Contribution of MTR A2756G polymorphism and MTRR A66G polymorphism to the risk of idiopathic male infertility

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Abstract

Background: Methionine synthase reductase gene (MTRR A66G) polymorphism and methionine synthase gene (MTR A2756G) polymorphism have shown an association with idiopathic male infertility risk in several ethnic populations. However, their small sample sizes and inconsistent outcomes have prevented strong conclusions. We performed a meta-analysis with published studies to evaluate the associations of the 2 single nucleotide polymorphisms (SNPs) and idiopathic male infertility risk.

Methods: A thorough literature search was performed up to Jun 21, 2019 with Medline, Embase, Web of Science, China National Knowledge Infrastructure (CNKI), China Biology Medical literature (CBM), China Science and Technology Journal Database (VIP), and Chinese literature (Wan Fang) databases. Odds ratio (OR) and 95% confidence interval (95% CI) were used to assess the strength of associations.

Results: Seventeen studies including 3269 cases and 3192 controls met the inclusion criteria. Our meta-analysis showed that the MTR A2756G mutation may contribute to genetic susceptibility to the risk of idiopathic male infertility in Non-Asians, but not to Asian population, whereas the MTRR A66G polymorphism may be unrelated to idiopathic male infertility in both Non-Asian and Asian populations. In the stratified analysis by infertility type, the MTR A2756G polymorphism was a risk factor for both non-obstructive azoospermia (NOA) and oligoasthenoteratozoospermia (OAT) patients. However, the MTRR A66G polymorphism is associated with risk for OAT in Asian, but not in Non-Asian population.

Conclusion: This meta-analysis suggested that the MTR A2756G and MTRR A66G polymorphisms were risk factors for idiopathic male infertility. Studies with larger sample sizes and representative population-based cases and well-matched controls are needed to validate our results.

Abbreviations: CBM = China Biology Medical literature, CI = confidence interval, CNKI = China National Knowledge Infrastructure, HWE = Hardy-Weinberg equilibrium, MS = methionine synthase, MTHFR = methylenetetrahydrofolate reductase, MTR = methionine synthase, MTRR = methionine synthase reductase, N = number of studies, NOA = non-obstructive azoospermia, NOCS = Newcastle-Ottawa Scale, OAT = oligoasthenoteratozoospermia, OR = odds ratio, PCR = polymerase chain reaction, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, SNPs = single nucleotide polymorphisms, VIP = China Science and Technology Journal Database.

Keywords: idiopathic male infertility, meta-analysis, MTR A2756G polymorphism, MTRR A66G polymorphism
1. Introduction

Infertility, defined as the failure of a couple to achieve pregnancy after 1 year of unprotected, regular sexual intercourse, affects approximately 15% of all couples attempting to conceive a child. It is known to all that male infertility is a multifactorial and multigenetic disease with a complex pathogenesis that involves lifestyle, environmental risk factors, and individual genetic background. Studies have shown that genetic abnormalities account for approximately 15% of male infertility. Over the years, a large number of studies focusing on the association between male infertility and folate-related enzyme genetic polymorphisms have been carried out.

Folate is essential in DNA synthesis, RNA synthesis, methylation reactions, and protein synthesis, which contribute to the maintenance of genome integrity. Several key enzymes, including methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), and methionine synthase reductase (MTRR) are involved in the folate metabolic pathway. MTHFR catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the dominant circulating form of folate. Methionine as a precursor for S-adenosylmethionine, is produced via the transfer of a methyl group from 5-methyltetrahydrofolate, which is catalyzed by MTR and MTRR. Variations in the genes encoding the mentioned enzymes could be potential risk factors for male infertility. The single-nucleotide polymorphisms (SNPs) in the mentioned enzyme genes, such as MTHFR gene polymorphisms (MTHFR C677T, rs1801133 and MTHFR A1298C, rs1801131), a MS gene polymorphism (MS A2756G, rs1805087, also known as MTR A2756G), and MTRR gene polymorphism (MTRR A66G, rs1801394) have been reported frequently. The MTHFR C677T and A1298C polymorphisms have been investigated in previous meta-analyses for their role as risk factors for male infertility. However, the role of MTR A2756G and MTRR A66G polymorphisms in the folate metabolic pathway has not yet been fully evaluated for association with the risk of male infertility.

