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The relevance of MTHFR C677T, A1298C, and MTRR A66G polymorphisms with response to male infertility in Asians

A meta-analysis

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Abstract

Although published studies have reported the association between MTHFR C677T (rs 1801133), A1298C (rs 1801131), and MTRR A66G (rs1801394) polymorphisms and male infertility in Asian populations, the results are conflicting. In order to accurately evaluate the relevance, a meta-analysis was performed.

We searched for potential studies in 4 databases, containing PubMed, ScienceDirect, China National Knowledge Infrastructure (CNKI), and Wanfang database until May 31, 2018. The summarized odds ratio (OR) with 95% confidence intervals (95% CI) were calculated to evaluate the relevance in 5 genetic models. The heterogeneity test, sensitivity analysis, and publication bias test was performed by Review Manager 5.3 software.

Overall, 22 case–control studies with 5049 cases and 4157 controls were included in this meta-analysis, which contained 20 studies of MTHFR C677T polymorphism, 12 studies of MTHFR A1298C polymorphism and 4 studies of MTRR A66G polymorphism. The results indicated that MTHFR C677T, A1298C, and MTRR A66G polymorphisms were significantly associated with male infertility in Asian populations (Dominant model: MTHFR CC + CT vs TT: OR = 0.60, 95% CI (0.53, 0.67), $P < .00001$; MTHFR AA + AC vs CC: OR = 0.62, 95% CI (0.49, 0.79), $P = .0001$; MTRR AA + AG vs GG: OR = 0.60, 95% CI (0.45, 0.81), $P = .001$. Recessive model: MTHFR CC vs CT + TT: OR = 0.67, 95% CI (0.61, 0.74), $P < .00001$; MTHFR AA vs AC + CC: OR = 0.79, 95% CI (0.70, 0.88), $P < .0001$; MTRR AA vs AG + GG: OR = 0.70, 95% CI (0.56, 0.88), $P = .002$. Heterozygote model: MTHFR CC vs CT: OR = 0.74, 95% CI (0.67, 0.82), $P < .00001$; MTHFR AA vs AC: OR = 0.83, 95% CI (0.73, 0.93), $P = .002$; MTRR AA vs AG: OR = 0.76, 95% CI (0.60, 0.92), $P = .02$. Homozygote model: MTHFR CC vs TT: OR = 0.48, 95% CI (0.41, 0.56), $P < .00001$; MTHFR AA vs CC: OR = 0.61, 95% CI (0.39, 0.93), $P = .02$; MTRR AA vs GG: OR = 0.51, 95% CI (0.36, 0.72), $P = .0001$. Allele model: MTHFR C vs T: OR = 0.70, 95% CI (0.66, 0.75), $P < .00001$; MTHFR A vsC: OR = 0.82, 95% CI (0.71, 0.95), $P = .01$; MTRR A vs G: OR = 0.76, 95% CI (0.66, 0.88), $P = .00003$). Stratified analyses by geographical location and source of controls showed the same results. Sensitivity analyses indicated that the final consequences of this meta-analysis were stable, and the publication biases test had not found obvious asymmetry.

This meta-analysis indicates that MTHFR C677T, A1298C, and MTRR A66G polymorphisms are the risk factors with susceptibility to male infertility in Asians.

Abbreviations: CI = confidence interval, CNKI = China National Knowledge Infrastructure, HB = hospital-based, HWE = Hardy–Weinberg equilibrium, MTHFR = methylene tetrahydrofolate reductase, MTRR = methionine synthase reductase, OR = odds ratio, PB = population-based.

Keywords: Asians, male infertility, MTHFR A1298C, MTHFR C677T, MTRR A66G, polymorphism

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1. Introduction

It had shown that about 10%~15% of married couples in the world were suffering from infertility, about half of which was attributed to male partner.[1] So far, male infertility has become a concern and urgent problem in the world. Many reasons such as environmental disruptors, genetic, testes pathologies, and sedentary lifestyle may affect spermatogenesis leading to male infertility,[2,3] but almost half of all male infertility patients are still undiagnosed for the complicated mechanism which may be associated with spermatogenesis process of gene mutations.[4] Folate plays an important role in cell metabolism, like the synthesis of nucleic acids and epigenetic regulation of gene expression through remethylation of homocysteine into methionine.[5] Once the folate is deficient, the proliferation of sperm cells will be reduced.[6] Methylene tetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) are the key enzymes in folate metabolism. The enzyme activities of

MTHFR and MTRR are influenced by gene polymorphisms.[7] So the polymorphisms of MTHFR and MTRR may be a potential risk factor for male infertility.[8]

Several studies have investigated the association between MTHFR C677T (rs 1801133), A1298C (rs 1801131) and MTRR A66G (rs1801394) polymorphisms, and male infertility, but the conclusions are controversial.[9] The reason may be partially attributed to racial difference. For Asians, only 4 meta-analyses have evaluated the impact of MTHFR C677T polymorphism on male infertility by far[10–13] Gupta's study with 522 cases and 315 controls was limited to Indian population.[10] Weiner's study with 275 men of idiopathic male infertility and 349 controls was limited to Russian population.[11] Ren's study including 1713 cases and 1104 controls was limited to Chinese population,[12] and Rai's research with 4392 breast infertile males and 3667 fertile males has not included the latest research data after March 2015.[13] Only Ren et al have evaluated the association between MTHFR A1298C and male infertility.[12] The system review with respect to MTRR A66G polymorphism specifically for Asian populations has not been reported till date. Therefore, it is necessary to collect more studies in a large sample size for further elucidating correlation between these polymorphisms and male infertility in Asians. In this present research, we performed a meta-analysis based on 22 studies with 5049 cases and 4157 controls to investigate the relationship between MTHFR C677T, A1298C, and MTRR A66G polymorphisms and risk of male infertility in Asians.

2. Materials and methods

2.1. Literature search and selection

The systematic search from PubMed, ScienceDirect, CNKI, and Wanfang databases updated on May 31, 2018 using the terms" (Methylenetetrahydrofolate reductase or MTHFR or methionine synthase reductase or MTRR or C677T or A1298C or A66G) and (polymorphism or variants or mutation) and (male infertility)" was conducted by 2 review authors (Shi and Wu). The languages were limited to English and Chinese. Furthermore, we manually searched references in the eligible articles to acquire more applicable information.

2.2. Criteria of inclusion and exclusion

Inclusion criteria were showed as following:

(1) case–control studies;
(2) evaluation of the association between MTHFR C677T and/or A1298C and/or MTRR A66G polymorphism and male infertility risk in Asian populations;
(3) all genotypes had complete data;
(4) published in English or Chinese language.

The reasons for excluding studies were:

(1) uncertain type of study or not case–control study;
(2) no detailed data on genotype distribution;
(3) not in Asian populations.

