

## Original Article

# MTHFR C677T and A1298C polymorphisms are not related to ventricular or atrial septal defect: a meta-analysis of 1272 cases and 1386 controls

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**Abstract:** Objective: Congenital heart defect (CHD), especially ventricular and atrial septal defects (VSD/ASD), is a severe neonatal disease affecting infants in both developing and developed countries. Ultrasonic exam is the best way to detect this disease. The study between related SNP (Single Nucleotide Polymorphism) has distinctly auxiliary value in CHD screening and diagnosis. Although many studies reported the association between MTHFR (methylenetetrahydrofolate reductase) polymorphisms and VSD/ASD risk, the results are controversial. Therefore, we conducted a meta-analysis of the published literature to identify the association between MTHFR Gene Polymorphism and Risk of VSD/ASD. Design: EMBASE, Web of science, PubMed and Chinese National Knowledge Infrastructure (CNKI) databases were searched for articles that reported the association between MTHFR polymorphisms and VSD/ASD risk. Setting: The studies that focused on the relationship between C677T and C1298C polymorphisms and VSD/ASD risk were extracted for analysis. Patients: 1272 cases and 1386 controls from 9 studies were included in our study. Interventions: Fixed-effect model or random-effect model was used to calculate the pooled odds ratio (ORs) and its corresponding 95% confidence interval (95% CI). The Begg's and Egger's test were used to assess publication bias. The forest plot was drawn by ethnicity. Sensitivity analyses were also performed to evaluate the robustness of the findings. Outcome Measures: The odd ratio of the association between MTHFR C677T and A1298C polymorphism and VSD/ASD risk was calculated in five genetic models. Results: The results of allele model (C vs. T) showed no significant association between MTHFR C677T and A1298C polymorphism and VSD/ASD risk (C677T Odd Ratio=0.96, 95% CI 0.74, 1.24, A1298C Odd Ratio=1.10, 95% CI 0.80, 1.51) in total population. Subgroup analysis by ethnicity and other four genetic models also did not show significant association. The limitation of the analysis was Hardy-Weinberg equilibrium in some studies demonstrated statistical significance, and Begg's funnel plot showed potential publication bias ( $P < 0.01$ ). Linkage disequilibrium analysis did not identify any polymorphism linkage with MTHFR C677T or A1298C. Conclusion: The meta-analysis suggested that MTHFR C677T and A1298C polymorphisms are not associated with the risk of ventricular or atrial septal defect.

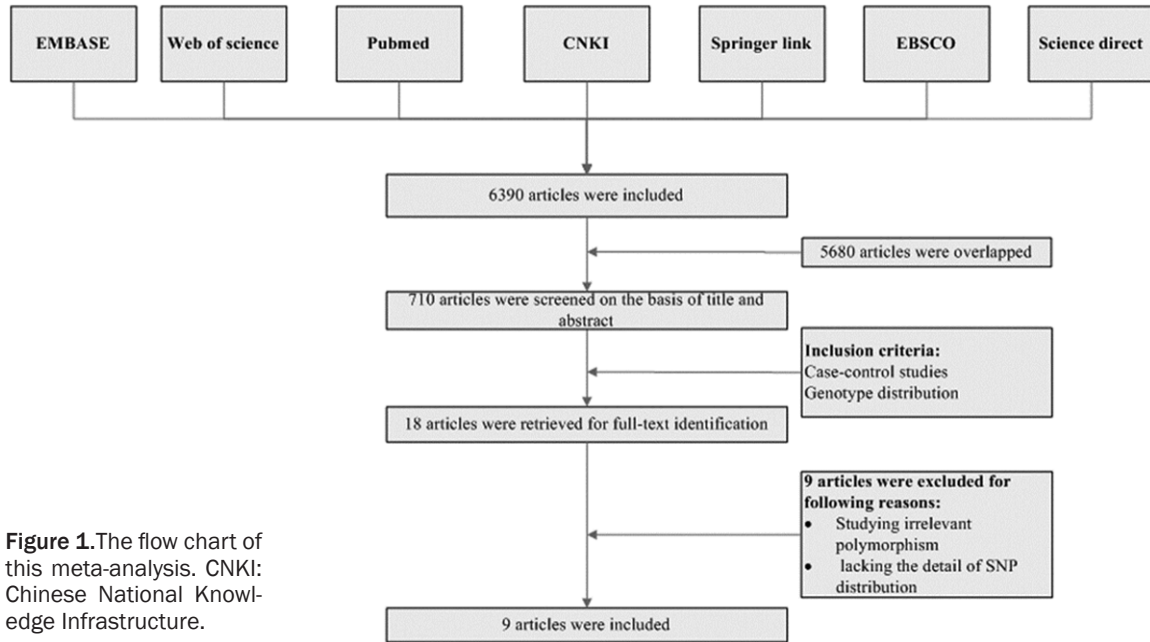
**Keywords:** Methylenetetrahydrofolate reductase, MTHFR, C677T, A1298C, polymorphism, ventricular septal defect, atrial septal defect

