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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 vs C: OR = 0.82, 95% CI (0.71, 0.95), P =.001. Allele model: MTHFR C vs T: OR = 0.70, 95% CI (0.66, 0.75), P <.00001; MTHFR A vs C: 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 metaanalyses 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 I² statistics. If there was no heterogeneity with $P \ge .1$ or $I^2 \le 50$, 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

							Ca	ses					Cont	rols				
Author	Year	Country	Geographical location	Source of controls	Total	CC	CT	TT	C	Т	Total	CC	CT	TT	C	Т	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	Α	C	Total	AA	AC	CC	Α	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 A6	6G polvi	norphism																

						Cases			Controls									
Reference	Year	Country	Geographical location	Source of controls	Total	AA	AG	GG	Α	G	Total	AA	AG	GG	Α	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.

CC+CT vs TT	-						
Study on Submann	Case) T-4-1	Contr	ol	14/-:	Odds Ratio	Odds Ratio
A ZC 2007	<u>_vents</u> 20∩	<u>1 otal</u> 355	<u>⊏vents</u> 223	<u>1 otal</u> 252	vveight 7 2%	0.58 [0.36, 0.93]	
Dhillon VS 2007	158	179	170	200	2.8%	1.33 [0.73, 2.42]	
Karimian M 2016	110	118	129	132	1.2%	0.32 [0.08, 1.23]	
Lee HC 2006	296	360	284	325	8.0%	0.67 [0.44, 1.02]	
Li SS 2014	50	82	97	133	4.3%	0.58 [0.32, 1.04]	
Li XY 2015	138	162	102	120	2.6%	1.01 [0.52, 1.97]	
LIU L 2011 Mfady DS 2014	130	/5 150	08 1/1	150	1.4%	0.38 [0.11, 1.28]	
Naiafipour R 2017	236	280	109	120	3.6%	0.54 [0.27, 1.09]	
Nagvi H 2014	601	637	354	364	3.8%	0.47 [0.23, 0.96]	
Park JH 2005	310	373	345	396	8.5%	0.73 [0.49, 1.08]	
Pei J 2013	177	290	71	90	6.4%	0.42 [0.24, 0.73]	
Qiu XF 2011	187	271	148	180	8.3%	0.48 [0.30, 0.76]	
Singn K 2005 Sup HT 2005	140	182	200	200	3.8%	0.06 [0.00, 1.00]	
Vani GT 2011	200	206	230	230	1.0%	0.07 [0.00, 1.20]	· · · · · · · · · · · · · · · · · · ·
Wang Y 2017	52	76	78	95	3.3%	0.47 [0.23, 0.96]	
Yang BH 2010	89	131	240	293	7.2%	0.47 [0.29, 0.75]	
Zhang WB 2010	296	491	300	430	19.1%	0.66 [0.50, 0.87]	
Zhang XJ 2007	134	165	108	132	3.4%	0.96 [0.53, 1.73]	
Total (95% CI)		4734		3967	100.0%	0.60 [0.53, 0.67]	•
Total events	3777		3440				
Heterogeneity: Chi ² = 26	.14, df =	: 19 (P	= 0.13); l ^a	= 27%			
Test for overall effect: Z	= 8.21 (I	P < 0.00	0001)				Favours [experimental] Favours [control]
A							
CC vs CT+T	Г						
00 10 01 1	• (.ae4	2	Contr	ol		Odds Ratio	Odds Ratio
Study or Subgroup	Events	<u>Total</u>	Events	Total	Weight	M-H, Fixed, 95% C	M-H. Fixed, 95% CI
