findings are in agreement with previous case-control reports that showed an association of *MTHFR* C677T polymorphism with schizophrenia [8–14]. These results strongly suggest that the *MTHFR* gene is involved in the pathogenesis of schizophrenia. On the other hand, there are also previous reports showing a lack of association between *MTHFR* C677T and schizophrenia [15–21]. This may be explained as followings, the subpopulation genetic heterogeneity, the clinical heterogeneity due to differences in diagnostic instruments and procedures, confounding by environmental risk factors, etc. We have checked the HapMap database (www.hapmap.org) and found that the frequency of allele T in our sample (T frequency of 0.551) was similar with that of the HapMap HCB sample (T frequency of 0.511). Moreover, according to the meta-analysis reported by the SZgene database (www.szgene.org) [5], the T allele of *MTHFR* C677T also showed a significant association

with schizophrenia (OR = 1.29, 95%CI = 1.07–1.56) in seven independent Asia populations [8,9,17,21,50–52]. But the heterogeneity test showed that $I^2 = 66\%$ for above meta-analysis, which suggested there might be subpopulation heterogeneity across different studies [53]. As a complex and chronic brain disorder, schizophrenia is thought to be caused by multiple interacting genes influenced by environmental factors [54]. However, Muntjewerff's report did not indicate the evidence for interaction between *MTHFR* 677TT genotype and winter birth in the development of schizophrenia [55]. It also required further exploration on the other confounding environmental risk factors and the methylation mechanism of *MTHFR* in pathogenesis of schizophrenia.

Since genetic risk for schizophrenia could affect functional activity in the brain and verbal EM deficits are putative endophenotypes for schizophrenia, we examine the effect of this variant in the *MTHFR* gene on memory functions. Our EM assessment revealed significant main effect of “diagnosis” and interaction effect of “diagnosis” by “genotype”. In agreement with previous studies of executive function in schizophrenia [31,56], we found that the *MTHFR* genotype (the risk T allele) had a load-dependent effect on EM in schizophrenic patients, but not in healthy controls. Schizophrenia is increasingly considered to be a neurodevelopmental disorder [57], with in-utero exposures and epigenetic mechanisms such as DNA methylation being important in its etiology [58–59]. *MTHFR* is a critical component of the 1-carbon cycle, and the *MTHFR* polymorphisms C677T affect both nucleotide synthesis and DNA methylation. Thus, it may be speculated that deviant DNA methylation is one possible mechanism by which aberrant one-carbon metabolism influences EM in schizophrenia. In addition, homocysteine may also be toxic to dopaminergic neurons and has been shown to affect adversely dopamine turnover in the striatum [60]. Muntjewerff et al. provided evidence for an association of homocysteine with schizophrenia [23]. We think that the disturbed homocysteine metabolism may be the second possible mechanism affecting EM in schizophrenia.

In this study, we found reduction of GMD in the bilateral frontal cortices, bilateral insula, left medial temporal cortex and bilateral occipital cortices in schizophrenic patients when compared to healthy controls. The findings are concordant with previous neuroimaging studies of schizophrenia [36–37,61].

We found significant genotype effects on brain GMD in the right insula, right inferior frontal gyrus, right rolandic opercula, right parahippocampal gyrus, and right medial temporal pole. In such brain regions, the CT carriers showed greater GMD than the homozygotes. Our results also revealed that the interaction effects of disease and *MTHFR* C677T polymorphism on GMD in the right temporal gyrus. Further analysis revealed that the interaction effect may come from the different effect of the *MTHFR* C677T polymorphism on the GMD in patients and controls, i.e., CT carriers in controls had higher GMD than homozygotes; CT carriers in patients had lower GMD than homozygotes, though non-significant.

Previous studies indicate that dopamine impacts on prefrontal cortex function in the light of an inverted U-shaped dose–response curve [62–63]. Roffman et al. recently demonstrated that the *MTHFR* 677T allele could exacerbate prefrontal dopamine deficiency through affecting methylation of the *COMT* promoter [30–31]. These studies might suggest that the response is optimized in participants with the CT genotype, whose dopamine level is optimal. Though we found no significant correlation between the EM and GMD in schizophrenic patients, some regions showing significant “diagnosis” effect, “genotype” effect and interaction effects of “diagnosis” by “genotype” in the current study have been proved to play an importance role in human episodic memory, including the hippocampus and adjacent medial temporal lobe structures regions, as well as dorsolateral prefrontal

