Genetic polymorphism of methylenetetrahydrofolate reductase as a potential risk factor for congenital heart disease
A meta-analysis in Chinese pediatric population
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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 performed 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\textsuperscript{[1]}.

CHD accounts for about one-third of all congenital anomalies seen in newborn that is responsible for infant mortality\textsuperscript{[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\textsuperscript{[3]}.

Recent studies revealed that methylene tetrahydrofolate reductase (MTHFR) gene may play an important role in increasing the risk of developing CHD\textsuperscript{[4,5]}. Single nucleotide polymorphisms of genes involved in the folate pathway can affect folate acid metabolism and thus, increases the risk of CHD\textsuperscript{[6]}. Folic acid may protect against development of CHD since its deficiency could be an important risk factor for CHD\textsuperscript{[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\textsuperscript{[8]}. Hyperhomocysteinemia is a significant risk factor for the development of heart defects\textsuperscript{[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\textsuperscript{[10]}. Based on this assumption, it has been proposed that polymorphisms of MTHFR in children population may predispose to the development of CHD\textsuperscript{[11]}. Previous reports suggested that children’s MTHFR genetic variants, such as C677T, can influence the development of CHD\textsuperscript{[12]}. However, these studies gave inconsistent results. Hence, the present study was performed to clarify possible relationship between C677T
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 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 on 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 subgroup, each subgroup 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; 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).\(^{1,2}\) 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 \(I^2\)-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 homogeneous. 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
its standard error. The severity of publication bias was estimated using Egger 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. Thus, a total of 20 eligible studies for MTHFR C677T polymorphism and the risk of CHD for each study signiﬁcant associations were observed in all genetic models: the dominant model (TT + CT versus CC: OR = 1.44, 95% CI: 1.11–1.87; P < .00001, Table 2), and the recessive model (TT versus 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

| Cases study | Controls year | Source of CC | CT | TT | CC | CT | TT | HWE | Methods | Main types of CHD | Quality score controls |
|-------------|---------------|--------------|----|----|----|----|----|-----|--------|------------------|-----------------------|
| Yuan [14]   | 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                     |
| Li [19]     | 2005          | HB           | 19 | 54 | 24 | 33 | 69 | 16  | 0.234  | PCR-RFLP          | Congenital heart defects | 6                 |
| Lee [15]    | 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          | CHD                   | 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                     |
| Xu [24]     | 2009          | HB           | 59 | 76 | 25 | 53 | 100| 35  | 0.377  | PCR-RFLP          | CHD                   | 6                     |
| Zhou [25]   | 2012          | HB           | 46 | 42 | 46 | 55 | 114| 39  | 0.164  | PCR-RFLP          | CHD                   | 6                     |
| Wang [26]   | 2012          | HB           | 45 | 123| 76 | 43 | 72 | 21  | 0.183  | PCR-RFLP          | CHD                   | 4                     |
| Zhang [27]  | 2012          | HB           | 23 | 59 | 25 | 53 | 100| 35  | 0.377  | PCR-RFLP          | CHD                   | 7                     |
| Jing [28]   | 2013          | HB           | 46 | 42 | 16 | 39 | 114| 39  | 0.164  | PCR-RFLP          | CHD                   | 6                     |
| Wang [29]   | 2013          | HB           | 33 | 92 | 111| 88 | 126| 63  | 0.183  | PCR-RFLP          | CHD                   | 6                     |
| Xu [30]     | 2013          | PB           | 16 | 42 | 46 | 55 | 114| 39  | 0.164  | PCR-RFLP          | CHD                   | 6                     |
| Xu [31]     | 2013          | HB           | 10 | 41 | 29 | 177| 40 | 23  | 0.900  | PCR-RFLP          | CHD                   | 4                     |
| Xu [32]     | 2010          | HB           | 162| 244| 96 | 151| 261| 115  | 0.900  | PCR-RFLP          | CHD                   | 6                     |
| Gong [33]   | 2011          | PB           | 16 | 42 | 46 | 55 | 114| 39  | 0.164  | PCR-RFLP          | CHD                   | 6                     |
| Zhu [34]    | 2013          | PB           | 15 | 13 | 2  | 34 | 28 | 2   | 1.71   | PCR-RFLP          | CHD                   | 6                     |
| Liu [35]    | 2007          | HB           | 30 | 68 | 34 | 46 | 48 | 13  | 0.829  | PCR-RFLP          | CHD                   | 7                     |
| Xu [36]     | 2013          | PB           | 59 | 76 | 25 | 53 | 100| 35  | 0.377  | PCR-RFLP          | CHD                   | 6                     |
| Chao [37]   | 2014          | HB           | 10 | 5  | 2  | 19 | 12 | 3   | 0.660  | PCR-RFLP          | CHD                   | 8                     |
| Yu [38]     | 2014          | HB           | 61 | 7  | 1  | 53 | 8  | 1   | 0.631  | PCR-RFLP          | CHD                   | 7                     |

AP Window = atrial pulmonary window, ASD = atrial septal defect, 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.
References retrieved by search (n=378) identified and screened through PubMed, Embase, Cochrane Library, CNKI, Wanfang and VIP databases

Excluded based on their titles and abstracts (n=322): not case-control trial or irrelevant studies or no desire polymorphism

Potentially relevant studies identified after the first selection (n=56)

Further not eligible studies excluded according to full-text studies:
- Studies with duplicated samples (n=10)
- Studies focusing on MTHFR gene other polymorphisms (n=10)
- Studies with data insufficiency (n=6)
- Studies no satisfying Hardy–Weinberg equilibrium (n=2)

Eligible articles included in the meta-analysis (n=20)

Study included: two different studies in one paper, we considered these studies separated (n=1)

Qualified studies based on full text in the meta-analysis (n=21)

