

Correlations between plasma homocysteine and *MTHFR* gene polymorphism and white matter lesions

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Abstract

This study aims to investigate the correlation between the plasma homocysteine (Hcy) level and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism, and white matter lesions (WML). The plasma Hcy level and MTHFR C677T gene polymorphism in 104 patients with white matter lesions and 74 controls were measured. The severity of cerebral white matter was scored on the magnetic resonance imaging (MRI) images by the modified Scheltens scale (score of 0-30). The plasma Hcy level in the WML group was remarkably higher than that in the control group ($p < 0.05$), and the proportion of the patients with a high Hcy level in the WML group was notably higher than that in the control group ($p < 0.05$). Moreover, the patients with TT genotype in MTHFR gene had a significantly higher plasma Hcy level than those with CT or CC genotype. In addition, the plasma Hcy level in the patients with CT genotype was also significantly different from that in the patients with CC genotype ($p < 0.05$). The severity of cerebral white matter lesions among the patients with different genotypes in MTHFR gene was not significantly different ($p > 0.05$). The plasma Hcy level is positively correlated with WML and significantly correlated with C677T gene polymorphism in MTHFR gene. The severity of white matter lesions is not correlated with MTHFR C677T gene polymorphism.

Key words: brain white matter disease, gene polymorphism, homocysteine, MTHFR, risk factors.

Introduction

The white matter lesion (WML) is a common type of cerebral small vessel disease. Cerebral white matter lesions in craniocerebral computed tomography (CT) or magnetic resonance imaging (MRI) are characterized by a patchy or diffusive shadow in the paraventricular and (or) semioval center, showing low density in CT and high T2 and Flair signals in MRI [9]. WML causes cognitive dysfunction, declined capability in memory, computing and execution, dysarthria and gait instability in the elderly population, which not only seriously

affects the life quality of the patients, but also brings a heavy burden to the family and society [5,11]. Thus, it is necessary to study the risk factors and pathogenesis of WML. Currently, the risk factors for WML and the mechanisms are still unclear. Previous studies show that there is a certain correlation between hyperhomocysteinemia and WML [16,18,26].

N-5, N-10-methylenetetrahydrofolate reductase (MTHFR) is the rate limiting enzyme in the metabolism of Hcy. Studies have found that MTHFR has a certain correlation with cerebrovascular disease [1,2]

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and has a predictive value in ischemic stroke [25]. However, the relationship between *MTHFR* gene C677T polymorphism and WML is still unclear. This study detected the plasma homocysteine levels and *MTHFR* C677T gene polymorphism in the patients with WML and evaluated the severity of WML by the modified Scheltens scale, aiming to explore the risk factors and pathogenesis of WML.

Material and methods

Subjects

The clinical data of 104 patients with WML who were hospitalized in the Department of Neurology of the Second People's Hospital of Lianyungang from March 2015 to June 2016 were collected. The patients included 64 males and 40 females, with an average age of 67.26 (\pm 8.32) years. Inclusion criteria were as follows: (1) patients aged 45-80 years; and (2) meeting the diagnostic standard for white matter lesions [22]. Exclusion criteria included: (1) patients with a previous history of stroke; (2) caused by other diseases such as immune, hypoxia, poisoning and genetics; (3) taking folic acid and vitamin B; (4) with Alzheimer's disease, Parkinson's disease, alcoholism and other cognitive function; (5) with a dysfunction or failure in heart, lung, liver, kidney and other important organs; (6) cannot have the head MRI scan. Seventy-four healthy individuals with normal head MRI results, aged 45-80 years, were collected as the control group from the outpatient department of our hospital in the same period. This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with an approval from the Ethics Committee of the Soochow University. Written informed consent was obtained from all participants.

General information

The age, gender, education (years), smoking and drinking history, histories of hypertension, diabetes, hyperlipidemia, coronary heart disease and plasma Hcy level of each subjects were recorded.

Hcy level detection

Extracted 2 ml from the median vein of the elbow, placed in EDTA anticoagulant tube and 3000 rpm centrifugation for 10 minutes were taken on an empty stomach. The level of plasma Hcy was detected by using the BeckMan AU5800 automatic biochemical

analyzer. The normal range of Hcy was 0~10 μ mol/l, and Hcy > 10 μ mol/l was defined as hyperhomocysteinemia.

