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REVIEW

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MTHFR C677T、MTHFR A1298C、MTRR A66G and MTR A2756G polymorphisms and male infertility risk: a systematic review and meta-analysis

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Abstract

Background Epidemiological studies have reported that polymorphisms of folate-metabolizing genes have a significant impact on male infertility. However, the results of published studies have come to different conclusions.

Objective To determine an association between folate-metabolizing gene polymorphisms and the risk of male infertility.

Methods The meta-analysis was conducted according to the PRISMA 2020 statement. The protocol was registered with PROSPERO (CRD42023412251). Studies were searched from PubMed, Google Scholar, Embase, Scopus, and the Cochrane Library up to 24st October2023. Articles that satisfied the inclusion criteria were evaluated for their quality using the Newcastle–Ottawa Scale. Data were extracted from the eligible studies and were analyzed for pooled up odds ratio (OR) with 95% confidence interval (CI). Meta-analysis was conducted using STATA 12.

Results Forty-six case–control studies were included in the meta-analysis which comprised 20,639 participants. The pooled analysis revealed that the MTHFR C677T polymorphism was significantly associated with male infertility and abnormospermia.Three-fifths of the model showed there was a significant association between the MTR A2756G polymorphism and male infertility. Both MTHFR A1298C and MTRR A66G polymorphisms were not significantly associated with male fertility. Furthermore, subgroup analysis revealed a significant association between the MTHFR C677T polymorphism and male fertility in Asian countries.

Conclusion This meta-analysis suggests that the MTHFR C677T and MTR A2756G polymorphisms may be a potential risk factor for male infertility.

Keywords Folate-metabolizing gene, MTHFR C677T, MTHFR A1298C, MTRR A66G, MTR A2756G, Male infertility

Introduction

Infertility is defined as the being unable to conceive after 1 year or more of regular unprotected sexual intercourse according to the World Health Organization [1] [2]. Approximately 15%–20% of newly married couples worldwide experience from fertility-related complications [3, 4], with the male factor playingan important role in these cases [3, 5]. Male infertility is a multifactorial disease resulting from various genetic and environmental

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factors. Many factors that contribute to male infertility have been identified, such as chromosomal abnormalities [6], Y-chromosome microdeletion [7, 8], cystic fibrosis transmembrane regulator mutations in men with congenital bilateral absence of the vas deferens [9, 10], history of neoplasia and related treatments [11, 12], varicocele [13, 14] and other factors [15, 16]. However, many factors that affect reproductive function remain unclear and contradictory.

Spermatogenesis is a very complex process that is influenced by many factors. Folic acid, an essential methyl donor, plays a critical role in nucleic acid synthesis, methylation and amino acid metabolism [17]. The folate metabolic pathway is speculated to play an important role in spermatogenesis as folate deficiency is linked to hyperhomocysteinaemia, a known risk factor for male infertility. Folate supplementation has been shown to have a beneficialeffect on male fertility. Emmanuelle et al. reported that high-dose folic acid supplementation in infertile men could improve the in-vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) outcomes [18]. Similarly, Wong et al. found that the total normal sperm count increased in both sub-fertile and fertile men after folic acid and zinc sulfate supplementation [19]. Methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), and methionine synthase reductase (MTRR) are three essential enzymes in the folate metabolism. The MTHFR gene is located at the end of the short arm of chromosome 1 (1p36.3) and has 33 exons [20]. The MTRR gene is located on chromosome 5 (5p15.2). It has 15 exons [21]. The MTR gene is located on chromosome 1 (1q43). It has 33 exons [22]. And study showed that variations in these genes may serve as vital risk factors for male infertility.

The association between folate-metabolizing gene polymorphisms and male fertility has been extensively reported; however, prior literature on the subject reports conflicting data. A study by S. Q. Ren et al. found that the MTRR A66G polymorphism was associated with an increased risk of male infertility [23]. However, a study by Tamjeed Tariq et al. found no significant association between MTRR A66G and male infertility [24]. B. Wei et al. showed that both the 677C/T and 1298A/C polymorphisms were not significantly associated with the risk of male infertility [25]. However, the results of the study by Fereshteh Aliakbari et al. were not consistent with those of the study by B. Wei et al. [26]. Therefore, identification of the association between folate-metabolizing gene polymorphisms and male fertility is crucial to improve our understanding of male infertility, thereby aiding in the establishment of potential interventions to mitigate the risk of male infertility. Several meta-analyses have tried to draw conclusions on the association between folate-metabolizing gene polymorphisms and male fertility; however, most of them focused on a specific folate-metabolizing gene or were conducted in specific geographic areas [27–31]. Additionally, the majority of available meta-analyses have focused on fertile and infertile populations, but not on normozoospermic and abnormospermic populations.

In this study, we performed a meta-analysis to evaluate the association between polymorphisms of MTHFR C677T (rs1801133), MTHFR A1298C (rs1801131), MTRR A66G (rs1801394) and MTR A2756G (rs1805087) and the risk of male infertility. The study population included not only fertile and infertile populations but also normospermic and abnormospermic populations.

Materials and methods

The meta-analysis was performed following the Preferred Reporting Item for Systematic Reviews and Meta-analysis (PRISMA) guidelines [32] and was prospectively registered in the International Prospective Register of Systematic Reviews (CRD42023412251). Ethical approval was not required as publicly available data were used for the analysis.

Search strategy

PubMed, Google Scholar, Embase, Scopus and the Cochrane Library were comprehensively searched for eligible studies from inception to 24st October2023. Search terms or exploded MeSH terms were derived from the following search words: ('methylenetetrahydrofolate reductase'or 'MTHFR' or 'methionine synthase reductase' or 'MTRR' or 'methionine synthase' or 'MTR' or 'polymorphism' or 'variant', 'C677T' or 'A1298C' or 'A66G' or 'A2756G' or 'folate-metabolizing gene')and ('male infertility' or 'male fertility'or 'sperm' or 'semen' or 'azoospermia' or 'oligozoospermia' or 'oligoasthenoteratozoospermia' or 'normozoospermia' or 'abnormospermia'). Search terms and functions were altered for each database. Articles published in languages other than English were translated by medical professionals of that specific language or those articles were sourced in the English language. Moreover, references to original articles and review articles were manually searched to identify any additional eligible studies.

Inclusion and exclusion criteria

Inclusion criteria were as follows: (1) studies on the association between MTHFR C677T, MTHFR A1298C, MTRR A66G or MTR A2756G polymorphism and male fertility; (2) human study; (3) case–control study; (4) sufficient published data to calculate the odds ratio (OR) and 95% confidence interval (CI).

Exclusion criteria were as follows: (1) studies not relevantto male infertility, (2) review articles, case reports, book chapters, animal studies and no case–control study design; (3) overlapping or repeated data from various studies. If the same data were used in more than one study, the study with the larger sample size was selected.

Data extraction

Three investigators (TF Y, F L, JJ Q) independently reviewed the articles and selected the eligible studies based on the inclusion and exclusion criteria. Disagreements were resolved by a fourth investigator (LX L). The following data were collected from the studies: first author, year of publication, genotyping method, country, sample size, age, Newcastle–Ottawa Scale (NOS), Hardy–Weinberg equilibrium (HWE), number of cases and controls and number of genotyped cases and controls.

Quality assessment

Quality assessment was performed independently by three investigators (TF Y, F L, JJ Q). Disagreements were resolved by a a fourth investigator (LX L). The Newcastle–Ottawa Scale (NOS) [33] was used to evaluate the risk of bias in each study. Studies were considered low quality if they received 0–6 stars and high quality if they received 7–9 stars.

