Systematic Review and Meta-Analysis

Medicine®

OPEN

Genetic polymorphism of methylenetetrahydrofolate reductase as a potential risk factor for congenital heart disease

A meta-analysis in Chinese pediatric population

Ye Yuan, MD[a], Xia Yu, MD[b], Fenglan Niu, MD[c,*], Na Lu, MD[b,*]

Abstract

Background: A meta-analysis of polymorphism C677T (rs1801133) of the methylene tetrahydrofolate reductase (MTHFR) gene as a potential risk factor for congenital heart disease (CHD) in Chinese paediatric population was studied in view of the previously reported controversial results.

Methods: We searched literature including PubMed, Embase, Cochrane Library, CNKI, Wanfang, and VIP databases that resulted in the identification of a total of 21 separate studies with 6414 subjects that met the inclusion criteria in the Chinese population. The quality assessment of the included studies was preformed and relevant information was collected. We chose the fixed-effect model or random-effect model to calculate the pooled odds ratio (ORs) and its corresponding 95% confidence interval (95% CI) where appropriate. Begg test was used to measure publication bias and sensitivity analyses were done to ensure authenticity of the outcome.

Results: We observed a significant association between MTHFR C677T polymorphism and CHD development in all the genetic models evaluated. The pooled ORs and 95% CIs in all genetic models indicated that children's MTHFR C677T polymorphism was significantly associated with CHD.

Conclusion: Our study results indicate that MTHFR gene 677T polymorphism is a genetic risk factor in the development of CHD in Chinese paediatric population.

Abbreviations: CHD = congenital heart disease, CI = confidence interval, CNKI = China National Knowledge Infrastructure, HWE = Hardy–Weinberg equilibrium, MTHFR = methylene tetrahydrofolate reductase, OR = odds ratio, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism.

Keywords: congenital heart disease, meta-analysis, MTHFR, polymorphism

1. Introduction

Congenital heart disease (CHD) is a common birth defect that is responsible for significant childhood morbidity and mortality.[1] CHD accounts for about one-third of all congenital anomalies seen in newborn that is responsible for infant mortality.[2] Genetic factors play an important role in increasing the risk of CHD though the exact cause for it is not clear. Some of the risk factors that may increase the risk of development of CHD include: ingestion of cardiotoxic medicines, alcohol, or drug abuse during pregnancy; maternal rubella (German measles) in the first trimester of pregnancy; drugs or environmental factors that can interfere with the metabolism of folic acid and genetic factors.[3] Recent studies revealed that methylene tetrahydrofolate reductase (MTHFR) gene may play an important role in increasing the risk of developing CHD.[4,5] Single nucleotide polymorphisms of genes involved in the folate pathway can affect folic acid metabolism and thus, increase the risk of CHD.[6] Folic acid may protect against development of CHD since its deficiency could be an important risk factor for CHD.[7] The flavin adenine dinucleotide-dependent enzyme 5,10-methylenetetrahydrofolate reductase catalyzes the reduction of methylenetetrahydrofolate to 5-methyltetrahydrofolate which is necessary for remethylation of homocysteine to methionine.[8] Hyperhomocysteinemia is a significant risk factor for the development of heart defects.[9] Availability of adequate amounts of 5-methyltetrahydrofolate, the major circulating metabolite of folic acid, reduces maternal homocysteine plasma levels and thus, aids in preventing development of CHD.[10] Based on this assumption, it has been proposed that polymorphisms of MTHFR in children population may predispose to the development of CHD. Previous reports suggested that children's MTHFR genetic variants, such as C677T, can influence the development of CHD.[11] However, these studies gave inconsistent results. Hence, the present study was performed to clarify possible relationship between C677T

Editor: Manal Elshmaa.

The authors have no conflicts of interest to disclose.

[a] Department of Anesthesiology, [b] Department of Pediatrics, First Hospital, Jilin University, [c] School of Public Health of Jilin University, Changchun, China.

[*] Correspondence: Fenglan Niu, School of Public Health, Jilin University, Changchun, Jilin 130021, China (e-mail: fenglanniu2000@sina.com); Na Lu, Department of Pediatrics, First Hospital, Jilin University, Changchun, Jilin 130021, China (e-mail: lunarmoo@163.com).

Copyright © 2017 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the Creative Commons Attribution-NoDerivatives License 4.0, which allows for redistribution, commercial and non-commercial, as long as it is passed along unchanged and in whole, with credit to the author.

Medicine (2017) 96:23(e7057)

Received: 18 November 2016 / Received in final form: 9 March 2017 / Accepted: 10 May 2017

http://dx.doi.org/10.1097/MD.0000000000007057

polymorphism and susceptibility to develop CHD in the paediatric population.

2. Materials and methods

This study was approved by the ethics committees of the First Bethune Hospital of Jilin University and conformed to the principles of the Declaration of Helsinki. Written informed consent was obtained from each participant before entry into the study, and all of the procedures were in accordance with institutional guidelines.

We searched PubMed, Embase, CNKI (China National Knowledge Infrastructure), Wanfang and VIP databases were to identify eligible publications. The search terms used were: MTHFR or methylenetetrahydrofolate reductase or folic acid and variant or polymorphism or SNP and congenital heart disease or defect or CHD or congenital anomalies. All publications in either Chinese or English language were considered suitable for inclusion in the analysis. In addition, all potentially relevant studies were reviewed including their titles and abstracts, and only those studies that matched the inclusion criteria were retrieved. The literature search was performed of all publications till Jun 6, 2016.