## Introduction

Congenital heart defect (CHD) is a common heart disease that affects neonates with abnormal cardiovascular development, leading to high mortality rate in newborns [1]. Ultrasonic exam is the best diagnostic test. In the developed countries about 3~12 per 1000 newborns are diagnosed as CHD [2]. But the prevalent rate is unclear in developing countries. Many hospitals in these countries might lack ultrasonic apparatus and instruments. Some poor patients are also unable to afford the

check-up charge. On the other hand, the cause of CHD can be both genetic and environmental. Some predisposing factors are shown to be associated with increased risk of heart defect, which include A. drug or alcohol abuse during pregnancy; B. maternal viral infection during pregnancy; C. subgroup of patients carrying high risk genotype [3]. Furthermore, evidence suggests that supplementation of folic acid reduces the risk of CHD [4]. In a case-control study in four populations with different maternal dietary habits, the offspring's risk of congenital heart defects was reduced when preg-

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**Figure 1.** The flow chart of this meta-analysis. CNKI: Chinese National Knowledge Infrastructure.

nant mother took dietary folic acid [5]. Folic acid may contribute to the cardiac neural crest cells migration, and regulate the truncus arteriosus formation and division, which may cause aorta and pulmonary artery defect [6]. 5,10-Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in homocysteine metabolism and catalyzes the reduction of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, the predominant circulating form of folate. This reaction provides methyl for downstream metabolism. In the MTHFR gene, a polymorphism mutation in exon 4 (C677T) results in a substitution of alanine with valine, and the mutation in exon 7 (A1298C) results in the substitution of glutamic acid with alanine, affecting MTHFR enzyme's function. In recent years, several studies have found an association between MTHFR C677T polymorphism and risk of congenital heart disease, which suggests patients could be easily screened for CHD by blood testing, a potential fast, convenient and inexpensive approach to diagnose CHDs. However, the congenital heart disease is a complex disease, which contains ventricular septal defect, tetralogy of Fallot, aortic valve stenosis, transposition of the great arteries, and atrial septal defect. Here we focus on septal defects, especially ventricular septal defect and atrial septal defect because they are the most prevalent types of congenital cardiac disease. Ventricular septal defect (VSD) accounts for 50% of patients with congenital heart dis-

ease [7], while atrial septal defect (ASD) accounts for 35% [8]. In the early stage, 4-5 weeks of embryonic heart development, atrial and ventricular are separated by septum primum and muscular part of interventricular septum. Ventricular or atrial septal defect may appear during this stage. The defect may affect the normal function of heart and have an impact on cardiovascular development [9]. Because the potential impact on septal defect, we exclude other studies focusing on tetralogy of Fallot, aortic valve stenosis, transposition of the great arteries. Thus, our meta-analysis that focused on the association between MTHFR C677T and A1298C polymorphism and the risk of CHDs, especially VSD and ASD, might be more reasonable and specific. Since many genome-wide association studies suggested no association between MTHFR C677T/A1298C and VSD/ASD risk in various populations, the results seem controversial. Here we performed a large literature review based on seven databases to evaluate the association in a broader global population.

### Methods

#### Literature review

We searched the EMBASE, Web of science, PubMed, Springer Link, Chinese National Knowledge Infrastructure (CNKI), EBSCO and Science Direct databases to identify the publications that reported the association between

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**Table 1.** Characteristics of the studies included in the analysis

Authors	Year of publication	Country	Host ethnicity	Age mean $\pm$ SD		Sample n		Genotyping method
				Case	Controls	Case	Controls	
Kocakap et al.	2015	Turkey	Caucasian	3.7 $\pm$ 7.6	8.7 $\pm$ 5.9	75	95	PCR-RFLP
Luciano C. Galdieri	2006	Brazil	Brazilians	3.27 $\pm$ 3.14	4.44 $\pm$ 3.31	58	38	PCR
Jing Xu	2010	China	Han population	6.50	6.69	308	527	PCR
Karen E. Christensen	2013	Canada	Caucasian	-	-	271	69	PCR-RFLP
Lydi M.J.W. van Driel	2008	Caucasian	Caucasian	1.4	1.35	229	251	PCR-RFLP
Seyyed R	2013	Iranian	Mid-Asian	4.51 $\pm$ 2.39	5.43 $\pm$ 0.51	123	125	PCR-RFLP
D. Ab-d	2014	Egypt	Mid-Asian	-	-	26	18	PCR-RFLP
Wenli L	2006	China	Chinese	-	-	22	103	PCR-RFLP
Haidy E Zidan	2013	Egypt	Mid-Asian	4.3 $\pm$ 2.32	4.88 $\pm$ 2.51	80	80	PCR-RFLP

