The relevance of MTHFR C677T, A1298C, and MTRR A66G polymorphisms with response to male infertility in Asians

A meta-analysis

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Abstract

Although published studies have reported the association between MTHFR C677T (rs1801133), A1298C (rs1801131), and MTRR A66G (rs1801394) polymorphisms and male infertility in Asian populations, the results are conflicting. In order to accurately evaluate the relevance, a meta-analysis was performed.

We searched for potential studies in 4 databases, containing PubMed, ScienceDirect, China National Knowledge Infrastructure (CNKI), and Wanfang database until May 31, 2018. The summarized odds ratio (OR) with 95% confidence intervals (95% CI) were calculated to evaluate the relevance in 5 genetic models. The heterogeneity test, sensitivity analysis, and publication bias test was performed by Review Manager 5.3 software.

Overall, 22 case-control studies with 5049 cases and 4157 controls were included in this meta-analysis, which contained 20 studies of MTHFR C677T polymorphism, 12 studies of MTHFR A1298C polymorphism and 4 studies of MTRR A66G polymorphism. The results indicated that MTHFR C677T, A1298C, and MTRR A66G polymorphisms were significantly associated with male infertility in Asian populations (Dominant model: MTHFR CC + CT vs TT: OR = 0.60, 95% CI (0.53, 0.67), P < .00001; MTRR AA + AC vs CC: OR = 0.62, 95% CI (0.49, 0.79), P = .0001; MTRR AA + AG vs GG: OR = 0.60, 95% CI (0.45, 0.81), P = .001. Recessive model: MTHFR CC vs CT + TT: OR = 0.67, 95% CI (0.61, 0.74), P < .00001; MTHFR AA vs AC + CC: OR = 0.70, 95% CI (0.67, 0.82), P < .00001; MTRR AA vs AG + GG: OR = 0.71, 95% CI (0.56, 0.88), P = .002. Homozygote model: MTHFR CC vs CT: OR = 0.73, 95% CI (0.67, 0.80), P < .00001; MTHFR AA vs AC: OR = 0.79, 95% CI (0.70, 0.88), P < .00001; MTRR AA vs AG: OR = 0.76, 95% CI (0.67, 0.89), P = .01. Homozygote model: MTHFR CC vs TT: OR = 0.48, 95% CI (0.41, 0.56), P < .00001; MTRR AA vs CC: OR = 0.51, 95% CI (0.43, 0.60), P = .001. Allele model: MTHFR C vs T: OR = 0.70, 95% CI (0.66, 0.75), P < .00001; MTHFR A vs C: OR = 0.82, 95% CI (0.71, 0.95), P = .01; MTRR A vs G: OR = 0.76, 95% CI (0.66, 0.88), P = .00003. Stratified analyses by geographical location and source of controls showed the same results. Sensitivity analyses indicated that the final consequences of this meta-analysis were stable, and the publication biases test had not found obvious asymmetry.

This meta-analysis indicates that MTHFR C677T, A1298C, and MTRR A66G polymorphisms are the risk factors with susceptibility to male infertility in Asians.

Abbreviations: CI = confidence interval, CNKI = China National Knowledge Infrastructure, HB = hospital-based, HWE = Hardy-Weinberg equilibrium, MTHFR = methylene tetrahydrofolate reductase, MTRR = methionine synthase reductase, OR = odds ratio, PB = population-based.

