Association of MTHFR C677T gene polymorphism with metabolic syndrome in a Chinese population: a case–control study

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Abstract

Objective: To investigate the association of the MTHFR C677T gene polymorphism with metabolic syndrome (MetS) in people in Hubei Province, China.

Methods: A case–control study was conducted with 651 subjects with MetS (MetS group) and 727 healthy controls (control group) at Renmin Hospital of Wuhan University between January and December 2016. The MTHFR C677T genotype was detected by the gene chip technique and clinical data were collected.

Results: Body mass index, waist circumference, the waist-hip-ratio, systolic and diastolic blood pressure, fasting blood glucose, fasting insulin, triglyceride, total cholesterol, low-density lipoprotein-cholesterol, and homocysteine levels, and the homeostasis model assessment of insulin resistance were higher in the MetS group than in controls. The risk of MetS was higher for the TT genotype and T allele carriers than for the CC genotype and C allele carriers. With MetS, the TT genotype increased the risk of elevated blood pressure, fasting glucose levels, and triglyceride levels. Patients with MetS and the TT genotype showed more severe abdominal obesity, dyslipidaemia, insulin resistance, elevated blood pressure, elevated fasting glucose levels, and hyperhomocysteinaemia compared with those with the CC genotype.

Conclusions: In this population, MTHFR C677T gene polymorphism may be a risk factor for MetS.
Introduction

Metabolic syndrome (MetS) occurs as a cluster of metabolic abnormalities, which involve abdominal obesity, dyslipidaemia, hyperglycaemia, and hypertension. MetS contributes to cardiovascular morbidity and mortality. Furthermore, MetS or its components may play a role in the aetiology, progression, or prognosis of certain cancers. Currently, in China, the prevalence of MetS is 11.9% in men and 10.1% in women (>18 years old). However, MetS is rapidly increasing worldwide. A complex mix of many genetic and environmental factors may be involved in developing MetS. In view of the potential effect of MetS and its associated health complications, studying the genetic factors as screening tools for identifying whether individuals are at high risk of MetS is important. Genome-wide association analyses have explained some of the genetic predispositions for MetS, but none of them have reached an accepted conclusion.

Some studies have found that homocysteine (Hcy) is associated with MetS. The MTHFR gene encodes 5,10-methylenetetrahydrofolate reductase (MTHFR) protein. MTHFR catalyses 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Therefore, MTHFR is an important enzyme involved in Hcy metabolism. The compound 5-methyltetrahydrofolate is the main active form of folic acid in vivo, and provides methyl to methylate Hcy to methionine, thereby maintaining normal Hcy levels. A series of mutations in the MTHFR gene cause the activity of MTHFR to decrease, but only MTHFR gene polymorphism at position 677 from C to T (alanine replaced by valine) is widely thought to be the leading cause of hyperhomocysteinaemia (HHcy). The C677T polymorphism of the MTHFR gene is located in the area encoding MTHFR’s catalytic region, and thus its reductase activity and thermal stability are decreased because of the mutation. This leads to increased Hcy levels in the blood.

Epidemiological studies showed that MTHFR 677 T carriers were at increased risk of suffering obesity, hypertension, insulin resistance or type 2 diabetes mellitus, and lipid disorders, and that these metabolic disturbances were components of MetS. Additionally, the polymorphism C677T in the MTHFR gene may affect DNA methylation, and DNA hypomethylation resulting from reduced MTHFR activity is also associated with MetS.

A direct relationship between MTHFR C677T gene polymorphism and MetS still remains controversial because results differ among different populations. However, one study of a population in North China showed an association between MTHFR C677T and MetS. Based on the above-mentioned evidence, we hypothesized that MTHFR gene polymorphism is associated with a genetic susceptibility for MetS in the Chinese population. We conducted a case–control study to investigate the association between MTHFR C677T gene polymorphism and MetS.
Materials and methods

Subjects

This study enrolled 651 patients with MetS and 727 healthy controls who were admitted to Renmin Hospital of Wuhan University from January 2016 to December 2016. Patients with MetS were recruited to the MetS group according to the following inclusion criteria: 1) patients with a diagnosis of MetS based on the National Cholesterol Education Program Adult Treatment Panel III, which was revised by the American Heart Association/National Heart, Lung, and Blood Institute in 2005; and 2) patients aged between 18 and 65 years.

