Systematic Review and Meta-Analysis

Medicine®

OPEN

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

Zheng-Ju Ren, MD[a], Yan-Ping Zhang, MD[b], Peng-Wei Ren, MD[c], Bo Yang, MD[a], Shi Deng, MD[a], Zhu-Feng Peng, MD[a], Liang-Ren Liu, MD[a], WuRan Wei, MD[a], Qiang Dong, MD[a,*]

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, NOS = 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

Editor: Ziyuan Zhou.

Z-JR and Y-PZ authors have contributed equally to this work and should be considered co-first authors.

This work was supported by Science and Technology Support Project of Science and Technology Department of Sichuan Province, Grant ID: 2016FZ0103; and Key Research and Development Project of Science and Technology Department of Sichuan Province, Grant ID: 2017SZ0067; and 1.3.5 project for disciplines of excellence, West China Hospital, Sichuan University; Grant ID: ZY2016104. The study sponsor had no role in the design and conduct of the study; collection, management, analysis and interpretation of the data; preparation, review or approval of the article; and decision to submit the article for publication.

The authors have no conflicts of interest to disclose.

[a] Department of Urology, Institute of Urology, West China Hospital, Sichuan University, Chengdu, Sichuan, [b] Department of Anesthesiology, Chongqing Traditional Chinese Medicine Hospital, Chongqing, [c] Department of Evidence-Based Medicine and Clinical Epidemiology, West China Hospital, Sichuan University, Chengdu, Sichuan, China.

[*] Correspondence: Qiang Dong, West China School of Medicine/West China Hospital, Sichuan University, 37, Guo Xue Road, Chengdu 610041, Sichuan Province, China (e-mail: dqiang666@163.com).

How to cite this article: Ren ZJ, Zhang YP, Ren PW, Yang B, Deng S, Peng ZF, Liu LR, Wei W, Dong Q. Contribution of MTR A2756G polymorphism and MTRR A66G polymorphism to the risk of idiopathic male infertility. Medicine 2019;98:51(e18273).

Received: 4 May 2018 / Received in final form: 2 September 2019 / Accepted: 7 November 2019

http://dx.doi.org/10.1097/MD.0000000000018273

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.[1–3] 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.[4,5] Studies have shown that genetic abnormalities account for approximately 15% of male infertility.[6] 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.[7,8] Several key enzymes, including methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), and methionine synthase reductase (MTRR) are involved in the folate metabolic pathway.[9] 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.[10,11] Variations in the genes encoding the mentioned enzymes could be potential risk factors for male infertility.[12] 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 several 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.[13] Six case–control studies involving 1150 cases and 1082 controls were include to assess the association between MTR A2756G polymorphism and idiopathic male infertility risk, and the results showed no 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.[13] In 2016, Karimian and Colagar[14] 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[15] 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," "MTRR 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).[16] 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.

