

Association of a Methylene Tetrahydrofolate Reductase C677T Polymorphism with Several Blood Chemical Levels in a Chinese Population

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Background: The methylene tetrahydrofolate reductase (*MTHFR*) C677T polymorphism is associated with hypertension in certain populations. This study investigated the relationship between the *MTHFR* polymorphism and hypertension and correlated blood lipid indexes, including homocysteine (HCY), lipoprotein (a) [Lp (a)], high-density lipoprotein (HDL), low-density lipoprotein (LDL), apolipoprotein A I (Apo AI), Apo B, glucose (GLU), total cholesterol (TC), and triglyceride (TG), in a Chinese population. **Materials and Methods:** A total of 174 patients with hypertension and 634 healthy control individuals from Jiangxi Province were recruited between June 2012 and September 2012 for genotyping of the *MTHFR* C677T polymorphism using polymerase chain reaction-restriction fragment length polymorphism. Biochemical parameters were also assessed in these subjects and statistically compared to the *MTHFR* C677T polymorphism and the risk for hypertension. **Results:** HCY and Lp (a) levels were significantly higher in subjects with a *MTHFR* 677TT genotype than in those with a CC/CT genotype, independent of hypertension. The frequency of the TT genotype and the T allele in hypertension patients was significantly higher than in the healthy controls. Furthermore, in the male hypertension patient group, the average levels of HCY, HDL, Apo AI, and TC were significantly different from those in female hypertension patients ($p_{\text{HCY}}=0.001$, $p_{\text{HDL}}=0.004$, $p_{\text{Apo AI}}<0.001$, $p_{\text{TC}}=0.012$). In the male control group, the average levels of HCY, HDL, Apo AI, GLU, and TC were significantly different from those of female controls ($p_{\text{HCY}}<0.001$, $p_{\text{HDL}}<0.001$, $p_{\text{Apo AI}}<0.001$, $p_{\text{GLU}}=0.001$, and $p_{\text{TC}}=0.004$). **Conclusion:** Our data demonstrate that the *MTHFR* C677T polymorphism is positively correlated with an increased risk of hypertension through an increase in HCY levels. The blood lipid correlative index was different between male and female hypertension patients and controls.

Introduction

CARDIOVASCULAR DISEASE ACCOUNTED for 30% of the 17 million global deaths in 2008 (Ala *et al.*, 2011). Hypertension and dyslipidemia are two major risk factors for cardiovascular disease (Ala *et al.*, 2011). Cardiovascular disease is frequently treated with medication and surgery. However, understanding the underlying mechanisms of cardiovascular disease may help to reduce disease incidence and to help prevention in worldwide populations. To this end, research regarding genetic and environmental interactions has indicated that individuals with specific gene polymorphisms have an increased risk for developing cardiovascular disease. For example, some studies indicate that the methylene tetrahydrofolate reductase (*MTHFR*) C677T polymorphism is associated with increased risk of cardiovascular disease and hypertension (Gupta *et al.*, 2012; Bayramoglu

et al., 2013; Kang *et al.*, 2013; Wang *et al.*, 2013; Xu *et al.*, 2013; Zhao and Jiang, 2013), although other reports have failed to confirm this association (Davis *et al.*, 2013). The *MTHFR* protein plays an important role in maintaining levels of folate and methionine, as well as controlling the blood concentration of homocysteine (HCY) in the human body (Wilson *et al.*, 2010). The *MTHFR* C677T polymorphism results in mutated CT or TT genotypes, leading to a decreased enzyme activity when compared to the wild-type (CC) enzyme (Taioli *et al.*, 2009).