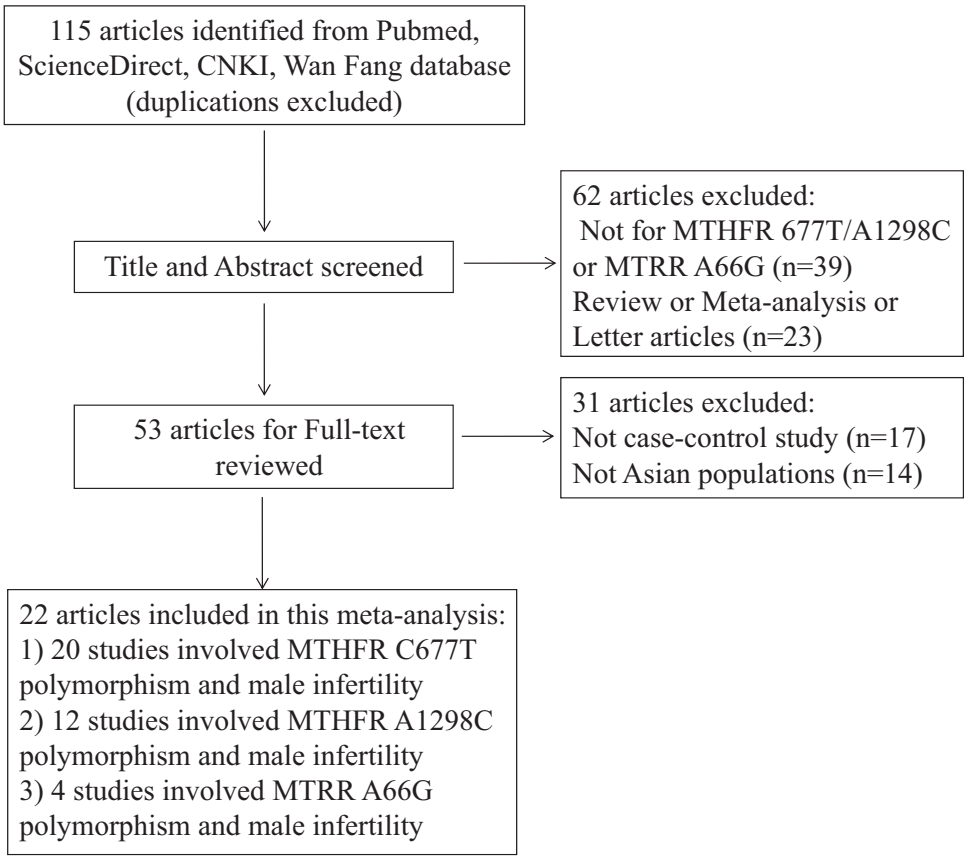


Figure 1. Flow chart of the included studies in the meta-analysis.

2.3. Data extraction

The following information was carefully and independently collected from each eligible study by 2 reviewers: the first author's name, publication year, country, geographical location, source of controls, and the count of persons with each genotype and allele. The *P* value of Hardy–Weinberg equilibrium test (HWE) was also calculated. If the clinical trial data is not complete, we try to contact the author as far as possible.

2.4. Methodological quality assessment

Two reviewers (Shi and Wu) independently assessed the methodological quality of included literature using Newcastle–Ottawa Scale (NOS). The maximum score was 9, and the score of studies ranged from 0 to 3, 4 to 6, and 7 to 9 were regarded as low-quality, moderate-quality, and high-quality, respectively.[14]

2.5. Statistical analysis

Review Manager 5.3 software was used for analyses. HWE in each study was calculated by Chi-squared test. The associations between MTHFR C677T, A1298C, and MTRR A66G polymorphisms and the risk of male infertility were estimated by odds ratios (OR) with 95% confidence interval (95% CI). The heterogeneity among studies was evaluated by Q and I^2 statistics. If there was no heterogeneity with $P \geq .1$ or $I^2 \leq 50$, the fixed-effect model was used. Conversely, the random-effect model was used. Subgroup analysis or sensitivity analysis was performed to exclude the possible causes of heterogeneity. Funnel plot was applied to detect publication bias in the included studies. The statistical significance was considered with *P* value less than .05.

This study was approved by the Ethics Committee of the First Affiliated Hospital of University of Science and Technology of China. It was conducted in accordance with the Declaration of Helsinki.

3. Results

3.1. Characteristics of included studies

A flow chart summarizing the process of literature selection is shown in Fig. 1. Based on the inclusion/exclusion criteria, 22 case–control studies were recruited in the final analysis.[15–36] 20 studies were concerned with the association between MTHFR C677T polymorphism and male infertility,[15–18,20,21,23–36] 12 studies evaluated the MTHFR A1298C polymorphism,[15–17,19,20,22,28,29,33,35,36] and only 4 studies evaluated the MTRR

Table 1

Main characteristics of included studies in the meta-analysis.

A: *MTHF C677T* polymorphism

					Cases						Controls							
Author	Year	Country	Geographical location	Source of controls	Total	CC	CT	TT	C	T	Total	CC	CT	TT	C	T	Hardy Weinberg-*P*	Quality score
Wang Y	2017	China	East Asia	PB	76	15	37	24	60	92	95	24	54	17	102	88	.399	7
Najafipour R	2017	Iran	West Asia	HB	280	113	123	44	349	211	120	66	43	11	75	65	.102	8
Karimian M	2016	Iran	West Asia	HB	118	51	59	8	161	75	132	77	52	3	206	58	.031	8
Li XY	2015	China	East Asia	PB	162	61	77	24	199	125	120	48	54	18	150	90	.661	7
Mfady DS	2014	Jordanian	Western Asia	HB	150	67	63	20	197	103	150	74	67	9	215	85	.221	7
Naqvi H	2014	Indian	South Asia	HB	637	447	154	36	1048	226	364	275	79	10	629	99	.145	7
Li SS	2014	China	East Asia	PB	82	14	36	32	64	100	133	36	61	36	133	133	.340	8
Pei J	2013	China	East Asia	PB	290	39	138	113	216	364	90	24	47	19	95	85	.651	7
Vani GT	2011	Indian	South Asia	HB	206	158	42	6	358	54	230	188	42	0	418	42	.128	7
Liu L	2011	China	East Asia	HB	75	27	38	10	92	58	72	40	28	4	108	36	.753	6
Qiu XF	2011	China	East Asia	NA	271	75	112	84	262	280	180	63	85	32	211	149	.720	7
Yang BH	2010	China	East Asia	HB	131	34	55	42	123	139	293	98	142	53	338	248	.901	8
Zhang WB	2010	China	East Asia	HB	491	43	253	195	339	643	430	87	213	130	387	473	.998	7
Dhillon VS	2007	Indian	South Asia	NA	179	81	77	21	239	119	200	70	100	30	240	160	.556	8
A ZC	2007	China	East Asia	HB	355	130	160	65	420	290	252	128	95	29	351	153	.085	6
Zhang XJ	2007	China	East Asia	HB	165	41	93	31	175	155	132	48	60	24	156	108	.492	8
Lee HC	2006	Korea	East Asia	Mixed	360	115	181	64	411	309	325	118	166	41	402	248	.138	7
Park JH	2005	Korea	East Asia	Mixed	373	105	205	63	415	331	396	145	200	51	490	302	.161	7
Singh K	2005	Indian	South Asia	PB	151	105	40	6	250	52	200	163	37	0	363	37	.149	7
Sun HT	2005	China	East Asia	PB	182	27	86	69	140	224	53	15	28	10	58	48	.630	6

