

Original Article

Methionine synthase reductase A66G polymorphism and risk of male infertility: a meta-analysis

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Abstract: This study aimed to evaluate the association between *MTRR* A66G polymorphism and male infertility risk. A search of PubMed, Embase, the Cochrane Library, and Web of Science was conducted up to March 31, 2018. Odds ratios (OR) and 95% confidence intervals (95% CI) were used to assess strength of associations. Twelve studies, including 2,367 cases and 2,380 controls, were included in this meta-analysis. Overall results indicated that *MTRR* A66G polymorphism might not be associated with increased risk of male infertility in the allele model (G vs. A: OR = 1.04, 95% CI = 0.96-1.14), additive model (GG vs. AA: OR = 1.14, 95% CI = 0.94-1.40), dominant model (GG+GA vs. AA: OR = 1.04, 95% CI = 0.91-1.20), and recessive model (GG vs. GA+AA: OR = 1.58, 95% CI = 1.31-1.90). However, according to subgroup analysis based on infertility type, significant association was found with oligoasthenozoospermia (OAT) in the allele model (G vs. AOR = 1.25, 95% CI = 1.07-1.45), additive model (GG vs. AA: OR = 1.61, 95% CI = 1.14-2.28), dominant model (GG+GA vs. AA: OR = 1.47, 95% CI = 1.13-1.92), and recessive model (GG vs. GA+AA: OR = 1.37, 95% CI = 1.05-1.78). Sensitivity analysis indicated that the results of this meta-analysis were relatively stable. In conclusion, this study suggests that *MTRR* A66G polymorphism may contribute to genetic susceptibility to OAT, but not to non-obstructive azoospermia (NOA). Studies with larger sample sizes and representative population-based cases are necessary to validate present results.

Keywords: Methionine synthase reductase, *MTRR* gene, polymorphism, male infertility, meta-analysis

Introduction

Infertility has been defined as the inability of a couple to conceive pregnancy after one year of unprotected and regular sexual intercourse, affecting 10%~20% of couples wishing to have children [1-3]. Of these, half of these cases are associated with male factors [4, 5]. The etiology of male infertility in approximately 50% of infertile men is still not well understood. It has been suggested that genetic factors contribute up to 15~30% in male factor infertility [6-8]. Previous studies have reported that some genetic mutations in folate-related enzyme genes, such as *MTHFR* C677T and *MTRR* A66G polymorphisms, may be associated with risk of male infertility [6, 9]. These findings have been supported by subsequent meta-analyses [10, 11]. Foliates are a group of inter-convertible co-enzymes that play essential roles in DNA synthesis, methylation reactions, and protein syn-

thesis [12]. Folate deficiency may impair the function of these metabolic pathways and result in homocysteine (Hcy) accumulation, considered a risk factor for male infertility [13]. In addition, variations in the gene encoding key enzymes involved in folate metabolism can affect the activity, stability, and levels of folate metabolism-related enzymes, which may affect folate metabolism and DNA synthesis [14, 15]. Moreover, folate metabolism disorder may lead to sperm DNA damage and spermatogenic failure, resulting in male infertility.

Many studies have investigated the association between *MTRR* A66G polymorphism and risk of male infertility. However, results have been controversial and previous studies have generally been small. In 2015, a meta-analysis based on 6 case-control studies, including 1,249 cases and 1,160 controls, was performed. The authors concluded that *MTRR* A66G polymor-