SD, Standard Deviation; PCR, Polymerase Chain Reaction; RFLP, restriction fragment length polymorphism.

the MTHFR polymorphism and risk for septal defect of CHD from August 1997 to November 2015. The keywords used were 'Septal Defect', methylenetetrahydrofolate reductase. Polymorphism', 'Septal Defect, methylenetetrahydrofolate reductase, SNP', 'Septal Defect, methylenetetrahydrofolate reductase, allele', 'Septal Defect, MTHFR, Polymorphism', 'Septal Defect, MTHFR, SNP', 'Septal Defect, MTHFR, allele', 'Congenital heart defect, methylenetetrahydrofolate reductase, Polymorphism', 'Congenital heart defect, methylenetetrahydrofolate reductase, SNP', 'Congenital heart defect, methylenetetrahydrofolate reductase, allele', 'Congenital heart defect, MTHFR, Polymorphism', 'Congenital heart defect, MTHFR, SNP', 'Congenital heart defect, MTHFR, allele', 'CHD, methylenetetrahydrofolate reductase, Polymorphism', 'CHD, methylenetetrahydrofolate reductase, SNP', 'CHD, methylenetetrahydrofolate reductase, allele', 'CHD, MTHFR, Polymorphism', 'CHD, MTHFR, SNP', 'CHD, MTHFR, allele'. Only the articles in English or Chinese with an English abstract were selected. After excluding duplicates, titles and abstracts were reviewed.

Studies were selected if they: 1) were case-control studies (compare the difference between patients and health controls); 2) reported an association between MTHFR genotype and VSD/ASD. The exclusion criteria were: 1) Review articles; 2) Not related to MTHFR; 3) Animal or in vitro study; 4) Not related to the association between CHD risk and MTHFR genotype.

The study selection process was summarized in **Figure 1**.

This study was performed with the approval of the Ethical Committee of Beijing Obstetrics and Gynecology Hospital.

### Data extraction

For each study, the following information was extracted from original article: the name of first author, the year of publication, country of origin, ethnic of study population, genotypes distribution for each polymorphism in both cases and controls, characteristics of the population (sample size, gender and age distribution of cases and controls), source of controls, and genotyping methods.

### Statistical analyses

Hardy-Weinberg Equilibrium (HWE) was examined in controls by asymptotic Pearson's Chi-square test for each polymorphism in each study. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to estimate the association between polymorphism and CHD risk. The heterogeneity of data was tested using  $I^2$  test.  $P$ -value  $<0.05$  indicated there was significant heterogeneity between studies. In this case, random-effects model was used to provide pooled estimates. Otherwise, fixed model was used [10]. The publication bias was evaluated using Begg's and Egger's test [11, 12].  $P < 0.05$  was considered statistically significant. Statistical analyses were conducted with Stata 13.0 (College Station, TX).

### Linkage disequilibrium analysis

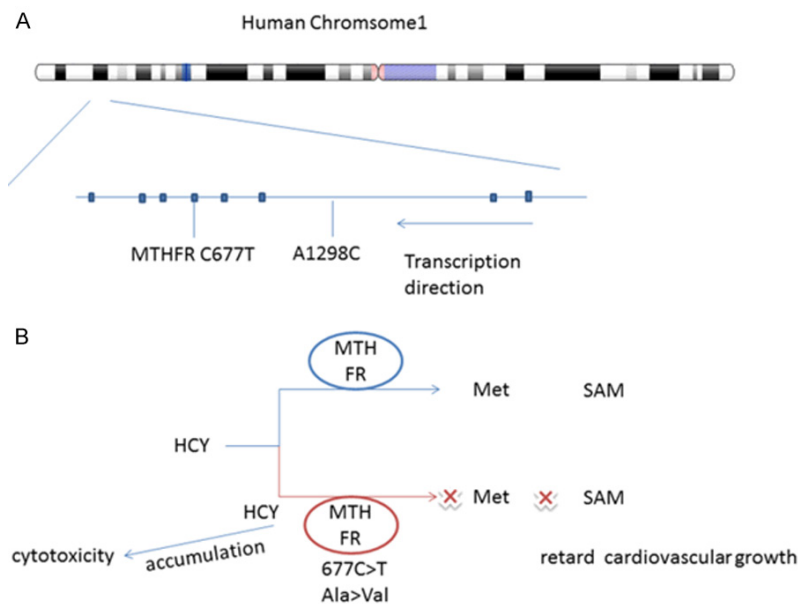
Linkage disequilibrium (LD) analysis was performed based on the database of International