B: *MTHFR A1298C* polymorphism

					Cases						Controls							
Reference	Year	Country	Geographical location	Source of controls	Total	AA	AC	CC	A	C	Total	AA	AC	CC	A	C	Hardy Weinberg-*P*	Quality score
Najafipour R	2017	Iran	West Asia	HB	280	129	116	35	374	186	120	57	50	13	164	76	.247	8
Karimian M	2016	Iran	West Asia	HB	118	59	44	15	162	74	132	70	48	14	188	76	.051	8
Li XY	2015	China	East Asia	PB	162	101	54	7	256	198	120	80	38	2	198	42	.290	7
Mfady DS	2014	Jordanian	West Asia	HB	150	71	61	18	203	97	150	59	75	16	193	107	.273	7
Li XY	2014	China	East Asia	PB	162	101	54	7	256	68	50	34	15	1	83	17	.656	7
Li SS	2014	China	East Asia	PB	82	49	29	4	127	37	133	88	36	9	212	54	.168	8
Singh K	2010	South Asia	South Asia	PB	151	66	76	9	208	94	140	64	74	2	202	78	.000	7
Zhang WB	2010	China	East Asia	HB	491	224	220	47	668	314	430	270	150	10	690	170	.262	7
Dhillon VS	2007	Indian	South Asia	NA	179	90	80	9	260	98	200	103	84	13	290	110	.451	8
Zhang XJ	2007	China	East Asia	HB	165	90	60	15	240	90	132	85	45	2	215	49	.142	8
Lee HC	2006	Korea	East Asia	Mixed	360	222	120	18	564	156	325	213	98	14	524	124	.526	7
Park JH	2005	Korea	East Asia	Mixed	373	237	118	18	592	154	396	269	111	16	649	143	.294	7

C: MTRR A66G polymorphism

					Cases						Controls							
Reference	Year	Country	Geographical location	Source of controls	Total	AA	AG	GG	A	G	Total	AA	AG	GG	A	G	Hardy Weinber-P	Quality score
Li XY	2015	China	East Asia	PB	162	83	65	14	231	93	120	70	44	6	184	56	.785	7
Mfady DS	2014	Jordanian	West Asia	HB	150	48	78	24	174	126	150	61	67	22	189	111	.608	7
Zhang XJ	2007	China	East Asia	HB	165	38	72	55	148	182	132	45	65	22	155	109	.857	8
Lee HC	2006	Korea	East Asia	Mixed	360	64	250	46	378	342	325	72	224	29	368	282	.000	7

CI = confidence interval, HB = hospital-based, MTHFR = methylene tetrahydrofolate reductase, MTRR = methionine synthase reductase, OR = odds ratio, PB = population-based.

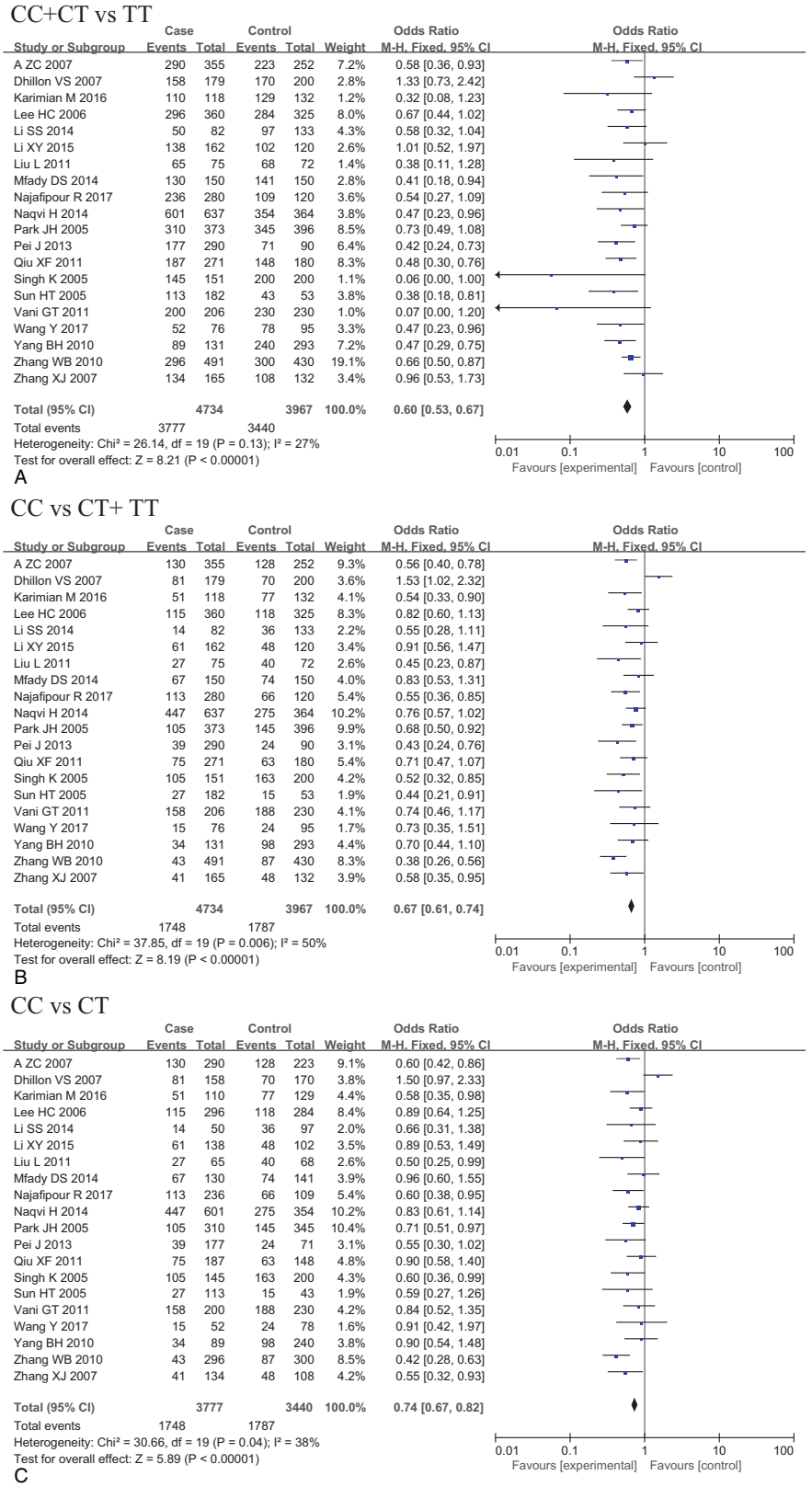


Figure 2. Forest plots for association of MTHFR C677T polymorphism with the risk of male infertility in Asians. MTHFR = methylene tetrahydrofolate reductase.

