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Meta-analysis

Methylenetetrahydrofolate reductase C677T gene polymorphism and coronary artery disease in a Chinese Han population: a meta-analysis

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ABSTRACT

Methylenetetrahydrofolate reductase (*MTHFR*) C677T gene polymorphism has been suggested to be associated with increased coronary artery disease (CAD) risk. To explore the relationship between *MTHFR* C677T gene polymorphism and CAD in the Chinese Han population, a meta-analysis was performed. Fourteen separate studies were included and 2981 subjects were involved in the current meta-analysis. The pooled odds ratio (OR) between CAD size to CAD size and control size (CAD/CAD + control) and the corresponding 95% confidence interval (95% CI) between the CC and TT genotype groups were estimated by a random-effects model. Meta-regression was performed to explore the heterogeneity source. The CAD/CAD + control values were 0.45 for the CC genotype group and 0.62 for the TT genotype group. The pooled OR for the CAD/CAD + control between the CC and TT genotype groups was 0.55 (95% CI, 0.37-0.83; $P_{heterogeneity}$ = .0004, I^2 = 64.7%). These results indicated that *MTHFR* C677T gene polymorphism and CAD were significantly associated (P = .005) in the Chinese Han population. Publication year was detected as the main heterogeneity source. In a stratified analysis by publication year, the pooled OR was 0.76 (95% CI, 0.37-1.57; $P_{heterogeneity}$ = .0002; I^2 = 79.6%) in subgroup 1 (publication years 1999-2004). No significant association between gene polymorphism and CAD was found in this subgroup (P = .46). In subgroup 2 (publication years 2005-2011), the pooled OR was 0.39 (95% CI, 0.28-0.55; $P_{heterogeneity}$ = .53; I^2 = 0); and the association between gene polymorphism and CAD was significant (P < .00001). In the Chinese Han population, the TT genotype for the *MTHFR* C677T gene appeared to be associated with increased CAD risk.

1. Introduction

Mild elevation of plasma homocysteine (Hcy) has been considered to be an independent risk factor for coronary artery disease (CAD) [1]. Methylenetetrahydrofolate reductase (*MTHFR*) is a key enzyme for Hcy metabolism. *MTHFR* catalyzes the demethylation of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, which provides methyl for Hcy

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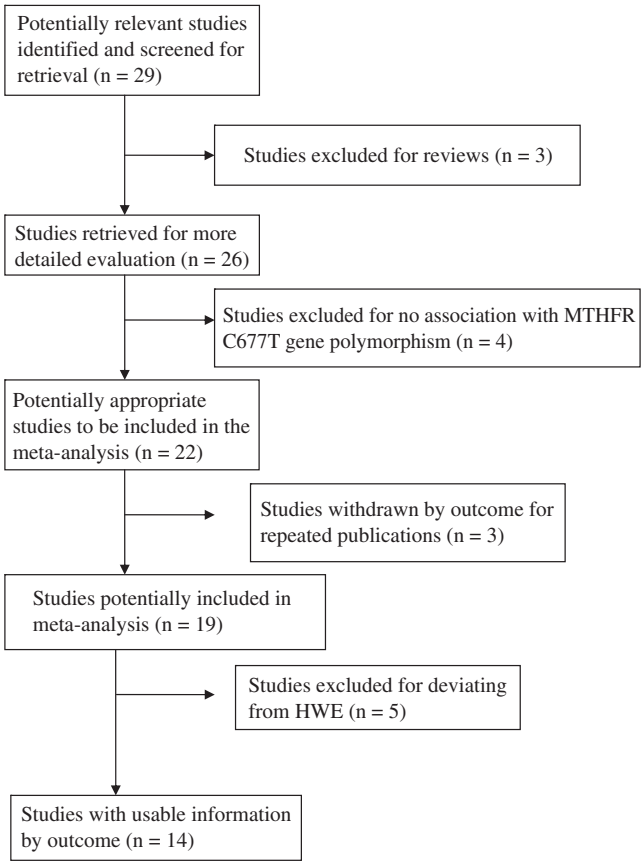

Fig. 1 – Flow diagram of article selection process for *MTHFR C677T* gene polymorphism and EH risk meta-analysis.

remethylation and methionine transformation. C677T is the most common mutation type in the Hcy metabolism pathway.

