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ORIGINAL ARTICLE

Association Between *MTHFR* Gene Common Variants, Serum Homocysteine, and Risk of Early-Onset Coronary Artery Disease: A Case–Control Study

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Abstract

The common variants of the *methylenetetrahydrofolate reductase* (*MTHFR*) gene are related to the activity of the MTHFR enzyme and the concentrations of blood homocysteine (Hcy). This study was designed to investigate the associations of *MTHFR* in Chinese populations with early-onset coronary artery disease (EOCAD). The two common variants of the *MTHFR* gene were genotyped in 875 EOCAD patients and 956 controls using PCR, followed by direct sequencing of the PCR product. Serum levels of Hcy were measured using an automatic biochemistry analyzer. A significant association between the *MTHFR*-677C/T variant and the risk of EOCAD was detected in CC versus TT (odds ratio (OR) 1.456, 95% confidence interval (CI) 1.120–1.892), dominant genetic model (OR 1.266, 95% CI 1.027–1.546), and recessive genetic model (OR 1.306, 95% CI 1.040–1.639). Hcy was most abundant in TT genotype (18.31 ± 7.22 μmol/L), least abundant in CC genotype (11.37 ± 5.23 μmol/L), and detectable at intermediate levels in heterozygotes (15.25 ± 6.58 μmol/L). Elevated serum Hcy levels were an independent risk factor for EOCAD (OR_{adjust} 1.431, 95% CI 1.135–1.763). Our findings indicated that the T allele of -677C/T *MTHFR* variant predisposes to high levels of Hcy, and that the T allele is an important risk factor for EOCAD in the Chinese population.

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Introduction

The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The latter provides methyl for the conversion of homocysteine (Hcy) to methionine (Biselli et al. 2010). Thus, there is a close relationship between MTHFR activity and Hcy metabolism. Hcy impairs endothelial function and leads to platelet activation and thrombus formation (Hanratty et al. 1998). Experimental evidence has demonstrated that elevated blood Hcy levels were significantly associated with an increased risk of cardiovascular events, which appears to be largely independent of other conventional risk factors (Refsum et al. 1998; Nygård et al. 1999).

Worldwide, coronary artery disease (CAD) remains the leading cause of death and disability. As a special type of CAD, early-onset coronary artery disease (EOCAD) has particular components of etiology, including family heredity, lipid metabolism, gender composition, and other risk factors. In our previous studies, we demonstrated that asymmetric dimethylarginine and uric acid associated with the presence and severity of EOCAD (Tian et al. 2018; Xuan et al. 2017). Familial aggregation strongly indicated the presence of genetic factors for increased susceptibility to the disease (Engert et al. 2008). EOCAD affects young and middle-aged individuals, and is more harmful than conventional CAD.

In several previous studies, it has been suggested that common variants of the *MTHFR* gene (rs1801131, rs1801133) and elevated serum Hcy levels are important risk factors for conventional CAD (Lewis et al. 2005; Biselli et al. 2010). In EOCAD patients, many countries and regions have also studied the relationships; however, the results were controversial (Hou et al. 2015). According to the principle of genetic diversity, there are differences in genes and phenotypes between different races due to evolutionary and environmental differences (Leimar 2005; Messer et al. 2016). Therefore, it is critical to investigate the association between these genetic variations and disease susceptibility in different populations. To our knowledge, in Chinese population, there are few studies considering the relationships between common variants of the *MTHFR* gene, serum Hcy, and risk of EOCAD. In this study, we aimed to investigate these associations.

Materials and Methods

Subjects

In this hospital-based case–control study, all the participants visited The Affiliated Hospital of Qingdao University between January 2013 and June 2018. A total of 875 patients who met CAD diagnostic criteria were enrolled in the study when their first onset of symptoms and hospitalization for coronary angiography occurred at age $\leq$50 years. The diagnosis and severity of EOCAD were assessed by a cardiologist

who used angiographic findings. Patients with other serious illnesses and/or who were taking drugs (folic acid, vitamin B12) that might interfere with the results of the study were excluded. The 956 controls were age and sex-matched who did not show any signs or symptoms of cardiovascular events. All patients and controls included in the study signed informed consent prior to the start of the study. The Ethics Committee of our hospital approved the study, and the protocol was conformed the ethical guidelines of the Helsinki Declaration of 1975.