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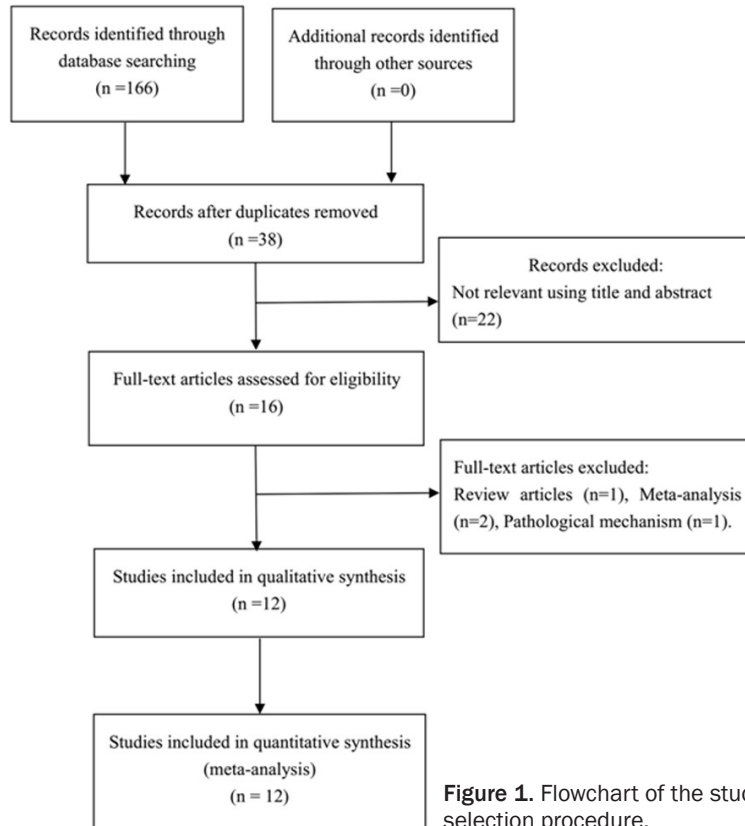


Figure 1. Flowchart of the study selection procedure.

phism was capable of causing male infertility susceptibility in Asians, but not in Caucasians [11]. In 2017, Xu et al. conducted a meta-analysis of 7 case-control studies, including 1,633 cases and 1,735 controls. Their results demonstrated that *MTRR* A66G polymorphism was not associated with male infertility in either Asians or Caucasians [10]. Subsequently, a series of novel studies have been performed. Therefore, the present meta-analysis based on 12 studies of *MTRR* A66G polymorphism (2,317 cases and 2,380 controls) was performed to clarify the effects of *MTRR* A66G polymorphisms on risk of male infertility.

Methods

Search strategy

PubMed, Embase, the Cochrane Library, and Web of Science (up to March 31, 2018) were searched for studies evaluating the association of *MTRR* A66G polymorphism with male infertility in humans. The following search terms were used: “folate-related enzyme gene” or “*MTRR*” or “methionine synthase reductase” and “SNP” or “polymorphism” or “mutation” or

“variant” and “male infertility”. In addition, a manual search was conducted to find more studies based on references listed in individual articles. The search strategy flowchart is shown in **Figure 1**.

Inclusion and exclusion criteria

Inclusion criteria of literature were as follows: 1) Full text of the article was available; 2) The study was a case-control study investigating the association between *MTRR* A66G polymorphism and male infertility; 3) Genotype distributions were available for both cases and controls; 4) There was no duplicate data. For studies that considered partially or fully duplicated data and that were by the same authors, the study with the most subjects was selected; 5) The published language

was English or Chinese; and 6) Genotypic distributions were available for the estimation of odds ratios (ORs) and 95% confidence intervals (CIs). An article was excluded if: 1) It was not a study of the association between *MTRR* A66G polymorphism and male infertility risk; and 2) The article was an animal study, review article, meta-analysis, conference abstract, or editorial article.

Quality assessment

The Newcastle-Ottawa Scale (NOS) [16] was used to assess the quality of included studies. NOS contains eight items for both cohort and case-control studies. The scale assesses the quality of case-control studies based on three areas: selection, comparability, and exposure. A star rating system was used to judge methodological quality. Selection had a maximum of 4 stars, comparability had a maximum of 2 stars, and exposure had a maximum of 3 stars. Total scores ranged from 0 stars (worst) to 9 stars (best) and the quality of each study was graded as low (0±3), moderate (4±6), or high (7±9). Discrepant opinions were resolved by discussion and consensus.