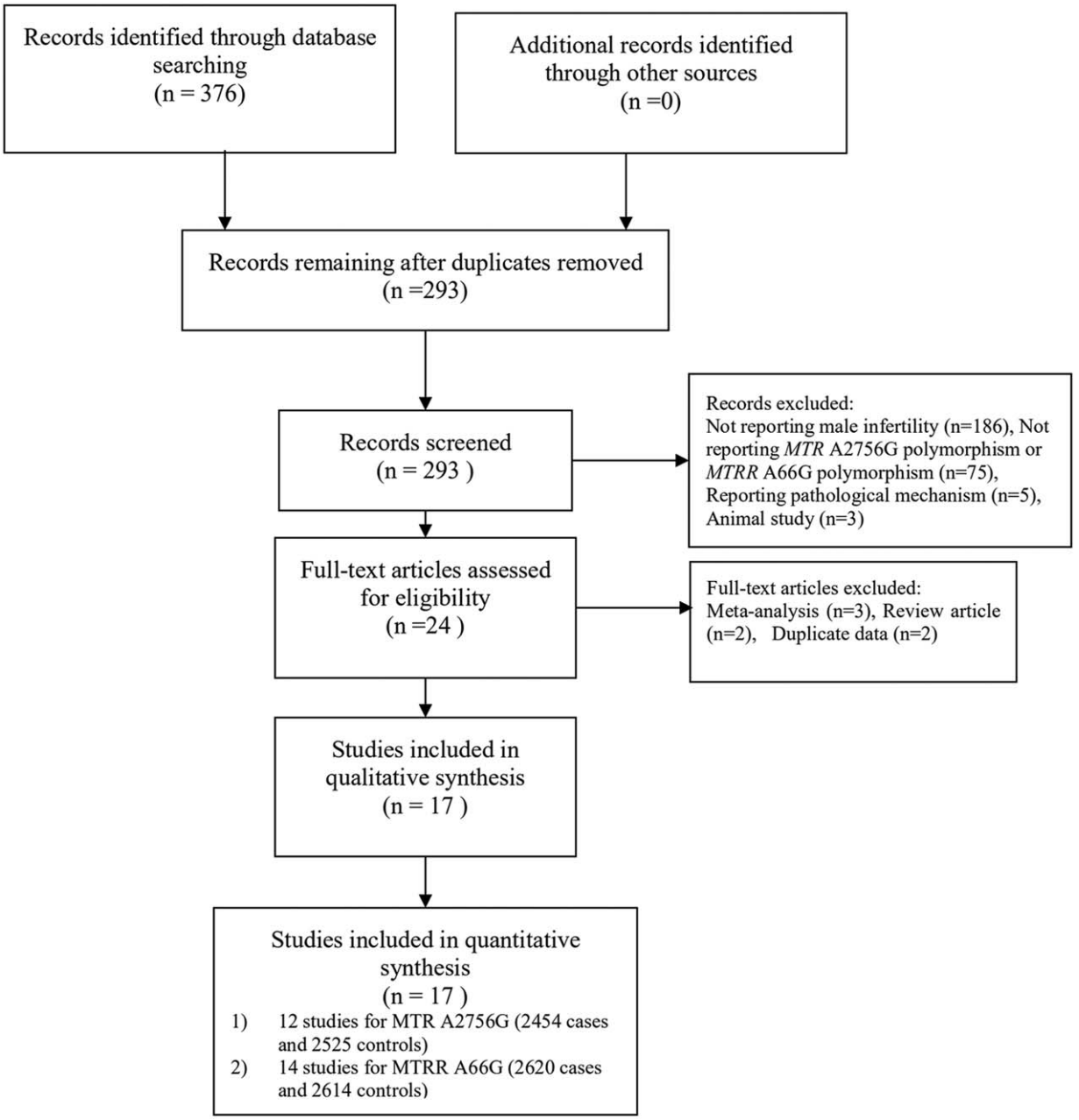

Figure 1. Flowchart showing the study selection.

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 I^2 metric were used to assess heterogeneity between studies. The heterogeneity was considered significant when $P < .10$ and $I^2 > 50\%$. If the P value for heterogeneity was $<.10$ and $I^2 > 50\%$, the pooled ORs were analyzed using the random-effect model. In contrast, we applied the fixed-effect model if $P \geq .10$ or $I^2 \leq 50\%$. 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, 359 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 *MTRR* A2756G with 2454 cases and 2525 controls[8,9,14,17–24] and 14 studies of *MTR* A66G with 2620 cases and 2614 controls.[8,9,17–23,25–29] In the studies eligible for *MTRR* A66G, 8 were conducted in Non-Asian populations, and 6 involved Asian populations. For *MTR* A2756G, 6 studies were conducted in Non-Asian populations, and 6 involved Asian populations. The studies were published between 2006 and 2017. The HWE was performed on all of the included studies, and the genotype distributions in the controls for all studies were consistent with the HWE, except for 3 studies. The detailed characteristics of all the included studies are shown in Table 1. And the quality of studies based on the NOS score is presented in Table 2.

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 significant association between the *MTR* A2756G polymorphism and idiopathic male infertility risks (G vs A: OR = 1.18, 95% CI = 1.00–1.40; GG vs AA: OR = 2.03, 95% CI = 1.48–2.77; GG + AG vs AA: OR = 1.16, 95% CI = 1.02–1.32; 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 population, 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	Case AA	Case AG	Case GG	Case G	Case A	Control AA	Control AG	Control GG	Control G	Control A	HWE
MTR A2756G																	
Farcas[22]	2009	Romania	Non-Asian	PCR-RFLP	65	67	40	23	2	27	103	42	24	1	26	108	>0.05
Gava[18]	2011	Brazil	Non-Asian	TaqMan	133	171	78	23	32	87	179	107	47	17	81	261	<0.05
Karimian[14]	2016	Iran	Non-Asian	PCR-RFLP	217	223	94	96	27	150	284	133	78	12	102	344	>0.05
Kim[19]	2015	Korea	Asian	PCR-RFLP	85	246	64	20	1	22	148	187	56	3	62	430	>0.05
Kurzawski[23]	2015	Poland	Non-Asian	TaqMan	284	352	178	93	13	119	449	218	125	9	143	561	>0.05
Lee[8]	2006	Korea	Asian	PCR	360	325	270	79	11	101	619	255	66	4	74	576	>0.05
Li[20]	2015	China	Asian	Sequencing	162	120	124	35	3	41	283	101	17	2	21	219	>0.05
Liu[3]	2012	China	Asian	PCR	75	72	60	14	1	16	134	61	11	0	11	133	>0.05
Murphy[17]	2011	Sweden	Non-Asian	PCR	147	181	100	41	6	53	241	116	57	8	73	289	>0.05
Ni[21]	2915	China	Asian	SNaPshot	296	204	245	47	4	55	537	163	37	4	45	363	>0.05
Weiner[9]	2014	Russia	Non-Asian	PCR	273	281	157	98	18	134	412	184	87	10	107	455	>0.05
Wen-Bo[24]	2010	China	Asian	TaqMan	357	283	340	17	0	17	697	261	22	0	22	544	>0.05
MTRR A66G																	
Balkan[26]	2013	Turkey	Non-Asian	TaqMan	108	125	36	52	20	92	124	35	61	29	119	131	>0.05
Farcas[22]	2009	Romania	Non-Asian	PCR-RFLP	65	67	13	46	6	58	72	18	42	7	56	78	<0.05
Gava[18]	2011	Brazil	Non-Asian	TaqMan	133	173	37	62	34	130	136	59	84	30	144	202	>0.05
Kim[19]	2015	Korea	Asian	PCR-RFLP	85	246	52	29	4	37	133	125	107	14	135	357	>0.05
Kurzawski[23]	2015	Poland	Non-Asian	TaqMan	284	352	51	139	94	327	241	70	171	111	393	311	>0.05
Lee[8]	2006	Korea	Asian	PCR	360	325	64	250	46	342	378	72	224	29	282	368	<0.05
Li[20]	2015	China	Asian	PCR	162	120	83	65	14	93	231	70	44	6	56	184	>0.05
Ma[28]	2017	China	Asian	PCR	138	102	66	60	12	84	192	42	48	12	72	132	>0.05
Mfady[25]	2014	Jordan	Non-Asian	PCR	150	150	48	78	24	126	174	61	67	22	111	189	>0.05
Murphy[17]	2011	Sweden	Non-Asian	PCR	150	180	50	68	32	132	168	60	88	32	152	208	>0.05
Ni[21]	2915	China	Asian	SNaPshot	296	204	158	119	19	157	435	99	91	14	119	289	>0.05
Ravel[27]	2009	France	Non-Asian	PCR-RFLP	252	114	27	145	80	292	186	12	60	42	141	81	>0.05
Weiner[9]	2014	Russia	Non-Asian	PCR	272	324	54	136	82	300	244	57	170	97	364	284	>0.05
Xian-Jun[29]	2007	China	Asian	PCR	165	132	38	72	55	182	148	45	65	22	109	155	>0.05