The *MTHFR* gene, located in 1p36.3, spans 2.22 kilobases and contains 11 exons and 10 introns [2]. The C677T gene polymorphism is located in the *MTHFR* gene-catalyzing region. When thymine (T base) substitutes for cytosine (C base) in the 677th base of the *MTHFR* gene, conservative alanine (Ala) is displaced by valine (Val) in the corresponding amino acid sequence; and the Hinfl restriction enzyme cutting site is simultaneously generated. C677T gene mutations could directly influence the activity and thermal resistance of the enzyme, thereby blocking sulfenyl transportation and remethylation. *MTHFR* C677T gene mutations contribute to decreasing Hinfl activities and elevating serum Hcy levels [3-5].

In a 2000 study, Ho [6] reported that the serum Hcy level in a CAD group was much higher than that in a control group. In addition, the prevalence of hypertension, smoking, and diabetes mellitus did not influence the Hcy levels of the 2 groups. Hence, it was implied that an increased Hcy level is an independent risk factor for CAD. In 2005, Kullo and Ballantyne [7] found that mild and moderate elevations of plasma Hcy could elevate cardiovascular risk by 60% in male subjects and by 80% in female subjects, similar to the elevation of risk when total serum cholesterol is increased to 20 mg/dL. Thus, Hcy is considered to be an independent risk factor for cardiovascular disease, paralleling other risk factors, such as smoking, hyperlipoidemia, and hypertension.

Table 1 – Characteristics of the investigated studies of the association between the *MTHFR C677T* gene polymorphism and CAD

Author	Year	Region	Ethnicity	CC		CT		TT		Genotyping	Study design	Matching criteria	Sample size (CAD/control)
				CAD	Control	CAD	Control	CAD	Control				
Xu [14]	1999	Beijing	Han	23	9	29	15	15	20	PCR-RFLP	Case-control	Age, sex, ethnicity	67/44
Dai [9]	2001	Hunan	Han	32	37	33	47	8	16	PCR-RFLP	Case-control	Ethnicity	73/100
Fang [15]	2002	Beijing	Han	34	44	80	60	47	21	PCR-RFLP	Case-control	Age, sex, ethnicity	161/125
Mao [10]	2002	Tianjin	Han	53	27	142	61	103	48	PCR-RFLP	Case-control	Ethnicity	298/136
Gao [20]	2004	Zhejiang	Han	22	40	48	32	26	10	PCR-RFLP	Case-control	Age, sex, ethnicity	96/82
Jiang [21]	2004	Beijing	Han	16	29	39	46	23	25	Molecular beacon	Case-control	Age, sex, ethnicity	78/100
Li [22]	2005	Hunan	Han	62	37	83	32	16	5	PCR-RFLP	Case-control	Sex ethnicity	161/74
Mu [23]	2005	Tianjin	Han	12	27	27	19	8	3	PCR-RFLP	Case-control	Ethnicity	47/49
Niu [24]	2005	Beijing	Han	18	19	28	23	12	3	PCR-RFLP	Case-control	Age, sex, ethnicity	58/45
Xu [11]	2005	Guangdong	Han	34	90	11	50	2	3	PCR-RFLP	Case-control	Ethnicity	47/143
Chen [25]	2007	Xinjiang	Han	23	34	65	32	26	17	PCR-RFLP	Case-control	Ethnicity	114/83
Luo [26]	2007	Beijing	Han	27	42	61	35	17	14	PCR-RFLP	Case-control	Ethnicity	105/91
Li [12]	2010	Yunnan	Han	36	10	51	15	27	6	PCR-RFLP	Case-control	Age, sex, ethnicity	114/31
Yang [27]	2011	Henan	Han	38	88	96	110	76	51	PCR-RFLP	Case-control	Ethnicity	210/249

PCR-RFLP indicates polymerase chain reaction–restriction fragment length polymorphism.

