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# Association between MTR A2756G polymorphism and susceptibility to congenital heart disease: A meta-analysis

**Wanru Liu, Jing Wang, Lin-jiao Chen***

Center for Reproductive Medicine, Center for Prenatal Genetics, First Hospital of Jilin University, Changchun, Jilin, China

* chenlinjiao@jlu.edu.cn



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**Abbreviations:** CHD, congenital heart disease; MTHFR, 5,10-methylenetetrahydrofolate reductase; MTR, methionine synthase; NOS, Newcastle-

## Abstract

The association between methionine synthase (MTR) A2756G (rs1805087) polymorphism and the susceptibility to congenital heart disease (CHD) has not been fully determined. A meta-analysis of case-control studies was performed to systematically evaluate the above association. Studies were identified by searching the PubMed, Embase, Web of Science, China National Knowledge Infrastructure, and WanFang databases from inception to June 20, 2021. Two authors independently performed literature search, data extraction, and quality assessment. Predefined subgroup analyses were carried out to evaluate the impact of the population ethnicity, source of healthy controls (community or hospital-based), and methods used for genotyping on the outcomes. A random-effects model was used to combine the results, and 12 studies were included. Results showed that MTR A2756G polymorphism was not associated with CHD susceptibility under the allele model (odds ratio [OR]: 0.96, 95% confidence interval [CI]: 0.86 to 1.07, P = 0.43, $I^2$ = 4%), heterozygote model (OR: 0.95, 95% CI: 0.84 to 1.07, P = 0.41, $I^2$ = 0%), homozygote model (OR: 1.00, 95% CI: 0.64 to 1.55, P = 0.99, $I^2$ = 17%), dominant genetic model (OR: 0.95, 95% CI: 0.84 to 1.07, P = 0.41, $I^2$ = 0%), or recessive genetic model (OR: 0.94, 95% CI: 0.62 to 1.43, P = 0.32, $I^2$ = 13%). Consistent results were found in subgroup analyses between Asian and Caucasian populations in studies with community and hospital-derived controls as well as in studies with PCR-RFLP and direct sequencing (all P values for subgroup differences > 0.05). In conclusion, current evidence does not support an association between MTR A2756G polymorphism and CHD susceptibility.

## Introduction

Congenital heart disease (CHD) is a common birth defect in newborns that has been associated with increased morbidity and mortality for infants [1, 2]. Accumulating evidence from clinical studies suggests that folic acid deficiency in women during pregnancy is associated with a higher risk of CHD for the fetus [3]. Folic acid supplementation has been associated with a series of birth defects, such as neural tube defects and CHD [4]. Moreover, genetic

Ottawa Scale; HWE, Hardy-Weinberg equilibrium; HR, hazard ratio.

studies also showed that the genotype status of genes that play important roles in the metabolic pathway for folic acid may affect the risk of CHD [5–7]. Previous studies confirmed that two classical variants of 5,10-methylenetetrahydrofolate reductase (MTHFR), namely MTHFR C677T (rs1801133) and A1298C polymorphisms, are associated with an increased risk of CHD [8, 9]. Previous association studies were focused on the influence of the methionine synthase (MTR) variant on CHD susceptibility [10–12]. Physiologically, MTR is a key enzyme involved in the metabolism of folic acid and catalyzes the remethylation of homocysteine to methionine during the removal of homocysteine [13, 14]. The MTR A2756G (rs1805087) polymorphism, a classical genetic variant, can lead to the deletion mutation of codon D919G and therefore affect the enzyme activity of MTR [13, 14]. A growing number of studies have been performed to evaluate the association between MTR A2756G polymorphism and CHD susceptibility [15–26]. However, a conclusion remains to be determined. Therefore, we performed a meta-analysis of case-control studies to summarize the potential associations between MTR A2756G polymorphism and CHD susceptibility. The possible influences of the study characteristics, such as the ethnicity of the participants, source of healthy controls, and methods used for genotyping, on the association were also explored in subgroup analyses.

## Materials and methods

The Meta-analysis of Observational Studies in Epidemiology [27] Statement and Cochrane's Handbook [28] were followed for the design, performance, and reporting of this meta-analysis. The protocols and analytical strategies for the meta-analysis were also in accordance with those for previous meta-analyses of studies related to SNPs [29–32].

### Search strategy

Electronic databases including PubMed, Embase, Web of Science, China National Knowledge Infrastructure, and WanFang were searched with a combination of the following terms: (1) "MTR" OR "Methionine synthase" OR "A2756G" OR "rs1805087"; (2) "Heart Defects, Congenital" OR "congenital heart abnormalities" OR "congenital heart abnormality" OR "congenital heart malformation" OR "congenital heart defect" OR "congenital heart disease" OR "congenital heart defects" OR "congenital heart diseases" OR "congenital anomalies" OR "birth defect"; and (3) "Polymorphism, Single Nucleotide" OR "Genotype" OR "Alleles" OR "polymorphism" OR "genetic variant" OR "genetic variants" OR "genetic polymorphism" OR "genetic" OR "Genetic Variation" OR "SNP" OR "mutation" OR "variation" OR "variant" OR "single nucleotide polymorphism". Studies published in English or Chinese were considered. The reference lists of related original and review articles were manually searched for potentially eligible studies. The final literature search was performed on June 20, 2021.