A ZC 2007	130	355	128	252	9.3%	0.56 [0.40, 0.78]	
Dhillon VS 2007	81	179	70	200	3.6%	1.53 [1.02, 2.32]	
Karimian M 2016	51	118	77	132	4.1%	0.54 [0.33, 0.90]	
Lee HC 2006	115	360	118	325	8.3%	0.82 [0.60, 1.13]	
Li SS 2014	61	162	30 48	120	2.2%	0.95 [0.26, 1.11]	
Liu L 2011	27	75	40	72	2.6%	0.45 [0.23, 0.87]	
Mfady DS 2014	67	150	74	150	4.0%	0.83 [0.53, 1.31]	
Najafipour R 2017	113	280	66	120	5.4%	0.55 [0.36, 0.85]	
Naqvi H 2014	447	637	275	364	10.2%	0.76 [0.57, 1.02]	
Park JH 2005	105	373	145	396	9.9%	0.68 [0.50, 0.92]	
Pei J 2013	39	290	24	180	3.1%	0.43 [0.24, 0.76]	·
Singh K 2005	105	151	163	200	4.2%	0.52 [0.32 0.85]	
Sun HT 2005	27	182	15	53	1.9%	0.44 [0.21, 0.91]	
Vani GT 2011	158	206	188	230	4.1%	0.74 [0.46, 1.17]	
Wang Y 2017	15	76	24	95	1.7%	0.73 [0.35, 1.51]	
Yang BH 2010	34	131	98	293	4.4%	0.70 [0.44, 1.10]	
Zhang WB 2010 Zhang XL 2007	43	491	87	430	8.3%	0.38 [0.26, 0.56]	· · · · · · · · · · · · · · · · · · ·
Zhang XJ 2007	41	105	40	132	3.9%	0.56 [0.55, 0.95]	
Total (95% CI)		4734		3967	100.0%	0.67 [0.61, 0.74]	•
Total events	1748		1787				
Heterogeneity: Chi ² = 37	.85, df =	: 19 (P	= 0.006);	$l^2 = 50^{\circ}$	%		0.01 0.1 1 10 100
Test for overall effect: Z =	= 8.19 (I	P < 0.00	0001)				Favours [experimental] Favours [control]
В							
CC vs CT							
	Case	9	Contr	ol		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% C	M-H. Fixed, 95% Cl
A ZC 2007	130	290	128	223	9.1%	0.60 [0.42, 0.86]	
Dhillon VS 2007	81	158	70	170	3.8%	1.50 [0.97, 2.33]	
Karimian M 2016	51	206	118	129	4.4% 8.4%	0.58 [0.35, 0.98]	
Li SS 2014	14	50	36	97	2.0%	0.66 [0.31, 1.38]	
Li XY 2015	61	138	48	102	3.5%	0.89 [0.53, 1.49]	
Liu L 2011	27	65	40	68	2.6%	0.50 [0.25, 0.99]	
Mfady DS 2014	67	130	74	141	3.9%	0.96 [0.60, 1.55]	
Najafipour R 2017	113	236	66	109	5.4%	0.60 [0.38, 0.95]	
Naqvi H 2014 Dork JH 2005	447	601	275	354	10.2%	0.83 [0.61, 1.14]	
Pei J 2003	39	177	24	71	3.1%	0.55 [0.30, 1.02]	
Qiu XF 2011	75	187	63	148	4.8%	0.90 [0.58, 1.40]	
Singh K 2005	105	145	163	200	4.3%	0.60 [0.36, 0.99]	
Sun HT 2005	27	113	15	43	1.9%	0.59 [0.27, 1.26]	
Vani GT 2011	158	200	188	230	4.2%	0.84 [0.52, 1.35]	
Wang Y 2017	15	52	24	78	1.6%	0.91 [0.42, 1.97]	
Tang DH 2010 Zhang WB 2010	34 12	296 296	98 87	240	3.8% 8.5%	0.30 [0.54, 1.48]	- - -
Zhang XJ 2007	43	290 134	48	108	4.2%	0.55 [0.32, 0.93]	
					,,,		
Total (95% CI)		3777		3440	100.0%	0.74 [0.67, 0.82]	•
Total events	1748	10.77	1787				
Heterogeneity: Chi ² = 30	.೮೮, df =	:19 (P :	= 0.04); l ^a	= 38%			0.01 0.1 1 10 100
C.	0.08 (I	- 0.00					Favours [experimental] Favours [control]
. /							

