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Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and male infertility risk

An updated meta-analysis

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Abstract

Background: 18 previous meta-analyses have been published on the methylenetetrahydrofolate reductase (*MTHFR)* C677T and A1298C polymorphisms with male infertility risk. However, results of the previous meta-analyses were still inconsistent. Moreover, their meta-analyses did not assess false-positive report probabilities except one study. Furthermore, many new studies have been published, and therefore an updated meta-analysis and re-analysis of systematic previous meta-analyses were performed to further explore these issues.

Objectives: To determine the association between *MTHFR* C677T and A1298C polymorphisms and male infertility risk.

Methods: Crude odds ratios and their 95% confidence intervals were used to assess the association between *MTHFR* C677T and A1298C polymorphisms and male infertility risk. We used the Bayesian false discovery probability (BFDP) to assess the credibility of statistically significant associations.

Results: Fifty-nine studies were included concerning the *MTHFR* C677T and 28 studies were found on the *MTHFR* A1298C with male infertility risk. Overall, the *MTHFR* C677T was associated with increased male infertility risk in overall populations, Africans, East Asians, West Asians, South Asians, azoospermia, and Oligoasthenoteratozoospermia (OAT). In further sensitivity analysis and BFDP test, the positive results were only considered as "noteworthy" in the overall population (TT vs CC: BFDP = 0.294, CT + TT vs CC: BFDP = 0.300, T vs C: BFDP = 0.336), East Asians (TT vs CC: BFDP = 0.089, TT vs CT + CC: BFDP = 0.020, T vs C: BFDP < 0.001), West Asians (TT vs CC: BFDP = 0.584), hospital-based studies (TT vs CC: BFDP = 0.726, TT vs CT + CC: BFDP = 0.126), and OAT (TT vs CT + CC: BFDP = 0.494) for *MTHFR* C677T. In addition, a significantly increased male infertility risk was found in East Asians and population-based studies for *MTHFR* A1298C. However, we did not find that the positive results were considered as "noteworthy" in the overall and all subgroup analyses for *MTHFR* A1298C.

Conclusions: In summary, this study indicates that the *MTHFR* C677T is associated with increased male infertility risk in East Asians, West Asians, and OAT. No significant association was observed on the *MTHFR* A1298C with male infertility risk.

Abbreviations: BFDP = Bayesian false discovery probability, CIs = confidence intervals, HWD = Hardy-Weinberg dis-equilibrium, HWE = Hardy-Weinberg equilibrium, *MTHFR* = methylenetetrahydrofolate reductase, OAT = oligoasthenoteratozoospermia, ORs = odds ratios.

Keywords: Bayesian false discovery probability, male infertility, meta-analysis, Methylenetetrahydrofolate reductase, polymorphism

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This is a meta-analysis, hence, ethical approval was waived or not necessary

This study was designed by Xiao-Feng He and Xiang-Hua Ye. Li-Juan Han and Xiao-Feng He did the literature search, study quality assessment, and data extraction. Xiao-Feng He performed the statistical analysis and drafted the tables and figures. Li-Juan Han wrote the first draft of this analysis, and Xiao-FH and XHY helped to finish the final version. All authors approved the conclusions of our study.

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Supplemental Digital Content is available for this article.

The datasets generated during and/or analyzed during the current study are publicly available.

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1. Introduction

Infertility, defined as the inability to conceive after one year of regular unprotected sexual intercourse by the World Health Organization, has been a major health problem which is multifactorial in nature and affected approximately 15% to 20% of all couples trying for pregnancy.[1–3] Male factors infertility accounts for 40% to 50% about the cases of infertility.[4–5] The etiological factors of male infertility are multifactorial syndrome with a very complex pathogenesis, involving lifestyle, organic diseases, genetic factors, environmental risk factors, and their interactions.[6–8]

Folate play much essential roles for the maintenance of genome integrity in Deoxyribonucleic acid synthesis, repair and methylation.[9] Methylenetetrahydrofolate reductase (*MTHFR)* gene has the chromosomal locus 1p36.6 and is 2.2 kb in length with a total of 11 exons, which is involved in folate and homocysteine metabolism. A change of C to T at nucleotide 677 in *MTHFR* C677T (Ala222Val, rs1801133) results in an amino acid substance change of an alanine to valine, and this substance is associated with reduced enzyme activity that leads to reduced plasma folate levels.[10,11] The *MTHFR* A1298C polymorphism, marked as rs1801131 in the NCBI database, is located at exon 7 and results in a 1298A-C mutation resulting in a glu429-to-ala (E429A) substitution at codon 429,[12] is also associated with decreased enzyme activity.[13,14]

To date, sixty-six studies have been published on the *MTHFR* C677T and A1298C polymorphisms with male infertility risk. However, the results of these studies were still contradictory. In addition, 15 previous meta-analyses[15,17,19–29,31,32] have been reported on the *MTHFR* C677T polymorphism with male infertility risk (as shown in Table 1). Among these publications, two studies[32] investigated this issue in Caucasians, two studies[22,27] in Asians, one study[25] in Chinese population, and 11 studies[15,17,19–21,23,24,26,28,29,31] in overall populations. Moreover, ten previous meta-analyses[15,16,18,20,24–27,30,32] have also been published on the *MTHFR* A1298C polymorphism with male infertility risk (as shown in Table 2). However, the previous meta-analysis results still inconsistent. Moreover, their meta-analyses did not assess false-positive report probabilities except Liu et al[26] by using the Benjamini-Hochberg methods, which control for false discovery rate, furthermore, many new studies have been published, and therefore an updated and high quality meta-analysis were performed to further explore the issues. For all we know, this is the first meta-analysis to further investigate the positive result using a Bayesian method.

2. Materials and methods

2.1. Search strategy

The eligible studies were searched (the deadline was April 9, 2020) to used three databases (PubMed, CNKI, and WangFang). Retrieval strategy was designed by the following keywords (methylenetetrahydrofolate reductase OR *MTHFR*) AND (polymorphism OR mutation OR variant) AND (infertility OR azoospermia OR oligoasthenoteratozoospermia OR oligozoospermia OR subinfertility). Language did not be restrict in this study. We send emails to the corresponding authors if data of a few studies did not be collect by full-text. In addition, the previous meta-analyses were also carefully examined by reference lists.

2.2. Inclusion and exclusion criteria

The inclusion criteria as following:

(1) human case-control or cohort studies (Infertility was defined as conception failure after at least 1 year of regular unprotected sexual intercourse among couples; Controls were healthy without a history of infertility, and had one child at least with normal sperm parameters. In addition, Cases and controls should be comparable),
(2) studies on the *MTHFR* C677T and A1298C polymorphisms and male infertility risk,
(3) If more than one study had been published using the same case series, we selected one study including the maximum sample size, and
(4) the genotype data or odds ratios (ORs) and their 95% confidence intervals (CIs) provided.

The exclusion criteria as following:

(1) data not listed,
(2) not human case–control or cohort studies, and
(3) reviews, meta-analyses, conference abstracts, letters, and editorials.

2.3. Data extraction and quality score assessment

Two authors independently extracted data from selected studies including the following information:

(1) first author's name,
(2) year of publication,
(3) country,
(4) ethnicity,
(5) source of controls,
(6) sample size, and
(7) genotype distribution of male infertility cases and controls.

Two investigators assessed independently the quality of eligible articles. The literature quality assessment criteria was shown in supplemental Table 1, http://links.lww.com/MD/F387. The biggest score value is eleven by the quality assessment; scoring ≥ 5 were considered as high quality studies. A third author adjudicated inconsistent scores.

2.4. Statistical analysis

We evaluated the association between the *MTHFR* C677T and A1298C polymorphisms and male infertility risk by pooled the crude ORs and their 95% CIs. The pooled ORs with the corresponding 95% CIs were performed by the following genetic models: a dominant model: (CT + TT) vs. CC for the *MTHFR* C677T polymorphism and (AC + CC) vs. AA for the *MTHFR* A1298C polymorphism, a recessive model: TT vs (CC + CT) for the C677T and (AC + CC) vs AA for the A1298C, a heterozygote model: CT vs. CC for the C677T and AC vs. AA for the A1298C, a homozygote model: TT vs CC for the C677T and CC vs. AA for the A1298C, and an allele model: T vs. C for the C677T and C vs. A for the A1298C. Heterogeneity among studies was checked according to the Cochran Q[94] and I^2 value[95]. The $P > .10$ and/or $I^2 < 50\%$ indicate a lack of heterogeneity among studies, hence, the pooled crude ORs was calculated using a fixed-effects model (Mantel–Haenszel method)[96]; otherwise, a random-effect model (DerSimonian and Laird method) was applied[97].

Table 1

Results of previous meta-analysis between MTHFR C677T polymorphism with male infertility risk.