### Study selection

Studies fulfilling all of the following criteria were included: (1) case-control studies published as full-length articles; (2) included patients with confirmed CHD diagnosis and healthy participants as controls; (3) MTR A2756G polymorphism was evaluated and regarded as exposure; and (4) reported the association between the MTR A2756G polymorphism status and susceptibility to CHD. Reviews, editorials, studies reporting the maternal genotype of the patients and controls, studies without healthy controls, and studies without detailed genotype data were excluded from the current meta-analysis.

### Data extraction and quality evaluation

The literature search, data extraction, and quality assessment of the included studies were performed by two authors independently according to the predefined criteria. Discrepancies were resolved by consensus. The extracted data were as follows: (1) name of the first author, publication year, and country; (2) participant characteristics, including the ethnicity of the population and source of healthy controls; (3) genotyping methods; and (4) distributions of participants with MTR A2756G genotype status (AA, AG, and GG). The quality of each study was evaluated using the Newcastle-Ottawa Scale (NOS) [33]. This scale ranges from 1 to 9 and judges the quality of case-control studies according to the selection of the study groups, comparability of the groups, and ascertainment of exposure.

### Statistical analyses

For each study, the Hardy-Weinberg equilibrium (HWE) was tested to examine possible biases in genotype distribution. Odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were applied as the general measure for the association between the MTR A2756G genotype status and CHD susceptibility. The pooled ORs and 95% CIs were calculated for five genetic models: allele model (G versus A), heterozygote model (AG versus AA), homozygote model (GG versus AA), dominant model (GG + AG versus AA), and recessive model (GG versus AG + AA). Cochrane's Q test was used to evaluate the heterogeneity among the included cohort studies as well as to estimate the $I^2$ statistic [34]. $I^2 > 50\%$ indicated significant heterogeneity. A random-effects model was used to synthesize the hazard ratio (HR) data because this model is considered a more generalized method that incorporates potential heterogeneity among the included studies [28]. Sensitivity analysis, conducted by excluding one study at a time, was performed to test the stability of the results [35]. Predefined subgroup analyses were carried out to evaluate the impact of the population ethnicity, source of healthy controls (community or hospital-based), and methods used for genotyping on the outcomes. Publication bias was assessed by visual inspection of the funnel plots for symmetry as well as by Egger's regression asymmetry test [36]. RevMan (Version 5.1; Cochrane Collaboration, Oxford, UK) software was used to perform the meta-analysis and statistical analysis.

## Results

### Literature search

**Fig 1** shows the process for the database search. Briefly, 351 articles were obtained during the initial literature search of the databases after excluding duplicates. Among them, 329 articles were excluded for lack of relevance after screening the titles and abstracts. Subsequently, 22 articles underwent full-text review. Of these, 10 articles were further excluded for the reasons listed in **Fig 1**. Finally, 12 studies were obtained for this meta-analysis [15–26].

### Study characteristics and quality evaluation

The characteristics of the included studies are summarized in **Table 1**. One article included three comparisons from different centers in China; therefore, the datasets were included in the meta-analysis separately [22]. Overall, 12 case-control studies including 3853 patients with CHD and 3776 healthy controls were obtained for the meta-analysis. These studies were published between 2004 and 2018, and were performed in China, India, Malaysia, Brazil, and the United States, separately. Most of the studies included community-based healthy controls, with the exception of two studies that included healthy controls recruited in a hospital setting who visited the clinics for health examinations [16, 19]. Regarding the methods used for