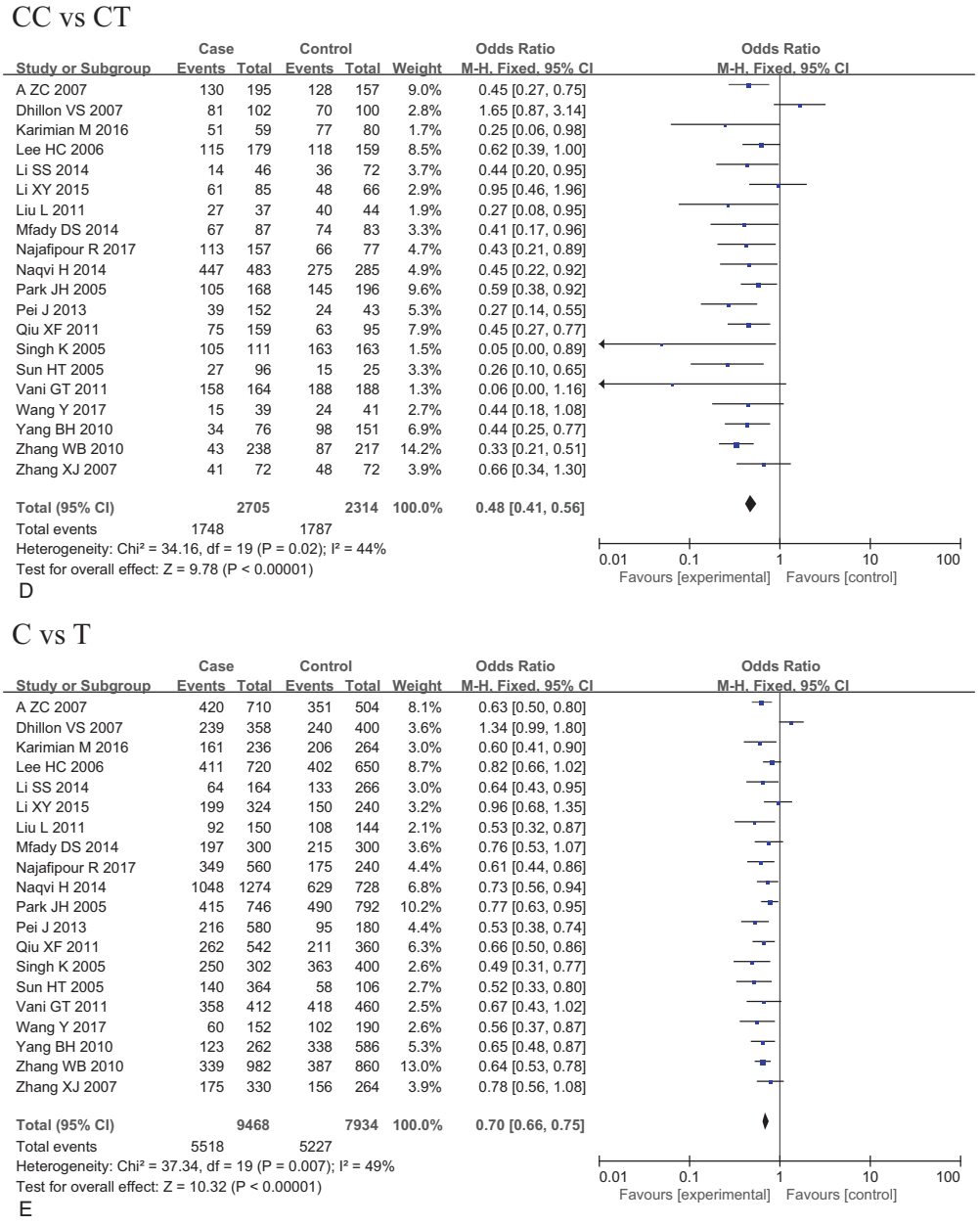


Figure 2. (Continued).

A66G polymorphism.[15,17,28,29] The main characteristics and quality score of each study were displayed in Table 1. All studies were stratified by geographical location, of which 14 studies were performed in East Asians[15,16,18,21,22,24–29,32–34] and the remaining 8 across South/West Asians.[17,19,20,23,30,31,35,36] When stratified by source of controls, the amount of hospital-based (HB) studies was 10,[17,18,21,25,28,30–32,35–36] population-based (PB) studies was 8,[19,22–24,27,29,33,34] and mixed population or uncertain source was 4.[15,16,20,26]

3.2. Results of meta-analysis and subgroup-analysis

3.2.1. MTHFR C677T polymorphism. After pooling 20 studies with 4734 cases and 3967 controls into 1 data set for meta-analysis, we found that the MTHFR C677T polymorphism had statistical association with the risk of male infertility in Asians (see Fig. 2; (A) Dominant model (CC + CT vs TT): OR = 0.60, 95% CI (0.53,0.67), $P < .00001$; (B) Recessive model (CC vs CT + TT): OR = 0.67, 95% CI (0.61, 0.74), $P < .00001$; (C) Heterozygote model (CC vs CT): OR = 0.74, 95% CI (0.67, 0.82), $P < .00001$; (D) Homozygote model (CC vs TT): OR = 0.48, 95% CI (0.41, 0.56), $P < .00001$; (E) Allele model (C vs T): OR = 0.70, 95% CI (0.66, 0.75), $P < .00001$.)

In the subgroup analysis of geographical location, we observed that a similar association existed both in East Asians and South/West Asians for the MTHFR C677T polymorphism with the male infertility risk. Further stratified analysis by the source of controls showed that the MTHFR C677T polymorphism was also significantly associated with male infertility both in HB and population-based studies. Table 2 summarized the results of overall and subgroup analysis in all of 5 genetic models.

Table 2

Subgroup analyses for MTHFR C677T polymorphism in 5 comparative genetic models.

Models	Population	No. of studies	Sample size (case/control)	I^2 (%)	OR (95% CI)	*P*
Dominant model (CC + CT vs TT)	overall	20	4734 /3967	27	0.60 (0.53,0.67)	<.00001
	East Asia	13	3013/2571	3	0.6 (0.53,0.69)	<.00001
	South/West Asia	7	1721/1396	57	0.56 (0.41,0.77)	.0003
	HB	10	2608/2175	0	0.58 (0.48,0.69)	<.00001
	PB	6	943/691	35	0.51 (0.38,0.67)	<.00001
	Others	4	1183/1101	57	0.70 (0.56,0.87)	.002
Recessive model (CC vs CT + TT)	overall	20	4734 /3967	50	0.67 (0.61,0.74)	<.00001
	East Asia	13	3013/2571	28	0.62 (0.57,0.70)	<.0001
	South/West Asia	7	1721/1396	65	0.76 (0.65,0.89)	.0005
	HB	10	2608/2175	26	0.61 (0.53,0.69)	<.00001
	PB	6	943/691	8	0.60 (0.47,0.76)	<.0001
	Others	4	1183/1101	72	0.84 (0.71,1.00)	.05
Heterozygote model (CC vs CT)	overall	20	3777 /3440	38	0.74 (0.67,0.82)	<.00001
	East Asia	13	2197/2107	20	0.69 (0.60,0.78)	<.00001
	South/West Asia	7	1580/1333	53	0.82 (0.70,0.97)	.02
	HB	10	2151/1902	34	0.67 (0.58,0.77)	<.00001
	PB	6	675/591	0	0.69 (0.53,0.89)	.004
	Others	4	951/947	60	0.91 (0.76,1.09)	.30
Homozygote model (CC vs TT)	overall	20	2705 /2314	44	0.48 (0.41,0.56)	<.00001
	East Asia	13	1542/1338	17	0.47 (0.40,0.55)	<.00001
	South/West Asia	7	1163/976	68	0.53 (0.38,0.74)	.0001
	HB	10	1568/1354	0	0.40 (0.33,0.50)	<.00001
	PB	6	529/410	48	0.41 (0.29,0.58)	<.00001
	Others	4	608/550	70	0.66 (0.52,0.85)	.001
Allele model (C vs T)	overall	20	9468 /7934	49	0.70 (0.66,0.75)	<.00001
	East Asia	13	6026/5142	20	0.69 (0.64,0.74)	<.00001
	South/West Asia	7	1721/1396	71	0.75 (0.66,0.86)	<.0001
	HB	10	5216/4350	0	0.66 (0.60,0.73)	<.00001
	PB	6	1886/1382	43	0.62 (0.53,0.73)	<.00001
	Others	4	2366/2202	77	0.83 (0.74,0.94)	.003

CI = confidence interval, HB = hospital-based, MTHFR = methylene tetrahydrofolate reductase, OR = odds ratio, PB = population-based.

