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Meta-analyses of Blood Homocysteine Levels for Gender and Genetic Association Studies of the *MTHFR* C677T Polymorphism in Schizophrenia

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Previous studies suggest that elevated blood homocysteine levels and the methylenetetrahydrofolate reductase (*MTHFR)* C677T polymorphism are risk factors for schizophrenia. However, the effects of gender and *MTHFR* C677T genotypes on blood homocysteine levels in schizophrenia have not been consistent. We first investigated whether plasma total homocysteine levels were higher in patients with schizophrenia than in controls with stratification by gender and by the *MTHFR* C677T genotypes in a large cohort (*N* = 1379). Second, we conducted a meta-analysis of association studies between blood homocysteine levels and schizophrenia separately by gender (*N* = 4714). Third, we performed a case-control association study between the *MTHFR* C677T polymorphism and schizophrenia (*N* = 4998) and conducted a meta-analysis of genetic association studies based on Japanese subjects (*N* = 10 378). Finally, we assessed the effect of plasma total homocysteine levels on schizophrenia by a mendelian randomization approach. The ANCOVA after adjustment for age demonstrated a significant effect of diagnosis on the plasma total homocysteine levels in all strata, and the subsequent meta-analysis for gender demonstrated elevated blood homocysteine levels in both male and female patients with schizophrenia although antipsychotic medication might influence the outcome. The meta-analysis of the Japanese genetic association studies demonstrated a significant association between the *MTHFR* C677T polymorphism and schizophrenia. The mendelian randomization analysis in the Japanese populations yielded an OR of 1.15 for schizophrenia per 1-SD increase in plasma total homocysteine. Our study suggests that increased plasma total homocysteine levels may be associated with an increased risk of schizophrenia.

Key words: mendelian randomization/SNP/Japanese/plasma homocysteine

Introduction

Schizophrenia is a devastating psychiatric disorder with a median lifetime prevalence rate of 0.7%–0.8%.[1] Accumulating evidence has shown that alterations in 1-carbon metabolism might play an important role in the pathogenesis of schizophrenia.[2,3] A number of studies have been conducted to evaluate the association between blood homocysteine levels and schizophrenia. The majority of these studies have demonstrated elevated blood homocysteine levels in patients with schizophrenia compared with controls.[4–24] However, several studies have reported no significant diagnostic differences in the blood homocysteine levels between the 2 groups.[25–31]

To date, 1 study has examined an association between blood homocysteine levels and schizophrenia by conducting a meta-analysis of 8 case-control studies (a total number of 812 cases with schizophrenia and 2113 control subjects) and demonstrated that a 5 μmol/l increase in homocysteine concentration was associated with a higher risk of schizophrenia (OR = 1.7; 95% CI = 1.27–2.29).[32]

However, this meta-analysis was performed without consideration of the effect of gender. Higher blood homocysteine levels in men than in women have been reported,[33] and the results of the previous association studies between blood homocysteine levels and schizophrenia with stratification by gender are inconclusive. In some studies, elevated blood homocysteine levels were observed in only male patients with schizophrenia and not in female patients,[5,6,10,19] whereas other studies have demonstrated that both male and female patients with schizophrenia had increased blood homocysteine levels.[11,17]

Blood homocysteine levels are also influenced by genetic variations.[34,35] Among these variants, 1 common functional single nucleotide polymorphism (SNP) of the methylenetetrahydrofolate reductase (*MTHFR*) gene, C677T (rs1801133), has been investigated well. The *MTHFR* C677T polymorphism results in amino acid substitution (Ala222Val) and causes a reduction of enzyme activity and higher homocysteine levels.[36] The results of the previous association studies between blood homocysteine levels and schizophrenia with stratification by C677T genotypes are inconclusive. A significant diagnostic difference in blood homocysteine levels has been found only in the subjects carrying the CT genotype or only in the subjects carrying the TT genotype.[7,18] However, Feng et al[16] showed a significant diagnostic difference for both CT and TT genotype carriers.