MRI scoring

A modified Scheltens scale (0-30 scores) was used to score the cranial MRI images of the patients with WML by two experienced radiologists. The basal ganglia and subtentorial lesions and the paraventricular and deep lesions were scored separately using the modified Scheltens scale (score of 0-30) [21]. The paraventricular high signals (0-6 scores) were scored as follows: the hat-shaped high intensity in the occipital lobe and frontal lobe were both scored as 0-2, and the banded high signals in the lateral ventricle were scored as 0-2. No lesion, lesions \leq 5 mm and lesions with a size of 6-10 mm were scored as 0, 1 and 2, respectively. Hyperintensities of the white matter were scored 0-24 as follows: WML in the frontal, parietal, occipital and temporal lobes was rated 0-6, respectively. No abnormality was scored 0; the lesions smaller than or equal to 3 mm and the number of less than or equal to 5 were scored 1; the lesions smaller than or equal to 3 mm and the number of more than 6 were scored 2; the lesions within 4-10 mm and the number of less than or equal to 5 were scored 3; the lesion within 4-10 mm and the number equal to or more than 6 were scored 4; the lesions larger than 11 mm and the number of more than 1 were scored 5; and the fused lesions were scored 6.

Detection of gene polymorphisms

Genomic DNA was extracted from 2 ml vein blood which had been placed in the EDTA anticoagulant tube and stored at -20°C . Blood genomic DNA Kit (Axygen, USA) was used for DNA extraction according to the instructions provided by the manufacturer.

The primer sequences used for PCR amplification were: (forward) 5'-TGAAGGAGAAGGTGTCTGCGGGA-3', (reverse) 5'-AGGACGGTGCGGTGAGAGTG-3' (Applied Biosystems, USA). The volume of the PCR reaction system was 25 μ l, which contained 1 μ l genome DNA, 2.5 μ l 10 \times PCR buffer (including Mg^{2+}), 2 μ l 2.5 mmol/l dNTP, 1 μ l of both the forward and reverse primers (100 pmol/ μ l), 5 U/ μ l Taq polymerase 0.125 μ l and H_2O 17.375 μ l. The PCR reactions were run in the following conditions: pre-denaturation at 94°C for 10 min, 39 cycles of denaturation at 94°C for 45 s,

annealing at 62°C for 40 s and extension at 72°C for 45 s, and a terminal extension at 72°C for 7 min.

The PCR products were analyzed for identification of variants MTHFR C677T using a TaqMan SNP Genotyping Assay (Applied Bio-systems Inc., Foster City, Calif., USA) according to the previous report [28]. All assays were performed in duplicate, and an automatic allele of quality value was used to determine genotype assignment. TaqMan assay was performed to genotype subjects with indeterminate results by direct sequencing of the PCR amplification containing the MTHFR C677T locus.

Statistical analysis

SPSSv.17 statistical software was used for statistical analysis. The measurement data were expressed as mean \pm standard deviation (SD) and analyzed using *t* test. Enumeration data were expressed as a percentage (%) and compared using χ^2 test. ANOVA was used to compare the mean of multiple samples. The influencing factors of WML were analyzed by multivariate Logistic regression. The difference was statistically significant when $p < 0.05$.

Results

Clinical data

MRI score of patients in WML and control group is shown in Figure 1A. There was no statistically significant difference in gender composition, education duration, smoking and drinking history, diabetes

mellitus, coronary heart disease (CHD) and hyperlipidemia between the WML group and the control group ($p > 0.05$), while the age, hypertension and Hcy level in the WML group were significantly higher than those in the control group ($p < 0.05$), indicating that age, hypertension and hyperhomocysteinemia were risk factors for WML (Table I, Fig. 1B).

Genotyping scatter plot of MTHFR

According to the scatter plot of genotyping, there were 50 cases with CC genotype including 26 cases in the WML group and 24 cases in the control group, 93 cases with CT heterozygous type including 56 cases in the WML group and 37 cases in the control group, and 35 cases with TT homozygous type including 22 cases in the WML group and 13 cases in the control group (Fig. 2).