Clinical Parameters and Biochemical Measurements

Data on physical examination including smoking and drinking habits, gender, age, height, weight, myocardial infarction (MI), hypertension, and diabetes mellitus (DM) were recorded. Whole blood was collected by vacuum blood collection without anticoagulant, and was centrifuged at 1500×*g* for 15 min. Serum concentrations of low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), serum creatinine (SCr), fasting blood glucose (FBG), Lipoprotein(a) (Lp(a)), and Hcy were determined in the morning after fasting of at least 8 h. Serum biochemical indicators were determined using an automatic biochemistry analyzer (Hitachi HCP-7600, Hitachi, Japan).

DNA Isolation and Genotyping

Whole blood was collected by vacuum blood collection using an EDTA-K_2 anticoagulant. Genomic DNA was isolated by a Blood Genomic DNA Extraction Kit (Tianlong Science and Technology, Xi'an, China) according to the instructions using an NP968 Nucleic Acid Extraction System (Tianlong Science and Technology, Xi'an, China), which was based on a magnetic bead separation method. DNA was extracted from 200 μL whole blood and stored at − 80 °C. Primers for the two common variants of *MTHFR* were as follows: Forward (rs1801133): 5′-CGGTGC ATGCCTTCACAA-3′ and reverse: 5′-CTGACCTGAAGCACTTGAAGGA-3′. Forward (rs1801131): 5′-CCCGAGAGGTAAAGAACAAAGACTT-3′ and reverse: 5′-GGAGGAGCTGCTGAAGATGTG-3′. The standard PCR protocol for amplifying targets was as follows: one cycle of 1 min at 95 °C, then 36 cycles of 30 s at 94 °C and 30 s at 65 °C/60 °C, followed by 10 min at 72 °C using a GeneAmp PCR machine (Tianlong Science And Technology, Xi'an. China). PCR products were directly sequenced using a genomic company (Genewiz Biotechnology, Suzhou, China). Common variants of the *MTHFR* gene were identified by Gene Tools, LLC (Philomath, OR, USA) according to the reference sequence (from NCBI).

Statistical Analysis

Unpaired *t* test was used to compare continuous variables, and the χ^2 test was used to compare categorical variables. A *Q* test with one degree of freedom was used to test the Hardy–Weinberg equilibrium (*HWE*) (Xuan et al. 2014; Xuan et al. 2016). In genetic models, the contrast of A versus a, AA versus aa, dominant genetic model

(AA+Aa vs. aa), and recessive genetic model (AA vs. Aa+aa) were also investigated. The associations between common variants of the *MTHFR* gene and the risk of EOCAD were estimated using the odds ratio (OR) and the 95% confidence interval (CI). Adjusted ORs and 95% CIs after adjustment for age, gender, BMI, hypertension, diabetes, smoking, and biochemical indicators were estimated by logistic regression. Analyses were performed using SPSS software version 11.0, and Stata software version 11.0 and $P < 0.05$ was considered statistically significant.

Results

Characteristics of Participants

A total of 875 EOCAD patients (mean age 46.20 ± 4.32; 91.20% men) and 956 controls (mean age 43.96 ± 5.52; 90.27% men) were enrolled in the present study. No significant differences were observed between EOCAD patients and controls regarding gender, age, hypertension, TC, and SCr. However, BMI, and levels of FBG, TG, HDL-C, LDL-C, and Lp(a) were significantly elevated in EOCAD patients when compared to controls. In addition, the patients group had higher diabetes, smoking and drinking rate compare with controls. In the EOCAD patients group, 285 patients were diagnosed with MI. The EOCAD patients group included 576 patients with single-vessel disease, 212 patients with double-vessel disease, and 87 patients with triple-vessels disease. Clinical characteristics of all participants are summarized in Table 1.

