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ORIGINAL ARTICLE

Associations of *methylenetetrahydrofolate reductase* (*MTHFR*) C677T and A1298C polymorphisms with genetic susceptibility to rheumatoid arthritis: a meta-analysis

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Abstract The aim of our study was to conduct a meta-analysis to assess whether combined evidence shows associations between C677T and A1298C polymorphisms of *methylenetetrahydrofolate reductase* (*MTHFR*) and genetic susceptibility to rheumatoid arthritis (RA). A total of 11 articles involving 20 comparisons were included, containing 12 comparisons for the *MTHFR* C677T polymorphism and 8 comparisons for the *MTHFR* A1298C polymorphism. Significant evidence was detected for the association of RA susceptibility with the *MTHFR* C677T polymorphism T allele under allelic contrast and dominant model in Asians (T versus C, OR = 1.300, 95 % CI = 1.104–1.531, $p = 0.002$; TT + CT versus CC, OR = 1.495, 95 % CI = 1.187–1.882, $p = 0.001$). Significant association between RA susceptibility and the *MTHFR* A1298C polymorphism A allele under recessive model was found in the overall meta-analysis (AA versus AC + CC, OR = 1.281, 95 % CI = 1.048–1.565, $p = 0.016$). Our meta-analysis results demonstrate that the *MTHFR* C677T polymorphism is involved in the genetic susceptibility of RA in Asians, and the *MTHFR* A1298C polymorphism is associated with genetic susceptibility to RA in the overall population. Given the paucity of studies, especially in non-Asian populations, further studies with larger sample sizes are required to elucidate the role of *MTHFR* polymorphisms in the genetic basis of RA in different ethnic populations.

Keywords Meta-analysis · Methylenetetrahydrofolate reductase · Polymorphism · Rheumatoid arthritis

Han Cen and Hua Huang contributed equally to this work and should be considered as co-first author.

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Introduction

Rheumatoid arthritis (RA) is a common systemic autoimmune disease characterized by the proliferation of synovial cells, the infiltration of inflammatory cells and angiogenesis [1]. Although the etiology of RA has not been precisely demonstrated, it has been widely accepted that both environmental and genetic risk factors have been implicated in the onset and progression of this disorder. Available data shows that the prevalence of RA varies geographically, with much higher prevalence having been reported in North America and North Europe [2]. Overall, about three quarters of RA patients are female. The development of RA could occur in any age, but much more cases develop in their middle or old age [1]. Although great improvements have been achieved in the field of RA treatment, uncontrolled disease could result in disability, decreased life quality, and several severe comorbidities. During the past few decades, our understanding of the genetic basis of RA has been rapidly prompted through the application of numerous large-scale candidate gene studies and genome-wide association studies (GWASs), and multiple

susceptible genes/loci associated with RA have been identified and confirmed [3].

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme involved in the folate metabolic pathway, catalyzing the irreversible conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, which is a methylation group donor and has been implicated in the methylation of genomic DNA. Notably, 5-methyltetrahydrofolate acts as the primary methylation donor for the remethylation of homocysteine into methionine [4]. Thus, impaired MTHFR might result in hypomethylation of genomic DNA and hyperhomocysteinemia, and both of these events have been suggested to be involved in the pathogenesis of RA [5–7], making MTHFR as a candidate susceptible gene for RA. The gene encoding MTHFR has been mapped to the chromosomal region 1p36, and multiple single nucleotide polymorphisms (SNPs) within *MTHFR* have been identified [8]. Remarkably, the chromosomal region 1p36 has been previously been shown to be associated with genetic susceptibility to RA using genome-wide linkage studies [9–11]. Besides, a recent meta-analysis shows that SNPs within the PADI4 gene have also been revealed to be associated with RA genetic predisposition in Asians and Caucasians, and this gene also locates in the chromosomal region 1p36 [12]. Among the SNPs within the MTHFR gene, the two most commonly studied polymorphisms are C677T (rs1801133) and A1298C (rs1801131), which are both nonsynonymous SNPs. The C to T change at nucleotide 677 leads to alanine to valine substitution at codon 222, rendering MTHFR more thermolabile and reducing its enzyme activity [13, 14]. Similar to the C677T polymorphism, the A to C change at nucleotide 1298 results in glutamine to alanine substitution, also leading to reduced enzyme activity [15–17]. The associations between C677T and A1298C polymorphisms within *MTHFR* and RA genetic susceptibility have been widely investigated in different populations with inconsistent results [18–35]. This discrepancy might be due to different sample sizes and genetic backgrounds, clinical heterogeneity, publication bias, etc. Meta-analysis is a useful tool to combine the results on the same topic to get a pooled estimation with increased statistical power [36]. Thus, the aim of our study was to conduct a meta-analysis to assess whether combined evidence shows associations between C677T and A1298C polymorphisms of *MTHFR* and genetic susceptibility to RA and to summarize the effect sizes of the polymorphisms associated with RA.