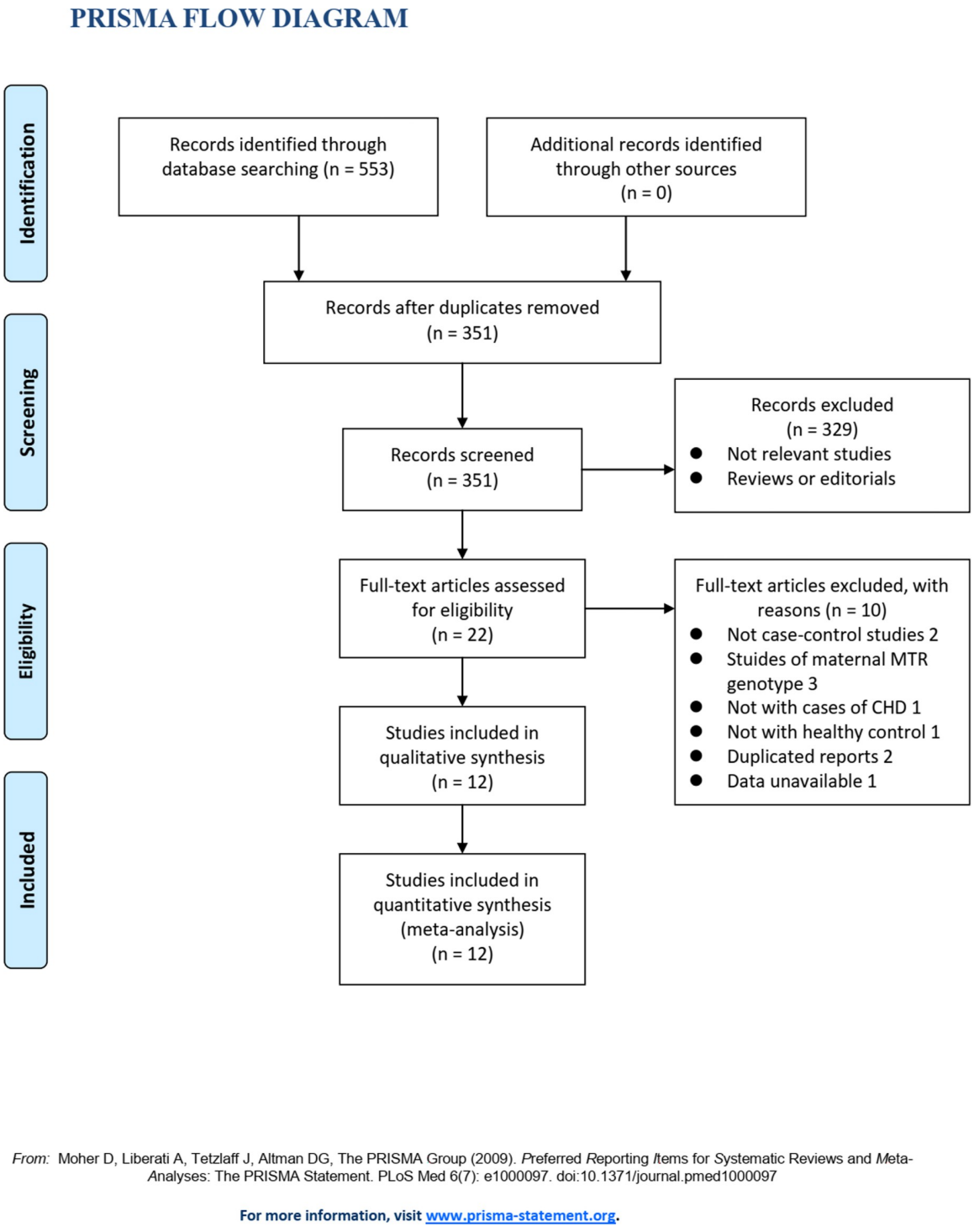


**Fig 1. Flowchart of the database search and study identification.**

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genotyping, polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) analyses were performed in six studies [15–17, 19, 21, 24], and direct sequencing was applied in the remaining studies [18, 20, 22, 23, 25, 26]. The sample size for the included

**Table 1. Characteristics and genotype status of MTR A2756G in patients and controls for the included studies.**

| Study | Country | Ethnicity | Source of control | Genotyping method | Patients | | | | Controls | | | | P for HWE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | AA | AG | GG | Total | AA | AG | GG | Total | |
| Zhu 2004 | China | Asian | Community-based | PCR-RFLP | 169 | 17 | 0 | 186 | 92 | 11 | 0 | 103 | 0.567 |
| Galdieri 2007 | Brazil | Caucasian | Hospital-based | PCR-RFLP | 36 | 20 | 2 | 58 | 22 | 13 | 3 | 38 | 0.588 |
| Liu 2007 | China | Asian | Community-based | PCR-RFLP | 120 | 12 | 0 | 132 | 97 | 10 | 0 | 107 | 0.612 |
| Shaw 2009 | USA | Caucasian | Community-based | Direct sequencing | 141 | 66 | 7 | 214 | 144 | 69 | 7 | 220 | 0.715 |
| Gong 2010 | China | Asian | Hospital-based | PCR-RFLP | 48 | 12 | 0 | 60 | 43 | 17 | 0 | 60 | 0.201 |
| Wang 2013 | China | Asian | Community-based | Direct sequencing | 132 | 27 | 1 | 160 | 153 | 33 | 2 | 188 | 0.883 |
| Zhao 2014a | China | Asian | Community-based | Direct sequencing | 513 | 80 | 9 | 602 | 567 | 87 | 6 | 660 | 0.251 |
| Zhao 2014b | China | Asian | Community-based | Direct sequencing | 627 | 103 | 5 | 735 | 459 | 97 | 8 | 564 | 0.998 |
| Zhao 2014c | China | Asian | Community-based | Direct sequencing | 891 | 104 | 8 | 1003 | 913 | 129 | 4 | 1046 | 0.252 |
| Mohamad 2014 | Malaysia | Asian | Community-based | PCR-RFLP | 106 | 41 | 3 | 150 | 114 | 36 | 0 | 150 | 0.104 |
| Shi 2015 | China | Asian | Community-based | Direct sequencing | 107 | 31 | 0 | 138 | 174 | 33 | 0 | 207 | 0.212 |
| Elizabeth 2017 | India | Asian | Community-based | PCR-RFLP | 11 | 14 | 7 | 32 | 18 | 9 | 5 | 32 | 0.078 |
| Su 2018 | China | Asian | Community-based | Direct sequencing | 87 | 82 | 14 | 183 | 96 | 78 | 27 | 201 | 0.088 |
| Duan 2018 | China | Asian | Community-based | Direct sequencing | 164 | 32 | 4 | 200 | 160 | 37 | 3 | 200 | 0.612 |

