The Roles of Reduced Folate Carrier-1 (RFC1) A80G (rs1051266) Polymorphism in Congenital Heart Disease: A Meta-Analysis

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Source of support:
Health Industry Scientific Research Project of Gansu Province (GSWSKY2016-04)

Background: We performed the present study to better elucidate the correlation of reduced folate carrier-1 (RFC1) A80G (rs1051266) polymorphism with the risk of congenital heart disease (CHD).

Material/Methods: According to the designed search strategy, a systematic literature search was performed through the PubMed, Cochrane Library, Web of Science, EMBASE, CNKI, VIP, and Wan Fang databases to collect published case-control studies on the correlation between RFC1 A80G polymorphism and CHD. All relevant studies up to October 1, 2019 were identified. The odds ratio (OR) and 95% confidence interval (CI) of the genotype distribution were used as the effect indicators.

Results: A total of 6 eligible studies was finally included in our meta-analysis, including 724 children with CHD, 760 healthy children, 258 mothers of the children with CHD, and 334 mothers of healthy control children. The meta-analysis revealed that for fetal analysis, only in the heterozygous model (GA vs GG, OR=1.36, 95% CI [1.06, 1.75], P=0.02) was RFC1 A80G polymorphism associated with risk of CHD. In maternal analysis, 3 genetic models of RFC1 A80G polymorphism increased the risk of CHD: the allelic model (A vs G, OR=1.36, 95% CI [1.07, 1.71], P=0.01), the homozygote model (AA vs GG, OR=2.99, 95%CI [1.06, 8.41], P=0.04), and the dominance model (GA+AA vs GG, OR=1.53, 95%CI [1.08, 2.16], P=0.02).

Conclusions: The maternal RFC1 A80G polymorphism has a strong correlation with CHD. Compared with the G allele, the A allele increases the risk of CHD by 0.36-fold.

Keywords: Heart Defects, Congenital • Meta-Analysis • Polymorphism, Single Nucleotide • Reduced Folate Carrier Protein • Review

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/929911
Background

Congenital heart disease (CHD) is a congenital malformation caused by abnormal embryonic development of heart blood vessels affecting nearly 10 to 12 per 1000 liveborn infants (1-1.2%) [1]. According to the World Health Organization, CHD accounts for 42% of infant deaths and has become the main cause of infant mortality [2]. There are many forms of CHD, and their severity varies widely. For example, atrial septal defect may be asymptomatic, whereas purpuric heart disease requires urgent surgery [3]. Advances in surgical and peripерative care, as well as catheter-based interventions, have greatly improved survival. However, for the most complex heart defects, the mortality rate is still as high as 20% [4]. Epidemiological studies show that genetic or environmental causes can be identified in 20% to 30% of CHD cases [5]; the unexplained remainder is presumed to be multifactorial (oligogenetic or some combination of genetic and environmental factors) [6].

CHD is considered a folic acid-sensitive birth defect because women who take folic acid-containing multivitamins early in pregnancy have a 30-40% lower risk of having offspring with these heart defects [7,8]. Folic acid is an essential B vitamin that the human body cannot synthesize; it can only be obtained from the diet. Studies have shown that folic acid plays an important role in embryonic development, including the development of the cardiovascular system [9]. If folic acid is metabolically disordered, it will cause the methionine cycle to be blocked. On the one hand, it affects the methylation reaction in the body, which in turn affects the metabolic growth of cells. On the other hand, it causes the metabolic disorder of homocysteine (Hcy) in the blood, which leads to an increase in Hcy levels [10]. Elevated Hcy is an independent risk factor for cardiovascular disease, which can damage or interfere with early cardiovascular growth and development [11]. If the metabolism of folate is affected, deoxyribonucleic acid synthesis and repair will be impaired, and the development of the neural crest in the embryo will be abnormal, which will eventually lead to the occurrence of CHD [12]. The reduced folate carrier (RFC) cooperates with the folate receptor in the process of folate absorption to complete the transport of folic acid from tissue to cell [13]. Moreover, reduced folic acid carrier-1 (RFC1) is considered an organic anion exchanger that can absorb folic acid and transports 5-methyltetrahydrofolate and thiamine monophosphate bidirectionally [14,15]. During the critical period of fetal development, RFC1 deficiency can reduce its affinity with folic acid, thus reducing the amount of folic acid transported into the cell. The folate deficiency of the developing embryo has a potential impact on the occurrence of CHD [16].