The retrieved articles were scrutinized with respect to their data to make sure that their contents included topic of interest of the study planned. In addition, references of the retrieved articles were also screened. To prevent data duplication, especially when a report overlapped with another study, only the one that contained the most detailed study was included in the analysis. If an article included in the study contained results on a different ethnic subpopulation, each subpopulation was treated as an independent study.

We ensured that studies included in the meta-analysis had met the following criteria: independently published case-control or cohort studies on the relationship between MTHFR gene polymorphism and CHD; contained genotype data of TT, TC, and CC and contained comprehensive statistical indicators directly or indirectly: OR values and 95% CI; studies had similar themes and methods. In other words, all the studies included in the analysis are case-control studies, which evaluated the association between the C677T polymorphism in Chinese pediatric population and susceptibility to CHD_S; no restriction on the source of the control group (general population or participants in a hospital) was imposed, and the study should satisfy Hardy–Weinberg equilibrium (HWE) among the controls.

Two authors independently preformed the quality assessment of included studies according to the Newcastle–Ottawa Scale (NOS).[12] The NOS method, with a maximum score of 9 points, includes 3 quality categories: selection, comparability, and exposure evaluation. Studies with more than 6 scores were identified as high quality. Any disagreement was resolved by reevaluation of the originally included studies.

The exclusion criteria employed in the present study were: reviews, case reports, editorials or comments in other words only original studies were eligible for inclusion in the study for analysis; duplicate studies were excluded; studies that contained insufficient data or data was not of sufficient quality; and studies that did not have appropriate control(s). In instances, wherein genotype frequency was not reported, the original authors of the study were contacted by emails to obtain the missing data.

To minimize selection bias, the data was collected independently by 2 investigators using the standard protocol adopted for this study. The data that was extracted from the studies meant for the present analysis included: first author's name; year of publication; study design; sample size of cases and controls; method of screening used for CHD; genotype distribution(s) of both cases and controls; and whether the included population of the study was in HWE. Studies that contained little or insufficient data, violation of the inclusion criteria, and repeated publication of the same data were considered of poor research quality and excluded from the analysis. In the event, the same research results were used in 2 or more different publications, only 1 result of such studies was considered for the present meta-analysis. If any discrepancy was noticed between 2 or more studies, the same was discussed among the authors or investigators and arrived at a decision based on mutual consensus. Whenever any insufficient and equivocal data was detected in any study elected for the analysis, efforts were made to obtain the missing information from the original authors. Such studies were included in the final analysis only if all the required information was obtained; otherwise, it was discarded from the final analysis. All the data included in the present analysis is given in Table 1.

Meta-analysis of possible association between MTHFR polymorphisms and risk of CHD was performed employed case-using ORs corresponding to a 95% CI as the present study used control studies. In order to assess the intensity of the association between MTHFR gene polymorphism and CHD, ORs were calculated according to the method described by Woolf.[13] The significance of pooled ORs was analyzed using Z test, and only when $P < .05$ the results were considered statistically significant.

To assess interstudy heterogeneity, we used an x^2-based Q statistic and the result was significant only if $P < .1$ since the power of statistics was low. Quantification of the heterogeneity was done employing the I^2 metric, which is independent of the number of studies used in the meta-analysis. I^2 used represents the percentage of the observations between various studies as the variability is due to heterogeneity rather than to chance. If it ranges between 0% and 100%; values above 75% implies large heterogeneity.[14] Heterogeneity between studies was not expected since the studies that were analyzed were both clinically and methodologically heterogeneous. In the present study, 2 models of meta-analysis were applied for dichotomous outcomes: the fixed-effects model and the random-effects model. The pooled OR was calculated by a fixed-effects model if the result of the Q test was $P > .1$, which suggests that the heterogeneity among the studies was insignificant. Otherwise, a random-effects model was used.

When the analysis of the outcomes was heterogeneous, prespecified subgroup comparisons were conducted to find out the influence of the following factors on the relationship between MTHFR gene C677T polymorphism and CHD: method of screening for CHD; source of controls (whether it is population based vs hospital based); sample size ($n < 100$ vs $n > 100$); and NOS score.

Since the present analysis included a wide variety of study designs such as (diverse methods, the source of controls, and sample size), sensitivity analyses were conducted to know the stability of the results by omitting only 1 study at a time and recalculating the pooled effect to see whether the pooled effect estimate was influenced by a certain individual study. Potential outliers (i.e., data points that are far outside the norm) were identified by a sensitivity analysis.

To evaluate the presence of publication bias a funnel plot was constructed by plotting the effect measure against the inverse of

Table 1

The detailed characteristics of all eligible studies for MTHFR C677T polymorphism.