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Table 1. Characteristics of the studies included in the meta-analysis and their genotype distributions of the MTRR A66G gene polymorphism

Author	Year	Contry	Ethnicity	Case					Control					HWE	NOS		
				Case	Control	AA	AG	GG	A	G	AA	AG	GG			A	G
Balkan et al.	2013	Turkey	Caucasian	108	125	36	52	20	124	92	35	61	29	131	119	> 0.05	7
Farcas et al.	2009	Romania	Caucasian	65	67	13	46	6	72	58	18	42	7	78	56	< 0.05	6
Gava et al.	2011	Brazil	Caucasian	133	173	37	62	34	136	130	59	84	30	202	144	> 0.05	6
Kim et al.	2015	Korea	Asian	85	246	52	29	4	133	37	125	107	14	357	135	> 0.05	7
Kurzawski et al.	2015	Poland	Caucasian	284	352	51	139	94	241	327	70	171	111	311	393	> 0.05	7
Lee et al.	2006	Korea	Asian	360	325	64	250	46	378	342	72	224	29	368	282	< 0.05	7
Li et al.	2015	China	Asian	162	120	83	65	14	231	93	70	44	6	184	56	> 0.05	6
Mfady et al.	2013	Jordan	Caucasian	150	150	48	78	24	174	126	61	67	22	189	111	> 0.05	7
Murphy et al.	2011	Sweden	Caucasian	150	180	50	68	32	168	132	60	88	32	208	152	> 0.05	7
Ni et al.	2015	China	Asian	296	204	158	119	19	435	157	99	91	14	289	119	> 0.05	6
Ravel et al.	2009	France	Caucasian	252	114	27	132	80	186	292	12	57	42	81	141	> 0.05	6
Weiner et al.	2014	Russia	Caucasian	272	324	54	136	82	244	300	57	170	97	284	364	> 0.05	7

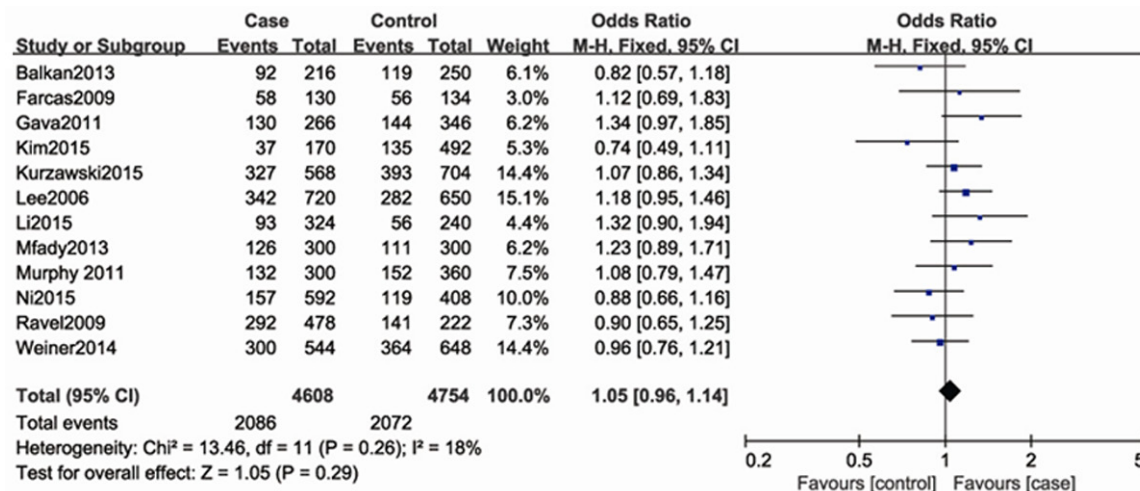


Figure 2. Forest plot of studies assessing the association between MTRR A66G, MTHFR polymorphism, and male infertility. (Allelic model: G vs. A).

Data extraction strategy

Two authors checked and extracted the relevant data, independently, in compliance with inclusion criteria. Extracted data was entered in a collection form and checked by a third author. Disagreements were solved by discussion and consensus. The following information was extracted: 1) First author's name, year of publication, country, and genotyping method; 2) Number of cases and controls; 3) Genotype and allele frequencies; and 4) Results of Hardy-Weinberg equilibrium testing.