First author/ year	Variable	n (Cases/ Controls)	CT vs. CC		TT vs. CC		(CT + TT) vs. CC		TT vs. (CC + CT)		T vs. C		Whether performed assessment of literature quality	Whether performed *P* adjust
			OR (95% CI)	P_h/I^2 (%)	OR (95% CI)	P_h/I^2 (%)	OR (95% CI)	P_h/I^2 (%)	OR (95% CI)	P_h/I^2 (%)	OR (95% CI)	P_h/I^2 (%)		
Ullah[32] 2019	Low income	8 (NA)	NA	NA	NA	NA	1.87 (0.96, 3.64)	NA	NA	NA	NA	NA	No	No
	Middle income	13 (NA)	NA	NA	NA	NA	**1.38 (1.02, 1.88)**	NA	NA	NA	NA	NA		
	High income	9 (NA)	NA	NA	NA	NA	1.26 (0.92, 1.71)	NA	NA	NA	NA	NA		
Shi[27] 2019	Asian	20 (4734/3967)	**1.35 (1.22, 1.49)**	NA/38	**2.08 (1.79, 2.44)**	NA/44	**1.49 (1.35, 1.64)**	NA/50	**1.67 (1.49, 1.89)**	NA/27	**1.43 (1.33, 1.52)**	NA/49	Yes	No
	East Asian	13 (3013/2571)	**1.45 (1.28, 1.67)**	NA/20	**2.13 (1.82, 2.50)**	NA/17	**1.61 (1.43, 1.75)**	NA/28	**1.67 (1.45, 1.89)**	NA/3	**1.45 (1.35, 1.56)**	NA/20		
	South/West Asia	7 (1721/1396)	**1.22 (1.03, 1.43)**	NA/53	**1.89 (1.35, 2.63)**	NA/68	**1.32 (1.12, 1.54)**	NA/65	**1.78 (1.29, 2.14)**	NA/57	**1.33 (1.16, 1.52)**	NA/71		
Hong[19] 2017	Overall	15 (3853/3613)	**1.34 (1.03, 1.74)**	<.001/80	**1.86 (1.36, 2.54)**	0.009/55	**1.46 (1.05, 2.04)**	<.001/89	**1.42 (1.19, 1.70)**	.03/49	**1.38 (1.18, 1.63)**	.0007/66	Yes	No
	Caucasian	2 (NA)	NA	NA	NA	NA	NA	NA	NA	NA	1.23 (0.85, 1.70)	.10/63		
	East-asian	5 (NA)	NA	NA	NA	NA	NA	NA	NA	NA	**1.39 (1.20, 1.61)**	.23/29		
	Middle-estern	2 (NA)	NA	NA	NA	NA	NA	NA	NA	NA	**1.30 (1.05, 1.63)**	.78/0		
	Indian	3 (NA)	NA	NA	NA	NA	NA	NA	NA	NA	1.25 (0.74, 2.13)	.0003/88		
	Mixed-race	1 (NA)	NA	NA	NA	NA	NA	NA	NA	NA	**1.96 (1.35, 2.85)**	.001/63		
Rai[22] 2017	Asian	17 (4392/3667)	**1.40 (1.18, 1.62)**	.005/52.7	**2.10 (1.61, 2.61)**	.02/47.4	**1.53 (1.30, 1.77)**	.005/53.7	**1.70 (1.38, 2.10)**	.03/43.7	**1.99 (1.58, 2.51)**	<.001/89.4	No	No
Ren[25] 2017	Chinese	9 (1713/1104)	NA	NA	**2.08 (1.68, 2.58)**	NA/35	**1.51 (1.30, 1.77)**	NA/29	**1.58 (1.31, 1.90)**	NA/0.0	**1.47 (1.32, 1.63)**	NA/42	Yes	No
Yang et al.[15] 2016	Overall	21 (4505/4024)	**1.21 (1.04, 1.41)**	.001/54.7	**1.63 (1.22, 2.18)**	<.001/69.4	**1.29 (1.09, 1.54)**	<.001/68.6	**1.46 (1.16, 1.85)**	<.001/60.3	**1.26 (1.10, 1.46)**	<.001/76.1	No	No
	Caucasian	13 (NA)	1.13 (0.90, 1.42)	NA	1.38 (0.84, 2.27)	NA	1.17 (0.90, 1.51)	NA	1.30 (0.86, 1.98)	NA	1.16 (0.92, 1.45)	NA		
	Asian	8 (NA)	**1.32 (1.14–1.53)**	NA	**1.90 (1.54, 2.35)**	NA	**1.47 (1.25, 1.73)**	NA	**1.63 (1.36, 1.96)**	NA	**1.40 (1.24, 1.59)**	NA		
Zhu[17] 2016	Overall	26 (5659/5528)	**1.09 (1.00, 1.19)**	.008/45	**1.83 (1.48, 2.26)**	<.001/74	**1.19 (1.04, 1.36)**	.0002/57	**1.54 (1.27, 1.88)**	.002/51	**1.23 (1.10,1.37)**	<.001/66	No	No
	Asian	16 (NA)	**1.22 (1.10, 1.36)**	.08/36	**2.43 (2.08, 2.83)**	.13/30	**1.36 (1.19, 1.56)**	.04/42	**1.81 (1.47, 2.23)**	.08/36	**1.37 (1.27, 1.47)**	.01/51		
	Caucasian	10 (NA)	0.89 (0.77, 1.03)	.28/17	1.08 (0.83, 1.41)	.04/49	0.93 (0.81, 1.06)	.15/32	1.14 (0.92, 1.41)	.05/48	0.99 (0.89, 1.09)	.03/51		
	Azoo*	12 (NA)	**1.67 (1.36, 2.06)**	.14/31	**1.17 (1.02, 1.35)**	.10/36	**1.26 (1.10, 1.44)**	.04/42	**1.50 (1.25, 1.82)**	.3/15	**1.25 (1.14, 1.38)**	.03/49		
	OAT†	14 (NA)	1.01 (0.90, 1.14)	.05/41	1.41 (1.00, 1.99)	<.001/69	1.10 (0.91, 1.33)	.15/32	**1.43 (1.04, 1.98)**	<.001/69	1.17 (0.98, 1.40)	<.001/76		
Gong[23] 2015	Overall	26 (5575/5447)	NA	NA	**1.76 (1.53, 2.01)**	NA/54.0	**1.34 (1.23, 1.46)**	NA/68.2	**1.60 (1.41, 1.81)**	NA/36.9	**1.32 (1.24, 1.41)**	NA/71.5	No	No
	Asian	10 (NA)	NA	NA	NA	NA	**1.54 (1.35, 1.76)**	NA/0.0	NA	NA	NA	NA		
	Caucasian	11 (NA)	NA	NA	NA	NA	**1.19 (1.05, 1.36)**	NA/48.1	NA	NA	NA	NA		
	Azoo*	1412/3532	NA	NA	NA	NA	**1.36 (1.18, 1.55)**	NA/49.1	NA	NA	NA	NA		
	OAT†	615/1865	NA	NA	NA	NA	**1.35 (1.11, 1.64)**	NA/44.7	NA	NA	NA	NA		
Liu[26] 2015	Overall	32 (NA)	**1.17 (1.03, 1.33)**	<.001/NA	**1.62 (1.29, 2.04)**	<.001/NA	**1.26 (1.10, 1.45)**	<.001/NA	**1.47 (1.23, 1.77)**	.002/NA	**1.25 (1.12, 1.40)**	<.001/NA	Yes	Yes (FDR)
	Asian	17 (NA)	**1.28 (1.13, 1.46)**	.153/NA	**2.15 (1.67, 2.75)**	.036/NA	**1.44 (1.24, 1.66)**	.019/NA	**1.79 (1.48, 2.16)**	.193/NA	**1.42 (1.27, 1.60)**	.005/NA		
	Azoo*	14 (NA)	**1.21 (1.04, 1.41)**	.298/NA	**1.64 (1.12, 2.42)**	.002/NA	**1.31 (1.06, 1.61)**	.015/NA	**1.49 (1.07, 2.06)**	.011/NA	**1.27 (1.05, 1.54)**	<.001/NA		
	OAT†	16 (NA)	1.17 (0.96, 1.44)	.001/NA	**1.52 (1.12, 2.06)**	.008/NA	**1.25 (1.01, 1.55)**	<.001/NA	**1.43 (1.13, 1.82)**	.098/NA	**1.24 (1.05, 1.47)**	<.001/NA		
Nikzad[28] 2015	Overall	23 (5174/5253)	NA	NA	**1.44 (1.09, 1.89)**	<.001/66	**1.21 (1.06, 1.39)**	<.001/60	**1.38 (1.14, 1.68)**	.017/43	**1.21 (1.08, 1.36)**	< .001/67	No	No
Weiner[29] 2014	Overall	17 (2972/3436)	NA	NA	NA	NA	1.05 (0.99, 1.11)	NA	NA	NA	NA	NA	No	No
	Azoo*	6 (NA)	NA	NA	NA	NA	1.18 (0.92, 1.51)	NA	NA	NA	NA	NA		
Gupta[31] 2013	Overall	13 (3094/2877)	NA	NA	NA	NA	**1.31 (1.17, 1.46)**	NA	NA	NA	**1.30 (1.20, 1.41)**	NA	No	No
	Azoo*	NA	NA	NA	NA	NA	**1.65 (1.36, 1.99)**	NA	NA	NA	**1.52 (1.32, 1.17)**	NA		
	OAT†	NA	NA	NA	NA	NA	1.08 (0.88, 1.34)	NA	NA	NA	**1.43 (1.18, 1.73)**	NA		
Wu[21] 2012	Overall	10 (2275/1958)	1.11 (0.86, 1.43)	.004/NA	1.39 (0.93, 2.07)	<.001/NA	1.15 (0.89, 1.49)	<.001/NA	1.34 (0.99, 1.81)	.012/NA	1.17 (0.95, 1.43)	<.001/NA	No	No
	Asian	6 (NA)	1.70 (0.97, 1.72)	.017/NA	**1.79 (1.08, 2.96)**	.003/NA	**1.42 (1.02, 1.96)**	.001/NA	**1.50 (1.21, 1.86)**	.055/NA	**1.36 (1.06, 1.75)**	<.001/NA		
	Caucasian	3 (NA)	0.71 (0.45, 1.12)	.932/NA	0.71 (0.45, 1.12)	.522/NA	0.75 (0.55, 1.01)	.774/NA	0.81 (0.53, 1.23)	.534/NA	0.81 (0.65, 1.01)	.534/NA		
	Azoo*	5 (NA)	**1.45 (1.18, 1.79)**	.340/NA	**1.89 (1.43, 2.51)**	.308/NA	**1.55 (1.28, 1.88)**	.257/NA	**1.51 (1.17, 1.95)**	.333/NA	**1.38 (1.20, 1.57)**	.155/NA		
	OAT†	7 (NA)	0.90 (0.74, 1.08)	.062/NA	1.02 (0.78, 1.32)	.064/NA	0.91 (0.69, 1.19)	.031/NA	1.08 (0.85, 1.38)	.119/NA	0.96 (0.78, 1.18)	.017/NA		
Wei[24] 2012	Overall	11 (2217/2312)	1.22 (0.96, 1.56)	.001/NA	1.40 (0.98, 2.00)	.001/NA	1.26 (0.97, 1.65)	<.001/NA	**1.28 (1.00, 1.64)**	.09/NA	NA	NA	No	No
	Caucasian	5 (635/611)	1.17 (0.67, 2.06)	.001/NA	1.18 (0.60, 2.34)	.01/NA	1.17 (0.65, 2.12)	<.001/NA	1.09 (0.70, 1.71)	.15/NA	NA	NA		
	Asian	6 (1582/1701)	1.26 (0.98, 1.62)	.03/NA	**1.57 (1.05, 2.37)**	.02/NA	**1.34 (1.01, 1.77)**	.004/NA	**1.40 (1.05, 1.86)**	.17/NA	NA	NA		
Tüttelmann[20] 2007	Overall	8 (1843/1791)	NA	NA	NA	NA	**1.39 (1.15, 1.69)**	NA	NA	NA	NA	NA	No	No

* Azoospermia.

† Including oligoasthenoteratozoospermia (OAT), severe OAT, oligozoospermia, and teratozoospermia. NA = not available.

Table 2

Results of previous meta-analysis between MTHFR A1298C polymorphism with male infertility risk.