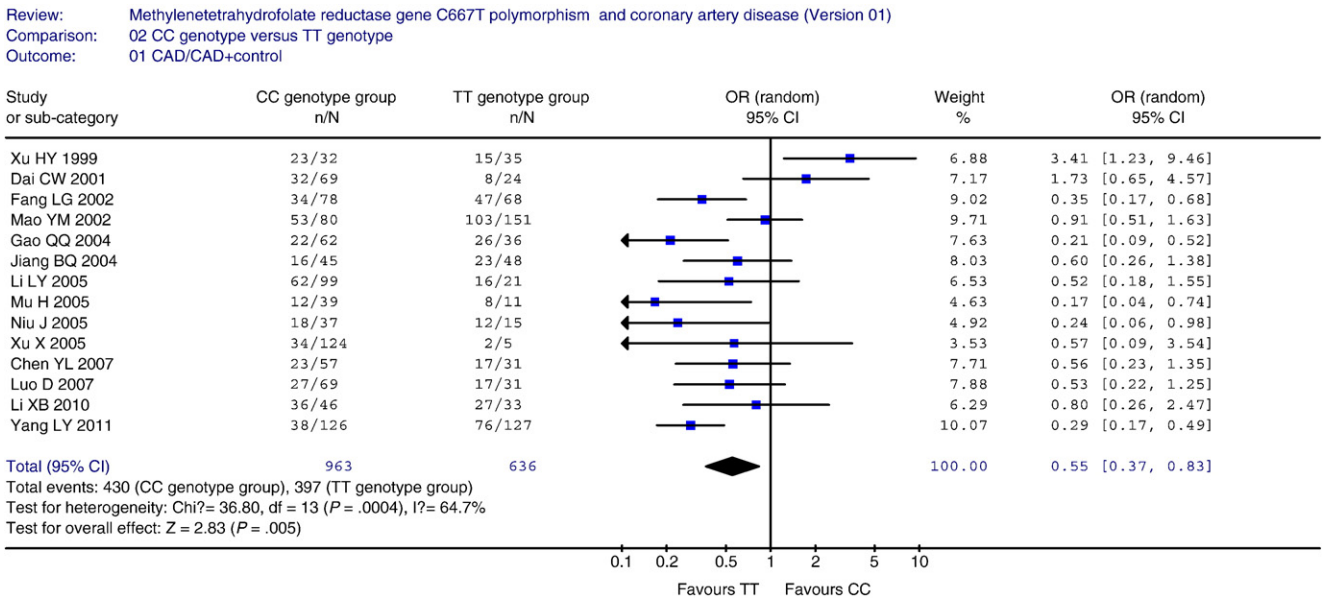

Fig. 2 – Forest plot of CAD associated with *MTHFR* C677T gene polymorphism (CAD/CAD + control).

In 2002, Ashavaid et al [8] found that mutations in *MTHFR* C677T could cause hyperhomocysteinemia, an independent risk factor for atherothrombosis. Routine assessments have been suggested to include *MTHFR* C677T gene polymorphism to aid the prediction of future coronary events in the Indian population. Although studies on *MTHFR* C677T gene polymorphism and CAD association have been extensively performed domestically, the results obtained remain controversial. In 2001, Dai and Zhang [9] reported that *MTHFR* C677T polymorphism is not associated with susceptibility to CAD in the Changsha region of China. Whereas the same conclusion was demonstrated in several studies [10-13], other works showed the opposite [14,15]. Thus, the present meta-analysis, which includes 2981 participants, is conducted to obtain a reasonable conclusion on the relationship between *MTHFR* C677T gene polymorphism and CAD in the Chinese Han population.

2. Materials and methods

2.1. *Publication search and inclusion criteria*

A search of electronic databases, such as PubMed, Embase, Web of Science, China Biological Medicine Database, and China National Knowledge Infrastructure, using MeSH terms such as *coronary artery disease* or *coronary heart disease*, *polymorphism*, *methylenetetrahydrofolate reductase*, *gene*, and *Chinese* was conducted to obtain studies published from 1999 to 2011 (last research updated on June 25, 2011).

The selected studies had to be consistent with the following criteria: (*a*) evaluation of the *MTHFR* C677T gene polymorphism and CAD in Chinese population was performed; and (*b*) CAD diagnosis was based on coronary arteriography and clinical symptoms combined with electrocardiogram, echocardiography, treadmill exercise test, and myocardial perfusion imaging in emission computed tomography results.