HWE = Hardy–Weinberg equilibrium, PCR = polymerase chain reaction, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism.

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
Gava[18]	2011	★★	★★	★★	6
Karimian[14]	2016	★★★	★★	★★	7
Kim[19]	2015	★★★	★★	★★	7
Kurzawski[23]	2015	★★★	★★	★★	7
Lee[8]	2006	★★★	★★	★★	7
Li[20]	2015	★★★	★★	★★	7
Liu[3]	2012	★★★	★★	★★	7
Fang-Fang and Hou-Zhao[28]	2017	★★	★	★★	5
Mfady[25]	2013	★★★	★★	★★	7
Murphy[17]	2011	★★★	★★	★★	7
Ni[21]	2915	★★★	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, 2.95]
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, 8.63]	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.

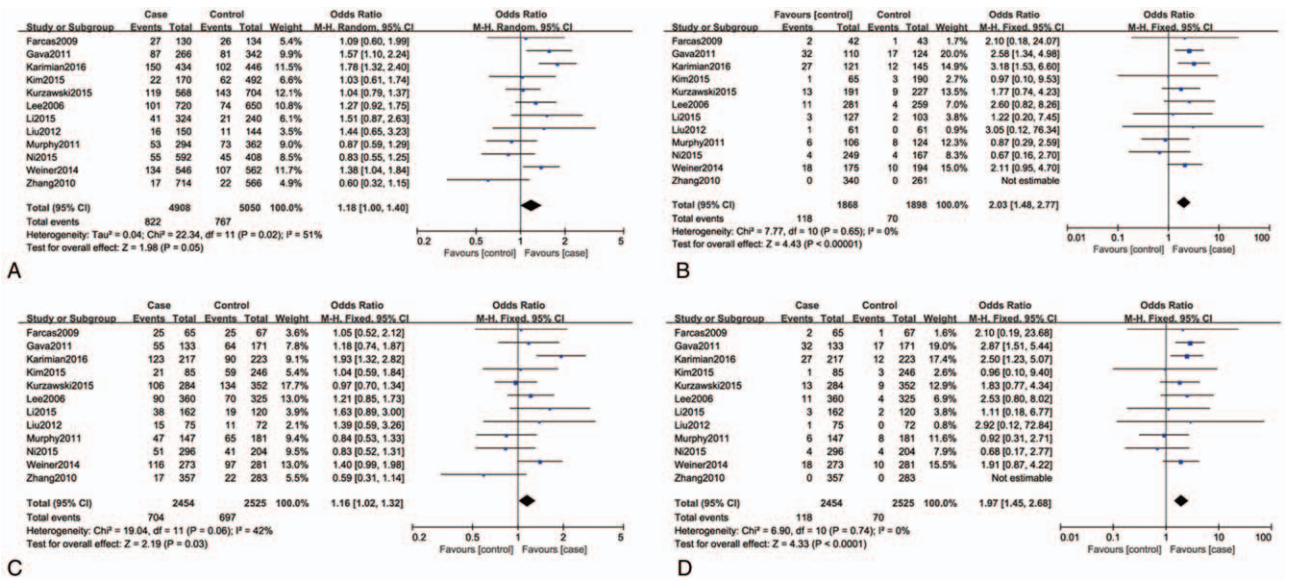

Figure 2. Forest plot of the studies assessing the association between *MTR* A2756G polymorphism and idiopathic male infertility. (A. Allelic model: G vs A; B. additive model: GG vs AA; C. dominant model: GG + AG vs AA; D. recessive model: GG vs AG + AA).

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, 2.25]	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.

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 OAT risks, however, no statistically significant increased risk of NOA was found in any of the genetic models. Furthermore, these data were further stratified by ethnicity, no significant risks were observed between *MTRR*