Cases study	Controls year	Source of	CC	CT	TT	CC	CT	TT	HWE	Methods	Main types of CHD	Quality score controls
Yan[16]	2003	HB	28	89	57	22	57	24	1.48	PCR-RFLP	CHD	7
Li[17]	2005	PB	32	94	61	20	57	25	0.320	PCR-RFLP	VSD, ASD, PDA, TOF,	8
Liu[18]	2005	HB	19	54	24	33	69	16	0.234	PCR-RFLP	Congenital heart defects	6
Lee[19]	2005	HB	110	89	14	114	68	13	0.556	PCR-DHPLC	AP Window, ASD, CoA, PS, DILV, DORV, ECD, IAA, LAI, PA, PDA, RAI, TGA, TOF, VSD	5
Zhu[20]	2006	PB	7	22	27	22	57	24	0.328	PCR-RFLP	ASD, PDA	7
Li[21]	2007	HB	16	42	46	55	114	39	0.14	PCR-RFLP	CHD	6
Zhang[22]	2007	PB	15	13	2	34	28	2	1.71	PCR-RFLP	CHD	6
Liu[23]	2007	HB	30	68	34	46	48	13	0.829	PCR-RFLP	CHD	7
Li[24]	2009	HB	26	52	66	49	84	35	Yes	PCR-RFLP	All types	6
Li[25]	2009	HB	16	42	46	55	114	39	Yes	PCR-RFLP	All types	6
Gong[26]	2009	HB	10	41	29	177	40	23	Yes	PCR-RFLP	CHD	4
Xu[27]	2010	HB	162	244	96	151	261	115	0.930	PCR-RFLP	Cyanotic Cardiac Disease, ASD, VSD, PDA, Left-sided Obstruction Defects	6
Gong [28]	2012	HB	45	123	76	43	72	21	Yes	MassArray	Conotruncal heart defects	4
Zhou [29]	2012	HB	23	60	53	88	126	63	0.183	PCR-RFLP	TOF	6
Wang [30]	2013	HB	59	76	25	53	100	35	0.377	SEQUENCE	CHD	7
Jing [31]	2013	HB	46	42	16	39	114	55	0.164	PCR-RFLP	CHD	6
Wang [32]	2013	HB	33	92	111	88	126	63	0.183	PCR-RFLP	VSD, ASD, PDA, TOF, DORV	6
Xu [15]	2013	PB	23	54	28	46	40	19	Yes	PCR-RFLP	All types	5
Xu [15]	2013	PB	50	52	21	78	34	13	Yes	PCR-RFLP	All types	5
Chao[33]	2014	HB	10	5	2	19	12	3	0.660	PCR-RFLP	PDA	8
Yu [34]	2014	HB	61	7	1	53	8	1	0.631	PCR-RFLP	All types	7

AP Window = atriopulmonary window, ASD = atriopulmonary window, CHD = congenital heart disease, CoA = coarctation of the aorta, DILV = double inlet of the left ventricle, DORV = double outlet of the right ventricle, ECD = endocardial cushion defect, IAA = interrupted aortic arch, LAI = left atrial isomerism, PA = pulmonary atresia, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, PDA = patent ductus arteriosus, PS = pulmonary stenosis, RAI = right atrial isomerism, TGA = transposition of the great artery, TOF = tetralogy of Fallot, VSD = ventricular septal defect.

its standard error. The severity of publication bias was estimated using Egger test. Statistical analysis was performed with Stata 11.0 (Stata Corp, College Station, TX) and Cochrane Collaboration Review Manager 5.2 software. All *P* values were 2-sided.

3. Results

Three hundred seventy-eight publications were selected for the present study based on preliminary analysis, which included 276 Chinese and 102 English publications. In total, 322 publications were excluded due to their unsuitability since some of them were duplicate publications while some were based on nonclinical-based work. Finally, 56 full-text publications were retrieved and the rest were excluded since some of them were duplicate publications; some focused on MTHFR gene polymorphisms other than C677T (rs1801133) and contained insufficient data. In addition, 2 studies not satisfying HWE were excluded from the final analysis. Figure 1 shows the process of study selection and exclusion, with reasons for the same. Based on all the criteria used for the selection of studies for the present analysis, we could finally select 20 articles that met the selection criteria. One publication that reported results based on 2 different data was included in the final meta-analysis.[15] Thus, a total of 20 eligible original reports that contained 21 separate studies [15–34] formed the basis of the present meta-analysis. A flow diagram of the study selection and their characteristics is given in Figure 1 and Table 1 respectively.

The 21 studies from China included in the present final meta-analysis were all case-control studies. It is noteworthy that 16 of the 21 studies included in the final analysis consisted of data obtained on hospital-based populations, while the other 5 were population-based studies. In 18 of the studies, genotyping of C677T was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, while in 3 studies by other DNA sequencing methods. All 21 studies contained full-length reports published in peer-reviewed journals. Five studies were divided into low quality with an NOS score of 4 or 5 points, and 16 with score 6 or greater were assigned as high quality.

It was noted that the genetic variant of MTHFR 677T in all the subjects of the 21 studies analyzed showed significantly higher 677T allele frequency in the CHD group (49.1%) compared with control (44.2%) ($P < .00001$). In addition, the prevalence of CC/CT/TT genotype was 27.5%/44.8%/27.7% in CHDs group and 33.1%/48.0%/18.9% in the control group respectively.

Furthermore, when we evaluated association between MTHFR C677T polymorphism and the risk of CHD for each study significant associations were observed in all genetic models: the dominant model (TT + CT vs CC: OR = 1.44, 95% CI: 1.11–1.87; $P < .00001$, Fig. 2), and the recessive model (TT vs TC + CC: OR = 1.78, 95% CI: 1.36–2.33; $P < .0001$, Table 2), T versus C (OR = 1.30, 95% CI: 1.06–1.59; $P = .010$, Fig. 3), TT versus CC (OR = 2.12, 95% CI: 1.44–3.11; $P = .0001$, Fig. 4), and TT versus TC (OR = 1.63, 95% CI: 1.30–2.05; $P < .0001$, Table 2). Pooled ORs were calculated by the random-effect method and are shown in Table 2.