3.2.2. MTHFR A1298C polymorphism. Twelve studies with 2673 cases and 2328 controls were included to examine the effect of MTHFR A1298C polymorphism on male infertility (see Fig. 3; (A) Dominant model (AA + AC vs CC): OR = 0.62, 95% CI (0.49, 0.79), *P* = .0001; (B) Recessive model (AA vs AC + CC): OR = 0.79, 95% CI (0.70, 0.88), *P* < .0001; (C) Heterozygote model (AA vs AC): OR = 0.83, 95% CI (0.73, 0.93), *P* = .002; (D) Homozygote model (AA vs CC): OR = 0.61, 95% CI (0.39, 0.93), *P* = .02; (E) Allele model (A vs C): OR = 0.82, 95% CI (0.71, 0.95), *P* = .01). The results showed the significantly increased risk of male infertility with MTHFR 1298C allele carriers.

In the subgroup analysis of geographical location, we observed that the statistic association existed in East Asians but not in South/West Asians. Further stratified analysis by the source of controls, no significant enhanced risk was observed in all of 3 subgroups. Table 3 showed the results of overall and subgroup analysis in all of 5 genetic models.

3.2.3. MTRR A66G polymorphism. Four studies with 837 cases and 727 controls were included to assess the association between MTRR A66G polymorphism and the risk of male infertility (See Fig. 4 (A) Dominant model (AA + AG vs GG): OR = 0.60, 95% CI (0.45, 0.81), *P* = .001; (B) Recessive model (AA vs AG + GG): OR = 0.70, 95% CI (0.56, 0.88), *P* = .002; (C) Heterozygote model (AA vs AG): OR = 0.76, 95% CI (0.60, 0.92), *P* = .02; (D) Homozygote model (AA vs GG): OR = 0.51, 95% CI (0.36, 0.72), *P* = .0001; (E) Allele model (A vs G): OR = 0.76, 95% CI (0.66, 0.88), *P* = .00003). *In short, the MTRR 66G allele carriers had a markedly increased risk of male infertility in Asian populations.*

3.3. Sensitivity analysis and publication bias

In sensitivity analysis, elimination of each study made no qualitative difference on the pooled OR values, which indicated that the final consequences of this meta-analysis were stable (Table 4).

The publication biases of the included studies were assessed by funnel plot. The shape of funnel plot in MTHFR C677T, A1298C, and MTRR A66G genotype comparison indicated no obvious asymmetry (Fig. 5).

4. Discussion

According to the present meta-analysis involving 5049 cases and 4157 controls from 22 published studies, the MTHFR C677T polymorphism has statistical impact on the risk of male infertility in Asian populations which was similarly supported by the prior 4 meta-analysis of Asians.[10–13] Compared with them, this meta-analysis has a bigger number of included studies and samples. Therefore, the results are more valuable for Asian populations. Previously, a meta-analysis had included 3 studies with a total of 898 individuals to assess the association between MTHFR A1298C polymorphism and male infertility risk in Chinese population and confirmed that MTHFR A1298C polymorphism

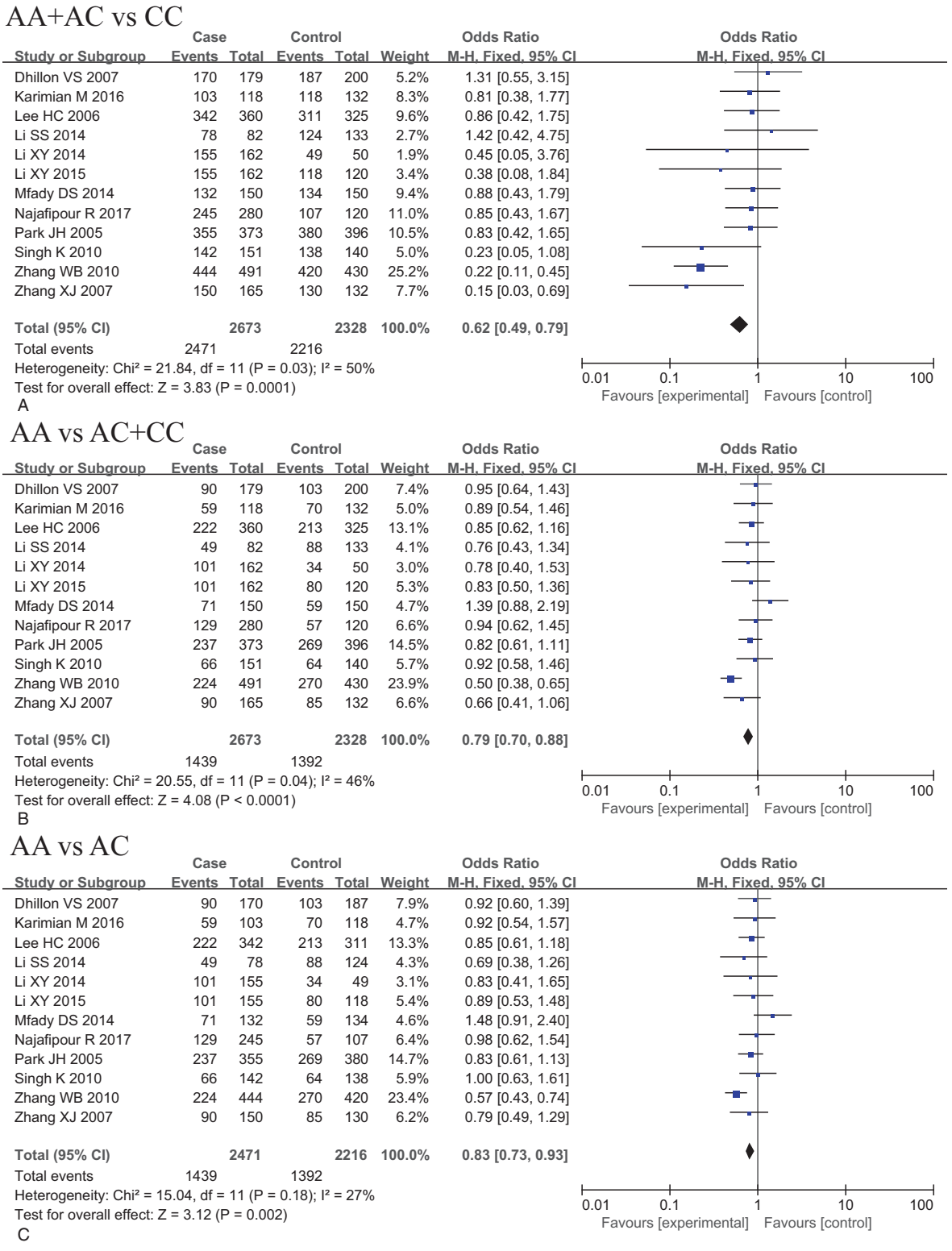
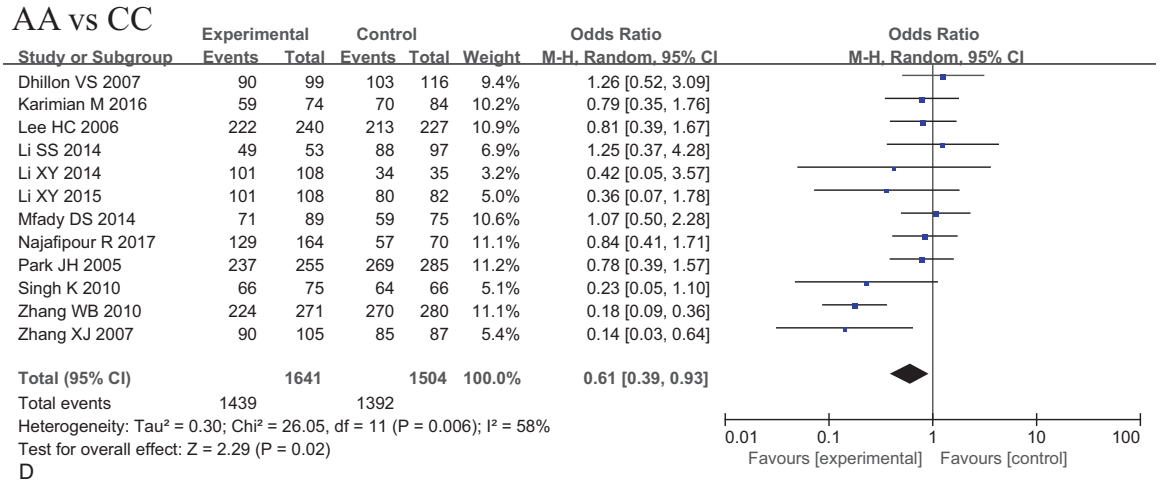