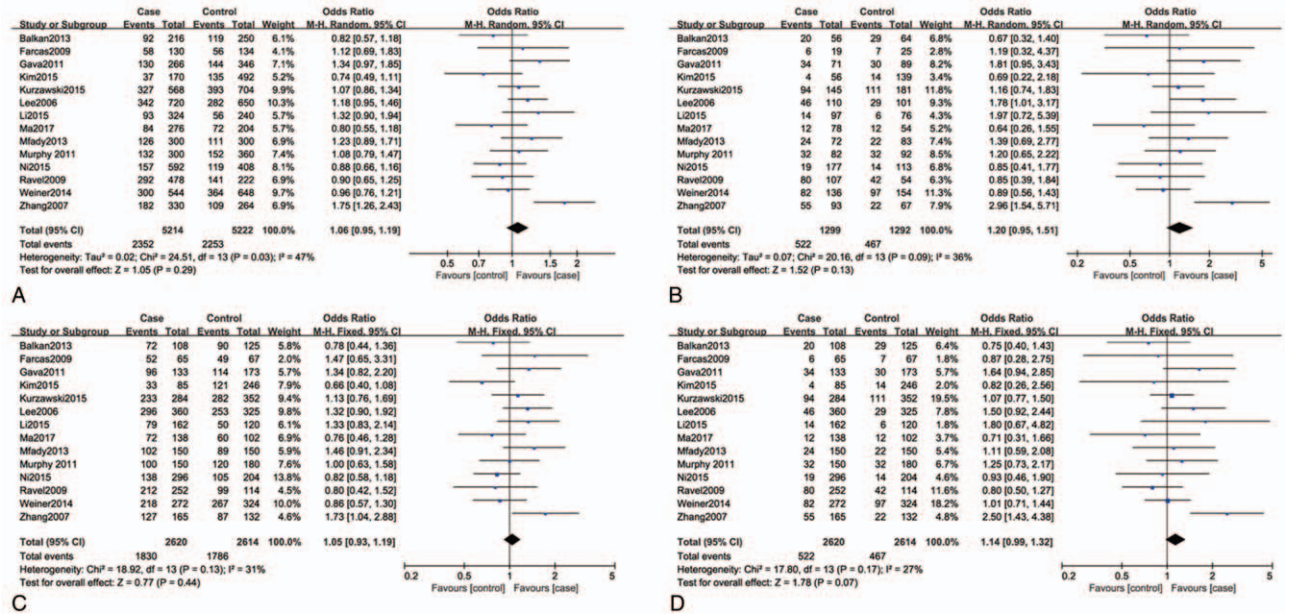

Figure 3. Forest plot of the studies assessing the association between *MTRR* A66G polymorphism and idiopathic male infertility. (A. Allelic model: G vs A; B. additive model: GG vs AA; C. dominant model: GG+AG vs AA; D. recessive model: GG vs AG+AA).

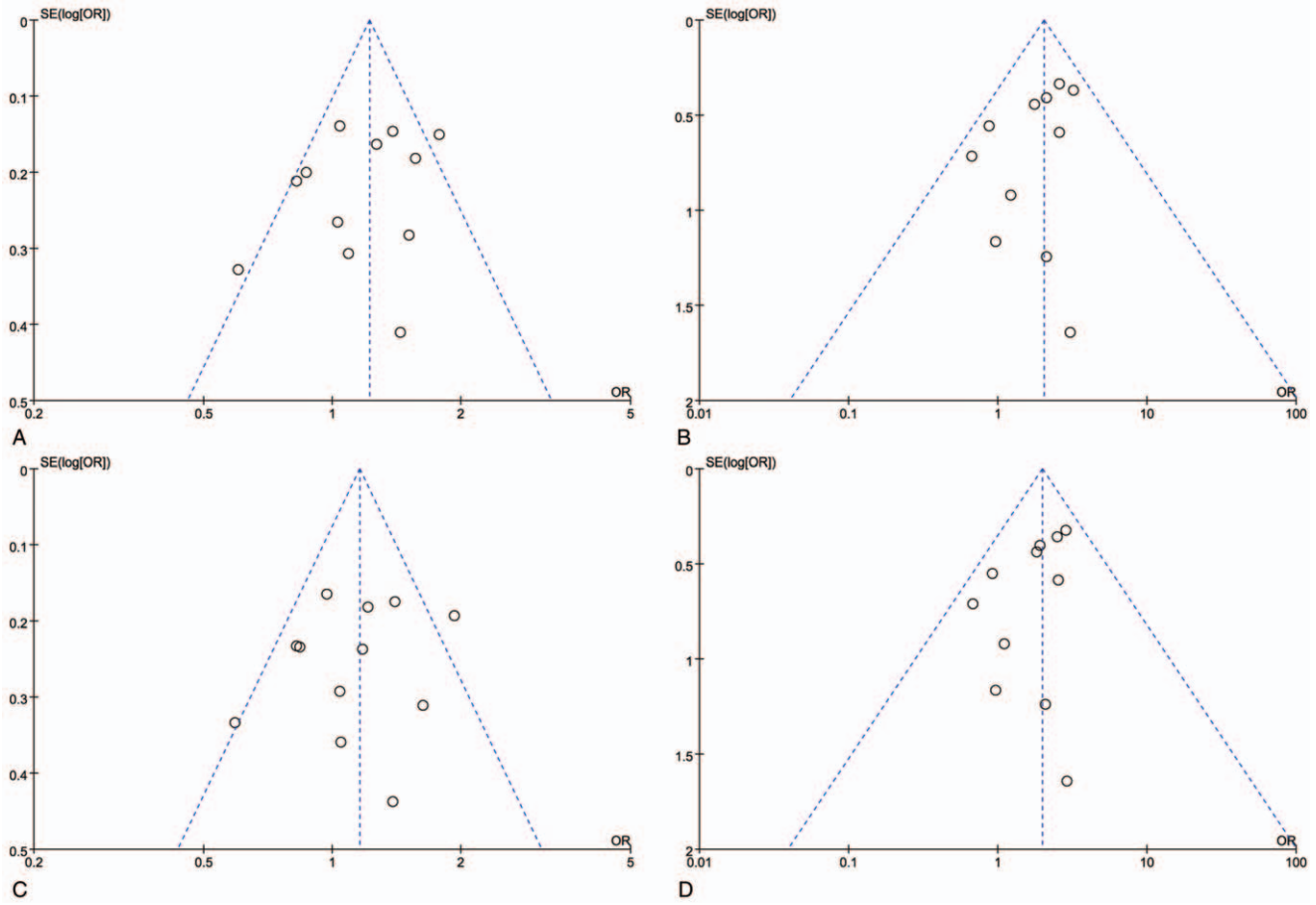

Figure 4. Funnel plots for the *MTR* A2756G polymorphism and idiopathic male infertility. (A. Allelic model: G vs A; B. additive model: GG vs AA; C. dominant model: GG + AG vs AA; D. recessive model: GG vs AG + AA).