Statistical analysis

The relationship between MTRR A66G polymorphism and male infertility was assessed by

determining pooled ORs and 95% CIs for the allele comparison model, dominant model, recessive model, and codominant model. Statistical heterogeneity among studies was estimated using the Q-test and I² statistics. I² statistics was used to measure the degree of heterogeneity (I² = 0%-20%, no heterogeneity; I² = 20%-50%, moderate heterogeneity; I² > 50%, obvious heterogeneity). A random-effects model was used to estimate pooled ORs and 95% CIs. Heterogeneity was found with P < 0.10 or I² > 50%. Statistical analysis was performed with Reviewer Manager 5.3 and STATA 12.0. Potential publication bias was estimated using funnel plots and Egger's regression test. Sensitivity analysis was performed to evaluate the stability of results.

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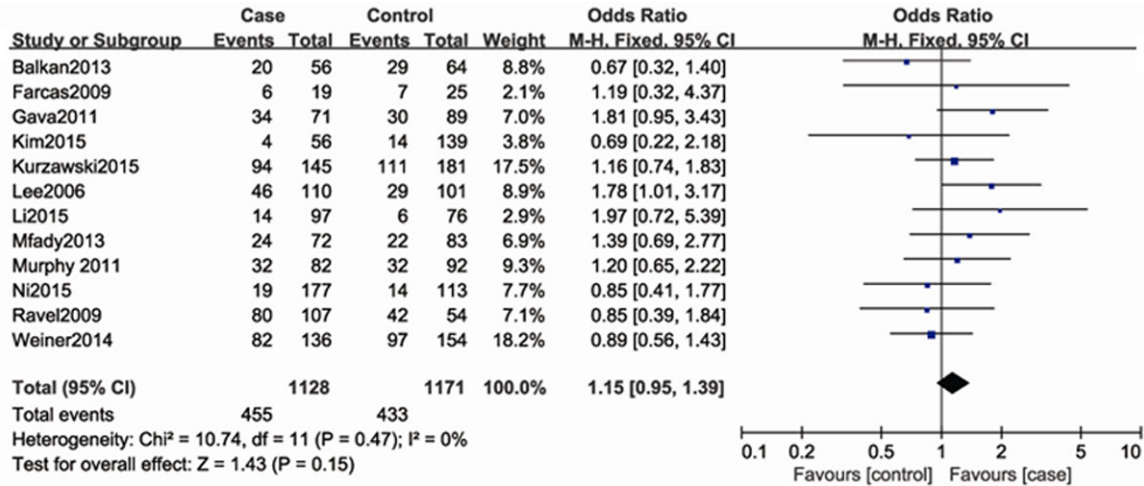


Figure 3. Forest plot of studies assessing the association between *MTRR* A66G, MTHFR polymorphism, and male infertility. (Additive model: GG vs. AA).

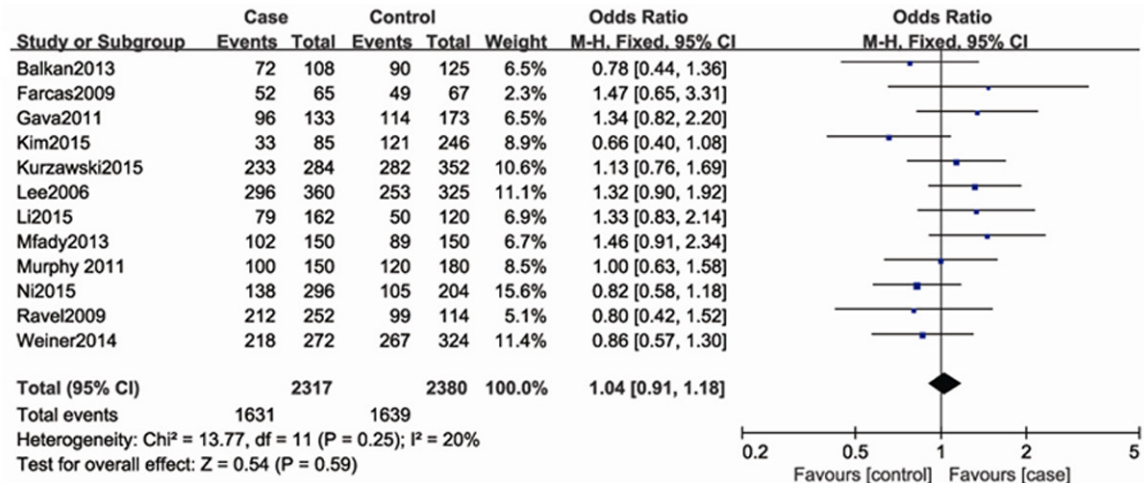


Figure 4. Forest plot of studies assessing the association between *MTRR* A66G, MTHFR polymorphism, and male infertility. (Dominant model: GG+AG vs. AA).