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

CC vs CT

	Case	9	Contr	ol		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% Cl	M-H, Fixed, 95% Cl
A ZC 2007	130	195	128	157	9.0%	0.45 [0.27, 0.75]	
Dhillon VS 2007	81	102	70	100	2.8%	1.65 [0.87, 3.14]	
Karimian M 2016	51	59	77	80	1.7%	0.25 [0.06, 0.98]	
Lee HC 2006	115	179	118	159	8.5%	0.62 [0.39, 1.00]	
Li SS 2014	14	46	36	72	3.7%	0.44 [0.20, 0.95]	
Li XY 2015	61	85	48	66	2.9%	0.95 [0.46, 1.96]	
Liu L 2011	27	37	40	44	1.9%	0.27 [0.08, 0.95]	
Mfady DS 2014	67	87	74	83	3.3%	0.41 [0.17, 0.96]	
Najafipour R 2017	113	157	66	77	4.7%	0.43 [0.21, 0.89]	
Naqvi H 2014	447	483	275	285	4.9%	0.45 [0.22, 0.92]	
Park JH 2005	105	168	145	196	9.6%	0.59 [0.38, 0.92]	
Pei J 2013	39	152	24	43	5.3%	0.27 [0.14, 0.55]	
Qiu XF 2011	75	159	63	95	7.9%	0.45 [0.27, 0.77]	_ _
Singh K 2005	105	111	163	163	1.5%	0.05 [0.00, 0.89]	·
Sun HT 2005	27	96	15	25	3.3%	0.26 [0.10, 0.65]	
Vani GT 2011	158	164	188	188	1.3%	0.06 [0.00, 1.16]	· · · · · · · · · · · · · · · · · · ·
Wang Y 2017	15	39	24	41	2.7%	0.44 [0.18, 1.08]	
Yang BH 2010	34	76	98	151	6.9%	0.44 [0.25, 0.77]	
Zhang WB 2010	43	238	87	217	14.2%	0.33 [0.21, 0.51]	
Zhang XJ 2007	41	72	48	72	3.9%	0.66 [0.34, 1.30]	
Total (95% CI)		2705		2314	100.0%	0.48 [0.41, 0.56]	▼
Total events	1748		1787				
Heterogeneity: Chi ² = 3	84.16, df =	19 (P	= 0.02); l ²	= 44%			
Test for overall effect: 2	Z = 9.78 (I	o < 0.0	0001)				Favours [experimental] Favours [control]
D							and the second s
~ -							
C vs T							
	Case	•	Contr	ol		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Fixed, 95% CI	M-H, Fixed, 95% CI
A ZC 2007	420	710	351	504	8.1%	0.63 [0.50, 0.80]	
Dhillon VS 2007	239	358	240	400	3.6%	1.34 [0.99, 1.80]	
Karimian M 2016	161	236	206	264	3.0%	0.60 [0.41, 0.90]	
Lee HC 2006	411	720	402	650	8.7%	0.82 [0.66, 1.02]	
Li SS 2014	64	164	133	266	3.0%	0.64 [0.43, 0.95]	_ _ _
Li XY 2015	199	324	150	240	3.2%	0.96 [0.68, 1.35]	-+-
Liu L 2011	92	150	108	144	2.1%	0.53 [0.32, 0.87]	
Mfady DS 2014	197	300	215	300	3.6%	0.76 [0.53, 1.07]	
Naiafipour R 2017	349	560	175	240	4.4%	0.61 [0.44, 0.86]	
Nagvi H 2014	1048	1274	629	728	6.8%	0.73 [0.56, 0.94]	
Park JH 2005	415	746	490	792	10.2%	0.77 [0.63, 0.95]	
Pei J 2013	216	580	95	180	4.4%	0.53 [0.38, 0.74]	