Genetic Variants Of The *MTHFR* Gene

The genotypes of variants were summarized in Table 2. The two common variants of the *MTHFR* gene (rs1801133 and rs1801131) were genotyped in all participants, including 875 EOCAD patients and 956 controls. The distribution of genotypes in controls was compatible with *HWE* ($P > 0.05$).

MTHFR Gene Variants and EOCAD Risk

In this study, we showed a significant association between the *MTHFR*-677C/T variant and the risk of EOCAD in contrast of CC versus TT (OR 1.456, 95% CI 1.120–1.892), dominant model (OR 1.266, 95% CI 1.027–1.546), and recessive model (OR 1.306, 95% CI 1.040–1.639). Allele T of the *MTHFR*-677C/T is a risk allele for EOCAD (OR 1.208, 95% CI 1.061–1.377). After adjusting confounding factors, including age, gender, BMI, hypertension, diabetes, smoking, and biochemical indicators, the allele T was identified as an independent risk factor for EOCAD (OR_{adjust} 1.182, 95% CI 1.035–1.396). The results are shown in Table 3.

The association between the *MTHFR*-1298A/C variant and EOCAD risk was also detected in AA versus CC (OR 1.613, 95% CI 1.039–2.503), the dominant genetic model (OR 1.615, 95% CI 1.045–2.495), but not in the recessive genetic model (OR

Table 1 Demographic and clinical characteristics of EOCAD patients and controls

Variable	EOCAD ($n=875$)	Control ($n=956$)	*P*-value
Gender, male *n* (%)[a]	798 (91.20)	863 (90.27)	0.494
Age, years[b]	46.20 ± 4.32	43.96 ± 5.52	0.324
BMI (kg/m^2)[b]	27.31 ± 3.88	24.65 ± 4.38	< 0.001
Hypertension, *n* (%)[a]	237 (27.09)	243 (25.42)	0.418
Diabetes, *n* (%)[a]	166 (18.97)	78 (8.16)	< 0.001
Smoking, *n* (%)[a]	389 (44.46)	380 (39.75)	0.041
Drinking, *n* (%)[a]	677 (61.94)	415 (43.41)	< 0.001
FBG, mmol/l[b]	5.86 ± 2.37	5.34 ± 1.83	< 0.001
TG, mmol/l[b]	2.18 ± 1.76	1.55 ± 1.32	< 0.001
TC, mmol/l[b]	4.34 ± 1.56	4.58 ± 2.03	0.256
HDL-C, mmol/l[b]	1.06 ± 0.29	1.29 ± 0.45	< 0.001
LDL-C, mmol/l[b]	2.66 ± 1.12	2.53 ± 0.86	0.012
Lp(a), mmol/l[b]	298.89 ± 345.65	200.35 ± 221.76	< 0.001
SCr, μmol/l[b]	75.65 ± 16.78	73.64 ± 15.32	0.231
Myocardial infarction, *n* (%)	285 (32.57)	–	–
Severity of EOCAD	–	–	–
Single-vessle disease, *n* (%)	576 (65.83)	–	–
Double-vessles disease, *n* (%)	212 (24.23)	–	–
Triple-vessles disease, *n* (%)	87 (9.94)	–	–
Hcy, μmol/l[b]	18.85 ± 6.93	13.56 ± 5.83	< 0.001
Male, μmol/l[b]	19.21 ± 6.67	13.95 ± 5.96	< 0.001
Female, μmol/l[b]	16.39 ± 6.21	10.51 ± 4.36	< 0.001

EOCAD early-onset coronary artery disease, *BMI* body mass index, *Hcy* Homocysteine, *FBG* fasting blood glucose, *LDL-C* low-density lipoprotein cholesterol, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *Lp(a)* lipoprotein(a), *TC* total cholesterol, *SCr* serum creatinine

[a]Categorical variables are expressed as percentages. The *P* value of the categorical variables was calculated by χ^2 test

[b]Continuous variables are expressed as mean ± SD. The *P* value of the continuous variables was calculated by the unpaired *t* test