Healthy volunteers were recruited from individuals who received regular physical examinations at the Medical Examination Centre of Renmin Hospital of Wuhan University. These volunteers were selected as subjects by the fixed point continuous sampling method and comprised the control group. The inclusion criteria for the control group were as follows: (1) no MetS component, including patients without central obesity (males: waist circumference < 90 cm; females: waist circumference < 80 cm), patients without dyslipidaemia (triglyceride [TG] levels < 1.7 mmol/L, high-density lipoprotein-cholesterol [HDL-C] levels > 1.03 mmol/L in men and HDL-C levels > 1.3 mmol/L in women), patients without hyper-tension (without a history of hypertension and blood pressure [BP] < 130/85 mmHg), patients without hyperglycaemia (without a history of hyperglycaemia and fasting plasma glucose [FPG] levels < 5.6 mmol/L); and (2) patients without a family history of diabetes mellitus and hypertension.

The exclusion criteria for the MetS and control groups were as follows: 1) patients who did not agree to participate in the study; 2) patient’s age < 18 years old or > 65 years old; 3) patients with severe hepatic or renal dysfunction; 4) patients with a severe infection, tumour, or thyroid dysfunction; and 5) patients who used vitamin B6 and/or vitamin B12 and/or folic acid for at least 1 month.

This study followed the principle of informed consent and patients/subjects provided verbal informed consent. The study was approved by the ethics committee of Renmin Hospital of Wuhan University.

Study design

The controls were frequency-matched according to age (± 5 years old) and sex with the MetS group. Diagnosis of MetS was made by identification of any three of the following five features: 1) elevated waist circumference defined as a waist circumference ≥ 90 cm (Asian man) or waist circumference ≥ 80 cm (Asian woman); 2) elevated TG levels defined as TG levels ≥ 1.70 mmol/L (150 mg/dl) or patients who had accepted treatment for elevated TG levels; 3) reduced HDL-C levels defined as HDL-C levels < 1.03 mmol/L (40 mg/dl) in men, HDL-C levels < 1.30 mmol/L (50 mg/dl) in women, or patients who had accepted treatment for reduced HDL-C levels; 4) elevated BP defined as systolic BP ≥ 130 mmHg, diastolic BP ≥ 85 mmHg, or patients who had accepted treatment for hypertension; and 5) elevated FPG levels defined as FPG levels ≥ 5.6 mmol/L (100 mg/dl) or patients who had accepted treatment for elevated FPG levels.

Data acquisition and detection

Data such as age, sex, history of past illness, and family medical history were collected. All of the subjects were fasted for more than 8 h. BP, height, weight, waist circumference (iliac crest plane), hip circumference, body mass index (BMI), and the waist-to-hip ratio (WHR) were measured.
after an overnight fast. TG, total cholesterol (TC), HDL-C, LDL-C, and FPG levels in samples were determined with a SIEMENS ADVIA2400 automatic biochemical analyser (Siemens Healthcare, Erlangen, Germany). Fasting serum insulin (FINS) and Hcy levels were determined by using the SIEMENS ADVIA Centaur XP chemiluminescence immunoassay system (Siemens Healthcare). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the following formula: HOMA-IR = FINS (mU/L) × FPG (mmol/L)/22.5.31

Gene detection

Genomic DNA from peripheral blood was extracted by the salting-out method. MTHFR C677T genotypes were analysed by gene chips (MTHFR gene detection kit; BaiO, Shanghai, China), and the assays were performed exactly according to manufacturer’s instructions. A sample of the polymerase chain reaction (PCR) system contained 2 µl of genomic DNA, 22 µl of amplification reagent that included labelled primer pairs (MTHFR gene detection kit; BaiO), and 1 µl of reaction solution A (MTHFR gene detection kit; BaiO). The positive controls (TT genotype) and negative controls (MTHFR gene detection kit; BaiO) were amplified at the same time. Asymmetric PCR amplification was performed in a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) and amplification steps were 50°C for 5 min, 94°C for 5 min, and 35 cycles of the following steps: 94°C for 25 s, 56°C for 25 s, and 72°C for 25 s. Finally, amplification was performed at 72°C for 5 min. Specific hybridization between amplification products and gene chip probes, and the chromogenic reaction were all completed in an e-Hyb automated hybridization instrument (BR-526-24; BaiO). Finally, signals on the chip were identified by a biochip reading system (BE-2.0; BaiO), which is based on the principle of a charge-coupled device, and Array Doctor gene chip image analysis software (V2.0; BaiO) provided the results of genotyping.