Method

Identification of eligible studies and data extraction

The present meta-analysis is performed in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) [37]. An exhaustive search on studies examining the association of *MTHFR* C677T and A1298C polymorphisms with RA susceptibility was performed. The literature search was made using PubMed, China National Knowledge Infrastructure (CNKI) database, and Wanfang database to identify relevant articles, applying the following medical subject heading (MeSH) terms and/or text words: 'methylenetetrahydrofolate reductase,' 'MTHFR,' 'polymorphism,' 'polymorphisms,' 'rheumatoid arthritis,' and 'RA.' No language restrictions were applied. The references in these studies were also reviewed to identify additional studies. The inclusion criteria were as follows: (a) being published before October 2015; (b) using case–control study design; (c) allele or genotype frequencies among RA patients and controls available, providing enough data to calculate odds ratio (OR); and (d) the genotype distribution among control group should conform to Hardy–Weinberg equilibrium (HWE), since deviation from HWE among controls could imply some potential bias in control selection or genotyping errors. When two or more studies published by the same authors contained overlapped data, we extracted data from the study with the largest sample size. When a study described the results in different subgroups, we treated them independently. Studies in which family members had been studied were excluded because its analysis was based on linkage consideration. The following information from each study was extracted: first author's name, year of publication, country, ethnicity, the number of cases and controls, and allele/genotype distribution in cases and controls. Two authors independently extracted these data, and discrepancy was addressed by discussion.

Evaluation of the statistical association

Allele frequencies of the *MTHFR* C677T and A1298C polymorphisms from each study were determined by the allele counting method. Chi-square test was applied to assess whether the observed genotype frequencies in control group conformed to HWE. In this study, we performed meta-analysis on (a) allelic contrast, (b) recessive model, and (c) dominant model. The heterogeneity between studies was assessed by Cochran's Q statistic, as well as I^2 statistic, which was used to quantify the effect of heterogeneity ($I^2 = 100\ \% \times (Q - \mathrm{df}) / Q$), measuring the proportion of total variation in study estimates due to heterogeneity [38]. Finally, the pooled estimate of risk was obtained by a random effects (DerSimonian–Laird) or a fixed effects model (Mantel–Haenszel) in the presence ($P \leq 0.1$ or $I^2 > 50\ \%$) or absence ($P > 0.1$ and $I^2 \leq 50\ \%$) of heterogeneity, respectively. Due to the genetic heterogeneity among different ethnicities, we performed subgroup meta-analysis stratified by ethnicity. Statistical analysis for this meta-analysis was performed by Stata version 10.0 (Stata Corporation, College Station, TX, USA).

Evaluation of publication bias

We assessed potential publication bias by funnel plot [39] and utilized the Egger's linear regression test to evaluate the funnel plot asymmetry, which is an approach to measure funnel plot asymmetry on the natural logarithm scale of the OR [40]. The significance of the intercept was assessed by the *t* test suggested by Egger, and the *p* value less than 0.05 was considered significant publication bias.

