Association between C677T and A1298C polymorphisms of the *MTHFR* gene and risk of male infertility: a meta-analysis

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ABSTRACT. Published studies on the association between the C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase (*MTHFR*) gene and male infertility risk are controversial. To obtain a more precise evaluation, we performed a meta-analysis based on published case-control studies. We conducted an electronic search of PubMed, EMBASE, the Cochrane Library, the Web of Science, and the China Knowledge Resource Integrated Database for papers on *MTHFR* gene C677T and A1298C polymorphisms and male infertility risk. Pooled odds ratios (ORs) with 95% confidence intervals (95%CIs) were used to assess the strength of association in homozygote, heterozygote, dominant, recessive, and additive models. Statistical heterogeneity, test of publication bias, and sensitivity analysis were carried out using the STATA software (Version 13.0). Overall, 21 studies of C677T (4505 cases and 4024 controls) and 13 studies of A1298C (2785 cases and

3094 controls) were included in this meta-analysis. For C677T, the homozygote comparison results were OR = 1.629, 95%CI (1.215-2.184), and the recessive model results were OR = 1.462 (1.155-1.850). For A1298C, the homozygote comparison results were OR = 1.289 (1.029-1.616), and the recessive model results were OR = 1.288 (1.034-1.604). In conclusion, the current meta-analysis showed that the *MTHFR* C677T polymorphism was associated with a significantly increased male infertility risk in the Asian and overall populations, but not in the Caucasian population, and there was a significant association between the A1298C polymorphism and male infertility risk in the Asian, Caucasian, and overall groups.

Key words: *MTHFR*; C677T; A1298C; Polymorphisms; Male infertility; Meta-analysis

INTRODUCTION

Infertility, defined as the inability to conceive after 1 year of regular unprotected intercourse, is a major health problem. It affects 10-15% of couples of reproductive age worldwide, and the inability to conceive in approximately half of these couples is due to male infertility (Gelbaya et al., 2014). Male infertility is a multifactorial syndrome with a complex pathogenesis that involves lifestyle, individual genetic background, and environmental risk factors. Despite significant advances in diagnostic methods in the field, the etiology of about half of all male infertility cases remains unknown. Infertile men with no past history and normal semen analysis results are designated as having “idiopathic infertility”. Several potential factors are responsible for the symptoms of idiopathic infertility, including oxidative stress induced by reactive oxygen species (ROS), sperm DNA damage, and molecular genetic abnormalities (Treulen et al., 2015).

Over the years, a large number of genetic studies focusing on the association between male infertility and genetic polymorphisms have been carried out (Lee et al., 2006). In these studies, a great deal of attention has been paid to the methylenetetrahydrofolate reductase (*MTHFR*) gene, which plays a key role in the folate metabolism pathway and regulates the intracellular folate pool for the synthesis and methylation of DNA (Ebisch et al., 2003). The *MTHFR* gene has the chromosomal locus 1p36.6 and is 2.2 kb in length with a total of 11 exons. The C-to-T transition at nucleotide 677 in exon 4 is a point mutation that converts a cytosine (C) to a thymine (T), resulting in an alanine-to-valine mutation, which can affect the thermal stability and reduce the activity of MTHFR. The *MTHFR* gene A1298C (rs1801131) mutation in exon 7, a glutamate-to-alanine substitution at codon 429 (E429A), can increase serum folate levels (Parle-McDermott et al., 2006). In adult male mice, MTHFR levels are highest in the testis, and in *Mthfr* gene knockout mice, the MTHFR deficiency resulting in abnormal spermatogenesis and infertility in males suggests an important role for MTHFR in spermatogenesis (Kelly et al., 2005).