Data analysis

Five models were used to analyze the relationships between the male infertility risks and the MTHFR C677T, MTHFR A1298C, MTRR A66G and MTR A2756G polymorphisms: additive model, homozygote model, recessive model, dominant model and heterozygote model.The pooled ORs and corresponding 95% CIs were estimated using a random-effects model to determine the strength of the association.

Hardy–Weinberg Equilibrium (HWE) was assessed to verify the representation of the population in each study. Heterogeneity was assessed using Cochran's Q test and quantified by the I2 index, with I2 R 50% indicating high heterogeneity [34, 35]. Subgroup analyses were performed based on country (Asian countries or non-Asian countries), sample size (<300 or ≥ 300), study quality (0–6 or 7–9 stars) and HWE (yes or no). Publication bias was assessed using Begg's test and Egger's test. $P<0.05$ in Begg's test or Egger's test was considered to be statistically significant [36]. To evaluate the stability of the results, sensitivity analyses were performed by repeating the meta-analysis while deleting one study per analysis. Statistical analysis was performed using STATA (version 12).

Results

Study characteristics

The flow diagram of the study selection for the meta-analysis is illustrated in Fig. 1. A total of 3,223 studies were initially identified based on the search criteria. However, 3,159 studies were excluded as 2,364 were duplicate records; 793 studies were excluded based on title and abstract; 15 studies did not address the association between MTHFR C677T, MTHFR A1298C, MTRR A66G or MTR A2756G polymorphism and male fertility; relevant data could not be extracted from three studies; one study was a letter and one was a review article. Finally, 46 case–control studies with 20,639 participants were selected for the systematic review and meta-analysis (Fig. 1).

The publication years of the eligible studies ranged from 2003 to 2022. The genotyping methods varied between studies, with the most common being polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) [20, 37–68]; other methods in the 32 studies included polymerase chain reaction (PCR) (one study) [69]; competitive allele-specific PCR(one study) [70]; SnaPshot (one study) [71]; PCR combined with mass-spectrography(one study) [72]; PCR combined with DNA sequencing(four studies) [73–76] and Real-time PCR(five studies) [77–81]. A total of 30 articles reported participants from Asian countries [20, 37, 38, 40–42, 44, 45, 47, 49, 51, 54–56, 58–65, 67, 68, 71–76] and 16 articles reported participants from non-Asian countries [39, 43, 46, 48, 50, 52, 53, 57, 66, 69, 70, 77–81]. Moreover, 21 studies reported a sample size of less than 300 [20, 40, 43, 46, 48, 51–54, 57, 59, 60, 63, 65, 66, 68, 69, 75, 76, 79, 81] and 25 studies reported a sample size of 300 or more [37–39, 41, 42, 44, 45, 47, 49, 50, 55, 56, 58, 61, 62, 64, 67, 70–74, 77, 78, 80]. A detailed summary of the included studies is presented in Table 1.

Quality Scores

Based on the NOS scores, 27 studies were assessed as low-quality (0–6 stars) [20, 37, 39, 41–44, 46, 49, 50, 52–54, 56, 60, 66, 68, 69, 71, 72, 74–77, 79–81] and 19 studies were assessed as high-quality (7–9 stars) [38, 40, 45, 47, 48, 51, 55, 57–59, 61–65, 67, 70, 73, 78]. A comprehensive account of the scoring system is provided in Table 2.

Synthesis of results

The results of this meta-analysis were divided into two main sections according to the study population. The first section compared the differences in MTHFR, MTR, and MTRR polymorphisms in fertile and infertile populations (Table 3), while the second section compared them in normospermic and abnormospermic populations

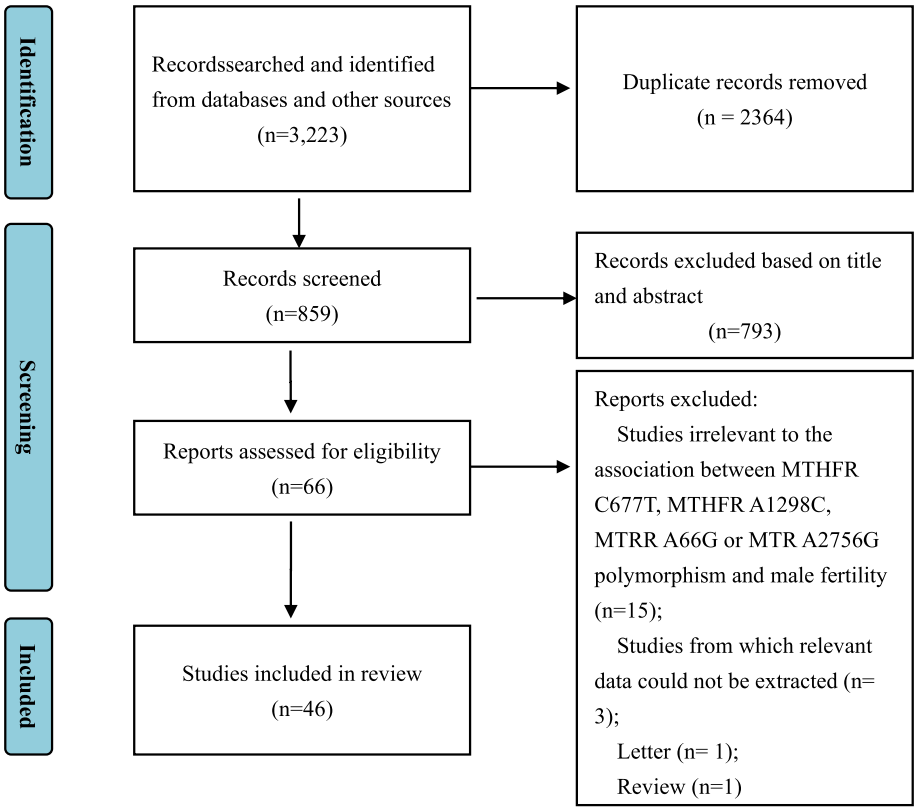

Fig. 1 The flow diagram of the study selection for the systematic review

(Table 4). The random-effects models were used to summarise the ORs with the corresponding 95% CIs and the corresponding forest plots are showed in the Figs. 2, 3, 4, 5, 6 and 7.

In fertile and infertile populations

A total of 34 studies, comprising 16,919, reported the association between the MTHFR C677T polymorphism and male infertility and the pooled results showed that the MTHFR C677T polymorphism was significantly associated with male infertility (the additive model T vs. C: OR = 1.25, 95% CI = 1.12–1.39; the homozygote model TT vs. CC: OR = 1.49, 95% CI = 1.20–1.84; the heterozygote model CT vs. CC: OR = 1.22, 95% CI = 1.08–1.38; the dominant model CT + TT vs. CC: OR = 1.28, 95% CI = 1.12–1.47; the recessive model TT vs. CC + CT: OR = 1.33, 95% CI = 1.12–1.58).

Twenty-one studies, comprising 12,548 participants, reported the association between the MTHFR A1298C polymorphism and male infertility.We attained that the MTHFR A1298C polymorphism was not associated with male infertility (the additive model C vs. A: OR = 1.10, 95% CI = 0.99–1.21; the homozygote model CC vs. AA: OR = 1.19, 95% CI = 0.92–1.53; the heterozygote model AC vs. AA: OR = 1.09, 95% CI = 0.97–1.23; the dominant model AC + CC vs. AA: OR = 1.11, 95% CI = 0.98–1.25; the recessive model CC vs. AA + AC: OR = 1.16, 95% CI = 0.92–1.47).