Results

Studies included in the meta-analysis

As shown in Fig. 1, a total of 18 relevant articles examining the association of *MTHFR* C677T polymorphism and/or A1298C polymorphism with genetic susceptibility to RA were identified through PubMed, CNKI database, and Wanfang database search and a review of the references [18–35], and seven articles were excluded [18, 20, 27, 29, 31–33]. Five articles were excluded since the detailed genotype, and allele information is unavailable [18, 20, 27, 29, 31]. Two articles were published by the same research group, so only the article with the largest sample size was included [26] and the other one was excluded [32]. One study in which the genotype distribution of *MTHFR* C677T polymorphism did not conform to HWE was excluded [33]. One article contained data on two different subgroups [34], and we treated them independently. Among the 11 eligible articles, there are 2 articles in which only *MTHFR* C677T polymorphism has been investigated [22, 23] and the other 9 articles in which both *MTHFR* C677T and A1298C polymorphisms have been studied [19, 21, 24–26, 28, 34, 35]. Among two eligible articles containing both *MTHFR* C677T and A1298C polymorphisms, the genotype distribution of *MTHFR* A1298C polymorphism deviated from HWE in the control group, so the data of *MTHFR* A1298C polymorphism in these two articles was excluded [19, 30]. Thus, we analyzed 20 separate comparisons in total, including 12 comparisons for the *MTHFR* C677T polymorphism and 8 comparisons for the *MTHFR* A1298C polymorphism. The patients with RA in all eligible studies were diagnosed according to the American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis [41]. The characteristics of the selected studies are summarized in Table 1 (detailed information about genotype and allele frequencies for each polymorphism in selected studies is available in Supplementary Tables 1 and 2).