The RFC1 (SLC19A1) gene is located on chromosome 21q22.3, which encodes a typical transporter with 12 transmembrane domains involved in the active transport of 5-methyltetrahydrofolate from plasma to the cytosol and regulation of intracellular folate concentration [17]. RFC1 has not been directly related to the increase of total homocysteine (tHcy), but it may limit the absorption of folic acid by the developing fetus, thus affecting the growth of the fetus. A80G (rs1051266) is the most common single nucleotide polymorphism (SNP) in RFC1. It affects plasma folate and Hcy levels alone or together with the C677T polymorphism in the methylenetetrahydrofolate reductase gene [18]. Shaw et al. [19] described the highly frequent A80G SNP, which results in the change of amino acid from histidine (encoded by CAG) to arginine (encoded by CGG) in the second exon, altering its metabolic pathways, and affecting the absorption rate of folic acid into the cell. Epidemiological investigations have shown that adequate folic acid supplementation in early pregnancy can reduce the risk of fetal CHD [20]. Any effect of RFC1 genotype on the risk of CHD may be mediated by the early uterine environment, which is mainly determined by the mother’s RFC1 genotype [21]. Therefore, RFC1 as a folate carrier may be considered as a genetic biomarker of CHD [22].

To date, several studies have been conducted on RFC1 genetic polymorphisms, particularly the association between A80G polymorphism and CHD. Some of these studies only analyzed the relationship between fetal RFC1 gene polymorphisms and CHD. Part of the literature started with children with CHD and examined the relationship between maternal RFC1 gene polymorphisms and CHD. On the one hand, most analyses only focus on fetal research or maternal research, which introduces statistical bias, making the research results less comprehensive, and it cannot be ruled out that the maternal genotype can independently cause the risk of fetal disease. On the other hand, these studies are inconsistent and controversial because of regional differences or small sample sizes. To illustrate this relationship, we conducted this meta-analysis from both the fetal and maternal perspectives to integrate the results of case-control studies to analysis of the association between RFC1 A80G (rs1051266) gene polymorphism and CHD risk.

Material and Methods

The study was reported according to Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines.

Literature Search

A systematic literature study was conducted on 7 databases including PubMed, the Cochrane Library, Web of Science, EMBASE, China National Knowledge Infrastructure, Wan Fang, and VIP to retrieve all relevant articles before October 1, 2019. The complete detailed search strategy in Web of Science is listed in Supplementary Table 1. We expanded the search
Inclusion and Exclusion Criteria

The inclusion criteria for this study were determined before the literature search. The included studies needed to meet the following criteria: (1) association studies between RFC1 A80G (rs1051266) polymorphisms and CHD; (2) case-control studies; (3) detailed genotype data can be obtained by calculated odds ratios (OR) and 95% confidence intervals (CIs); (4) distribution of genotypes in the control group is consistent with Hardy-Weinberg equilibrium (HWE).

The exclusion criteria were as follows: (1) reviews, comments, letters, expert opinions, case reports, and family-based association studies; (2) repetition of previous publications; (3) animal-based studies or cell line research; (4) CHD patients with other diseases.

Data Extraction and Risk of Bias

The following data were independently extracted according to inclusion and exclusion criteria: first author's last name, publication year, country and region of study, genotyping method, type of CHD, source of control population, case and control sample size, genotype frequencies of RFC1 gene polymorphisms in case and control, and results of the HWE test.

The risk of bias in the included literature was referenced to the Newcastle-Ottawa scale scoring standard. The scoring system evaluated the included studies from 3 aspects: (1) the selectivity of the case and the control group; (2) the comparability of the case and the control group; (3) the exposure of the risk factors [23]. The scale is 0-9, and when the score is ≥7, it is considered to be a study with low risk of bias [24].

The screening of documents, the extraction of data, and the risk of bias evaluation work are completed independently by the 2 individuals. When there is a disagreement, they will discuss the solution together or negotiate with a third person until an agreement is reached.