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

Qiu XF 2011

Singh K 2005

Sun HT 2005

Vani GT 2011

Wang Y 2017

Yang BH 2010

Zhang WB 2010

Zhang XJ 2007

Total (95% CI)

Total events

Е

262 542

250 302

140 364

358 412

60 152

123 262

339 982

175 330

5518

Heterogeneity: Chi² = 37.34, df = 19 (P = 0.007); l² = 49%

Test for overall effect: Z = 10.32 (P < 0.00001)

9468

211 360

363 400

58 106

418 460

102 190

338 586

387 860

156 264

5227

6.3%

2.6%

2.7%

2.5%

2.6%

5.3%

13.0%

3.9%

7934 100.0%

0.66 [0.50, 0.86]

0.49 [0.31, 0.77]

0.52 [0.33, 0.80]

0.67 [0.43, 1.02]

0.56 [0.37, 0.87]

0.65 [0.48, 0.87]

0.64 [0.53, 0.78]

0.78 [0.56, 1.08]

0.70 [0.66, 0.75]

Figure 2. (Continued).

0.01

0.1

Favours [experimental] Favours [control]

3.2.1. MTHFR C677T polymorphism. After pooling 20 studies with 4734 cases and 3967 controls into 1 data set for metaanalysis, 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.)

10

100

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.

Models	Population	No. of studies	Sample size (case/control)	<i>l</i> ² (%)	OR (95% CI)	Р
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

Table 2

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 metaanalysis 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