Gene frequency distribution of MTHFR C677T genotypes

The gene frequency distribution of *MTHFR C677T* genotypes showed no statistical difference between the WML group and the control group ($p > 0.05$, Table II).

Comparisons of plasma Hcy levels among the MTHFR C677T genotypes

According to the carrying of MTHFR C677T genotypes, samples were divided into TT, CT and CC groups, and then the plasma Hcy levels were compared among them. The plasma Hcy level was the highest in

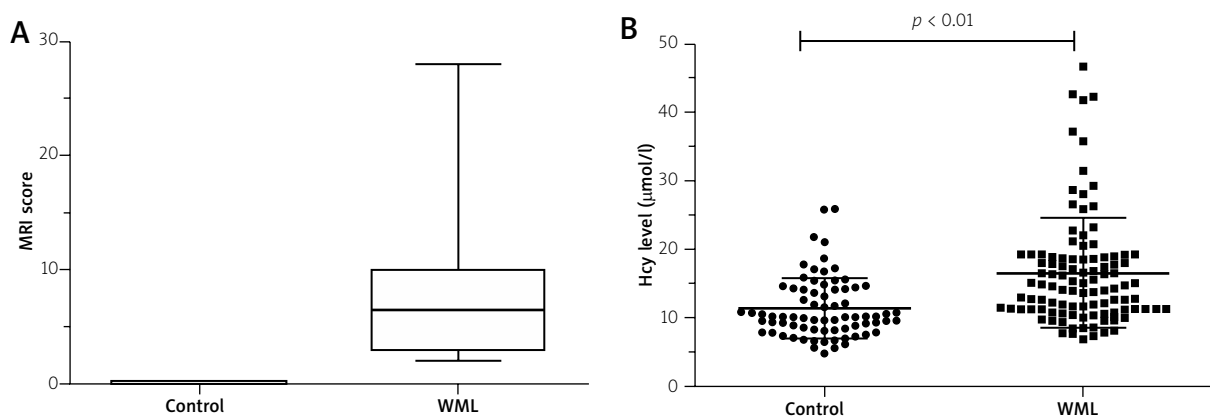


Fig. 1. MRI score and plasma Hcy levels of patients. MRI scores of patients are shown in **A**). $n = 104$ in the white matter lesions (WML) group and $n = 74$ in the control group. **B**) Plasma Hcy levels of WML and control patients were measured. The number of samples in both WML and control groups are consistent with patients with MRI score.

Table I. Comparison of the general data and the risk factors for stroke between the two groups

General data	WML group	Control group	p value
Age (years)*	67.26 ± 8.32	59.45 ± 7.16	0.001
Gender (male %)	61 (58.7)	43 (58.1)	0.942
Education duration*	9.7 ± 3.2	9.2 ± 2.9	0.472
Smoking (%)	47 (45.2)	29 (39.2)	0.308
Drinking (%)	23 (22.1)	11 (14.9)	0.225
Hypertension (%)	75 (76.0)	22 (29.7)	0.001
Diabetes (%)	17 (16.3)	9 (12.2)	0.412
Hyperlipidemia*	4.56 ± 1.13	4.46 ± 1.07	0.682
CHD (%)	8 (7.7)	4 (5.4)	0.549
Hcy (mmol/l)*	16.81 ± 5.18	11.40 ± 3.72	0.005

*Data are shown as mean ± SD. WML – white matter lesions, CHD – coronary heart disease, Hcy – homocysteine.