			AC vs. AA		CC vs. AA		(AC + CC) vs. AA		CC vs. (AA + AC)		C vs. A			
First author	Variable	n (Cases/ Controls)	OR (95% CI)	P_h/I^2 (%)	OR (95% CI)	P_h/I^2 (%)	OR (95% CI)	P_h/I^2 (%)	OR (95% CI)	P_h/I^2 (%)	OR (95% CI)	P_h/I^2 (%)	Whether performed assessment of literature quality	Whether performed *P* adjust
Shi[27] 2019	Asian	12 (2673/2328)	1.20 (1.08, 1.37)	NA/27	1.64 (1.08, 2.56)	NA/ 58	1.27 (1.14, 1.43)	NA/46	1.61 (1.27, 2.04)	NA/50	1.22 (1.05, 1.41)	NA/57	Yes	No
	East Asian	7 (1759/1586)	1.35 (1.16, 1.56)	NA/0	2.17 (1.11, 4.17)	NA/ 65	1.43 (1.25, 1.67)	NA/38	2.04 (1.47, 2.86)	NA/59	1.37 (1.12, 1.67)	NA/56		
	South/West Asia	5 (878/742)	0.96 (0.78, 1.19)	NA/0	1.14 (0.78, 1.67)	NA/0	1.00 (0.82,1.22)	NA/0	1.20 (0.84, 1.72)	NA/0	1.03 (0.77, 1.20)	NA/0		
Ullah[32] 2019	Low income	6 (NA)	NA	NA	NA	NA	NA	NA	1.71 (1.19, 2.47)	NA	NA	NA	No	No
	Middle income	10 (NA)	NA	NA	NA	NA	NA	NA	1.02 (0.81, 1.28)	NA	NA	NA		
	High income	4 (NA)	NA	NA	NA	NA	NA	NA	0.86 (0.62, 1.19)	NA	NA	NA		
Zhang[16] 2017	Overall	20 (4293/4507)	1.02 (0.93, 1.12)	.165/NA	1.01 (0.85, 1.20)	.100/NA	1.02 (0.93, 1.12)	.157/NA	1.01 (0.86, 1.19)	.111/NA	1.02 (0.95, 1.09)	.148/NA	Yes	No
	Caucasian	15 (NA)	0.95 (0.85, 1.06)	.142/NA	0.94 (0.78, 1.14)	.063/NA	0.95 (0.86, 1.06)	.177/NA	0.96 (0.80, 1.14)	.056/NA	0.96 (0.89, 1.04)	.202/NA		
	Asian	5 (NA)	1.20 (1.01, 1.44)	.994/NA	1.41 (0.93, 2.15)	.860/NA	1.23 (1.04, 1.45)	.996/NA	1.33 (0.88, 2.02)	.846/NA	1.20 (1.04, 1.39)	.985/NA		
Ren[25] 2017	Chinese	3 (540/457)	NA	NA	1.34 (0.66, 2.71)	NA/0.0	1.27 (0.95, 1.65)	NA/0.0	1.44 (0.72, 2.88)	NA/9	1.22 (0.97, 1.53)	NA/0.0	Yes	No
Yang[15] 2016	Overall	13 (2785/3094)	1.02 (0.91, 1.14)	.216/22.5	1.29 (1.03, 1.62)	.330/11.5	1.06 (0.95, 1.18)	.224/21.7	1.29 (1.03, 1.60)	.345/10.0	1.08 (0.99, 1.18)	.294/15.0	No	No
	Caucasian	8 (NA)	0.90 (0.78, 1.04)	NA	1.31 (1.00, 1.72)	NA	0.95 (0.83, 1.09)	NA	1.35 (1.04, 1.74)	NA	1.02 (0.92, 1.14)	NA		
	Asian	5 (NA)	1.24 (1.03, 1.48)	NA	1.24 (0.82, 1.88)	NA	1.24 (1.04, 1.47)	NA	1.16 (0.77, 1.75)	NA	1.19 (1.03, 1.37)	NA		
Liu[26] 2015	Overall	17 (NA)	NA	NA	NA	NA	NA	NA	1.11 (0.87, 1.41)	NA	NA	NA	Yes	Yes (FDR)
Gupta[30] 2013	Overall	10 (2734/2737)	NA	NA	NA	NA	1.05 (0.89, 1.23)	.058/45.4	NA	NA	NA	NA	No	No
	Azoo*	NA	NA	NA	NA	NA	0.97 (0.79, 1.18)	.697/0.0	NA	NA	NA	NA		
	OAT†	NA	NA	NA	NA	NA	0.96 (0.74, 1.24)	.006/66.6	NA	NA	NA	NA		
Shen[18] 2012	Overall	7 (1633/1735)	1.10 (0.95, 1.27)	.855/NA	1.29 (0.97, 1.72)	.087/NA	1.13 (0.98, 1.30)	.578/NA	1.26 (0.95, 1.65)	.119/NA	1.12 (1.00, 1.26)	.215/NA	No	No
	Asian	5 (NA)	1.11 (0.94, 1.31)	.919/NA	1.18 (0.84, 1.66)	.435/NA	1.12 (0.96, 1.31)	.917/NA	1.14 (0.82, 1.59)	.415/NA	1.10 (0.97, 1.25)	.841/NA		
	Caucasian	2 (NA)	1.06 (0.77, 1.45)	.206/NA	1.55 (0.42, 5.72)	.012/NA	1.15 (0.85, 1.55)	.052/NA	1.52 (0.49, 4.71)	.023/NA	0.81 (0.65, 1.01)	.011/NA		
	Azoo*	4 (NA)	1.01 (0.78, 1.31)	.840/NA	1.66 (1.01, 2.73)	.124/NA	1.08 (0.85, 1.38)	.965/NA	1.67 (1.03, 2.71)	.078/NA	1.14 (0.94, 1.38)	.625/NA		
	OAT†	5 (NA)	1.10 (0.91, 1.34)	.401/NA	1.15 (0.82, 1.63)	.140/NA	1.12 (0.93, 1.34)	.177/NA	1.12 (0.81, 1.56)	.290/NA	1.09 (0.95, 1.26)	.079/NA		
Wei[24] 2012	Overall	7 (1633/1735)	1.30 (0.87, 1.95)	.09/NA	1.10 (0.95, 1.27)	.86/NA	1.13 (0.98, 1.30)	.58/NA	1.26 (0.95, 1.65)	.12/N1	NA	NA	No	No
	Caucasian	2 (406/346)	1.55 (0.42, 5.72)	.01/NA	1.06 (0.77, 1.45)	.21/NA	1.15 (0.85, 1.55)	.05/NA	1.54 (0.94, 2.54)	.02/N1	NA	NA		
	Asian	5 (1227/1389)	1.16 (0.82, 1.64)	.44/NA	1.11 (0.94, 1.31)	.92/NA	1.12 (0.96, 1.32)	.92/NA	1.14 (0.82, 1.59)	.42/NA	NA	NA		
Tüttelmann[20] 2007	Overall	2 (539/525)	NA	NA	NA	NA	0.97 (0.54, 1.74)	NA	NA	NA	NA	NA	No	No

* Azoospermia, [2] Including oligoasthenoteratozoospermia (OAT), severe OAT, oligozoospermia, and teratozoospermia.

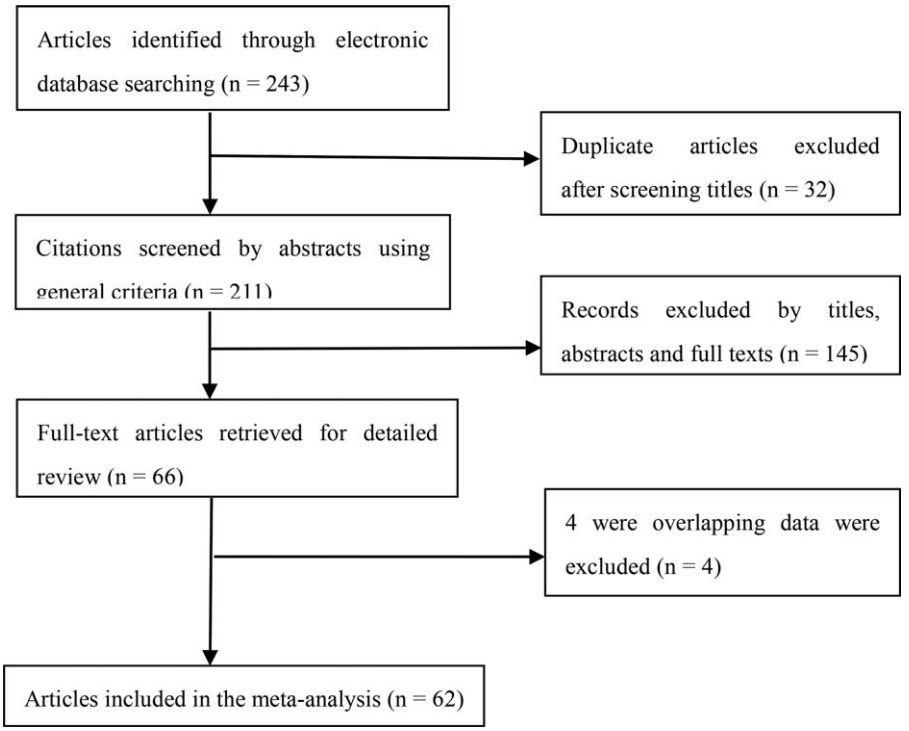

Figure 1. OA_ Guidelines Flow Diagram Study selection flowchart in the current meta-analysis.

A meta-regression analysis was used to explore sources of heterogeneity[98] if heterogeneity among studies was significant. Subgroup analyses were conducted according to ethnicity, source of controls and type of male infertility. Sensitivity analyses were also performed to estimate the robustness of the pooled results. We used the following methods to perform the sensitivity analyses: excluded the studies of Hardy-Weinberg dis-equilibrium (HWD) and quality scores < 5. Hardy-Weinberg equilibrium (HWE) was calculated by chi-square goodness-of-fit test, and significant deviation was considered in control groups if the P value < .05. The publication bias was assessed to using Begg funnel[99] and Egger test.[100] Last, a Bayesian false discovery probability (BFDP: a cutoff value was set up to be a level of 0.8 and a prior probability of 0.001)[101] was used to evaluate positive results whether were noteworthy or not. All statistical analyses were conducted using STATA version 12.0 (STATA Corporation, College Station, TX).

3. Results

3.1. Study characteristics

A flowchart of study selection is listed in Figure 1. Overall, we retrieved 243 publications by several databases. Among these publications, sixty-six articles were selected after filtering titles, abstracts, and full texts. In addition, the sample size of four publications[54,75,86,92] overlapped with those of another four publications.[2,57,61,88] Therefore, sixty-two publications were involved in the final analysis. Table 3 lists the main characteristics of the selected studies. Fifty-nine studies[2,28,29,31–43,46–53,55–63,65–74,76–85,87–91] were included concerning the *MTHFR* C677T polymorphism (11,767 male infertility cases and 10,591 controls; two studies on Africans, thirteen on Caucasians, twenty-seven on East Asians, seven on West Asians, eight on South Asians, and two mixed populations; fifty-three hospital-based studies and six population-based studies; twenty-four azoospermia studies and thirty-seven Oligoasthenoteratozoospermia (OAT) studies) with male infertility risk. Twenty-eight studies were found on the *MTHFR* A1298C polymorphis[2,29,30,32,37,38,41,43–45,49,50,52,53,55–59,61,63–65,72,73,80,81,91] (5,976 male infertility cases and 5,774 controls; four studies on South Asians, 7 on West Asians, nine on East Asians, six on Caucasians, one on Africans, and one mixed populations; twenty-five hospital-based studies and three population-based studies; twelve azoospermia studies and twelve OAT studies) with male infertility risk. In addition, HWD of controls was observed in six studies[2,32,43,69,76,80] for C677T polymorphism and six studies[32,44,49,63,81,91] for A1298C polymorphism.