AA vs CC								
	Experim	ental	Contr	ol	147.1.1.1	Odds Ratio	Odds Ratio	
Study or Subgroup	Events	lotal	Events	Iotal	weight	M-H, Random, 95% CI	M-H, Random, 95% Cl	—
Dhillon VS 2007	90	99	103	116	9.4%	1.26 [0.52, 3.09]		
Karimian M 2016	59	74	70	84	10.2%	0.79 [0.35, 1.76]		
Lee HC 2006	222	240	213	227	10.9%	0.81 [0.39, 1.67]		
Li SS 2014	49	53	88	97	6.9%	1.25 [0.37, 4.28]		
LI XY 2014	101	108	34	35	3.2%	0.42 [0.05, 3.57]		
Li XY 2015	101	108	80	82	5.0%	0.36 [0.07, 1.78]		
Mfady DS 2014	/1	89	59	75	10.6%	1.07 [0.50, 2.28]		
Najafipour R 2017	129	164	57	70	11.1%	0.84 [0.41, 1.71]		
Park JH 2005	237	255	269	285	11.2%	0.78 [0.39, 1.57]		
Singh K 2010	66	75	64	66	5.1%	0.23 [0.05, 1.10]		
Zhang WB 2010	224	271	270	280	11.1%	0.18 [0.09, 0.36]		
Zhang XJ 2007	90	105	85	87	5.4%	0.14 [0.03, 0.64]		
Total (95% CI)		1641		1504	100.0%	0.61 [0.39, 0.93]	•	
Total events	1439		1392					
Heterogeneity: Tau ² =	0.30; Chi ²	= 26.05	, df = 11 (l	P = 0.0	06); l ² = 58	3%		4
Test for overall effect: 2	Z = 2.29 (F	e = 0.02)		,.		0.01 0.1 1 10 100	J
D	```		, ,				Favours [experimental] Favours [control]	
Λ T								
A vs C	Case	÷	Contro	ы		Odds Ratio	Odds Ratio	
A vs C Study or Subgroup	Case Events	e Total	Contro Events	ol Total	Weight	Odds Ratio M-H, Random, 95% CI	Odds Ratio M-H, Random, 95% Cl	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007	Case Events 260	<u>Total</u> 358	Contro Events 290	ol <u>Total</u> 400	<u>Weight</u> 9.0%	Odds Ratio <u>M-H, Random, 95% Cl</u> 1.01 [0.73, 1.39]	Odds Ratio M-H, Random, 95% Cl	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016	Case Events 260 162	• <u>Total</u> 358 236	Contro Events 290 188	0 1 Total 400 264	<u>Weight</u> 9.0% 7.6%	Odds Ratio <u>M-H, Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30]	Odds Ratio M-H, Random, 95% Cl	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016 Lee HC 2006	Case Events 260 162 564	Total 358 236 720	Contro Events 290 188 524	Total 400 264 650	<u>Weight</u> 9.0% 7.6% 10.4%	Odds Ratio <u>M-H. Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13]	Odds Ratio <u>M-H, Random, 95% Cl</u>	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016 Lee HC 2006 Li SS 2014	Case Events 260 162 564 127	Total 358 236 720 164	Contro Events 290 188 524 212	ol <u>Total</u> 400 264 650 266	<u>Weight</u> 9.0% 7.6% 10.4% 6.0%	Odds Ratio <u>M-H. Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13] 0.87 [0.55, 1.40]	Odds Ratio <u>M-H, Random, 95% Cl</u>	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016 Lee HC 2006 Li SS 2014 Li XY 2014	Case Events 260 162 564 127 256	Total 358 236 720 164 324	Contro Events 290 188 524 212 83	Total 400 264 650 266 100	Weight 9.0% 7.6% 10.4% 6.0% 4.5%	Odds Ratio <u>M-H. Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13] 0.87 [0.55, 1.40] 0.77 [0.43, 1.39]	Odds Ratio M-H, Random, 95% Cl 	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016 Lee HC 2006 Li SS 2014 Li XY 2014 Li XY 2015	Case Events 260 162 564 127 256 256	Total 358 236 720 164 324 324	Contro Events 290 188 524 212 83 198	Total 400 264 650 266 100 240	Weight 9.0% 7.6% 10.4% 6.0% 4.5% 6.8%	Odds Ratio <u>M-H, Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13] 0.87 [0.55, 1.40] 0.77 [0.43, 1.39] 0.80 [0.52, 1.22]	Odds Ratio M-H, Random, 95% Cl	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016 Lee HC 2006 Li SS 2014 Li XY 2014 Li XY 2015 Mfady DS 2014	Case <u>Events</u> 260 162 564 127 256 256 203	Total 358 236 720 164 324 324 300	Contro Events 290 188 524 212 83 198 193	Total 400 264 650 266 100 240 300	Weight 9.0% 7.6% 10.4% 6.0% 4.5% 6.8% 8.6%	Odds Ratio <u>M-H, Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13] 0.87 [0.55, 1.40] 0.77 [0.43, 1.39] 0.80 [0.52, 1.22] 1.16 [0.83, 1.63]	Odds Ratio M-H, Random, 95% Cl	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016 Lee HC 2006 Li SS 2014 Li XY 2014 Li XY 2015 Mfady DS 2014 Naiafipour R 2017	Case Events 260 162 564 127 256 256 203 374	Total 358 236 720 164 324 300 560	Contro Events 290 188 524 212 83 198 193 164	Total 400 264 650 266 100 240 300 240	Weight 9.0% 7.6% 10.4% 6.0% 4.5% 6.8% 8.6% 8.9%	Odds Ratio <u>M-H, Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13] 0.87 [0.55, 1.40] 0.77 [0.43, 1.39] 0.80 [0.52, 1.22] 1.16 [0.83, 1.63] 0.93 [0.67, 1.29]	Odds Ratio M-H, Random, 95% Cl	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016 Lee HC 2006 Li SS 2014 Li XY 2014 Li XY 2014 Li XY 2014 Majafipour R 2017 Park JH 2005	Case Events 260 162 564 127 256 256 203 374 592	Total 358 236 720 164 324 324 300 560 746	Contro 290 188 524 212 83 198 193 164 649	Total 400 264 650 266 100 240 300 240 792	Weight 9.0% 7.6% 10.4% 6.0% 4.5% 6.8% 8.6% 8.9% 10.7%	Odds Ratio <u>M-H. Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13] 0.87 [0.55, 1.40] 0.77 [0.43, 1.39] 0.80 [0.52, 1.22] 1.16 [0.83, 1.63] 0.93 [0.67, 1.29] 0.85 [0.66, 1.09]	Odds Ratio M-H, Random, 95% Cl	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016 Lee HC 2006 Li SS 2014 Li XY 2014 Li XY 2014 Li XY 2015 Mfady DS 2014 Najafipour R 2017 Park JH 2005 Singh K 2010	Case <u>Events</u> 260 162 564 127 256 203 374 592 208	Total 358 236 720 164 324 324 300 560 746 302	Contro Events 290 188 524 212 83 198 193 164 649 202	Total 400 264 650 266 100 240 300 240 792 280	Weight 9.0% 7.6% 10.4% 6.0% 4.5% 6.8% 8.6% 8.9% 10.7% 8.2%	Odds Ratio <u>M-H, Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13] 0.87 [0.55, 1.40] 0.77 [0.43, 1.39] 0.80 [0.52, 1.22] 1.16 [0.83, 1.63] 0.93 [0.67, 1.29] 0.85 [0.66, 1.09] 0.85 [0.60, 1.22]	Odds Ratio <u>M-H, Random, 95% CI</u>	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016 Lee HC 2006 Li SS 2014 Li XY 2014 Li XY 2015 Mfady DS 2014 Najafipour R 2017 Park JH 2005 Singh K 2010 Zhang WB 2010	Case Events 260 162 564 127 256 256 203 374 592 208 668	Total 358 236 720 164 324 324 300 560 746 302 982	Contro 290 188 524 212 83 198 193 164 649 202 690	I 400 264 650 266 100 240 300 240 792 280 860	Weight 9.0% 7.6% 10.4% 6.0% 4.5% 6.8% 8.6% 8.6% 8.9% 10.7% 8.2% 11.7%	Odds Ratio <u>M-H, Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13] 0.87 [0.55, 1.40] 0.77 [0.43, 1.39] 0.80 [0.52, 1.22] 1.16 [0.83, 1.63] 0.93 [0.67, 1.29] 0.85 [0.66, 1.09] 0.85 [0.66, 1.22] 0.52 [0.42, 0.65]	Odds Ratio M-H, Random, 95% CI 	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016 Lee HC 2006 