MTR, methionine synthase; PCR-RFLP: polymerase chain reaction followed by restriction fragment length polymorphism; HWE, Hardy-Weinberg equilibrium.

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studies ranged from 64 to 2049 and the distributions of the MTR A2756G genotype status for all of the included studies were in agreement with HWE (P all > 0.05). The NOS scores were 7~9 for all of the included studies, indicating good study quality (Table 2).

## MTR A2756G polymorphism and CHD susceptibility

Pooled results from the 14 datasets on 12 case-control studies showed that MTR A2756G polymorphism was not associated with CHD susceptibility under the allele model (OR: 0.96, 95% CI: 0.86 to 1.07, P = 0.43, $I^2$ = 4%; Fig 2A), heterozygote model (OR: 0.95, 95% CI: 0.84 to 1.07, P = 0.41, $I^2$ = 0%; Fig 2B), homozygote model (OR: 1.00, 95% CI: 0.64 to 1.55, P = 0.99, $I^2$ =

**Table 2. Quality evaluation for the included case-control studies via the Newcastle-Ottawa Scale.**

| Study | Adequate definition of cases | Representativeness of cases | Selection of control | Definition of control | Control for age and sex | Control for other factors | Ascertainment of exposure | Same method of ascertainment for case and control | None response rate | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Zhu 2004 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 8 |
| Galdieri 2007 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 7 |
| Liu 2007 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 8 |
| Shaw 2009 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 8 |
| Gong 2010 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 7 |
| Wang 2013 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 8 |
| Zhao 2014 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 9 |
| Mohamad 2014 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 7 |
| Shi 2015 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 7 |
| Elizabeth 2017 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 8 |
| Su 2018 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 8 |
| Duan 2018 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 7 |

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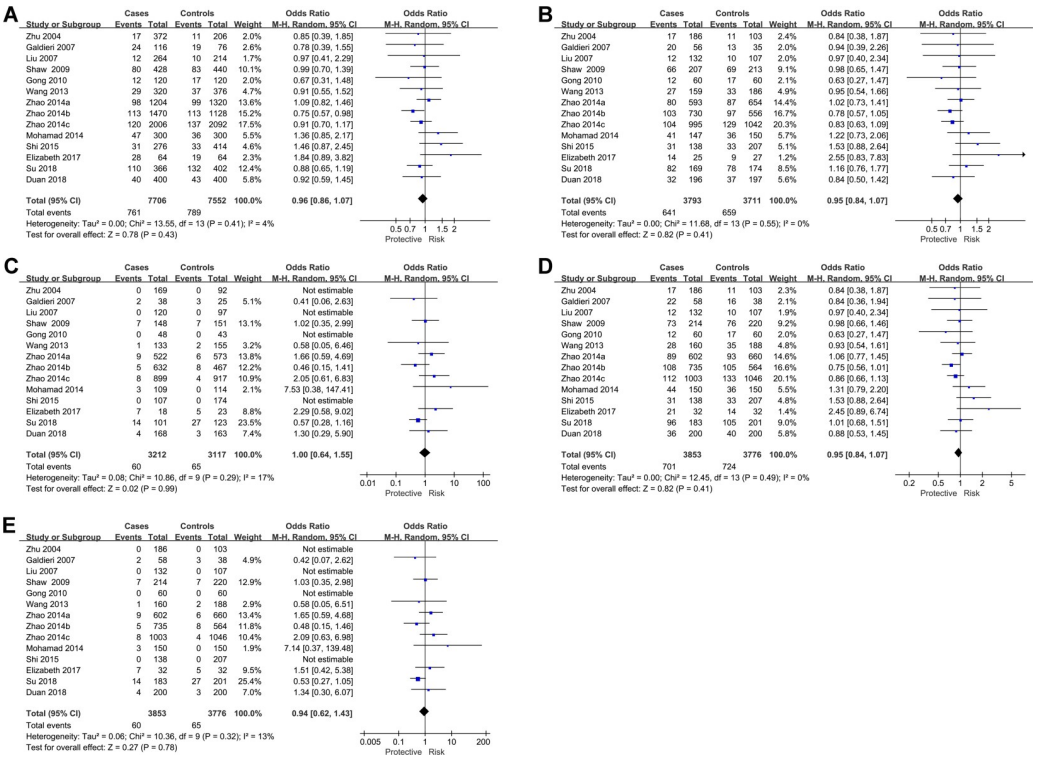