Figure 3. Forest plots for association of MTHFR A1298C polymorphism with the risk of male infertility in Asians. MTHFR = methylene tetrahydrofolate reductase.

was not the risk factor of male infertility (C vs A: OR = 1.22, 95% CI (0.97, 1.53), $I^2 = 0$; CC + AC vs AA: OR = 1.27, 95% CI (0.98, 1.65), $I^2 = 0$; CC vs AA: OR = 1.34, 95% CI (0.66, 2.77), $I^2 = 0$; CC vs AC + AA: OR = 1.44, 95% CI (0.72,2.88), $I^2 = 9$),[12] which was in contrast to the conclusion of present meta-analysis. This difference may be caused by sample sizes or population substructure. Regarding the MTRR A66G polymorphism, our results provided strong evidence of the association with male infertility risk. For Asians, NCBI database has shown that the allelic frequencies of MTHFR C677T, A1298C, and MTRR A66G are 0.51, 0.24, and 0.30 respectively. Basing on present study, we reached the following conclusion that men carrying the



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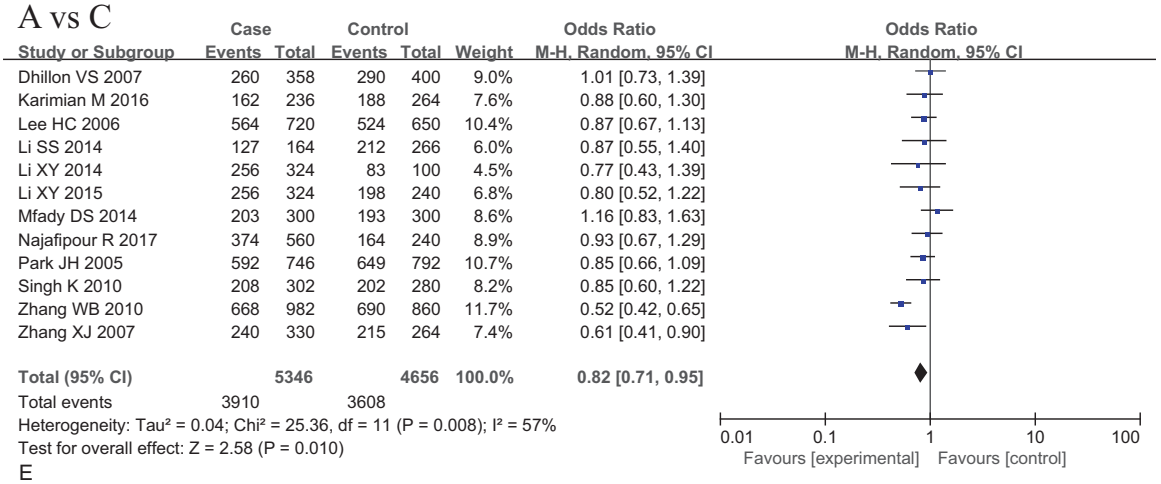

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Figure 3. (Continued).

Table 3

Subgroup analyses for MTHFR A1298C polymorphism in 5 comparative genetic models.

Models	Population	No. of studies	Sample size (case/control)	I^2 (%)	OR (95% CI)	P
Dominant model (AA + AC vs CC)	overall	12	2673/2328	50	0.62 (0.49,0.79)	.0001
	East Asia	7	1759/1586	59	0.49 (0.35,0.68)	<.0001
	South/West Asia	5	878/742	0	0.83 (0.58,1.19)	.31
	HB	5	1204/964	71	0.51 (0.36,0.71)	<.0001
	PB	4	557/443	22	0.55 (0.27,1.10)	.09
	Others	3	912/921	0	0.94 (0.61,1.44)	.78
Recessive model (AA vs AC + CC)	overall	12	2673/2328	46	0.79 (0.70,0.88)	<.0001
	East Asia	7	1759/1586	38	0.70 (0.60,0.80)	<.00001
	South/West Asia	5	878/742	0	1.00 (0.82,1.22)	.98
	HB	5	1204/964	77	0.72 (0.60,0.85)	.0001
	PB	4	557/443	0	0.83 (0.64,1.09)	.18
	Others	3	912/921	46	0.86 (0.71,1.04)	.12
Heterozygote model (AA vs AC)	overall	12	2471/2216	27	0.83 (0.73,0.93)	.002
	East Asia	7	1679/1532	0	0.74 (0.64,0.86)	<.0001
	South/West Asia	5	792/684	0	1.04 (0.84,1.28)	.74
	HB	5	1074/909	70	0.78 (0.65,0.94)	.008
	PB	4	530/429	0	0.87 (0.66,1.14)	.32
	Others	3	867/878	0	0.86 (0.70,1.04)	.13
Homozygote model (AA vs CC)	overall	12	1641/1504	58	0.61 (0.39,0.93)	.02
	East Asia	7	1140/1093	65	0.46 (0.24,0.90)	.02
	South/West Asia	5	501/411	0	0.88 (0.60,1.28)	.50
	HB	5	703/596	78	0.48 (0.22,1.08)	.08
	PB	4	344/280	8	0.53 (0.24,1.19)	.12
	Others	3	594/628	0	0.89 (0.57,1.38)	.60
Allele model (Avs C)	overall	12	5346/4656	57	0.82 (0.71,0.95)	.01
	East Asia	7	3590/3172	56	0.73 (0.60,0.89)	.001
	South/West Asia	5	1756/1484	0	0.97 (0.83,1.13)	.68
	HB	5	2408/1928	80	0.78 (0.57,1.08)	.13
	PB	4	1114/886	0	0.83 (0.67,1.04)	.10
	Others	3	1824/1842	0	0.89 (0.76,1.05)	.16

CI = confidence interval, HB = hospital-based, MTHFR = methylene tetrahydrofolate reductase, MTRR = methionine synthase reductase, OR = odds ratio, PB = population-based.