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**Table 2.** Genotype and Allele Distribution of Polymorphisms in VSD/ASD Patients and controls

SNP	Study	Case					Control					Total		HWE	
		CC	CT	TT	C	T	CC	CT	TT	C	T	case	control	Chi	p-value
MTHFR C677T	Beyza D. SayinKocakap	40	33	2	113	37	43	44	8	130	60	75	95	0.49	0.48
	D. M. El-Abd et al.	7	12	7	26	26	13	5	0	31	5	26	18	0.47	0.49
	Haidy E. Zidan	18	21	41	57	103	32	21	27	85	75	80	80	17.89	2.34E-05
	Haidy E. Zidan et al.	32	21	27	85	75	18	21	41	57	103	80	80	14.63	0.00
	Jing Xu	95	147	66	337	279	151	261	115	563	491	308	527	0.01	0.91
	Karen E. Christensen	136	97	38	369	173	35	26	8	96	42	271	69	0.84	0.36
	Luciano C. Galdieri	30	21	7	81	35	18	14	6	50	26	58	38	1.25	0.26
	Lydi M.J.W. van Driel	99	103	27	301	157	119	107	25	345	157	229	251	0.02	0.90
	Seyyed Reza Pishva et al.	63	60	0	186	60	71	54	0	196	54	123	125	9.49	0.00
	Wenli L. Zhu1 et al.	3	7	12	13	31	22	57	24	101	105	22	103	1.18	0.28
MTHFR A1298C		AA	AC	CC	A	C	AA	AC	CC	A	C	case	control	Chi	p-value
	Beyza D. SayinKocakap	20	36	13	76	62	51	37	11	139	59	69	99	1.13	0.29
	Luciano C. Galdieri	35	21	1	91	23	19	16	3	54	22	57	38	0.02	0.88
	Haidy E. Zidan	16	27	37	59	101	30	26	24	86	74	80	80	9.60	0.00
	Jing Xu	190	102	6	482	114	326	185	16	837	217	298	527	2.85	0.09
	Karen E. Christensen	146	104	21	396	146	38	26	5	102	36	271	69	0.04	0.85
Lydi M.J.W. van Driel	112	90	27	314	144	97	129	25	323	179	229	251	3.62	0.06	

MTHFR, methylenetetrahydrofolate reductase; HWE, Hardy-Weinberg equilibrium.



**Figure 2.** The location and function of MTHFR C677T polymorphism. A. MTHFR locates on chromosome 1 p36.3, and the C677T polymorphism is also known as rs1801131. B. MTHFR provides Methyl during the conversion of HCY to Met, following the synthesis of SAM. The mutation of C677T changes Ala to Val, resulted in 50% reduction of enzymatic activity. The accumulation of HCY can also leads to cytotoxicity and the lacking of SAM can result in cardiovascular defect. MTHFR: Methylene tetrahydrofolate reductase; HCY: Homocysteine; Met: Methionine; SAM: S-adenosyl-L-methionine; Ala: Alanine; Val: Valine.

HapMap Project (HapMap3 Genome Browser release #2, Phase 3 - genotypes, frequencies & LD). Utah residents with Northern and Western European ancestry population (CEU) were cho-

sen in this LD analysis. A total of 20 kbp D' and R2 values for MTHFR SNPs was calculated using Haploview 4.2 (Mark Daly's lab, Broad Institute).  $R^2 > 0.8$  was considered significant linkage disequilibrium.