Table 2 Genotype frequencies of *MTHFR* gene in EOCAD and control groups

Groups	-677 C/T(rs1801133)				-1298 A/C(rs1801131)			
	Genotype			*HWE*	Genotype			*HWE*
	CC	CT	TT		AA	AC	CC	
EOCAD ($n=875$)	163	424	288	–	567	256	52	–
Control ($n=956$)	220	469	267	0.605	633	287	36	0.597

EOCAD Early-onset coronary artery disease, *HWE* Hardy–Weinberg equilibrium

Table 3 The results of *MTHFR* gene polymorphisms (rs1801133 and rs1801131) and EOCAD risk

	All				Men				Women			
	OR 95% CI	*P*	OR_{adjust} 95% CI	P_{adjust}	OR 95% CI	*P*	OR_{adjust} 95% CI	P_{adjust}	OR 95% CI	*P*	OR_{adjust} 95% CI	P_{adjust}
rs1801133												
C versus T	1.208 (1.061–1.377)	**0.004**	1.182 (1.035–1.396)	**0.028**	1.265 (1.103–1.452)	**0.001**	1.238 (1.100–1.489)	**0.005**	0.729 (0.471–1.128)	0.156	0.715 (0.453–1.146)	0.157
CC versus TT	1.456 (1.120–1.892)	**0.005**	1.412 (1.103–1.932	**0.016**	1.639 (1.235–2.174)	**0.001**	1.611 (1.203–2.196)	**0.002**	0.633 (0.279–1.437)	0.274	0.612 (0.255–1.488)	0.275
Dominant model	1.266 (1.027–1.546)	**0.024**	1.241 (1.022–1.572)	**0.049**	1.304 (1.059–1.606)	**0.012**	1.288 (1.035–1.638)	**0.031**	0.829 (0.394–1.746)	0.623	0.818 (0.356–1.792)	0.626
Recessive model	1.306 (1.040–1.639)	**0.021**	1.289 (1.028–1.703)	**0.048**	1.468 (1.145–1.882)	**0.002**	1.455 (1.121–1.925)	**0.007**	0.600 (0.322–1.118)	0.108	0.585 (0.306–1.153)	0.113
rs1801131												
A versus C	1.120 (0.952–1.319)	0.174	1.095 (0.899–1.465)	0.466	1.116 (0.942–1.321)	0.205	1.102 (0.926–1.352)	0.314	1.125 (0.604–2.096)	0.711	1.112 (0.583–2.108)	0.746
AA versus CC	1.613 (1.039–2.503)	**0.033**	1.592 (0.991–2.611)	0.060	1.576 (0.994–2.499)	0.053	1.535 (0.984–2.523)	0.074	2.000 (0.459–8.714)	0.356	1.952 (0.434–8.918)	0.386
Dominant model	1.615 (1.045–2.495)	**0.031**	1.601 (0.998–2.601)	0.054	1.574 (0.998–2.483)	0.051	1.531 (0.982–2.516)	0.076	2.083 (0.482–9.011)	0.326	2.005 (0.451–9.231)	0.363
Recessive model	1.065 (0.878–1.291)	0.522	1.052 (0.831–1.319)	0.667	1.066 (0.872–1.313)	0.532	1.043 (0.855–1.362)	0.723	0.971 (0.470–2.007)	0.937	0.959 (0.432–2.075)	0.917

Bold values indicate statistical significance ($P < 0.05$)

1.065, 95% CI 0.878–1.291). No significant association was observed between allele C and EOCAD risk (OR 1.120, 95% CI 0.952–1.319). The adjusted results (OR_{adjust} 1.095, 95% CI 0.899–1.465) were consistent with the original results. The results are shown in Table 3.

MTHFR Gene Variants and Serum Hcy Concentrations

Our study showed a trend in correlation between serum Hcy levels and the *MTHFR*-677C/T genotype in controls. In general, the Hcy concentration successively decreased in the homozygous mutant, heterozygous mutant, and wild type. When comparing the serum Hcy concentrations in the CC genotype (11.37 ± 5.23 μmol/L), the serum Hcy concentrations in the TT genotype (18.31 ± 7.22 μmol/L, $P < 0.01$, one-way ANOVA, Fig. 1) and CT genotype (15.25 ± 6.58 μmol/L, $P < 0.01$, one-way ANOVA, Fig. 1) were significantly increased. The significant increase was not observed in the *MTHFR*-1298A/C variant.