In the stratified analysis by source of controls, significant association was found when all the studies were pooled with fixed models for T versus C (OR 1.48, 95% CI: 1.25–1.75; $P = .25$), and for TT versus CC (OR 2.39, 95% CI: 1.62–3.53; $P = .67$), dominant model (OR 1.74, 95% CI:1.31–2.32; $P = .39$) and

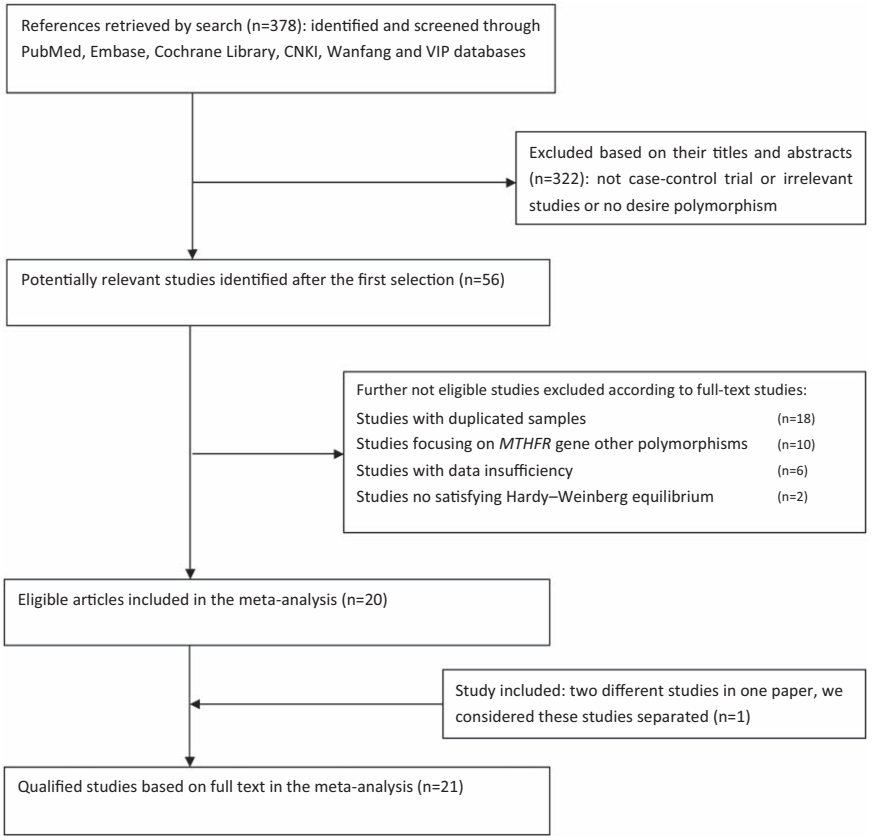

Figure 1. Flowchart of study selection.

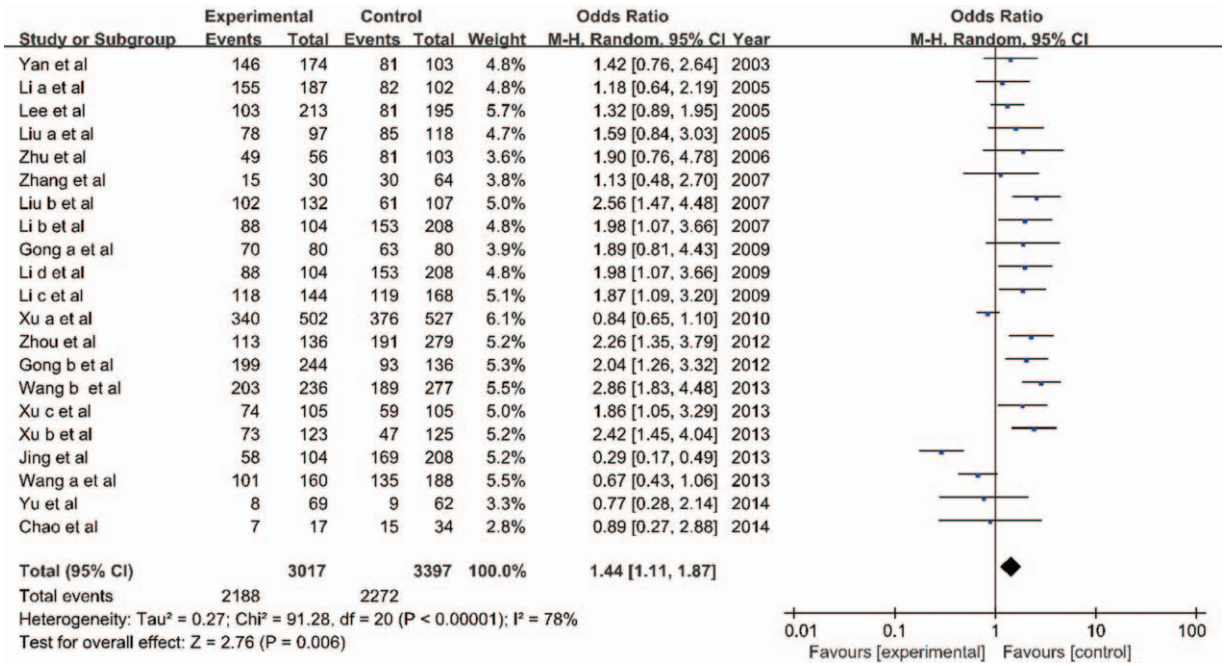

Figure 2. Forest plot on the association between the C677T polymorphism and the risk for CHD in the Chinese fetal population (TT + CT vs CC). CHD = congenital heart disease.

Table 2

Summary ORs and 95% CI of the studies included in meta-analysis.