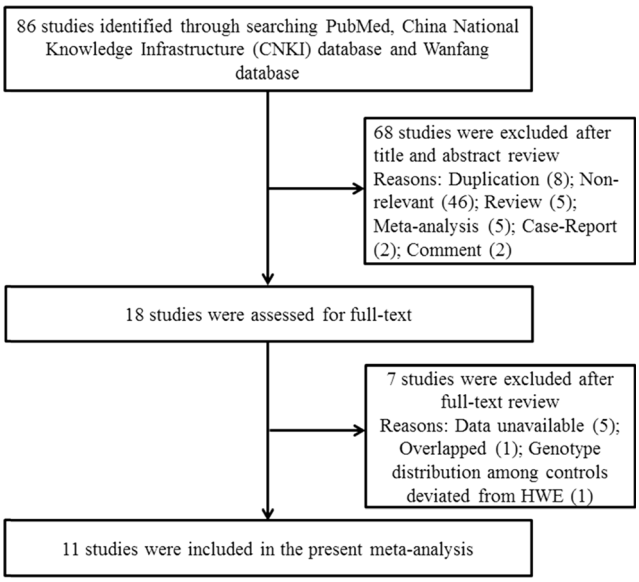


Fig. 1 The flowchart for study selection process

Quantitative synthesis

Meta-analysis of the MTHFR C677T polymorphism with RA susceptibility

A total of 1744 cases and 1599 controls in 12 case–control studies were eligible for meta-analysis of association between the *MTHFR* C677T polymorphism and genetic susceptibility to RA (Table 2). Six studies were from Asian, two from European, one from African, one from African-American, one from Caucasian-American, and one from Jewish. Overall meta-analysis revealed that non-significant evidence for the association of the *MTHFR* C677T polymorphism with RA susceptibility was detected (T versus C, OR = 1.221, 95 % CI = 0.999–1.493, I^2 = 64.2 %, p = 0.051; TT + CT versus CC, OR = 1.271, 95 % CI = 0.975–1.658, I^2 = 61.7 %, p = 0.076; TT versus CT + CC, OR = 1.183, 95 % CI = 0.943–1.484, I^2 = 30.2 %, p = 0.146) (Figs. 2 and 3). When we combined results by ethnicity, significant evidence was detected for the association of RA susceptibility with the *MTHFR* C677T polymorphism T allele under allelic contrast and dominant model in Asians (T versus C, OR = 1.300, 95 % CI = 1.104–1.531, I^2 = 28.4 %, p = 0.002; TT + CT versus CC, OR = 1.495, 95 % CI = 1.187–1.882, I^2 = 0, p = 0.001) but not in Europeans (T versus C, OR = 1.150, 95 % CI = 0.587–2.255, I^2 = 83.4 %, p = 0.684; TT + CT versus CC, OR = 0.954, 95 % CI = 0.453–2.013, I^2 = 70.5 %, p = 0.902).

Meta-analysis of the MTHFR A1298C polymorphism with RA susceptibility

A total of 1094 cases and 1026 controls in 8 case–control studies were included for meta-analysis of association between the *MTHFR* A1298C polymorphism and RA susceptibility (Table 3). Four studies were from Asian, one from

Table 1 Characteristics of individual study included in the present meta-analysis

Author	Reference	Year	Country	Ethnicity	Numbers		Polymorphisms	HWE (*p* value)[a]
					Case	Control		
Saad et al.	[19]	2015	Egypt	African	105	80	C677T[b]	0.17
Saleh et al.	[21]	2015	Jordan	Asian	159	170	C677T, A1298C	0.72, 0.17
Inanir et al.	[22]	2013	Turkey	Asian	147	150	C677T	0.26
Shi et al.	[23]	2013	China	Asian	183	100	C677T	0.06
Plaza-Plaza et al.	[24]	2012	Spain	European	67	67	C677T, A1298C	0.88, 0.18
Taşbaş et al.	[25]	2011	Turkey	Asian	64	31	C677T, A1298C	0.85, 0.82
Xiao et al.	[26]	2011	China	Asian	110	180	C677T, A1298C	0.63, 0.69
Cai et al.	[28]	2009	China	Asian	86	101	C677T, A1298C	0.82, 0.08
Rubini et al.	[30]	2008	Italy	European	217	251	C677T[b]	0.32
Hughes et al.	[34]	2006	America	African-American	138	53	C677T, A1298C	0.95, 0.61
Hughes et al.	[34]	2006	America	Caucasian-American	393	50	C677T, A1298C	0.52, 0.43
Berkun et al.	[35]	2004	Israel	Jewish	93	377	C677T, A1298C	0.28, 0.27

[a] HWE *p* value was among control group in each eligible study

[b] Data on the *MTHFR* A1298C polymorphism was excluded due to the genotype distribution among controls deviated from HWE

European, one from African-American, one from Caucasian-American, and one from Jewish. Overall meta-analysis showed that RA susceptibility was significantly associated with the *MTHFR* A1298C polymorphism A allele under recessive model (AA versus AC + CC, OR = 1.281, 95 % CI = 1.048–1.565, I^2 = 0, p = 0.016) (Fig. 4). However,

Table 2 Meta-analysis of the *MTHFR* C677T and A1298C polymorphisms with RA susceptibility