Keywords: Asians, male infertility, MTHFR A1298C, MTHFR C677T, MTRR A66G, polymorphism

1. Introduction

It had shown that about 10%~15% of married couples in the world were suffering from infertility, about half of which was attributed to male partner.[1] So far, male infertility has become a concern and urgent problem in the world. Many reasons such as environmental disruptors, genetic, testes pathologies, and sedentary lifestyle may affect spermatogenesis leading to male infertility.[2,3] but almost half of all male infertility patients are still undiagnosed for the complicated mechanism which may be associated with spermatogenesis process of gene mutations.[4]

Folate plays an important role in cell metabolism, like the synthesis of nucleic acids and epigenetic regulation of gene expression through remethylation of homocysteine into methionine.[5] Once the folate is deficient, the proliferation of sperm cells will be reduced.[6] Methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) are the key enzymes in folate metabolism. The enzyme activities of
MTHFR and MTRR are influenced by gene polymorphisms.\(^7\) So the polymorphisms of MTHFR and MTRR may be a potential risk factor for male infertility.\(^8\)

Several studies have investigated the association between MTHFR C677T (rs 1801133), A1298C (rs 1801131) and MTRR A66G (rs1801394) polymorphisms, and male infertility, but the conclusions are controversial.\(^9\) The reason may be partially attributed to racial difference. For Asians, only 4 meta-analyses have evaluated the impact of MTHFR C677T polymorphism on male infertility by far\(^{10-13}\) Gupta's study with 522 cases and 315 controls was limited to Indian population.\(^{10}\) Weiner's study with 275 men of idiopathic male infertility and 349 controls was limited to Russian population.\(^{11}\) Ren's study including 1713 cases and 1104 controls was limited to Chinese population,\(^{12}\) and Rai's research with 4392 breast infertile males and 3667 fertile males has not included the latest research data after March 2015.\(^{13}\) Only Ren et al have evaluated the association between MTHFR A1298C and male infertility.\(^{12}\) The system review with respect to MTRR A66G polymorphism specifically for Asian populations has not been reported till date. In this present research, we performed a meta-analysis based on 22 studies with 5049 cases and 4157 controls to investigate the relationship between MTHFR C677T, A1298C, and MTRR A66G polymorphisms and risk of male infertility in Asians.

### 2. Materials and methods

#### 2.1. Literature search and selection

The systematic search from PubMed, ScienceDirect, CNKI, and Wanfang databases updated on May 31, 2018 using the terms "(Methylenetetrahydrofolate reductase or MTHFR or methionine synthase reductase or MTRR or C677T or A1298C or A66G) and (polymorphism or variants or mutation) and (male infertility)" was conducted by 2 review authors (Shi and Wu). The languages were limited to English and Chinese. Furthermore, we manually searched references in the eligible articles to acquire more applicable information.

#### 2.2. Criteria of inclusion and exclusion

Inclusion criteria were showed as following:

1. case–control studies;
2. evaluation of the association between MTHFR C677T and/or A1298C and/or MTRR A66G polymorphism and male infertility risk in Asian populations;
3. all genotypes had complete data;
4. published in English or Chinese language.

The reasons for excluding studies were:

1. uncertain type of study or not case–control study;
2. no detailed data on genotype distribution;
3. not in Asian populations.

| 115 articles identified from Pubmed, ScienceDirect, CNKI, Wan Fang database (duplications excluded) |
|---|
| **62 articles excluded:** Not for MTHFR 677T/A1298C or MTRR A66G (n=39) Review or Meta-analysis or Letter articles (n=23) |
| **53 articles for Full-text reviewed** |
| **31 articles excluded:** Not case-control study (n=17) Not Asian populations (n=14) |
| **22 articles included in this meta-analysis:** 1) 20 studies involved MTHFR C677T polymorphism and male infertility 2) 12 studies involved MTHFR A1298C polymorphism and male infertility 3) 4 studies involved MTRR A66G polymorphism and male infertility |

Figure 1. Flow chart of the included studies in the meta-analysis.
2.3. Data extraction

The following information was carefully and independently collected from each eligible study by 2 reviewers: the first author’s name, publication year, country, geographical location, source of controls, and the count of persons with each genotype and allele. The \( P \) value of Hardy–Weinberg equilibrium test (HWE) was also calculated. If the clinical trial data is not complete, we try to contact the author as far as possible.