Eight studies, comprising 3,695 participants, reported the association between the MTRR A66G polymorphism and male infertility. However, no significant association between MTRR A66G polymorphism and male infertility was observed (the additive model G vs. A: OR = 1.05, 95% CI = 0.94–1.18; the homozygote model GG vs. AA: OR = 1.17, 95% CI = 0.94–1.46; the heterozygote model AG vs. AA: OR = 1.01, 95% CI = 0.84–1.21; the dominant model AG + GG vs. AA: OR = 1.04, 95% CI = 0.86–1.25; the recessive model GG vs. AA + AG: OR = 1.13, 95% CI = 0.94–1.35).

Nine studies, comprising 3,901 participants, reported the association between the MTR A2756G polymorphism and male infertility. Three fifths of the model showed that there is a significant association between MTR A2756G polymorphism with male infertility(the additive model G vs. A: OR = 1.26, 95% CI = 1.03–1.56; the homozygote model GG vs. AA: OR = 1.82, 95% CI = 1.29–2.57; the heterozygote model AG vs. AA: OR = 1.05, 95% CI = 0.91–1.22; the dominant model GG + AG vs. AA: OR = 1.13, 95% CI = 0.98–1.31; the recessive model GG vs. AA + AG: OR = 1.85, 95% CI = 1.32–2.59).

Table 1 Characteristics of the included articles

Study	Genotyping method	country	Sample size	Age
Inge M. W. Ebisch 2003	PCR	Netherlands	190	-[a]
L. Stuppia 2003	PCR–RFLP	Italy	198	27-52[b]
Jung Hoon Park 2005	PCR–RFLP	Korea	769	38.14 ± 8.13[c]
KIRAN SINGH 2005	PCR and DNA sequencing	India	351	30 ± 3
Han-Chul Lee 2006	PCR–RFLP	Korea	685	42.8 ± 8.6
Paracchini V 2006	PCR–RFLP	Italy	105	-
Varinderpal S.Dhillon 2007	PCR–RFLP	India	379	25–35
Zhou-Cun A 2007	PCR–RFLP	China	607	control group 26–51; patient group 25–38
Celia Ravel 2009	PCR–RFLP	French	366	-
Singh K 2010	PCR–RFLP	India	291	30 ± 3
Aleksandra Nikolic 2010	PCR–RFLP	Serbia	108	-
Laurel E Murphy 2011	competitive allele-specific PCR	Sweden	337	32.72 ± 4.5
Kishlay Kumar 2011	PCR–RFLP	India	200	33 ± 3.68
Mohammad Reza Safarinejad 2011	PCR–RFLP	Iran	492	32.17 ± 4.54
Nishi Gupta 2011	PCR and DNA sequencing	India	837	-
Marcello Machado Gava 2011	Real-time PCR	Brazil	389	-
Abdelmajid Eloualid 2012	PCR–RFLP	morocco	1034	control group 30–55; patient group 25–50
Ling Liu 2012	PCR–RFLP	China	147	control group 35.8 ± 5.5; patient group 37.0 ± 6.9
Djalila Chellat 2012	PCR–RFLP	Algeria	158	24–48
G. T. Vani 2012	PCR–RFLP	India	436	25–45
Cristina Camprubí 2013	PCR–RFLP	Spain	132	control group 19–45; patient group 26–53
Stangler Herodež 2013	PCR–RFLP	Slovenia	211	-
Nishi Gupta 2013	PCR–RFLP	India	747	-
Hena Naqvi 2014	PCR–RFLP	India	1001	22–40
Doaa S. Mfady 2014	PCR–RFLP	Jordan	300	Age in control group and patient group origin-matched
Alexandra S. Weiner 2014	Real-time PCR	Russia	624	control group 18–58; patient group20-45
S.-S. Li1 2014	PCR–RFLP	China	215	31.5 ± 4.45
Tarek M 2014	PCR–RFLP	Egypt	214	control group 21–49; patient group25-57
H. Gurkan 2015	Real-time PCR	Turkey	271	32.84 ± 6.5
Shin Young Kim 2015	PCR–RFLP	Korea	331	40.29 ± 8.6
X.Y. Li 2015	PCR and DNA sequencing	China	282	-
Mateusz Kurzawski 2015	Real-time PCR	Polish	636	control group 21–56; patient group22-49
Wuhua Ni 2015	SNaPshot	China	500	-
Hossein Nikzad 2015	PCR–RFLP	Iran	497	31.17 ± 3.7
Mohammad Karimian 2016	PCR–RFLP	Iran	250	30.98 ± 3.71
Muhammad Irfan 2016	PCR–RFLP	Pakistan	655	-
K. Louie 2016	PCR–RFLP	Canada	52	-
Shu-Yuan Liu 2017	PCR and mass-spectrography	China	592	33.29 ± 5.23
Tao Wang 2017	PCR–RFLP	China	3555	32.31 ± 7.18
Shiva Poorang 2018	PCR–RFLP	Iran	50	control group40 ± 7.9; patient group37.24 ± 4.6
Chong Xie 2019	PCR and DNA sequencing	China	245	control group 25–38; patient group22-44
Noor Ullah 2019	PCR–RFLP	Pakistan	346	-
Asghar Tanoomand 2019	PCR–RFLP	Iran	200	35 ± 5
Nina Kulchenko 2020	Real-time PCR	Russia	195	-
Mozhgan Raigani 2021	PCR–RFLP	Iran	331	35.97 ± 6.94
Tasneem Fatima 2022	PCR–RFLP	Pakistan	128	control group 26–67; patient group22-67

[a] "-" indicates "related data is not available"

[b] "27–52" indicates "The age ranges from 27 to 52"

[c] "38.14 ± 8.13" indicates " The mean age is 38.14 and the SD is 8.13"

Table 2 The results of the quality assessment

Article	1	2	3	4	5	6	7	8	NOS Scores
Inge M. W. Ebisch 2003	1	0	0	1	0	1	1	1	5
L. Stuppia 2033	1	0	1	1	1	1	1	1	7
Jung Hoon Park 2005	1	1	1	1	1	1	1	1	8
KIRAN SINGH 2005	1	0	1	1	2	1	1	1	8
Han-Chul Lee 2006	1	1	0	1	0	1	1	1	6
Paracchini V 2006	1	1	1	1	0	1	1	1	7
Varinderpal S.Dhillon 2007	1	0	1	1	1	1	1	1	7
Zhou-Cun A 2007	1	1	0	1	0	1	1	1	6
Celia Ravel 2009	1	0	1	1	0	1	1	1	6
Singh K 2010	1	0	1	1	2	1	1	1	8
Aleksandra Nikolic 2010	0	0	1	0	0	1	1	1	4
Laurel E Murphy 2011	1	1	1	1	2	1	1	1	9
Kishlay Kumar 2011	0	0	1	1	0	1	1	1	5
Safarinejad MR. 2011	1	0	1	1	0	1	1	1	6
Nishi Gupta 2011	1	0	1	1	0	1	1	1	6
Marcello Machado Gava 2011	1	0	0	1	0	1	1	1	5
Abdelmajid Eloualid 2012	0	0	0	1	0	1	1	1	4
Ling Liu 2012	1	1	1	1	1	1	1	1	8
Djalila Chellat 2012	1	1	0	1	0	1	1	1	6
G. T. Vani 2012	0	0	1	1	0	1	1	1	5
Cristina Camprubí 2013	1	0	0	1	0	1	1	1	5
Hena Naqvi 2014	1	1	1	1	2	1	1	1	9
Stangler Herodež 2013	1	0	0	1	0	1	1	1	5
Nishi Gupta 2013	1	0	1	1	0	1	1	1	6
Mohammad Karimian 2016	1	0	0	1	0	1	1	1	5
Doaa S. Mfady 2014	0	0	1	1	2	1	1	1	7
Alexandra S. Weiner 2014	1	1	1	1	0	1	1	1	7
S.-S. Li 2014	1	1	1	1	0	1	1	1	7
H. Gurkan 2015	0	1	1	1	0	1	1	1	6
Tarek M 2014	0	0	0	1	0	1	1	1	4
Shin Young Kim 2015	1	1	0	1	1	1	1	1	7
X.Y. Li 2015	0	1	0	1	0	1	1	1	5
Mateusz Kurzawski 2015	1	0	0	0	2	1	1	1	6
Wuhua Ni 2015	0	0	0	1	0	1	1	1	4
Hossein Nikzad 2015	0	1	0	1	2	1	1	1	7
Muhammad Irfan 2016	1	1	0	1	1	1	1	1	7
K. Louie 2016	1	0	0	1	1	1	1	1	6
Shu-Yuan Liu 2017	0	0	0	1	1	1	1	1	5
Tao Wang 2017	1	0	1	1	2	1	1	1	8
Shiva Poorang 2018	0	0	1	1	2	1	1	1	7
Chong Xie 2019	1	1	0	0	1	1	1	1	6
Noor Ullah 2019	1	0	0	1	1	1	1	1	6
Nina Kulchenko 2020	0	0	1	0	2	1	1	1	6
Mozhgan Raigani 2021	1	0	1	1	2	1	1	1	8
Tasneem Fatima 2022	1	1	1	0	1	1	1	1	7