A number of studies have investigated the association between the two common *MTHFR* polymorphisms and the risk of male infertility. However, the results remain controversial and previous studies have generally been small. In 2012, a meta-analysis based on 10 case-control studies including 2275 cases and 1958 controls was performed, and the

authors concluded that the *MTHFR* C677T polymorphism is capable of causing male infertility susceptibility in Asians, but not in Caucasians (Wu et al., 2012). Shen et al. (2012) conducted a meta-analysis of seven case-control studies including 1633 cases and 1735 controls, and their results demonstrated that the *MTHFR* A1298C polymorphism is capable of causing male infertility susceptibility. Subsequently, a series of novel studies have been performed, so an updated meta-analysis based on 21 studies of C677T (4505 cases and 4024 controls) and 13 studies of A1298C (2785 cases and 3094 controls) was performed to clarify the effect of *MTHFR* polymorphisms on the risk of male infertility.

MATERIAL AND METHODS

Search strategy

PubMed, EMBASE, the Cochrane Library, the Web of Science, and the China Knowledge Resource Integrated Database (up to March 1, 2015) were searched using the terms: "(C677T or A1298C or MTHFR) and (polymorphism or variants or mutation) and (male infertility)". Studies published in English or Chinese were selected. Case-control studies containing available genotype frequencies of C677T and A1298C were chosen. Related reference articles were also searched to identify other relevant publications. Unpublished data were not included.

Inclusion and exclusion criteria

A study was considered eligible if it met the following inclusion criteria: 1) it was about *MTHFR* C677T or A1298C polymorphisms and male infertility; 2) it had a human case-control design; 3) it reported the frequency of the *MTHFR* C677T or A1298C polymorphisms as the number of infertile males and controls, according to the three variant genotypes of either polymorphism; and 4) it was published in English or Chinese. A study was considered ineligible if it met the following exclusion criteria: 1) it was not related to the *MTHFR* C677T or A1298C polymorphisms and male infertility; 2) it was not a primary case-control study; 3) no usable or sufficient genotype data were reported; 4) the allele frequency in the control population deviated from the Hardy-Weinberg equilibrium (HWE) at a P value equal to or less than 0.01; and 5) the study was a case report, a letter to the editor, a book chapter, or a review. If more than one article had been published by the same author using the same case series, we selected one paper and excluded the others. The study inclusion and exclusion procedures are summarized in Figure 1.

Data extraction

Two investigators independently extracted the data from all qualifying studies according to the selection standards listed above. Discrepancies were solved through discussion until agreement was reached. The following information was extracted: the first author's name, the year of publication, the country in which the study was conducted, the evidence for HWE agreement in the controls, the sample size, and the number of cases and controls with the CC/CT/TT or AA/AC/CC genotypes.

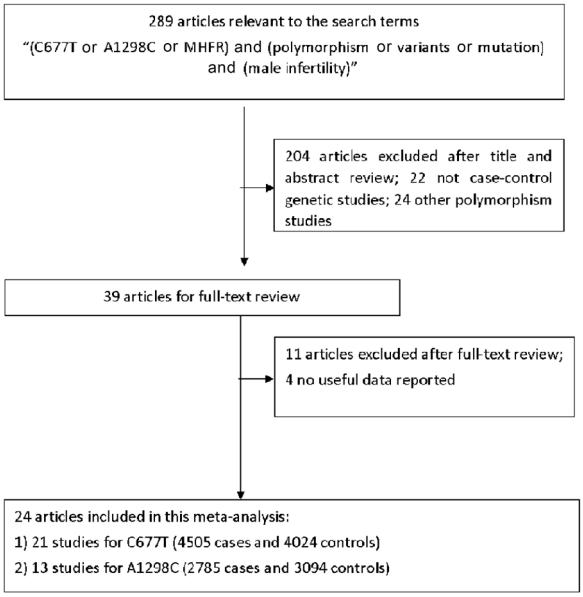

Figure 1. Inclusion and exclusion procedures.