2.4. Methodological quality assessment

Two reviewers (Shi and Wu) independently assessed the methodological quality of included literature using Newcastle-Ottawa Scale (NOS). The maximum score was 9, and the score of studies ranged from 0 to 3, 4 to 6, and 7 to 9 were regarded as low-quality, moderate-quality, and high-quality, respectively.\[14\]

2.5. Statistical analysis

Review Manager 5.3 software was used for analyses. HWE in each study was calculated by Chi-squared test. The associations were estimated by odds ratio (OR) with 95% confidence interval (95% CI). The heterogeneity among studies was evaluated by \( Q \) and \( I^2 \) statistics. If there was no heterogeneity with \( P \geq 0.1 \) or \( I^2 \leq 50\% \), the fixed-effect model was used. Conversely, the random-effect model was used. Subgroup analysis or sensitivity analysis was performed to exclude the possible causes of heterogeneity. Funnel plot was applied to detect publication bias in the included studies. The statistical significance was considered with \( P \) value less than 0.05.

This study was approved by the Ethics Committee of the First Affiliated Hospital of University of Science and Technology of China. It was conducted in accordance with the Declaration of Helsinki.

3. Results

3.1. Characteristics of included studies

A flow chart summarizing the process of literature selection is shown in Fig. 1. Based on the inclusion-exclusion criteria, 22 case-control studies were recruited in the final analysis.\[15–36\] 20 studies were concerned with the association between MTHFR C677T polymorphism and male infertility,\[15–18,20,21,23–36\] 12 studies evaluated the MTHFR A1298C polymorphism,\[15–17,19,20,22,28,29,33,35,36\] and only 4 studies evaluated the MTRR A66G polymorphism.\[13,25–27\] The characteristics of included studies in the meta-analysis are presented in Table 1.

### Table 1

Main characteristics of included studies in the meta-analysis.

#### A: MTHFR C677T polymorphism

| Author     | Year | Country       | Geographical location | Source of controls | Cases | Controls |
|------------|------|---------------|-----------------------|-------------------|------|----------|
| Wang Y     | 2017 | China         | East Asia             | PB                | 76   | 15       |
| Najafpour R| 2017 | Iran          | West Asia             | HB                | 280  | 113      |
| Karimian M | 2016 | Iran          | West Asia             | HB                | 118  | 51       |
| Li XY      | 2015 | China         | East Asia             | PB                | 162  | 61       |
| Mhady OS   | 2014 | Jordanian     | Western Asia          | HB                | 150  | 67       |
| Naghi H    | 2014 | Indian        | South Asia            | HB                | 637  | 447      |
| Li SS      | 2014 | China         | East Asia             | PB                | 82   | 14       |
| Pet J      | 2013 | China         | East Asia             | PB                | 290  | 39       |
| Vardi GT   | 2011 | Italy         | South Asia            | HB                | 206  | 156      |
| Liu L      | 2011 | China         | East Asia             | HB                | 75   | 27       |
| Gao X      | 2011 | China         | East Asia             | NA                | 271  | 75       |
| Yang BH    | 2010 | China         | East Asia             | HB                | 131  | 34       |
| Zhang WB   | 2010 | China         | East Asia             | HB                | 491  | 43       |
| Dhillon VS | 2007 | India         | South Asia            | NA                | 179  | 81       |
| Ali J      | 2007 | China         | East Asia             | HB                | 351  | 130      |
| Zhang XI   | 2007 | China         | East Asia             | HB                | 165  | 41       |
| Li XY      | 2014 | China         | East Asia             | PB                | 373  | 105      |
| Park JH    | 2005 | Korea         | South Asia            | Mixed             | 151  | 105      |
| Singh K    | 2005 | India         | South Asia            | Mixed             | 151  | 105      |
| Sun HF     | 2005 | China         | East Asia             | PB                | 182  | 27       |

