# Effect of MTHFR A1298C and MTRR A66G genetic mutations on homocysteine levels in the Chinese population: a systematic review and meta-analysis

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## **ABSTRACT**

Background and Objectives: The Chinese population typically has inadequate folate intake and no mandatory folic acid fortification. Methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) are the two key regulatory enzymes in the folate/ homocysteine (Hcy) metabolism. Hcy has been implicated in the pathogenesis of cardiovascular disease. We conducted a meta-analysis to assess whether the MTHFR gene A1298C and the MTRR gene A66G polymorphisms affect Hcy levels in the Chinese population. Methods: This analysis included 13 studies with Hcy levels reported as one of the study measurements. Summary estimates of weighted mean differences and 95% confidence intervals (CIs) were obtained using random-effect models. Results: Overall, there were no significant differences in Hcy concentrations between participants with the MTHFR 1298 CC (12 trials, n = 129), AA  $(n = 2166; \beta, -0.51 \mu mol/L; 95\%Cl: -2.14, 1.11; P = 0.53)$ , or AC genotype  $(n = 958; \beta, 0.55)$ µmol/L; 95%CI: −0.72, 1.82; P = 0.40). Consistently, compared to those with the MTRR 66 GG genotype (6 trials, n = 156), similar Hcy concentrations were found in participants with the AA  $(n = 832; \beta, -0.43 \mu \text{mol/L}; 95\%\text{CI}: -1.04, 0.17; P = 0.16)$  or AG  $(n = 743; \beta, -0.57 \mu \text{mol/L}; 95\%\text{CI}: -1.04, 0.17; P = 0.16)$ 95%CI: -1.46, 0.31; P = 0.21) genotype. Similar results were observed for the dominant and recessive models. Conclusions: Neither the MTHFR A1298C polymorphism nor the MTRR A66G polymorphism affects Hcy levels in the Chinese population.

Key words: homocysteine; MTHFR gene A1298C polymorphism; MTRR gene A66G polymorphism; Meta-analysis

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# **INTRODUCTION**

Homocysteine (Hcy) is a sulfur-containing amino acid.<sup>[1]</sup> Elevated Hcy concentration has been reported to be an independent risk factor for a variety of cardiovascular diseases, including coronary heart disease and stroke.<sup>[2,3]</sup> A previous meta-analysis of genetic and prospective studies estimated that, if causal, a 3-µmol/L reduction in serum Hcy concentrations would decrease the risk of stroke by 24%.<sup>[3]</sup> Possible mechanisms by which Hcy contributes to vascular damage include inhibiting endothelial cell (EC) growth and post-injury endothelial repair,<sup>[4,5]</sup> inducing endothelial dysfunction,<sup>[6]</sup> promoting vascular remodeling,<sup>[7]</sup> and inflammatory monocyte differentiation.<sup>[8,9]</sup>

Methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) are the two key enzymes responsible for the remethylation of Hcy to methionine in the one-carbon metabolic pathway.<sup>[10]</sup> MTHFR converts 5,10-methylene-tetrahydrofolate (THF) into 5-methyl-THF, the dominant circulating form of folate. The 5-methyl-THF product donates a methyl group to Hcy in the generation of S-adenosylmethionine, a major source of methyl groups used for DNA methylation.<sup>[11]</sup> MTRR regenerates a functional methionine synthase via reductive methylation, which is associated with the Hcy transfer to methionine, [12] Two common polymorphisms of the MTHFR gene are C677T and A1298C. The MTHFR 677C→T polymorphism leads to a reduction in enzyme activity, resulting in increased concentrations of plasma Hcy and lower levels of serum folate. [13,14] While the effect of the MTHFR C677T gene polymorphism on Hcy levels is very clear, the relationship between the gene polymorphisms MTHFR A1298C and MTRR A66G and Hcy levels are still controversial.

We conducted a meta-analysis to examine whether the MTHFR gene A1298C and the MTRR gene A66G could affect the serum Hcy concentrations in the Chinese population.