Many genetic case-control association studies between the *MTHFR* C677T polymorphism and schizophrenia have been performed in various populations, and the results of these association studies are not consistent. Only 1 study of the Japanese population reported a significant association between the *MTHFR* C677T polymorphism and schizophrenia, while the other 3 studies of the Japanese population have not replicated this positive finding.[37–40] However, several meta-analyses of association studies have revealed a significant association between this SNP and schizophrenia.[39,41–47]

In this study, we first investigated whether plasma total homocysteine levels were higher in patients with schizophrenia than in nonpsychiatric controls with stratification by gender and by the *MTHFR* C677T genotypes in a large cohort (N = 1379). Second, we conducted a meta-analysis of association studies between the blood homocysteine levels and schizophrenia separately by gender to evaluate a precise estimation of the association (N = 4714). Third, we performed a case-control association study between the *MTHFR* C677T polymorphism and schizophrenia (N = 4998) and carried out a meta-analysis of genetic association studies of this SNP with schizophrenia based on Japanese subjects to determine whether the *MTHFR* C677T polymorphism was genetically implicated in schizophrenia in the Japanese population (N = 10 378). Finally, we assessed the effect of plasma total homocysteine levels on schizophrenia by a mendelian randomization (MR) approach, a useful tool for assessing causal associations in observational data.[48,49]

Methods

Subjects of the Association Study Between the Plasma Total Homocysteine Levels and Schizophrenia

Three hundred and eighty-one patients with schizophrenia (225 men, mean age: 58.2 ± 9.3 y; 156 women, mean age: 59.4 ± 9.7 y) were recruited from Tokushima University Hospital in Japan. The diagnosis of schizophrenia was made according to Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria by at least 2 expert psychiatrists on the basis of extensive clinical interviews and a review of medical records. None of the patients had any psychiatric comorbidity or cardiovascular diseases. All patients were treated with various antipsychotic drugs. The mean chlorpromazine equivalent dose was 689.6 ± 581.3 mg/d. Nine hundred and ninety-eight control subjects (331 men, mean age: 38.3 ± 12 y; 667 women, mean age: 43.0 ± 11.9 y) were selected from volunteers who were recruited from hospital staff, students, and company employees documented to be free from psychiatric problems and past histories of mental illness. All subjects who participated in this study were of Japanese origin. All subjects signed written informed consent approved by the institutional ethics committees of the University of Tokushima Graduate School. Of 1379 subjects used in this association study, 1357 with genomic DNA (377 patients and 980 controls) were used in the next genetic association study.

Subjects of the Association Study Between the MTHFR C677T Polymorphism and Schizophrenia

Two case-control sets were used: the Tokushima sample set (A southern island of Japan) and the Osaka sample set (Midwestern Japan). Both sets have been described in previous studies.[50,51] For the Tokushima sample set, 1149 patients with schizophrenia (676 males, 473 females, mean age: 54.6 ± 14.9 y) were recruited from the Tokushima and Kochi University Hospitals in Japan. The diagnosis of schizophrenia was made according to DSM-IV criteria. A total of 2742 control subjects (1230 males, 1512 females, mean age: 38.8 ± 12.6 y) were selected from volunteers. For the Osaka sample set, 621 patients with schizophrenia (302 males, 319 females, mean age: 46.5 ± 15.8 y) were recruited from Osaka University Hospital in Japan. The diagnosis of schizophrenia was made according to DSM-IV criteria. A total of 486 control subjects (231 males, 255 females, mean age: 35.0 ± 12.7 y) were selected from volunteers. All subjects signed written informed consent approved by the institutional ethics committees of the University of Tokushima Graduate School, Kochi Medical School, and University of Osaka Graduate School.

Plasma Total Homocysteine Analysis

Plasma total homocysteine levels were measured by high-performance liquid chromatography. Homocysteine was labeled with 4-fluoro-7-sulfamoylbenzofurazan and detected by a fluorescent detector according to the method of a previous study.[52]

MTHFR Genotyping

We genotyped the *MTHFR* C677T polymorphism by using a commercially available TaqMan probe with the Applied Biosystems 7500 Fast Real Time PCR System, according to the protocol recommended by the manufacturer (Applied Biosystems, California, USA). Twelve percent of the genotypes were genotyped again, and there were no mismatches between the 2 genotyping steps.