Alteration in the blood lipid chemical index, including HCY, lipoprotein (a) [Lp (a)], high-density lipoprotein (HDL), low-density lipoprotein (LDL), apolipoprotein A I (Apo AI), Apo B, glucose (GLU), total cholesterol (TC), and triglyceride (TG) levels, can increase the risk of developing hypertension and cardiovascular disease. HCY is an important intermediate in human sulfur-containing amino acid

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metabolism and is the product of S-adenosine homocysteine hydrolysis in the methionine cycle. HCY contributes to the remethylation and trans-sulfuration pathways. Remethylation is the primary pathway of HCY metabolism, in which HCY is catalyzed by methionine synthase. The pathway requires vitamin and 5-methyl-tetrahydrofolate to synthesize tetrahydrofolate and methionine. Alteration of this pathway is associated with cardiovascular, neurodegenerative, and kidney disorders (Welch and Loscalzo, 1998; Hankey and Eikelboom, 1999; Smulders and Blom, 2011; Malinowska *et al.*, 2012) and stroke (Furie *et al.*, 2011). Lp (a) was first described in 1963 and is an independent plasma lipoprotein; it includes an Apo B-containing LDL protein and Apo A. Previous studies have shown that Lp (a) is a risk factor for various diseases, including arterial ischemic stroke, coronary artery disease, intrauterine growth restriction, and certain cancers (Nagaraj *et al.*, 2011; Nasr *et al.*, 2011; Ashfaq *et al.*, 2013; Goldenberg *et al.*, 2013; Greif *et al.*, 2013; Kronenberg and Utermann, 2013; Marrer *et al.*, 2013; Suzuki *et al.*, 2013; Gerhardt *et al.*, 2014). Another study reported a positive association between vascular adhesion protein-1 (VAP-1) expression and Lp (a) (Nemati *et al.*, 2013). However, the exact function and contribution of Lp (a) to cardiovascular disease are still unknown (Angelin, 2013).

In this study, we detected the *MTHFR* C677T polymorphism using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 174 patients with hypertension and 634 healthy controls from Jiangxi Province, China. We collected biochemical data to determine the blood lipid correlative index, including HCY, Lp (a), HDL, LDL, Apo AI, Apo B, GLU, TC, and TG, for association with the *MTHFR* C677T polymorphism and risk of hypertension.

Materials and Methods

Study population

A total of 174 patients with hypertension (blood pressure $\geq 140/90$ mmHg) and 634 healthy control subjects from Jiangxi province, China, were enrolled in this study between June 2012 and September 2012. The mean age of the hypertension group was 68 years old (ranging from 28 to 90 years) and included 86 males and 88 females. The mean age of the healthy control group was 64 years old (ranging from 29 to 90 years) and included 313 males and 321 females. Individuals with any conditions, such as liver and kidney diseases, diabetes, autoimmune diseases or other malignant diseases, pregnancy or lactation, were excluded from the study. This study was approved by our hospital review board and each subject provided a written consent form before

participation in this study. Five milliliters of venous blood was obtained from each individual after overnight fasting. The plasma was separated within an hour for biochemical parameter analyses, and white blood cells were collected for genotyping the *MTHFR* C677T polymorphism.

Genomic DNA extraction and PCR-RFLP analysis of the *MTHFR* genotype

Genomic DNA was extracted from white blood cells using the TIANamp Blood DNA Kit (Tiangen). The DNA concentration was measured with an ultraviolet spectrophotometer and then adjusted to 10 ng/ μ L with Tris/EDTA buffer. These DNA samples were stored at -20°C until use. For detection of the *MTHFR* C677T polymorphism, we performed PCR-RFLP using the following PCR primers: 5'-ATA AAT AAT AAA TAA AAT AAT AAA TAA ATA ATT ATT GGC AGG TTA CCC CAA A-3' and 5'-CTC ACC TGG ATG GGA AAG AT-3'. PCR amplification was carried out in 20 μ L PCR mixture in a PCR Amplifier (Long Gene) as follows: an initial denaturation at 94°C for 3 min and then 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 20 s, and a final extension at 72°C for 3 min. Next, the PCR products were digested by *Hinf*I (Thermo) at 37°C for 1 h and then separated by 2% agarose gel electrophoresis stained with ethidium bromide (Fig. 1). Every sample was repeated at least once.

Analysis of blood lipid correlative index parameters

Plasma was separated from 5 mL of venous blood less than 1 h after being drawn and analyzed by an automatic biochemical analyzer (AU5400; Olympus) using commercially available kits (HCY kit was from AUSA; kits for Lp (a), HDL, LDL, Apo AI, Apo B, GLU, TC, and TG were from Biotech).