## MATERIALS AND METHOD

#### Search strategy and selection criteria

Our study followed the Meta-analysis Of Observational Studies in Epidemiology guidelines. [15] A systematic literature search of published studies was undertaken. We searched the databases PubMed, CNKI, Wangfang, and Weipu for studies published up to May 2017 with the following Standard Medical Subject Headings terms: (MTHFR or methylenetetrahydrofolate reductase or MTRR or Methionine Synthase Reductase) and (Homocysteine or total homocysteine or Hcy) and (China or Chinese). Manual searches of bibliographies for all relevant studies

and review articles were also conducted. There were no language restrictions. A team of experts in the relevant field was assembled. The results of the search strategy are summarized in Figure 1.

A standard protocol for study selection and data extraction was developed by our multidisciplinary team with relevant expertise in epidemiology, clinical medicine, and biostatistics. Studies that met the following criteria were included: (1) sufficient data for Hcy concentrations (sample size, mean, and standard deviation [SD]) and frequencies of the MTHFR gene A1298C or the MTRR gene A66G; (2) when the same data were used in more than one publication, the latest or largest study was selected.

#### Data extraction

All data from eligible trials were independently extracted in duplicate by two independent investigators using the standard protocol and reviewed by a third investigator. Discrepancies were resolved by discussion with the multidisciplinary research team who developed the protocol.

The following information was extracted: first author's name, year of publication, mean age in each group, percentage of female participant, participant sample size, study design, genotyping method, mean and SD of plasma or serum Hcy concentrations in the genotype categories, and concomitant diseases.

Cross-sectional studies (including baseline evaluation of cohort studies and pre-intervention evaluations of

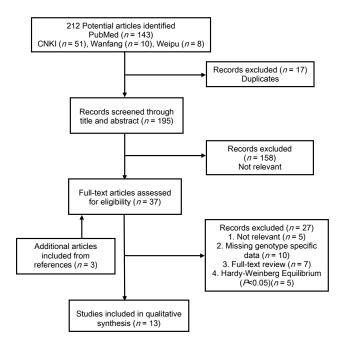


Figure 1. Flow chart of the study selection procedure. CNKI: China National Knowledge Infrastructure

randomized controlled trials) and case–control studies were included in the analysis. The methodological quality of the cross-sectional studies was assessed using an 11-item checklist as recommended by the Agency for Healthcare Research and Quality. An item received a score of "0" if answered as "NO" or "UNCLEAR"; if answered as "YES," the item received a score of "1." The quality of an article was assessed based on the score as follows: low quality = 0–3; moderate quality = 4–7; high quality = 8–11. The quality of case–control studies was assessed by the Newcastle-Ottawa Scale. It In brief, a maximum of 9 points was assigned to each study: 4 for selection, 2 for comparability, and 3 for outcomes. A final score of >6 was regarded as high quality.

## Statistical analysis

Our primary outcome was Hcy concentrations in participants within the different genotypes. Summary estimates of weighted mean differences and 95% confidence intervals (CIs) were obtained using random-effect (DerSimonian and Laird) models. Heterogeneity between studies was assessed by Cochran's Q test with a significance level set at 0.10. The I² statistics was also examined, and I² > 50% was considered as relevant heterogeneity. The presence of publication bias was assessed by Egger's linear regression test. Sensitivity analyses were performed by removing one study at a time to calculate overall homogeneity. The Hardy–Weinberg equilibrium was assessed using Fisher's exact test.

All statistical analyses were performed using Review Manager (Revman) 5.3.0. A two-tailed *P* value of <0.05 was considered statistically significant unless otherwise stated.

# **RESULTS**

#### Characteristics of the included studies

A total of 212 articles were retrieved from the initial searches. From these, 17 were duplicates, leaving 195 articles that required closer inspection by examining the abstract and full text when necessary. From these, there were 40 potential studies for the meta-analysis. Of the 40 studies, 10 were excluded because of lack of genotyping data, 7 were review articles, 5 were not relevant, and 5 deviated from the Hardy–Weinberg Equilibrium (P < 0.05). Finally, 13 studies<sup>[10,12,14,18-27]</sup> were suitable for the meta-analysis (Figure 1).