Note: 1. Is the case definition adequate; 2. Representativeness of the cases; 3. Selection of Controls; 4. Definition of Controls; 5. Comparability of cases and controls on the basis of the design or analysis; 6. Ascertainment of exposure; 7. Same method of ascertainment for cases and controls; 8. Non-Response rate

Table 3 Detailed summary of the results of the meta-analysis in infertility and fertility populations

Genetic models	MTHFR C677T	MTHFR A1298C	MTRR A66G	MTR A2756G
the additive model	1.25(1.12–1.39), < 0.001	1.10(0.99–1.21),0.072	1.05(0.94–1.18),0.363	1.26(1.03–1.56),0.027
the homozygote model	1.49(1.20–1.84), < 0.001	1.19(0.92–1.53),0.190	1.17(0.94–1.46),0.163	1.82(1.29–2.57),0.001
the heterozygote model	1.22(1.08–1.38), < 0.001	1.09(0.97–1.23),0.156	1.01(0.84–1.21),0.911	1.05(0.91–1.22),0.508
the dominant model	1.28(1.12–1.47), < 0.001	1.11(0.98–1.25),0.092	1.04(0.86–1.25),0.689	1.13(0.98–1.31),0.086
the recessive model	1.33(1.12–1.58),0.001	1.16(0.92–1.47),0.213	1.13(0.94–1.35),0.190	1.85(1.32–2.59), < 0.001

Table 4 Detailed summary of the results of the meta-analysis in abnormospermia and normozoospermia populations

Genetic models	MTHFR C677T	MTHFR A1298C
the additive model	1.28(1.04–1.59),0.023	1.12(0.97–1.30),0.117
the homozygote model	1.57(1.07–2.31),0.021	1.30(0.89–1.90),0.174
the heterozygote model	1.31(1.04–1.65),0.023	1.15(1.02–1.30),0.027
the dominant model	1.36(1.05–1.76),0.021	1.16(1.01–1.34),0.040
the recessive model	1.38(1.03–1.87),0.034	1.28(0.94–1.75),0.123

Note: The analyses of the association between the MTRR A66G or MTR A2756G polymorphism and male abnormospermia have not been carried out due to the small number of literature included

In normospermic and abnormospermic populations

A total of 16 studies, comprising 8,287 participants, reported the association between the MTHFR C677T polymorphism and male abnormospermia. The pooled results showed that the MTHFR C677T polymorphism was significantly associated with male abnormospermia (the additive model T vs. C: OR = 1.28, 95% CI = 1.04–1.59; the homozygote model TT vs. CC: OR = 1.57, 95% CI = 1.07–2.31; the heterozygote model CT vs. CC: OR = 1.31, 95% CI = 1.04–1.65; the dominant model CT + TT vs. CC: OR = 1.36, 95% CI = 1.05–1.76; the recessive model TT vs. CC + CT: OR = 1.38, 95% CI = 1.03–1.87).

Five studies, comprising 4,754 participants, reported the association between the MTHFR A1298C polymorphism and male abnormospermia. And the MTHFR A1298C polymorphism was not associated with male abnormospermia (the additive model C vs. A: OR = 1.12, 95% CI = 0.97–1.30; the homozygote model CC vs. AA: OR = 1.30, 95% CI = 0.89–1.90; the heterozygote model AC vs. AA: OR = 1.15, 95% CI = 1.02–1.30; the dominant model AC + CC vs. AA: OR = 1.16, 95% CI = 1.01–1.34; the recessive model CC vs. AA + AC: OR = 1.28, 95% CI = 0.94–1.75).

In this section, the analyses of the association between the MTRR A66G or MTR A2756G polymorphism and male abnormospermia were not performed due to the limited number of literature included. Both studies reported that no association was found between the MTRR A66G polymorphism and male abnormospermia, and the only study included showed that the MTR A2756G polymorphism was not associated with male abnormospermia.

Subgroup analysis

Subgroup analyses were performed based on country, sample size, study quality and HWE. No significant differences were observed in the subgroup analyses with the exception of the correlation between MTHFR C677T polymorphism and male fertility in the subgroup analysis based on country.

Subgroup analysis revealed that the MTHFR C677T polymorphism was significantly associated with male infertility (T vs. C: OR = 1.33, 95% CI = 1.16–1.52; TT vs. CC: OR = 1.68, 95% CI = 1.26–2.23; CT vs. CC: OR = 1.29, 95% CI = 1.12–1.49; CT + TT vs. CC: OR = 1.37, 95% CI = 1.17–1.60; TT vs. CC + CT: OR = 1.46, 95% CI = 1.15–1.84) and abnormospermia (T vs. C: OR = 1.40, 95% CI = 1.09–1.80; TT vs. CC: OR = 1.88, 95% CI = 1.19–2.98; CT vs. CC: OR = 1.43, 95% CI = 1.10–1.86; CT + TT vs. CC: OR = 1.51, 95% CI = 1.24–2.04; TT vs. CC + CT: OR = 1.58, 95% CI = 1.09–2.27) in Asian countries (Table 5).

Evaluation of heterogeneity

True heterogeneity was observed between all models in terms of the association between the MTHFR C677T polymorphism and male infertility (T vs. C: $I^2 = 75.0\%$, $P < 0.001$; TT vs. CC: $I^2 = 62.4\%$, $P < 0.001$; CT vs. CC: $I^2 = 59.4\%$, $P < 0.001$; CT + TT vs. CC: $I^2 = 69.5\%$, $P < 0.001$; TT vs. CC + CT: I2 = 50.1%, $P < 0.001$) and abnormospermia (T vs. C: $I^2 = 85.1\%$, $p < 0.001$; TT vs. CC: $I^2 = 76.4\%$, $P < 0.001$; CT vs. CC: $I^2 = 73.1\%$, $P < 0.001$; CT + TT vs. CC: $I^2 = 81.5\%$, $P < 0.001$; TT vs. CC + CT: $I^2 = 65.2\%$, $P < 0.001$).