### Results

#### Characteristics of studies included in the meta-analysis

A total of 1462 articles were identified from 7 databases. A total of 616 abstracts were reviewed after excluding the duplicates (**Figure 1**). In these publications, 18 research articles reported the association of MTHFR gene polymorphisms and congenital heart defect risk. In the next full-text-retrieval process, seven articles were excluded since they did not refer

to VSD or ASD risk. We next extracted the articles on VSD/ASD by calculating the percentage of VSD and ASD cases in the total patients. The articles were included in our study if the major-

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**Table 3.** Meta-analysis by Genetic Models for Polymorphisms

Polymorphism	Genetic model	Participants	OR (95% CI)	Z	P value	I <sup>2</sup> %	P <sub>het</sub>	Effect model	Begg's test p> z	Egger's test p> t
MTHFR C677T	CC vs CT+TT	2326	0.80 (0.532, 1.141)	1.28	0.20	74.7	<0.01	random	1	0.877
	TT vs CT+CC	2326	1.299 (0.793, 2.127)	1.04	0.30	68.3	0.001	random	0.917	0.682
	CC vs CT	2341	0.981 (0.799, 1.203)	0.19	0.85	17.3	0.284	fixed	0.472	0.374
	CC vs TT	1623	0.911 (0.535, 1.551)	0.34	0.73	70.4	0.001	random	0.463	0.376
	C vs T	5642	0.96 (0.743, 1.239)	0.31	0.75	73.6	<0.01	random	0.59	0.399
MTHFR A1298C	AA vs AC+CC	1736	0.948 (0.584, 1.54)	0.22	0.83	80.1	<0.01	random	0.348	0.136
	CC vs AC+AA	1736	0.838 (0.365, 1.925)	0.42	0.676	81.3	<0.01	random	0.188	0.36
	AA vs AC	2389	1.15 (0.901, 1.47)	1.12	0.26	40	0.139	fixed	0.85	0.62
	AA vs CC	1635	1.32 (0.56, 3.13)	0.63	0.53	82.1	<0.01	random	0.57	0.48
	A vs C	5194	1.10 (0.80, 1.51)	0.57	0.57	80.5	<0.01	random	0.85	0.814

MTHFR, methylenetetrahydrofolate reductase; OR, odd ratio; CI, confidence interval; P<sub>het</sub>, p value of heterogeneity.

ity diseases were VSD or ASD. Genome-wide association study was also perused [13-15]. But these articles were excluded due to lack of details of genotype distribution. Finally, 9 articles were included in this analysis [16-24]. Seven articles were published in English and two articles were published in Chinese. Overall, four MTHFR SNPs, including C677T, C524T, A1298C and A66G were studied in these research articles; however, the number of studies on C524T and A66G allele is too small for a meta-analysis. Therefore, we chose MTHFR SNP C677T (rs1801133) and A1298C (rs1801131) as the target gene in our final analysis. As shown in **Table 1**, the race of study population included Caucasian, Mid-Asian and Chinese. The pooled population of C677T, rs1801133 from 10 studies in 9 articles consisted of 2658 subjects (1272 cases and 1386 controls) and the pooled population of A1298C, rs1801131 from 6 studies consisted of 2064 subjects (1004 cases and 1064 controls). The details of each polymorphism were shown in **Table 2**. In all studies, the genotype distribution met the law of HWE. The location of each SNP was shown in **Figure 2** based on the information from CNBI SNPs database.

### Data synthesis by polymorphism

MTHFR polymorphism C677T, rs1801133: Ten case-control studies in 9 articles (1272 cases and 1386 controls) investigated the relationship between C677T polymorphism and VSD/ASD risk. As shown in **Table 3** and **Figure 3A**, the heterogeneity of allele model (C vs. T) was significant (Phet<0.001, I<sup>2</sup>=73.6%). The overall OR using random-effect model was 0.96 (95% CI 0.74, 1.24). Analyses for heterozygote, homo-

zygote, dominant and recessive models were also performed, but no significant association was observed (**Table 3**). The publication bias was also negligible in these genetic models (**Table 3**). In the subgroup analyses for five genetic models by ethnicity, none of them was significant. The allele model (C vs. T) was plotted in **Figure 3C**. The funnel plot for allele model was shown as **Figure 4A**. Heterozygote, homozygote, dominant and recessive models were also performed and no significant result was found.