Serum Hcy Concentrations and Risk of EOCAD

In this study, serum Hcy concentrations were determined in all participants, and the data showed that the serum Hcy concentration was closely related to the risk of EOCAD. In EOCAD patients, the mean concentration of serum Hcy was 18.85 ± 6.93 μmol/L. Serum Hcy levels were significantly elevated in EOCAD patients when compared to controls (13.56 ± 5.83 μmol/L, $P < 0.001$, Fig. 2a). After further adjusting all conventional factors, serum Hcy concentrations remained significantly associated with the risk of EOCAD (OR_{adjust} 1.431, 95% CI 1.135–1.763).

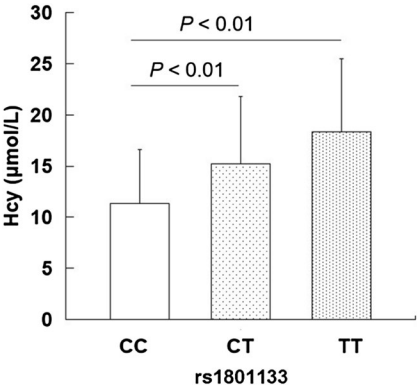

Fig. 1 Influence of the *MTHFR* gene polymorphism (rs1801133) on serum homocysteine (Hcy) concentrations in controls. The serum Hcy concentrations in TT genotype of *MTHFR* gene-677C/T polymorphism (18.31 ± 7.22 μmol/L, $n = 267$) shows significantly increase to compare with the concentrations in CC genotype (11.37 ± 5.23 μmol/L, $P < 0.01$, $n = 220$, one-way ANOVA) and CT genotype (15.25 ± 6.58 μmol/L, $P < 0.01$, $n = 469$, one-way ANOVA)

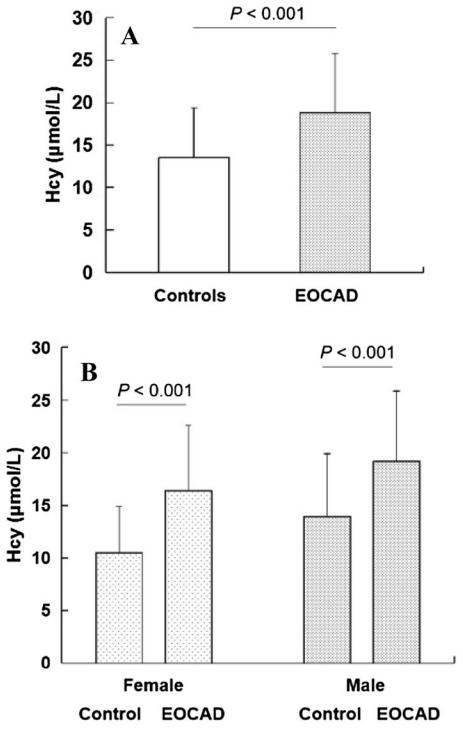

Fig. 2 Serum Hcy concentrations in patient and control groups. **a** Serum Hcy levels in healthy controls (13.56 ± 5.83 μmol/L, $n = 956$) were significantly increased when compared with the EOCAD patients (18.85 ± 6.93 μmol/L, $n = 875$, un-paired t test, $P < 0.001$). **b** Serum Hcy levels were significantly lower in female controls (10.51 ± 4.36 μmol/L, $n = 93$) compared with female patients (16.39 ± 6.21 μmol/L, $n = 77$, un-paired t test, $P < 0.001$). The same relationship was also detected in male EOCAD patients (19.21 ± 6.67 μmol/L, $n = 798$) and male controls (13.95 ± 5.96 μmol/L, $n = 863$, un-paired t test, $P < 0.001$)

Subgroup analysis was performed by gender, and serum Hcy levels were significantly lower in female controls (10.51 ± 4.36 μmol/L) when compared with female EOCAD patients (16.39 ± 6.21 μmol/L, $P < 0.001$, Fig. 2b). A similar finding was observed in male EOCAD patients (19.21 ± 6.67 μmol/L) versus male controls (13.95 ± 5.96 μmol/L, $P < 0.001$, Fig. 2b).