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.[14,30,31] 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.[9,32] *MTR* is critical for homocysteine metabolism, and *MTRR* is required to maintain *MTR* in its active state.[33] 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.[34] The mechanisms of pathogenesis may involve changes of enzyme structure and mRNA properties that are due to these folate-related enzymes gene polymorphisms.[35]

There is increasing evidence investigating the association between *MTR* A2756G and *MTRR* A66G polymorphisms and risk of idiopathic male infertility. Gava et al[18] 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[20] 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[22] 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[23] found that the *MTR* A2756G and *MTRR* A66G polymorphism are not major risk factors for non-obstructive idiopathic male infertility in the Polish population.

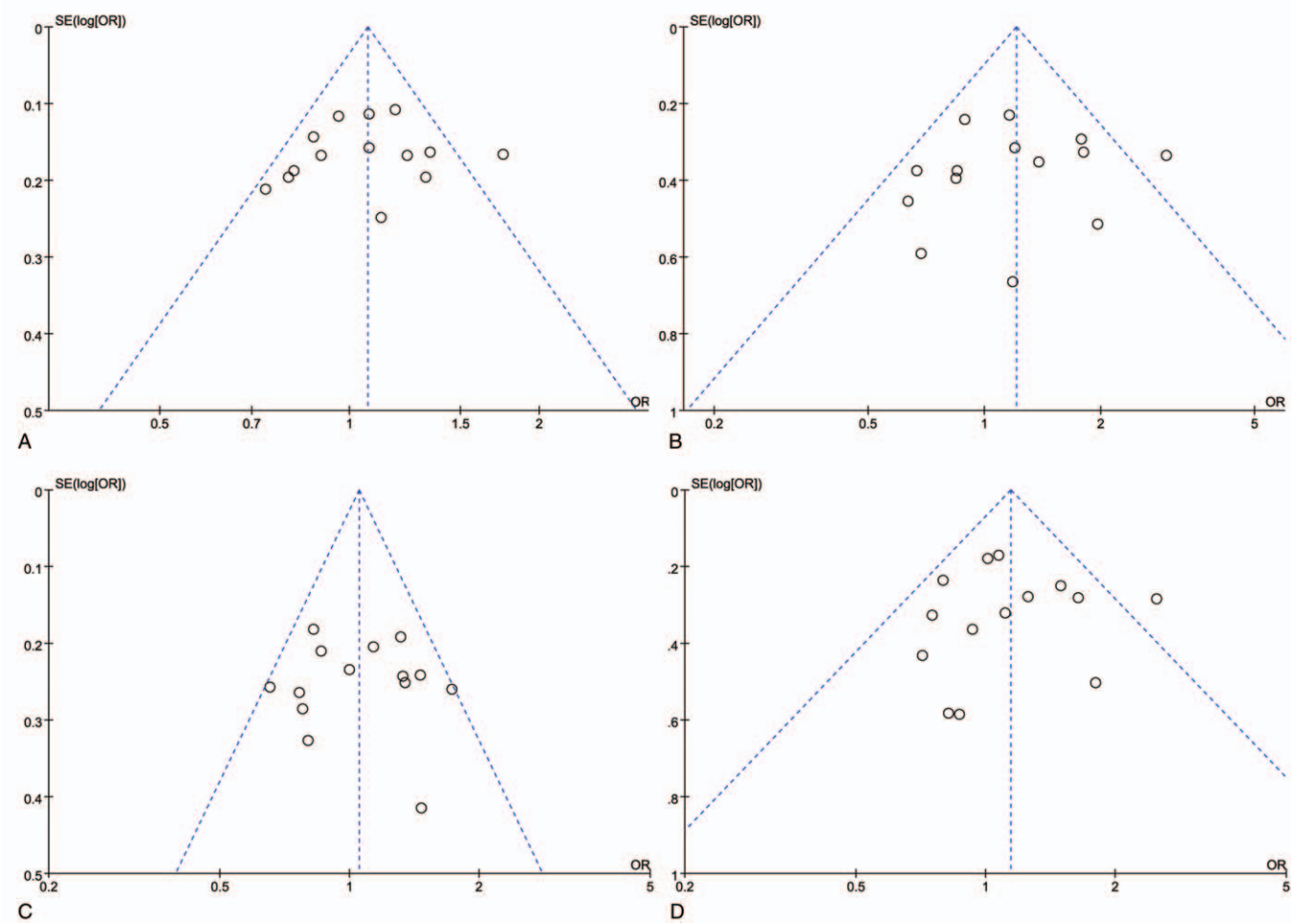

Figure 5. Funnel plots for the *MTRR* A66G polymorphism and idiopathic male infertility. (A. Allelic model: G vs A; B. additive model: GG vs AA; C. dominant model: GG + AG vs AA; D. recessive model: GG vs AG + AA).

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

Publication bias test for the *MTR* A2756G and *MTRR* A66G polymorphisms.

	Egger test			Begg test
Comparisons	**Coefficient**	***P* value**	**95% CI**	***P* value**
MTR A2756G				
G vs A	−1.430	.309	−4.402–1.541	.373
GG vs AA	−0.905	.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.