However, a lack of heterogeneity was also observed among all the models regarding the association between MTHFR A1298C polymorphism and male abnormospermia(C vs. A: $I^2 = 26.5\%$, $P = 0.245$; CC vs. AA: $I^2 = 33.9\%$, $P = 0.195$; AC vs. AA: $I^2 < 0.01\%$, $P = 0.527$; AC + CC vs. AA: $I^2 = 8.2\%$, $P = 0.360$; CC vs. AA + AC: $I^2 = 20.6\%$, $P = 0.283$) and MTRR A66G polymorphism

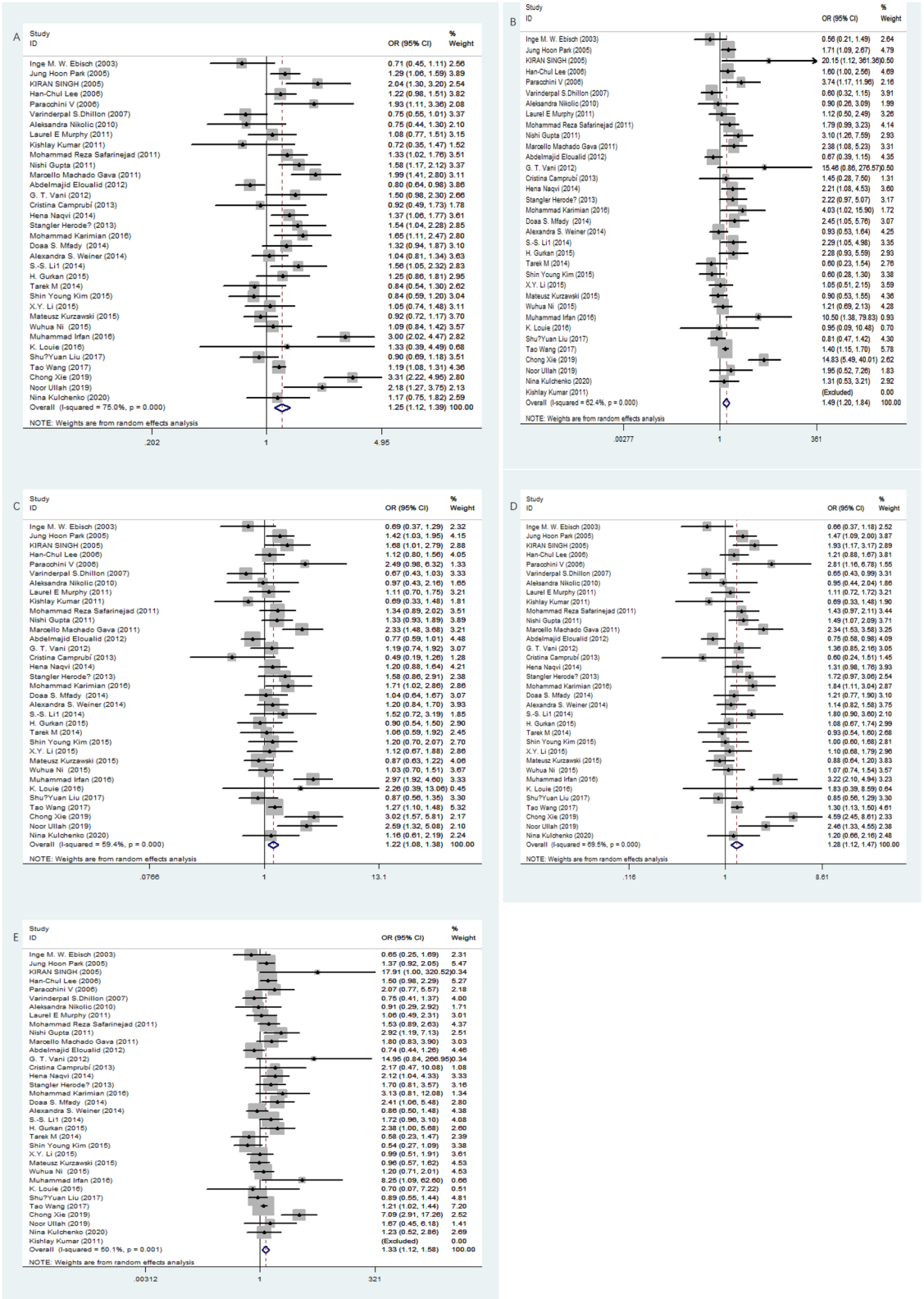

Fig. 2 Forest plot of the association between MTHFR C677T polymorphism and male infertility. **A** the additive model T vs. C; **B** the homozygote model TT vs. CC; **C** the heterozygote model CT vs. CC; **D** the dominant model CT + TT vs. CC; **E** the recessive model TT vs. CC + CT

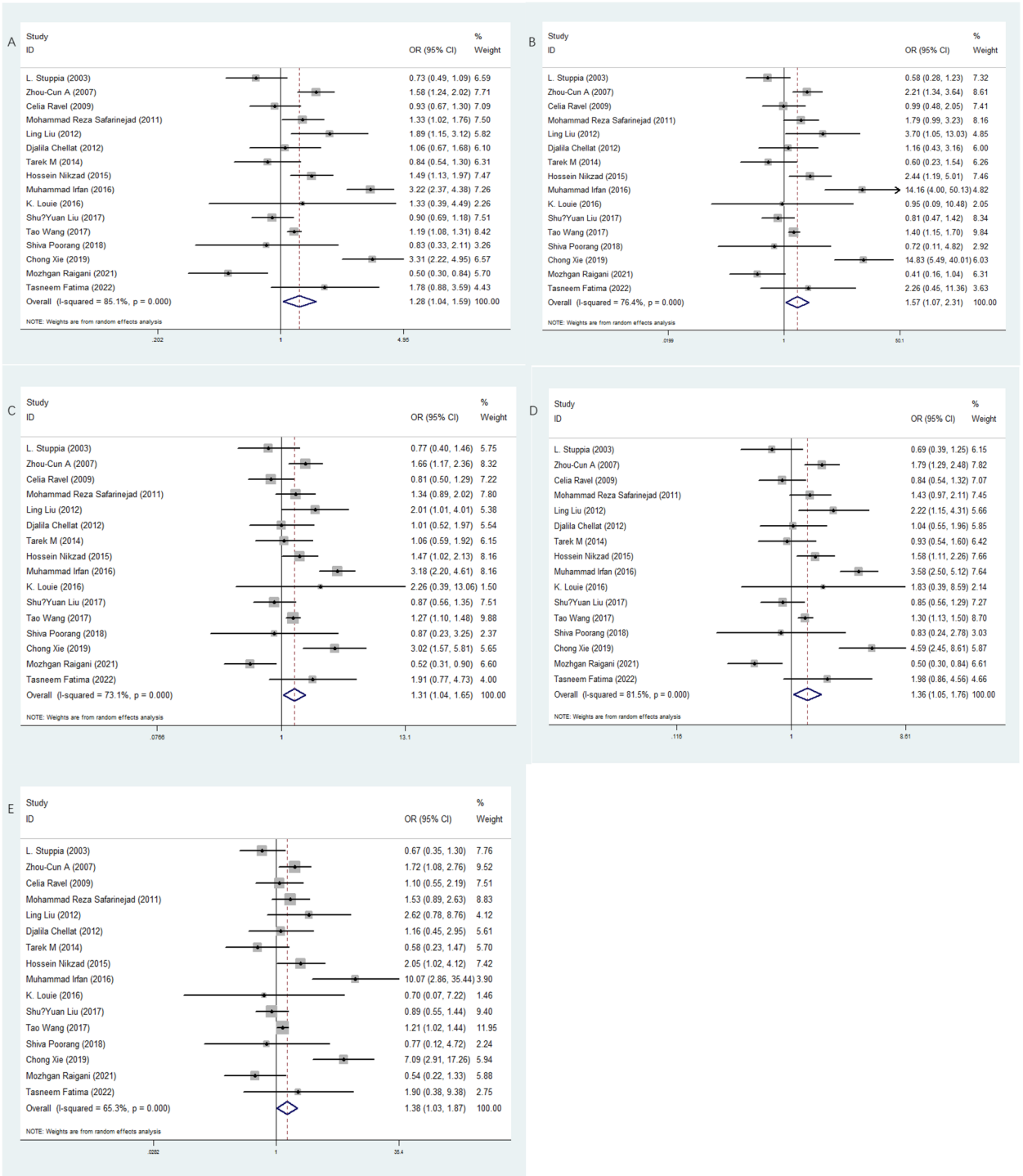

Fig. 3 Forest plot of the association between MTHFR C677T polymorphism and male abnormospermia. **A** the additive model T vs. C; **B** the homozygote model TT vs. CC; **C** the heterozygote model CT vs. CC; **D** the dominant model CT + TT vs. CC; **E** the recessive model TT vs. CC + CT