Study Selection for the Meta-analysis of Association Studies Between Blood Homocysteine Levels and Schizophrenia

Eligible studies were identified using the PubMed search engine with the terms "homocysteine," "hyperhomocysteinemia," and "schizophrenia." We also conducted an additional manual search of reference lists and review articles. Studies meeting the following criteria were included for further meta-analysis: (1) included laboratory assessment of serum or plasma homocysteine levels, (2) performed a case-control study (schizophrenia vs control), (3) provided raw data of homocysteine levels separately by gender, and (4) published in an English language. The 2 reviewers (N.S. and K.K.) selected the articles independently according to the inclusion criteria, and then discussed the articles until they reached a consensus on every study used for the meta-analysis.

Statistical Methods

A linear regression analysis was used to examine the effects of diagnosis, age, gender, and the *MTHFR* C677T genotypes on the plasma total homocysteine. An ANCOVA was performed to examine the presence of the differences in the plasma total homocysteine between the 2 groups (schizophrenia vs control) separately by gender and by the 3 *MTHFR* C677T genotypes (total of 6 strata). Allelic and genotypic frequencies of the *MTHFR* C677T polymorphism in patients and control subjects were compared using the χ^2 test. In order to quantify the strength of association between plasma total homocysteine and schizophrenia, an MR approach was used, as in a previous study.[53] The risk estimate in gene-schizophrenia association for the TT genotypes of the *MTHFR* C677T polymorphism (vs the CC genotypes; $OR_{SCZ/TT}$) was from the current meta-analysis of the Japanese genetic association studies. For gene-homocysteine association, the effect of the TT genotypes (vs the CC genotypes) on plasma total homocysteine levels ($beta_{hcy/TT}$) was estimated using the Japanese control subjects from the Tokushima homocysteine study under a multivariate linear regression model including age and gender as covariates. The effect for the TT genotypes in the gene-homocysteine association was expressed as 1-SD increase in plasma total homocysteine. From these 2 estimates, an MR estimate of the effect of plasma total homocysteine on the risk of schizophrenia ($OR_{SCZ/hcy}$) was calculated as follows: log $OR_{SCZ/hcy}$ = (log $OR_{SCZ/TT}$)/$beta_{hcy/TT}$. The MR estimate represented the OR for schizophrenia risk per 1-SD increase in plasma total homocysteine. The standard error (SE) of the MR estimate was calculated by the Delta method.[54,55]

Meta-analysis

The meta-analysis of association studies between the blood homocysteine levels and schizophrenia was performed on the standardized mean differences (SMD). The meta-analysis of association studies between the *MTHFR* C677T polymorphism and schizophrenia was performed for 5 genetic models, recessive (CC/CT vs TT genotypes), dominant (CC vs CT/TT genotypes), codominant (CC vs TT genotypes), codominant (CT vs TT genotypes), and allele frequency (C-allele vs T-allele), as had been done in a previous study.[45] Heterogeneity was assessed using the I^2 statistic. If heterogeneity across studies was found, then a random-effects model was applied; otherwise, a fixed-effects model was applied. Publication bias was assessed using funnel plots and a regression test.[56] OR and 95% CI were calculated by "metafor," an R package.

Results

Differences in the Plasma Total Homocysteine Levels Between Patients With Schizophrenia and Controls

A linear regression analysis showed significant effects of diagnosis (higher in schizophrenic patients than in controls), *MTHFR* C677T genotypes (higher in CT carriers than in CC carriers, and higher in TT carriers than in CC carriers), age (decreases with age), and gender (higher in males than in females) on the plasma total homocysteine levels (diagnosis $P = 3.4 \times 10^{-29}$, genotype P [CT vs CC] $= 4.7 \times 10^{-3}$, genotype P [TT vs CC] $= 5.8 \times 10^{-43}$, age $P = 1.0 \times 10^{-2}$, and gender $P = 1.3 \times 10^{-26}$). Next, an ANCOVA was performed to examine the presence of the differences between the 2 groups in the plasma total homocysteine separately by gender and by the 3 *MTHFR* C677T genotypes (total of 6 strata), and a significant effect of diagnosis (higher in patients with schizophrenia than in the control) was still observed in all strata (diagnosis P of male-genotype CC $= 2.4 \times 10^{-8}$, diagnosis P of male-genotype CT $= 3.2 \times 10^{-10}$, diagnosis P of male-genotype TT $= 2.3 \times 10^{-4}$, diagnosis P of female-genotype CC $= 1.1 \times 10^{-8}$, diagnosis P of female-genotype CT $= 3.2 \times 10^{-8}$, and diagnosis

P of female-genotype TT = 1.2×10^{-5}, respectively) after adjustment for age (figure 1).