The baseline characteristics of the study participants by MTHFR A1298C are shown in Table 1 and MTRR A66G genotype in Table 2. Among the 13 studies, 7 were case—control studies<sup>[12,14,23-27]</sup> and 6 were cross-sectional studies.<sup>[10,18-22]</sup> In addition, five manuscripts<sup>[10,12,14,21-22]</sup> were published in English, and eight manuscripts<sup>[18-20,23-27]</sup> were

published in Chinese. Hcy was measured by fluorescent polarization immunoassay, [14,22,24] high-performance liquid chromatography, [10,23,27] enzymatic cycle method, [12,19-20,26] chemiluminescence method, [18] and enzyme-linked immunosorbent assay. [25] Genotype was analyzed by fluorescence quantitative polymerase chain reaction, [12,18,26] polymerase chain reaction—restriction fragment length polymorphism, [10,14,22-25,27] ligase detection reaction, [19] iPLEX gold chemistry, [21] and taqman-minor groove binder. [20]

In general, much of the eligible data from both cases and controls group were included in the current analysis. However, some of the case or control groups from the included case—control studies were excluded because of the following reasons: (i) the population deviated from the Hardy—Weinberg equilibrium<sup>[23]</sup> or (ii) either the case or the control group did not provide sufficient data according to the inclusion criteria.<sup>[12]</sup> In addition, some case—control studies only depicted the gene and Hcy data in the total population.<sup>[24-27]</sup>

# Quality analysis

The quality of the 13 included studies ranged from 5 to 7 (Supplemental Tables 1 and 2), suggesting that the overall quality of the included studies was acceptable. The mean scores were 6 for the case—control studies and 5.5 for the cross-sectional studies.

# Main outcome and subgroup analyses

A total of 12 studies were examined for the association between Hcy levels and the MTHFR A1298C genotype. Figure 2 shows that there were no significant differences in Hcy concentrations between participants with the MTHFR 1298 CC (12 trials, n = 129), AA (n = 2166;  $\beta$ , -0.51 µmol/L; 95%CI: -2.14, 1.11; P = 0.53), or AC genotype (n = 958;  $\beta$ , 0.55 µmol/L; 95%CI: -0.72, 1.82; P = 0.40).

A total of 6 studies were examined for the association between Hcy levels and the MTRR A66G genotype. As shown in Figure 3, compared to those with the MTRR 66 GG genotype (6 trials, n = 156), similar Hcy concentrations were found in participants with the AA (n = 832;  $\beta$ , -0.43  $\mu$ mol/L; 95%CI: -1.04, 0.17; P = 0.16) or AG (n = 743;  $\beta$ , -0.57  $\mu$ mol/L; 95%CI: -1.46, 0.31; P = 0.21) genotype.

Similar results were observed for the dominant and recessive models (Figures 4 and 5).

## Publication bias and sensitivity analysis

Egger's test results suggested the absence of publication bias for the meta-analysis evaluating the association between the MTHFR 1298 CC/AA genotype (P = 0.53), the MTHFR 1298 CC/CA genotype (P = 0.72), the MTHFR 1298 CC+AC/AA genotype (P = 0.37), the MTHFR 1298