**Fig 2. Forest plots for the meta-analysis of the association between MTR A2756G polymorphism and CHD susceptibility in different genetic models.** A, meta-analysis under the allele model; B, meta-analysis under the heterozygote model; C, meta-analysis under the homozygote model; D, meta-analysis under the dominant genetic model; and E, meta-analysis under the recessive genetic model.

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17%; Fig 2C), dominant genetic model (OR: 0.95, 95% CI: 0.84 to 1.07, P = 0.41, $I^2$ = 0%; Fig 2D), or recessive genetic model (OR: 0.94, 95% CI: 0.62 to 1.43, P = 0.32, $I^2$ = 13%; Fig 2E). No significant heterogeneity was observed for the above meta-analyses (P for Cochrane's Q test = 0.41, 0.55, 0.29, 0.49, and 0.32, respectively). Further sensitivity analyses by excluding one dataset at a time showed consistent results (ORs under allele model: 0.93~1.00, ORs under heterozygote model: 0.93~0.99, ORs under homozygote model: 0.90~1.21, ORs under dominant genetic model: 0.93~1.00, ORs under recessive genetic model: 0.85~1.17; all P > 0.05). Consistent results were found in subgroup analyses between Asian and Caucasian populations in studies with community and hospital-derived controls as well as in studies with PCR-RFLP and direct sequencing (Table 3; all P values for subgroup differences > 0.05).

## Publication bias

Funnel plots for the association between MTR A2756G polymorphism and susceptibility to CHD in different models are shown in Fig 3A to 3E. The funnel plots were symmetrical on visual inspection, suggesting a low risk of publication bias. The results of Egger's regression tests also did not indicate significant publication bias underlying the meta-analyses (all P > 0.10).

## Discussion and conclusion

In this meta-analysis of case-control studies, we found that the MTR A2756G polymorphism is not significantly associated with CHD susceptibility. The reliability of the findings was

**Table 3. Subgroup analysis for the association between MTR A2756G and CHD susceptibility.**

| | Datasets | OR (95% CI) | P for subgroup effect | $I^2$ | P for subgroup difference |
|---|---|---|---|---|---|
| Allele | | | | | |
| Ethnicity | | | | | |
| Asian | 12 | 0.97 [0.85, 1.10] | 0.62 | 17% | |
| Caucasian | 2 | 0.94 [0.70, 1.28] | 0.71 | 0% | 0.89 |
| Source of control | | | | | |
| Community-based | 12 | 0.97 [0.86, 1.10] | 0.66 | 12% | |
| Hospital-based | 2 | 0.73 [0.44, 1.23] | 0.24 | 0% | 0.30 |
| Genotyping method | | | | | |
| PCR-RFLP | 6 | 1.07 [0.80, 1.45] | 0.64 | 12% | |
| Direct sequencing | 8 | 0.93 [0.83, 1.05] | 0.24 | 0% | 0.38 |
| Heterozygote | | | | | |
| Ethnicity | | | | | |
| Asian | 12 | 0.95 [0.83, 1.09] | 0.49 | 6% | |
| Caucasian | 2 | 0.97 [0.67, 1.41] | 0.87 | 0% | 0.93 |
| Source of control | | | | | |
| Community-based | 12 | 0.96 [0.84, 1.09] | 0.51 | 0% | |
| Hospital-based | 2 | 0.77 [0.42, 1.41] | 0.39 | 0% | 0.48 |
| Genotyping method | | | | | |
| PCR-RFLP | 6 | 1.05 [0.76, 1.44] | 0.78 | 0% | |
| Direct sequencing | 8 | 0.93 [0.82, 1.07] | 0.31 | 0% | 0.51 |
| Homozygote | | | | | |
| Ethnicity | | | | | |
| Asian | 12 | 1.09 [0.63, 1.87] | 0.76 | 30% | |
| Caucasian | 2 | 0.81 [0.32, 2.06] | 0.66 | 0% | 0.60 |
| Source of control | | | | | |
| Community-based | 12 | 1.05 [0.66, 1.66] | 0.83 | 20% | |
| Hospital-based | 2 | 0.41 [0.06, 2.63] | 0.35 | NA | 0.33 |
| Genotyping method | | | | | |
| PCR-RFLP | 6 | 1.57 [0.37, 6.73] | 0.54 | 41% | |
| Direct sequencing | 8 | 0.90 [0.58, 1.39] | 0.62 | 8% | 0.47 |
| Dominant | | | | | |
| Ethnicity | | | | | |
| Asian | 12 | 0.96 [0.83, 1.10] | 0.57 | 11% | |
| Caucasian | 2 | 0.95 [0.67, 1.36] | 0.79 | 0% | 0.97 |
| Source of control | | | | | |
| Community-based | 12 | 0.97 [0.85, 1.10] | 0.59 | 4% | |
| Hospital-based | 2 | 0.73 [0.40, 1.32] | 0.30 | 0% | 0.37 |
| Genotyping method | | | | | |
| PCR-RFLP | 6 | 1.06 [0.77, 1.47] | 0.71 | 8% | |
| Direct sequencing | 8 | 0.93 [0.82, 1.06] | 0.29 | 0% | 0.46 |
| Recessive | | | | | |
| Ethnicity | | | | | |
| Asian | 12 | 1.02 [0.61, 1.71] | 0.94 | 27% | |
| Caucasian | 2 | 0.82 [0.33, 2.06] | 0.67 | 0% | 0.69 |
| Source of control | | | | | |
| Community-based | 12 | 0.99 [0.64, 1.54] | 0.97 | 17% | |
| Hospital-based | 2 | 0.42 [0.07, 2.62] | 0.35 | NA | 0.37 |