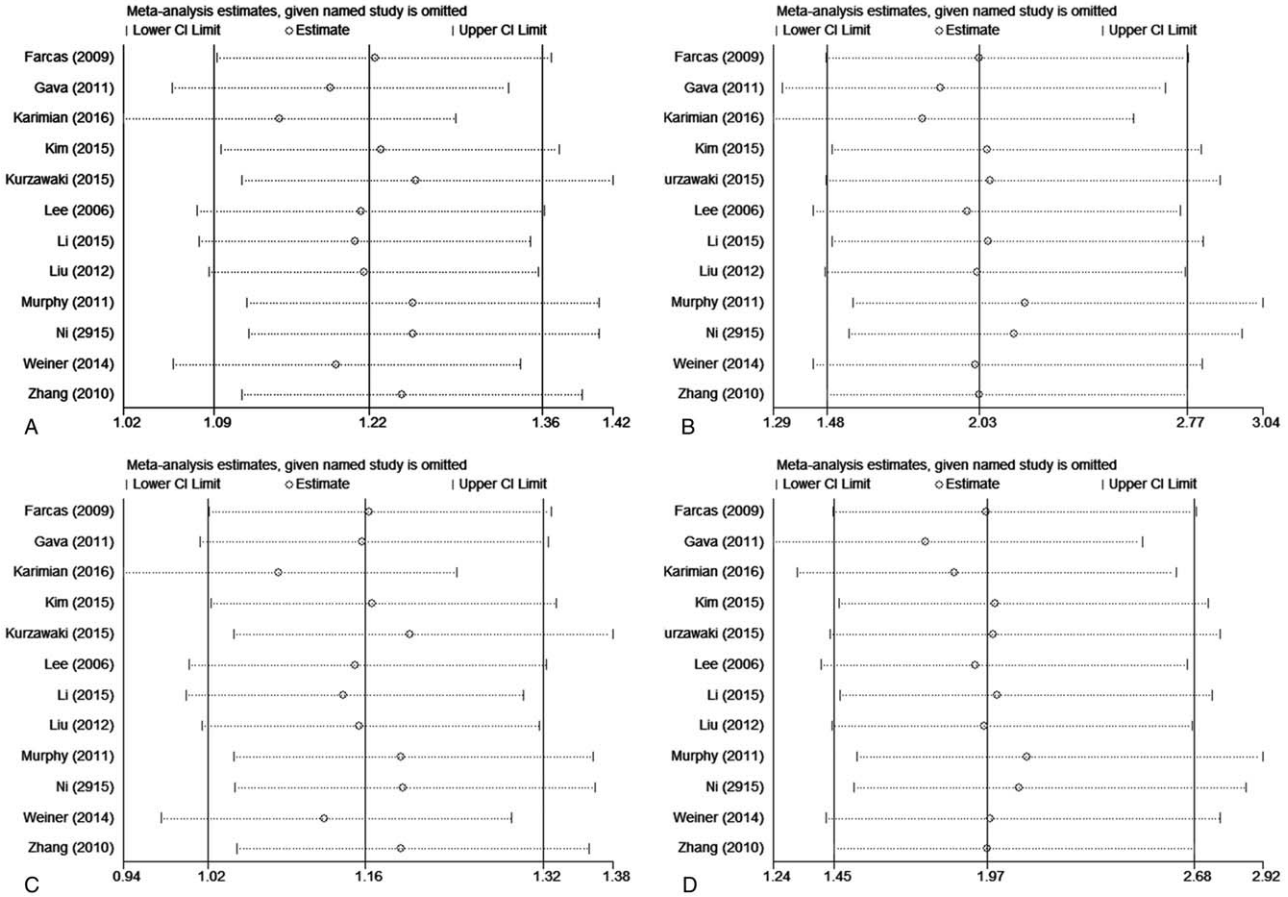

Figure 6. Sensitivity analysis diagram for each study used to assess the relative risk estimates for the *MTR* A2756G polymorphism and idiopathic male infertility. (A. Allelic model: G vs A; B. additive model: GG vs AA; C. dominant model: GG + AG vs AA; D. recessive model: GG vs AG + AA).

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.[36] 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,[14] 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 the *MTRR* A66G polymorphism associated with OAT in Asians but not Non-Asians. A previous meta-analysis conducted by Xu et al[10] 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[37] 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