Statistical analysis

The STATA software (Version 13.0) was used for all statistical analyses. Two-sided P values less than 0.05 were considered statistically significant. For the control groups for each study, the observed genotype frequencies of the *MTHFR* C677T and A1298C polymorphisms were evaluated for HWE. The strength of the association between the *MTHFR* C677T and A1298C polymorphisms and male infertility risk was assessed by the odds ratios (ORs) with 95% confidence intervals (95%CIs). Pooled ORs were calculated for homozygote, heterozygote, dominant, recessive, and additive models (Chen et al., 2013; Guan et al., 2014). Cochran's Q-statistic and the I^2 metric were used to assess heterogeneity between studies; $P < 0.10$ and $I^2 > 50\%$ were considered to indicate significant heterogeneity (Jackson et al., 2012). If the heterogeneity test results returned $P > 0.1$, the pooled ORs were analyzed using the random-effect model, otherwise the fixed-effect model was used. Sensitivity analyses were also performed after sequential removal of each study. Finally, Begg's funnel plots and the Egger test were used to statistically examine any publication bias.

RESULTS

Characteristics of the included studies

Overall, 24 case-control articles (Bezold et al., 2001; Ebisch et al., 2003; Stuppia et al., 2003; Park et al., 2005; Lee et al., 2006; Paracchini et al., 2006; A et al., 2007; Dhillon et al., 2007; Sun et al., 2007; Ravel et al., 2009; Singh et al., 2005, 2010; Gava et al., 2011; Liu, 2011; Qiu et al., 2011; Safarinejad et al., 2011; Eloualid et al., 2012; Vani et al., 2012;

Karimian and Colagar, 2014; Li et al., 2014a,b; Mfady et al., 2014; Naqvi et al., 2014; Ni et al., 2015) were included in this meta-analysis. There were 21 studies of C677T with 4505 cases and 4024 controls, and 13 studies of A1298C with 2785 cases and 3094 controls. In the studies eligible for C677T, 13 were conducted in Caucasian populations, and eight involved Asian populations. For A1298C, eight studies were conducted in Caucasian populations, and five involved Asian populations. The studies were published between 2001 and 2015. The characteristics of the case-control studies included in the meta-analysis are summarized in Table 1. The genotype distributions in the controls for all studies were consistent with the HWE (Norton and Neel, 1965), except for four studies (Ravel et al., 2009; Singh et al., 2010; Gava et al., 2011; Eloualid et al., 2012).

Table 1. Characteristics of studies included in this meta-analysis.