Statistical analyses

The SPSS 19.0 software (SPSS) was used for all statistical analyses. The blood lipid and correlative index parameters are expressed as the mean \pm standard deviation. The confidence intervals of the *MTHFR* allele and the genotype were computed according to a normal approximation with correction for continuity. The association between the blood lipid and correlative index parameters in the experiments and the controls was statistically analyzed using a Student *t*-test. The chi-square test was used to analyze the enumeration data. A *p*-value of <0.05 was considered to be statistically significant.

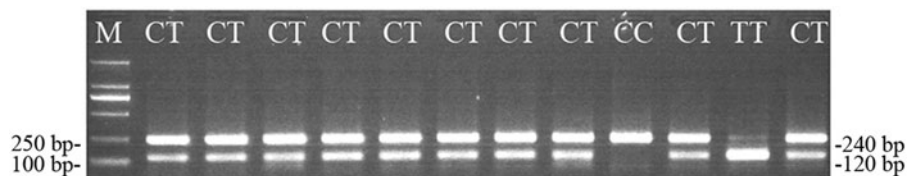


FIG. 1. DNA gel electrophoresis after polymerase chain reaction (PCR) restriction products were digested by *Hinf*I. The *Hinf*I-digested PCR products were separated by 2% agarose gel electrophoresis and stained with ethidium bromide. The genotype TT product shows two 120 bp bands, whereas the genotype CC shows a 240 bp band. In contrast, the digested genotype CT shows 120 and 240 bp bands.

TABLE 1. *MTHFR* C677T GENOTYPE AND ALLELE FREQUENCIES IN CASE AND CONTROL SUBJECTS

	Number of persons	<i>MTHFR</i> C677T genotype (%)				Allele frequency		
		CC	CT	TT	95% CI	C	T	95% CI
Case	174	45 (25.9)	53 (30.4)	76 (43.7)	51.1–36.3	41.1	58.9	64.1–53.7
Control	634	258 (40.7)	291 (45.9)	85 (13.4)	10.9–16.1	63.6	36.4	33.8–39.0

The *MTHFR* 677TT genotype and 677T allele were significantly different between case and control subjects ($\chi^2=78.411$, $p<0.001$ and $\chi^2=57.319$, $p<0.001$, respectively).

MTHFR, methylene tetrahydrofolate reductase.

Results

Genotype and allele frequencies of the *MTHFR* C677T polymorphism between cases and controls

In this study, we detected the genotype and allele frequencies of the *MTHFR* C677T polymorphism in 174 patients with hypertension and 634 healthy control individuals (Table 1). There was a significant difference between the case and control groups ($\chi^2=78.411$, $p<0.001$ and $\chi^2=57.319$, $p<0.001$, respectively).

Blood lipid and correlative index between cases and controls

The biochemical characteristics of blood lipid and correlative indexes are shown in Table 2. Specifically, there were significant differences in HCY ($p<0.001$), TG ($p=0.006$), LDL ($p=0.013$), Apo-AI ($p<0.001$), and Lp (a) ($p<0.001$) levels between the hypertensive and control groups. In the hypertensive group, HCY ($p=0.001$), HDL ($p=0.004$), Apo AI ($p<0.001$), and TC ($p=0.012$) were significantly dif-

ferent between males and females, while differences in the GLU ($p=0.174$), Lp (a) ($p=0.668$), LDL ($p=0.359$), Apo B ($p=0.095$), and TG ($p=0.073$) levels were not statistically significant. In addition, there was a significant difference in HCY ($p<0.001$), HDL ($p<0.001$), Apo AI ($p<0.001$), GLU ($p=0.001$), and TC ($p=0.004$) levels, but not Lp (a) ($p=0.789$), LDL ($p=0.278$), Apo B ($p=0.600$), or TG ($p=0.575$) levels between males and females in the control group.