(*Continued*)

**Table 3.** (Continued)

| | Datasets | OR (95% CI) | P for subgroup effect | $I^2$ | P for subgroup difference |
|---|---|---|---|---|---|
| Genotyping method | | | | | |
| PCR-RFLP | 6 | 1.26 [0.36, 4.45] | 0.72 | 29% | |
| Direct sequencing | 8 | 0.89 [0.57, 1.42] | 0.64 | 16% | 0.62 |

MTR, methionine synthase; CHD, congenital heart disease; OR, odds ratio; CI, confidence interval; PCR-RFLP: polymerase chain reaction followed by restriction fragment length polymorphism; NA, not applicable.

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evidenced by the consistency of the results for five genetic models, as well as the results of sensitivity and subgroup analyses. Specifically, sensitivity analysis by excluding one study at a time showed that the results were not primarily driven by any of the included studies. Subgroup

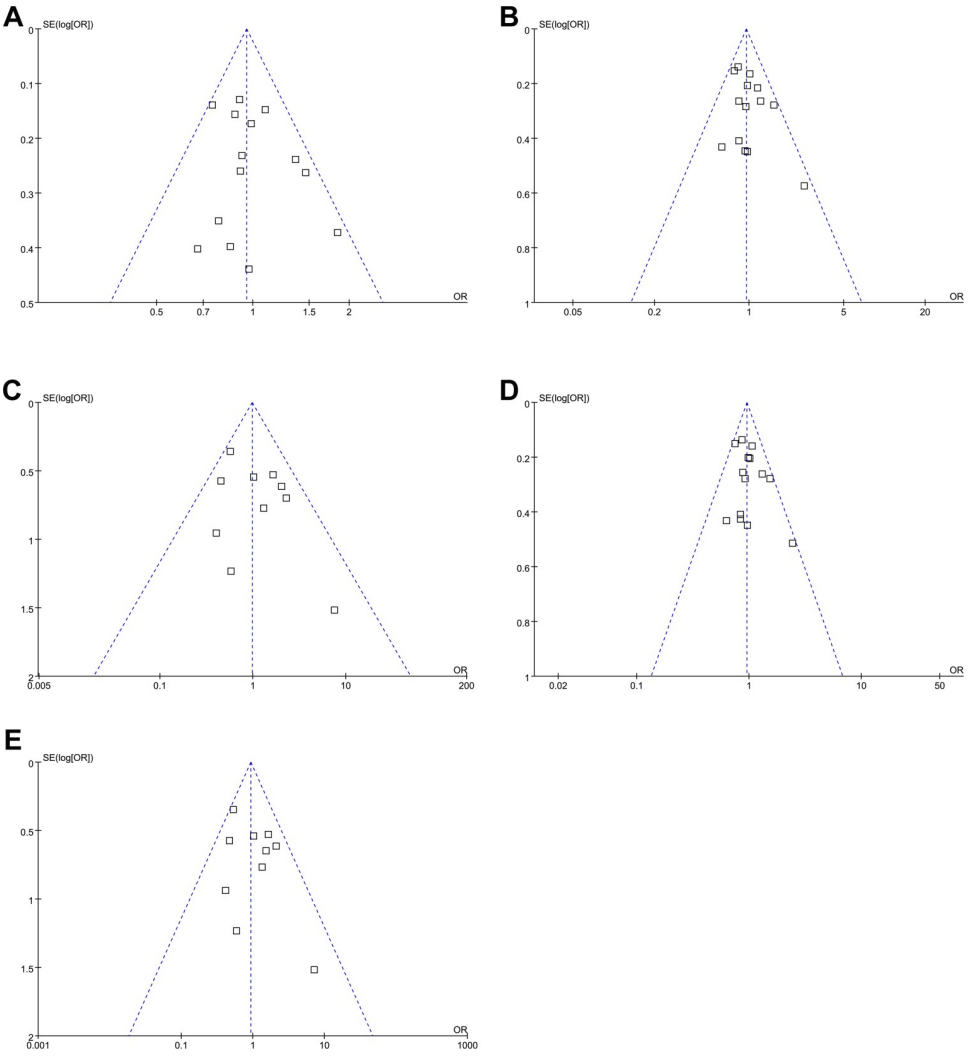


**Fig 3. Funnel plots for the publication bias underlying the meta-analysis of the association between MTR A2756G polymorphism and CHD susceptibility in different genetic models.** A, funnel plots for the meta-analysis under the allele model; B, funnel plots for the meta-analysis under the heterozygote model; C, funnel plots for the meta-analysis under the homozygote model; D, funnel plots for the meta-analysis under the dominant genetic model; and E, funnel plots for the meta-analysis under the recessive genetic model.

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analyses showed that there was no significant influence from the predefined study characteristics on the association, including the ethnicity of the participants, source of healthy controls, and genotyping methods. Taken together, the current evidence from case-control studies does not support a significant association between MTR A2756G polymorphism and CHD susceptibility.

To the best of our knowledge, only one previous meta-analysis evaluated the potential association between the MTR A2756G polymorphism and CHD susceptibility [10]. The previous meta-analysis included four case-control studies published before 2014 (482 participants) and showed that A2756G in MTR was not significantly associated with susceptibility to CHD [10]. Although the results of the previous meta-analysis were consistent with those of the current study, the limited