	MTHFR gene C677T polymorphism												
No.	Author	Year	Country	Ethnicity			Case				Control		Hardy-Weinberg (P)
					Total	CC	CT	TT	Total	CC	CT	TT	
1	Bezold et al.	2001	Germany	Caucasian	255	114	93	48	200	92	89	19	0.705
2	Stuppia et al.	2003	Italy	Caucasian	93	37	37	19	105	33	43	29	0.065
3	Ebisch et al.	2003	Netherlands	Caucasian	77	42	28	7	113	50	48	15	0.522
4	Singh et al.	2005	India	Caucasian	151	105	40	6	200	163	37	0	0.149
5	Park et al.	2005	Korea	Asian	373	105	205	63	396	145	200	51	0.161
6	Lee et al.	2006	Korea	Asian	360	115	181	64	325	118	166	41	0.138
7	Paracchini et al.	2006	Italy	Caucasian	59	11	32	16	46	18	21	7	0.830
8	A et al.	2007	China	Asian	355	130	160	65	252	128	95	29	0.085
9	Dhillon et al.	2007	India	Caucasian	179	81	77	21	200	70	100	30	0.556
10	Sun et al.	2007	China	Asian	182	27	86	69	53	15	28	10	0.630
11	Ravel et al.	2009	France	Caucasian	250	118	101	31	132	49	52	31	**0.023**
12	Gava et al.	2011	Brazil	Caucasian	156	81	60	15	233	167	53	13	**0.003**
13	Safarinejad et al.	2011	Iran	Caucasian	156	50	80	26	328	144	148	36	0.825
14	Liu et al.	2011	China	Asian	75	27	38	10	72	40	28	4	0.753
15	Qiu et al.	2011	China	Asian	271	75	112	84	180	63	85	32	0.720
16	Vani et al.	2012	India	Caucasian	230	188	42	0	206	158	42	6	0.132
17	Mfady et al.	2014	Jordan	Caucasian	150	67	63	20	150	74	67	9	0.221
18	Naqvi et al.	2014	India	Caucasian	637	447	154	36	364	275	79	10	0.145
19	Karimian et al.	2014	Iran	Caucasian	118	51	59	8	132	77	52	3	0.087
20	Li et al.	2014a	China	Asian	82	14	36	32	133	36	61	36	0.340
21	Ni et al.	2015	China	Asian	296	117	135	44	204	84	94	26	0.970
	MTHFR gene A1298C polymorphism												
No.	Author	Year	Region	Ethnicity			Case				Control		Hardy–Weinberg (P)
					Total	AA	AC	CC	Total	AA	AC	CC	
1	Park et al.	2005	Korea	Asian	373	237	118	18	396	269	111	16	0.294
2	Lee et al.	2006	Korean	Asian	360	222	120	18	325	213	98	14	0.526
3	Dhillon et al.	2007	India	Caucasian	179	90	80	9	200	103	84	13	0.451
4	Ravel et al.	2009	French	Caucasian	250	131	94	25	113	54	46	13	0.507
5	Singh et al.	2010	India	Caucasian	151	66	76	9	140	64	74	2	**0.000**
6	Safarinejad et al.	2011	Iran	Caucasian	164	75	70	19	328	149	141	38	0.599
7	Gava et al.	2011	Brazil	Caucasian	156	71	62	23	233	130	89	14	0.811
8	Eloualid et al.	2012	Morocco	Caucasian	344	205	122	17	690	370	303	17	**0.000**
9	Mfady et al.	2014	Jordan	Caucasian	150	71	61	18	150	59	75	16	0.273
10	Karimian et al.	2014	Iran	Caucasian	118	59	44	15	132	70	48	14	0.194
11	Li et al.	2014a	China	Asian	82	49	29	4	133	88	36	9	0.059
12	Li et al.	2014b	China	Asian	162	101	54	7	50	34	15	1	0.656
13	Ni et al.	2015	China	Asian	296	181	106	9	204	137	62	5	0.515

Results of the overall meta-analysis

The main results of the meta-analysis on the association between the *MTHFR* C677T and A1298C polymorphisms and risk of male infertility are listed in detail in Table 2. The

MTHFR C677T polymorphism showed pooled ORs for the homozygote comparison [TT *vs* CC: OR = 1.629, 95%CI (1.215-2.184)], the heterozygote comparison [CT *vs* CC: 1.212, 95%CI (1.042-1.411)], the dominant model [CT+TT *vs* CC: OR = 1.294, 95%CI (1.089-1.537)], the recessive model [TT *vs* CT+CC: OR = 1.462, 95%CI (1.155-1.850)], and the additive model [T *vs* C: OR = 1.263, 95%CI (1.095-1.458)]. The *MTHFR* A1298C polymorphism showed pooled OR for the homozygote comparison [CC *vs* AA: OR = 1.289 (1.029-1.616)], the heterozygote comparison [AC *vs* AA: OR = 1.022 (0.914-1.143)], the dominant model [AC+CC *vs* AA: OR = 1.056 (0.949-1.176)], the recessive model [CC *vs* AC+AA: OR = 1.288 (1.034-1.604)], and the additive model [C *vs* A: OR = 1.078 (0.988-1.175)]. The forest plots for the overall association between the *MTHFR* gene C677T and A1298C polymorphisms and risk of male infertility are shown in Figures 2 and 3.

Table 2. Results of the overall meta-analysis.