Genetic models	Model	Test of heterogeneity			Test of association				Test of publication bias	
		Q	P	I^2	OR	95% CI	Z	P	Z	P
T vs C	Random-effects	117.16	<.00001	85%	1.30	1.06–1.59	2.56	.010	−0.84	.709
TT vsTC	Random-effects	50.96	.0002	61%	1.63	1.30–2.05	4.24	<.0001	−1.17	.659
TT vs CC	Random-effects	106.25	<.0001	81%	2.12	1.44–3.11	2.32	.0001	−0.36	.384
TT+CT vs CC	Random-effects	91.28	<.00001	78%	1.44	1.11–1.87	2.76	.006	−0.84	.340
TT vs TC+CC	Random-effects	81.01	<.0001	75%	1.78	1.36–2.33	4.22	<.0001	−1.36	.794

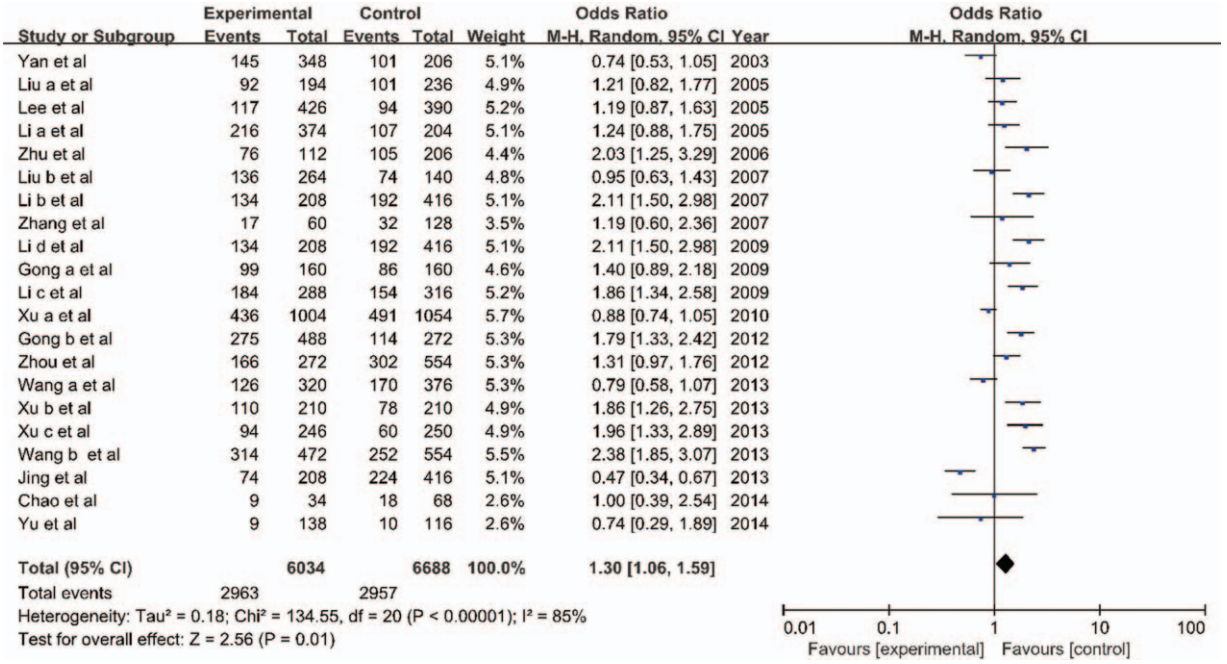

Figure 3. Forest plot on the association between the C677T polymorphism and the risk for CHD in the Chinese fetal population (T vs C).

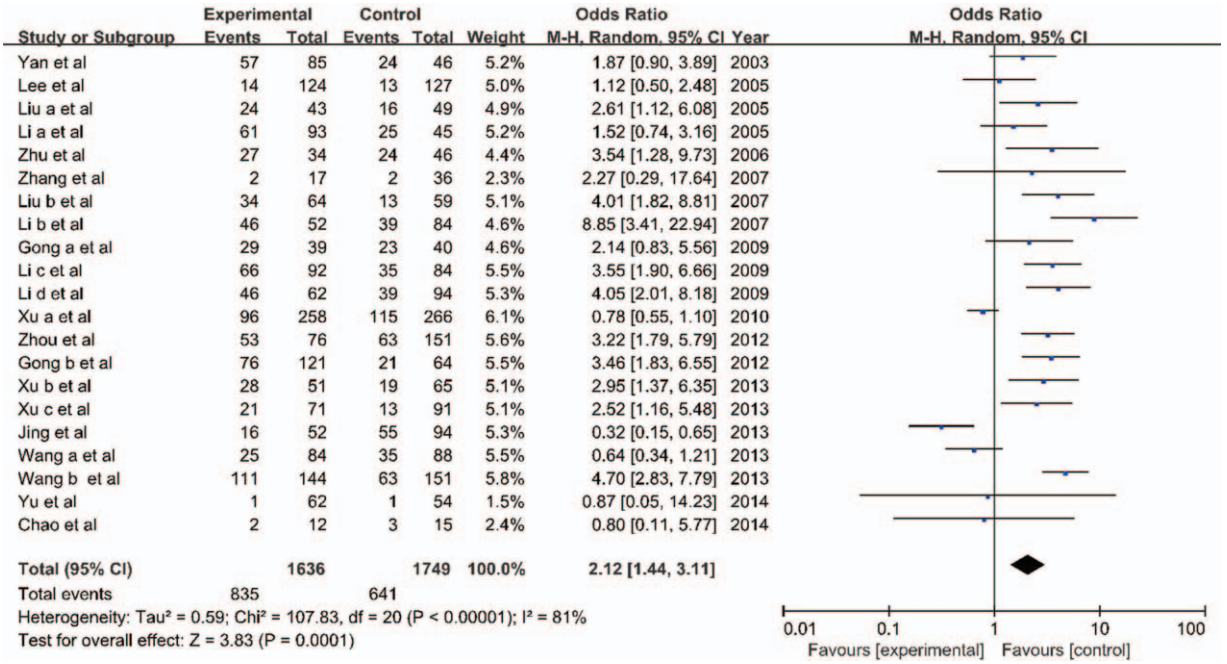

Figure 4. Forest plot on the association between the C677T polymorphism and the risk for CHD in the Chinese fetal population (TT vs CC).