number of datasets and small sample size included in the previous meta-analysis may lead to an inadequate statistical power for the detection of a potentially significant association between MTR A2756G polymorphism and CHD susceptibility. Moreover, only one genetic model was applied (allele) and subgroup analyses could not be performed in the previous study [10]. Our meta-analysis, on the other hand, was performed with 7629 participants following an intensive literature search and the inclusion of up-to-date studies. The remarkably larger sample size guaranteed adequate statistical power and the feasibility of multiple sensitivity and subgroup analyses. Collectively, the results of the main meta-analysis with five genetic models and subgroup analyses in our study consistently showed that MTR A2756G polymorphism was not significantly associated with CHD susceptibility. This is consistent with the previous meta-analyses that evaluated the association between MTR A2756G polymorphism and folic acid deficiency-related birth defects. Early meta-analyses including 10–13 studies showed that MTR A2756G polymorphism is not associated with neural tube defect risk, and the results were consistent in the overall and Caucasian populations [37–39]. Similarly, a meta-analysis of nine studies also showed that maternal gene polymorphism of MTR A2756G did not significantly affect the risk of Down syndrome in the offspring [40]. Additionally, a recent meta-analysis of 12 case-control studies indicated that MTR A2756G polymorphism may not be associated with the risk of nonsyndromic cleft lip with or without a cleft palate (NSCL/P) [41]. These findings may reflect the complexity of environmental and genetic interactions during the pathogenesis of birth defects. A previous study suggested a significant gene–gene interaction between the MTR A2756G polymorphism and MTHFR (rs1801133) in determining the susceptibility to NSCL/P, which was not significant if only MTR A2756G polymorphism was considered [42]. Similarly, a recent study showed that a haplotype CAA (rs1770449-rs1805087-rs1050993) in MTR rather than MTR A2756G alone was associated with the total CHD risk [43]. Future studies that incorporate gene–gene and gene–environment interactions are needed to determine the role of certain variants in the pathogenesis of CHD.