Associations of the *MTHFR* C677T polymorphism with blood biochemical data in cases versus controls

We then combined *MTHFR* C677T polymorphisms with blood biochemical data from case and control subjects for analysis. We found that the average HCY and Lp (a) levels were significantly higher in patients with a *MTHFR* 677CC/CT or 677TT genotype in the hypertensive group than controls ($p_{HCY}<0.001$, $p_{Lp(a)}<0.001$) (Table 3). In the hypertension group, HCY and Lp (a) levels in patients with the TT genotype were significant higher than in patients with a CC/

TABLE 2. AVERAGE BLOOD LIPID CONTENT AND CORRELATIVE INDEXES BY GENDER IN CASE AND CONTROL SUBJECTS

	Male	95% CI	Female	95% CI	Total	95% CI
Case						
HCY (μM) ^a	27.27±8.60	25.63–29.31	23.50±6.02	22.29–24.81	25.36±7.62	24.25–26.55
Lp (a) (g/L)	37.71±20.21	33.66–42.20	34.37±21.89	30.16–39.15	36.02±25.14	32.97–39.59
HDL (mM) ^a	0.97±0.22	0.93–1.02	1.08±0.26	1.02–1.13	1.03±0.24	0.99–1.06
LDL (mM)	2.79±0.77	2.64–2.95	2.90±0.88	2.73–3.08	2.85±0.83	2.73–2.97
Apo AI (g/L) ^b	1.04±0.17	1.01–1.07	1.16±0.20	1.12–1.20	1.10±0.19	1.07–1.13
Apo B (g/L)	0.76±0.18	0.72–0.79	0.81±0.21	0.76–0.85	0.78±0.20	0.75–0.81
GLU (mM)	5.40±1.34	5.15–5.68	5.14±1.16	4.90–5.41	5.26±1.26	5.09–5.45
TC (mM) ^c	4.31±0.97	4.11–4.51	4.70±1.05	4.49–4.90	4.51±1.03	4.35–4.67
TG (mM)	1.59±1.144	1.36–1.83	1.99±1.77	1.66–2.38	1.79±1.50	1.58–2.02
Control						
HCY (μM) ^b	19.79±9.96	18.76–21.00	14.82±7.70	14.02–15.69	17.27±9.22	16.60–18.00
Lp (a) (g/L)	19.63±15.54	17.96–21.55	19.29±16.65	17.45–21.13	19.46±16.10	18.25–20.70
HDL (mM) ^b	0.94±0.21	0.92–0.96	1.04±0.24	1.01–1.06	0.99±0.23	0.97–1.01
LDL (mM)	2.64±0.83	2.55–2.73	2.71±0.74	2.62–2.79	2.67±0.79	2.62–2.73
Apo AI (g/L) ^b	0.93±0.17	0.92–0.95	1.06±0.34	1.02–1.10	1.00±0.27	0.98–1.02
Apo B (g/L)	0.80±0.21	0.78–0.83	0.81±0.24	0.79–0.84	0.81±0.23	0.79–0.83
GLU (mM) ^a	5.07±1.32	4.94–5.21	5.55±2.13	5.31–5.79	5.31±1.79	5.18–5.46
TC (mM) ^a	4.30±1.01	4.19–4.41	4.52±0.94	4.41–4.62	4.41±0.98	4.34–4.49
TG (mM)	1.53±0.85	1.44–1.63	1.57±0.84	1.48–1.66	1.55±0.85	1.49–1.61

There were significant differences between males and females in HCY ($p=0.001$), HDL ($p=0.004$), Apo AI ($p<0.001$), and TC ($p=0.012$) levels among the case patients. There were also significant differences between males and females in HCY ($p<0.001$), HDL ($p<0.001$), Apo AI ($p<0.001$), GLU ($p=0.001$), and TC ($p=0.004$) among the control subjects.

^a $p<0.01$, ^b $p<0.001$, ^c $p<0.05$.

Apo AI, apolipoprotein AI; GLU, glucose; HCY, homocysteine; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lp (a), lipoprotein (a); TC, total cholesterol; TG, triglyceride.