To date, a number of studies have investigated the association between MTR A2756G and MTRR A66G polymorphisms and idiopathic male infertility risk; however, the results remain controversial and previous studies have small patient sample sizes. In 2015, a meta-analysis was performed to explore the association between folate metabolism genes and idiopathic male infertility risk. Six case-control studies involving 1150 cases and 1082 controls were included to assess the association between MTR A2756G polymorphism and idiopathic male infertility risk in both Non-Asian and Asian populations. And no association was observed between MTR A2756G polymorphism and non-obstructive azoospermia (NOA) risk, whereas significant association was only observed in oligoasthenoteratozoospermia (OAT) subgroup in the homozygous genetic model. With regard to the MTRR A66G mutation, 7 case-control studies involving 1369 cases and 1330 controls were included in the meta-analysis. They did not detect any outstanding association between the A66G mutation and idiopathic male infertility risk in the overall analyses for all of the genetic models, and no significant risks were observed among the NOA and OAT types. However, in the subgroup analysis by ethnicity, an increased risk was observed in Asians but not in Europeans in the homozygous genetic model. In 2016, Karimian and Colagar conducted a meta-analysis of 7 case-control studies including 1724 cases and 1678 controls, and their results demonstrated that the MTR A2756G polymorphism is capable of causing idiopathic male infertility susceptibility in both Non-Asian and Asian populations. In 2017, Xu et al. conducted a meta-analysis of 7 case-control studies including 1438 cases and 1363 controls, and their results demonstrated that the MTRR A66G polymorphism may be unrelated to idiopathic male infertility in both Non-Asian and Asian populations. And in the stratified analysis by infertility type, the MTRR A66G polymorphism may be associated with OAT risk. Subsequently, a series of novel studies have been performed. In addition, the majority of relevant studies in the Chinese population published in local Chinese journals were not included in these meta-analyses. Therefore, to derive a more precise estimation of these associations, an updated meta-analysis based on 12 studies of MTR A2756G (2454 cases and 2525 controls) and 14 studies of MTR A66G (2620 cases and 2614 controls) was performed.

2. Materials and methods

2.1. Searching strategy

Medline, Embase, Web of Science, CNKI, CBM, VIP, and Wan Fang databases (up to Jun 21, 2019) were searched using the terms: “folate-related enzyme gene,” “MTR or methionine synthase reductase,” “MTR, MS, or methionine synthase polymorphism, mutation, or variant,” “male infertility.” In addition, the references of reviews and retrieved articles were also searched to identify other relevant publications. The search was limited to studies published in English or Chinese language. The search strategy flowchart is shown in Fig. 1. All analyses were based on previous published studies, thus no ethical approval and patient consent are required.

2.2. Inclusion criteria and exclusion criteria

The studies included in the present meta-analysis were required to meet the following criteria: studies with full text articles, published in English or Chinese; investigating the association between MTR A2756G or MTRR A66G polymorphism and idiopathic male infertility susceptibility; availability of genotype frequencies or allele frequencies for both cases and controls; case-control studies; sufficient genotype data were available for the estimation of odds ratios (ORs) and 95% confidence intervals (CIs). Studies were excluded for the following reasons: non-case-control studies; duplicated publications, animal studies, reviews, abstracts, and comments; lack of sufficient data for calculating genotype frequency.

2.3. Quality assessment

The quality of the included studies was assessed by the Newcastle-Ottawa Scale (NOS). The NOS contains 8 items for case-control studies. It is categorized into 3 aspects: selection, comparability, and exposure. The methodological quality is judged by a “star” rating system. Selection has a maximum of 4 stars, comparability has a maximum of 2 stars, and exposure has a maximum of 3 stars. Scores ranged from 0 stars (worst) to 9 stars (best). Studies were graded as “high quality” if the score was 7 to 9, “moderate quality” if the score was 4 to 6, and “low quality” if the score was <3.
2.4. Data extraction strategy

The following information was extracted from each study by 2 investigators independently: the first author’s name, year of publication, country, genotyping method; sample size of the study case and control groups; results of the Hardy-Weinberg equilibrium test (HWE). Extracted data were entered into a collection form and checked by a third author. Disagreements were resolved by discussion between the 2 investigators (BY and SD).