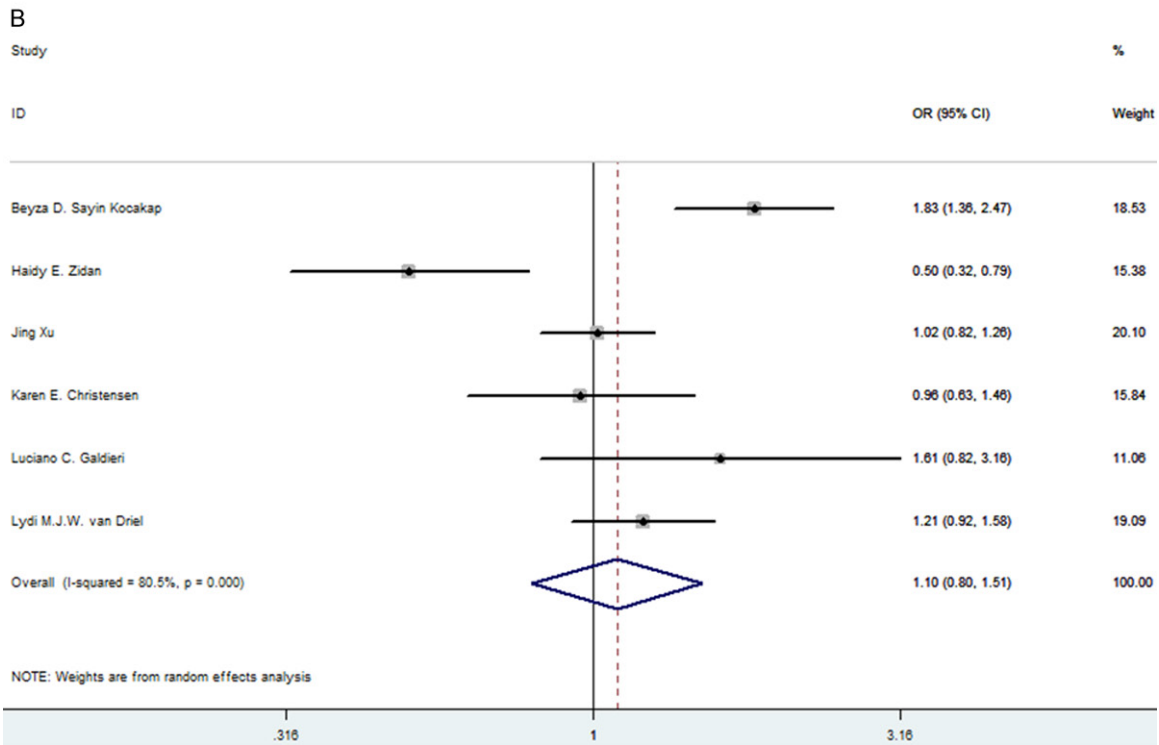
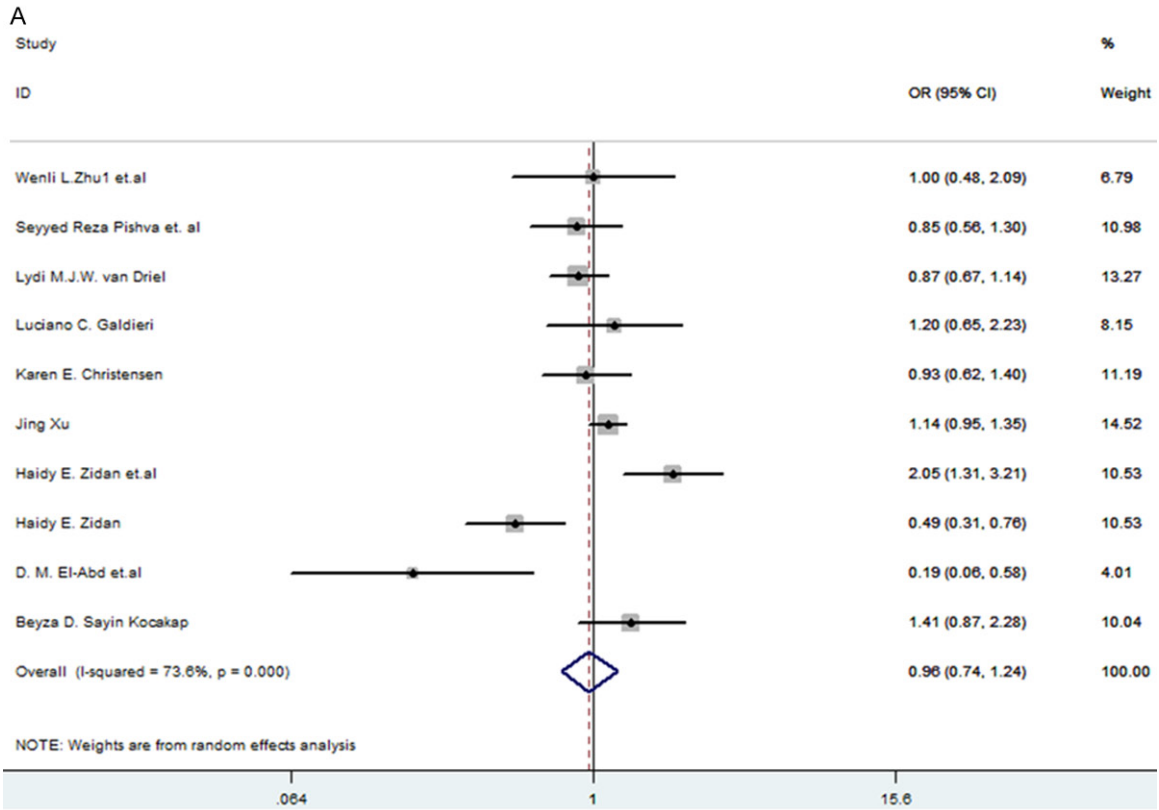
MTHFR polymorphism A1298C, rs1801131: Six case-control studies (1004 cases and 1064 controls) investigated the relationship between C677T polymorphism and the risk of CHD. As shown in **Table 3** and **Figure 3B**, the heterogeneity was not significant (Phet<0.001, I<sup>2</sup>=80.5%). The overall OR (C vs. T alleles) using random-effect model was 1.10 (95% CI 0.80, 1.51). Analyses of other genetic models were also performed, but no association was identified (**Table 3**). The publication bias was also negligible in these genetic models (**Table 3**). Subgroup analysis by ethnicity in allele comparison (C vs. T) was performed, no significant odd ratio was identified (**Figure 3D**). The funnel plot for allele model was shown as **Figure 4B**. Heterozygote, homozygote, dominant and recessive models were also performed and no significant result was found.