Study	Year	Design	Sex (female)	Age	Sample size	Method of plasma Homeasurement	y MTHFR analysis	Method of plasma Hcy MTHFR analysis Concomitant disease measurement
Hou <i>et al.</i> [12]	2016	Case-control	195 (100%)	30.6 (5.3)	195	Enzymatic cycle method	FQ-PCR	Healthy controls
Ji <i>et al.</i> <sup>[24]</sup>	2004	Case-control	55 (31.4%)	67.5	177	FPIA	PCR-RFLP	CHD/healthy controls
Kasiman <i>et al.</i> [22]	2009	Cross-sectional	145 (34.6%)	61.9 (11.6)	419	FPIA	PCR-RFLP	Ischemic stroke
Li et al. [10]	2017	Cross-sectional	268 (57.3%)	56.7	468	HPLC	PCR-RFLP	Hypertensive patients
Liu <i>et al.</i> [23]	2006	Case-control	17 (42.9%)	51.9 (7.5)	44	HPLC	PCR- RFLP	Diabetic retinopathy
Nan <i>et al.</i> [27]	2007	Case-control	57 (28.5%)	40.0	200	HPLC	PCR-RFLP	Ischemic stroke/Healthy controls
Wang et al. [25]	2010	Case-control	220 (53.9%)	45.1	408	ELISA	PCR-RFLP	Hypertensive patients/Healthy controls
Wang <i>et al.</i> [18]	2014	Cross-sectional	110 (100%)	26.7 (4.6)	110	Chemiluminescence method	FQ-PCR	General population
Wu <i>et al.</i> <sup>[19]</sup>	2016	Cross-sectional	257 (58.7%)	38 (8.6)	438	Enzymatic cycle method	LDR	General population
Xing <i>et al.</i> [20]	2014	Cross-sectional	425 (100%)	NA	425	Enzymatic cycle method	Taqman-MGB	General population
Ye <i>et al.</i> [26]	2016	Case-control	192 (100%)	28.1	192	Enzymatic cycle method	FQ-PCR	Incipient abortion/healthy controls
Yuan <i>et al.</i> [14]	2009	Case-control	31 (64.6%)	71.8 (10.3)	48	FPIA	PCR-RFLP	Parkinson's disease
Yuan <i>et al.</i>			17 (60.7%)	70.6 (9.1)	28			Parkinson's disease
Yuan <i>et al.</i>			73 (66.4%)	70.0 (8.5)	110			Healthy controls

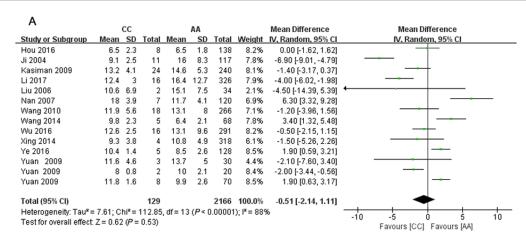
\*For continuous variables, values are presented as mean ±SD;

FPIA: fluorescent polarization immunoassay; LDR: ligase detection reaction; HPLC: high-performance liquid chromatography; FQ-PCR: fluorescence quantitative polymerase chain reaction reaction reaction regenent length polymorphism; Taqman-MGB: taqman-minor groove binder.

Table 2: Characte	ristics o	f studies included	in the meta-a	inalyses on the	association	between MTRR A66G	genotypes and	Table 2: Characteristics of studies included in the meta-analyses on the association between MTRR A66G genotypes and homocysteine levels*
Study	Year	Year Design	Sex (female)	e) Age	Sample size	Sample size Method of plasma Hcy MTRR analysis Concomitant disease Measurement	MTRR analysis	Concomitant disease
Hou <i>et al.</i> [12]	2016	2016 Case-control	195 (100%)	30.6 (5.3)	195	Enzymatic cycle methodFQ-PCR	IFQ-PCR	Healthy controls
Li et al. [10]	2017	Cross-sectional	268 (57.3%)	56.7	468	HPLC	PCR-RFLP	Patients with hypertension
Ni e <i>t al.</i> <sup>[21]</sup>	2017	Cross-sectional	ΑN	26.8 (10.6)	95	NA	IPLEX gold chemistry	Patients with Epilepsy
Wang <i>et al.</i> <sup>[18]</sup>	2014	Cross-sectional	110 (100%)	26.7 (4.6)	110	Chemiluminescence method	FQ-PCR	General population
Wu <i>et al.</i> [19]	2016	Cross-sectional	257 (58.7%)	38 (8.6)	438	Enzymatic cycle methodLDR	ILDR	General population
Xing et al. [20]	2014	2014 Cross-sectional	425 (100%)	ΑN	425	Enzymatic cycle methodTaqman-MGB	Taqman-MGB	General population

\*For continuous variables, values are presented as mean  $\pm$  SD.

HPLC: high-performance liquid chromatography; FQ-PCR: fluorescence quantitative polymerase chain reaction; PCR-RFLP: polymerase chain reaction, reaction-restriction fragment length polymorphism; LDR: ligase detection reaction; Taqman-MGB: taqman-minor groove binder.