Li SS 2014 Li XY 2014 Li XY 2015 Mfady DS 2014 Najafipour R 2017 Park JH 2005 Singh K 2010 Zhang WB 2010 Zhang XJ 2007	Case Events 260 162 564 256 256 203 374 592 208 668 240	Total 358 236 720 164 324 324 300 560 746 302 982 330	Contro 290 188 524 212 83 198 193 164 649 202 690 215	Image: system 400 264 650 266 100 240 300 240 792 280 860 264	Weight 9.0% 7.6% 10.4% 6.0% 4.5% 6.8% 8.6% 8.8% 10.7% 8.2% 11.7% 7.4%	Odds Ratio <u>M-H, Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13] 0.87 [0.55, 1.40] 0.77 [0.43, 1.39] 0.80 [0.52, 1.22] 1.16 [0.83, 1.63] 0.93 [0.67, 1.29] 0.85 [0.66, 1.09] 0.85 [0.66, 1.22] 0.52 [0.42, 0.65] 0.61 [0.41, 0.90]	Odds Ratio <u>M-H, Random, 95% CI</u> 	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016 Lee HC 2006 Li SS 2014 Li XY 2014 Li XY 2014 Li XY 2015 Mfady DS 2014 Najafipour R 2017 Park JH 2005 Singh K 2010 Zhang WB 2010 Zhang XJ 2007	Case Events 260 162 564 256 256 203 374 592 208 668 240	Total 358 236 720 164 324 324 300 560 746 302 982 330	Contro Events 290 188 524 212 83 198 193 164 649 202 690 215	I 400 264 650 266 100 240 300 240 792 280 860 264	Weight 9.0% 7.6% 10.4% 6.0% 4.5% 6.8% 8.9% 10.7% 8.2% 11.7% 7.4%	Odds Ratio <u>M-H, Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13] 0.87 [0.55, 1.40] 0.77 [0.43, 1.39] 0.80 [0.52, 1.22] 1.16 [0.83, 1.63] 0.93 [0.67, 1.29] 0.85 [0.66, 1.09] 0.85 [0.66, 1.22] 0.52 [0.42, 0.65] 0.61 [0.41, 0.90]	Odds Ratio M-H, Random, 95% CI	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016 Lee HC 2006 Li SS 2014 Li XY 2014 Li XY 2015 Mfady DS 2014 Najafipour R 2017 Park JH 2005 Singh K 2010 Zhang WB 2010 Zhang XJ 2007 Total (95% CI)	Case Events 260 162 564 256 203 374 592 203 668 240	Total 358 236 720 164 324 324 300 560 746 302 982 330 5346	Contro Events 290 188 524 212 83 198 193 164 649 202 690 215	400 264 650 266 100 240 300 240 792 280 860 264 4656	Weight 9.0% 7.6% 6.0% 4.5% 6.8% 8.6% 8.6% 8.2% 10.7% 8.2% 11.7% 7.4% 100.0%	Odds Ratio <u>M-H, Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13] 0.87 [0.55, 1.40] 0.77 [0.43, 1.39] 0.80 [0.52, 1.22] 1.16 [0.83, 1.63] 0.93 [0.67, 1.29] 0.85 [0.66, 1.29] 0.85 [0.66, 1.22] 0.52 [0.42, 0.65] 0.61 [0.41, 0.90] 0.82 [0.71, 0.95]	Odds Ratio M-H, Random, 95% CI	
A vs C <u>Study or Subgroup</u> Dhilon VS 2007 Karimian M 2016 Lee HC 2006 Li SS 2014 Li XY 2014 Li XY 2015 Mfady DS 2014 Najafipour R 2017 Park JH 2005 Singh K 2010 Zhang WB 2010 Zhang XJ 2007 Total (95% CI) Total events	Case Events 260 162 564 127 256 256 203 374 592 208 668 240 3910	Total 358 236 720 164 324 324 324 300 560 746 302 982 330 5346	Contro Events 290 188 524 212 83 198 193 164 649 202 690 215 3608	J 400 264 650 266 100 240 300 240 792 280 860 264 4656	Weight 9.0% 7.6% 10.4% 6.0% 4.5% 6.8% 8.6% 8.9% 10.7% 8.2% 11.7% 7.4% 100.0%	Odds Ratio <u>M-H, Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13] 0.87 [0.55, 1.40] 0.77 [0.43, 1.39] 0.80 [0.52, 1.22] 1.16 [0.83, 1.63] 0.93 [0.67, 1.29] 0.85 [0.66, 1.09] 0.85 [0.66, 1.22] 0.52 [0.42, 0.65] 0.61 [0.41, 0.90] 0.82 [0.71, 0.95]	Odds Ratio M-H, Random, 95% CI 	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016 Lee HC 2006 Li SS 2014 Li XY 2014 Li