Statistical analysis

IBM SPSS Statistics, version 21.0 (IBM Corp., Armonk, NY, USA) was used to analyse the data. All significance tests were two-tailed tailed and P < 0.05 was considered a significant difference. The Kolmogorov–Smirnov test was used to determine whether the measurement data were normally distributed. Measurement data that were normally distributed are shown as mean ± SD. Analysis of variance was used for comparison between groups. Logarithmic transformation was performed on data that did not have a normal distribution. If the converted data still did not have a normal distribution, they are shown by the median (interquartile range). The Mann–Whitney U test was used to compare two independent samples of two groups. The Kruskal–Wallis test was used for comparison between multiple groups. The Student–Newman–Keuls q test was used for pairwise comparison within a group. The chi-square test or Fisher’s exact test was performed to identify departure from the Hardy–Weinberg equilibrium, and to compare the differences between the two groups regarding the sex ratio, and allelic and genotypic frequencies. Unconditional logistic regression analysis was used to analyse the effect of MTHFR C677T gene polymorphisms on MetS and the components in the MetS group.

Results

Comparison of clinical data between the MetS and control groups

The demographic characteristics, anthropometric measurements, and clinical biochemical indices of the subjects are shown in Table 1.
There were no significant differences in the proportions of men between the MetS group and control group. The mean age was also not significantly different between the groups. The BMI, waist circumference, WHR, systolic BP, diastolic BP, and FPG, FINS, TG, TC, LDL-C, and Hcy levels in the MetS group were significantly higher, and HDL-C levels were significantly lower than those in the control group (all \( P < 0.01 \)). The HOMA-IR in the MetS group was significantly higher than that in the control group (\( P < 0.001 \)). This finding indicated that insulin resistance was present in the MetS group, which is consistent with the characteristics of MetS.

### Analysis of the association between MTHFR C677T gene polymorphism and MetS

The results of genotype distribution in the populations of the two groups are shown in Table 2. The frequencies of CC, CT, and TT genotypes in the control group were 29.02%, 49.38%, and 21.60%, respectively. The frequencies of the CC, CT, and TT genotypes in the MetS group were 20.58%, 47.62%, and 31.80%, respectively. Genotype distribution of this polymorphism conformed to Hardy–Weinberg equilibrium in the control group (chi-square value = 0.035, \( P = 0.852 \)) and MetS group (chi-square value = 0.820, \( P = 0.365 \)), which meant the distribution frequencies reached genetic equilibrium. There was a significant difference in genotype distribution between the two groups (\( P < 0.001 \)). After age, sex, and BMI were adjusted, logistic regression analysis showed that individuals who carried the TT genotype had a higher risk of developing MetS than did those who carried the CC genotype (odds ratio [OR] = 1.59, 95% confidence interval [CI] = 1.05–2.41, \( P = 0.028 \)). However, there was no significant difference in the risk of developing