Statistical Analysis

All data analysis was performed using RevMan5.3 software. The HWE was evaluated for each study by a chi-square test in the control group, and \( P < 0.05 \) was considered a significant departure from HWE. The OR and 95% CIs in the fetal and maternal groups were calculated among 5 genetic models including allele model (A vs G), heterozygous model (GA vs GG), homozygous model (AA vs GG), dominant model (GA+AA vs GG), and recessive model (AA vs GA+GG). In addition, a subgroup analysis based on the source region of the sample was used to further investigate the correlation between the two. A heterogeneity test was performed on the included studies using the Q test and the \( I^2 \) test. The fixed-effect model was used for analysis only when \( P > 0.10 \) and \( I^2 \leq 50\% \). Otherwise, the heterogeneity of the study was considered significant and the random-effects model was used for analysis. A sensitivity analysis was performed to detect the heterogeneity by omitting 1 study in each turn. Publication bias was assessed by funnel plots and Egger’s test.
Results

Characteristics of Included Studies

The literature search identified 188 citations, 153 remaining after removing duplicates. By reading the title and abstract, 145 irrelevant documents were eliminated; we read the full text of the remaining 8 articles. Among them, the data of Pei et al [25] were duplicated, and Christensen et al [26] could not submit the data. As a result, a total of 6 studies [18,19,22,28,29] that met the inclusion criteria was finally included in our meta-analysis (Figure 1). After pooling the data, our meta-analysis contained 724 fetal cases, 760 fetal controls, 258 maternal cases, and 334 maternal controls. All the data in these studies related to an association between the RFC1 A80G polymorphism and CHD. The characteristics of all the included articles are summarized in Table 1. The genotype characteristics of included studies are represented in Table 2. Table 3 shows the risk of bias results for the 6 included studies.

Table 1. Characteristics of included studies.

| First author | Year | Country | Region | Genotyping method | Case type | Controls source | PHWE |
|--------------|------|---------|--------|-------------------|-----------|-----------------|------|
| Fetal group  |      |         |        |                   |           |                 |      |
| Wang BJ [27] | 2013 | China   | East Asian | SNaPshot multiple PCR | CHD        | HB              | 0.142 |
| Shaw GM [19] | 2003 | USA     | North America | PCR-RFLP | CHD        | PB              | 0.0085 |
| Gong DX [28] | 2012 | China   | East Asian | MALDI-ToF-MS | TOF, TGA  | HB              | 0.189 |
| Pei LJ [29]  | 2006 | China   | East Asian | PCR-RFLP | CHD        | PB              | 0.9   |
| Koshy T [30] | 2015 | India   | South Asian | ABI 3730 automated sequencer | CTD | PB | 0.00036 |
| Maternal group |     |         |        |                   |           |                 |      |
| Wang XK [18] | 2018 | China   | East Asian | Taqman SNP Genotyping Assay | CTD | HB | 0.0000584 |
| Pei LJ [29]  | 2006 | China   | East Asian | PCR-RFLP | CHD        | PB              | 0.601 |

CHD – congenital heart disease; HWE – Hardy Weinberg equilibrium; NA – not available; TGA – transposition of the great arteries; TOF – tetralogy of fallot; PB – population-based; HB – hospital-based; CTD – conotruncal heart defects.

Table 2. Genotype characteristics of included studies.

| First author | Cases | Controls | Allele frequencies cases | Allele frequencies controls |
|--------------|-------|----------|--------------------------|-----------------------------|
|              | Total | GG | GA | AA | Total | GG | GA | AA | G | A | G | A |
| Fetal group  |       |    |   |    |       |    |   |    |    |   |   |   |
| Wang BJ [27] | 160  | 31 | 87 | 42 | 188  | 33 | 103 | 52 | 0.466 | 0.534 | 0.449 | 0.551 |
| Shaw GM [19] | 163  | 47 | 90 | 26 | 239  | 75 | 99  | 65 | 0.564 | 0.436 | 0.521 | 0.479 |
| Gong DX [28] | 238  | 56 | 129 | 53 | 134  | 43 | 59  | 32 | 0.506 | 0.494 | 0.541 | 0.459 |
| Pei LJ [29]  | 67   | 13 | 42 | 12 | 99   | 27 | 50  | 22 | 0.507 | 0.493 | 0.525 | 0.475 |
| Koshy T [30] | 96   | 39 | 30 | 27 | 100  | 48 | 30  | 22 | 0.5625 | 0.4375 | 0.63 | 0.37 |
| Maternal group |     |    |   |    |       |    |   |    |    |   |   |   |
| Wang XK [18] | 193  | 68 | 69 | 56 | 234  | 102 | 82  | 50 | 0.531 | 0.469 | 0.611 | 0.389 |
| Pei LJ [29]  | 65   | 12 | 39 | 14 | 100  | 31 | 47  | 22 | 0.485 | 0.515 | 0.545 | 0.455 |