#### B: MTHFR A1298C polymorphism

| Author     | Year | Country       | Geographical location | Source of controls | Cases | Controls |
|------------|------|---------------|-----------------------|-------------------|------|----------|
| Najafpour R| 2017 | Iran          | West Asia             | HB                | 280  | 113      |
| Karimian M | 2016 | Iran          | East Asia             | PB                | 118  | 51       |
| Li XY      | 2015 | China         | East Asia             | HB                | 162  | 101      |
| Mhady OS   | 2014 | Jordanian     | West Asia             | HB                | 150  | 71       |
| Li XY      | 2014 | China         | East Asia             | PB                | 162  | 101      |
| Li SS      | 2014 | China         | East Asia             | PB                | 82   | 49       |
| Singh K    | 2005 | India         | East Asia             | Mixed             | 151  | 66       |
| Lee HC     | 2006 | Korea         | East Asia             | Mixed             | 373  | 137      |

#### C: MTRR A66G polymorphism

| Author     | Year | Country       | Geographical location | Source of controls | Cases | Controls |
|------------|------|---------------|-----------------------|-------------------|------|----------|
| Li XY      | 2015 | China         | East Asia             | PB                | 162  | 83       |
| Mhady OS   | 2014 | Jordanian     | West Asia             | HB                | 150  | 46       |
| Zhang WB   | 2010 | China         | East Asia             | HB                | 491  | 224      |
| Lee HC     | 2006 | Korea         | East Asia             | Mixed             | 360  | 222      |
| Park JH    | 2005 | Korea         | East Asia             | Mixed             | 373  | 137      |

\( CI = \) confidence interval, \( HB = \) hospital-based, \( MTHFR = \) methylene tetrahydrofolate reductase, \( MTRR = \) methionine synthase reductase, \( OR = \) odds ratio, \( PB = \) population-based.
Figure 2. Forest plots for association of MTHFR C677T polymorphism with the risk of male infertility in Asians. MTHFR=methylene tetrahydrofolate reductase.
A66G polymorphism.\cite{15,17,28,29} The main characteristics and quality score of each study were displayed in Table 1. All studies were stratified by geographical location, of which 14 studies were performed in East Asians\cite{11,16,18,21,22,24,29} and the remaining 8 across South/West Asians.\cite{17,19,20,23,30,31,35,36} When stratified by source of controls, the amount of hospital-based (HB) studies was 10,\cite{11,17,18,21,25,28,30,32,33,35,36} and population-based (PB) studies was 8,\cite{11,22,24,27,29,33,34} and mixed population or uncertain source was 4.\cite{15,16,20,26}

3.2. Results of meta-analysis and subgroup-analysis

3.2.1. MTHFR C677T polymorphism. After pooling 20 studies with 4734 cases and 3967 controls into 1 data set for meta-analysis, we found that the MTHFR C677T polymorphism had statistical association with the risk of male infertility in Asians (see Fig. 2; (A) Dominant model (CC+CT vs TT): OR = 0.60, 95% CI (0.53, 0.67), P < 0.00001; (B) Recessive model (CC vs CT + TT): OR = 0.67, 95% CI (0.61, 0.74), P < 0.00001; (C) Heterozygote model (CC vs CT): OR = 0.74, 95% CI (0.67, 0.82), P < 0.00001; (D) Homozygote model (CC vs TT): OR = 0.48, 95% CI (0.41, 0.56), P < 0.00001; (E) Allele model (C vs T): OR = 0.70, 95% CI (0.66, 0.75), P < 0.00001.)

In the subgroup analysis of geographical location, we observed that a similar association existed both in East Asians and South/West Asians for the MTHFR C677T polymorphism with the male infertility risk. Further stratified analysis by the source of controls showed that the MTHFR C677T polymorphism was also significantly associated with male infertility both in HB and population-based studies. Table 2 summarized the results of overall and subgroup analysis in all of 5 genetic models.