### LD analysis

The LD pattern in the 20-kb region including MTHFR C677T and A1298C in chromosome 1 was investigated. However, there was no SNP linkage with MTHFR C677T and A1298C.



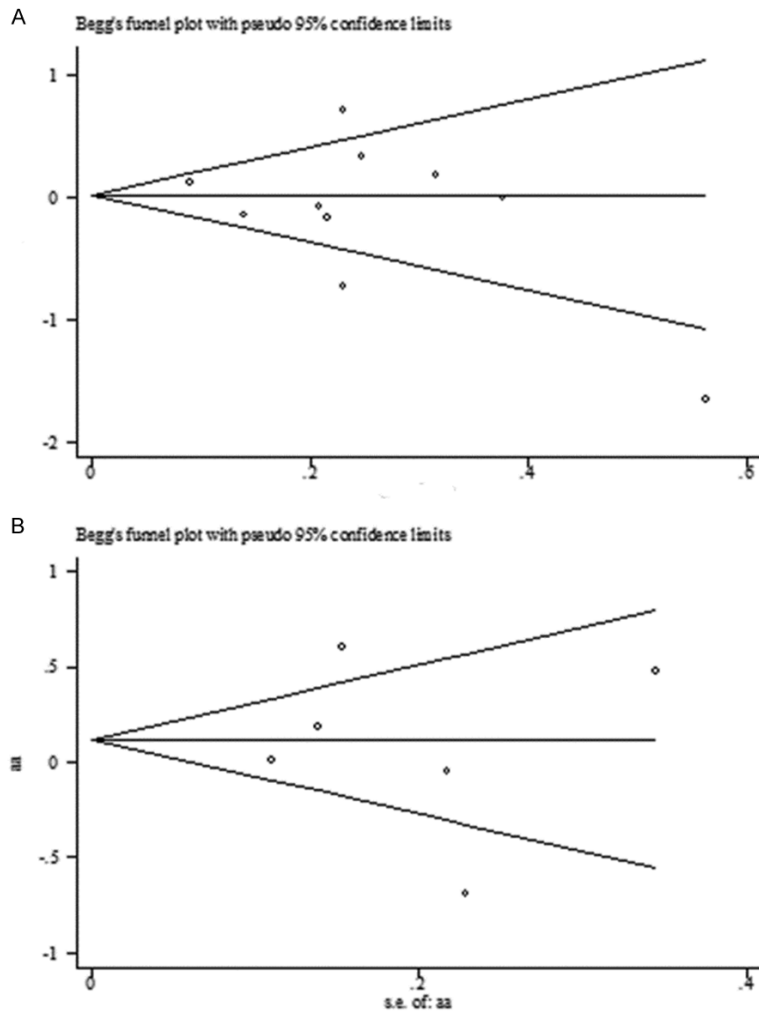
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**Figure 3.** The forest plot for MTHFR SNPs. A. Allele model plot for C677T. B. Allele model plot for A1298C. C. Subgroup plot for C677T. D. Subgroup plot for A1298C. 1. MTHFR: Methylenetetrahydrofolate reductase. 1 Mid-Asian; 2 Brazil; 3 Caucasian; 4 Chinese.