3.2. Quantitative synthesis

3.2.1. MTHFR C677T polymorphism. Table 4 shows the results of the association between the *MTHFR* C677T polymorphism and male infertility risk. Overall, a significantly increased male infertility risk (CT vs CC: OR = 1.27, 95% CI: 1.15–1.40, P_h < .001, I^2 = 54.1%; TT vs CC: OR = 1.74, 95% CI: 1.47–2.07, P_h < .001, I^2 = 65.3%; CT + TT vs CC: OR = 1.38, 95% CI: 1.24–1.54, P_h < .001, I^2 = 66.6%; TT vs CC + CT: OR = 1.52, 95% CI: 1.33–1.74, P_h < .001, I^2 = 56.4%; T vs C: OR = 1.33, 95% CI: 1.22–1.45, P_h < .001, I^2 = 73.1%) was observed in all eligible studies.

In subgroup analyses by ethnicity and source of controls, a significantly increased male infertility risk was found in Africans (CT + TT vs CC: OR = 0.78, 95% CI: 0.62–0.99,

Table 3

Characteristics of studies included in the current meta-analysis.

First Author/Year	Country	Ethnicity	SC	Sample size	Case									Control			HWE	Quality score
					Azoospermia			OAT			Total							
					CC	CT	TT	CC	CT	TT	CC	CT	TT	CC	CT	TT		
MTHFR C667T																		
Bezold[33] 2001	German	Caucasian	HB	255/200	–	–	–	–	–	–	114	93	48	92	89	19	0.705	5
Stuppia[34] 2003	Italy	Caucasian	HB	93/105	8	6	7	29	31	12	37	37	19	33	43	29	0.066	4
Ebisch[35] 2003	Netherlands	Caucasian	PB	77/113	–	–	–	–	–	–	42	28	7	50	48	15	0.522	4
Singh[36] 2005	India	South Asian	PB	151/200	–	–	–	–	–	–	105	40	6	163	37	0	0.149	6
Park[37] 2005	Korea	East Asian	HB	373/396	75	164	47	28	40	17	105	205	63	145	200	51	0.161	5
Lee[38] 2006	Korea	East Asian	HB	360/325	44	100	30	71	81	34	115	181	64	118	166	41	0.138	5
Paracchini[39] 2006	Italy	Caucasian	HB	59/46	–	–	–	–	–	–	11	32	16	18	21	7	0.83	4
A[40] 2007	China	East Asian	HB	355/252	83	97	48	47	63	17	130	160	65	128	95	29	0.085	5
Dhillon[41] 2007	India	South Asian	HB	179/200	–	–	–	81	77	21	81	77	21	70	100	30	0.556	5
Sun[42] 2007	China	East Asian	NR	182/53	–	–	–	22	75	52	27	86	69	15	28	10	0.63	3
Zhang[73] 2007	China	East Asian	HB	165/132	–	–	–	–	–	–	41	93	31	48	60	24	0.492	4
Ravel[43] 2009	French	Caucasian	HB	250/113	33	31	6	85	70	25	118	101	31	49	52	31	0.024	3
Yang[72] 2010	China	East Asian	HB	131/293	–	–	–	34	55	42	34	55	42	98	142	53	0.901	4
Đorđević[61] 2010	Serbia	Caucasian	HB	52/56	–	–	–	–	–	–	22	24	6	23	26	7	0.934	5
Zhang[82] 2010	China	East Asian	HB	491/430	–	–	–	–	–	–	43	253	195	87	213	130	0.988	5
Gava[45] 2011	Brazil	Mixed	HB	156/233	27	15	7	54	45	8	81	60	15	167	53	13	0.003	3
Safarinejad[46] 2011	Iran	West Asian	HB	164/328	–	–	–	58	80	26	58	80	26	144	148	36	0.826	7
Liu[47] 2011	China	East Asian	HB	75/72	–	–	–	27	38	10	27	38	10	40	28	4	0.753	3
Qiu[48] 2011	China	East Asian	HB	271/180	42	66	50	33	46	34	75	112	84	63	85	32	0.72	4
Murphy[64] 2011	Swede	Caucasian	HB	153/184	–	–	–	–	–	–	73	63	13	94	73	15	0.876	6
Kumar[67] 2011	India	South Asian	HB	100/100	–	–	–	–	–	–	86	14	0	81	19	0	0.294	4
Gupta[31] 2011	India	South Asian	HB	522/315	49	15	4	144	46	10	378	116	28	251	58	6	0.229	5
Vani[49] 2012	India	South Asian	HB	206/230	–	–	–	–	–	–	158	42	6	188	42	0	0.128	4
Eloualid[50] 2012	Morocco	African	HB	344/690	65	37	8	134	88	12	199	125	20	351	286	53	0.611	6
Chellat[69] 2012	Algeria	Mixed	HB	74/84	20	19	7	11	14	3	31	33	10	36	38	10	0.995	3
Liu[68] 2012	China	East Asian	HB	75/72	–	–	–	27	38	10	27	38	10	40	28	4	0.753	3
Stangler[66] 2013	Slovene	Caucasian	PB	100/111	–	–	–	–	–	–	29	51	20	47	50	14	0.902	6
Camprubi[71] 2013	Spain	Caucasian	HB	107/25	–	–	–	42	36	14	47	43	17	8	15	2	0.172	3
Pei J[75] 2013	China	East Asian	HB	290/90	–	–	–	–	–	–	39	138	113	24	47	19	0.651	4
Balkan[81] 2014	Turkey	West Asian	NR	108/125	57	40	11	–	–	–	57	40	11	78	36	11	0.032	3
Mfady[51] 2014	Jordan	West Asian	HB	150/150	–	–	–	–	–	–	67	63	20	74	67	9	0.221	5
Naqvi[52] 2014	India	South Asian	HB	637/364	34	11	4	413	143	33	447	154	36	275	79	10	0.145	7
Li SS[54] 2014	China	East Asian	HB	82/133	–	–	–	–	–	–	14	36	32	36	61	36	0.34	4
Weiner[29] 2014	Russia	Caucasian	PB	271/301	49	41	8	40	31	11	129	116	26	153	115	33	0.113	7
Vardarli[62] 2014	Turkey	Caucasian	HB	100/50	23	22	5	21	22	7	44	44	12	30	20	0	0.077	4
Hussein[77] 2014	Egypt	African	HB	107/107	64	35	8	–	–	–	64	35	8	62	32	13	0.012	3
Ng[78] 2014	Canada	Caucasian	NR	39/19	10	10	2	12	4	1	22	14	3	8	5	3	0.219	2
Ni W[56] 2015	China	East Asian	PB	296/204	–	–	–	–	–	–	117	135	44	84	94	26	0.97	7
Gurkan[57] 2015	Turkey	West Asian	HB	137/134	41	25	9	29	24	9	70	49	18	71	55	8	0.533	5
Li XY[58] 2015	China	East Asian	HB	162/120	36	49	15	25	28	9	61	77	24	48	54	18	0.661	5
Kurzawski[59] 2015	Poland	Caucasian	HB	284/352	–	–	–	–	–	–	143	113	28	166	150	36	0.806	5
Kim[60] 2015	Korea	East Asian	HB	85/246	30	44	11	–	–	–	30	44	11	87	106	53	0.057	4
Nikzad[28] 2015	Iran	West Asian	HB	242/255	47	49	11	62	60	13	109	109	24	144	98	13	0.48	5
Karimian[53] 2016	Iran	West Asian	HB	118/132	–	–	–	51	59	8	51	59	8	77	52	3	0.087	4
Irfan[79] 2016	Pakistan	South Asian	PB	437/218	36	18	3	249	118	3	285	136	16	187	30	1	0.862	9
Najafipour[74] 2017	Iran	West Asian	HB	280/120	25	34	11	88	89	33	113	123	44	66	43	11	0.31	4
Ma FF[86] 2017	China	East Asian	HB	140/96	40	30	4	36	22	8	76	52	12	44	44	8	0.514	4
Hu[70] 2017	China	East Asian	HB	186/131	–	–	–	68	80	38	68	80	38	72	41	18	0.005	3
Wang Y[83] 2018	China	East Asian	HB	76/95	–	–	–	14	34	11	15	37	24	24	54	17	0.163	3
Hu LL[84] 2018	China	East Asian	HB	145/88	–	–	–	23	60	62	23	60	62	11	48	29	0.194	4
Zhou SH[85] 2018	China	East Asian	HB	145/88	3	18	8	4	13	11	15	90	40	11	48	29	0.194	4
Cai[63] 2018	China	East Asian	HB	90/90	–	–	–	13	40	37	13	40	37	26	47	17	0.602	3
Zuo YJ[80] 2018	China	East Asian	HB	154/294	–	–	–	33	59	62	33	59	62	95	138	61	0.406	4
Ullah[32] 2019	Pakistan	South Asian	HB	232/114	–	–	–	–	–	–	169	53	10	99	12	3	0.003	3
Xie C[88] 2019	China	East Asian	HB	167/78	–	–	–	23	82	62	23	82	62	33	39	6	0.229	4
Suo F[89] 2019	China	East Asian	HB	715/572	–	–	–	126	326	264	126	326	264	134	272	166	0.272	6
Shao LJ[90] 2019	China	East Asian	HB	167/65	–	–	–	52	71	44	52	71	44	30	28	7	0.903	4
Song N[91] 2019	China	East Asian	HB	100/100	–	–	–	–	–	–	31	46	23	32	52	16	0.501	4

(*continued*)

Table 3

(continued).