![Figure 2. (Continued).]
3.2.2. **MTHFR A1298C polymorphism.** Twelve studies with 2673 cases and 2328 controls were included to examine the effect of MTHFR A1298C polymorphism on male infertility (see Fig. 3; (A) Dominant model (AA + AC vs CC): OR = 0.62, 95% CI (0.49, 0.79), *P* = .0001; (B) recessive model (AA vs AC + CC): OR = 0.79, 95% CI (0.70, 0.88), *P* < .0001; (C) Heterozygote model (AA vs AC): OR = 0.83, 95% CI (0.73, 0.95), *P* = .001; (D) Homozygote model (AA vs CC): OR = 0.61, 95% CI (0.39, 0.93), *P* = .02; (E) Allele model (A vs C): OR = 0.82, 95% CI (0.71, 0.95), *P* = .01). The results showed the significantly increased risk of male infertility with MTHFR 1298C allele carriers.

In the subgroup analysis of geographical location, we observed that the statistic association existed in East Asians but not in South/West Asians. Further stratified analysis by the source of controls, no significant enhanced risk was observed in all of 3 subgroups. Table 3 showed the results of overall and subgroup analysis in all of 5 genetic models.

3.2.3. **MTRR A66G polymorphism.** Four studies with 837 cases and 727 controls were included to assess the association between MTRR A66G polymorphism and the risk of male infertility (see Fig. 4 (A) Dominant model (AA + AG vs GG): OR = 0.60, 95% CI (0.45, 0.81), *P* = .001; (B) recessive model (AA vs AG + GG): OR = 0.70, 95% CI (0.56, 0.88), *P* = .002; (C) Heterozygote model (AA vs AG): OR = 0.76, 95% CI (0.60, 0.92), *P* = .02; (D) Homozygote model (AA vs GG): OR = 0.51, 95% CI (0.36, 0.72), *P* = .0001; (E) Allele model (A vs G): OR = 0.76, 95% CI (0.66, 0.88), *P* = .0003). In short, the MTRR 66G allele carriers had a markedly increased risk of male infertility in Asian populations.

3.3. **Sensitivity analysis and publication bias**

In sensitivity analysis, elimination of each study made no qualitative difference on the pooled OR values, which indicated that the final consequences of this meta-analysis were stable (Table 4).

The publication biases of the included studies were assessed by funnel plot. The shape of funnel plot in MTHFR C677T, A1298C, and MTRR A66G genotype comparison indicated no obvious asymmetry (Fig. 5).

4. **Discussion**

According to the present meta-analysis involving 3049 cases and 4157 controls from 22 published studies, the MTHFR C677T polymorphism has statistical impact on the risk of male infertility in Asian populations which was similarly supported by the prior 4 meta-analysis of Asians.[10–13] Compared with them, this meta-analysis has a bigger number of included studies and samples. Therefore, the results are more valuable for Asian populations. Previously, a meta-analysis had included 3 studies with a total of 898 individuals to assess the association between MTHFR A1298C polymorphism and male infertility risk in Chinese population and confirmed that MTHFR A1298C polymorphism...
was not the risk factor of male infertility (C vs A; OR = 1.22, 95% CI (0.97, 1.53), \(I^2 = 0\); CC + AC vs AA; OR = 1.27, 95% CI (0.98, 1.65), \(I^2 = 0\); CC vs AA; OR = 1.34, 95% CI (1.66, 2.77), \(I^2 = 0\); CC vs AC + AA; OR = 1.44, 95% CI (0.72, 2.88), \(I^2 = 9\).\(^{[12]}\) which was in contrast to the conclusion of present meta-analysis. This difference may be caused by sample sizes or population substructure. Regarding the MTRR A66G polymorphism, our results provided strong evidence of the association with male infertility risk. For Asians, NCBI database has shown that the allelic frequencies of MTHFR C677T, A1298C, and MTRR A66G are 0.51, 0.24, and 0.30 respectively. Basing on present study, we reached the following conclusion that men carrying the
Table 3

Subgroup analyses for MTHFR A1298C polymorphism in 5 comparative genetic models.