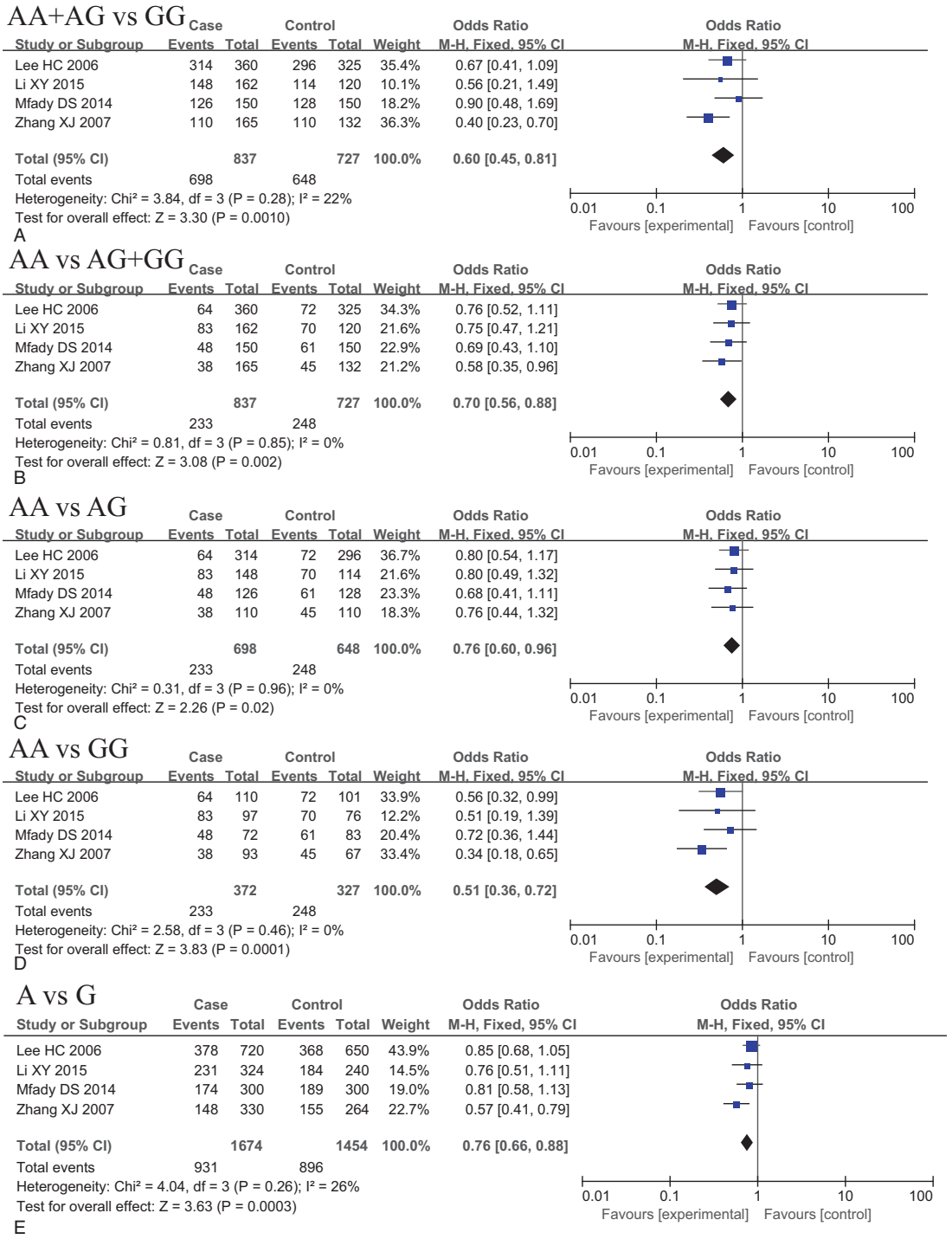

Figure 4. Forest plots for association of MTRR A66G polymorphism with the risk of male infertility in Asians. MTRR = methionine synthase reductase.

alleles of MTHFR 677T, 1298C, and MTRR 66G were likely to become infertile. Therefore, the analysis of these 3 key mutations would be helpful in the prognostication and screening of male infertility.

Although the precise mechanism by which MTHFR C677T, A1298C, and MTRR A66G polymorphisms have effect on fertility is unclear, previous researches have put forward some potential mechanisms. The folate-mediated 1-carbon metabolism is very important for many reactions in human sperm cells,[37,38] such as the methylation, repair, and synthesis of DNA. As one of the key enzymes in DNA synthesis, MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolic acid which participates in the exchange of deoxyuridine triphosphate (dUTP) for deoxythymidine monophosphate (dTMP) to 5-methyl-tetrahydrofolic acid with a biological function.[39] As a major regulatory enzyme in the pathway of homocysteine metabolism, MTRR plays a vital role in folate and vitamin B_{12}-dependent remethylation of homocysteine to methionine.

Table 4

Sensitivity analysis for the MTHFR C677T, A1298C, and MTRR A66G polymorphism.

A: MTHFR C677T polymorphism

	Heterogeneity		Effect size
Eliminated study	I^2	***P***	**OR (95%)**
A ZC 2007	31	.10	0.60 (0.52,0.68)
Dhillon VS 2007	6	.38	0.57 (0.51,0.65)
Karimian M 2016	29	.12	0.60 (0.52,0.68)
Lee HC 2006	31	.10	0.59 (0.52,0.68)
Li SS 2014	31	.10	0.60 (0.52,0.68)
Li XY 2015	24	.16	0.58 (0.51,0.66)
Liu L 2011	30	.11	0.60 (0.52,0.68)
Mfady DS 2014	29	.12	0.60 (0.53,0.68)
Najafipour R 2017	31	.10	0.60 (0.53,0.68)
Naqvi H 2014	30	.11	0.60 (0.53,0.68)
Park JH 2005	29	.12	0.58 (0.51,0.66)
Pei J 2013	26	.14	0.61 (0.53,0.69)
Qiu XF 2011	28	.12	0.61 (0.53,0.69)
Singh K 2005	23	.17	0.60 (0.53,0.68)
Sun HT 2005	27	.14	0.60 (0.53,0.68)
Vani GT 2011	25	.16	0.60 (0.53,0.68)
Wang Y 2017	30	.11	0.60 (0.53,0.68)
Yang BH 2010	28	.13	0.60 (0.53,0.69)
Zhang WB 2010	30	.11	0.60 (0.50,0.67)
Zhang XJ 2007	24	.17	0.58 (0.51,0.66)

B: MTHFR A1298CT polymorphism

	Heterogeneity		Effect size
Eliminated study	I^2	***P***	**OR (95%)**
Dhillon VS 2007	48	.04	0.58 (0.45,0.75)
Karimian M 2016	54	.02	0.60 (0.47,0.78)
Lee HC 2006	53	.02	0.60 (0.46,0.77)
Li SS 2014	51	.03	0.60 (0.47,0.77)
Li XY 2014	54	.02	0.62 (0.49,0.80)
Li XY 2015	53	.02	0.63 (0.49,0.81)
Mfady DS 2014	53	.02	0.59 (0.46,0.77)
Najafipour R 2017	53	.02	0.59 (0.46,0.77)
Park JH 2005	53	.02	0.60 (0.46,0.77)
Singh K 2010	50	.03	0.64 (0.50,0.82)
Zhang WB 2010	6	.38	0.75 (0.58,0.90)
Zhang XJ 2007	44	.06	0.66 (0.51,0.85)

C: MTRR A66G polymorphism

	Heterogeneity		Effect size
Eliminated study	I^2	***P***	**OR (95%)**
Lee HC 2006	17	.30	0.70 (0.57,0.85)
Li XY 2015	50	.13	0.77 (0.65,0.90)
Mfady DS 2014	49	.14	0.75 (0.64,0.89)
Zhang XJ 2007	0	.88	0.82 (0.70,0.97)

MTHFR = methylene tetrahydrofolate reductase, MTRR = methionine synthase reductase, OR = odds ratio.