First Author/Year	Country	Ethnicity	SC	Sample size	Case									Control			HWE	Quality score
					Azoospermia			OAT			Total							
					CC	CT	TT	CC	CT	TT	CC	CT	TT	CC	CT	TT		
Xu JJ[92] 2019	China	East Asian	HB	104/108	–	–	–	–	–	–	38	41	29	50	44	14	0.386	4
MTHFR A1298C																		
Park[37] 2005	Korea	East Asian	HB	373/396	–	–	–	–	–	–	237	118	18	269	111	16	0.294	5
Lee[38] 2006	Korea	East Asian	HB	360/325	109	57	8	113	63	10	222	120	18	213	98	14	0.526	5
Dhillon[41] 2007	India	South Asian	HB	179/200	–	–	–	90	80	9	90	80	9	103	84	13	0.451	5
Zhang[73] 2007	China	East Asian	HB	165/132	–	–	–	–	–	–	90	65	15	85	45	2	0.142	4
Ravel[43] 2009	French	Caucasian	HB	250/113	34	28	7	97	66	18	131	94	25	54	46	13	0.501	4
Farcas[65] 2009	Romania	Caucasian	HB	66/67	–	–	–	–	–	–	35	29	2	39	26	2	0.34	4
Singh[44] 2010	India	South Asian	HB	151/141	66	76	9	–	–	–	66	76	9	64	74	2	0.0002	3
Zhang[82] 2010	China	East Asian	HB	491/430	–	–	–	–	–	–	224	220	47	270	150	10	0.039	4
Gava[45] 2011	Brazil	Mixed	HB	156/233	26	14	9	45	48	14	71	62	23	130	89	14	0.811	4
Safarinejad[46] 2011	Iran	West Asian	HB	164/328	–	–	–	75	70	19	75	70	19	149	141	38	0.599	7
Murphy[64] 2011	Swede	Caucasian	HB	153/184	–	–	–	–	–	–	58	77	11	87	62	27	0.007	5
Eloualid[50] 2012	Morocco	African	HB	344/690	67	39	4	138	83	13	205	122	17	370	303	17	<0.001	5
Gupta[30] 2013	India	South Asian	HB	611/136	–	–	–	–	–	–	165	320	126	27	74	35	0.283	7
Stangler[66] 2013	Slovene	Caucasian	PB	100/111	–	–	–	–	–	–	44	35	21	48	50	13	0.997	6
Weiner[29] 2014	Russia	Caucasian	PB	275/349	37	54	8	42	32	9	126	125	23	142	142	30	0.52	7
Mfady[51] 2014	Jordan	West Asian	HB	150/150	–	–	–	–	–	–	71	61	18	59	75	16	0.273	5
Vardarli[62] 2014	Turkey	West Asian	HB	100/50	21	23	6	24	18	8	45	41	14	19	22	9	0.556	4
Balkan[81] 2014	Turkey	West Asian	NR	108/125	47	42	19	–	–	–	47	42	19	45	56	24	0.383	4
Li SS[54] 2014	China	East Asian	HB	82/133	–	–	–	–	–	–	49	29	4	88	36	9	0.059	4
Ni W[56] 2015	China	East Asian	PB	296/204	–	–	–	–	–	–	181	106	9	137	62	5	0.514	7
Gurkan[57] 2015	Turkey	West Asian	HB	137/134	34	34	7	29	25	8	63	59	15	49	66	19	0.668	5
Li XY[58] 2015	China	East Asian	HB	162/120	66	31	3	35	23	4	101	54	7	80	38	2	0.29	5
Kurzawski[59] 2015	Poland	Caucasian	HB	284/352	–	–	–	–	–	–	128	130	26	156	156	40	0.916	5
Kim[60] 2015	Korea	East Asian	HB	85/246	52	28	5	–	–	–	52	28	5	184	56	6	0.486	4
Karimian[53] 2016	Iran	West Asian	HB	118/132	–	–	–	59	44	15	59	44	15	70	48	14	0.194	4
Najafipour[74] 2017	Iran	West Asian	HB	280/120	27	30	13	102	114	22	129	116	35	57	50	13	0.683	4
Ullah[32] 2019	Pakistan	South Asian	HB	235/109	–	–	–	–	–	–	59	133	43	47	59	3	0.002	3
Xu JJ[92] 2019	China	East Asian	HB	104/108	–	–	–	–	–	–	77	14	13	78	15	15	<0.001	4

[1]Including Oligoasthenoteratozoospermia (OAT), severe OAT, oligozoospermia, and teratozoospermia. HB = hospital-based studies, PB = population-based studies.

$P_h = .507$, $I^2 = 0.0\%$; T vs C: OR = 0.80, 95% CI: 0.67–0.97, $P_h = .818$, $I^2 = 0.0\%$), East Asians (CT vs CC: OR = 1.37, 95% CI: 1.21–1.56, $P_h = .038$, $I^2 = 35.2\%$, Fig. 2; TT vs CC: OR = 2.07, 95% CI: 1.70–2.51, $P_h < .001$, $I^2 = 57.0\%$; CT + TT vs CC: OR = 1.57, 95% CI: 1.37–1.80, $P_h = .001$, $I^2 = 52.1\%$; TT vs CC + CT: OR = 1.70, 95% CI: 1.44–1.96, $P_h = .001$, $I^2 = 51.5\%$; T vs C: OR = 1.45, 95% CI: 1.31–1.60, $P_h < .001$, $I^2 = 63.2\%$), West Asians (CT vs CC: OR = 1.36, 95% CI: 1.14–1.61, $P_h = .471$, $I^2 = 0.0\%$; TT vs. CC: OR = 2.15, 95% CI: 1.60–2.90, $P_h = .879$, $I^2 = 0.0\%$, Fig. 3; CT + TT vs. CC: OR = 1.47, 95% CI: 1.25–1.74, $P_h = .653$, $I^2 = 0.0\%$; TT vs CC + CT: OR = 1.86, 95% CI: 1.40–2.48, $P_h = .823$, $I^2 = 0.0\%$; T vs C: OR = 1.42, 95% CI: 1.26–1.62, $P_h = .895$, $I^2 = 0.0\%$), South Asians (TT vs CC: OR = 2.70, 95% CI: 1.14–6.40, $P_h = .002$, $I^2 = 71.6\%$; TT vs CC + CT: OR = 2.42, 95% CI: 1.14–5.13, $P_h = .011$, $I^2 = 63.9\%$), and hospital-based studies (CT vs CC: OR = 1.25, 95% CI: 1.13–1.38, $P_h < .001$, $I^2 = 50.2\%$; TT vs CC: OR = 1.77, 95% CI: 1.48–2.12, $P_h < .001$, $I^2 = 65.3\%$; CT + TT vs CC: OR = 1.37, 95% CI: 1.23–1.53, $P_h < .001$, $I^2 = 64.5\%$; TT vs CC + CT: OR = 1.54, 95% CI: 1.34–1.77, $P_h < .001$, $I^2 = 56.5\%$; T vs C: OR = 1.33, 95% CI: 1.22–1.45, $P_h < .001$, $I^2 = 71.4\%$). In subgroup analysis by infertility type, the *MTHFR* C677T polymorphism was also associated with increased azoospermia (CT vs. CC: OR = 1.27, 95% CI: 1.13–1.42, $P_h = .101$, $I^2 = 28.1\%$; TT vs CC: OR = 1.45, 95% CI: 1.09–1.93, $P_h = .001$, $I^2 = 55.7\%$; (CT + TT) vs. CC: OR = 1.30, 95% CI: 1.11–1.53, $P_h = .003$, $I^2 = 50.1\%$; TT vs (CC + CT): OR = 1.29, 95% CI: 1.00–1.66, $P_h = .002$, $I^2 = 51.6\%$; T vs C: OR = 1.23, 95% CI: 1.07–1.42, $P_h < .001$, $I^2 = 65.4\%$) and OAT risk (CT vs CC: OR = 1.25, 95% CI: 1.09–1.44, $P_h < .001$, $I^2 = 58.0\%$; TT vs. CC: OR = 1.75, 95% CI: 1.39–2.19, $P_h < .001$, $I^2 = 63.4\%$; CT + TT vs CC: OR = 1.37, 95% CI: 1.18–1.59, $P_h < .001$, $I^2 = 67.9\%$; TT vs (CC + CT): OR = 1.59, 95% CI: 1.33–1.89, $P_h < .001$, $I^2 = 52.1\%$; T vs C: OR = 1.35, 95% CI: 1.20–1.52, $P_h < .001$, $I^2 = 73.7\%$).

Obvious heterogeneity was observed in the current meta-analysis, as also shown in Table 2. $I^2 > 75\%$ was found in South Asians (CT vs. CC: $I^2 = 77.3\%$, (CT + TT) vs. CC: $I^2 = 80.6\%$, T vs. C: $I^2 = 82.8\%$) and population-based studies (CT vs. CC: $I^2 = 75.5\%$, (CT + TT) vs CC: $I^2 = 81.4\%$, T vs C: $I^2 = 85.0\%$). Then, a meta-regression analysis method was applied to explore the sources of heterogeneity and the results indicate that ethnicity (TT vs CC: $P = .014$; TT vs (CC + CT): $P = .008$; T vs C: $P = .021$) and HWE (TT vs CC: $P = .041$; TT vs (CC + CT): $P = .020$) were sources of heterogeneity.

The results of sensitivity analysis were shown in Table 3. It is not clear whether the *MTHFR* C677T polymorphism is associated with increased male infertility risk in South Asians. The results did not pool because $I^2 > 75\%$ was observed in any

Table 4

The results of the association of MTHFR C667T polymorphism with male infertility.

Variable	n (Cases/Controls)	CT vs. CC			TT vs. CC			(CT + TT) vs. CC			TT vs. (CC + CT)			T vs. C		
		OR (95% CI)	P_h/I^2 (%)	BFDP	OR (95% CI)	P_h/I^2 (%)	BFDP	OR (95% CI)	P_h/I^2 (%)	BFDP	OR (95% CI)	P_h/I^2 (%)	BFDP	OR (95% CI)	P_h/I^2 (%)	BFDP
Overall	59 (11767/10591)	1.27 (1.15–1.40)*	<.001/54.1	0.106	1.74 (1.47–2.07)*	<.001/65.3	<0.001	1.38 (1.24–1.54)*	<.001/66.6	0.001	1.52 (1.33–1.74)*	<.001/56.4	<0.001	1.33 (1.22–1.45)*	<.001/73.1	<0.001
Ethnicity																
African	2 (451/797)	0.82 (0.64–1.04)	.340/0.0	–	0.65 (0.40–1.04)	.843/0.0	–	0.78 (0.62–0.99)*	.507/0.0	0.998	0.70 (0.44–1.11)	.661/0.0	–	0.80 (0.67–0.97)*	.818/0.0	0.998
Caucasian	13 (1836/1689)	0.99 (0.86–1.15)	.235/20.7	–	1.06 (0.71–1.57)*	.002/62.1	–	1.01 (0.83–1.24)*	.032/46.8	–	1.04 (0.74–1.47)*	.006/56.6	–	1.03 (0.86–1.24)*	.001/64.4	–
East Asian	27 (5587/4803)	1.37 (1.21–1.56)*	.038/35.2	0.111	2.07 (1.70–2.51)*	<.001/57.0	<0.001	1.57 (1.37–1.80)*	.001/52.1	<0.001	1.70 (1.44–1.96)*	.001/51.5	<0.001	1.45 (1.31–1.60)*	<.001/63.2	<0.001
West Asian	7 (1199/1244)	1.36 (1.14–1.61)	.471/0.0	0.928	2.15 (1.60–2.90)*	.879/0.0	0.031	1.47 (1.25–1.74)	.653/0.0	0.268	1.86 (1.40–2.48)*	.823/0.0	0.479	1.42 (1.26–1.62)	.895/0.0	0.012
South Asian	8 (2,464/1,741)	–	<.001/77.3	–	2.70 (1.14–6.40)	.002/71.6	0.997	–	<.001/80.6	–	2.42 (1.14–5.13)*	.011/63.9	0.997	–	<.001/82.8	–
Source of controls																
HB	53 (10435/9444)	1.25 (1.13–1.38)*	<.001/50.2	0.408	1.77 (1.48–2.12)*	<.001/65.3	<0.001	1.37 (1.23–1.53)*	<.001/64.5	0.002	1.54 (1.34–1.77)*	<.001/56.5	<0.001	1.33 (1.22–1.45)*	<.001/71.4	<0.001
PB	6 (1,332/1,147)	–	.001/75.5	–	1.50 (0.79–2.86)	.15/64.4	–	–	<.001/ 81.4	–	1.31 (0.76–2.24)	.049/ 55.0	–	–	<.001/85.0	–
Infertility type																
Azoospermia	24 (2,241/4,952)	1.27 (1.13–1.42)*	.101/28.1	0.619	1.45 (1.09–1.93)*	.001/55.7	0.995	1.30 (1.11–1.53)*	.003/50.1	0.981	1.29 (1.00–1.66)*	.002/51.6	0.999	1.23 (1.07–1.42)*	<.001/65.4	0.993
OAT*	37 (5670/7062)	1.25 (1.09–1.44)*	<.001/58.0	0.986	1.75 (1.39–2.19)*	<.001/63.4	0.049	1.37 (1.18–1.59)*	<.001/67.9	0.619	1.59 (1.33–1.89)*	<.001/52.1	0.009	1.35 (1.20–1.52)*	<.001/73.7	0.047

*Including Oligoasthenoteratozoospermia (OAT), severe OAT, oligozoospermia, and teratozoospermia.

genetic model. Another results did not change, such as overall population, Africans, East Asians, West Asians, and so on.