Regarding disease severity, serum Hcy concentrations were 17.55 ± 6.41 μmol/L ($n = 476$), 18.21 ± 6.83 μmol/L ($n = 279$), and 20.11 ± 7.21 μmol/L ($n = 120$) in EOCAD patients with single, double, and triple-vessels disease, respectively. We detected significant differences between the three groups (one-way ANOVA, $P < 0.01$). Levels of serum Hcy in the group with triple-vessels disease were significantly higher when compared to those in groups with single-vessel disease and double-vessels disease (one-way ANOVA, $P_{1\ vs.\ 3} < 0.01$, and $P_{2\ vs.\ 3} < 0.01$, Fig. 3a). In addition, as one of the most serious forms of CAD, serum Hcy levels in the MI group (22.53 ± 7.41 μmol/L, n = 285) were significantly higher compared to that in the non-MI group (17.23 ± 6.34 μmol/L, $n = 590$, $P < 0.001$, Fig. 3b).

Discussion

In the present study, we demonstrated that (1) the -677C/T common variant of the *MTHFR* gene was closely related to EOCAD risk in the Chinese population, and that the allele T of the variant was a risk allele. (2) The C677T variant of the

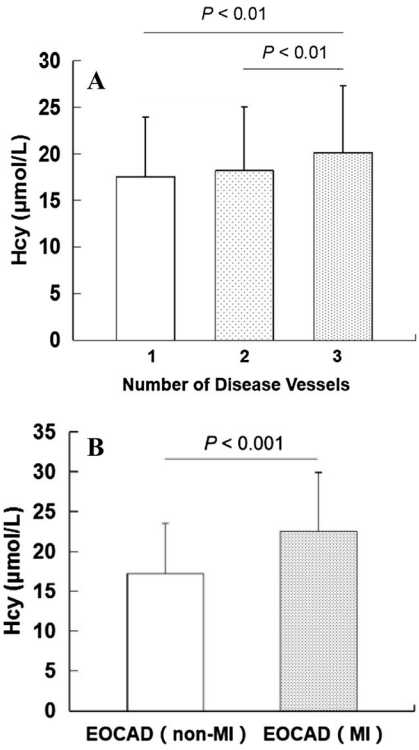

Fig. 3 Serum Hcy concentrations associated with severity of the disease. **a** Serum Hcy levels were 17.55 ± 6.41 μmol/L (n = 476), 18.21 ± 6.83 μmol/L (n = 279), and 20.11 ± 7.21 μmol/L (n = 120) in EOCAD patients with single, double and triple-vessel disease. Significant difference in the three groups was detected (one-way ANOVA, $P < 0.01$). **b** Serum Hcy levels in the MI group (22.53 ± 7.41 μmol/L, n = 285) was significantly higher than that in the non-MI group (17.23 ± 6.34 μmol/L, n = 590, un-paired t test, $P < 0.001$)

MTHFR gene affected the serum Hcy concentrations in controls. (3) Elevated serum Hcy levels were an independent risk factor for EOCAD, and were associated with disease severity.

The *MTHFR* gene has been located on 1p36.3 (Goyette et al. 1994). The MTHFR enzyme, which is expressed by the gene, catalyzes 5,10-methylenetetrahydrofolate reduction to 5-methylte trahydrofolate, and the latter serves as a methyl donor for the remethylation of Hcy to methionine. Thus, MTHFR has been considered the key enzyme of Hcy metabolism.