**Figure 4.** The funnel plot for MTHFR SNPs. A. Funnel plot for C677T. B. Funnel group for A1298C. MTHFR: methylenetetrahydrofolate reductase.

### Discussion

Genetic polymorphisms that can alter the level of protein expression could have a substantial influence on disease activity [25]. Although the relationship between MTHFR C677T and A1298C polymorphism and ventricular septal defect and atrial septal defect risk has been well studied, the results are still controversial. The objective of this meta-analysis is to synthesize the information from these studies to better understand the potential association between MTHFR C677T and A1298C polymor-

phism and ventricular septal defect and atrial septal defect risk and explore whether there is any difference by ethnicity. In our analysis that included 10 studies, the study population included Caucasian, Mid-Asian and Chinese. But no of them showed an increased risk of ventricular septal defect and atrial septal defect associated with MTHFR C677T and A1298C polymorphism. To date, our study, with an analysis of 1272 cases and 1386 controls, provides the most comprehensive evaluation on the association between the C677T variant and CHDs. The results showed that the Chinese population with T genotype is more susceptible to CHDs than C genotype. Although the ultrasonic exam is the optimal method for detecting CHDs, detecting SNP genotype provides a fast, convenient and inexpensive way to screen high-risk individuals. Patients could easily be tested using blood sample during check-up to estimate their potential CHDs risk. But the publication bias exists in this meta-analysis for the C677T polymorphism.

As the number of eligible studies was limited in the meta-analysis, these results still need further investigation.

Previous meta-analysis mainly focusing on Europeans did not identify significant association between C677T polymorphism and CHDs [26]. A potential explanation is that maternal periconceptional use of folic acid and multivitamin supplements might be more prevalent in Europe than in China [26, 27]. Folic acid supplement in diet has been shown to increase folate concentration in maternal serum during preg-



nancy [28]. van Beynum et al. [26] studied the interaction between the MTHFR polymorphisms and folic acid intake on the risk of CHDs. They found mothers with MTHFR 677CT and TT genotypes had increased risk of CHDs in their offspring when folic acid was insufficient [26]. Furthermore, trimethoprim, an antagonist to folic acid, could increase the risk of CHDs when it was abused during pregnancy [29]. It is suggested it is possible to supplement folic acid for decreasing the risk of CHDs.

The mechanism of how the MTHFR polymorphism relates to VSD/ASD risk is still unclear. The existing theory hypothesizes that the homocysteine (HCY) is converted to Methionine (Met) by the catalytic effect of MTRR (5-methyltetrahydrofolate-homocysteine methyltransferase reductase). This process needs MTHFR as the Methyl donor catalyst. The metabolite is then converted to SAM, S-adenosyl-L-methionine, which plays an important role for organs or whole body health. The transition of C to T in 677 amino acid residue changes the amino acid from Alanine (Ala) to Valine (Val) in MTHFR protein and the mutation in exon 7 (A1298C) results in the substitution of glutamic acid (Glu) with Alanine (Ala) (**Figure 2B**). As a result, the enzymatic activity reduces by 50% at least. The immediate impact is that HCY may not be converted to SAM, and the baby's cardiovascular growth is delayed. On the other hand, the HCY could be accumulated, causing cytotoxicity [30]. This mechanism suggests that the C677T polymorphism might play an important role in the development of CHDs.

In the Genome-wide association studies, Cordell, Flaquer and Hu focused on the population in the United Kingdom, German and China respectively [13-15]. They did not identify SNPs in MTHFR loci that might contribute to the risk of VSD or ASD. Here, we performed a meta-analysis of studies that included broader and more representative of global population to draw a more confirmative conclusion on the association between MTHFR SNPs and VSD/ASD risk.

There are several limitations in this meta-analysis. Firstly, because of the limitation of search database, some relevant studies in non-English or non-Chinese are not included in our analysis. Secondly, the number of published studies was not sufficient for a comprehensive analysis,

and of the small sample size in these studies also limited the statistical power to detect the real association between the C677T and A1298C polymorphism and susceptibility to CHDs. Finally, some main confounding variables were not always available in the original papers, such as age, gender, ethnicity, and exposures.

In conclusion, the result of meta-analysis showed no significant association between MTHFR C677T and A1298C polymorphisms and CHD risk in all the population we studied. It suggested patients with other polymorphisms in MTHFR gene should be selected for ultrasonic examination in the VSD and ASD diagnosis. As long as folate fortification is not mandated, periconceptional folate supplementation should be promoted. Further studies with consideration of gene-gene and gene-environment interactions should also be conducted to further investigate this association.

### Disclosure of conflict of interest

None.

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