| Models | Population | No. of studies | Sample size (case/control) | I² (%) | OR (95% CI) | P |
|--------|------------|----------------|----------------------------|--------|-------------|---|
| Dominant model (AA vs CC) overall | 12 | 2673/3238 | 50 | 0.62 (0.49, 0.79) | .001 |
| East Asia | 7 | 1759/1856 | 59 | 0.49 (0.35, 0.68) | <.0001 |
| South/West Asia | 5 | 876/742 | 0 | 0.86 (0.56, 1.38) | .31 |
| HB | 5 | 1204/964 | 71 | 0.51 (0.36, 0.71) | <.0001 |
| PB | 4 | 557/443 | 22 | 0.55 (0.27, 1.10) | .09 |
| Others | 3 | 912/201 | 0 | 0.94 (0.61, 1.44) | .78 |
| Recrecessive model (AA vs CC) overall | 12 | 2673/3238 | 46 | 0.70 (0.50, 0.98) | <.0001 |
| East Asia | 7 | 1759/1856 | 38 | 0.70 (0.50, 0.98) | <.0001 |
| South/West Asia | 5 | 876/742 | 0 | 1.00 (0.82, 1.22) | .98 |
| HB | 5 | 1204/964 | 77 | 0.72 (0.50, 1.06) | .0001 |
| PB | 4 | 557/443 | 0 | 0.83 (0.64, 1.09) | .16 |
| Others | 3 | 912/201 | 46 | 0.86 (0.71, 1.04) | .12 |
| Heterozygote model (AA vs AC) overall | 12 | 2471/2216 | 27 | 0.83 (0.73, 0.93) | .002 |
| East Asia | 7 | 1678/1532 | 0 | 0.74 (0.64, 0.86) | <.0001 |
| South/West Asia | 5 | 763/684 | 0 | 1.04 (0.94, 1.15) | .74 |
| HB | 5 | 1074/909 | 70 | 0.78 (0.65, 0.94) | .008 |
| PB | 4 | 530/429 | 0 | 0.87 (0.66, 1.16) | .32 |
| Others | 3 | 867/878 | 0 | 0.86 (0.70, 1.04) | .13 |
| Homozygote model (AA vs CC) overall | 12 | 1641/1504 | 58 | 0.61 (0.39, 0.93) | .02 |
| East Asia | 7 | 1142/1093 | 65 | 0.46 (0.24, 0.90) | .02 |
| South/West Asia | 5 | 501/411 | 0 | 0.88 (0.60, 1.28) | .50 |
| HB | 5 | 703/596 | 78 | 0.48 (0.22, 1.08) | .08 |
| PB | 4 | 344/280 | 8 | 0.53 (0.24, 1.19) | .12 |
| Others | 3 | 594/628 | 0 | 0.89 (0.57, 1.38) | .60 |
| Allele model (A vs C) overall | 12 | 5346/4656 | 57 | 0.82 (0.71, 0.95) | .01 |
| East Asia | 7 | 3593/3172 | 56 | 0.73 (0.60, 0.89) | .001 |
| South/West Asia | 5 | 1756/1484 | 0 | 0.97 (0.83, 1.13) | .68 |
| HB | 5 | 2408/1928 | 80 | 0.78 (0.57, 1.08) | .13 |
| PB | 4 | 1114/886 | 0 | 0.83 (0.67, 1.04) | .10 |
| Others | 3 | 1842/1842 | 0 | 0.89 (0.76, 1.05) | .16 |

CI = confidence interval, HB = hospital-based, MTHFR = methylene tetrahydrofolate reductase, MTR = methionine synthase reductase, OR = odds ratio, PB = population-based.
alleles of MTHFR 677T, 1298C, and MTRR 66G were likely to become infertile. Therefore, the analysis of these 3 key mutations would be helpful in the prognostication and screening of male infertility.