Table 3

Pooled ORs and 95% CIs of the association between fetal MTHFR C677T polymorphism and CHD following by subgroup.

Contrasts	No. of studies	Total case/control	Model	T vs C				TT vs CC				TT + TC vs CC				TT vs TC + CC			
				OR	95% CI	I^2	P_H	OR	95% CI	I^2	P_H	OR	95% CI	I^2	P_H	OR	95% CI	I^2	P_H
Source of controls																			
HB	16	2516/2898	Random-effects	1.21	0.96–1.54	88%	<.01	2.02	1.26–3.25	86%	<.01	1.39	1.01–1.90	82%	<.01	1.74	1.25–2.42	81%	<.01
PB	5	501/499	Fixed-effects	1.48	1.25–1.75	27%	.25	2.39	1.62–3.53	0%	.67	1.74	1.31–2.32	3%	.39	1.84	1.34–2.54	0%	.59
Sample size																			
Large	17	2821/3157	Random-effects	1.33	1.07–1.67	88%	<.01	2.20	1.45–3.35	85%	<.01	1.50	1.12–2.01	82%	<.01	1.82	1.36–2.44	80%	<.01
Small	4	867/758	Fixed-effects	1.20	0.87–1.66	0%	.21	1.73	0.82–3.68	0%	.58	1.18	0.74–1.89	0%	.38	1.46	0.98–2.18	0%	.96
Method of screening																			
PCR-RFLP	18	2400/2878	Random-effects	1.32	1.05–1.66	86%	<.01	2.30	1.51–3.51	81%	<.01	1.49	1.11–2.01	79%	<.01	1.89	1.41–2.54	76%	<.01
Others	3	617/519	Random-effects	1.19	0.74–1.91	86%	<.01	1.36	0.48–3.85	86%	<.01	1.21	0.66–2.22	82%	<.01	1.28	0.61–2.69	77%	<.01
Quality																			
Low	5	820/724	Random-effects	1.33	0.96–1.85	78%	<.01	1.72	0.86–3.42	77%	<.01	1.49	0.93–2.40	77%	<.01	1.38	0.90–2.11	55%	.06
High	16	2197/2673	Random-effects	1.29	1.00–1.65	87%	<.01	2.27	1.42–3.63	83%	<.01	1.42	1.03–1.97	80%	<.01	1.95	1.40–2.70	78%	<.01

CI = confidence interval, HB = hospital-based, OR = odds ratio, PB = population-based, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism.

recessive model (OR 1.84, 95% CI: 1.34–2.54; $P = .59$) in population-based control subgroup. However, no significant association was found in hospital-based control subgroups in all genetic models. Results of meta-analysis shown in Table 3 revealed a significant association between MTHFR C677T polymorphism and the risk of CHD when all the studies were pooled with fixed-effects models in small sample size subgroup rather than in large sample size subgroup (Table 3). Performance of the stratified analysis based on the method of screening, no significant association was found whether MTHFR C677T polymorphism was detected using the PCR-RFLP method or by other methods. Similarly, no significant association was found in the stratification analyses according to NOS score (low quality and high quality). The results of meta-analysis are given in Table 3.

Begg funnel plot and Egger test revealed that points are evenly distributed and symmetrical, and most of the points are within the 95% CI and the shape of funnel plots showed no obvious asymmetry suggesting that there is no publication bias, and emphasized that the result of the study is credible and dependable. Absence of statistical significance based on Egger test about the value of the funnel plot ($P = .340$) suggested absence of publication bias in this model (Fig. 5). In other genetic models, also no publication biases were seen and *P* values were showed in Table 2.

Deletion of 1 single study from the overall pooled analysis each time to check the influence of the removed data set to the overall ORs to assess the sensitivity analysis did not alter or impact the overall ORs (Fig. 6).

4. Discussion

Based on the studies published before April, 2011, Yin and coworkers inferred that MTHFR C667T gene may enhance the risk of CHD.[35] In contrast, analysis of 7698 cases and 13,159 controls published till 2010 led Mamasoula and coworkers to conclude that MTHFR C667T gene polymorphism is not associated with increased risk for CHD.[1] Several other meta-analyses studies that evaluated association between MTHFR polymorphism and CHD [9,11,36] also gave inconclusive results. These controversial results could be attributed to small sample size used in these studies. In the present meta-analysis, we combined several studies that reported similar kinds of studies to increase the sample size to 6414 and statistical power and evaluated the association between MTHFR C677T and susceptibility of CHD in the paediatric population. Based on this evaluation, we noticed that C677T polymorphism in the MTHFR gene is significantly associated with increased susceptibility to CHD in Han Chinese population. This implies that decreased children's MTHFR enzyme activity may result in an

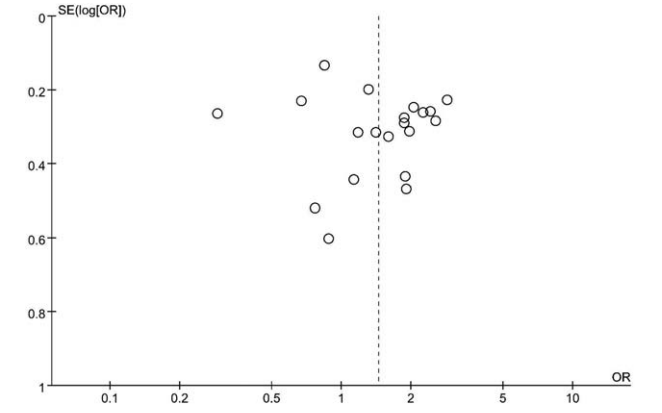

Figure 5. Begg funnel plot for publication bias in studies on *MTHFR* C677T polymorphism and CHD (TT+CT vs CC). Each point represents a separate study for the indicated association. Log or represents natural logarithm of OR. Vertical line represents the mean effects size.