Table 2 – Summary of meta-analysis of association of *MTHFR* C677T gene polymorphism and CAD risk (CAD/CAD + control between CC and TT genotypes)

	Pooled OR (95% CI)	$P_{heterogeneity}$	Z (P)	I^2 (%)
CAD/CAD + control	0.55(0.37-0.83)	.0004	2.83(P = .005)	64.7

Table 3 – The confounding factors for the potential sources of heterogeneity studied by meta-regression

Study	Year	Region	Case size	Control size	Total size	RR	LnOR
Xu [14]	1999	1	67	44	111	1.52	1.23
Dai [9]	2001	2	73	100	173	0.73	0.55
Fang [15]	2002	1	161	125	286	1.29	−1.05
Mao [10]	2002	1	298	136	434	2.19	−0.09
Gao [23]	2004	2	96	82	178	1.17	−1.56
Jiang [21]	2004	1	78	100	178	0.78	−0.51
Li [22]	2005	2	161	74	235	2.18	−0.65
Mu [23]	2005	1	47	49	96	0.96	−1.77
Niu [24]	2005	1	58	45	103	1.29	−1.43
Xu [11]	2005	2	47	143	190	0.33	−0.56
Chen [25]	2007	1	114	83	197	1.37	−0.58
Luo [26]	2007	1	105	91	196	1.15	−0.63
Li [12]	2010	2	114	31	145	3.68	−0.22
Yang [27]	2011	1	210	249	459	0.84	−1.24

Region 1: northern China; Region 2: southern China. Case size: CAD group sample size; control size: control group sample size; total size: Total sample size. LnOR indicates the natural logarithm of OR for CAD/CAD + control between CC and TT genotype groups.

Table 4 – The meta-regression results among 14 studies

Item	Coefficient	Standard error	T value	P value	95% CI
Publication year	−0.2496638	0.0852966	−2.93	.019*	−0.4463582 to −0.0529695
Ratio of CAD and control group size	1.346536	0.6140945	2.19	.06	−0.0695684 to 2.762641
Region	−0.2504056	0.463921	−0.54	.604	−1.320209 to 0.819398
Control sample size	0.0304142	0.0165341	1.84	.103	−0.0077134 to 0.0685419
Total sample size	−0.0122628	0.0071768	−1.71	.126	−0.0288125 to 0.0042868
Summation	498.053	170.4503	2.92	.019	104.9938 to 891.1121

* $P < .10$.

2.2. Data extraction

The data were abstracted using a standard protocol. In the present meta-analysis, repeated publications, poor–research quality articles, and studies violating the inclusion criteria or providing little information were excluded. If the same result appeared in different studies, the result was adopted only once. The data drawn included the following: first author's name, publication year, region, number of genotypes, genotyping, study design, matching criteria, total number of cases, and controls.

2.3. Statistical analysis

Using the odds ratio (OR) corresponding to a 95% confidence interval (CI), the CAD/CAD + control values of the CC and TT genotype groups of the *MTHFR* C677T gene were compared. The χ^2-based Q test was used to determine significant heterogeneity between studies (significance was set to $P < .10$) [16]. The variation caused by heterogeneity was assessed by calculating the inconsistency index I^2. If heterogeneity existed among the studies, the pooled OR was estimated by a random-effects model (the DerSimonian and Laird method) [17]. Otherwise, a fixed-effects model was used (the Mantel-Haenszel method) [18]. The pooled OR was determined by Z tests with significance set to $P < .05$.

The Hardy-Weinberg equilibrium was assessed using Fisher exact test with significance set to $P < .05$. A funnel plot was adopted to estimate potential publication bias. The funnel plot asymmetry on the natural logarithm scale of the OR was assessed by Egger linear regression test (significance was set to $P < .05$) [19]. Statistical analysis was performed using STATA 10.0 software (StataCorp, College Station, TX).

3. Results

3.1. Studies and populations

Twenty-nine articles were acquired through the literature search, of which 14 complied with the inclusion criteria. Of the 15 excluded studies, 3 were repeated studies, 3 were reviews, 4 were not associated with *MTHFR* C677T gene polymorphism, and 5 deviated from the Hardy-Weinberg equilibrium (Fig. 1). Data were collected from 1629 CAD patients and 1352 controls of Han ethnicity. The regions investigated included the provinces of Beijing, Hunan, Tianjin, Zhejiang, Guangdong, Xinjiang, Yunnan, and Henan (Table 1) [20-27].

3.2. Pooled analyses

The CAD/CAD + control value was 0.45 for the CC genotype group and 0.62 for the TT genotype group. The pooled OR for the CAD/CAD + control between the CC and TT genotype groups was 0.55 (95% CI, 0.37-0.83; $P_{heterogeneity} = .0004$; $I^2 = 64.7\%$). The association between *MTHFR* C677T gene polymorphism and CAD in the Chinese Han population was significant ($P = .005$) (Fig. 2, Table 2).