Therefore, the polymorphisms of MTHFR C677T, A1298C, and MTRR A66G may influence the activity and stability of the above enzymes leading to imbalance of folate-related metabolism.[40] Then, the abnormal metabolism may give rise to the risk of male infertility.

For Asians, our meta-analysis again indicated the significant association between MTHFR C677T polymorphism and male infertility which kept consistent with previous meta-analysis. Instead, as to MTHFR A1298C polymorphism, the conclusions were not the same. Ren et al suggested it was not the risk factor of male infertility in Chinese population.[12] However, the present meta-analysis observed the statistic association existing in Asians especially for East Asians. This discordant finding may be due to the more included studies and a larger sample size for our research. Most importantly, this is the first meta-analysis specifically for Asian populations assessing the correlation between MTRR A66G polymorphism and male infertility. It showed that the genotypes and mutant allele of MTRR A66G were significantly related with male infertility in Asians. Liu et al and Xu et al have performed meta-analyses to investigate the association between MTRR A66G polymorphism and male infertility in overall population, and they failed to draw any statistic conclusions.[38,41] When restricting the subgroup analysis to ethnicity, Liu et al observed an increased risk in Asians but

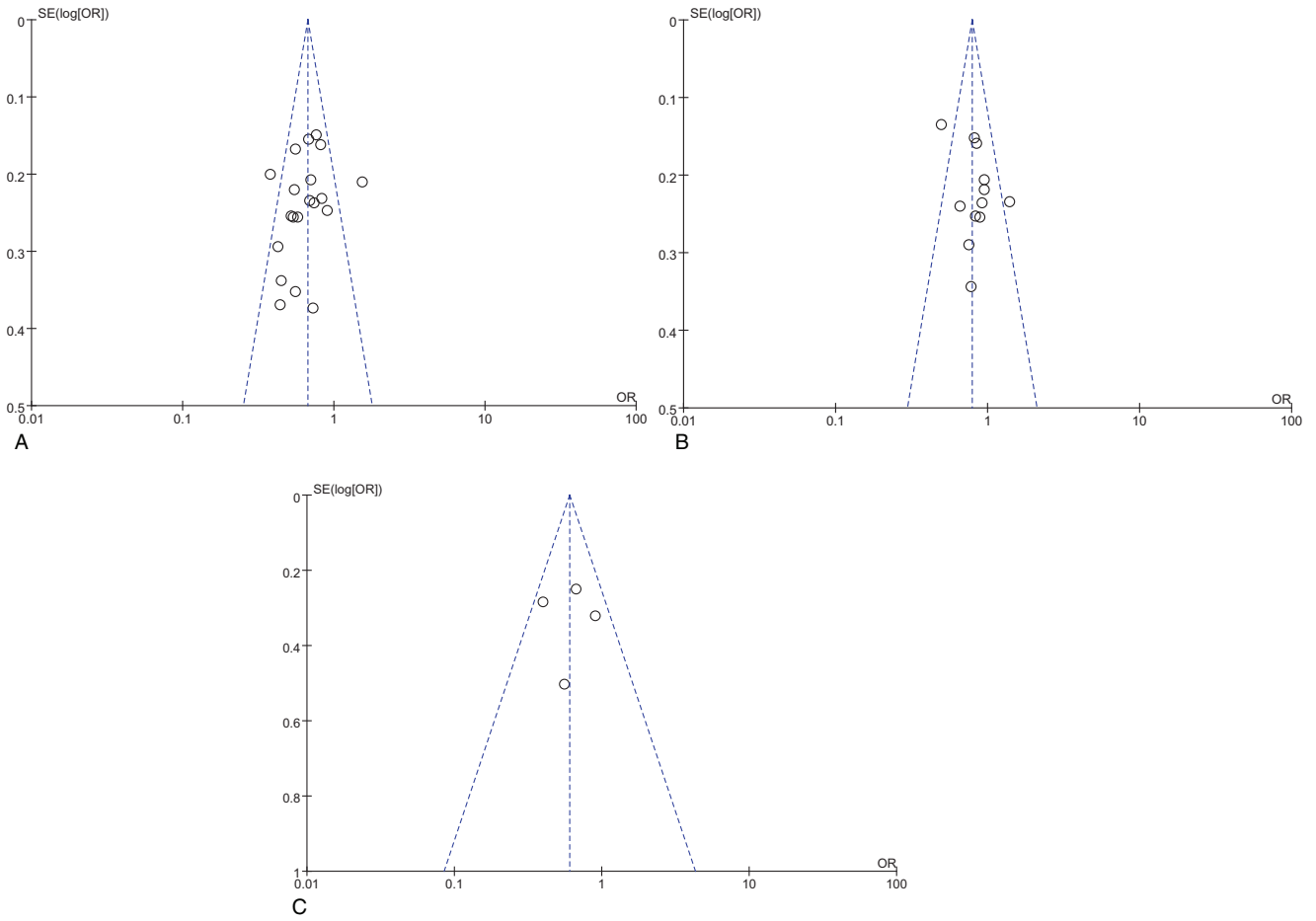

Figure 5. Publication bias test for MTHFR C677T, A1298C, and MTRR A66G polymorphism (A: MTHFR C677T polymorphism, B: MTHFR A1298C polymorphism, C: MTRR A66G polymorphism). MTHFR = methylene tetrahydrofolate reductase, MTRR = methionine synthase reductase.

not in Europeans in homozygous, dominant and allele genetic models.[38] In addition, there were available data analyzing these 3 polymorphisms within certain patients. Zhang et al [28] have enrolled 165 infertile patients and 132 healthy fertile males in China to evaluate the impact of MTHFR and MTRR gene polymorphisms on idiopathic male infertility. The findings discovered that: first, the heterozygous genotype (CT) and combined genotype (CT + TT) were present at statistical significances in male infertility ($P = .026$, $P = .031$) for MTHFR C677T polymorphism. Second, the frequencies of allele C and homozygous genotype (CC) were significantly different between case group and control group ($P = .013$, $P = .004$) for MTHFR A1298C polymorphism. Third, the prevalence of GG genotype and combined genotype (AG + GG) showed significant difference in the 2 groups ($P = .001$, $P = .035$) for MTRR A66G. These data are in consistent with our research revealing that the 3 polymorphisms might play an important role in the occurrence of male infertility. However, further studies are still needed to reveal the correlation between polymorphisms of MTHFR C677T, A1298C, and MTRR A66G with Asian male infertility.

On the other hand, some inherent limitations of this meta-analysis should be admitted. First, there may be some language bias since the included literatures are given priority to Chinese and English. Second, the sources of controls among the studies were different from each other. Some studies were HB studies, some studies were PB studies, and others were mixed population or uncertain. Third, our analysis was merely based on single-factor estimation ignoring the interactions of gene-gene and gene-environmental in the development of male infertility. Finally, the sample size was relatively small in part of the included studies.

5. Conclusion

In short, our meta-analysis provides further evidence indicating that MTHFR C677T, A1298C, and MTRR A66G polymorphisms are the risk factors with susceptibility to male infertility in Asian populations. In the future, studies with larger sample sizes will be performed to confirm it, and to explore the relationship between potential gene-gene, gene-environment interactions and male infertility with purpose of providing an important basis for the prevention and treatment of male infertility.

Author contributions

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Writing – original draft: Tianlu Shi.
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