Results

Study characteristics

A total of 12 case-control articles, considering 2,367 cases and 2,380 controls, were included in this meta-analysis [9, 17-25]. Seven studies were conducted in Caucasian populations and five involved Asian populations. The studies were published between 2006 and 2017. Hardy-Weinberg testing (HWE) was performed on all included studies. HWE of the *MTRR* A66G polymorphism was violated in one study. Characteristics of the case-control studies included in this meta-analysis are summarized in **Table 1**.

Association of *MTRR* A66G polymorphism with male infertility

Figures 2-5 and **Table 2** summarize the results of the meta-analysis. The between-study heterogeneity of four genetic models of *MTRR* A66G polymorphism was low (I² range: 0-27%; P-value range: 0.19-0.59). Therefore, a fixed-effects model was used for pooling the association between *MTRR* A66G polymorphism and risk of male infertility. Overall, no significant association was found between *MTRR* A66G polymorphism and male infertility in any of the comparison models used. ORs and 95% CIs for each model were as follows: 1.04 [0.96, 1.14] in the allele model (G vs. A), 1.14 [0.94-1.40] in

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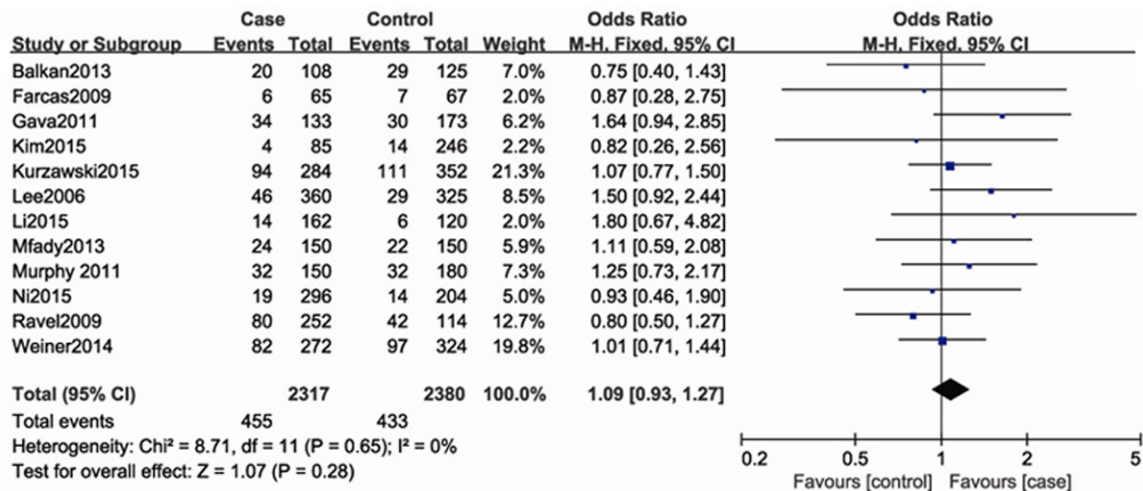


Figure 5. Forest plot of studies assessing the association between *MTRR* A66G, *MTHFR* polymorphism, and male infertility. (Recessive model: GG vs. AG+AA).

Table 2. Meta-analysis of the association of *MTRR* A66G polymorphism with male infertility