A Meta-analysis of the Blood Homocysteine Levels in Schizophrenia

We performed a meta-analysis of previous association studies between the blood homocysteine levels and schizophrenia separately by gender. The studies included in this meta-analysis are shown in the supplementary table 1. For the meta-analysis of males, data were obtained from 12 association studies,[5,6,8,10,11,13,17,20,21,25,28] including our data, for a total of 1079 patients with schizophrenia and 1559 control subjects. As shown in figure 2A, the random-effects model showed that the blood homocysteine levels were significantly higher in male patients with schizophrenia than in the male controls (SMD = 0.76; 95% CI = 0.30–1.22; $P = 1.2 \times 10^{-3}$) with significant heterogeneity among studies ($I^2 = 96.3\%$; $P < .05$). The funnel plot analysis indicated no evidence of publication bias in the male association studies ($P = .13$; supplementary figure 1). For the meta-analysis of females, data were obtained from 10 association studies,[5,6,8,10,11,13,17,25,28] including our data, for a total of 615 patients with schizophrenia and 1461 control subjects. As shown in figure 2B, the random-effects model showed that the blood homocysteine levels were significantly higher in female patients with schizophrenia than in the female controls (SMD = 0.50; 95% CI = 0.31–0.70; $P = 5.9 \times 10^{-7}$) with significant heterogeneity among the studies ($I^2 = 65.7\%$; $P < .05$). The funnel plot analysis indicated no evidence of publication bias in the female association studies ($P = .73$; supplementary figure 2).

A Case-Control Association Study Between the MTHFR C677T Polymorphism and Schizophrenia

Two case-control data sets were evaluated: one is the Tokushima sample set (case = 1149, control = 2742), and the other is the Osaka sample set (case = 621, control = 486). The genotypic distributions of rs1801133 did not deviate significantly from the Hardy-Weinberg equilibrium (HWE) in the control groups of these 2 sample sets ($P > .05$). Significant difference was observed between the controls and patients with schizophrenia in the allelic frequencies of the Tokushima sample set ($P = .025$). On the other hand, no significant differences were observed in the genotypic and allelic frequencies of the Osaka sample set (genotype $P = .98$, and allele $P = .97$, respectively).

A Meta-analysis of Genetic Association Studies Between the MTHFR C677T Polymorphism and Schizophrenia

Six association studies on Japanese subjects, including the 2 data sets from this study, were used for the meta-analysis.[37–40] The genotypic distributions and allelic frequencies of rs1801133 in each study are shown in the supplementary table 2. The diagnosis of schizophrenia was made according to DSM-IV criteria in 5 studies, and it was made according to DSM-III criteria in the remaining study. A total of 4316 cases and 6062 controls were included in this analysis. Genotypic distribution of this SNP did not deviate significantly from the HWE in any control group across the 6 studies ($P > .05$). Significant heterogeneity was not detected at this SNP among the studies for the 5 genetic models ($P > .05$). The funnel plot analysis indicated no evidence of publication bias for all genetic models ($P > .05$). The results of ORs and CI analyzed by the fixed-effects model for all genetic models and the risk of schizophrenia are shown in table 1. Of these 5 genetic models, significant associations were found in 4 models. The highest OR was observed in the codominant model (CC vs TT genotypes; figure 3; OR = 1.16, 95% CI = 1.03–1.31, $P = 1.4 \times 10^{-2}$, in the fixed-effects model).