Meta-regression was conducted to explore the potential sources of heterogeneity. The confounding factors included publication year, study regions, CAD group sample size, control group sample size, total sample size, and ratio of CAD group sample size to control group sample size (RR). Publication year was detected as the main heterogeneity source ($P = .019$), and RR was regarded as a minor heterogeneity source ($P = .06$). The remaining confounding factors were not associated with heterogeneity ($P > .10$) (Tables 3 and 4).

In the stratified analysis by publication year, the pooled OR was 0.76 (95% CI, 0.37-1.57; $P_{heterogeneity} = .0002$; $I^2 = 79.6\%$) in subgroup 1 (publication years 1999-2004). No significant association between *MTHFR* C677T gene polymorphism and CAD in subgroup 1 ($P = .46$). In subgroup 2 (publication years 2005-2011), the pooled OR was 0.39 (95% CI, 0.28-0.55; $P_{heterogeneity} = .53$; $I^2 = 0$). The association between *MTHFR* C677T gene polymorphism and CAD was significant in subgroup 2 ($P < .00001$) (Table 5, Fig. 3).

3.3. Bias diagnostics

The funnel plot and Egger test were used to assess the publication bias of the studies. No visual publication bias was found in the

Table 5 – Subgroup analysis summary by publication year (CAD/CAD + control between CC and TT genotypes)

Subsection by control group age	Literature number	Weight (%)	Pooled OR (95% CI)	Z (P)	I^2 (%)
Subgroup 1 (publication years 1999-2004)	6	48.45	0.76 (0.37-1.57)	0.74 ($P = .46$)	79.6
Subgroup 2 (publication years 2005-2011)	8	51.55	0.39 (0.28-0.55)	5.56 ($P < .00001$)	0
Whole population	14	100.0	0.55 (0.37 to 0.83)	2.83 ($P = .005$)	64.7