Although the cause of the majority of CHD cases is unknown, advances in genetic CHD studies provide increasing evidence for genetic causes underlying CHD and have identified critical biological pathways involved in CHD, including chromatin remodeling, Notch signaling, cilia function, RAS signaling, and gut immunodeficiency etc. [5, 44–47]. In the present manuscript, we evaluated the correlation between MTR A2756G and susceptibility to CHD by meta-analysis based on 12 studies. Although only one SNP (MTR A2756G) was investigated, the role of MTR in the development of human diseases is critical because it has been a hot spot in recent genetic studies. MTR A2756G is a common nonsynonymous polymorphism in the gene that encodes MTR, a key enzyme in the pathway leading to DNA methylation that catalyzes the remethylation of homocysteine to methionine [48]. It has been reported that MTR A2756G increases the risk of cancer, such as pediatric acute lymphoblastic leukemia [49], prostate cancer [50], breast cancer etc. [51]. MTR A2756G is also involved in the regulation of

folate metabolism, which is profoundly implicated in the DNA methylation pathway. Multiple maternal factors are thought to contribute to CHD development, including folate intake [4]. Maternal DNA methylation, which is dependent on folate metabolism, may also impact the risk of CHDs. For example, there was a report that revealed the association between maternal DNA methylation and CHD risk [52]. Taken together, considering the important role of MTR A2756G in the biological process, we believe that a meta-analysis to evaluate the correlation between MTR A2756G and susceptibility to CHD is reasonable and meaningful. Although we used an extensive search strategy in this study, almost all studies aiming to evaluate the SNPs of MTR and CHD focused on the MTR A2756G polymorphism. Only one study included other SNPs of MTR, which showed that two regulatory variants of MTR, -186T>G and +-905G>A, were associated with an increased risk of CHD [22]. Due to the limited datasets available for other SNPs of MTR, a meta-analysis was not performed for other variants of the MTR gene. We also acknowledge the necessity and value of multi-gene SNPs or multi-SNPs of the same gene for their associations with CHD, and future studies are warranted.

The meta-analysis conducted in this study was based on association studies only. Despite significant progress in dissecting the genetic architecture of complex diseases by genome-wide association studies (GWAS), the genetic variants identified by GWAS can only explain a small proportion of the heritability of complex diseases. Association analysis (including SNP studies) is a major tool for genomic studies of complex diseases and has been used for decades. Although novel technologies have been developed to uncover hidden genetic variants, association analysis still lacks the power to determine the mechanisms of diseases due to its inability to identify causal signals, which are quite different from association signals. Another reason is that the widespread networks established from integrated omics analysis are undirected. Because association analysis has limited power to unravel the mechanisms of complex diseases, it is necessary to shift the paradigm of genomic analysis from association analysis to causal inference analysis [53]. Causal inference is the process of determining the independent, actual effect of a particular phenomenon that is a component of a larger system [54]. Causal inference analysis includes several algorithms, such as intervention, domain shift learning, temporal structure, and counterfactual thinking, all of which are used as major concepts to understand causation and reasoning. Moreover, Mendelian randomization (MR) is an analytic technique that uses genetic variants as instrumental variables to test for the causative association between an exposure and an outcome [55]. Therefore, bidirectional MR analysis is becoming increasingly efficient and cost-effective (with strong power) for analyzing GWAS data with multiple genetic variants [55]. In this regard, future studies with causal inference analysis or Mendelian randomization analysis are warranted to address the possible causative associations between MTR A2756G and CHD.

Artificial intelligence (AI) and machine learning (ML) have recently received enormous attention due to the successful application of deep neural networks in many fields, including medical science [55, 56]. However, the essential components of causal inference analysis are often overlooked by ML, leading to some failures in deep learning. This suggests that AI coupling with causal inference analysis is still under-developed in the current stage; for example, deep learning for nonlinear mediation and instrumental variable causal analysis or the construction of causal networks as a continuous optimization problem. Future studies evaluating the feasibility of genetic polymorphism-based ML models for predicting the risk of CHD may also be performed.

Our study also has limitations. Firstly, eight of the included studies were performed in China and the results were mostly from Chinese populations. Only two studies included Caucasian populations; therefore, the results should be validated in large-scale studies. The association between MTR A2756G polymorphism and CHD susceptibility in other ethnic groups

such as Africans should be investigated in the future. Secondly, outcomes according to the individual forms of CHD were rarely reported among the included studies. Therefore, we were unable to evaluate the possible associations between MTR A2756G polymorphism and individual forms of CHD. Future studies are warranted. In addition, this is a meta-analysis based on study-level data rather than data for individual patients. Therefore, the influence of age, sex, and other characteristics on the association between MTR A2756G polymorphism and susceptibility to CHD remains unknown. Finally, the results were based on estimates with univariate analysis. An imbalance in participant characteristics between the patients and controls may confound the results.

In conclusion, the results of this meta-analysis indicated that current evidence from case-control studies does not support an association between MTR A2756G polymorphism and CHD susceptibility.

## Author Contributions

**Data curation:** Wanru Liu.

**Formal analysis:** Wanru Liu, Lin-jiao Chen.

**Investigation:** Jing Wang.

**Methodology:** Wanru Liu.

**Project administration:** Wanru Liu.

**Visualization:** Jing Wang.

**Writing – original draft:** Wanru Liu, Lin-jiao Chen.

**Writing – review & editing:** Wanru Liu, Jing Wang, Lin-jiao Chen.

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