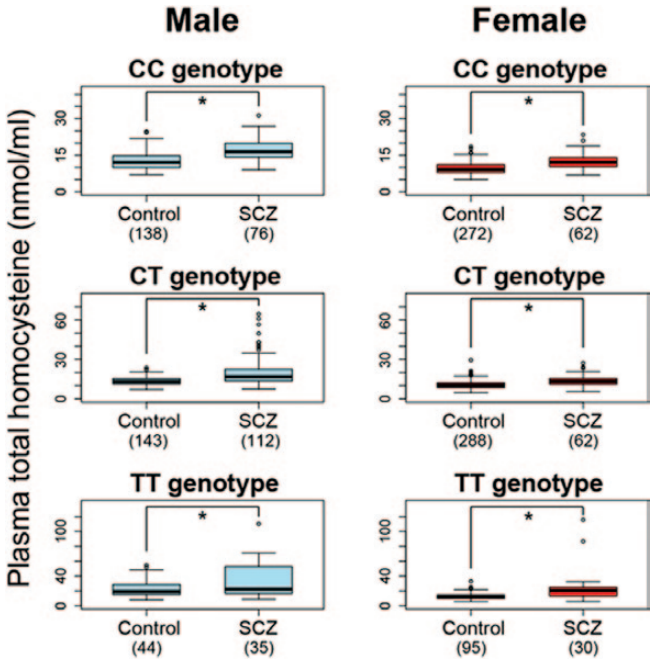

Fig. 1. Differences in the plasma total homocysteine levels between patients with schizophrenia and controls separately by gender and by the methylenetetrahydrofolate reductase (*MTHFR*) C677T genotypes. The ANCOVA demonstrated that the plasma total homocysteine levels were significantly higher in patients with schizophrenia than in controls in each of the 6 strata (*age-adjusted $P < .001$).

Effect of Plasma Total Homocysteine Levels on Schizophrenia Risk in an MR Study

From the current meta-analysis of the Japanese genetic association studies, the pooled OR (the TT vs CC genotypes) for the effect of the *MTHFR* C677T polymorphism on schizophrenia risk was 1.16 (95% CI = 1.03–1.31). In multivariate gene-homocysteine association analysis in the