Results
For the fetal group, the aggregated data were from 5 studies, including a total of 724 cases and 760 controls. The included literature was not significantly heterogeneous, so we applied the Mantel-Haenszel fixed-effects model. The results of meta-analysis of the association between RFC1 A80G polymorphism and fetal CHD risks are summarized in Table 4.

The results showed that RFC1 A80G polymorphism was associated with the risk of CHD only under the heterozygous model (GA vs GG, OR=1.36, 95% CI [1.06, 1.75], P=0.02) (Figure 2). However, no significant correlation was found in other models.

Subgroup analysis was performed on the basis of ethnicity. No correlation was found between RFC1 A80G polymorphism and CHD under 5 models including the allele model, the heterozygous model, the homozygous model, the dominant model, and the invisibility model (Figure 3).

### Table 3. Results of Newcastle-Ottawa scale quality evaluation included in the study.

| Inclusion study | Study population selection | Group-to-group | Comparison of exposure factors | Total (minutes) |
|-----------------|----------------------------|----------------|--------------------------------|-----------------|
| Wang BJ [27]    | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲           | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ | 7               |
| Wang XK [18]    | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲           | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ | 8               |
| Shaw GM [19]    | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲           | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ | 8               |
| Gong DX [28]    | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲           | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ | 7               |
| Pei LJ [29]     | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲           | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ | 8               |
| Koshy T [30]    | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲           | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ | ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ | 8               |

1) The case definition is adequate with independent validation; 2) Consecutive or obviously representative series of cases; 3) Community controls; 4) Controls with no history of disease (endpoint); 5) Cases and controls with comparable ages and comparability on any other factors; 6) Ascertainment of exposure using secure records (eg surgical records) or structured interviews with blinding to case/control; 7) Ascertainment of exposure using the same method for cases and controls; 8) Ascertainment of exposure with non-response rate for both groups.

### Table 4. Meta-analysis of reduced folate carrier-1 (RFC1) A80G polymorphism and fetal congenital heart disease risk.

| Type             | OR (95%CI) | z   | P   | Test of heterogeneity | Analysis model |
|------------------|------------|-----|-----|------------------------|----------------|
| Overall (5)      |            |     |     |                        |                |
| GA VS GG         | 1.36 [1.06, 1.75] | 2.4 | 0.02 | 0                      | Fixed-effects model |
| AA VS GG         | 0.99 [0.74, 1.34] | 0.04 | 0.97 | 11                     | Fixed-effects model |
| GA+AA VS GG      | 1.24 [0.98, 1.57] | 1.79 | 0.07 | 0                      | Fixed-effects model |
| AA VS GG+GA      | 1.83 [0.65, 1.06] | 1.52 | 0.13 | 38                     | Fixed-effects model |
| A VS G           | 1.02 [0.88, 1.18] | 0.21 | 0.84 | 9                      | Fixed-effects model |
| Asian (4)        |            |     |     |                        |                |
| GA VS GG         | 1.33 [0.98, 1.79] | 1.84 | 0.07 | 5                      | Fixed-effects model |
| AA VS GG         | 1.16 [0.83, 1.64] | 0.87 | 0.39 | 0                      | Fixed-effects model |
| GA+AA VS GG      | 1.29 [0.97, 1.70] | 1.78 | 0.08 | 0                      | Fixed-effects model |
| AA VS GG+GA      | 0.97 [0.73, 1.29] | 0.19 | 0.85 | 0                      | Fixed-effects model |
| A VS G           | 1.09 [0.92, 1.30] | 0.98 | 0.32 | 0                      | Fixed-effects model |

**Overall and Subgroup Analyses for RFC1 A80G Polymorphisms in Fetal Analysis**

For the fetal group, the aggregated data were from 5 studies, including a total of 724 cases and 760 controls. The included literature was not significantly heterogeneous, so we applied the Mantel-Haenszel fixed-effects model. The results of meta-analysis of the association between RFC1 A80G polymorphism and fetal CHD risks are summarized in Table 4.