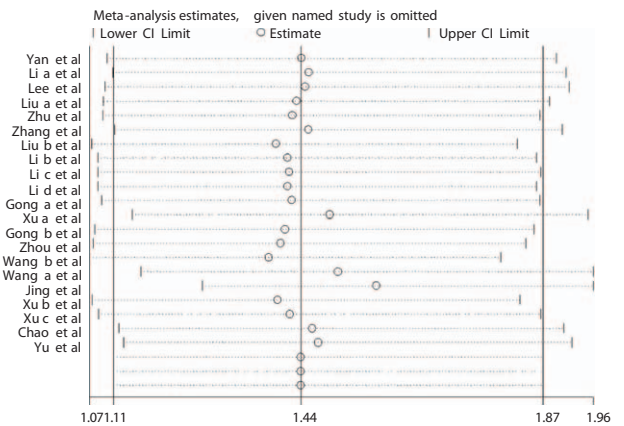

Figure 6. Potential outliers (i.e., data points that are far outside the norm) were identified by a sensitivity analysis.

increase in local hyperhomocystein environment resulting in an increase in the risk of CHD.[37]

It is known that genetic and environmental risk factors may have a complex interaction among them. Previous studies reported that MTHFR gene is a significant risk factor in the susceptibility to develop a variety of diseases including CHD.[38] In the MTHFR enzyme, C to T substitution at position 677 (C677T) results in the substitution of alanine to valine that results in impaired folate binding and reduced activity of the MTHFR enzyme. It is likely that C677T mutation of MTHFR renders the enzyme thermolabile with ~50% reduced activity that leads to an increase in plasma homocysteine concentrations.[39] Hence, variants of the MTHFR gene can alter the activity of MTHFR, which may lead to an increase in the susceptibility to develop CHD. Even though the heterogeneity between MTHFR C667T polymorphism and CHDs was significant in hospital-based control group, this was not significantly different from the population-based control ($I^2=0$). This suggests that hospital-based and population-based controls were not homogenous. Additional analysis of the subgroup analysis showed a significant association between CHD and C677T in all the genetic models in both small and large sample studies. Interestingly, we found that heterogeneity was significant in large sample subgroup, while no heterogeneity was detected in small sample subgroup. This led us to conclude that the number of published studies was not sufficiently large for a comprehensive analysis, and some included studies of small size might not have had enough statistical power to explore the association between the C677T polymorphism and susceptibility to CHD.

Although we made these findings in this meta-analysis, there were several limitations. Our study was mainly based on unadjusted odd ratios, and the potential covariates including gender, age, vitamin supplement, or other environmental factors, which might influence the final results, were unable to control. Second, significant heterogeneity in the study was presented in overall and subgroup analysis. We have also investigated the study heterogeneity including method of screening for CHD, source of controls, and sample size between studies by meta-regression (data not shown); however, none of them was identified as the potential source of heterogeneity. We estimated that other unknown confounding factors may help explain the between-study heterogeneity. We estimated that other unknown confounding factors may help explain the between-study heterogeneity. Third, it was known that there were several subtypes of congenital heart diseases. In addition, only a few studies included in our meta-analysis have classified their cases by types of CHD. To analyze this issue, we need more studies involving CHD cases with clear subtypes.

5. Conclusions

Based on our meta-analysis of possible association between MTHFR C677T genetic variant and the risk of CHD presented here suggests that MTHFR C677T genetic variant is significantly associated with increased risk of CHD in the paediatric population. Hence, we propose that children's MTHFR C677T polymorphism could be used as a molecular biomarker for evaluating the susceptibility to CHD.