2.5. Statistical analysis

The relationships between MTRR A66G and MTR A2756G gene polymorphism with the idiopathic male infertility risks were analyzed by using 4 models including allele comparison model, dominant model, recessive model, and codominant model. The strength of the association between MTRR A66G and MTR A2756G gene polymorphism with the idiopathic male infertility risks were assessed by the OR with 95% CI. Chi-square-based Q test and the I² metric were used to assess heterogeneity between studies. The heterogeneity was considered significant when P < .10 and I² > 50%. If the P value for heterogeneity was <.10 and I² > 50%, the pooled ORs were analyzed using the random-effect model. In contrast, we applied the fixed-effect model if P ≥ .10 or I² ≤ 50%. The significance of the pooled OR was determined by the Z-test, and P < .05 was considered as statistically significant. The Reviewer Manager 5.3 (Cochrane Collaboration, http://ims.cochrane.org/revman) and Stata 12.0 (StataCorp LP, College Station, TX, USA) were used for all
statistical analyses. Begg test, Egger test, and funnel plots were used to statistically examine any publication bias. Sensitivity analysis was performed to evaluate the stability of the results by sequential removal of each study.

### 3. Results

#### 3.1. Study characteristics

A total of 376 results were retrieved after first search in Medline, Embase, Web of Science, CNKI, CBM, VIP, and Wan Fang databases. Of these studies, after the first screening, 339 studies were excluded based on inclusion and exclusion criteria. Finally, after our careful selection, 17 case–control studies considering 3269 cases and 3192 controls were included in this meta-analysis. Of these, there were 12 studies of $MTR_{A2756G}$ with 2454 cases and 2525 controls[^8-14,17-24] and 14 studies of $MTR_{A66G}$ with 2620 cases and 2614 controls[^8,9,17-21,23-29].

#### 3.2. Association of the $MTR_{A2756G}$ polymorphism with idiopathic male infertility

Twelve studies included 4979 individuals, which totally evaluated the influence of $MTR_{A2756G}$ polymorphism on the risk of idiopathic male infertility. Table 3 listed the main results of the meta-analysis of the associations between the $A2756G$ mutation and idiopathic male infertility risk. Figure 2 shows the meta-analysis results for the allele model (G vs A), additive model (GG vs AA), dominant model (GG+AG vs AA), and recessive model (GG vs AG+AA), for which the $I^2$ value, representing the among-study heterogeneity, was 52%, 0%, 42%, and 0%, respectively. Thus, random-effects models were applied in the allele model. Overall, the results revealed a significant association between the $MTR_{A2756G}$ polymorphism and idiopathic male infertility risks (G vs A: OR = 1.16, 95% CI = 1.00–1.32; GG vs AA: OR = 1.45, 95% CI = 1.40–2.37; GG+AG vs AA: OR = 1.40, 95% CI = 1.32–2.43; GG vs AG+AA: OR = 1.97, 95% CI = 1.45–2.68). Sub-group analyses were performed on data stratified by ethnicity, we found that the $MTR_{A2756G}$ polymorphism was associated with a significantly increased idiopathic male infertility risk in Non-Asian populations, but not in Asian population. In the stratified analysis by infertility type, we observed a significant association between $MTR_{A2756G}$ polymorphism and NOA risks, and similar results were observed in OAT risks. Furthermore, these data were further stratified by ethnicity, significant risks were observed between $MTR_{A2756G}$ polymorphism and OAT risk in both Non-Asian and Asian populations. However, we found that the

### Table 1

Characteristics of studies included in meta-analysis.