Although the precise mechanism by which MTHFR C677T, A1298C, and MTRR A66G polymorphisms have effect on fertility is unclear, previous researches have put forward some potential mechanisms. The folate-mediated 1-carbon metabolism is very important for many reactions in human sperm cells,[37,38] such as the methylation, repair, and synthesis of DNA. As one of the key enzymes in DNA synthesis, MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate acid which participates in the exchange of deoxyuridine triphosphate (dUTP) for deoxythymidine monophosphate (dTMP) to 5-methyl-tetrahydrofolic acid with a biological function.[39] As a major regulatory enzyme in the pathway of homocysteine metabolism, MTRR plays a vital role in folate and vitamin B12-dependent remethylation of homocysteine to methionine.
Therefore, the polymorphisms of MTHFR C677T, A1298C, and MTRR A66G may influence the activity and stability of the above enzymes leading to imbalance of folate-related metabolism.\(^{40}\) Then, the abnormal metabolism may give rise to the risk of male infertility.

For Asians, our meta-analysis again indicated the significant association between MTHFR C677T polymorphism and male infertility which kept consistent with previous meta-analysis.\(^{12}\) Instead, as to MTHFR A1298C polymorphism, the conclusions were not the same. Ren et al suggested it was not the risk factor of male infertility in Chinese population.\(^{12}\) However, the present meta-analysis observed the statistic association existing in Asians especially for East Asians. This discordant finding may be due to the more included studies and a larger sample size for our research. Most importantly, this is the first meta-analysis specifically for Asian populations assessing the correlation between MTRR A66G polymorphism and male infertility. It showed that the genotypes and mutant allele of MTRR A66G were significantly related with male infertility in Asians. Liu et al and Xu et al have performed meta-analyses to investigate the association between MTRR A66G polymorphism and male infertility in overall population, and they failed to draw any statistic conclusion.\(^{38,41}\) When restricting the subgroup analysis to ethnicity, Liu et al observed an increased risk in Asians but

| Sensitivity analysis for the MTHFR C677T, A1298C, and MTRR A66G polymorphism. |
|-------------------------------------|------------------|-----------------|------------------|
| **A: MTHFR C677T polymorphism**    | **Heterogeneity** | **Effect size**  | **OR (95%)**     |
| Eliminated study                   | \(I^2\) | \(P\) | OR (95%)          |
|------------------------------------|--------|--------|-------------------|
| A Z C 2007                         | 31     | .10    | 0.60 (0.52,0.68)  |
| Dhillon VS 2007                    | 6      | .38    | 0.57 (0.51,0.65)  |
| Karimian M 2016                    | 29     | .12    | 0.60 (0.52,0.68)  |
| Lee HC 2006                        | 31     | .10    | 0.59 (0.52,0.68)  |
| Li SS 2014                         | 31     | .10    | 0.60 (0.52,0.68)  |
| Li XY 2015                         | 24     | .16    | 0.58 (0.51,0.66)  |
| Liu L 2011                         | 30     | .11    | 0.60 (0.52,0.68)  |
| Miftadi DS 2014                    | 29     | .12    | 0.60 (0.53,0.68)  |
| Najaipour R 2017                   | 31     | .10    | 0.60 (0.53,0.68)  |
| Napei H 2014                       | 30     | .11    | 0.60 (0.53,0.68)  |
| Park JH 2005                       | 29     | .12    | 0.58 (0.51,0.66)  |
| Pei J 2013                         | 26     | .14    | 0.61 (0.53,0.69)  |
| Qiu XF 2011                        | 28     | .12    | 0.61 (0.53,0.69)  |
| Singh K 2005                       | 23     | .17    | 0.60 (0.53,0.68)  |
| Sun HT 2005                        | 27     | .14    | 0.60 (0.53,0.68)  |
| Vare GT 2011                       | 25     | .16    | 0.60 (0.53,0.68)  |
| Wang Y 2017                        | 30     | .11    | 0.60 (0.53,0.68)  |
| Yang BH 2010                       | 28     | .13    | 0.60 (0.53,0.69)  |
| Zhang WB 2010                      | 30     | .11    | 0.60 (0.50,0.67)  |
| Zhang XJ 2007                      | 24     | .17    | 0.58 (0.51,0.66)  |