XY 2014 Li XY 2015 Mfady DS 2014 Najafipour R 2017 Park JH 2005 Singh K 2010 Zhang WB 2010 Zhang WB 2010 Zhang XJ 2007 Total (95% CI) Total events Heterogeneity: Tau ² =	Case Events 260 162 564 127 256 203 374 592 208 668 240 3910 0.04; Chi ²	Total 358 236 720 164 324 300 560 746 302 982 330 5346 = 25,36	Contro Events 290 188 524 212 83 198 193 164 649 202 690 215 3608 5, df = 11 (400 264 650 266 100 240 300 264 4656 P = 0.0	Weight 9.0% 7.6% 10.4% 6.0% 4.5% 6.8% 8.6% 8.9% 10.7% 8.2% 11.7% 7.4% 100.0% 008); l ² = 5	Odds Ratio <u>M-H. Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13] 0.87 [0.55, 1.40] 0.77 [0.43, 1.39] 0.80 [0.52, 1.22] 1.16 [0.83, 1.63] 0.93 [0.67, 1.29] 0.85 [0.66, 1.09] 0.85 [0.66, 1.09] 0.85 [0.66, 1.09] 0.52 [0.42, 0.65] 0.61 [0.41, 0.90] 0.82 [0.71, 0.95]	Odds Ratio M-H, Random, 95% CI 	
A vs C <u>Study or Subgroup</u> Dhillon VS 2007 Karimian M 2016 Lee HC 2006 Li SS 2014 Li XY 2014 Li XY 2014 Li XY 2015 Mfady DS 2014 Najafipour R 2017 Park JH 2005 Singh K 2010 Zhang WB 2010 Zhang WB 2010 Zhang VB 2010 Zhang VB 2010 Zhang VB 2010 Zhang VB 2010 Zhang S 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect:	Case <u>Events</u> 260 162 564 127 256 203 374 592 208 668 240 3910 0.04; Chi ² Z = 2.58 (F	Total 358 236 720 164 324 300 560 746 302 982 330 5346 2 2 3	Contro Events 290 188 524 212 83 198 193 164 649 202 690 215 3608 5, df = 11 (0)	Image: bit state 400 264 650 266 100 240 300 240 792 280 860 264 4656 (P = 0.0)	Weight 9.0% 7.6% 10.4% 6.0% 4.5% 8.6% 10.7% 8.2% 11.7% 7.4% 100.0% \u08); l ² = 5	Odds Ratio <u>M-H. Random, 95% CI</u> 1.01 [0.73, 1.39] 0.88 [0.60, 1.30] 0.87 [0.67, 1.13] 0.87 [0.55, 1.40] 0.77 [0.43, 1.39] 0.80 [0.52, 1.22] 1.16 [0.83, 1.63] 0.93 [0.67, 1.29] 0.85 [0.66, 1.09] 0.85 [0.66, 1.09] 0.85 [0.66, 1.09] 0.85 [0.60, 1.22] 0.52 [0.42, 0.65] 0.61 [0.41, 0.90] 0.82 [0.71, 0.95]	Odds Ratio M-H, Random, 95% CI 	

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>f</i> ² (%)	OR (95% CI)	Р
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 B₁₂-dependent remethylation of homocysteine to methionine.

Table 4

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

A: MTHFR C677T polymorphism

	Hetero	Effect size	
Eliminated study	ŕ	Р	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

	Hetero	Effect size			
Eliminated study	ľ	Р	OR (95 <i>%</i>)		
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					

	Hetero	Effect size	
Eliminated study	ŕ	Р	OR (95 <i>%</i>)
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 metaanalysis 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 singlefactor 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

Conceptualization: Tianlu Shi. Data curation: Tianlu Shi. Funding acquisition: Tianlu Shi. Investigation: Yan Wu, Yu Li, Zhen-Feng Chen, Yi-Ni Ma. Methodology: Zhe-Tao Zhang, Yong-Huang Zhang, Lei Zhang. Project administration: Tianlu Shi. Software: Yan Wu. **Supervision:** Tianlu Shi. **Validation:** Tianlu Shi.

validation: manu Sni.

Visualization: Tianlu Shi.

Writing – original draft: Tianlu Shi.

Writing - review & editing: Tianlu Shi, Yan Wu, Yu Li.

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