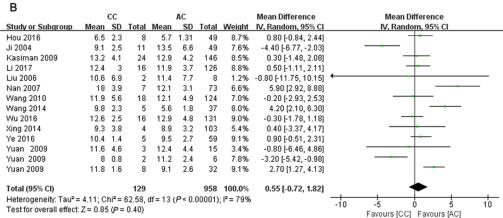


Figure 2. Random-effects meta-analysis of weighted mean differences (95% CI) in homocysteine levels with the MTHFR 1298 CC genotype compared with the AA or the AC genotype

Α		GG			AA			Mean Difference		Mean Di	ference	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI		IV, Rando	m, 95% CI	
Hou 2016	5.9	1.4	17	6.6	2.2	100	58.1%	-0.70 [-1.49, 0.09]				
Li 2017	16.1	10.4	78	16	13.5	153	3.7%	0.10 [-3.05, 3.25]				
Ni 2017	11.3	3.3	6	10.7	5.3	52	4.0%	0.60 [-2.41, 3.61]			•	
Wang 2014	5	2.7	8	5	1.8	60	9.9%	0.00 [-1.93, 1.93]				
Wu 2016	12.3	2.9	28	12.8	8.1	224	16.0%	-0.50 [-2.01, 1.01]				
Xing 2014	10.1	4.5	19	9.8	4.5	243	8.3%	0.30 [-1.80, 2.40]			•	-
Total (95% CI)			156			832	100.0%	-0.43 [-1.04, 0.17]		•		
Heterogeneity: Tau <sup>2</sup> =	= 0.00; C	hi² = 1	.67, df	= 5 (P =	0.89);	l <sup>2</sup> = 0%	,		-4	1	+	<del></del>
Test for overall effect:	Z = 1.41	(P=(	0.16)						-4	Favours [GG]	Favours (A	A]

В		GG			AG			Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Hou 2016	5.9	1.4	17	6.2	1.7	78	39.7%	-0.30 [-1.07, 0.47]	<del>-</del>
Li 2017	16.1	10.4	78	14	9.2	237	9.7%	2.10 [-0.49, 4.69]	<del>  •</del>
Ni 2017	11.3	3.3	6	11.8	6.2	37	6.4%	-0.50 [-3.81, 2.81]	<del></del>
Wang 2014	5	2.7	8	7.1	3.2	42	13.5%	-2.10 [-4.21, 0.01]	-
Wu 2016	12.3	2.9	28	13.5	9.6	186	17.7%	-1.20 [-2.95, 0.55]	<del></del>
Xing 2014	10.1	4.5	19	11.1	4.8	163	13.0%	-1.00 [-3.15, 1.15]	<del></del>
Total (95% CI)			156			743	100.0%	-0.57 [-1.46, 0.31]	•
Heterogeneity: Tau <sup>2</sup> =	0.36; C	hi² = 7	.18, df	= 5 (P =	0.21)	); I <sup>2</sup> = 30	0%		-10 -5 0 5 10
Test for overall effect:	Z = 1.28	6 (P= (	0.21)						-10 -5 0 5 10 Favours [GG] Favours [AG]

Figure 3. Random-effects meta-analysis of weighted mean differences (95% CI) in homocysteine levels with the MTRR 66 GG genotype compared with the AA or the AG genotype

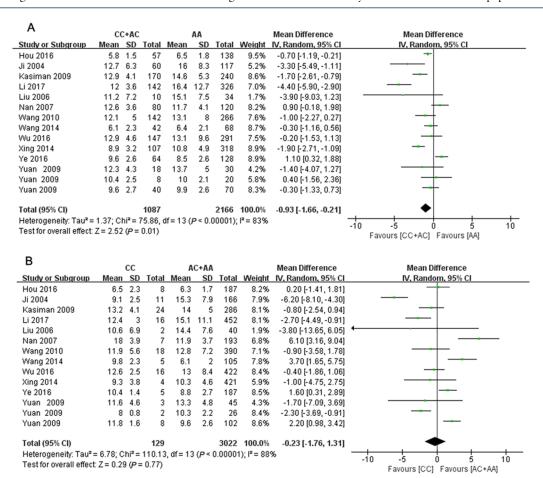


Figure 4. Random-effects meta-analysis of weighted mean differences (95% CI) in homocysteine levels with the MTHFR 1298 CC genotype compared with the AC/AA genotype and the MTHFR 1298 CC/AC genotype compared with the AA genotype