|                  | MetS group | Control group | \( P \) value |
|------------------|------------|---------------|---------------|
| No. of cases     | 651        | 727           | –             |
| Sex (male/female)| 425 (65%)/226 (35%) | 442 (61%)/285 (39%) | 0.085         |
| Age (years)      | 50.40 ± 10.74 | 51.23 ± 11.55 | 0.166         |
| BMI (kg/m²)      | 26.78 ± 2.98 | 22.35 ± 2.44  | <0.001        |
| Waist circumference (cm) | 91.00 (85.00–97.00) | 77.00 (71.00–81.00) | <0.001        |
| WHR              | 0.89 ± 0.05 | 0.82 ± 0.05   | <0.001        |
| Systolic BP (mmHg)| 134.00 (126.00–144.00) | 112.00 (104.00–120.00) | <0.001        |
| Diastolic BP (mmHg)| 82.00 (74.00–88.00) | 68.00 (62.00–74.00) | <0.001        |
| FPG (mmol/L)     | 5.65 (5.21–6.20) | 5.02 (4.77–5.25) | <0.001        |
| FINS (mU/L)      | 9.51 (7.17–13.61) | 6.42 (4.75–9.03) | <0.001        |
| TG (mmol/L)      | 2.14 (1.62–2.84) | 0.98 (0.70–1.28) | <0.001        |
| TC (mmol/L)      | 4.85 (4.27–5.38) | 4.50 (4.03–5.11) | <0.001        |
| HDL-C (mmol/L)   | 1.03 (0.91–1.20) | 1.44 (1.28–1.65) | <0.001        |
| LDL-C (mmol/L)   | 2.78 ± 0.82 | 2.57 ± 0.71   | 0.007         |
| Hcy (μmol/L)     | 12.66 (10.34–16.18) | 10.36 (8.31–12.73) | <0.001        |
| HOMA-IR          | 2.41 (1.76–3.53) | 1.40 (0.92–1.89) | <0.001        |

Values are mean ± SD or median (interquartile range).
MetS = metabolic syndrome; BMI = body mass index; WHR = waist-to-hip ratio; BP = blood pressure; FPG = fasting plasma glucose; FINS = fasting serum insulin; TG = triglycerides; TC = total cholesterol; HDL-C = high-density lipoprotein-cholesterol; LDL-C = low-density lipoprotein-cholesterol; Hcy = homocysteine; HOMA-IR = homeostasis model assessment of insulin resistance.
MetS between the CT and CC genotypes. In comparison of allele frequency, the OR of the T allele was 1.27 (95% CI = 1.03–1.55, \( P = 0.024 \)) by using the C allele as a reference. These results suggest that the \textit{MTHFR} C677T gene polymorphism may be a genetic risk factor for MetS.

### Comparison of clinical data of different \textit{MTHFR} C677T genotypes in the MetS group

Wx|x| WHR, BP, FPG, TC, FINS, TG, and Hcy levels, and HOMA-IR of TT genotype carriers in the MetS group were significantly higher, and HDL-C levels were significantly lower than those of CC genotype carriers (all \( P < 0.05 \)) (Table 3).

### Association between different \textit{MTHFR} C677T genotypes and the components of MetS

In the MetS group, after age, sex, and BMI were adjusted, logistic regression analysis showed that the risks of developing elevated blood pressure (OR = 3.86, 95% CI = 2.26–6.62, \( P < 0.001 \)), elevated fasting glucose levels (OR = 2.83, 95% CI = 1.79–4.48, \( P < 0.001 \)), and elevated triglyceride levels (OR = 1.57, 95% CI = 1.03–2.66, \( P = 0.043 \)) in individuals who carried the TT genotype increased more significantly than those in individuals who carried the CC genotype. However, there was no significant difference in the risk of elevated waist circumference or reduced HDL-C levels between the genotypes (Table 4). Individuals who carried the CT genotype showed higher risks of developing elevated blood pressure (OR = 1.61, 95% CI = 1.04–2.50, \( P = 0.034 \)) and elevated fasting glucose levels (OR = 1.76, 95% CI = 1.16–2.67, \( P = 0.008 \)) compared with individuals who carried the CC genotype, but there were no significant differences in the risks of other MetS components (Table 4). Hierarchical analysis showed some confounding factors for MetS (Table 5), and there was collinearity between waist circumference and BMI.

The proportion of the TT genotype significantly increased with the number of components of MetS, while the proportions of the CC/CT genotypes decreased (all \( P < 0.05 \), Table 6). In the five components group, the proportion of the TT genotype was much higher than that of the CC/CT genotypes (both \( P < 0.05 \)) (Table 6).