and male infertility(G vs. A: $I^2 = 20.3\%$, $P = 0.268$; GG vs. AA: $I^2 < 0.01\%$, $P = 0.524$; AG vs. AA: $I^2 = 22.9\%$, $P = 0.247$; AG + GG vs. AA: $I^2 = 32.5\%$, $P = 0.168$; GG vs. AA + AG: $I^2 < 0.01\%$, $P = 0.855$).

Heterogeneity was high among four models (C vs. A: $I^2 = 59.8\%$, $P < 0.001$; CC vs. AA: $I^2 = 59.4\%$, $P < 0.001$; AC + CC vs. AA: $I^2 = 52.8\%$, $P = 0.002$; CC vs. AA + AC: $I^2 = 56.7\%$, $P = 0.001$) in the studies of the association

Fig. 4 Forest plot of the assoassociation between MTHFR A1298C polymorphism and male infertility. **A** the additive model C vs. A; **B** the homozygote model CC vs. AA; **C** the heterozygote model AC vs. AA; **D** the dominant model AC + CC vs. AA; **E** the recessive model CC vs. AA + AC

between the MTHFR A1298C polymorphism and male infertility, and one model showed low heterogeneity (AC vs. AA: $I^2 = 56.7\%$, $P = 0.010$).

Heterogeneity was high among four models (GG vs. AA: $I^2 = 0.00\%$, $P = 0.654$; AG vs. AA: $I^2 = 20.3\%$, $P = 0.262$; GG + AG vs. AA: $I^2 = 10.4\%$, $P = 0.348$; GG vs. AA + AG: $I^2 = 0.00\%$, $P = 0.604$) in the studies of the association between the MTR A2756G polymorphism and male infertility, and one model showed low heterogeneity (G vs. A: $I^2 = 61.0\%$, $P = 0.008$).

Publication bias

Begg's test and Egger's test were performed to assess the publication bias in the literature. Egger's tests also revealed no publication bias. However, according to the results of Begg's test, publication bias was observed

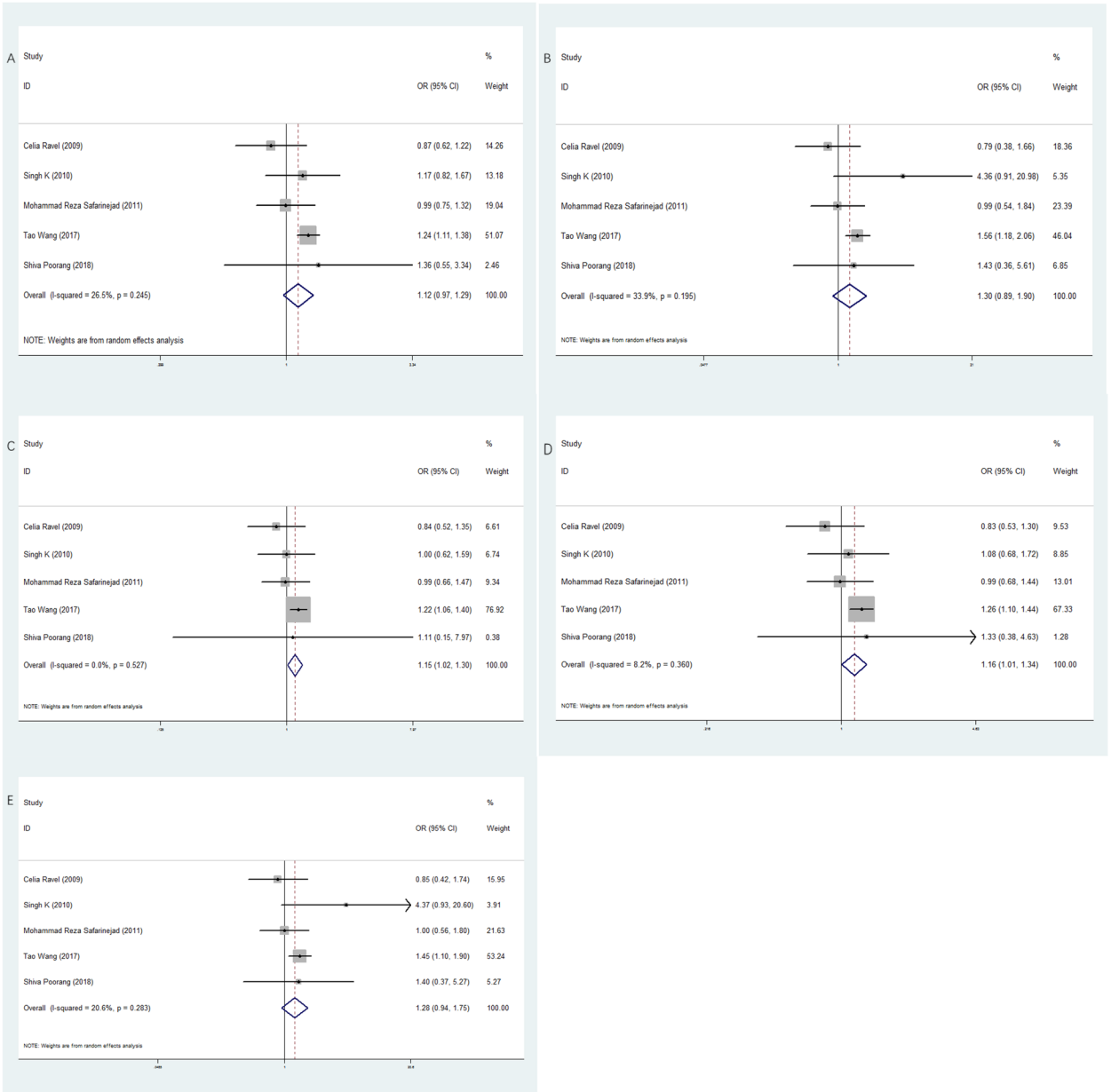

Fig. 5 Forest plot of the assoassociation between MTHFR A1298C polymorphism and male abnormospermia. **A** the additive model C vs. A; **B** the homozygote model CC vs. AA; **C** the heterozygote model AC vs. AA; **D** the dominant model AC + CC vs. AA; **E** the recessive model CC vs. AA + AC

in two genetic models for the association between the MTHFR C677T polymorphism and male infertility (TT vs. CC: Begg's $P = 0.011$; TT vs. CC + CT: Begg's $P = 0.015$) and one genetic model for the association between the MTR A2756G polymorphism and male infertility (GG vs AA + AG: Begg's $P = 0.043$).

Based on Begg's analysis, the trim-and-fill method was performed for the three models. As a result, five articles were corrected and added to the TT vs. CC model (OR = 1.31, 95% CI = 1.04–1.66); six articles were corrected and added to the TT vs. CC + CT model (OR = 1.19, 95% CI = 0.99–1.45); no trimming was performed in the GG vs AA + AG model and the data remained unchanged. There were no changes in the TT vs. CC model GG vs AA + AG model after the trim-and-fill method, suggesting that despite the presence of publication bias, it had no effect on the analysis results. However, contrary results were observed in the TT vs.