The conversion of amino acid Ala-to-Val at position 226 of the MTHFR protein is caused by the common C677T variant (rs1801133) in exon 4 of the *MTHFR* gene. The variant causes a half reduction of enzyme activity and leads directly to increased Hcy concentrations, and a decrease in folic acid concentration in human blood. The other common polymorphism (A1298C, rs1801131) is located on exon 7 within the presumptive regulatory domain and results in a Glu-to-Ala change, which also decreases activity of the enzyme (Ueland et al. 2001; Moll and Varga (2015). Animal experiments have demonstrated that Hcy plays important roles in atherosclerosis and thrombosis. (Lentz 2005). Several retrospective and prospective studies have investigated the effects of Hcy in cardiovascular diseases, and the results indicated a significant relationship between elevated Hcy levels and increased risk of cardiovascular events (Knekt et al. 2001; Albert et al. 2002; Hu et al. 2015). In mechanisms, several potential sites

where hyperhomocysteinemia may induce vascular lesions, including connective tissue and smooth-muscle cells, platelets, endothelial cells, vessel wall, blood lipids, coagulation factors, and nitric oxide have been identified (Olszewski and McCully 1993; Nishinaga et al. 1993; Majors et al. 1997). In 1976, Wilcken et al. published the first report on the association between CAD patients and abnormal Hcy metabolism (Wilcken and Wilcken 1976). In 1988, Kang and coworkers were the first to detect a mutation of the *MTHFR*. They found that the mutation was associated with decreased enzyme activity, and increased Hcy concentrations (Kang et al. 1988). The mutation was first identified as C677T of the *MTHFR* gene by Frosst and co-workers in 1995 (Frosst et al. 1995), and has so far been the most common and best studied *MTHFR* variant. The second important mutation of the *MTHFR* gene (A1298C) was first described in 1998 (van der Put et al. 1998). Subsequently, its effect on Hcy, the folate metabolism, and its potential role as a risk factor for cardiovascular disease was investigated. In the following decades, many studies focused on the relation between common mutations of the *MTHFR* gene, Hcy levels, and diverse disease, including cardiovascular diseases (Lewis et al. 2005; Luo et al. 2018).

According to our knowledge, only few studies have been described on common *MTHFR* gene variants, serum homocysteine levels, and EOCAD risk in the Chinese population. Late-onset CAD and EOCAD may have some differences in pathogenesis (Benfante et al. 1989; Christiansen et al. 2017). Our findings also indicated that an abnormal lipid metabolism and genetic factors may play more important roles in the pathogenesis of EOCAD (Xuan et al. 2011, 2018). In addition, according to the principle of genetic diversity, differences in genes and phenotypes between different races are due to evolutionary and environmental differences. Therefore, it is critical to study these associations in the Chinese population.

In the current study, we included 875 EOCAD patients and 956 controls, and evaluated the association between the two common variants of the *MTHFR* gene (rs1801131, rs1801133), serum Hcy concentrations, and risk of EOCAD, and observed a positive result. We believed that the *MTHFR*-677C/T variant was significantly related with an increased risk of EOCAD, and individuals carrying allele T have a significant increased risk of EOCAD disease (OR_{adjust} 1.182, 95% CI 1.035–1.396). The genotype of the *MTHFR* gene-677C/T also affected the serum concentrations of Hcy. The Hcy concentrations significantly decreased in the homozygous mutant, heterozygous mutant, and wild type. In addition, we demonstrated an independent risk relationship between elevated serum Hcy concentrations and EOCAD. The elevated serum Hcy levels also positively associated with disease severity.

Our study has some limitations. First, although we selected gender—and age-matched individuals without signs or symptoms of CAD as the control group—it should be noted that the controls did not undergo angiography. Second, a cohort design with the capability of tracking Hcy changes would better show the impact of Hcy on the process of atherosclerosis and EOCAD development. However, we only measured serum Hcy levels in patients prior to angiography. Third, geographic variations in the prevalence of *MTHFR* variants in the Chinese population may bias the results of the single-center case–control studies.

In conclusion, we observed that the common C677T variant in the *MTHFR* gene was significantly associated with the risk of EOCAD. Moreover, its genotype was closely related to serum concentrations of Hcy in the Chinese population. In addition, elevated serum Hcy levels are an independent risk factor for EOCAD and associated with disease severity.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

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