Polymorphism	Comparison	Population	Sample size		No. of studies	Test of association			Model	Test of heterogeneity		
			Case	Control		OR	95 % CI	*p* value		*Q*	*p* value	I^2 (%)
MTHFR C677T	T vs. C	Overall	1744	1599	12	1.221	0.999–1.493	0.051	R	30.75	0.001	64.2
		Asian	731	723	6	1.300	1.104–1.531	0.002	F	6.98	0.222	28.4
		European	284	318	2	1.150	0.587–2.255	0.684	R	6.04	0.014	83.4
		African	105	80	1	2.308	1.392–3.826	0.001	NA	NA	NA	NA
		African-American	138	52	1	0.813	0.414–1.599	0.549	NA	NA	NA	NA
		Caucasian-American	393	50	1	1.110	0.699–1.763	0.658	NA	NA	NA	NA
		Jewish	93	376	1	0.764	0.542–1.077	0.124	NA	NA	NA	NA
	TT + CT vs. CC	Overall	1744	1599	12	1.271	0.975–1.658	0.076	R	28.72	0.003	61.7
		Asian	731	723	6	1.495	1.187–1.882	0.001	F	1.96	0.854	0
		European	284	318	2	0.954	0.453–2.013	0.902	R	3.39	0.066	70.5
		African	105	80	1	3.179	1.712–5.902	<0.001	NA	NA	NA	NA
		African-American	138	52	1	0.798	0.377–1.689	0.555	NA	NA	NA	NA
		Caucasian-American	393	50	1	1.005	0.558–1.811	0.986	NA	NA	NA	NA
		Jewish	93	376	1	0.732	0.464–1.154	0.179	NA	NA	NA	NA
	TT vs. CT + CC	Overall	1744	1599	12	1.183	0.943–1.484	0.146	F	15.76	0.150	30.2
		Asian	731	723	6	1.279	0.915–1.787	0.150	F	8.32	0.140	39.9
		European	284	318	2	1.471	0.553–3.915	0.440	R	4.24	0.039	76.4
		African	105	80	1	1.567	0.455–5.400	0.477	NA	NA	NA	NA
		African-American	138	52	1	0.750	0.067–8.451	0.816	NA	NA	NA	NA
		Caucasian-American	393	50	1	1.775	0.528–5.966	0.353	NA	NA	NA	NA
		Jewish	93	376	1	0.674	0.330–1.377	0.279	NA	NA	NA	NA

No. number, *OR* odds ratio, *CI* confidence interval, *F* fixed effects model, *R* random effects model, *NA* not applicable