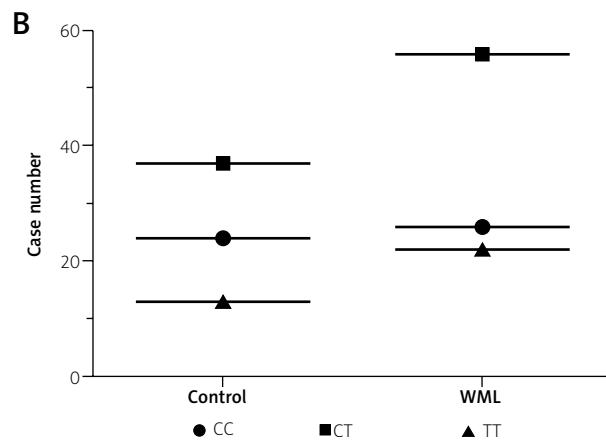
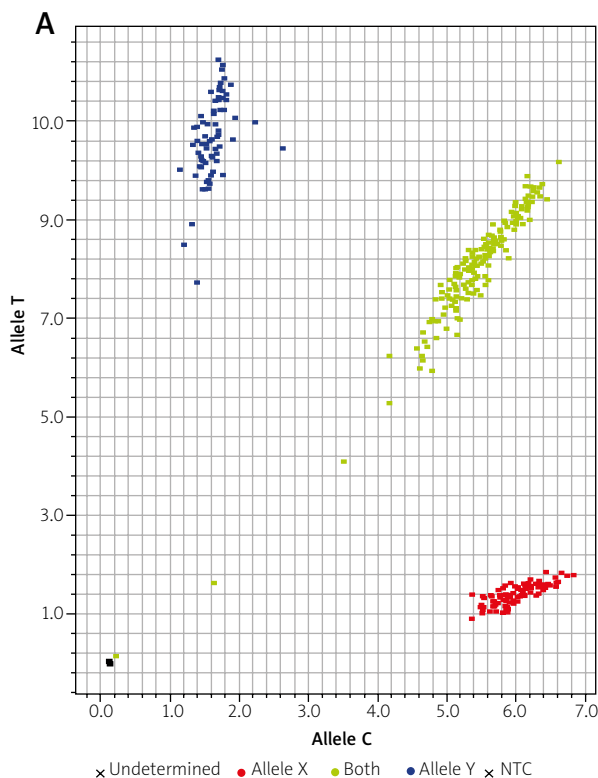


Fig. 2. Genotyping scatter plot of MTHFR. **A)** Y axis – T alleles; X axis – C alleles; the blue dots near the Y axis belong to TT genotype, the central green dots belong to CT genotype, and the red dots near the X axis belong to CC genotype. **B)** Case numbers of CC, CT and TT in the control and white matter lesions (WML) groups.

the TT group, followed by the CT group and then the CC group, with statistically significant differences ($p < 0.05$, Table III).

Risk factors of WML

Logistic regression analysis of age, sex, smoking and drinking history, hypertension, diabetes and hyperlipidemia showed that age and hypertension were independent risk factors for WML.

Taking WML as the dependent variable and the genotypes of *MTHFR* C677T and Hcy level as the independent variables, the multivariate Logistic regression analysis was carried out. The results showed that a high Hcy level was significantly correlated with the severity of WML lesions (Wald = 6.278, $p = 0.021$, OR = 2.423, 95% CI = 1.136-5.817), and notably associated with *MTHFR* gene polymorphism. However, there was no significant correlation

Table II. Comparisons of the gene frequency distribution of *MTHFR* C677T in the two groups

Groups	Case number	Genotypes			χ^2 value	p value
		CC	CT	TT		
WML	104	26	56	22	1.397	0.443
Control	74	24	37	13	–	–

Data were compared with the control group.
WML – white matter lesions

Table III. Plasma Hcy levels among the three genotypes of *MTHFR* C677T gene locus

Genotypes	Case number	Mean ($\mu\text{mol/l}$)	SD	F value	p value
MTHFR C677T	CC	26	13.55	0.538	
	CT	56	16.02	0.762	1.286
	TT	22	18.37	0.774	

between the polymorphism of *MTHFR* gene and the severity of WML ($p > 0.05$).

Discussion

With the coming of the aging society and the popularization of head MRI, the detection rate of WML is getting higher and higher. The etiology of WML may be related to endothelial dysfunction, chronic cerebral hypoperfusion, blood-brain barrier destruction, and auto-regulatory dysfunction of small vessels [10,15].