MTHFR C677T			
Contrast	OR [95%CI]	Heterogeneity	z and P
TT *vs* CC	1.629 [1.215-2.184]	χ^2 = 65.30 (d.f. = 20); P = 0.000; I^2 = 69.4%	z = 3.26; P = 0.001
CT *vs* CC	1.212 [1.042-1.411]	χ^2 = 44.12 (d.f. = 20); P = 0.001; I^2 = 54.7%	z = 2.49; P = 0.013
CT+TT *vs* CC	1.294 [1.089-1.537]	χ^2 = 64.13 (d.f. = 20); P = 0.000; I^2 = 68.8%	z = 2.93; P = 0.003
TT *vs* CT+CC	1.462 [1.155-1.850]	χ^2 = 50.42 (d.f. = 20); P = 0.000; I^2 = 60.3%	z = 3.16; P = 0.002
T *vs* C	1.263 [1.095-1.458]	χ^2 = 83.56 (d.f. = 20); P = 0.000; I^2 = 76.1%	z = 3.20; P = 0.001
MTHFR A1298C			
Contrast	OR [95%CI]	Heterogeneity	z and P
CC *vs* AA	1.289 [1.029-1.616]	χ^2 = 13.56 (d.f. = 12); P = 0.330; I^2 = 11.5%	z = 2.21; P = 0.027
AC *vs* AA	1.022 [0.914-1.143]	χ^2 = 15.49 (d.f. = 12); P = 0.216; I^2 = 22.5%	z = 0.38; P = 0.703
AC+CC *vs* AA	1.056 [0.949-1.176]	χ^2 = 15.33 (d.f. = 12); P = 0.224; I^2 = 21.7%	z = 1.00; P = 0.319
CC *vs* AC+AA	1.288 [1.034-1.604]	χ^2 = 13.33 (d.f. = 12); P = 0.345; I^2 = 10.0%	z = 2.26; P = 0.024
C *vs* A	1.078 [0.988-1.175]	χ^2 = 14.11 (d.f. = 12); P = 0.294; I^2 = 15.0%	z = 1.70; P = 0.090

d.f. = degrees of freedom.

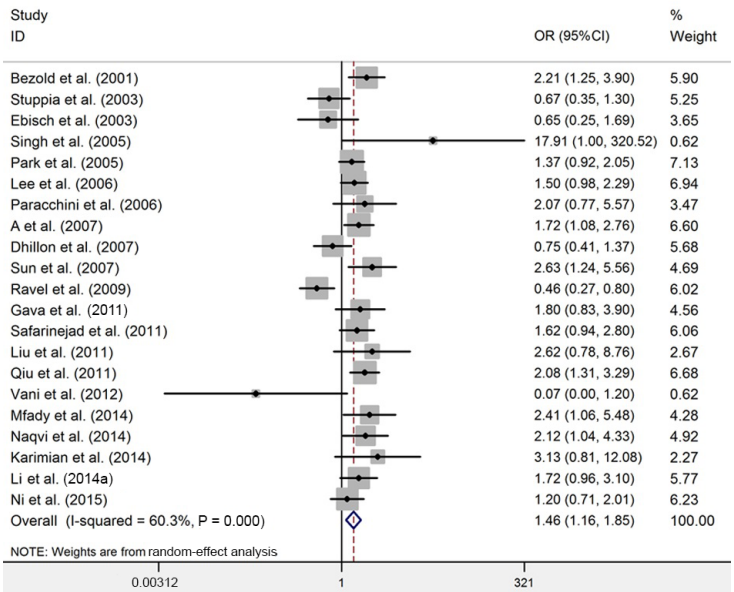

Figure 2. Random-effect forest plot of recessive model (TT *vs* CT+CC) of the *MTHFR* C677T polymorphism.