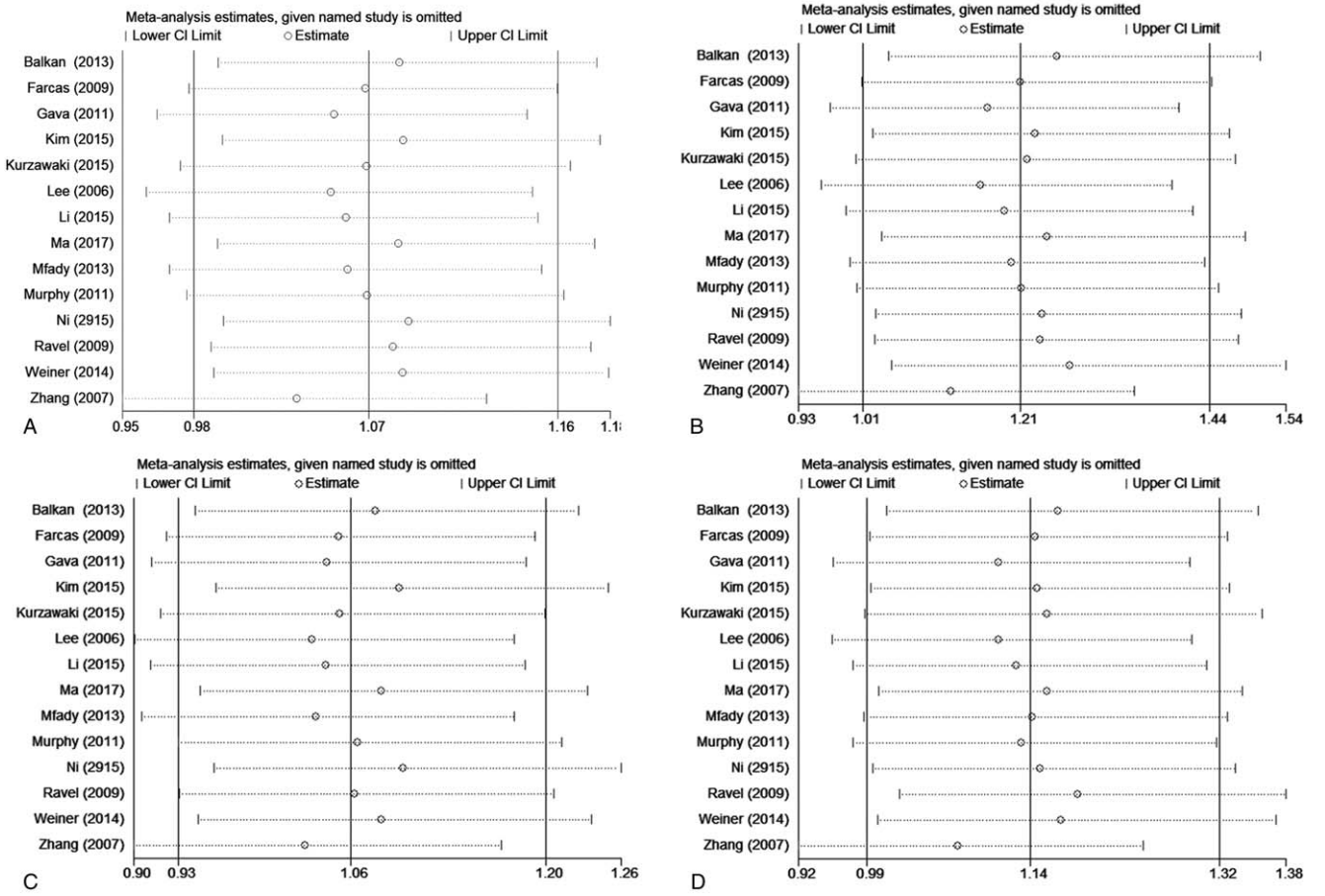

Figure 7. Sensitivity analysis diagram for each study used to assess the relative risk estimates for the *MTRR* A66G polymorphism and idiopathic male infertility. (A. Allelic model: G vs A; B. additive model: GG vs AA; C. dominant model: GG + AG vs AA; D. recessive model: GG vs AG + AA).

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

Conceptualization: Zheng-Ju Ren, Qiang Dong.
Data curation: Shi Deng.
Formal analysis: Bo Yang.
Methodology: Zheng-Ju Ren, Peng-Wei Ren, Zhu-Feng Peng.
Software: Yan-Ping Zhang, Peng-Wei Ren, Bo Yang.
Supervision: Zhu-Feng Peng, Liang-Ren Liu.
Writing – original draft: Zheng-Ju Ren, Yan-Ping Zhang.
Writing – review & editing: Liang-Ren Liu, Wuran Wei, Qiang Dong.

References

[1] Razavi SM, Sabbaghian M, Jalili M, et al. Comprehensive functional enrichment analysis of idiopathic male infertility. Sci Rep 2017;7:15778.
[2] Sharma RK, Said T, Agarwal A. Sperm DNA damage and its clinical relevance in assessing reproductive outcome. Asian J Androl 2004;6:139–48.
[3] Liu L, Cai Z, Leng H, et al. [Association of MTHFR C677T and MS A2756G polymorphism with semen quality]. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2012;37:1054–9.
[4] Chang J, Pan F, Tang Q, et al. eNOS gene T786C, G894T and 4a4b polymorphisms and idiopathic male infertility susceptibility: a meta-analysis. Andrologia 2017;4:49.
[5] Miyamoto T, Minase G, Shin T, et al. Human idiopathic male infertility and its genetic causes. Reprod Med Biol 2017;16:81–8.
[6] Tahmasbpour E, Balasubramanian D, Agarwal A. A multi-faceted approach to understanding idiopathic male infertility: gene mutations, molecular defects and assisted reproductive techniques (ART). J Assist Reprod Genet 2014;31:1115–37.
[7] Crider KS, Yang TP, Berry RJ, et al. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv Nutr 2012;3:21–38.
[8] Lee HC, Jeong YM, Lee SH, et al. Association study of four polymorphisms in three folate-related enzyme genes with non-obstructive idiopathic male infertility. Hum Reprod 2006;21:3162–70.
[9] Weiner AS, Boyarskikh UA, Voronina EN, et al. Polymorphisms in folate-metabolizing genes and risk of idiopathic idiopathic male infertility: a study on a Russian population and a meta-analysis. Fertil Steril 2014;101:87.e3–94.e3.
[10] Xu L, Qin Z, Wang F, et al. Methylenetetrahydrofolate reductase C677T polymorphism and colorectal cancer susceptibility: a meta-analysis. Biosci Rep 2017;37:pii: BSR20170917.
[11] Zhao Y, Chen ZX, Ma YS, et al. Lack of association between methionine synthase A2756G polymorphism and digestive system cancer risk: evidence from 39327 subjects. PLoS One 2013;8:e61511.
[12] Young SS, Eskenazi B, Marchetti FM, et al. The association of folate, zinc and antioxidant intake with sperm aneuploidy in healthy non-smoking men. Hum Reprod 2008;23:1014–22.