TABLE 3. LEVELS OF BLOOD LIPIDS AND CORRELATED INDEXES BY GENE POLYMORPHISM BETWEEN CASE AND CONTROL SUBJECTS

	Case		Control	
	CC+CT	TT	CC+CT	TT
HCY (μM)	26.58 \pm 8.36	29.69 \pm 7.75	16.48 \pm 7.79	22.41 \pm 14.61
Lp (a) (g/L)	31.55 \pm 19.12	42.50 \pm 19.22	18.78 \pm 14.99	24.00 \pm 21.53
HDL (mM)	0.98 \pm 0.22	1.01 \pm 0.24	0.99 \pm 0.23	0.98 \pm 0.23
LDL (mM)	2.81 \pm 0.77	2.91 \pm 0.86	2.65 \pm 0.76	2.83 \pm 0.92
Apo AI (g/L)	1.05 \pm 0.17	1.09 \pm 0.20	1.00 \pm 0.28	0.99 \pm 0.20
Apo B (g/L)	0.76 \pm 0.18	0.79 \pm 0.21	0.81 \pm 0.23	0.83 \pm 0.21
GLU (mM)	5.39 \pm 1.29	5.45 \pm 1.50	5.30 \pm 1.77	5.35 \pm 1.92
TC (mM)	4.39 \pm 0.99	4.50 \pm 1.13	4.39 \pm 0.95	4.57 \pm 1.14
TG (mM)	1.68 \pm 1.24	1.73 \pm 1.55	1.55 \pm 0.85	1.55 \pm 0.79

HCY and Lp (a) levels in those with the TT genotype were significantly higher than in those with the CC/CT genotype, regardless of whether they were case or control subjects. HCY: $p < 0.001$, Lp (a): control, $p = 0.005$; hypertensive, $p = 0.003$.

CT genotype ($p_{\text{HCY}} < 0.001$, $p_{\text{Lp (a)}} = 0.003$). There were similar findings in the control group ($p_{\text{HCY}} < 0.001$, $p_{\text{Lp (a)}} = 0.005$).

As shown in Table 4, we divided the hypertensive and control groups by gender. We discovered that only the HCY level in patients with a TT genotype was significantly higher than in patients with a CC/CT genotype ($p_{\text{HCY}} < 0.001$) between males and females in both the hypertensive and control groups.

Discussion

Due to variable genetic polymorphisms, geographic residencies, and lifestyle, an individual's predisposition and susceptibility to hypertension may vary, especially in China

(Yang *et al.*, 2013). There have been no reports regarding the *MTHFR* C677T polymorphism and hypertension in Jiangxi, China. Thus, in this study, we compared the rate of the 677TT genotype and the T allele between hypertensive and control subjects in this region. The genotype rate was significantly higher in the hypertensive group than in the healthy control group, suggesting that the *MTHFR* C677T gene polymorphism may be a risk factor for hypertension. These findings are consistent with some similar studies in other geographical locations (Marinho *et al.*, 2007; Alghasham *et al.*, 2012). However, it is different than other gene polymorphisms. For example, the eNOS T786C polymorphism is associated with hypertension risk in Beijing but not in Jilin (Li *et al.*, 2011).

The *MTHFR* protein is an important enzyme in HCY metabolism (Huang *et al.*, 2012). A 677C \rightarrow T mutation