No significant publication bias was found by Begg funnel plot shape (supplemental Figs. 1, http://links.lww.com/MD/F380, http://links.lww.com/MD/F381, http://links.lww.com/MD/F382, http://links.lww.com/MD/F383, –5, http://links.lww.com/MD/F384) and Egger test (CT vs. CC: $P = .418$,TT vs CC: $P = .203$, CT + TT vs CC: $P = .274$, CT + TT vs CC: $P = .179$, T vs C: $P = .402$) in the overall analysis.

An BFDP test was used to further investigate significant associations in this study, as shown in Tables 4 and 5. Significantly increased male infertility risk was considered as "noteworthy" in the overall population (CT vs CC: BFDP = 0.106, TT vs CC: BFDP < 0.001, CT + TT vs. CC: BFDP = 0.001, TT vs. CT + CC: BFDP < 0.001, T vs. C: BFDP < 0.001), East Asians (CT vs. CC: BFDP = 0.111, TT vs. CC: BFDP < 0.001, CT + TT vs. CC: BFDP < 0.001, TT vs CT + CC: BFDP < 0.001, T vs C: BFDP < 0.001), West Asians (TT vs. CC: BFDP = 0.031, CT + TT vs CC: BFDP = 0.268, TT vs. CT + CC: BFDP = 0.479, T vs. C: BFDP = 0.012), hospital-based studies (CT vs CC: BFDP = 0.408, TT vs. CC: BFDP < 0.001, CT + TT vs. CC: BFDP = 0.002, TT vs. CT + CC: BFDP < 0.001, T vs C: BFDP < 0.001), azoospermia (CT vs. CC: BFDP = 0.619), and OAT (TT vs. CC: BFDP = 0.049, CT + TT vs CC: BFDP = 0.619, TT vs CT + CC: BFDP = 0.009, T vs. C: BFDP = 0.047) for *MTHFR* C677T polymorphism.

However, the positive results by sensitivity analysis (Table 5) were only considered as "noteworthy" in the overall population (TT vs. CC: BFDP = 0.294, CT + TT vs. CC: BFDP = 0.300, T vs. C: BFDP = 0.336), East Asians (TT vs. CC: BFDP = 0.089, TT vs. CT + CC: BFDP = 0.020, T vs. C: BFDP < 0.001), West Asians (TT vs. CC: BFDP = 0.584), hospital-based studies (TT vs. CC: BFDP = 0.726, TT vs. CT + CC: BFDP = 0.126), and OAT (TT vs. CT + CC: BFDP = 0.494) for *MTHFR* C677T polymorphism.

3.2.2. MTHFR A1298C polymorphism. Table 6 shows the results of meta-analysis on the association between the *MTHFR* A1298C polymorphism and male infertility risk. No significantly increased male infertility risk was found in all eligible studies. In subgroup analyses by ethnicity and source of controls, a significantly increased male infertility risk was found in East Asians (AC vs. AA: OR = 1.37, 95% CI: 1.20–1.56, $P_h = 0.515$, $I^2 = 0.0\%$; CC vs. AA: OR = 1.88, 95% CI: 1.10–3.20, $P_h = 0.006$, $I^2 = 62.7\%$; (AC + CC) vs. AA: OR = 1.42, 95% CI: 1.25–1.62, $P_h = 0.106$, $I^2 = 39.3\%$; CC vs. (AA + AC): OR = 1.69, 95% CI: 1.04–2.75, $P_h = 0.020$, $I^2 = 55.8\%$;C vs. A: OR = 1.35, 95% CI: 1.13–1.60, $P_h = 0.016$, $I^2 = 57.3\%$) and population-based studies (C vs. A: OR = 1.53, 95% CI: 1.28–1.83, $P_h = 0.767$, $I^2 = 0.0\%$). Moreover, no significant association was observed in subgroup analysis by infertility type.

Obvious heterogeneity was observed in the current meta-analysis, as also shown in Table 6.

The results indicate that quality score of the eligible studies (AC vs. AA: $P = .038$, CC vs. AA: $P = .013$, (AC + CC) vs. AA: $P = .009$, CC vs. (AA +AC): $P = .024$,C vs. A: $P = .003$) was source of heterogeneity by a meta-regression analysis method.

The results of sensitivity analysis was shown in Table 7 indicating that the results are stable except in West Asians. Significant increased male infertility risk was observed in West Asians (AC vs AA: OR = 0.79, 95% CI: 0.62–1.00, $P_h = .586$, $I^2 = 0.0\%$).

Significant publication was observed by the Begg funnel plot shape (Figures not shown) and Egger test (CC vs. AA: $P = 0.032$;

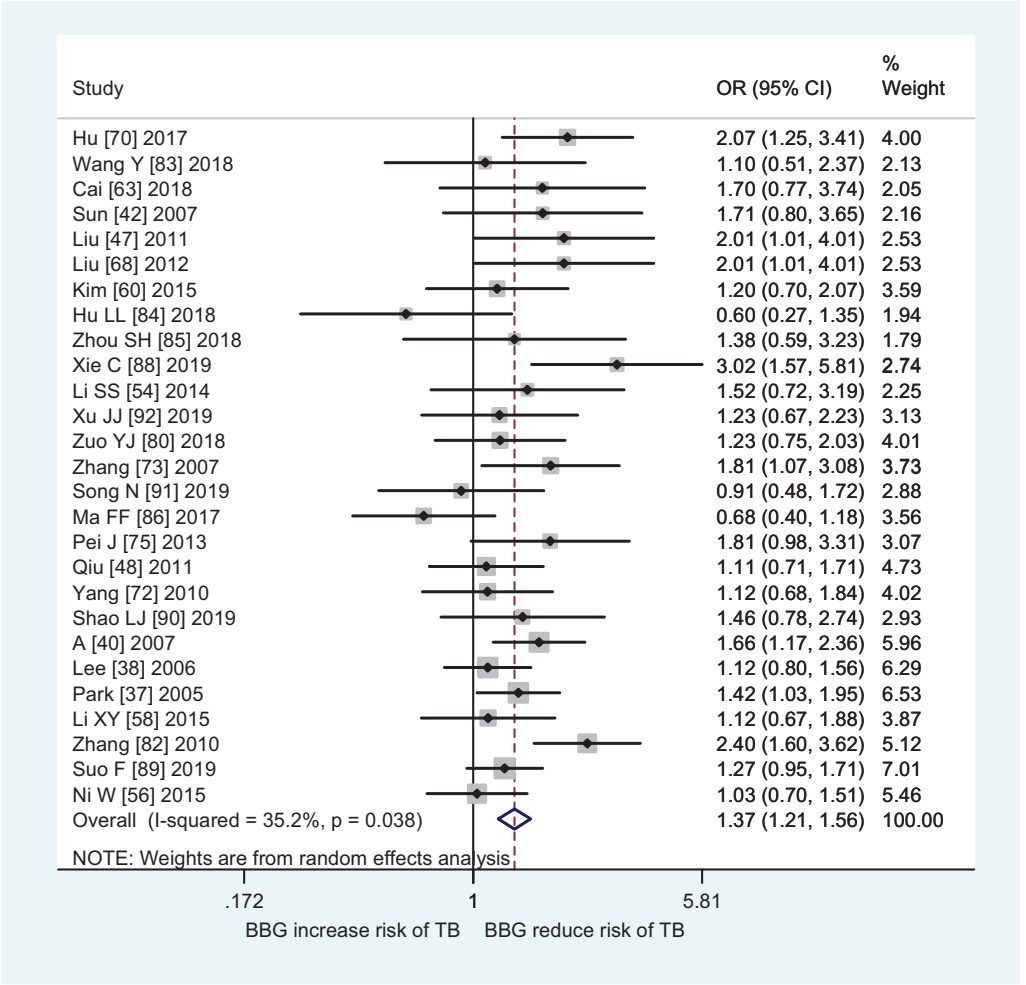

Figure 2. Forest plot of MTHFR C677T polymorphism and male infertile risk in East Asians (CT vs CC).

CC vs. (AA + AC): $P = .024$) in the overall analysis. Supplemental Figs.6, http://links.lww.com/MD/F385 –7, http://links.lww.com/MD/F386 list the Begg's funnel plots by the trim and fill method. Notably, log OR and 95% CI did not change.

An BFDP test was also applied to further investigate significant associations between MTHFR A1298C and male infertility risk, as shown in Tables 4 and 5. Significantly increased male infertility risk was considered as "noteworthy" in the East Asians (AC vs AA: BFDP = 0.111, AC + CC vs AA: BFDP = 0.012) and population-based studies (C vs A: BFDP = 0.139). However, we did not find that the positive results of sensitivity analysis were considered as "noteworthy" in the overall and all subgroup analyses.

4. Discussion

In 2001, Bezold et al.[33] first investigated the association between the *MTHFR* C667T polymorphism and male infertility risk. In 2005, Park et al.[37] first explored the *MTHFR* A1298C polymorphism with male infertility risk. Since then a lot of case–control studies have investigated the associations but the results are still inconsistent. Here, an updated and high quality meta-analysis was carried out to explore the above two gene polymorphism with male infertility risk.