**B: MTHFR A1298CT polymorphism**

| Eliminated study                   | \(I^2\) | \(P\) | Effect size |
|------------------------------------|--------|--------|-------------|
| Dhillon VS 2007                    | 48     | .04    | 0.58 (0.45,0.75) |
| Karimian M 2016                    | 54     | .02    | 0.60 (0.47,0.78) |
| Lee HC 2006                        | 53     | .02    | 0.60 (0.46,0.77) |
| Li SS 2014                         | 51     | .03    | 0.60 (0.47,0.77) |
| Li XY 2014                         | 54     | .02    | 0.62 (0.49,0.80) |
| Li XY 2015                         | 53     | .02    | 0.63 (0.49,0.81) |
| Miftadi DS 2014                    | 53     | .02    | 0.59 (0.46,0.77) |
| Najaipour R 2017                   | 53     | .02    | 0.59 (0.46,0.77) |
| Park JH 2005                       | 53     | .02    | 0.60 (0.46,0.77) |
| Singh K 2010                       | 50     | .03    | 0.64 (0.50,0.82) |
| Zhang WB 2010                      | 6      | .38    | 0.75 (0.58,0.90) |
| Zhang XJ 2007                      | 44     | .06    | 0.66 (0.51,0.85) |

**C: MTRR A66G polymorphism**

| Eliminated study                   | \(I^2\) | \(P\) | Effect size |
|------------------------------------|--------|--------|-------------|
| Lee HC 2006                        | 17     | .30    | 0.70 (0.57,0.85) |
| Li XY 2015                         | 50     | .13    | 0.77 (0.65,0.90) |
| Miftadi DS 2014                    | 49     | .14    | 0.75 (0.64,0.89) |
| Zhang XJ 2007                      | 0      | .88    | 0.82 (0.70,0.97) |

MTHFR = methylene tetrahydrofolate reductase, MTRR = methionine synthase reductase, OR = odds ratio.
not in Europeans in homozygous, dominant and allele genetic models. In addition, there were available data analyzing these 3 polymorphisms within certain patients. Zhang et al have enrolled 165 infertile patients and 132 healthy fertile males in China to evaluate the impact of MTHFR and MTRR gene polymorphisms on idiopathic male infertility. The findings discovered that: first, the heterozygous genotype (CT) and combined genotype (CT+TT) were present at statistical significances in male infertility ($P = 0.026$, $P = 0.031$) for MTHFR C677T polymorphism. Second, the frequencies of allele C and homozygous genotype (CC) were significantly different between case group and control group ($P = 0.013$, $P = 0.004$) for MTHFR A1298C polymorphism. Third, the prevalence of GG genotype and combined genotype (AG+GG) showed significant difference in the 2 groups ($P = 0.001$, $P = 0.035$) for MTRR A66G. These data are in consistent with our research revealing that the 3 polymorphisms might play an important role in the occurrence of male infertility. However, further studies are still needed to reveal the correlation between polymorphisms of MTHFR C677T, A1298C, and MTRR A66G with Asian male infertility.

On the other hand, some inherent limitations of this meta-analysis should be admitted. First, there may be some language bias since the included literatures are given priority to Chinese and English. Second, the sources of controls among the studies were different from each other. Some studies were HB studies, some studies were PB studies, and others were mixed population or uncertain. Third, our analysis was merely based on single-factor estimation ignoring the interactions of gene-gene and gene-environmental in the development of male infertility. Finally, the sample size was relatively small in part of the included studies.

5. Conclusion
In short, our meta-analysis provides further evidence indicating that MTHFR C677T, A1298C, and MTRR A66G polymorphisms are the risk factors with susceptibility to male infertility in Asian populations. In the future, studies with larger sample sizes will be performed to confirm it, and to explore the relationship between potential gene-gene, gene-environment interactions and male infertility with purpose of providing an important basis for the prevention and treatment of male infertility.

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