[13] Liu K, Zhao RZ, Shen M, et al. Role of genetic mutations in folate-related enzyme genes on idiopathic male infertility. Sci Rep 2015;5:15548.
[14] Karimian M, Colagar AH. Methionine synthase A2756G transition might be a risk factor for idiopathic male infertility: evidences from seven case-control studies. Mol Cell Endocrinol 2016;425:1–0.
[15] Xu WH, Zhang L, Wu XL, et al. Association between methionine synthase reductase A66G polymorphism and idiopathic male infertility: a meta-analysis. Crit Rev Eukaryot Gene Expr 2017;27:37–46.
[16] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010;25:603–5.
[17] Murphy LE, Mills JL, Molloy AM, et al. Folate and vitamin B12 in idiopathic idiopathic male infertility. Asian J Androl 2011;13:856–61.
[18] Gava MM, Kayaki EA, Bianco B, et al. Polymorphisms in folate-related enzyme genes in idiopathic infertile Brazilian men. Reprod Sci 2011;18:1267–72.
[19] Kim SY, Lim JW, Kim JW, et al. Association between genetic polymorphisms in folate-related enzyme genes and infertile men with non-obstructive azoospermia. Syst Biol Reprod Med 2015;61:286–92.
[20] Li XY, Ye JZ, Ding XP, et al. Association between methionine synthase reductase A66G polymorphism and primary infertility in Chinese males. Genet Mol Res 2015;14:3491–500.
[21] Ni WH, Li HY, Wu AH, et al. Lack of association between genetic polymorphisms in three folate-related enzyme genes and idiopathic male infertility in the Chinese population. J Assist Reprod Gen 2015;32: 369–74.
[22] Farcas MF, Trifa AP, Militaru M, et al. Association of methionine synthase A2756G SNP, methionine synthase reductase A66G and idiopathic male infertility. Revista Romana De Medicina De Laborator 2009;17:17–24.
[23] Kurzawski M, Wajda A, Malinowski D, et al. Association study of folate-related enzymes (MTHFR, MTR, MTRR) genetic variants with non-obstructive idiopathic male infertility in a Polish population. Genet Mol Biol 2015;38:42–7.
[24] Wen-Bo Z, Chao-Jun L, Mei L. Association Study of the Level of Folic Acid in Seminal Plasma and Single Nucleotide Polymorphisms in the Genes of Folate Metabolism and Idiopathic male infertility. [D]. Nanjing: Nanjing Normal University; 2010.
[25] Mfady DS, Sadiq MF, Khabour OF, et al. Associations of variants in MTHFR and MTRR genes with idiopathic male infertility in the Jordanian population. Gene 2014;536:40–4.
[26] Balkan M, Atar M, Erdal ME, et al. The possible association of polymorphisms in MTHFR, MTRR, and MTHFD1 Genes with Idiopathic male infertility. Int Med J 2013;20:404–8.
[27] Ravel C, Chantot-Bastaraud S, Chalmey C, et al. Lack of association between genetic polymorphisms in enzymes associated with folate metabolism and unexplained reduced sperm counts. PLoS One 2009;4: e6540.
[28] Fang-Fang M, Hou-Zhao W. Association of MTRR A66G polymorphism with male infertile. Chinese J Birth Health Hered 2017;25: 120–1.
[29] Xian-Jun Z, Chao-Jun L. Association Study of Single Nucleotide Polymorphisms in the Genes of Folate Metabolism and Idiopathic Idiopathic Male Infertility. [D]. Nanjing: Nanjing Normal University; 2007.
[30] Mc Auley MT, Mooney KM, Salcedo-Sora JE. Computational modelling folate metabolism and DNA methylation: implications for understanding health and ageing. Brief Bioinform 2018;19:303–17.
[31] Luo WP, Li B, Lin FY, et al. Joint effects of folate intake and one-carbon-metabolizing genetic polymorphisms on breast cancer risk: a case-control study in China. Sci Rep 2016;6:29555.
[32] Liu K, Zhao R, Shen M, et al. Role of genetic mutations in folate-related enzyme genes on Idiopathic male infertility. Sci Rep 2015;5:15548.
[33] Hiraoka M, Kagawa Y. Genetic polymorphisms and folate status. Congenit Anom (Kyoto) 2017;57:142–9.
[34] Stover PJ. Polymorphisms in 1-carbon metabolism, epigenetics and folate-related pathologies. J Nutrigenet Nutrigenomics 2011;4:293–305.
[35] Nikzad H, Karimian M, Sareban K, et al. MTHFR-Ala222Val and idiopathic male infertility: a study in Iranian men, an updated meta-analysis and an in silico-analysis. Reprod Biomed Online 2015;31: 668–80.
[36] Ren Z, Ren P, Yang B, et al. MTHFR C677T, A1298C and MS A2756G gene polymorphisms and male infertility risk in a Chinese population: a meta-analysis. PLoS One 2017;12:e0169789.
[37] Shi TL, Wu Y, Li Y, et al. The relevance of MTHFR C677T, A1298C, and MTRR A66G polymorphisms with response to male infertility in Asians: A meta-analysis. Medicine (Baltimore) 2019;98:e14283.