The results showed that RFC1 A80G polymorphism was associated with the risk of CHD only under the heterozygous model (GA vs GG, OR=1.36, 95% CI [1.06, 1.75], P=0.02) (Figure 2). However, no significant correlation was found in other models.

**Subgroup Analysis of RFC1 A80G in Maternal Analysis**

Since any effect of RFC1 genotype on CHD risk may be mediated by the early uterine environment, this is mainly determined by the mother’s RFC1 genotype. Therefore, by obtaining the genotype of RFC1 A80G of mothers of children with CHD, we explored the correlation between the mother’s RFC1 A80G polymorphism and the risk of CHD.
| Study or subgroup | Events | Total | Weight | Odds ratio M-H, Fixed, 95% CI | Odds ratio M-H, Fixed, 95% CI |
|------------------|--------|-------|--------|-----------------------------|-----------------------------|
| **1.1.1 A vs G** |        |       |        |                             |                             |
| Gong D X 2012    | 235    | 476   | 123    | 268                         | 22.6%                       |
| Koshy T 2015     | 84     | 192   | 74     | 200                         | 11.6%                       |
| Pei LJ 2006      | 66     | 134   | 94     | 198                         | 10.9%                       |
| Wang BJ 2013     | 142    | 326   | 229    | 478                         | 29.7%                       |
| **Subtotal (95% CI)** | 1448   |        | 1520   | 100.0%                      | 1.02 [0.88, 1.18]           |
| Total events     | 698    | 1520  | 727    |                             |                             |
| **Heterogeneity:** | Chi$^2$=4.39, df=4 (P=0.36); I$^2$=9% | Test for overall effect: Z=0.21 (P=0.84) | | | |

| **1.1.2 GA vs GG** |       |       |        |                             |                             |
| Gong D X 2012    | 129    | 185   | 59     | 102                         | 22.2%                       |
| Koshy T 2015     | 30     | 69    | 30     | 78                          | 15.3%                       |
| Pei LJ 2006      | 42     | 55    | 50     | 77                          | 9.5%                        |
| Wang BJ 2013     | 90     | 137   | 99     | 174                         | 28.8%                       |
| **Subtotal (95% CI)** | 564    |        | 567    | 100.0%                      | 1.36 [1.06, 1.75]           |
| Total events     | 378    | 567   | 341    |                             |                             |
| **Heterogeneity:** | Chi$^2$=3.27, df=4 (P=0.51); I$^2$=0% | Test for overall effect: Z=2.40 (P=0.02) | | | |

| **1.1.3 AA vs GG** |       |       |        |                             |                             |
| Gong D X 2012    | 53     | 109   | 32     | 75                          | 21.9%                       |
| Koshy T 2015     | 27     | 66    | 22     | 70                          | 14.2%                       |
| Pei LJ 2006      | 12     | 25    | 22     | 49                          | 8.7%                        |
| Wang BJ 2013     | 26     | 73    | 65     | 140                         | 32.3%                       |
| **Subtotal (95% CI)** | 346    |        | 419    | 100.0%                      | 0.99 [0.74, 1.34]           |
| Total events     | 160    | 419   | 193    |                             |                             |
| **Heterogeneity:** | Chi$^2$=4.51, df=4 (P=0.34); I$^2$=11% | Test for overall effect: Z=0.04 (P=0.97) | | | |

| **1.1.4 GA+AA vs GG** |       |       |        |                             |                             |
| Gong D X 2012    | 182    | 238   | 91     | 134                         | 21.9%                       |
| Koshy T 2015     | 57     | 96    | 52     | 100                         | 16.3%                       |
| Pei LJ 2006      | 54     | 67    | 72     | 99                          | 9.0%                        |
| Wang BJ 2013     | 116    | 163   | 164    | 239                         | 30.6%                       |
| **Subtotal (95% CI)** | 724    |        | 760    | 100.0%                      | 1.24 [0.98, 1.57]           |
| Total events     | 538    | 760   | 534    |                             |                             |
| **Heterogeneity:** | Chi$^2$=2.89, df=4 (P=0.58); I$^2$=0% | Test for overall effect: Z=1.79 (P=0.07) | | | |