| Author     | Year | Country | Ethnicity | Genotyping Method | Case          | Control       | HWE   |
|------------|------|---------|-----------|------------------|---------------|---------------|-------|
| Fastc       | 2009 | Romania | Non-Asian | PCR-RFLP         | 65            | 67            | >0.05 |
| Gava       | 2011 | Brazil  | Non-Asian | TaqMan           | 133           | 171           | >0.05 |
| Kurzawski   | 2015 | Korea   | Asian     | PCR-RFLP         | 45            | 119           | >0.05 |
| Wu         | 2006 | Korea   | Asian     | PCR              | 360           | 325           | >0.05 |
| Li         | 2015 | China   | Asian     | Sequencing       | 162           | 120           | >0.05 |
| Murphy     | 2011 | Sweden  | Asian     | PCR              | 75            | 72            | >0.05 |
| Ni         | 2015 | China   | Asian     | SnaPshot         | 296           | 204           | >0.05 |
| Weirn      | 2014 | Russia  | Non-Asian | PCR-RFLP         | 273           | 281           | >0.05 |
| Wen-Bu      | 2010 | China   | Asian     | TaqMan           | 357           | 283           | >0.05 |
| MTR A66G    |      |         |           |                  |               |               |       |
| Balkan     | 2013 | Turkey  | Non-Asian | TaqMan           | 108           | 125           | >0.05 |
| Fastc       | 2009 | Romania | Non-Asian | PCR-RFLP         | 65            | 67            | >0.05 |
| Gava       | 2011 | Brazil  | Non-Asian | TaqMan           | 133           | 173           | >0.05 |
| Kurzawski   | 2015 | Korea   | Asian     | PCR-RFLP         | 85            | 246           | >0.05 |
| Lee        | 2006 | Korea   | Asian     | PCR              | 360           | 325           | >0.05 |
| Wu         | 2017 | China   | Asian     | PCR              | 138           | 102           | >0.05 |
| Mfady      | 2014 | Jordan  | Non-Asian | PCR             | 150           | 150           | >0.05 |
| Murphy     | 2011 | Sweden  | Non-Asian | PCR-RFLP         | 150           | 180           | >0.05 |
| Ni         | 2015 | China   | Asian     | SnaPshot         | 296           | 204           | >0.05 |
| Rawel      | 2009 | France  | Non-Asian | PCR-RFLP         | 252           | 114           | >0.05 |
| Weirn      | 2014 | Russia  | Non-Asian | PCR              | 272           | 324           | >0.05 |
| Xian-ju     | 2007 | China   | Asian     | PCR-RFLP         | 165           | 132           | >0.05 |

HWE = Hardy–Weinberg equilibrium, PCR = polymerase chain reaction, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism.
Figure 2. Forest plot of the studies assessing the association between MTR A2756G polymorphism and idiopathic male infertility. (A. Allelic model: G vs A; B. additive model: GG vs AA; C. dominant model: GG + GA vs AA; D. recessive model: GG vs GA + AA).

Table 2
Quality assessment for all of the included studies.

| First author          | Publishing year | Selection | Comparability | Exposure | Total |
|-----------------------|-----------------|-----------|---------------|----------|-------|
| Balkan[26]            | 2013            | ★★★       | ★★           | ★★       | 7     |
| Farcas[22]            | 2009            | ★★       | ★★             | ★★       | 6     |
| Garou[16]             | 2011            | ★★       | ★★             | ★★       | 6     |
| Karimian[14]          | 2016            | ★★★      | ★              | ★★       | 7     |
| Kim[19]               | 2015            | ★★★      | ★              | ★★       | 7     |
| Kurzawski[23]         | 2015            | ★★★      | ★★★           | ★★       | 7     |
| Lee[8]                | 2006            | ★★★      | ★★★           | ★          | 7     |
| LI[9]                 | 2015            | ★★★      | ★★★           | ★          | 7     |
| Liu[3]                | 2012            | ★★★      | ★★★           | ★          | 7     |
| Fang-Fang and Hsu-Zhao[28] | 2017        | ★★★       | ★★★           | ★          | 5     |
| Mtady[25]             | 2013            | ★★★      | ★★★           | ★          | 7     |
| Murphy[17]            | 2011            | ★★★      | NA            | ★         | 5     |
| Ni[8]                 | 2015            | ★★★      | NA            | ★         | 5     |
| Ravel[27]             | 2009            | ★★★      | ★★             | ★        | 6     |
| Weiner[9]             | 2014            | ★★★      | ★              | ★        | 8     |
| Xian-Jun and Chao-Jun[29] | 2007        | ★★★      | ★★★           | ★          | 7     |
| Wen-Bo et al[24]      | 2010            | ★★★      | ★★★           | ★          | 7     |

Table 3
Meta-analysis of the association of MTR A2756G polymorphism with idiopathic male infertility.