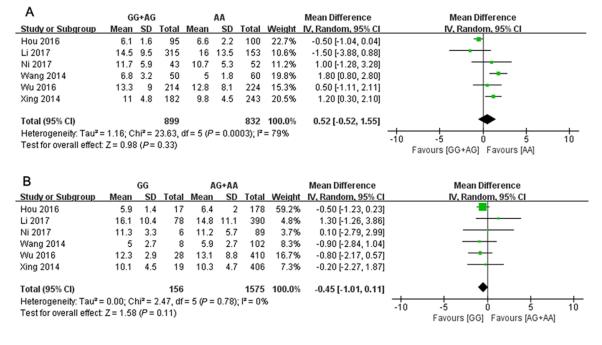


Figure 5. Random-effects meta-analysis of weighted mean differences (95% CI) in homocysteine levels with the MTRR 66 GG genotype compared with the AG/AA genotype and the MTRR 66 GG/AG genotype compared with the AA genotype

CC/AC+AA genotype (P = 0.74), the MTRR 66 GG/AG genotype (P = 0.85), the MTRR 66 GG+AG/AA genotype (P = 0.55), or the MTRR 66 GG/AG+AA genotype (P = 0.34) and Hcy levels. However, the test (P = 0.01) did not provide sufficient evidence against publication bias for the meta-analysis evaluating the relationship between the MTRR 66 GG/AA genotype and Hcy levels.

Sensitivity analyses showed that the results did not substantially alter after removing any one study.

## **DISCUSSION**

Increasing attention has been paid to the association of the MTHFR and MTRR gene polymorphisms with Hcy levels. Our meta-analysis is the first study to examine the relationship between the MTHFR A1298C and MTRR A66G genotypes and plasma Hcy levels in the Chinese population. We found that neither the MTHFR A1298C nor MTRR A66G polymorphism affected the plasma Hcy levels in Chinese populations.

As early as 1969, Hey, the sulfur-containing amino acid, was postulated to affect atherosclerotic processes. [28] However, findings from trials on Hcy-lowering therapy with folic acid or B-vitamins (containing folic acid) in stroke prevention have been mostly negative. [29-31] A meta-analysis of relevant trials revealed that most previous trials were conducted in countries with high folic acid consumption[32,33] and that mandatory folate fortification of the grain supply in those countries may have reduced the Hcy levels in the total population and affected the power of the trials to detect a positive effect. Consistently, our recent meta-analysis found that lowering Hcy concentration by folic acid supplementation significantly reduced the progression of carotid intima-media thickness, [34] the risk of cardiovascular diseases in patients with chronic kidney disease, [35] and the risk of stroke, [36] particularly in those without dietary folic acid fortification. More importantly, a positive association was found between reduction in Hcy levels and the beneficial effect associated with folic acid therapy. [34,37] There is substantial variability in Hcy levels even within the population without folic acid fortification. [13] Therefore, from a research and population-health perspective, it is important to clarify whether there are certain subgroups of individuals who had higher Hcy levels and might benefit from Hcy-lowering therapy.

Uncertainty remains about the relationship between the MTHFR A1298C and MTRR A66G gene polymorphisms and Hcy concentrations because of limited and inconsistent data. Oliveira *et al.* suggested that the A1298C polymorphism of the MTHFR gene is not associated with Hcy levels in Brazilian patients with Turner syndrome.<sup>[38]</sup> Hanson