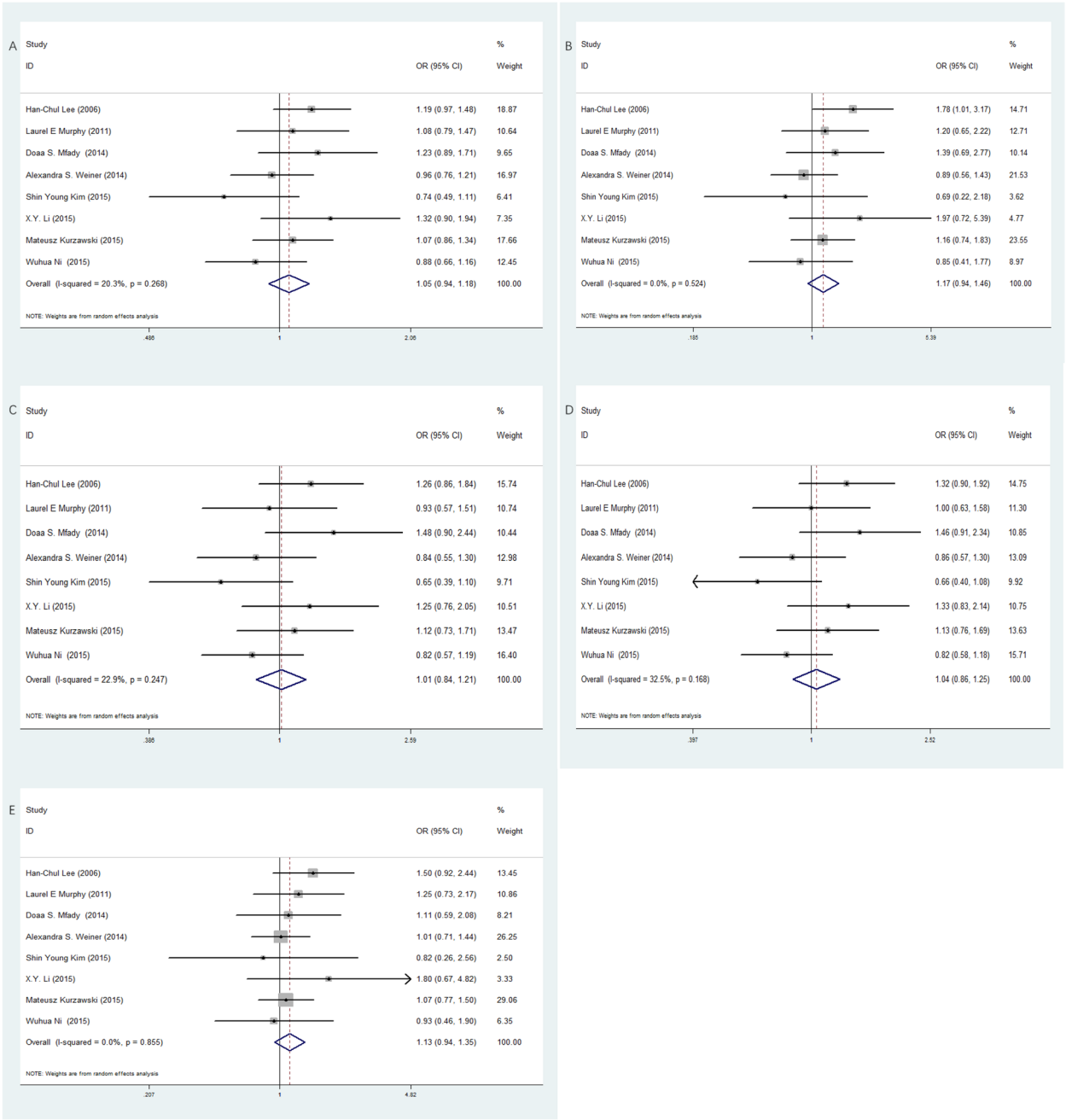

Fig. 6 Forest plot of the association between MTRR A66G polymorphism and male infertility. **A** the additive model G vs. A; **B** the homozygote model GG vs. AA; **C** the heterozygote model AG vs. AA; **D** the dominant model AG + GG vs. AA; **E** the recessive model GG vs. AA + AG

CC + CT model after the trim-and-fill method, suggesting that the analysis results should be considered with caution.

Sensitivity analysis

In the sensitivity analysis, the effect of each study on the pooled OR was evaluated by repeating the meta-analysis while deleting one study at a time. The results reveal the stability of our total results.

Discussions

In this study, we conducted a meta-analysis to comprehensively evaluate the association between MTHFR C677T, MTHFR A1298C, MTRR A66G and MTR A2756G polymorphisms and male fertility in the