The present study suggested that hyperhomocysteinemia is associated with WML, and is one of the risk factors for WML [3,17,24]. Vermeer *et al.* [24] found that the WML risk in the patients with the plasma Hcy level in the highest 5 percentile was 2.3 times higher than that in the lowest 5 percentile after correcting other risk factors, indicating that Hcy is an independent risk factor for WML. Pavlovic *et al.* [17] have found that a high plasma Hcy level are positively correlated with the severity of brain white matter disease, which can be used as an independent predictor for WML. Kloppenborg *et al.* [13] applied MRI to measure the volume of WML and found that hyperhomocysteinemia is positively correlated with the volume of WML, and has a significant correlation with the risk of its progression (OR = 2.4, $p < 0.001$). Feng *et al.* [7] studied the relationship between hyperhomocysteinemia and cerebral macroangiopathy and small vessel disease and found that hyperhomocysteinemia is closely related to cerebral small vessel disease and it is an independent risk factor for cerebral small vessel disease (OR = 1.315, $p < 0.001$). The results in this study showed that the Hcy level in the WML group was significantly higher than that

in the control group, indicating that hyperhomocysteinemia is an independent risk factor for WML, which is consistent with the domestic and foreign research results. The possible mechanism is that Hcy causes endothelial function injury in small blood vessels of brain via the pathways such as oxidative stress and influencing the vasomotor function.

Hcy level is mainly affected by the activity of its rate-limiting enzyme N-5, N-10-methylenetetrahydrofolate reductase (*MTHFR*). The main reason for the increase in the Hcy level is attributed to the polymorphism of *MTHFR* gene. C677T polymorphism is the most common polymorphism in *MTHFR* gene, in which C base is substituted by T base, leading to the encoding alanine substituted by valine. The substitution of alanine by valine results in the decrease in *MTHFR* activity and leads to the elevated level of Hcy. Studies have found that the enzyme activity of *MTHFR* with homozygous TT genotype is only 30% of the normal, and that with heterozygous CT genotype is only 65% of the normal, resulting in the plasma Hcy level in the patients with homozygous TT genotype increases significantly as compared with that in the patients with heterozygous CT genotype or wild-type (CC). The frequency of T alleles is different to a great extent in different countries and nationalities. It is significantly higher in the Chinese population than in the European and American population [27,29]. Whether the gene polymorphism of *MTHFR* C677T is associated with WML remains controversial. Rajagopalan *et al.* [19] followed up 359 patients with mild cognitive impairment for twelve months and found that T allele in *MTHFR* gene is significantly related to the volume of white matter. De Lau *et al.* [23] found that the *MTHFR* C677T gene

polymorphism is only related to Hcy level, but has no significant correlation with the brain white matter lesions. A study of Jeon *et al.* [12] has shown that a high Hcy level is associated with cerebral small vessel disease, and TT genotype of *MTHFR* is an important determinant of hyperhomocysteinemia, but *MTHFR* gene polymorphism is not correlated with cerebral small vessel disease. Rutten-Jacobs *et al.* [20] found that *MTHFR* C677T polymorphism is closely related to the volume of white matter lesions, which influences the white matter lesions by increasing their sensitivity to hypertension or increasing their interaction with hypertension. In this study, we found that the polymorphism of *MTHFR* C677T gene was significantly correlated with the Hcy level, but had no remarkable relationship with the severity of white matter lesions, which was consistent with the research results of Tran *et al.* [23]. Our results were only from a cross-sectional survey, thus further epidemiological and prospective studies are needed to demonstrate our findings.

It was reported that *MTHFR* C677T polymorphisms is a risk factor for autism [8], biological role of *MTHFR* gene polymorphism in pregnancy [30] and association between allergic rhinitis and polymorphisms of C677T for *MTHFR* gene in children [6] needed further study. *MTHFR* polymorphisms (C677T) may be not associated with pancreatic cancer risk indicated by meta-analysis [14]. In conclusion, the roles of *MTHFR* polymorphisms in many diseases need further study.

At present, the diagnosis of WML mainly depends on MRI. There are still many problems in the studies on WML. Early identification of WML using imaging, Hcy, *MTHFR* and other relevant indicators and early intervention for the risk factors of WML can help to predict the incidence risk of WML and prevent or delay the occurrence of WML, so as to reduce the severity of WML, improve the quality of life of the patients and reduce the burden of family and society.

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Conflicts of interest

The authors report no conflict of interest.

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