| Infertility | N   | G vs A [OR, 95% CI] | GG vs AA [OR, 95% CI] | GG + GA vs AA [OR, 95% CI] | GG vs GA + AA [OR, 95% CI] |
|-------------|-----|---------------------|------------------------|-----------------------------|-----------------------------|
| Non-Asian   | 6   | 1.28 [1.02, 1.59]   | 2.18 [1.54, 3.09]      | 1.21 [0.93, 1.55]           | 2.12 [1.51, 2.98]           |
| Asian       | 6   | 1.08 [0.89, 1.31]   | 1.49 [0.73, 3.02]      | 1.06 [0.86, 1.31]           | 1.45 [0.72, 2.98]           |
| Overall     | 12  | 1.18 [1.00, 1.40]   | 2.03 [1.48, 2.77]      | 1.16 [1.02, 1.32]           | 1.97 [1.45, 2.68]           |
| NOA         |     |                     |                        |                             |                             |
| Non-Asian   | 3   | 1.56 [1.25, 1.95]   | 2.53 [1.54, 4.15]      | 1.51 [1.14, 2.01]           | 2.36 [1.46, 3.82]           |
| Asian       | 3   | 1.04 [0.77, 1.39]   | 0.93 [0.28, 3.07]      | 1.05 [0.76, 1.45]           | 0.92 [0.28, 3.02]           |
| Overall     | 6   | 1.34 [1.12, 1.59]   | 2.15 [1.36, 3.37]      | 1.29 [1.04, 1.59]           | 2.04 [1.31, 3.17]           |
| OAT         |     |                     |                        |                             |                             |
| Non-Asian   | 3   | 1.63 [1.30, 2.05]   | 3.12 [1.90, 5.11]      | 1.48 [1.11, 1.99]           | 2.97 [1.84, 4.81]           |
| Asian       | 3   | 1.59 [1.19, 2.14]   | 3.31 [1.27, 8.03]      | 1.55 [1.11, 2.15]           | 3.08 [1.19, 8.00]           |
| Overall     | 6   | 1.62 [1.35, 1.94]   | 3.16 [2.03, 4.91]      | 1.51 [1.21, 1.88]           | 3.00 [1.95, 4.61]           |

NOA = non-obstructive azoospermia, OAT = including oligoasthenoteratozoospermia, oligozoospermia, asthenozoospermia, and teratozoospermia.
MTR A2756G polymorphism was associated with a significantly increased NOA risk in Non-Asian population, but not in Asian population.

### 3.3. Association of the MTRR A66G polymorphism with idiopathic male infertility

Fourteen studies included 5234 individuals, which totally evaluated the influence of MTRR A66G polymorphism on the risk of idiopathic male infertility. The main results of the meta-analysis on the association between the MTRR A66G polymorphism and risk of idiopathic male infertility were listed in detail in Table 4. Figure 3 shows the overall meta-analysis results for the allele model (G vs A), additive model (GG vs AA), dominant model (GG+AG vs AA), and recessive model (GG vs AG+AA), for which the $I^2$ value, representing the among-study heterogeneity, was 47%, 36%, 31%, and 26%, respectively. Thus, random-effects models were applied in the allele model. Overall, the results revealed no significant association between MTRR A66G polymorphism and idiopathic male infertility risks (G vs A: OR = 1.06, 95% CI = 0.95–1.19; GG vs AA: OR = 1.20, 95% CI = 0.95–1.51; GG+AG vs AA: OR = 1.05, 95% CI = 0.93–1.19; GG vs AG+AA: OR = 1.13, 95% CI = 0.98–1.31). Subgroup analyses were performed by ethnicity, we observed a significant association between MTRR A66G polymorphism and idiopathic male infertility risks in Asian population in recessive model (GG vs AG+AA: OR = 1.44, 95% CI = 1.09–1.90), whereas there was no significantly elevated infertility risks associated with the MTRR A66G polymorphism and idiopathic male infertility in Non-Asian population in any of the genetic models. Furthermore, in the stratified analysis by infertility type, we observed a significant association between MTRR A66G polymorphism and idiopathic male infertility in Non-Asian population in any of the genetic models. Furthermore, these data were further stratified by ethnicity, no significant risks were observed between MTRR