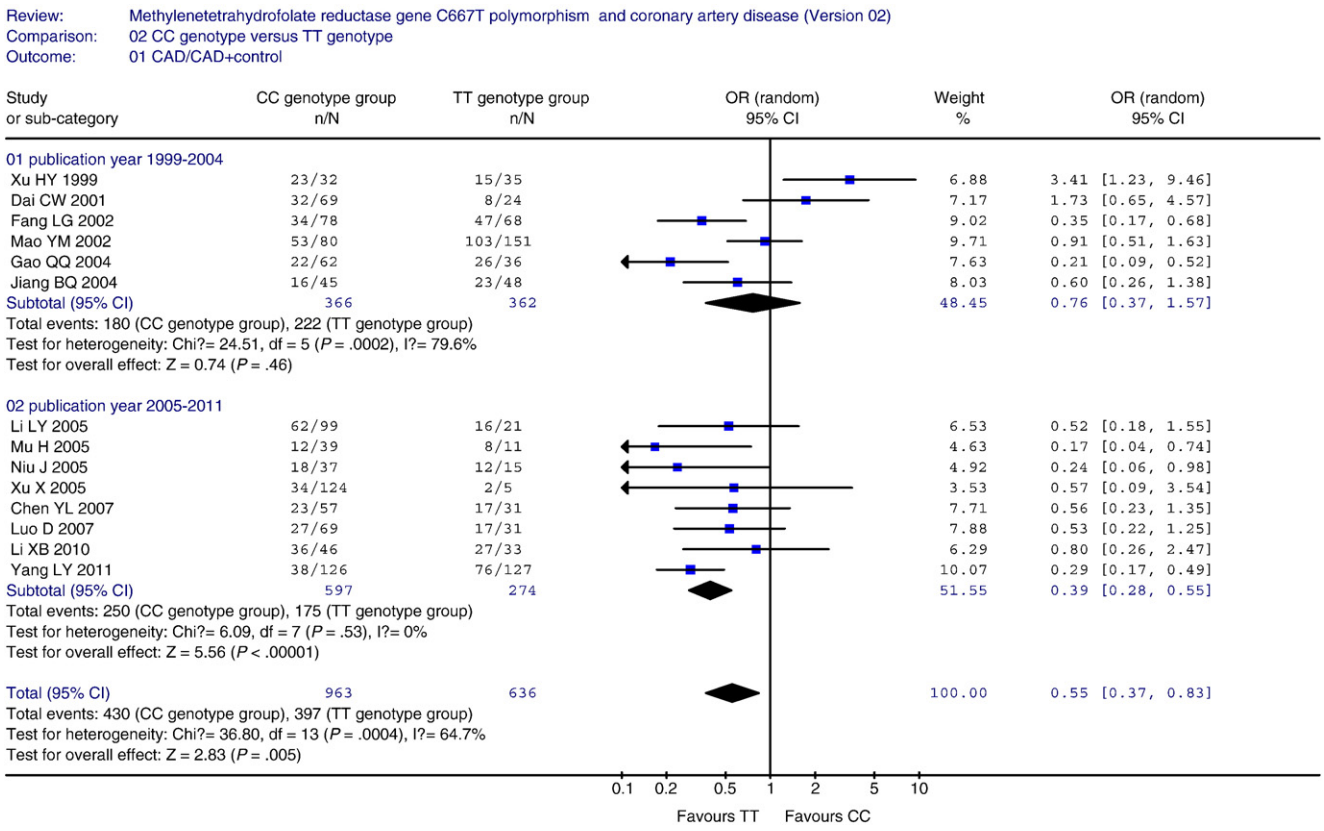

Fig. 3 – Coronary artery disease associated with *MTHFR* C677T gene polymorphism (CAD/CAD + control) stratified by publication year.

funnel plot (Fig. 4). The difference was not statistically significant in the Egger test, which implied that the present meta-analysis had low publication bias ($P = .516$, $T = 0.67$).

4. Discussion

In the current study involving 2981 Chinese Han subjects, *MTHFR* C677T gene polymorphism was found to be associated with CAD susceptibility. The TT genotype carriers of the *MTHFR* C677T gene were more prone to have CAD than the CC genotype carriers, suggesting that the T allele was the predisposing gene for CAD and that it was probably associated with elevated Hcy levels, which promote atherosclerosis.

MTHFR, a flavin-dependent enzyme with a relative molecular weight of 74.5 kd, mainly exists in the liver. It is the key enzyme in methionine and folate metabolism. The 5-methylenetetrahydrofolate generated, the main active form of the tissue and serum folate, is the primary methyl donor in the metabolic process. As the indirect methyl donor, *MTHFR* participates in purine and thymidine syntheses, which are the methylation processes of DNA, RNA, and proteins. Thus, *MTHFR* influences DNA metabolism and maintains the proper Hcy levels in vivo. *MTHFR* C677T gene mutation contributes to the *MTHFR* famine or activity dip, which hinders Hcy from being converted into methionine, causing the serum folate level to decrease, the Hcy level to increase, and DNA hypomethylation. These result in a series of pathological changes and various diseases [28,29].

The contribution of Hcy to atherosclerosis predisposition may be explained through the following mechanisms. First, the released Hcy is auto-oxidated; and Hcy disulphide is generated. Homocysteine, when mixed with disulfide and Hcy ester sulfur milk, is accompanied by a large amount of superoxide anions, generating peroxide, which injures the vascular endothelial cells, oxidizes low-density lipoproteins, causes the vascular smooth muscle to contract continuously, and accelerates the atherosclerosis process. Moreover, Hcy damages the nitric oxide system, which also causes injuries to endothelial cells and oxidation of lipids. Second, a high homocysteine level phosphorylates lipids, activates protein kinase C, and promotes the expression of Clos and Cmyb genes in vascular smooth muscle cells, causing vascular smooth muscle and endothelial cells to proliferate and participate in atherosclerosis. Third, activated Hcy aggregates platelets, which can form a dense compound with apolipoprotein B and easily be engulfed by vascular wall macrophagocytes to cause fat to accumulate in the vascular wall. Fourth, Hcy may influence many thrombosis factors, which could increase the activity of procoagulant substances in endothelial cells, decrease anticlotting substances activities, and promote thrombosis [30,31]. Recent research has demonstrated that Hcy injures endothelial cells; accelerates atherosclerosis onset and progress; and contributes to the formation of unstable plaque through inflammatory factors, oxidative stress, endoplasmic reticulum stress, and immune responses [32].