TABLE 4. LEVELS OF BLOOD LIPIDS AND CORRELATED INDEXES BY GENE POLYMORPHISM AND GENDER IN CASE AND CONTROL SUBJECTS

	Case		Control	
	CC+CT	TT	CC+CT	TT
Male				
HCY (μM) ^a	23.62 \pm 6.53	30.29 \pm 8.99	18.78 \pm 8.74	26.68 \pm 14.30
Lp (a) (g/L)	30.15 \pm 15.32	43.99 \pm 21.72	18.96 \pm 14.55	24.20 \pm 20.72
HDL (mM)	0.99 \pm 0.21	0.96 \pm 0.23	0.94 \pm 0.21	0.94 \pm 0.20
LDL (mM)	2.80 \pm 0.74	2.78 \pm 0.79	2.60 \pm 0.78	2.92 \pm 1.08
Apo AI (g/L)	1.05 \pm 0.17	1.03 \pm 0.18	0.94 \pm 0.17	0.93 \pm 0.15
Apo B (g/L)	1.05 \pm 0.17	1.03 \pm 0.18	0.79 \pm 0.21	0.86 \pm 0.24
GLU (mM)	5.33 \pm 1.20	5.45 \pm 1.46	5.06 \pm 1.33	5.08 \pm 1.21
TC (mM)	4.36 \pm 0.97	4.27 \pm 0.98	4.25 \pm 0.96	4.63 \pm 1.25
TG (mM)	1.54 \pm 0.99	1.62 \pm 1.26	1.53 \pm 0.86	1.55 \pm 0.82
Female				
HCY (μM) ^a	20.94 \pm 4.60	28.70 \pm 5.17	14.20 \pm 5.91	18.62 \pm 13.96
Lp (a) (g/L)	32.46 \pm 24.67	38.26 \pm 14.30	18.55 \pm 15.43	23.81 \pm 22.45
HDL (mM)	1.07 \pm 0.27	1.09 \pm 0.23	1.04 \pm 0.23	1.02 \pm 0.26
LDL (mM)	2.80 \pm 0.84	3.11 \pm 0.95	2.70 \pm 0.74	2.75 \pm 0.76
Apo AI (g/L)	1.14 \pm 0.20	1.19 \pm 0.18	1.06 \pm 0.35	1.03 \pm 0.23
Apo B (g/L)	0.78 \pm 0.19	0.85 \pm 0.25	0.81 \pm 0.25	0.81 \pm 0.17
GLU (mM)	4.98 \pm 0.85	5.45 \pm 1.59	5.54 \pm 2.09	5.59 \pm 2.38
TC (mM)	4.61 \pm 0.93	4.87 \pm 1.26	4.52 \pm 0.92	4.51 \pm 1.05
TG (mM)	2.03 \pm 1.69	1.92 \pm 1.95	1.57 \pm 0.86	1.56 \pm 0.77

The HCY levels with the TT genotype were significantly higher than with the CC/CT genotype in both case and control subjects.

^a $p < 0.001$.

results in a switch from alanine to valine. This mutation leads to decreased MTHFR enzymatic activity and increases the enzyme's sensitivity to heat (Zonouzi *et al.*, 2012). Thus, *MTHFR* gene mutations that are associated with higher levels of HCY are considered to be risk factors for cardiovascular disease (Yin *et al.*, 2012; Kevere *et al.*, 2013; Xu *et al.*, 2013). However, there is controversy about the relationship between the mutation and the rise in HCY levels. Our studies show that the *MTHFR* C677T mutation alters the serum level of HCY, which is consistent with some previous reports (Zong *et al.*, 2011; Zhang *et al.*, 2012; Nienaber-Rousseau *et al.*, 2013; Bharatkumar *et al.*, 2014) but contrasts with other studies (Yasar *et al.*, 2012; Creus *et al.*, 2013; de Carvalho *et al.*, 2013). Our results indicate that there are variations in the HCY levels among various *MTHFR*677 genotypes. In this study, the HCY level and the frequency of the TT genotype in the hypertensive group were both significantly higher than in the control group. Thus, the *MTHFR* C677T polymorphisms may be a risk factor for hypertension through increased HCY levels.

Studies have indicated a correlation between the *MTHFR* C677T polymorphism and the Lp (a) level in coronary artery disease (Balogh *et al.*, 2012) as well as in unexplained recurrent miscarriages (Krause *et al.*, 2005) and other diseases. However, few studies have analyzed the relationship between the *MTHFR* C677T polymorphism and Lp (a). *MTHFR* may indirectly affect Lp (a) metabolism. Therefore, further studies using a larger population are necessary to confirm our discovery.

In summary, this study showed significant differences in the *MTHFR* C677T polymorphism distribution in Chinese hypertensive patients. In addition, the *MTHFR* C677T polymorphism may be a risk factor for hypertension. Our findings may be helpful for designing more effective treatment measures for hypertension patients in specific populations. Furthermore, HCY levels are likely associated with the *MTHFR* C677T polymorphism and gender. In addition, we found that the Lp (a) level is separated from the genotype polymorphism, which has rarely been reported in previous studies.

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Author Disclosure Statement

All the authors declare that they have no conflicts of interest, financial or otherwise, in the publication of this study or its results.

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