### Table 4

**Meta-analysis of the association of MTRR A66G polymorphism with idiopathic male infertility.**

| Infertility | N   | G vs A [OR, 95% CI] | GG vs AA [OR, 95% CI] | GG + GA vs AA [OR, 95% CI] | GG vs GA + AA [OR, 95% CI] |
|-------------|-----|---------------------|----------------------|-----------------------------|-----------------------------|
| Non-Asian   | 8   | 1.05 [0.94, 1.17]   | 1.10 [0.88, 1.37]    | 1.06 [0.89, 1.27]           | 1.03 [0.87, 1.23]           |
| Asian       | 6   | 1.07 [0.84, 1.37]   | 1.33 [0.80, 2.22]    | 0.99 [0.71, 1.38]           | 1.44 [1.09, 1.90]           |
| Overall     | 14  | 1.06 [0.95, 1.19]   | 1.20 [0.95, 1.51]    | 1.05 [0.93, 1.19]           | 1.13 [0.98, 1.31]           |
| NOA         |     |                     |                      |                             |                             |
| Non-Asian   | 4   | 0.94 [0.77, 1.13]   | 0.89 [0.60, 1.32]    | 0.96 [0.70, 1.33]           | 0.94 [0.69, 1.27]           |
| Asian       | 4   | 0.90 [0.75, 1.08]   | 0.93 [0.58, 1.49]    | 0.82 [0.63, 1.05]           | 1.01 [0.65, 1.57]           |
| Overall     | 8   | 0.92 [0.81, 1.05]   | 0.91 [0.67, 1.23]    | 0.87 [0.71, 1.06]           | 0.96 [0.75, 1.23]           |
| OAT         |     |                     |                      |                             |                             |
| Non-Asian   | 3   | 1.11 [0.90, 1.36]   | 1.24 [0.81, 1.91]    | 1.15 [0.80, 1.66]           | 1.21 [0.89, 1.65]           |
| Asian       | 3   | 1.33 [1.09, 1.63]   | 2.02 [1.20, 3.38]    | 1.62 [1.17, 2.29]           | 1.59 [1.02, 2.48]           |
| Overall     | 6   | 1.22 [1.05, 1.40]   | 1.51 [1.08, 2.10]    | 1.39 [1.09, 1.78]           | 1.32 [1.02, 1.70]           |

N = number of studies, NOA = non-obstructive azoospermia, OAT = including oligoasthenoteratozoospermia, oligozoospermia, asthenozoospermia, and teratozoospermia.
A66G polymorphism and NOA risk both in Non-Asian and Asian populations. However, significant association was observed between MTRR A66G polymorphism and OAT risk in Asian population, but not in Non-Asian population.

3.4. Sensitivity analyses and publication bias

No evidence of obvious asymmetry was observed in the funnel plots in any of the models on the 2 SNPs (Figs. 4 and 5). In addition, Begg tests and Egger tests were used to calculate the potential publication bias, and no indication of publication bias was identified in the studies on the 2 SNPs (Table 5). The sensitivity analyses were performed to investigate the pooled ORs through excluding one study each time, and the results showed no individual study had substantial influence on the overall pooled ORs in all genetic models (Figs. 6 and 7). That is to say, the results of this meta-analysis are relatively stable.

4. Discussion

The folate metabolism pathway plays an important role in DNA methylation, DNA repair, and DNA synthesis. Abnormal folate metabolism has been proposed as a factor in male infertility. MTR and MTRR are the key enzymes implicated in the folate metabolic pathways and are crucial for DNA methylation and spermatogenesis. MTR is critical for homocysteine metabolism, and MTRR is required to maintain MTR in its active state. The SNPs of these folate-related enzymes gene can impair folate absorption or disturb the balance between folate derivatives by impacting the activity, stability, or level of the corresponding enzymes. The mechanisms of pathogenesis may involve changes of enzyme structure and mRNA properties that are due to these folate-related enzymes gene polymorphisms.

There is increasing evidence investigating the association between MTR A2756G and MTRR A66G polymorphisms and risk of idiopathic male infertility. Gava et al. reported that the combinatory analysis of the MTRR A66G polymorphism did not show difference between cases and controls, and the MTR A2756G polymorphism could be an important genetic factor predisposing to idiopathic infertility in Brazilian men. Li et al. revealed an association between the SNP A66G in the MTRR gene and idiopathic male infertility, no significant association was found between SNP A2756G in the MS gene and idiopathic male infertility risk in Chinese population. However, Farcas et al. found there is no significant association of SNP A2756G in the MS gene or SNP A66G in the MTRR gene with idiopathic male infertility risk in Romanian population group. Similarly, Kurzawski et al. found that the MTR A2756G and MTRR A66G polymorphism are not major risk factors for non-obstructive idiopathic male infertility in the Polish population.
This difference among studies may be due to small sample sizes, study differences in genotyping method or population substructure, or other factors. Despite the fact that several meta-analyses have been performed to evaluate the association between MTR A2756G, MTRR A66G polymorphisms and risk of idiopathic male infertility, to the best of our knowledge, this is the most updated meta-analysis which included 12 studies of MTRR A2756G with 4979 individuals and 14 studies of MTR A66G with 5234 individuals. In the present study, significant association was observed between MTR A2756G polymorphism and idiopathic male infertility in the overall population. In a subgroup analysis by nationality, we have found a significant association.