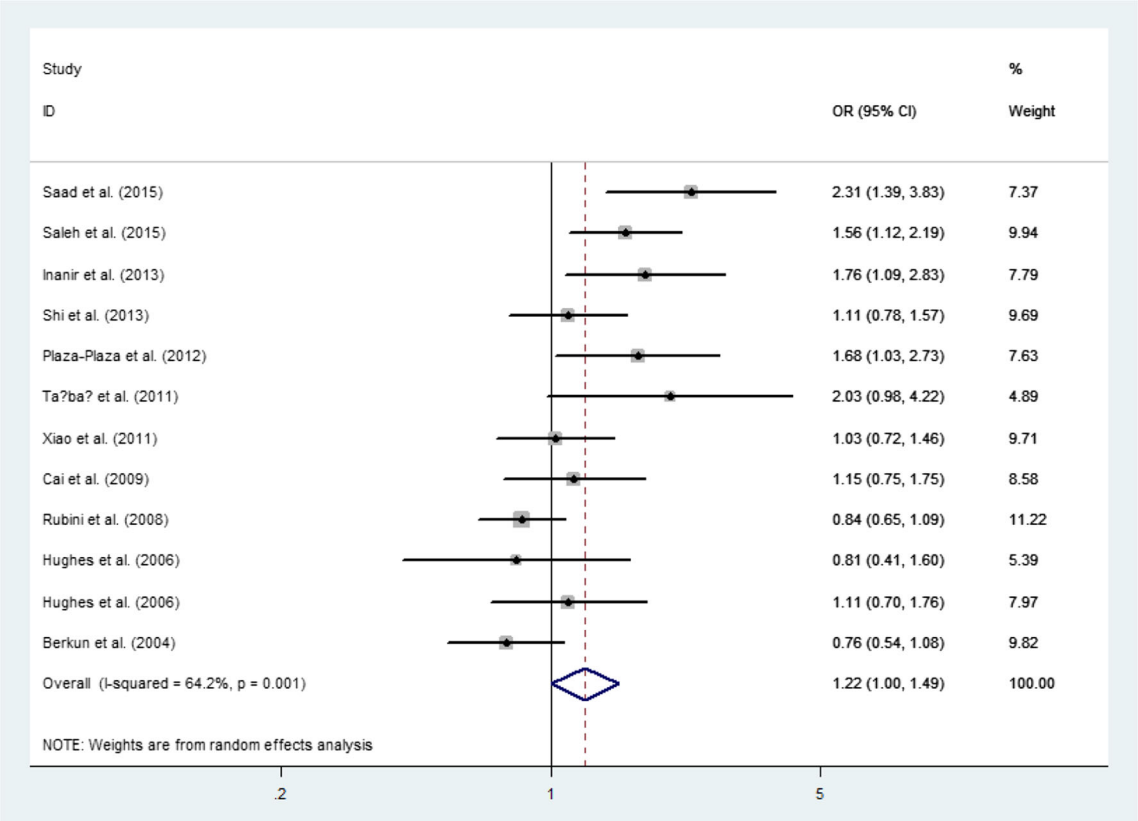


Fig. 2 ORs and 95 % CIs of individual studies and pooled data for the association between rheumatoid arthritis (RA) and *MTHFR* C677T polymorphism T allele under allelic contrast

non-significant evidence for the association of RA susceptibility with the *MTHFR* A1298C polymorphism A allele under allelic contrast or dominant model was found in the overall meta-analysis (A versus C, OR = 1.117, 95 % CI = 0.955–1.306, I^2 = 13.6 %, p = 0.167; AA + AC versus CC, OR = 0.973, 95 % CI = 0.572–1.657, I^2 = 49.4 %, p = 0.921) (Fig. 5). Only the number of study performed in Asian population was more than two, so we combined the results from Asian. However, the pooled results revealed non-significant association between RA susceptibility and the *MTHFR* A1298C polymorphism in Asians (A versus C, OR = 1.174, 95 % CI = 0.943–1.463, I^2 = 18.0 %, p = 0.152; AA + AC versus CC, OR = 0.997, 95 % CI = 0.589–1.685, I^2 = 41.4 %, p = 0.990; AA versus AC + CC, OR = 1.296, 95 % CI = 0.980–1.714, I^2 = 23.5 %, p = 0.069).

Evaluation of heterogeneity and publication bias

Heterogeneity was detected for the association of RA with the *MTHFR* C677T polymorphism T allele under allelic contrast and dominant model in the overall meta-analysis. When we pooled results according to the ethnicity of study populations, the heterogeneity disappeared in the subgroup analysis of Asians but remained significant in Europeans. In addition, heterogeneity was found for the association of RA with the *MTHFR* C677T polymorphism T allele under recessive model in the subgroup analysis of Europeans.