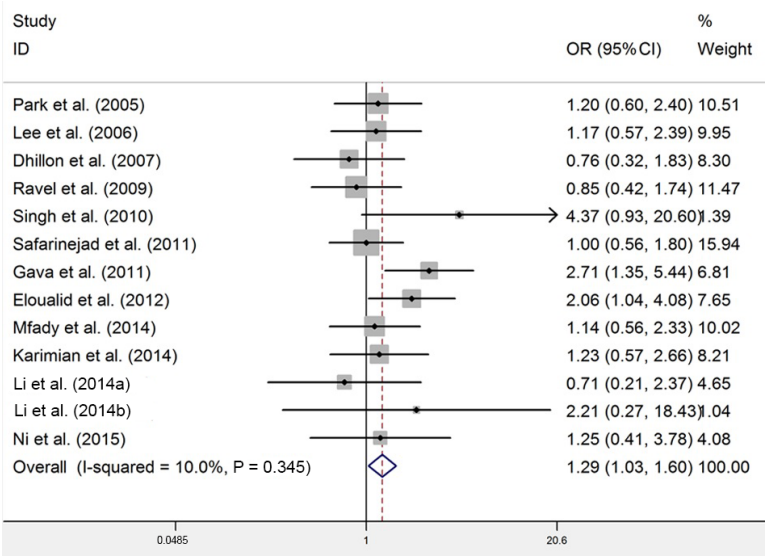

Figure 3. Fixed-effect forest plot of recessive model (CC *vs* AC+AA) of the *MTHFR* A1298C polymorphism.

Sub-group analysis

Sub-group analyses were performed on data stratified by ethnicity to determine possible factors that might have influenced the results. We found that the *MTHFR* C677T polymorphism was associated with a significantly increased male infertility risk in the Asian and overall populations, but not in the Caucasian population. In the homozygote comparison (TT *vs* CC), the pooled OR was 1.903 [95%CI (1.539-2.353)] for the Asian population. In the recessive comparison (TT *vs* CT+CC), the pooled OR was 1.402 [95%CI (1.236-1.589)] for the Asian population. We found no significant association between the *MTHFR* C677T polymorphism and male infertility risk in the Caucasian group in any of the genetic models. Significant association between the A1298C polymorphism and male infertility risk was observed in the Asian, Caucasian, and overall groups. The results of the sub-group analyses for all the genetic models are listed in detail in Table 3.

Table 3. Results of sub-group analysis stratified by ethnicity.

MTHFR C677T						
Ethnicity	Comparisons	TT *vs* CC (OR, 95%CI)	CT *vs* CC (OR, 95%CI)	CT+TT *vs* CC (OR, 95%CI)	TT *vs* CT+CC (OR, 95%CI)	T *vs* C (OR, 95%CI)
Caucasian	13	1.380 [0.839-2.268]	1.134 [0.903-1.423]	1.167 [0.902-1.509]	1.303 [0.858-1.977]	1.155 [0.921-1.450]
Asian	8	1.903 [1.539-2.353]	1.321 [1.137-1.534]	1.470 [1.246-1.734]	1.633 [1.361-1.959]	1.402 [1.236-1.589]
Overall	21	1.629 [1.215-2.184]	1.212 [1.042-1.411]	1.294 [1.089-1.537]	1.462 [1.155-1.850]	1.263 [1.095-1.458]
MTHFR A1298C						
Ethnicity	Comparisons	CC *vs* AA (OR, 95%CI)	AC *vs* AA (OR, 95%CI)	AC+CC *vs* AA (OR, 95%CI)	CC *vs* AC+AA (OR, 95%CI)	C *vs* A (OR, 95%CI)
Caucasian	8	1.310 [1.001-1.715]	0.903 [0.782-1.043]	0.954 [0.831-1.095]	1.345 [1.037-1.743]	1.022 [0.918-1.139]
Asian	5	1.242 [0.820-1.882]	1.235 [1.033-1.477]	1.236 [1.041-1.467]	1.157 [0.767-1.745]	1.186 [1.025-1.372]
Overall	13	1.289 [1.029-1.616]	1.022 [0.914-1.143]	1.056 [0.949-1.176]	1.288 [1.034-1.604]	1.078 [0.988-1.175]

Evaluation of heterogeneity

For the C677T group there was significant heterogeneity: for the homozygote comparison (TT *vs* CC), $\chi^2 = 65.30$ (degrees of freedom (d.f.) = 20), P = 0.000, and $I^2 = 69.4\%$; for the recessive model (TT *vs* CT+CC), $\chi^2 = 50.42$ (d.f. = 20), P = 0.000, and $I^2 = 60.3\%$. We assessed the source of heterogeneity by region, publication year, and sample size. However, we did not observe any sources that contributed to the substantial heterogeneity.