**Table 5**

| Comparisons | Coefficient | Egger test | 95% CI | Begg test |
|-------------|-------------|------------|--------|-----------|
|             |             | P value    |        | P value   |
| **MTR A2756G** |             |            |        |           |
| G vs A      | −1.430      | .309       | −4.402–1.541 | .373      |
| GG vs AA    | −0.906      | .165       | −2.260–0.449 | .276      |
| GG+AG vs AA | −0.929      | .526       | −4.082–2.223 | .837      |
| GG vs AG+AA | −0.896      | .135       | −2.131–0.338 | .350      |
| **MTRR A66G** |             |            |        |           |
| G vs A      | −0.586      | .725       | −4.139–2.967 | .228      |
| GG vs AA    | −0.499      | .699       | −3.245–2.248 | .443      |
| GG+AG vs AA | 0.552       | .744       | −3.047–4.150 | 1.000     |
| GG vs AG+AA | −0.015      | .988       | −2.067–2.037 | .913      |

*P < .05 was considered as statistically significant.*
between the MTR A2756G polymorphism and idiopathic male infertility in Non-Asians, but not in Asian population, which was consistent with a previous meta-analysis based on Chinese population.

Similarly, the MTR A2756G polymorphism was significantly associated with NOA and OAT in Non-Asian, but not in Asian population in any of the genetic models. In a more recent meta-analysis by Karimian and Colagar, they have suggested that MTR A2756G polymorphism was associated with idiopathic male infertility in both Non-Asian and Asian populations. In addition, they have found that MTR A2756G polymorphism significantly associated with NOA and OAT risk in the overall population. As regard to the MTRR A66G polymorphism, no significant association was found with idiopathic male infertility in the overall population, however, we have observed that MTRR A66G polymorphism was significantly associated with OAT in Asians but not Non-Asians. A previous meta-analysis conducted by Xu et al have suggested MTRR A66G polymorphism was not associated with idiopathic male infertility in both Non-Asian and Asian populations, however, in a subgroup analysis by infertility type, significant association was found between MTRR A66G polymorphism and OAT. A more recent meta-analysis conducted by Shi et al have suggested MTRR A66G polymorphism was significantly associated with idiopathic male infertility in Asian populations. The present study, however, has several advantages compared with the previous meta-analyses. First, more recently-published studies were included in the present meta-analysis, which may underscore the reliability of our findings. Second, Chinese database were searched to more comprehensively assess studies in Chinese populations. Third, the present study added additional subgroup analyses by ethnicity to evaluate the relationship of the 2 SNPs with NOA and OAT. These advantages allowed us to more precisely assess the MTR A2756G, MTRR A66G polymorphisms and idiopathic male infertility risk associations than previous meta-analyses.

Some limitations of the present study should be considered when interpreting the results. First, for subgroup analysis, the number of included studies was relatively small, therefore, limited data were available. Second, we did not estimate the potential gene–gene and gene–environment interactions due to the lack of information available in the original studies. Third, other clinical data, such as sources of control, subject age, and semen quality, were not considered here due to a lack of information. Finally, our meta-analysis was based on published articles, and there was no sufficient data for adjustment for individual level factors including diet, obesity, and smoking, which might affect the genetic effect.

5. Conclusion

In summary, this meta-analysis provides evidence that the MTR A2756G polymorphism may contribute to genetic susceptibility
to the risk of idiopathic male infertility in Non-Asian, but not to Asian population. The MTRR A66G polymorphism is associated with risk for OAT in Asian, but not in Non-Asian population. Nevertheless, more large sample and representative population-based cases and well-matched controls are needed to validate our results.

Author contributions

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