<i>MTRR</i> A66G					
Ethnicity	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)
Caucasian	7	1.07 [0.95, 1.19]	1.12 [0.89, 1.41]	1.09 [0.91, 1.31]	1.09 [0.91, 1.31]
Asian	5	1.02 [0.89, 1.16]	1.21 [0.86, 1.70]	0.98 [0.80, 1.19]	1.08 [0.82, 1.43]
Overall	12	1.05 [0.96, 1.14]	1.15 [0.95, 1.39]	1.04 [0.91, 1.18]	1.09 [0.93, 1.27]
Azoospermia					
Ethnicity	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)
Caucasian	3	0.97 [0.78, 1.20]	0.93 [0.61, 1.42]	0.91 [0.65, 1.28]	1.01 [0.71, 1.42]
Asian	4	0.93 [0.77, 1.11]	0.99 [0.62, 1.58]	0.91 [0.69, 1.19]	0.95 [0.65, 1.41]
Overall	7	0.94 [0.82, 1.08]	0.95 [0.70, 1.31]	0.91 [0.74, 1.13]	0.98 [0.76, 1.27]
OAT					
Ethnicity	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)
Caucasian	2	1.21 [0.93, 1.56]	1.43 [0.65, 3.14]	1.12 [0.73, 1.72]	1.44 [0.97, 2.14]
Asian	3	1.27 [0.94, 1.73]	1.76 [0.79, 3.91]	1.74 [1.24, 2.44]	1.32 [0.93, 1.87]
Overall	5	1.25 [1.07, 1.45]	1.61 [1.14, 2.28]	1.47 [1.13, 1.92]	1.37 [1.05, 1.78]

the additive model (GG vs. AA), 1.04 [0.91-1.20] in the dominant model (GG+GA vs. AA), and 1.58 [1.31-1.90] in the recessive model (GG vs. GA+AA).

Subgroup analyses were performed on data stratified by ethnicity. No association was found between *MTRR* A66G polymorphism and male infertility risk in both Caucasians and Asians in any of the genetic models (**Table 2**). Subgroup analyses were performed on data stratified by male infertility type. Significant association was found between *MTRR* A66G polymorphism and increased OAT risk, whereas no significant association between *MTRR* A66G polymorphism and NOA risk was found in any of

the genetic models. Results of subgroup analyses for all genetic models are listed in detail in **Table 2**. There was no significant heterogeneity in any genotype contrasts among the studies and fixed-effects models were applied.

Sensitivity and publication bias

Publication bias was assessed for the *MTRR* A66G polymorphism by funnel plots and Egger's test under all contrast models. The shape of the funnel plot did not indicate any evidence of obvious asymmetry in any contrast model for the *MTRR* A66G polymorphism (**Figure 6**). In addition, Egger's linear regression analysis suggested no evidence of publication bias (P =

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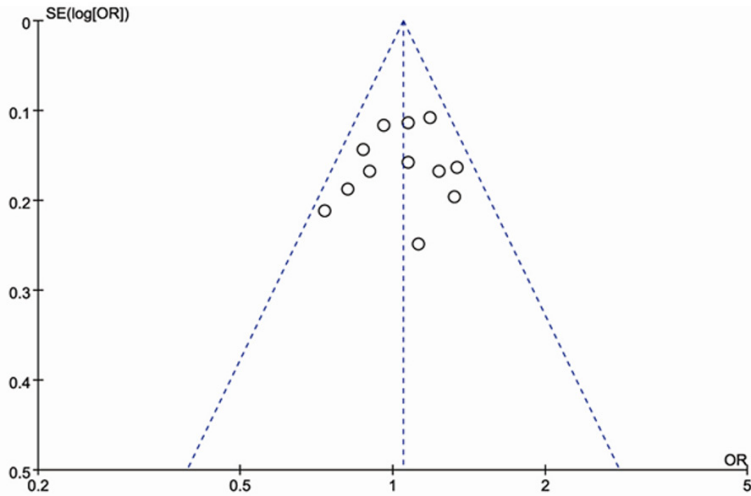


Figure 6. Funnel plot for the *MTRR* A66G polymorphism and male infertility risk. (Allelic model: G vs. A).