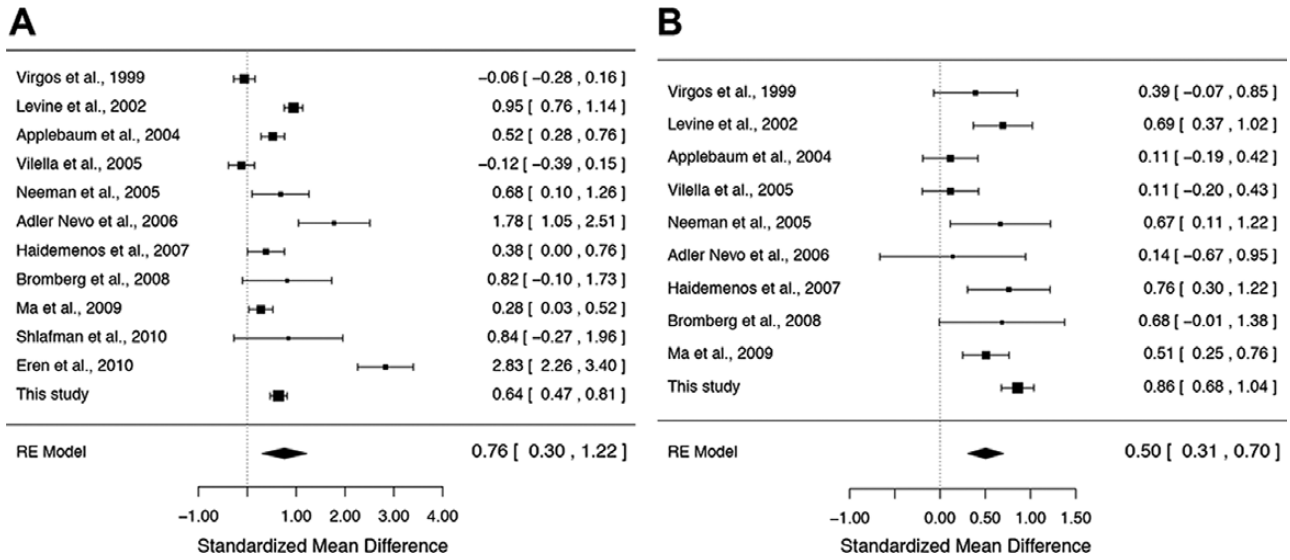

Fig. 2. Meta-analyses of association studies between the blood homocysteine levels and schizophrenia. (A) The result of the meta-analysis of 12 male association studies ($N = 2638$). The blood homocysteine levels were significantly higher in male patients with schizophrenia than in the male controls (standardized mean difference [SMD] = 0.76; 95% CI = 0.30–1.22; $P = 1.2 \times 10^{-3}$ in the random-effects model). (B) The result of the meta-analysis of 10 female association studies ($N = 2076$). The blood homocysteine levels were significantly higher in female patients with schizophrenia than in the female controls (SMD = 0.50; 95% CI = 0.31–0.70; $P = 5.9 \times 10^{-7}$ in the random-effects model).

Table 1. The Results of OR and 95% CI analyzed by the Fixed-Effects Model for All Genetic Models and each *P* Value in the Meta-analysis for methylenetetrahydrofolate reductase C677T

Model	OR	95% CI	*P* Value
Recessive (CC/CT vs TT)	1.14	1.03–1.27	.016
Dominant (CC vs CT/TT)	1.06	0.98–1.16	.147
Codominant (CC vs TT)	1.16	1.03–1.31	.014
Codominant (CT vs TT)	1.13	1.00–1.26	.042
Allelic (C vs T)	1.07	1.01–1.13	.022

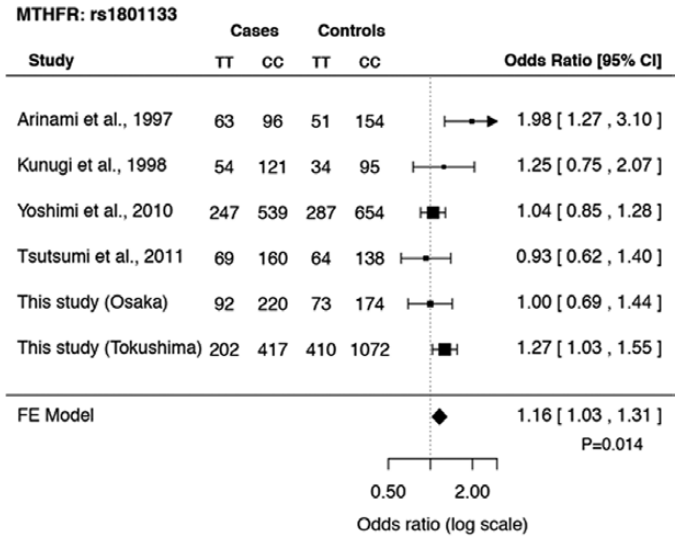

Fig. 3. A meta-analysis of genetic association studies on Japanese subjects between the *MTHFR* C677T polymorphism and schizophrenia. Six association studies on Japanese subjects, including the 2 data sets from this study, were used for the meta-analysis ($N = 10\,378$). Significant associations between the *MTHFR* C677T polymorphism and schizophrenia were found in 4 models, and the result of the codominant model (CC vs TT genotypes) is shown (OR = 1.16, 95% CI = 1.03–1.31, $P = 1.4 \times 10^{-2}$ in the fixed-effects model).

Japanese control subjects genotyped ($n = 980$ with a SD for plasma total homocysteine levels of 4.84 nmol/ml), the effect on plasma total homocysteine levels, expressed as 1-SD increase in homocysteine, was estimated to be 1.14 (95% CI = 0.96–1.33; $P = 1.1 \times 10^{-29}$) for the TT genotypes of the *MTHFR* C677T polymorphism (vs the CC genotypes), after adjustment for age and gender. When combining these 2 estimates by an MR approach, the effect of plasma total homocysteine on schizophrenia risk was statistically significant, representing the OR of 1.14 (95% CI = 1.03–1.27; $P = 1.6 \times 10^{-2}$) for schizophrenia per 1-SD increase in plasma total homocysteine (figure 4).