Overall, the *MTHFR* C677T polymorphism was associated with increased male infertility risk in overall populations, Africans, East Asians, West Asians, South Asians, hospital-based studies, azoospermia and OAT. In addition, a significantly increased male infertility risk was also found in East Asians and population-based studies for the *MTHFR* A1298C polymorphism. The pooled data was analyzed using five different genetic models and several subgroup analyses in this study. Under the circumstances, the *P*-value must be adjusted to explain the multiple comparisons.[93] In addition, random error and bias were common in the studies with small sample sizes so that the results were unreliable, especially in molecular epidemiological studies. Wakefield et al.[101] in 2007 proposed a more precise Bayesian measure of false discovery in genetic epidemiology studies, for determining the "noteworthiness" of the positive association. Hence, we used BFDP test to assess the false discovery in the current meta-analysis. Finally, the positive results by sensitivity analysis were only considered as "noteworthy" in the overall population and OAT for *MTHFR* C677T polymorphism. We did not find that the positive results of sensitivity

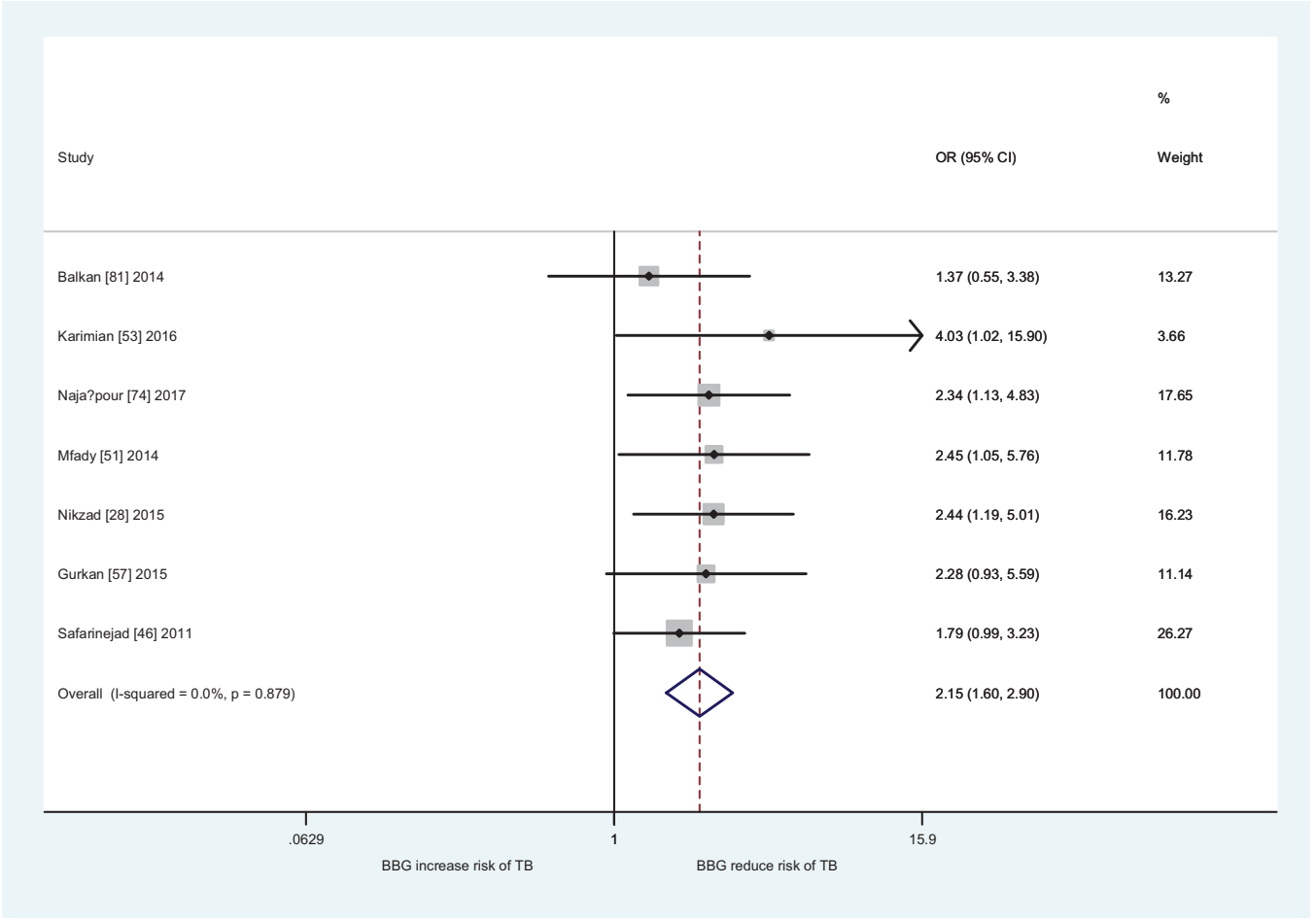

Figure 3. Forest plot of MTHFR C677T polymorphism and male infertile risk in West Asians (TT vs CC).

analysis were considered as "noteworthy" in the overall and all subgroup analyses for *MTHFR* A1298C.

Based on biochemical properties described for *MTHFR* C677T and A1298C polymorphisms, we expected that the two genes were associated with risk of male infertility risk risk in all races. However, we only observed that *MTHFR* C677T is associated with increased male infertility risk in East Asians and West Asians, but not other races (such as Caucasians and Africans). Moreover, no significant association was observed on *MTHFR* A1298C polymorphism with male infertility risk in any race. Hence, an ethnic variant in the frequency of *MTHFR* C677T polymorphism was demonstrated in different populations. The frequency of the 677T allele ranges from 30.5 to 42% among Asian population, from 32.2 to 44% in Caucasians. African population shows a lower frequency of T allele, ranging from 6 to 10.3%.[102,103] These results indicated that the same genes may play different roles in different races and countries, because infertility is a complicated multigenetic disease, and different genetic backgrounds and environmental factor (smoking or life style) may contribute to the discrepancy. Another possible explanation for the difference suggested the influence of the genetic variant might be masked by the presence of other as-yet unidentified causal genes involved in male infertility. The current studies demonstrated a clear north-to-south gradient in the effect of the *MTHFR* C677T variant in the determination of hyperhomocysteinemia, suggesting that diet is a relevant environmental agent, being the presence of folates in the food higher in the South of Europe than in the North. In addition, there was also the presence of folates in the food higher in the Caucasians than in the Asians. Obvious heterogeneity was observed in the current meta-analysis, as also shown in Tables 2 and 4. Ethnicity and HWE were sources of heterogeneity for *MTHFR* C677T polymorphism and quality score of the eligible studies was source of heterogeneity by a meta-regression analysis method. HWD may be genotyping errors and selection bias in molecular epidemiological studies. Small sample studies were easier to accept if there were positive reports as they tend to yield false-positive results because they may be not rigorous and are often of low-quality. Supplemental Fig. 3, http://links.lww.com/MD/F382 indicated that the asymmetry of the funnel plot was caused by studies of low-quality small samples. Therefore, we performed a sensitivity analysis restricted to studies that only included high-quality articles and controls in HWE.

15 previous meta-analyses[15,17,19–29,31,32] have been reported on the *MTHFR* C677T polymorphism with male infertility risk (as shown in Table 6). Yang et al.[15] and Wei et al.[24] showed that the *MTHFR* C677T polymorphism was associated with a significantly increased male infertility risk in the overall and Asian populations. Zhu et al.[17] suggested the *MTHFR* C677T polymorphism is capable of causing male infertility susceptibility,

Table 5

The results of sensitivity analysis between MTHFR C667T polymorphism with male infertility.

Variable	n (Cases/ Controls)	CT vs. CC			TT vs. CC			(CT + TT) vs. CC			TT vs. (CC + CT)			T vs. C		
		OR (95% CI)	P_h/I^2	BFDP	OR (95% CI)	P_h/I^2	BFDP	OR (95% CI)	P_h/I^2	BFDP	OR (95% CI)	P_h/I^2	BFDP	OR (95% CI)	P_h/I^2	BFDP
Quality score ≥ 5 and HWE																
Overall	23 (6827/6355)	1.22 (1.06–1.41)*	<.001/65.1	0.995	1.60 (1.29–1.97)*	<.001/59.6	0.294	1.31 (1.13–1.52)*	<.001/72.1	0.937	1.41 (1.21–1.64)*	.035/37.9	0.300	1.27 (1.14–1.41)*	<.001/72.4	0.336
African	1 (344/690)	0.77 (0.59–1.01)	–	–	0.67 (0.39–1.15)	–	–	0.75 (0.58–0.98)	–	0.998	0.74 (0.44–1.26)	–	–	0.80 (0.64–0.98)	–	0.998
Caucasian	6 (1111/1202)	1.03 (0.87–1.23)	.405/1.7	–	1.24 (0.95–1.64)	.188/33.0	–	1.07 (0.91–1.26)*	.426/0.0	–	1.23 (0.95–1.59)	.167/36.0	–	1.09 (0.96–1.24)	.271/21.7	–
East Asian	7 (2753/2299)	1.37 (1.13–1.68)*	.046/53.1	0.985	1.77 (1.39–2.24)*	.092/44.8	0.089	1.47 (1.19–1.81)*	.016/61.7	0.903	1.43 (1.25–1.64)	.864/ 0.0	0.020	1.33 (1.22–1.44)	.148/ 36.7	<0.001
West Asian	4 (693/867)	1.22 (0.99–1.52)	.397/0.0	–	2.16 (1.50–3.11)	.898/0.0	0.584	1.36 (1.11–1.66)	.584/0.0	0.985	1.93 (1.37–2.73)*	.743/0.0	0.861	1.36 (1.17–1.59)	.876/0.0	0.829
South Asian	5 (1926/1297)	–	<.001/83.3	–	–	<.001/78.5	–	–	<.001/86.4	–	2.35 (0.97–5.68)*	.007/ 71.7	–	–	<.001/88.5	–
HB	18 (5572/5321)	1.15 (1.00–1.33)*	.001/59.8	0.999	1.59 (1.27–2.00)*	<.001/60.4	0.726	1.23 (1.06–1.44)*	<.001/69.1	0.996	1.43 (1.22–1.66)*	.083/ 33.5	0.126	1.21 (1.09–1.35)*	<.001/67.4	0.969
PB	5 (1255/1034)	1.57 (1.07–2.30)*	.005/73.4	0.996	1.86 (0.92–3.76)*	.024/64.3	–	–	.001/79.6	–	1.53 (0.83–2.81)*	.049/58.2	–	–	<.001/84.6	–
Azoospermia	11 (1352/3370)	1.31 (1.06–1.64)*	.021/52.3	0.997	1.79 (1.30–2.48)*	.074/41.3	0.927	1.40 (1.12–1.77)*	.004/ 61.3	0.991	1.48 (1.20–1.82)	.144/31.9	0.873	1.34 (1.12–1.61)*	.001/65.2	0.981
OAT*	14 (3191/4470)	1.19 (0.98–1.44)*	<.001/66.0	–	1.46 (1.14–1.87)*	.045/ 42.7	0.984	1.25 (1.03–1.53)*	<.001/69.9	0.998	1.38 (1.19–1.60)	.226/ 21.0	0.494	1.24 (1.07–1.44)*	<.001/70.1	0.993

* Including Oligoasthenoteratozoospermia (OAT), severe OAT, oligozoospermia, and teratozoospermia.