### Discussion

The occurrence and development of MetS are associated with a complex mix of many genetic and environmental factors.\(^4\)–\(^6\) An increasing amount of indirect evidence has

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Table 2. Association of \textit{MTHFR} C677T gene polymorphism with MetS.

| Genotype, n (%) | MetS group (n = 651) | Control group (n = 727) | P value (single factor analysis) | OR value (95% CI) | P value |
|----------------|---------------------|------------------------|---------------------------------|------------------|--------|
| **Genotype, n (%)** |                      |                        |                                 |                  |        |
| CC             | 134 (20.58)         | 211 (29.02)            | <0.001                          | 1                |        |
| CT             | 310 (47.62)         | 359 (49.38)            | 1.17 (0.93–1.70)                | 0.309            |        |
| TT             | 207 (31.80)         | 157 (21.60)            | 1.59 (1.05–2.41)                | 0.028            |        |
| **Alleles, n (%)** |                      |                        |                                 |                  |        |
| C              | 578 (44.39)         | 781 (53.71)            | <0.001                          | 1                |        |
| T              | 724 (55.61)         | 673 (46.29)            | 1.27 (1.03–1.55)                | 0.024            |        |

MetS = metabolic syndrome; n (%) = frequency; OR = odds ratio; CI = confidence interval.
shown a correlation between HHcy and DNA hypomethylation with MetS.8–14 HHcy may produce oxygen-free radicals by stimulating proliferation of vascular smooth muscle cells, inducing insulin resistance, and damaging vascular endothelial cell function. This results in increased plasma C-reactive protein levels, promotion of lipid peroxidation, a decrease in Apo-A1 expression, and effects on other pathophysiological processes.10–14 These lead to hypertension, diabetes mellitus, and abnormal lipid metabolism, and all of these factors are related to MetS. Mutations of the MTHFR gene at position 677 may affect plasma Hcy levels and DNA methylation has been well-studied.15–18,24 Therefore, we hypothesized that MTHFR C677T gene polymorphisms are an important genetic risk factor for MetS. This study aimed to investigate MTHFR C677T gene polymorphism in people with MetS in Hubei Province, which is located in central China. We found that the frequencies of the TT genotype and T allele in patients with MetS were significantly higher than those in healthy controls. The TT genotype carriers had a 1.59 times higher risk of developing MetS than did CC genotype carriers. These findings indicated an association between MTHFR C677T gene polymorphism and MetS in this Chinese population. Additionally, for patients with MetS, the TT genotype carriers had a higher risk of elevated BP, elevated FPG, and elevated TG, and they had more severe abdominal obesity, elevated BP, elevated FPG levels, dyslipidaemia, elevated Hcy levels, and insulin resistance than did CC genotype carriers. Furthermore, the proportion of the TT genotype increased with the number of the components of MetS, while

Table 3. Comparison of clinical data of different genotypes of MTHFR C677T in the MetS group.

|                  | CC   | CT   | TT   | P value |
|------------------|------|------|------|---------|
| No. of cases     | 134  | 310  | 207  | –       |
| Sex (male/female)| 73/41| 206/114| 146/71| 0.749   |
| Age (years)      | 49.37±10.17 | 49.90±11.05 | 51.80±10.55 | 0.067   |
| BMI (kg/m²)      | 26.44±2.53 | 26.86±3.00 | 26.86±3.20 | 0.333   |
| Waist circumference (cm) | 90.00 (84.00–95.00) | 91.00 (86.75–97.00)* | 91.00 (85.00–99.00)* | 0.026   |
| WHR              | 0.89±0.05 | 0.89±0.05 | 0.90±0.06## | 0.017   |
| Systolic BP (mmHg) | 130.00 (114.00–134.00) | 135.00 (126.00–146.00)* | 138.00 (132.00–148.00)## | <0.001  |
| Diastolic BP (mmHg) | 77.00 (68.00–84.00) | 82.00 (74.00–90.00)* | 86.00 (78.00–90.00)## | <0.001  |
| FPG (mmol/L)     | 5.40 (5.11–5.72) | 5.63 (5.19–6.12)* | 6.02 (5.34–7.27)## | <0.001  |
| FINS             | 7.53 (6.92–9.70) | 9.62 (7.25–12.91)* | 12.76 (8.98–15.60)## | <0.001  |
| TG (mmol/L)      | 1.89 (1.54–2.24) | 2.15 (1.52–2.90)* | 2.38 (1.78–3.33)## | <0.001  |
| TC (mmol/L)      | 4.76±0.77 | 4.85±0.99 | 4.99±1.10# | 0.095   |
| HDL-C (mmol/L)   | 1.07 (0.94–1.21) | 1.03 (0.93–1.19) | 0.99 (0.86–1.19) | 0.011   |
| LDL-C (mmol/L)   | 2.78±0.67 | 2.82±0.84 | 2.72±0.87 | 0.392   |
| Hcy (µmol/L)     | 10.55 (8.62–14.33) | 11.98 (10.54–13.27) | 15.62 (13.62–17.97)## | <0.001  |
| HOMA-IR          | 1.81 (1.55–2.46) | 2.37 (1.59–3.42)* | 3.44 (2.25–5.01)## | <0.001  |