Discussion

In this study, we performed an association study between the plasma total homocysteine and schizophrenia with stratification by gender and by the *MTHFR* C677T genotypes and demonstrated significantly elevated plasma total homocysteine levels in patients with schizophrenia compared with controls, in both male and female subjects. The subsequent meta-analysis for gender supported this finding. To our knowledge, this is the first study to conduct a meta-analysis according to gender. The significant decrease in plasma total homocysteine levels with age in our multivariate linear regression

analysis was not consistent with a previous finding.[33] This discrepancy might be caused by disease status and gender of the subjects analyzed. When we examined the relationship between age and the plasma total homocysteine levels in a univariate linear regression model, a significant negative correlation was observed only in male subjects with schizophrenia (supplementary figure 3). Consistent with this finding, Levine et al[5] reported that the difference in the plasma total homocysteine levels between patients and controls was attributable to young male patients with schizophrenia.

We also conducted a meta-analysis of genetic association studies between the *MTHFR* C677T polymorphism and schizophrenia based on Japanese subjects and demonstrated that the *MTHFR* C677T polymorphism was a risk factor for developing schizophrenia in the Japanese population, which is consistent with the results of previous meta-analyses.[39,41–47,57] On the other hand, this polymorphism has not been identified as a risk locus for schizophrenia in the large genome-wide association studies.[58,59] This discrepancy might be caused by ethnic differences (risk allele [T] frequencies from HapMap; Japanese 0.39, Caucasian 0.31, African American 0.12, and Mexican American 0.41) and a lack of adequate statistical power to detect the relatively small genetic effect of this polymorphism on schizophrenia at the genome-wide significant threshold.

Importantly, we demonstrated that increased homocysteine levels may be associated with an increased risk of developing schizophrenia by an MR approach. Hyperhomocysteinemia has been proposed as being part of the pathophysiology of schizophrenia due to its various biological effects, such as acting as a partial antagonist of the glutamate site of the *N*-methyl-D-aspartate receptor[23] and causing subtle placental vascular damage that interferes with oxygen delivery to the fetus,[60] DNA damage and cell cytotoxicity,[61] neuronal apoptosis,[62] and mitochondrial nitric oxide accumulation.[63] Homocysteine acts as a methyl donor when it is converted to *S*-adenosyl-methionine, and we recently demonstrated a significant association between the plasma total homocysteine and DNA methylation in schizophrenia, which suggests that homocysteine might play a role in the pathogenesis of schizophrenia via alterations to DNA methylation.[64] Homocysteine, *S*-adenosyl-methionine, DNA methylation, *MTHFR*, folate, and vitamin B12 are involved in 1-carbon metabolism, and abnormalities of these components in schizophrenia have been reported in previous studies.[2,3,65] These lines of evidence suggest that disrupted 1-carbon metabolism may be an important role in the pathophysiology of schizophrenia.

The benefits of homocysteine-reducing strategies in schizophrenia have been shown in several studies. Levine et al[66] reported an improvement in the clinical symptoms of schizophrenic patients with hyperhomocysteinemia who were treated with folate, vitamin B12, and pyridoxine. Hill et al[67] reported an improvement in the negative symptoms