| **1.1.5 AA vs GG+GA** |       |       |        |                             |                             |
| Gong D X 2012    | 53     | 238   | 32     | 134                         | 22.5%                       |
| Koshy T 2015     | 27     | 96    | 22     | 100                         | 10.9%                       |
| Pei LJ 2006      | 12     | 67    | 22     | 99                          | 10.3%                       |
| Wang BJ 2013     | 26     | 163   | 65     | 239                         | 31.3%                       |
| **Subtotal (95% CI)** | 724    |        | 760    | 100.0%                      | 0.83 [0.65, 1.06]           |
| Total events     | 160    | 760   | 193    |                             |                             |
| **Heterogeneity:** | Chi$^2=6.41, df=4 (P=0.17); I$^2=38% | Test for overall effect: Z=1.52 (P=0.13) | | | |
## Study or subgroup

| Study or subgroup | Case Events | Control Events | Total Weight |
|-------------------|-------------|----------------|-------------|
| **1.2.1 A vs G** |             |                |             |
| Gong DX 2012      | 235         | 476            | 32.2%       |
| Koshy T 2015      | 84          | 192            | 16.5%       |
| Pei LJ 2006       | 66          | 134            | 15.6%       |
| Wang BJ 2013      | 171         | 320            | 35.8%       |
| Subtotal (95% CI) | 1122        | 2042           | 100.0%      |
| Total events      | 556         | 498            |             |
| Heterogeneity: Chi²=2.00, df=3 (P=0.57); I²=0% | |
| Test for overall effect: Z=0.98 (P=0.32) | |

| **1.2.2 GA vs GG** |             |                |             |
| Gong DX 2012      | 129         | 185            | 31.1%       |
| Koshy T 2015      | 30          | 69             | 21.5%       |
| Pei LJ 2006       | 42          | 55             | 13.3%       |
| Wang BJ 2013      | 87          | 118            | 34.0%       |
| Subtotal (95% CI) | 427         | 539            | 100.0%      |
| Total events      | 280         | 242            |             |
| Heterogeneity: Chi²=3.17, df=3 (P=0.37); I²=5% | |
| Test for overall effect: Z=1.84 (P=0.07) | |

| **1.2.3 AA vs GG** |             |                |             |
| Gong DX 2012      | 153         | 109            | 32.3%       |
| Koshy T 2015      | 27          | 66             | 20.9%       |
| Pei LJ 2006       | 12          | 25             | 12.8%       |
| Wang BJ 2013      | 42          | 73             | 33.9%       |
| Subtotal (95% CI) | 273         | 393            | 100.0%      |
| Total events      | 134         | 128            |             |
| Heterogeneity: Chi²=1.48, df=3 (P=0.69); I²=0% | |
| Test for overall effect: Z=0.87 (P=0.39) | |

| **1.2.4 AG+AA vs GG** |             |                |             |
| Gong DX 2012      | 182         | 238            | 31.5%       |
| Koshy T 2015      | 57          | 96             | 23.8%       |
| Pei LJ 2006       | 54          | 67             | 13.0%       |
| Wang BJ 2013      | 129         | 160            | 31.7%       |
| Subtotal (95% CI) | 561         | 521            | 100.0%      |
| Total events      | 422         | 370            |             |
| Heterogeneity: Chi²=2.63, df=3 (P=0.45); I²=0% | |
| Test for overall effect: Z=1.78 (P=0.08) | |

| **1.2.5 AA vs GG+GA** |             |                |             |
| Gong DX 2012      | 53          | 238            | 32.8%       |
| Koshy T 2015      | 27          | 96             | 15.9%       |
| Pei LJ 2006       | 12          | 67             | 15.0%       |
| Wang BJ 2013      | 42          | 160            | 36.3%       |
| Subtotal (95% CI) | 561         | 521            | 100.0%      |
| Total events      | 134         | 128            |             |
| Heterogeneity: Chi²=1.61, df=3 (P=0.66); I²=0% | |
| Test for overall effect: Z=0.19 (P=0.85) | |

**Figure 3.** Forest plot of Asian analysis in different genetic models.
Table 5. Meta-analysis of fetal reduced folate carrier-1 (RFC1) A80G polymorphism and maternal risk of congenital heart disease.