References

[1] Mamasoula C, Prentice RR, Pierscionek T, et al. Association between C677T polymorphism of methylene tetrahydrofolate reductase and congenital heart disease: meta-analysis of 7697 cases and 13,125 controls. Circ Cardiovasc Genet 2013;6:347–53.
[2] Tikkanen J, Heinonen OP. Risk factors for ventricular septal defect in Finland. Public Health 1991;105:99–112.
[3] Bruneau BG. The developmental genetics of congenital heart disease. Nature 2008;451:943–8.
[4] Zidan HE, Rezk NA, Mohammed D. MTHFR C677T and A1298C gene polymorphisms and their relation to homocysteine level in Egyptian children with congenital heart diseases. Gene 2013;529:119–24.
[5] Zhang Q, Zha D, Dong P, et al. Association analysis between MTHFR genetic polymorphisms and the risk of congenital heart diseases in Chinese Han population. J Pharm Pharmacol 2014;66:1259–64.
[6] Locke AE, Dooley KJ, Tinker SW, et al. Variation in folate pathway genes contributes to risk of congenital heart defects among individuals with Down syndrome. Genet Epidemiol 2010;34:613–23.
[7] Hoffman JI, Kaplan S. The incidence of congenital heart disease. J Am Coll Cardiol 2002;39:1890–900.
[8] Ueland PM, Hustad S, Schneede J, et al. Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmacol Sci 2001;22:195–201.
[9] Verkleij-Hagoort A, Bliek J, Sayed-Tabatabaei F, et al. Hyperhomocysteinemia and MTHFR polymorphisms in association with orofacial clefts and congenital heart defects: a meta-analysis. Am J Med Genet A 2007;143A:952–60.
[10] Lamers Y, Prinz-Langenohl R, Moser R, et al. Supplementation with [6S]-5-methyltetrahydro-folate or folic acid equally reduces plasma total homocysteine concentrations in healthy women. Am J Clin Nutr 2004;79:473–8.
[11] Nie Y, Gu H, Gong J, et al. Methylenetetrahydrofolate reductase C677T polymorphism and congenital heart disease: a meta-analysis. Clin Chem Lab Med 2011;49:2101–8.
[12] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010;25:603–5.
[13] Woolf B. On estimating the relation between blood group and disease. Ann Hum Genet 1955;19:251–3.
[14] Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–60.
[15] Xu R, Zhang L, Ma L, et al. The association study on MTHFR gene polymorphism C677 and simple congenital heart disease in Uyghur and Han population in Xinjiang. J Xinjiang Med Univ 2013;36:748–51.
[16] Yan L, Li Y. Association between MTHFR C677T polymorphism and congenital heart disease. J Peking Univ 2003;35:448–1448.
[17] Li Y, Cheng J, Zhu WL, et al. Study of serum Hcy and polymorphisms of Hcy metabolic enzymes in 192 families affected by congenital heart diseas. J Peking Univ 2005;37:75–80.
[18] Liu F, Bai P, Chen SB, et al. Association between 5, 10-methylenetetrahydrofolate reduetase C677T Polymorphisms and conotruncal heart defects in Chinese children. Chin J Contemp Pediatr 2005;7:99–102.
[19] Lee CN, Su YN, Cheng WF, et al. Association of the C677T methylenetetrahydrofolate reductase mutation with congenital heart diseases. Acta Obstet Gynecol Scand 2005;84:1134–40.
[20] Zhu WL, Li Y, Yan L, et al. Maternal and offspring MTHFR gene C677T polymorphism as predictors of congenital atrial septal defect and patent ductus arteriosus. Mol Hum Reprod 2006;12:51–4.
[21] Li Z, Ren A, Zhang L, et al. Periconceptional use of folic acid in Shanxi Province of northern China. Public Health Nutr 2007;10:471–6.
[22] Zhang J. Study on the relations between genetic polymorphisms of folic metabolic enzymes and the risk of congenital heart defect. Central South University 2007.
[23] Liu YS, Yin XG. Relation ship between genetic polymorphism of homocysteinemetabolism enzyme and congenital heart disease. Chin J Cardio Rev 2007;15:210–3.
[24] Li D, Jing X, Wang H, et al. Correlations between MTHFR gene polymorphism, exposure to chemicals during pregnancy andcongenita l heart disease. Chin J Public Health 2009;25:1081–3.
[25] Li D, Jing X, Wang H, et al. Study of correlationship between congenital heart disease and 5,10-methylenetetra hydrofolate reductase gene polymorphism or folacin intakes. Chin J Prev Med 2009;43:3117–21.
[26] Gong T, Li F, Shi J. Relationship between risk factors during pregnancy, maternal MTHFR gene 677C-T, plasma Hcy levels and congenital heart disease in children. Matern Child Health Care China 2009;24:2117–21.
[27] Xu J, Xu X, Xue L, et al. MTHFR c.1793G>A polymorphism is associated with congenital cardiac disease in a Chinese population. Cardiol Young 2010;20:318–26.
[28] Gong D, Gu H, Zhang Y, et al. Methylenetetrahydrofolate reductase C677T and reduced folate carrier 80 G>A polymorphisms are associated with an increased risk of conotruncal heart defects. Clin Chem Lab Med 2012;50:1455–61.

[29] Zhou SY, Zhou JP, Wang LN. Study on the association between MTHFR gene polymorphism and tetralogy of fallot. Shandong Med J 2012; 52:1–3.
[30] Wang B, Liu M, Yan W, et al. Association of SNPs in genes involved in folate metabolism with the risk of congenital heart disease. J Matern Fetal Neonatal Med 2013;26:1768–77.
[31] Jing XA, Wang HY, Li D, et al. Associations of MTHFR gene polymorphism and environmental factors with congenital heart disease. Chin J Public Health 2013;3:347–9.
[32] Wang LN. Relationship between 5,10-methylenetetrahydrofolate gene polymorphism and congenital heart disease in nuclear family. Chin J Appl Clin Pediatr 2013;1:32–5.
[33] Chao CS, Wei J, Huang HW, et al. Correlation between methyltetrahydrofolate reductase (MTHFR) polymorphisms and isolated patent ductus arteriosus in Taiwan. Heart Lung Circ 2014;23:655–60.
[34] Yu KK. Research of the relationship between the genes in floate metabolic pathway and congenital heart disease. Taishan Medical University 2014.
[35] Yin M, Dong L, Zheng J, et al. Meta analysis of the association between MTHFR C677T polymorphism and the risk of congenital heart defects. Ann Hum Genet 2012;76:9–16.
[36] Van Gorp V, Verfaillie G, Verborgh C, et al. Frey's syndrome after elective thyroidectomy: a case report. Acta Chir Belg 2008;108:613–5.
[37] Lu Y, Wang H, Wang X. Relationship of hyperhomocysteinemia in pregnant rats and congenital heart defects in the newborn rats. J Central South Univ 2011;36:68–73.
[38] Long S, Yang X, Liu X, et al. Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and susceptibility for cervical lesions: a meta-analysis. PLoS One 2012;7:e52381.
[39] Huhta JC, Hernandez-Robles JA. Homocysteine, folate, and congenital heart defects. Fetal Pediatr Pathol 2005;24:71–9.