Values are mean±SD or median (interquartile range). *P<0.05, compared with the CC genotype; †P<0.05, compared with the CT genotype. The Student–Newman–Keuls test was used for comparative analysis between the two groups. MetS = metabolic syndrome; BMI = body mass index; WHR = waist-to-hip ratio; BP = blood pressure; FPG = fasting plasma glucose; FINS = fasting serum insulin; TG = triglycerides; TC = total cholesterol; HDL-C = high-density lipoprotein-cholesterol; LDL-C = low-density lipoprotein-cholesterol; Hcy = homocysteine; HOMA-IR = homeostasis model assessment of insulin resistance.
the proportions of the CC/CT genotype decreased. In the five components group, the proportion of the TT genotype was much higher than that of the CC/CT genotypes. These results indicated that, for patients with MetS, TT genotype carriers were more severely affected than CC/CT genotype carriers. All of these findings suggested that MTHFR C677T gene polymorphism and MetS were associated in a Chinese population, which is similar to a previous study.27

The results of this study should be considered in relation to previous investigations in which the association of MTHFR C677T gene polymorphism with MetS remained inconclusive. Ellingrod et al.25 found that for schizophrenic patients, the risk of developing MetS in TT genotype carriers was 3.7 times higher than that in CC genotype carriers. Additionally, in a Greek population study, Vasilopoulos et al.27 found that the 677T allele increased the risk of MetS by 4.02 times. However, a study in Korean patients with colorectal cancer showed that the MTHFR C677T gene polymorphism was not associated with MetS.26 Moreover, van Winkel et al.28 showed that MetS was associated with the A1298C locus of the MTHFR gene, but not with C677T. Many factors may lead to these conflicting conclusions of genetic predisposition to disease between those published studies and our study. First, our study was conducted in the general population without any severe diseases, while most other studies

### Table 4. Association between different MTHFR C677T genotypes and the components of MetS.

|                        | No. of cases, n (%) | Adjusted ORa (95% CI) | P value |
|------------------------|---------------------|-----------------------|---------|
| Elevated waist circumference |                     |                       |         |
| CC                     | 116 (21.60)         | 1                     |         |
| CT                     | 260 (48.42)         | 0.96 (0.45–2.01)      | 0.904   |
| TT                     | 161 (29.98)         | 0.60 (0.27–1.32)      | 0.204   |
| Elevated BP            |                     |                       |         |
| CC                     | 77 (16.38)          | 1                     |         |
| CT                     | 216 (45.96)         | 1.61 (1.04–2.50)      | 0.034   |
| TT                     | 177 (37.66)         | 3.86 (2.26–6.62)      | <0.001  |
| Elevated FPG           |                     |                       |         |
| CC                     | 52 (14.90)          | 1                     |         |
| CT                     | 163 (46.70)         | 1.76 (1.16–2.67)      | 0.008   |
| TT                     | 134 (38.40)         | 2.83 (1.79–4.48)      | <0.001  |
| Elevated TG            |                     |                       |         |
| CC                     | 96 (20.08)          | 1                     |         |
| CT                     | 219 (45.82)         | 0.94 (0.59–1.49)      | 0.776   |
| TT                     | 163 (34.10)         | 1.57 (1.03–2.66)      | 0.043   |
| Reduced HDL-C          |                     |                       |         |
| CC                     | 98 (21.26)          | 1                     |         |
| CT                     | 217 (47.07)         | 0.96 (0.60–1.52)      | 0.850   |
| TT                     | 146 (31.67)         | 1.07 (0.65–1.77)      | 0.781   |

*aAdjusted for age, sex, and BMI.