| Type             | OR (95% CI) | z    | P     | Test of heterogeneity | Analysis model |
|------------------|-------------|------|-------|-----------------------|----------------|
| GA VS GG         | 1.44 [0.98, 2.11] | 1.86 | 0.06  | 24 0.25               | Fixed-effects model |
| AA VS GG         | 2.99 [1.06, 8.41] | 2.08 | 0.06  | 72 0.06               | Random-effects model |
| GA+AA VS GG      | 1.53 [1.08, 2.16] | 2.4  | 0.02  | 0 0.44                | Fixed-effects model |
| AA VS GG+GA      | 1.35 [0.92, 1.97] | 1.54 | 0.12  | 0 0.33                | Fixed-effects model |
| A VS G           | 1.36 [1.07, 1.71] | 2.56 | 0.01  | 0 0.75                | Fixed-effects model |

Figure 4. Meta-analysis of maternal genotypes (homozygous, allele, and dominant models), fixed-effects model.

For the maternal analysis, the aggregated data came from 2 studies, including 258 cases and 334 controls. Among them, the homozygous model ($I^2=72\%$, $P=0.06$) has high heterogeneity, so the random-effects model is used for analysis. The other 4 models have low heterogeneity, so we use the fixed-effects model for analysis (Table 5).

The meta-analysis results showed that RFC1 A80G polymorphism was significantly associated with an increased risk of CHD in the homozygous models (AA vs GG, OR=2.99, 95% CI [1.06, 8.41], $P=0.04$) (Figure 4), allele models (A vs G, OR=1.36, 95% CI [1.07, 1.71], $P=0.01$), and dominant models (GA+AA vs GG, OR=1.53, 95% CI [1.08, 2.16], $P=0.02$). There was no significant correlation between the homozygous models (GA vs GG, OR=1.44, 95% CI [0.98, 2.11], $P=0.06$) and invisible models (AA vs GG+GA, OR=1.35, 95% CI [0.92, 1.97], $P=0.12$) (Figure 5).

**Heterogeneity Test and Publication Bias**

Because of the small number of included articles, less than 10, we did not evaluate the publication bias; the heterogeneity of the included studies was low, so sensitivity analysis was not performed.

**Discussion**

To the best of our knowledge, this study is the first meta-analysis to explore the association between RFC1 A80G (rs1051266) gene polymorphism and CHD risk. We detected all the relevant literature and as far as possible, summarized and analyzed whether the fetal risk of CHD increased if the fetus and mother had mutations at this site. The research status of this field was systematically evaluated to provide reference for clinical research in this field in the future.

In this meta-analysis, the fetal analysis of 724 children with CHD and 760 controls from 5 studies showed that compared with individuals with the GG genotype, the GA genotype had a 36% higher OR of CHD risk ($P=0.02$), with better homogeneity and stable results. In other gene models, no effect of genotype was observed. Among the 5 included studies, only 1 study population was from North America, and the remaining 4 were from Asia. A subgroup analysis was carried out according to the source area of the samples, and there was no correlation between RFC1 A80G polymorphism and CHD. In terms of mechanism, the fetal RFC1 A80G gene mutation affects the transport of folic acid in the fetus, causing the developing embryo to lack folic acid and increasing the risk of fetal CHD. However, the current meta-analysis results did not support the association between fetal RFC1 A80G polymorphism and CHD susceptibility. These 2 contradictory views may be related to the differences in the disease phenotype, gender ratio, and matching conditions of the control group in the included literature samples, or it may be that this site caused folate transport and absorption disorders but failed to cause abnormal embryo development, which did not cause the fetus to develop CHD.
than those of the infant. Women with AA genotype might lead to reduced folate affinity; maternal plasma folate levels decreased, which in turn affected embryo development and increased the risk of fetal CHD.

Epidemiological studies have shown that adequate folic acid supplementation in early pregnancy can reduce the risk of fetal CHD [21,34,35]. This was first started in a case-control study in Hungary [36]. Through the analysis of national medical data, 3567 children with CHD from 1980 to 1991 in this country and 5395 normal controls were included in the study. The study found that the risk of CHD in the folic acid group was significantly reduced. Subsequently, the research group conducted a cohort study [37], with a total of 3056 birth outcomes. The study found that the risk of CHD in offspring of folic acid use group was significantly reduced. Several other studies [38-40] also found that standardized supplementation of folic acid was a protective factor for CHD. However, the interaction between maternal folic acid supplementation and folate-related gene polymorphisms showed no consistent effect on fetal CHD risk.