For the A1298C group there was no significant heterogeneity: for the homozygote comparison (CC *vs* AA), $\chi^2 = 13.56$ (d.f. = 12), P = 0.330, and $I^2 = 11.5\%$; for the recessive model (CC *vs* AC+AA), $\chi^2 = 13.33$ (d.f. = 12), P = 0.345, and $I^2 = 10.0\%$.

Sensitivity analysis

To evaluate the influence of each individual study on the overall results, sensitivity analysis was performed by deleting one study at a time from the pooled analysis. The results show that no single study had substantial power to affect the pooled ORs significantly (Figures 4 and 5).

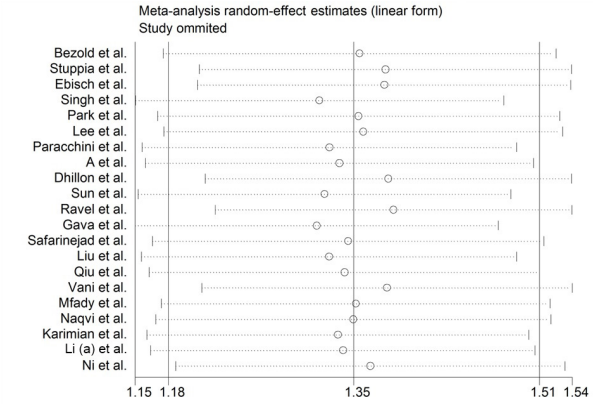

Figure 4. Sensitivity analysis for the *MTHFR* C677T polymorphism.

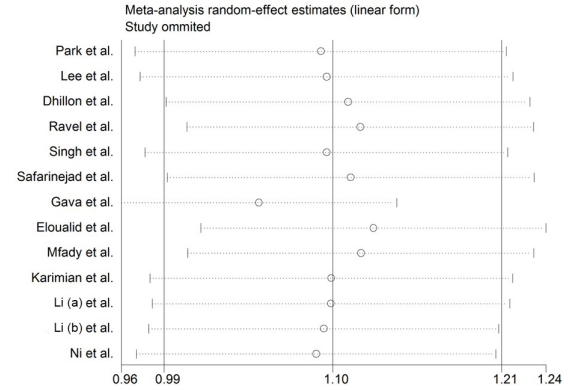

Figure 5. Sensitivity analysis for the *MTHFR* A1298C polymorphism.

Publication bias

To assess the publication bias of the currently available literature, both Begg's funnel plots and the Egger test were used. The results suggested that no publication bias on the pooled OR analysis existed: Begg test, P > |z| = 0.291 for the C677T group and 0.760 for the A1298C group; Egger test, P > |t| = 0.436 for the C677T group and 0.421 for the A1298C group (Figures 6 and 7).

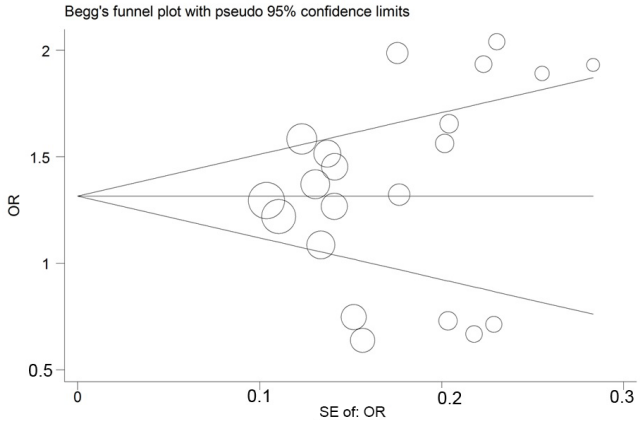

Figure 6. Publication bias test for the *MTHFR* C677T polymorphism.