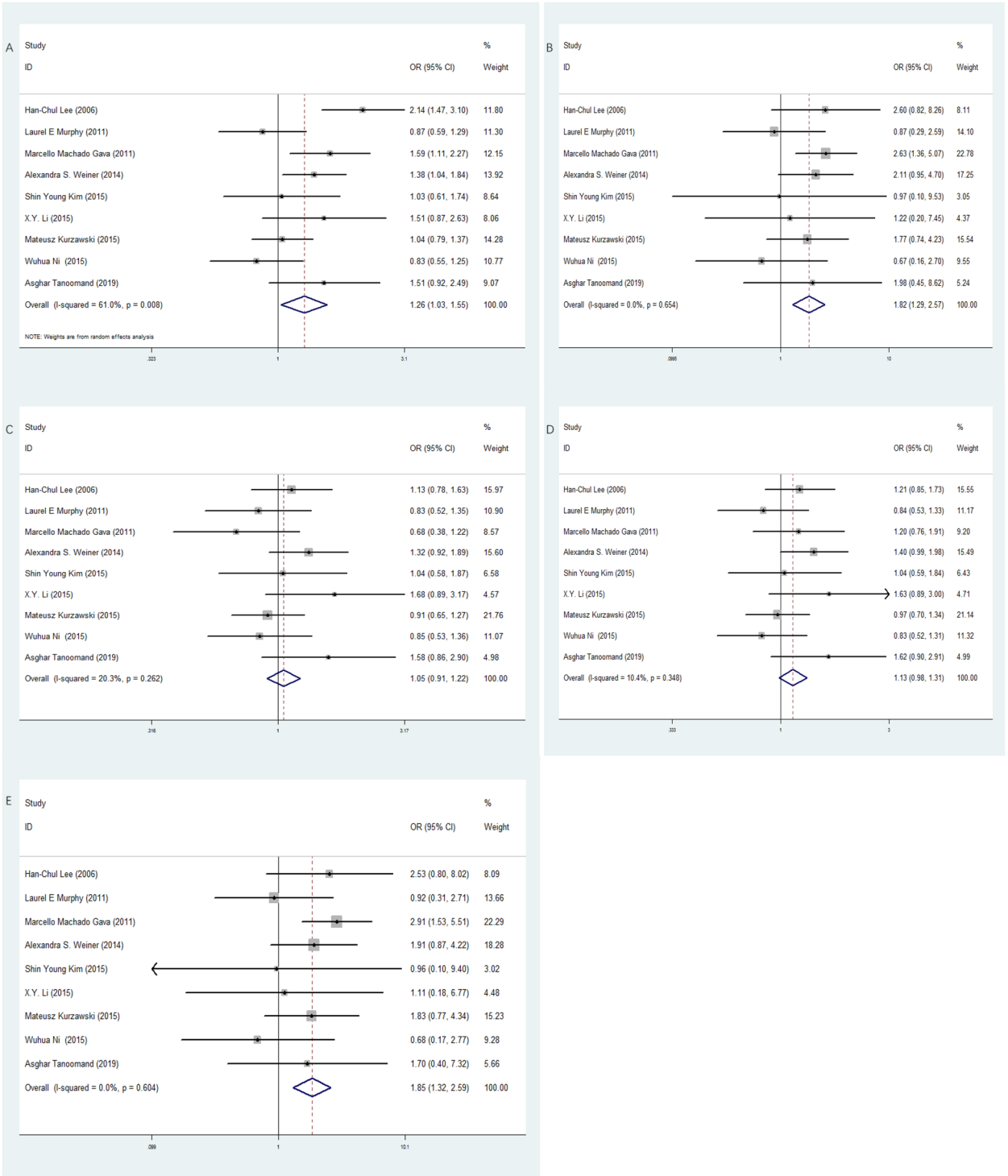

Fig. 7 Forest plot of the association between MTR A2756G polymorphism and male infertility. **A** the additive model G vs. A; **B** the homozygote model GG vs. AA; **C** the heterozygote model AG vs. AA; **D** the dominant model GG + AG vs. AA; **E** the recessive model GG vs. AA + AG

population of not only infeitility/feitility but also normozoospermia/abnormospermia. Our findings indicated a significant association between the MTHFR C677T polymorphisms and male infertilityand abnormospermia/. The MTR A2756G polymorphism may be a potential risk factor for male infertility. However, owing

Table 5 Subgroup analysis results in the Asian country for MTHFR C677T

Genetic models	Infertility/fertility populations	Normozoospermia / abnormospermia populations
T vs C	1.33(1.16–1.52), < 0.001	1.40(1.09–1.80),0.008
TT vs CC	1.68(1.26–2.23), < 0.001	1.88(1.19–2.98),0.007
CT vs CC	1.29(1.12–1.49), < 0.001	1.43(1.10–1.86),0.007
CT + TT vs CC	1.37 (1.17–1.60), < 0.001	1.51(1.12–2.04),0.006
TT vs CC + CT	1.46(1.15–1.84),0.002	1.58(1.09–2.27),0.015

to publication bias, as indicated by Begg's test, a controversial result was observed after the trim-and-fill methods in the TT vs. CC + CT model of the MTHFR C677T polymorphism in fertile and infertile populations. Further more, subgroup analysis revealed a significant association between the MTHFR C677T polymorphism and male fertility in Asian countries.

No significant association was observed between the MTHFR A1298C polymorphism and male fertility in either fertile and infertile populations or normospermic and abnormospermic populations. Moreover, the MTRR A66G polymorphisms were not significantly associated with male fertility in fertile and infertile populations. Additionally, the meta-analysis of the association between the MTRR A66G polymorphism and male fertility in normospermic and abnormospermic populations could not be performed owing to the limited number of eligible studies available.

One of the most auspicious fields of study in the realm of male infertility genetics is the intricate phenomenon of spermatogenesis, where in haploid spermatozoa are produced through mitotic and meiotic divisions of germ cells. Research has established a correlation between male infertility and aberrant folate metabolism. Empirical evidence strongly supports the notion that specific enzymes involved in folate metabolism play a crucial role in male infertility. The evaluation of tolate-metabolizing gene polymorphisms is more and more important in the male fertility assessment.

To date, most of the studies are observational and the research on the mechanisms underlying the association between folate-metabolizing genes polymorphisms and male fertility is still limited. The MTHFR gene plays a crucial role in the folate-metabolism pathway, which is involved in the conversion of 5-methyltetrahydrofolate to 5, 10-methylenetetrahydrofolate. This conversion provides a methyl group to convert homocysteine to methionine. Methionine then provides a methyl group for the formation of S-adenosylmethionine DNA, which is essential for spermatogenesis [82–84]. As a principal regulatory enzyme in the homocysteine metabolism pathway, the MTRR and MTR genes are instrumental in the folate- and vitamin B12-dependent remethylation of homocysteine to methionine [45, 85]. Thus, the MTRR and MTR genes have been implicated in the accumulation of homocysteine, resulting in homocysteinemia, a risk factor for DNA synthesis and methylation [86]. Notably, DNA methylation and DNA synthesis have important effects on spermatogenesis [87]. Additionally, homocysteinemia could increase oxidative stress to cause DNA damage and result in vascular disease to reduce testicular blood flow [88, 89]. The MTRR gene performs reductive methylation of the MTR gene, thereby maintaining the viability of the latter. This process ensures the maintenance of sufficient intracellular folate pools [22]. However, contrasting results on the association between MTHFR C677T, MTHFR A1298C, MTR A2756G and MTRR A66G polymorphisms and male fertility were observed in this study. The specific-pathogenesis for different folate-metabolizing gene polymorphisms remains unexplored.

This study has several strengths, including its pragmatic design. To our knowledge, this is the first systematic review and meta-analysis describing the association between folate-metabolizing gene polymorphisms and male fertility in normospermic and abnormospermic populations, therefore providing the most up-to-date evidence on the association between folate-metabolizing genes polymorphisms and male fertility. The large sample size of 20,439 participants enhances the robustness of our findings. Standard methodologies were used to assess the risk of bias and potential sources of heterogeneity using trim-and-fill methods and subgroup and sensitivity analyses.

However, there are several limitations to consider. Significant heterogeneity was observed in some models, even after subgroup analysis was performed. The high heterogeneity in observational studies is attributed to the high risk of confounding and selection bias that is built into the study design according to GRADE (Grading of Recommendations, Assessment Development and Evaluations). There are multiple definitions of infertility in the included studies, with most studies defining infertility as the failure to conceive a child after one year of regular

unprotected intercourse, whereases some studies extend this to two or more years, which could have significantly affected the meta-analysis heterogeneity. Moreover, the included studies were not consistent in their adjustment of confounding factors. Some studies did not identify the risk factors of male infertility, such as age, alcohol, smoking, dietary parameters and so on. Those studies that did control for confounding factors adjusted for different covariates, thereby increasing the heterogeneity.

In conclusion, this systematic review and meta-analysis suggests that men with the MTHFR C677T polymorphism have an increased risk of infertility and abnormospermia, especially in Asian populations. However, no risk was found in men with the MTHFR A1298C and MTRR A66G polymorphisms. Evaluation of folate-metabolizing gene polymorphisms is a routine part of the female fertility assessment, and it should also be considered in male fertility evaluations. It is recommended that genetic screening for MTHFR C677T polymorphisms be conducted in clinical practice, particularly among men preparing for pregnancy or experiencing infertility. Furthermore, it is recommended that individuals with MTHFR C677T polymorphisms receive appropriate medical care and nutritional management and folic acid management to reduce the risk of infertility. Moreover, further research is required to validate our findings and to investigate the specific aetiology of the association between MTHFR C677T polymorphisms and male infertility in greater depth.

Abbreviations

MTHFR	Methylene tetrahydrofolatereductase
MTRR	Methionine synthase reductase
MTR	Methionine synthase
IVF	In-vitro fertilization
ICSI	Intracytoplasmic sperm injection
NOS	Newcastle–Ottawa Scale
HWE	Hardy–Weinberg Equilibrium
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
GRADE	Grading of Recommendations, Assessment Development and Evaluations
OR	Odds ratio
SD	Standard deviation

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Authors' contributions
TF Y contributed to the conception of the study and was a major contributor in writing the manuscript. TF Y, F L, JJ Q independently selected the eligible studies and performed the quality assessment. Discrepancies were resolved by LX L. All authors read and approved the final manuscript.

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Data availability
All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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