Figure 1. Flowchart of study selection.

| Study or Subgroup | Experimental Events | Control Events | Total | Weight | Odds Ratio M-H. | Random. 95% CI | Year |
|-------------------|---------------------|---------------|-------|--------|----------------|----------------|------|
| Yan et al         | 146                 | 174           | 320   | 81     | 103            | 4.8%           | 2003 |
| Li et al          | 155                 | 182           | 337   | 102    | 102            | 4.8%           | 2005 |
| Lee et al         | 103                 | 213           | 316   | 81     | 195            | 5.7%           | 2005 |
| Liu et al         | 78                  | 97            | 175   | 85     | 118            | 4.7%           | 2005 |
| Zhu et al         | 49                  | 56            | 105   | 81     | 103            | 3.6%           | 2006 |
| Zhang et al       | 15                  | 30            | 45    | 30     | 64             | 3.8%           | 2007 |
| Liu et al         | 102                 | 132           | 234   | 61     | 107            | 5.0%           | 2007 |
| Li et al          | 88                  | 104           | 192   | 153    | 208            | 4.8%           | 2007 |
| Gong et al        | 70                  | 80            | 150   | 63     | 80             | 3.9%           | 2009 |
| Li et al          | 68                  | 104           | 172   | 153    | 208            | 4.8%           | 2009 |
| Li et al          | 118                 | 144           | 262   | 119    | 168            | 5.1%           | 2009 |
| Xu et al          | 340                 | 502           | 842   | 376    | 527            | 6.1%           | 2010 |
| Zhou et al        | 113                 | 136           | 249   | 131    | 179            | 5.2%           | 2012 |
| Gong et al        | 199                 | 244           | 443   | 93     | 136            | 5.5%           | 2012 |
| Wang et al        | 203                 | 236           | 439   | 189    | 277            | 5.5%           | 2013 |
| Xu et al          | 74                  | 105           | 179   | 59     | 105            | 5.0%           | 2013 |
| Xu et al          | 73                  | 123           | 196   | 47     | 125            | 5.2%           | 2013 |
| Jing et al        | 58                  | 104           | 162   | 69     | 169            | 5.2%           | 2013 |
| Wang et al        | 101                 | 160           | 261   | 135    | 188            | 5.5%           | 2013 |
| Yu et al          | 8                   | 69             | 77    | 6      | 214            | 3.3%           | 2014 |
| Chao et al        | 7                   | 17             | 24    | 15     | 34             | 2.8%           | 2014 |

Total (95% CI) | 3817 | 3387 | 100.0% | 1.44 [1.11, 1.87] |

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 | OR 95% CI | Test of association | Test of publication bias |
|----------------|-------------|-----------------------|-----------|---------------------|-------------------------|
| T vs C         | Random-effects | 117.16 < .00001 85%  | 1.30 1.06–1.59 2.56 .010 | –0.84 .709             |
| TT vs TC       | 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 < .0001 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).
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 C677T gene may enhance the risk of CHD.\(^\text{11}\) 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.\(^\text{14}\) Several other meta-analyses studies that evaluated association between MTHFR polymorphism and CHD\(^\text{9,11,36}\) 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

Table 3

| Contrasts | No. of studies | Total case/control | Model | OR | 95% CI | H | OR | 95% CI | H | OR | 95% CI | H |
|-----------|---------------|--------------------|-------|----|--------|----|----|--------|----|----|--------|----|
| T vs C    |               |                    |       |    |        |    |    |        |    |    |        |    |
| T vs TC   |               |                    |       |    |        |    |    |        |    |    |        |    |
| T vs TC+CC|               |                    |       |    |        |    |    |        |    |    |        |    |
| Source of controls |       |                    |       |    |        |    |    |        |    |    |        |    |
| HB        | 16            | 2516/2686          | Random-effects | 1.21 | 0.96–1.54 | 88%  | <.01 | 2.02 | 1.07–3.97 | 86%  | <.01 | 1.39 | 1.01–1.90 | 82%  | <.01 | 1.74 | 1.25–2.42 | 81%  | <.01 |
| PB        | 5             | 601/459            | Fixed-effects | 1.48 | 1.25–1.75 | 27%  | <.01 | 2.53 | 1.62–3.53 | 0%   | <.01 | 1.74 | 1.31–2.25 | 3%   | <.01 | 1.84 | 1.34–2.54 | 0%   | <.01 |
| Sample size |             |                    |       |    |        |    |    |        |    |    |        |    |
| Large     | 17            | 2821/3157          | Random-effects | 1.33 | 1.07–1.67 | 88%  | <.01 | 2.20 | 1.45–3.35 | 85%  | <.01 | 1.49 | 1.12–2.01 | 82%  | <.01 | 1.82 | 1.36–2.44 | 80%  | <.01 |
| Small     | 4             | 867/736            | Fixed-effects | 1.20 | 0.87–1.66 | 0%   | <.01 | 2.13 | 0.82–3.38 | 0%   | <.01 | 1.73 | 0.74–3.99 | 0%   | <.01 | 1.46 | 0.98–2.18 | 0%   | <.01 |
| Method of screening |       |                    |       |    |        |    |    |        |    |    |        |    |
| PCR-RFLP  | 18            | 2400/2678          | 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 | 0.84–2.35 | 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 | 1.30 | 0.90–2.11 | 55%  | <.01 |
| High      | 16            | 2197/10273         | Random-effects | 1.29 | 1.00–1.65 | 87%  | <.01 | 2.27 | 1.42–3.63 | 83%  | <.01 | 1.42 | 1.00–1.97 | 86%  | <.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.

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.\textsuperscript{[17]}

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.\textsuperscript{[18]}

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.\textsuperscript{[19]} 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 C677T 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 odds 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. First, 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.

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