MetS = metabolic syndrome; n (%) = frequency; OR = odds ratio; CI = confidence interval; BP = blood pressure; FPG = fasting plasma glucose; TG = triglycerides; HDL-C = high-density lipoprotein-cholesterol.
were carried out in patients with MetS in combination with other diseases, such as schizophrenia and cancer. These diseases and the treatment for them may affect the clinical data. Second, different diagnostic criteria for MetS were used in these studies, which may have affected the results of grouping and statistical analysis. Third, the frequency of C677T mutation in MTHFR is significantly different in different races and regions. The frequencies of the MTHFR 677T allele were found to be 24.1%–64.3% in Europe, 0%–35.5% in Africa, and 14.7%–20.4% in Asia, which may result in statistical discrepancy. Fourth, different genetic backgrounds affect disease susceptibility. Finally, different dietary habits (e.g., intake of folic acid, vitamin B6, vitamin B12, and calories) may affect the association between polymorphisms and MetS through epigenetic mechanisms.

This is the first study to examine the relationships of MTHFR C677T polymorphism with MetS in a population in central China. However, several limitations should be acknowledged in relation to this study. This study was based in a single centre. Therefore, the results may not be directly applicable to the Chinese population as a whole. Only one polymorphism was investigated in our study. Therefore, we need to examine the interactions between other polymorphisms in the future. We also did not investigate the effect of environmental factors. Factors, such as diet and behaviour, affect development of MetS. Therefore, we cannot make any conclusions about the interactions between the environment and these genetic polymorphisms.

**Conclusion**

Mutation of MTHFR C677T is more commonly identified in Chinese patients with MetS than in controls. The risk of

| Table 5. Confounding factors for MetS. |
|--------------------------------------|
|                                   | No. of cases | Percentage |
|-------------------------------------|--------------|------------|
| Waist circumference                 |              |            |
| Normal                              | 114          | 17.51      |
| Elevated                            | 537          | 82.49      |
| BP                                  |              |            |
| Normal                              | 181          | 27.80      |
| Elevated                            | 470          | 72.20      |
| FPG                                 |              |            |
| Normal                              | 302          | 46.39      |
| Elevated                            | 349          | 53.61      |
| TG                                  |              |            |
| Normal                              | 173          | 26.57      |
| Elevated                            | 478          | 73.43      |
| HDL-C                               |              |            |
| Normal                              | 190          | 29.19      |
| Reduced                             | 461          | 70.81      |

MetS = metabolic syndrome; BP = blood pressure; FPG = fasting plasma glucose; TG = triglycerides; HDL-C = high-density lipoprotein-cholesterol.

| Table 6. Association between different MTHFR C677T genotypes and the severity of MetS. |
|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
|                                     | Three components (n = 379)          | Four components (n = 203)           | Five components (n = 69)            | P value                             |
| Genotype, n (%)                     |                                     |                                     |                                     |                                     |
| CC                                  | 98 (25.86)                          | 34 (16.75)                          | 2 (2.90)                            | <0.001                              |
| CT                                  | 190 (50.13)*                        | 98 (48.28)*                         | 22 (31.88)*                         | 0.020                               |
| TT                                  | 91 (24.01)#                         | 71 (34.97)*                         | 45 (65.22)*                         | <0.001                              |

MetS = metabolic syndrome; n (%) = frequency

*P < 0.05, compared with the CC genotype; **P < 0.05, compared with the CT genotype.
developing MetS is higher for the TT genotype and T allele carriers than for the CC genotype and C allele carriers. In patients with MetS, abdominal obesity, elevated BP, elevated FPG levels, dyslipidaemia, elevated Hcy levels, and insulin resistance are more severe in TT genotype carriers than in CC genotype carriers. Moreover, the TT genotype increases the risks of elevated BP, elevated FPG levels, and elevated TG levels compared with the CC genotype in patients with MetS, and TT genotype carriers suffer from more components. Therefore, we suggest that in this Chinese population, the MTHFR C677T gene polymorphism could be a genetic risk factor for MetS.

Declaration of conflicting interest
The authors declare that there is no conflict of interest.

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