Table 6

Meta-analysis of the association of MTHFR A1298C polymorphism with male infertility.

Variable	n (Cases/ Controls)	AC vs AA			CC vs AA			(AC + CC) vs. AA			CC vs. (AA + AC)			C vs. A		
		OR (95% CI)	P_h/I^2	BFDP	OR (95% CI)	P_h/I^2	BFDP	OR (95% CI)	P_h/I^2	BFDP	OR (95% CI)	P_h/I^2	BFDP	OR (95% CI)	P_h/I^2	BFDP
Overall	28 (5,976/5,774	1.08 (0.96–1.22)*	.002/48.6	–	1.28 (0.99–1.67)*	<.001/63.5	–	1.11 (0.98–1.26)*	<.001/59.3	–	1.25 (0.99–1.58)*	<.001/58.4	–	1.11 (0.99–1.24)*	<.001/66.5	–
Ethnicity																
South Asian	4 (1,176/585)	1.08 (0.75–1.55)*	.065/58.5	–	–	<.001/87.2	–	–	.004/77.3	–	–	<.001/83.6	–	–	<.001/87.9	–
West Asian	7 (1057/1039)	0.86 (0.71–1.04)	.702/0.0	–	0.91 (0.69–1.21)	.816/ 0.0	–	0.87 (0.73–1.04)	.601/ 0.0	–	0.99 (0.76–1.29)*	.940/ 0.0	–	0.93 (0.81–1.06)	.646/ 0.0	–
East Asian	9 (2123/2094)	**1.37 (1.20–1.56)**	.515/0.0	**0.111**	**1.88 (1.10–3.20)***	.006/ 62.7	0.996	**1.42 (1.25–1.62)**	.106/ 39.3	**0.012**	**1.69 (1.04–2.75)***	.020/ 55.8	0.997	**1.35 (1.13–1.60)***	.016/ 57.3	0.949
Caucasian	6 (1120/1133)	1.06 (0.89–1.26)	.158/37.3	–	0.88 (0.65–1.17)	.551/0.0	–	1.02 (0.87–1.21)	.541/0.0	–	0.86 (0.65–1.13)	.159/37.2	–	0.98 (0.86–1.11)	.822/0.0	–
Source of controls																
HB	25 (5,306/5145)	1.09 (0.96–1.24)*	.001/52.1	–	1.30 (0.97–1.74)*	<.001/66.7	–	1.11 (0.97–1.28)*	<.001/62.9	–	1.26 (0.97–1.63)*	<.001/61.2	–	1.11 (0.99–1.25)*	<.001/69.4	–
PB	3 (670/629)	1.05 (0.83–1.33)	.309/14.7	–	1.15 (0.75–1.77)	.356/3.1	–	1.08 (0.86–1.35)	.471/ 0.0	–	1.19 (0.79–1.80)	.219/ 34.1	–	**1.53 (1.28–1.83)**	.767/ 0.0	**0.139**
Infertility type																
Azoospermia	12 (1,140/2,610)	1.01 (0.86–1.18)	.316/13.1	–	1.21 (0.91–1.61)	.117/ 34.2	–	1.04 (0.90–1.21)	.212/ 23.5	–	1.21 (0.92–1.58)	.131/ 32.4	–	1.06 (0.95–1.19)	.109/35.1	–
OAT*	12 (1,664/2,759)	0.98 (0.86–1.12)	.198/25.0	–	1.16 (0.91–1.47)	.248/19.9	–	1.01 (0.89–1.15)	.141/31.3	–	1.17 (0.93–1.47)	.375/7.2	–	1.04 (0.94–1.15)	.173/27.2	–

* Including Oligoasthenoteratozoospermia (OAT), severe OAT, oligozoospermia, and teratozoospermia.

Table 7

The results of sensitivity analysis between MTHFR A1298C polymorphism with male infertility.

Variable	n (Cases/ Controls)	AC vs AA			CC vs AA			(AC + CC) vs AA			CC vs (AA + AC)			C vs A		
		OR (95% CI)	P_h/I^2 (%)	BFDP	OR (95% CI)	P_h/I^2 (%)	BFDP	OR (95% CI)	P_h/I^2 (%)	BFDP	OR (95% CI)	P_h/I^2 (%)	BFDP	OR (95% CI)	P_h/I^2 (%)	BFDP
Quality score ≥ 5 and HWE																
Overall	13 (3198/2895)	1.00 (0.89–1.11)	.371/7.5	–	0.92 (0.76–1.12)	.562/0.0	–	1.00 (0.89–1.11)*	.296/14.7	–	0.96 (0.80–1.16)	.689/0.0	–	0.99 (0.91–1.08)	.292/15.1	–
South Asian	2 (790/336)	0.90 (0.66–1.23)	.182/43.8	–	0.64 (0.40–1.02)	.581/0.0	–	0.85 (0.55–1.32)*	.148/52.2	–	0.75 (0.51–1.11)	.975/ 0.0	–	0.86 (0.70–1.06)	.257/ 22.1	–
West Asian	4 (559/737)	**0.79 (0.62–1.00)**	.586/0.0	0.999	0.83 (0.58–1.18)	.786/0.0	–	0.80 (0.64–1.00)	.579/ 0.0	0.999	0.94 (0.68–1.31)	.862/ 0.0	–	0.87 (0.74–1.03)	.681/ 0.0	–
East Asian	4 (1191/1045)	**1.20 (1.00–1.45)**	.973/0.0	0.999	1.36 (0.88–2.11)	.834/0.0	–	**1.22 (1.03–1.46)**	.985/ 0.0	0.998	1.28 (0.83–1.98)*	.822/ 0.0	–	**1.19 (1.03–1.38)**	.981/ 0.0	0.998
Caucasian	3 (658/777)	0.97 (0.78–1.21)	.701/0.0	–	0.96 (0.67–1.37)	.248/28.3	–	0.97 (0.79–1.20)	1.000/ 0.0	–	1.04 (0.62–1.75)*	.114/ 53.9	–	0.98 (0.84–1.15)	.546/ 0.0	–
HB	10 (2528/2266)	0.98 (0.86–1.11)	.321/13.2	–	0.87 (0.70–1.08)	.608/0.0	–	0.97 (0.86–1.10)	.214/ 24.9	–	0.92 (0.75–1.12)	.844/0.0	–	0.97 (0.88–1.06)	.273/ 18.5	–
Azoospermia	5 (556/1018)	1.03 (0.82–1.29)	.292/19.2	–	0.86 (0.57–1.30)	.700/0.0	–	1.01 (0.82–1.26)	.267/ 23.1	–	0.88 (0.60–1.30)	.848/0.0	–	0.98 (0.83–1.16)	.403/0.5	–
OAT*	6 (736/1421)	1.01 (0.84–1.23)	.427/0.0	–	1.03 (0.73–1.46)	.531/0.0	–	1.03 (0.85–1.23)	.349/10.4	–	1.06 (0.76–1.49)	.659/0.0	–	1.03 (0.89–1.19)	.370/7.3	–

* Including Oligoasthenoteratozoospermia (OAT), severe OAT, oligozoospermia, and teratozoospermia.

especially in Asians, azoospermia and OAT. Hong et al[19] demonstrated that the *MTHFR* C677T polymorphism is associated with male infertility in East-asian populations, Middle-eastern populations, and mixed-race. Tüttelmann et al[20] and Nikzad et al[28] indicated that the *MTHFR* C677T polymorphism is associated with male infertility in overall populations. Wu et al.[21] supported that the *MTHFR* C677T polymorphism was capable of causing male infertility susceptibility in Asians and azoospermia. Gong et al[23] and Liu et al.[26]indicated that the *MTHFR* polymorphism was associated with an increased risk of male infertility in overall populations, especially in Asians and Caucasians and subgroups of azoospermia and OAT. Weiner et al.[29] suggested that the *MTHFR* C677T polymorphism was associated with an increased risk of male infertility in overall populations and subgroup of azoospermia. Gupta et al[31] supported that the *MTHFR* C677T polymorphism was associated with an increased risk of male infertility in overall populations and subgroups of azoospermia and OAT. Ullah et al[32] indicated that the *MTHFR* C677T polymorphism was associated with an increased risk of male infertility in Caucasians for middle income countries. Rai et al[22] and Shi et al[27] supported an association between C677T polymorphism and male infertility in Asians. Ren et al[25] suggested that the *MTHFR* C667T polymorphism may contribute to the genetic susceptibility to male infertility in the Chinese population. In addition, ten previous meta-analyses[15,16,18,20,24–27,30,32] have also been published on the *MTHFR* A1298C polymorphism with male infertility risk (as shown in Table 7). Among these publications, one study[32] investigated this issue in Caucasians, one study[27] in Asians, one study[25] in Chinese population, and seven studies[15,16,18,20,24,26,30] in overall populations. Ullah et al[32] indicated that the *MTHFR* A1298C polymorphism was associated with an increased risk of male infertility in Caucasians for low income countries. Shi et al[27] supported that *MTHFR* A1298C polymorphism was the risk factor with susceptibility to male infertility in Asians, especially in East Asians. Ren et al[25] demonstrated that *MTHFR* A1298C polymorphism may be unrelated to male infertility risk in Chinese population. Yang et al[15] suggested that there was a significant association between the A1298C polymorphism and male infertility risk in the Asian, Caucasian, and overall groups. Zhang et al[16] indicated that the *MTHFR* A1298C polymorphism may be a potential risk factor for male infertility, especially in the Asian population. Shen et al[18] and supported that the *MTHFR* A1298C polymorphism was capable of causing male infertility susceptibility, especially azoospermia. Tüttelmann et al,[20] Wei et al,[24] Gupta et al,[30]and Liu et al[26] indicated that the *MTHFR* A1298C polymorphism was not associated with male infertility susceptibility. However, quality assessment of the eligible studies was not performed in 13 previous meta-analyses.[15,17,18,20–24,28–32] In addition, the false-positive report probabilities of statistically significant association and statistical power was not evaluated in all previous meta-analyses except the study of Liu et al.[26] Moreover, many new studies have been published, therefore, an updated meta-analysis should be carried out.

This study has several advantages over previous meta-analyses.[15–32] First, the sample size was much larger, 59 studies on *MTHFR* C677T (11,767 male infertility cases and 10,591 controls) and 28 studies on *MTHFR* A1298C (5,976 male infertility cases and 5,774 controls) were identified in overall population. Second, this is the first meta-analysis to explore a

false-positive report probability by BFDP method. Third, an important sensitivity analysis was performed on studies that were high-quality and HWE. Although we have put considerable effort and resources into testing possible associations between *MTHFR* C677T and A1298C polymorphisms and male infertility risk, there are still some limitations inherited from the published studies. First, the controls were not uniformly defined. Second, no data were extracted on exploring interaction between gene and environment.

In summary, this study indicates that the *MTHFR* C677T polymorphism is associated with increased male infertility risk in East Asians, West Asians, and OAT. Other significant association should be interpreted with caution and may most likely result from false-positive results, rather than from true associations or biological factors.

Author contributions

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