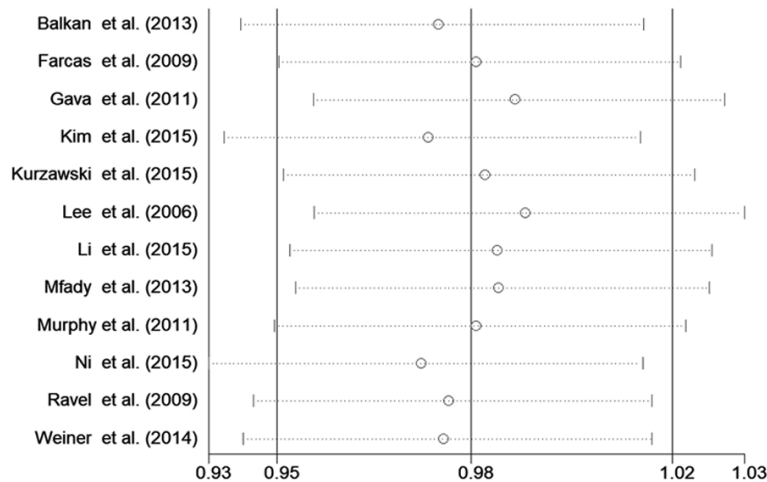


Figure 7. Sensitivity analysis diagram for each study used to assess the relative risk estimates for the *MTRR* A66G polymorphism and male infertility in all included studies. (Allelic model: G vs. A).

0.658 for an allelic contrast model, $P = 0.847$ for an additive model, $P = 0.936$ for a recessive model, and $P = 0.792$ for a dominant model). Sensitivity analyses were conducted to calculate the pooled ORs by omitting one study each time. Results revealed that no individual study influenced the overall pooled ORs (**Figure 7**), indicating that the results of this meta-analysis were relatively stable.

Discussion

There is considerable experimental evidence that key enzymes in the folate metabolism are

essential for male infertility. Folate-related enzymes are key enzymes implicated in the folate metabolic pathways, crucial for DNA methylation and spermatogenesis [26, 27]. The single nucleotide polymorphisms (SNPs) of these folate-related enzymes gene can impair folate absorption or disturb the balance between folate derivatives by impacting the activity, stability, or levels of the corresponding enzymes [28, 29]. Recent studies have revealed that *MTRR* A66G polymorphisms are associated with an increased risk of male infertility. Two meta-analyses reported associations between *MTRR* A66G polymorphism and male infertility risk [10, 11]. However, only seven studies were included in their study, the sample size was small, and the results were inconsistent. Hence, the present meta-analysis was performed to obtain a more precise evaluation. The present meta-analysis, including 12 studies and involving 2,367 cases and 2,380 controls, comprehensively evaluated associations between *MTRR* A66G polymorphism and risk of male infertility. Overall analyses showed that no significant association was found between *MTRR* A66G

polymorphism and male infertility risk in both Caucasian and Asian populations. Subgroup analyses showed that *MTRR* A66G polymorphism was associated with increased OAT risk. According to a meta-analysis conducted in 2015, association between the A66G mutation and male infertility risk was observed in Asians but not in Europeans, whereas no significant risks were observed among the azoospermia and OAT types. According to another meta-analysis conducted in 2017, no association between the A66G mutation and male infertility risk was observed both in Asians and Europeans, while significant association was found with OAT.

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These inconsistent results may be due to different search strategies. The two meta-analyses did not include all relevant studies.

Balkan et al. found no association between *MTRR* A66G polymorphism and both the NOA group and SO group in Brazilian men. However, Lee et al. found a significant association observed between *MTRR* A66G polymorphism control and male infertility in Korean men, especially for OAT. The present study revealed that *MTRR* A66G polymorphism significantly associated with OAT risk. Inconsistencies between the studies could have arisen from geographic variations, racial, and ethnic differences in the distribution of polymorphisms in *MTRR* gene. However, only 12 studies were included in the present meta-analysis. Five studies reported the relationship between *MTRR* A66G polymorphism and OAT risk, thus the sample size was small. Studies with high-quality and larger sample sizes are necessary to further investigate the potential relationship of *MTRR* A66G polymorphism with male infertility risk.

There were some limitations to the present meta-analysis. First, only eleven studies were included. The sample size of included published articles was small, thus ample data was unavailable. Second, absence of original data, such as age, smoking, drinking, and family history, may have affected the precision of association of *MTRR* A66G transition and male infertility. Third, this study did not estimate potential interactions among gene-gene and gene-environment due to the lack of information in the original studies. Fourth, this meta-analysis lacked data from African populations.

Conclusion

In summary, the present meta-analysis provides evidence that *MTRR* A66G polymorphisms may contribute to genetic susceptibility to OAT risk. However, large-scale, well-designed, and population-based studies are necessary to confirm present findings.

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Disclosure of conflict of interest

None.

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