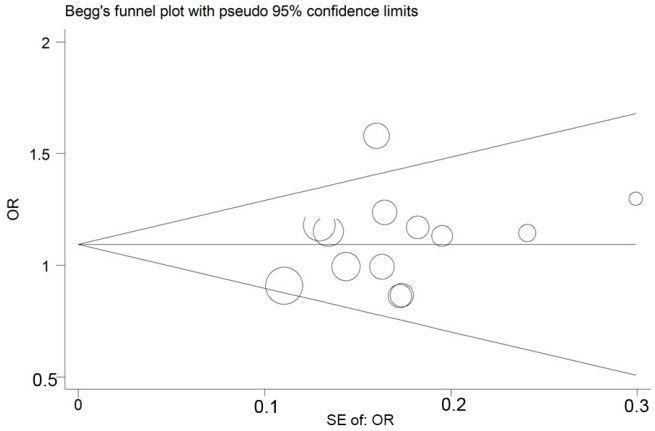

Figure 7. Publication bias test for the *MTHFR* A1298C polymorphism.

DISCUSSION

A number of studies have investigated the genetic effect of *MTHFR* polymorphisms on susceptibility to human diseases, such as coronary artery disease, pre-eclampsia, stroke, and infertility (Dell'Api et al., 2001; Kapoor, 2012). Studies concerning the association between

MTHFR polymorphisms and susceptibility to male infertility have been reported in different populations. However, results from published studies on the association between the *MTHFR* gene C677T and A1298C polymorphisms and male infertility risk remain controversial. Many factors, including varying study population recruitment procedures and differences in the genetic and environmental backgrounds, are responsible for this inconsistency. Considering the limitations of individual studies, we performed this meta-analysis based on published case-control studies to obtain a more precise evaluation.

The results of our meta-analysis demonstrate that the *MTHFR* C677T polymorphism was associated with a significantly increased male infertility risk in the Asian and overall populations, but not in the Caucasian population. A significant association between the A1298C polymorphism and male infertility risk was observed in the Asian, Caucasian, and overall groups. For the C677T group, there was significant heterogeneity, and for the A1298C group no significant heterogeneity was detected. The sensitivity analyses and publication bias results confirmed the reliability of these conclusions.

Some previous studies have attempted to explore the specific mechanism underlying the relationship between *MTHFR* polymorphisms and infertility risk. Wallock et al. (2001) conducted a study to evaluate relationships between seminal plasma folate levels and both folate nutriture and semen quality measurements, and found that total seminal plasma folate concentrations were on average 1.5 times higher than blood plasma folate concentrations in all the subjects. Ebisch et al. (2003) reported that, in contrast to heterozygotes and homozygotes of the C677T *MTHFR* polymorphism, sperm concentrations in the wild-type genotype significantly improved after folic acid and zinc sulfate intervention. However, the mechanism by which this takes place is still not entirely clear and research that focuses not only on individual genes, but also on gene-gene interactions, genetic-environmental interactions, and other single nucleotide polymorphisms is required.

We conducted a sub-group analysis stratified by ethnicity, but did not perform a sub-group analysis stratified by sperm concentration in the case group because some of the studies reported only the total genotype frequencies of the case group. The present study has several limitations, such as: 1) selection bias may have occurred because only studies in English or Chinese were included; and 2) there was significant heterogeneity in the C677T group. However, our meta-analysis has some clear advantages: 1) this meta-analysis was based on the most updated information; 2) we performed a sub-group analysis stratified by ethnicity; 3) sensitivity analysis showed that no individual study had a significant effect on the overall results; 4) the scientific search and selection method significantly increased the reality of this meta-analysis; and 5) no publication bias was detected.

In summary, the current meta-analysis, based on the most updated information, showed that the *MTHFR* C677T polymorphism was associated with a significantly increased male infertility risk in the Asian and overall populations, but not in the Caucasian population, and a significant association between the A1298C polymorphism and male infertility risk was found in the Asian, Caucasian, and overall groups.

Conflicts of interest

The authors declare no conflict of interest.

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