The mother provides the developmental environment for the embryo, and its folic acid level will affect embryonic development to a certain extent [30]. Many studies have shown that compared with women with RFC1-80GG genotype, women with GA and AA genotypes had higher plasma folic acid concentrations [31-33]. We further explored whether the presence of the maternal 80GG genotype increased the risk of giving birth to a child with CHD. Analysis of mothers of 258 cases and 334 controls from 2 studies showed that compared with the G allele, the putative dangerous allele A increased the risk of CHD by 36% (P = 0.01). GA+AA genotype made the OR with CHD risk increased, which in turn affected embryo development and increased the risk of fetal CHD.
This systematic review explored the relationship between folic acid supplementation and RFC1 A80G polymorphism. Folic acid gene testing has not yet been widely used. In some institutions with testing capabilities, the overall coverage rate is not high. Only some people will accept a doctor’s recommendation for this test. Therefore, in most studies, information about the use of conceptual folic acid supplements and the mother’s dietary folic acid intake is missing. In this meta-analysis, only Pei et al [28] described detailed information about the mother’s folic acid supplementation, and the data obtained were not sufficient to analyze folic acid supplementation. The relationship between the effects of folic acid supplements and the RFC1 A80G polymorphism should be studied in the future, so as to form certain normative guidelines to better guide women’s oral folic acid to prevent birth defects.

Our research also has some limitations. First of all, the number of studies we included is limited, especially for the maternal group. There are only 2 included studies, the sample size and the number of studies included are small, and the results are very uncertain, resulting in inaccurate risk estimates. Second, part of the control population included in the study came from hospitals, so the recruited subjects may not be representative of the general population. Third, in the maternal group, studies by Wang et al [18] lack information on the folic acid status of pregnant women, and it is impossible to determine whether the genetic polymorphism will affect the risk of CHD if the mother consumes enough folic acid early in the pregnancy. Fourth, our research only studied 1 gene polymorphism of RFC1, namely A80G (rs1051266). The result may lack stability in the overall relationship, and the interaction with multiple genes and environmental factors may change the relevance of the results. Considering these limitations, the results of this study should be interpreted carefully.

**Conclusions**

There is no correlation between the fetal RFC1 A80G polymorphism and CHD susceptibility, whereas the maternal RFC1 A80G polymorphism has a strong correlation with CHD. Compared with the G allele, the A allele increases the risk of CHD 0.36-fold. Additional replication with larger sample size is warranted.

**Conflicts of interest**

None.
Supplementary Data

Supplementary Table 1. The full detailed search strategy and searching terms.

| Set | Query |
|-----|-------|
| #1  | TS=("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") |
| #2  | TS=("atrial septal defects" OR "atrial septal defect") |
| #3  | TS=("ventricular septal defect" OR "ventricular septal defects") |
| #4  | TS=("Trilogy of Fallot" OR "Tetralogy of Fallot") |
| #5  | TS=("patent ductus arteriosus" OR "scimitar syndrome" OR "anomalous pulmonary venous connection") |
| #6  | TS=(foramen oval* OR lutembacher* syndrome) |
| #7  | TS=(single ventricle* OR univentricular heart*) |
| #8  | TS=("double inlet left ventricle" OR "double outlet right ventricle") |
| #9  | TS=("persistent truncus arteriosus" OR "persistent ostium primum" OR "interrupted aortic arch") |
| #10 | TS=("pulmonary valve stenoses" OR "pulmonary valve stenosis" OR "pulmonary stenoses" OR "pulmonary stenosis" OR "pulmonary valve stenosis" OR "pulmonic stenosis" OR "pulmonic stenoses") |
| #11 | TS=(tricuspid atresia* OR valve atresia*) |
| #12 | TS=("pulmonary atresia" OR "absent right atrioventricular connection") |
| #13 | #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 |
| #14 | TS=("solute carrier family 19 member 1" OR "Reduced folate carrier" OR "folate transporter 1" OR "intestinal folate carrier 1" OR "placental folate transporter" OR "reduced folate carrier protein" OR SLC19A1 OR RFC OR RFC-1 OR IFC1 OR IFC-1) |
| #15 | TS=("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") |
| #16 | #13 AND #14 AND #15 |

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