There is much controversy on the relationship between *MTHFR* C677T gene polymorphism and CAD risk. In 2009, Biselli

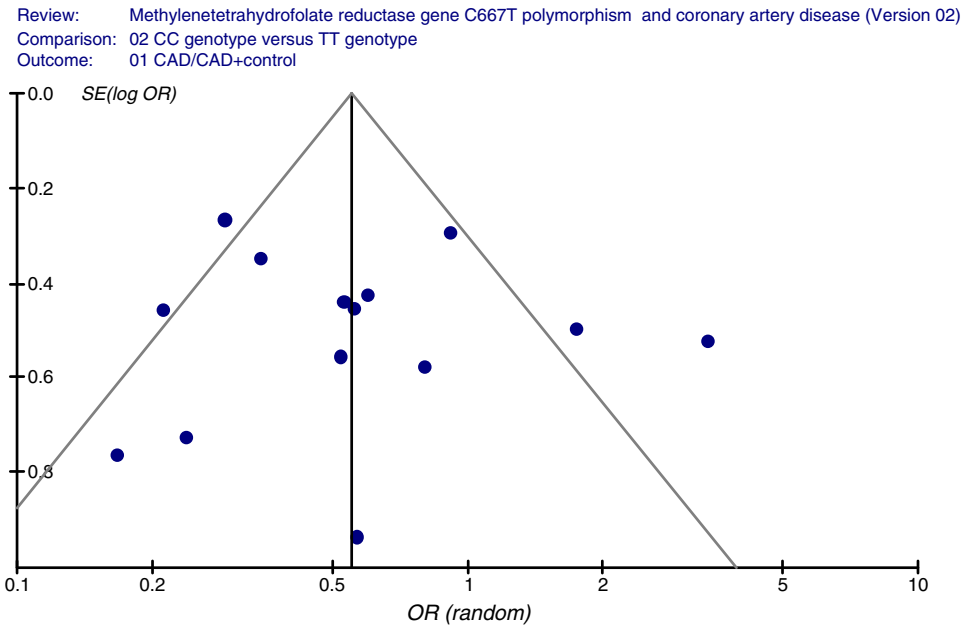

Fig. 4 – Funnel plot for studies of the association of CAD and *MTHFR* C677T gene polymorphism (CAD/CAD + control). The horizontal and vertical axes correspond to the OR and confidence limits. SE indicates standard error.

et al [33] reported that there was no association between *MTHFR* C677T polymorphisms and presence, extension, or severity of CAD in Portugal. Guerzoni et al [34] found that *MTHFR* C677T polymorphism showed no direct association with hyperhomocysteinemia or increased mean plasma concentrations of Hcy in Brazil. Rahimi et al [35] reported that *MTHFR* C677T polymorphism was not associated with CAD in western Iran.

In 2008, Mager et al [36] reported that the risk of early development of CAD associated with the TT genotype of the *MTHFR* C677T gene among non-Oriental women was 5.84-fold of that among Ashkenazi women (95% CI, 1.76-19.34). They thus concluded that the age of onset of CAD in Israeli women is influenced by the *MTHFR* genotype, their ethnic origin, and other coronary risk factors. Similarly, in 2011, Vijaya et al [37] found that *MTHFR* 677T increased the risk of developing CAD by 1.61-fold (95% CI, 1.04-2.50) in a case-controlled study of an Indian population. Belkahla et al [38] also reported that the *MTHFR* 677 TT genotype increased Hcy concentrations and coronary stenosis risks in a Tunisian population, especially in combination with low folatemia. The results obtained in the current research showed a similar conclusion.

In the subsequent meta-regression, the confounding factor, that is, the publication year, was considered to be the main heterogeneity source, suggesting that nonuniformity in the publication year could contribute to the heterogeneity among individual studies. Among the studies published before 2004, no significant association was found between *MTHFR* C677T gene polymorphism and CAD ($P > .05$). However, a significant association was found between them in studies published after 2005 ($P < .05$). Besides, RR could also partly explain the heterogeneity determined, indicating that the case and control sample size should be better balanced in further studies.

Several limitations exist in the current research. Defective and inadequate large-scale reports on the relationship between atherosclerosis and *MTHFR* C677T gene polymorphism must be considered. As well, the interference of factors, such as environmental and genetic factors, pharmaceuticals, and so on, requires further study.

The results of the current meta-analysis imply that the TT genotype of the *MTHFR* C677T gene is associated with increased CAD risks in the Chinese Han population. This finding may potentially be important when considering individual CAD therapies. Considering the findings and limitations discussed, our conclusion requires further verification by subsequent studies.

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Conflict of Interest

None.

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