cortex [64]. In those specific brain regions, the GMD reduction in patients with schizophrenia might be associated with the activation alternation and accordingly influence the relevant cognitive function, such as EM. Thus, we inferred that the *MTHFR* 677T risk allele may play a role on brain GMD, and further affect EM function in schizophrenia. Several limitations to this study are important to consider. First, only a few of patients and controls were selected to administer the EM and MRI examinations. And the genotyping of *MTHFR* 677T allele further reduced the sample size, especially the CC genotype group. Although the number of participants in each diagnosis and genotype may be enough for a neuroimaging study on GMD, the group sizes were relatively small for a cognitive function study on EM. Second, because of limited sample size, we cannot introduce other factors to interpret further different effects of *MTHFR* C677T polymorphism on the GMD in schizophrenic patients and healthy controls, e.g., other SNP sites or genes [30]. Therefore, our findings regarding effect of *MTHFR* C677T polymorphism on the EM and brain morphology, and correlation analysis between EM and GMD are tentative and need independent replication. We conclude that the *MTHFR* C677T polymorphism is associated with an increased risk for schizophrenia, and can affect EM function and brain GMD in schizophrenic patients. The deviant DNA methylation is important in the etiology of schizophrenia, while the *MTHFR* C677T polymorphism cannot play an independent role on the DNA methylation. The interaction of *MTHFR* C677T polymorphism with other genes or genotypes on the cognition and brain morphology needs to be further investigated in a larger sample in our future study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

MTHFR C677T polymorphism might be a risk factor for schizophrenia in Chinese.

MTHFR 677T might play a role on the gray matter density in schizophrenia.

MTHFR 677T might be associated with the episodic memory in schizophrenia.

Figure 1. Load-dependent effects of *MTHFR* C677 genotypes on EM in schizophrenic patients but not in healthy controls. Error bars ± 1.00 SD.

Figure 2. Interaction effects of "diagnosis" by "genotypes" on gray matter density (GMD). Statistical parametric maps were superimposed on the MNI152 template. Result was corrected at $P < 0.05$ and shown in warm color. Color bar indicated the F value. L, left hemisphere; R, right hemisphere.

Table 1

Demographic and clinical details.

Groups	Healthy controls N = 29		Schizophrenia patients N = 33		X ²	P (2-tailed)
	N	%	N	%		
Sex (female)	12	41.4	14	42.4	0.007	0.934
Age (years)	Mean	SD	Mean	SD	t	P (2-tailed)
Age (years)	23.2	3.0	23.5	4.0	0.307	0.760
Education (years)	14.4	2.0	13.7	2.1	-1.366	0.177
Age at onset of illness (years)			19.5	3.4		
Duration of illness (months)			41.3	33.1		
Medication dose (mg)			437.3	341.9		
PANSS_T ^a			67.8	11.5		
PANSS_P ^b			19.6	4.5		
PANSS_N ^c			15.9	4.5		
PANSS_G ^d			32.2	5.4		

^aPANSS_T, total score of PANSS;

^bPANSS_P, score of PANSS positive subscale;

^cPANSS_N, score of PANSS negative subscale;

^dPANSS_G, score of PANSS general psychopathology subscale.

Comparison of genotype and allele frequencies of the *MTHFR* C677T polymorphism between schizophrenia cases and healthy control participants.

Table 2

rs1801133	Cases N (Freq.) ^a	Controls N (Freq.) ^a	$\chi^2(df)$	P-value	OR (95% CI) ^b
Genotypes					
CC	166(0.166)	213(0.206)			
CT	450(0.451)	505(0.487)	14.41 (2)	7.52×10 ⁻⁴	
TT	384(0.383)	318(0.307)			
Alleles					
C	784(0.391)	931(0.449)			
T	1220(0.609)	1141(0.551)	14.11 (1)	1.74×10 ⁻⁴	0.79(0.70–0.89)

^aNumber and frequencies of the genotype or alleles in schizophrenic patients and healthy control participants.

^bOR, odds ratio; 95% CI, 95% confidence intervals.

The P- values of Hardy-Weinberg equilibrium tests (HWEP) were 0.09 for cases (n=1,002), and 0.62 for controls (n=1,036), respectively.

The simple effect analysis of the *MTHFR* genotype effects on EM in schizophrenic patients and healthy control participants.

Table 3

Group	Genotype carriers (N ^a)	EM ^b			F-value	P-value
		Contrast	S.E. ^c	Estimate		
Schizophrenia patients	TT (11) vs. CT (16)	-1.534	0.699	4.810	0.033	
	TT (11) vs. CC (5)	-2.909	0.963	9.122	0.004	
	CT (16) vs. CC (5)	-1.375	0.915	2.258	0.139	
Healthy controls	TT (8) vs. CT (17)	0.500	0.766	0.426	0.516	
	TT (8) vs. CC (4)	1.250	1.094	1.306	0.258	
	CT (17) vs. CC (4)	0.750	0.992	0.571	0.453	

^aNumber of the genotype in schizophrenic patients and healthy control participants.

^bEM, episodic memory.

^cS.E., standard error.

Table 4

Genotype-diagnosis interaction effects on gray matter density.

Regions	BA ^a	Cluster size	F value	Coordinates ^b
Temporal_Sup_R	21	5278	6.24	[66, -3, -6]
Temporal_Mid_R	21		6.14	[71, -32, -5]
Temporal_Mid_R	37		6.06	[68, -47, -5]

^aBA, Brodmann area;^bThe peak voxel in MNI coordinates (x, y, z).