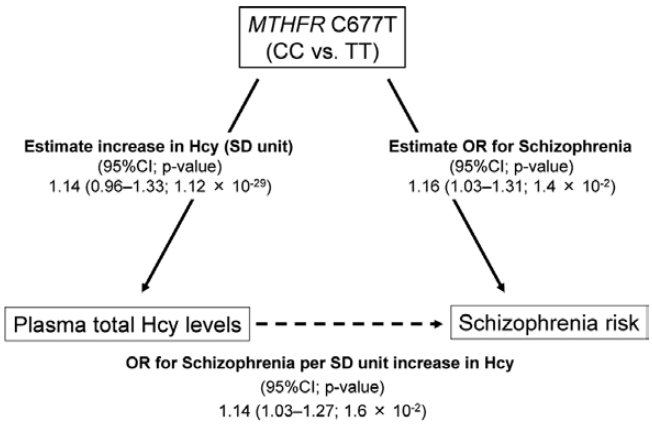

Fig. 4. Graphical representation of the mendelian randomization approach. The pooled OR (the TT vs CC genotypes) for the effect of the *MTHFR* C677T polymorphism on schizophrenia risk was 1.16 (95% CI = 1.03–1.31). The OR (the TT vs CC genotypes) for the effect of the *MTHFR* C677T polymorphism on plasma total homocysteine levels, expressed as 1-SD increase in homocysteine, was 1.14 (95% CI = 0.96–1.33; $P = 1.1 \times 10^{-29}$). The effect of plasma total homocysteine on schizophrenia risk by a mendelian randomization analysis was statistically significant, representing the OR of 1.14 (95% CI = 1.03–1.27; $P = 1.6 \times 10^{-2}$) for schizophrenia per 1-SD increase in plasma total homocysteine.

of schizophrenic patients who were treated with folate when the *MTHFR* C677T genotype was taken into account. Roffman et al[68] reported an improvement in the negative symptoms of schizophrenic patients who were treated with folate and vitamin B12 when 4 variants in the *FOLH1*, *MTHFR*, *MTR,* and *COMT* genes were taken into account. Further research will be necessary to identify the features of patients with schizophrenia who would benefit from homocysteine-reducing treatments. In cardiovascular diseases, which are also associated with hyperhomocysteinemia, clinical trials to identify a subgroup that appeared to benefit from homocysteine-lowering intervention have been performed[69,70] although no benefits of homocysteine-lowering intervention on cardiovascular outcomes have been reported in randomized controlled trials.[71]

Many studies have indicated the potential contributions of the *MTHFR* C677T polymorphism to the pathophysiology of schizophrenia. This risk SNP has been associated with schizophrenic negative symptoms, aggressive behavior, and various phenotypes related to schizophrenia, such as cognitive function, episodic memory, gray matter density, and prefrontal function.[72–80] Interestingly, pharmacogenetic studies have demonstrated that this risk SNP has also been involved in the antipsychotic drug response and metabolic syndrome treated with antipsychotics in schizophrenia.[81–84]

There are some limitations to this study. First, we did not obtain genomic DNA from all participants in the association study between the plasma total homocyateine levels and schizophrenia. Second, all patients were treated with various antipsychotic drugs, and these medications might

influence the outcome. When we examined the relationship between equivalent dose and the plasma total homocysteine levels in subjects with schizophrenia by a univariate linear regression model, a positive correlation was observed in female subjects ($P = .03$). However, it did not reach statistical significance after correction for multiple comparisons. Third, there was heterogeneity among the studies in the meta-analysis for blood homocysteine, while significant heterogeneity was not observed in the meta-analysis for genetic association studies. This heterogeneity might be caused by other genetic variations, the clinical heterogeneity of the patients included, medications, and environmental factors, such as folic acid, vitamin B6, vitamin B12, obesity, smoking status, and caffeine consumption, although we did not take these confounding factors into consideration in our analysis. Fourth, the use of "well controls" in this case-control analysis might accentuate such confounding influences.[85] Fifth, this is a cross-sectional study, and MR has limitations on the ability to establish causal relationships between risk factors and outcomes.[86] So, the causality between schizophrenia and blood homocysteine levels must be still cautious. Notably, elevated maternal levels of homocysteine during the third trimester have been found to increase the risk of schizophrenia in the offspring.[60] Finally, hyperhomocysteinemia has been identified as an independent risk factor for several neurological disorders in addition to schizophrenia, such as depression and dementia.[87,88] Further studies to examine how hyperhomocysteinemia is involved in the pathophysiology of each disease will be necessary.

In conclusion, to the best of our knowledge, this is the first meta-analysis of association studies between blood homocysteine levels and schizophrenia according to gender, and we demonstrated elevated blood homocysteine levels in both male and female subjects with schizophrenia. The meta-analysis of genetic association studies using the Japanese subjects provided stringent evidence of association between the *MTHFR* C677T polymorphism and schizophrenia. Our MR analysis using the Japanese subjects suggests that increased plasma total homocysteine levels may be associated with an increased risk of developing schizophrenia.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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