et al. also found that the A1298C polymorphism was not associated with either fasting or post-methionine load Hcy in vascular disease. [39] However, Zappacosta et al. [40] found that Hcy was higher in those with the wild type and lower in those who were homozygous. A report showed negative correlation between MTRR A66G and Hcy levels, [41] whereas other studies showed no significant correlation. [42,43] These discrepancies may primarily be due to study sample size and/or marked ethnic and geographic variation of the different study cohorts. Furthermore, a recent collaborative study,[44] which analyzed 237 datasets including 59,995 individuals (20,885 stroke events), suggested that the effect of the MTHFR gene variants on Hcy level and stroke risk might be subject to effect modification by prevailing folate concentrations in the population. Typically, the Chinese population has inadequate folate intake and no mandatory folic acid fortification. Such a population provides a unique opportunity to observe the true association between the polymorphisms and the key enzymes in folate/Hcy metabolism. Although our previous studies on Chinese cohorts found that the MTHFR gene C677T polymorphism was a major determinant for both Hcy and folate levels, [45,46] the current meta-analysis suggests that neither the MTHFR A1298C polymorphism nor the MTRR A66G polymorphism affects plasma Hcy levels in the Chinese population. Hey has been implicated in the pathogenesis of cardiovascular diseases, cancer, and birth defects. [2,47,48] Consistent with our results, Yang et al. found that it was the MTHFR C677T polymorphism, but not the MTHFR A1289C polymorphism, that was associated with most clinical disorders, including cardiovascular diseases, cancer, and birth defects.[49]

The present study has several potential limitations. First, our study was conducted in Chinese populations. Although inadequate folate intake is prevalent in most countries lacking mandatory folic acid fortification of foods, including Asia and other continents, the generalizability of our findings to populations in other countries without folic acid fortification remains to be determined. Second, the MTRR A66G polymorphism is not extensively studied. Therefore, we could not provide sufficient evidence against a publication bias for the meta-analysis regarding the relationship of the MTRR 66 GG/AA genotype with Hcy levels. Furthermore, in general, meta-analyses have some inherent problems, including their retrospective and aggregate nature, moderate to high heterogeneity of some of the analyses, and the inability to adjust for individual variables.

In conclusion, the results of the meta-analysis suggest that both the MTHFR A1298C and MTRR A66G genotypes have no effect on the Hcy levels in the Chinese population. Further confirmatory studies on other ethnic groups are needed.

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#### **Conflict of Interest**

On behalf of all authors, the corresponding author states that there are no conflicts of interest.

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# SUPPLEMENT MATERIALS

Supplemental Table 1: Quality assessment of cross-sectional studies based on the Healthcare Research and Qual	ity
(AHRQ) recommendations	

Authors (References)	Wang	et al.Xing et a	al. <b>Ni</b> et al.	Li et al.	Kasiman et al.	Wu et al.
1. Define the source of information (survey, record review)	1	1	1	1	1	1
List inclusion and exclusion criteria for exposed and unexposed subjects (cases and controls) or refer to previous publications	1	1	1	1	1	1
3. Indicate time period used for identifying patients	1	1	1	1	1	1
4. Indicate whether or not subjects were consecutive if not population based	0	1	0	1	1	0
5. Indicate if evaluators of subjective components of study were masked to other aspects of the status of the participants	0	0	0	0	0	0
6. Describe any assessments undertaken for quality assurance purposes (e.g., test/retest of primary outcome measurements)	0	0	0	0	1	0
7. Explain any patient exclusions from analysis	1	1	1	0	0	1
8. Describe how confounding was assessed and/or controlled	1	1	1	1	1	1
9. If applicable, explain how missing data were handled in the analysis	0	0	0	0	0	0
10. Summarize patient response rates and completeness of data collection	0	0	0	0	0	0
11. Clarify what follow-up, if any, was expected and the percentage of patients for which incomplete data or follow-up was obtained	0	0	0	0	1	0

Supplemental Table 2: Quality assessment of the case control studies based on the Newcastle-Ottawa Quality
Assessment Scale (NOS)

Assessment Scal	e (NOS)				
Study	Selection	Comparability	Outcome	Total	
Hou <i>et al.</i>	**	**	*	5	
Ji et al.	***	**	**	7	
Liu <i>et al.</i>	***	**	*	6	
Nan <i>et al.</i>	***	*	*	5	
Wang et al.	***	*	**	6	
Ye et al.	***	*	**	6	
Yuan <i>et al.</i>	***	**	**	7	