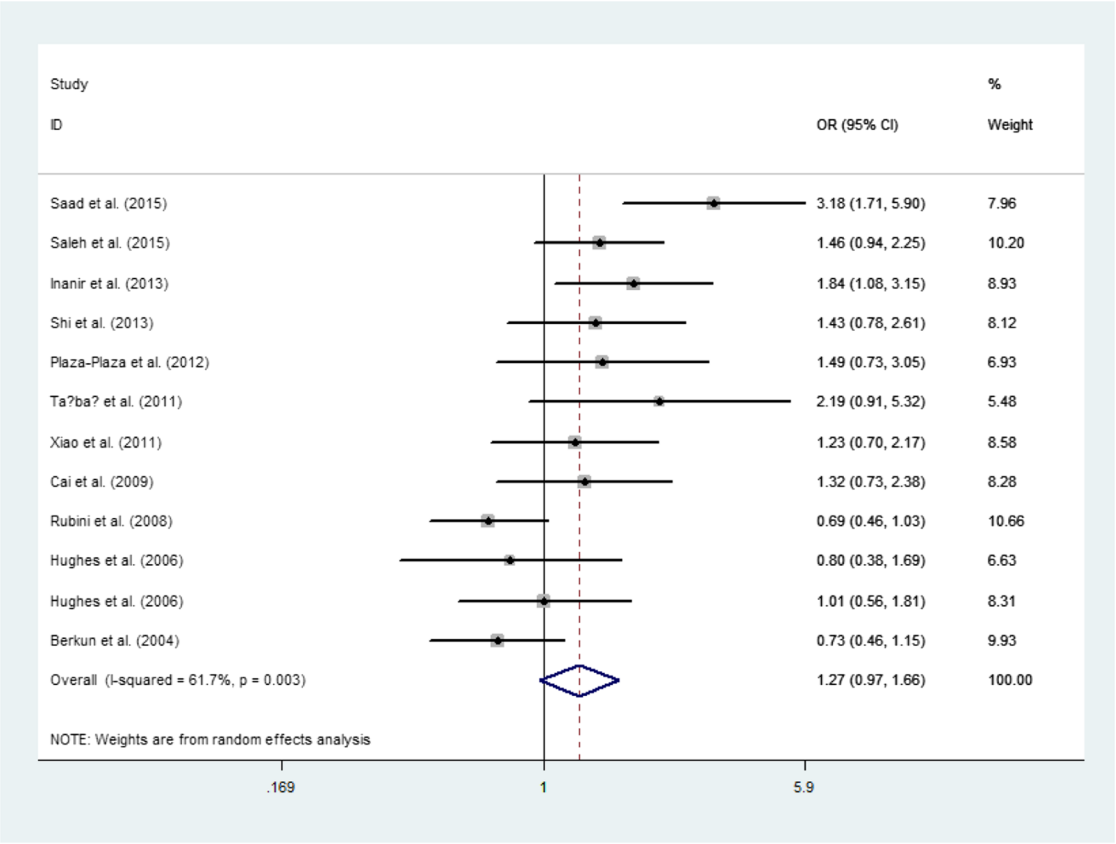
The heterogeneity was significant in the overall meta-analysis for the association of RA susceptibility with the *MTHFR* A1298C polymorphism A allele under dominant model. When we performed subgroup meta-analysis according to the ethnicity, heterogeneity disappeared in the pooled analysis of Asians.

We performed publication bias analysis on the *MTHFR* C677T and A1298C polymorphisms. No obvious asymmetry was found according to the shapes of the funnel plots (data not shown). We applied Egger's linear regression test to assess the funnel plot asymmetry, and the results indicated no publication bias ($p > 0.05$).

Discussion

MTHFR is a key enzyme involved in the metabolism of folate, which catalyzes the conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, providing methylation group for its substrate [4]. Methotrexate (MTX), a structural analog of folic acid, has become the anchor drug for the treatment of RA based on its best-known efficacy–toxicity ratio and long-term affordability [42]. Although the actual

a


Study
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OR (95% CI)
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Weight
Saad et al. (2015) 3.18 (1.71, 5.90) 7.96
Saleh et al. (2015) 1.46 (0.94, 2.25) 10.20
Inanir et al. (2013) 1.84 (1.08, 3.15) 8.93
Shi et al. (2013) 1.43 (0.78, 2.61) 8.12
Plaza-Plaza et al. (2012) 1.49 (0.73, 3.05) 6.93
Ta?ba? et al. (2011) 2.19 (0.91, 5.32) 5.48
Xiao et al. (2011) 1.23 (0.70, 2.17) 8.58
Cai et al. (2009) 1.32 (0.73, 2.38) 8.28
Rubini et al. (2008) 0.69 (0.46, 1.03) 10.66
Hughes et al. (2006) 0.80 (0.38, 1.69) 6.63
Hughes et al. (2006) 1.01 (0.56, 1.81) 8.31
Berkun et al. (2004) 0.73 (0.46, 1.15) 9.93
Overall (I-squared = 61.7%, p = 0.003) 1.27 (0.97, 1.66) 100.00
NOTE: Weights are from random effects analysis
.169
1
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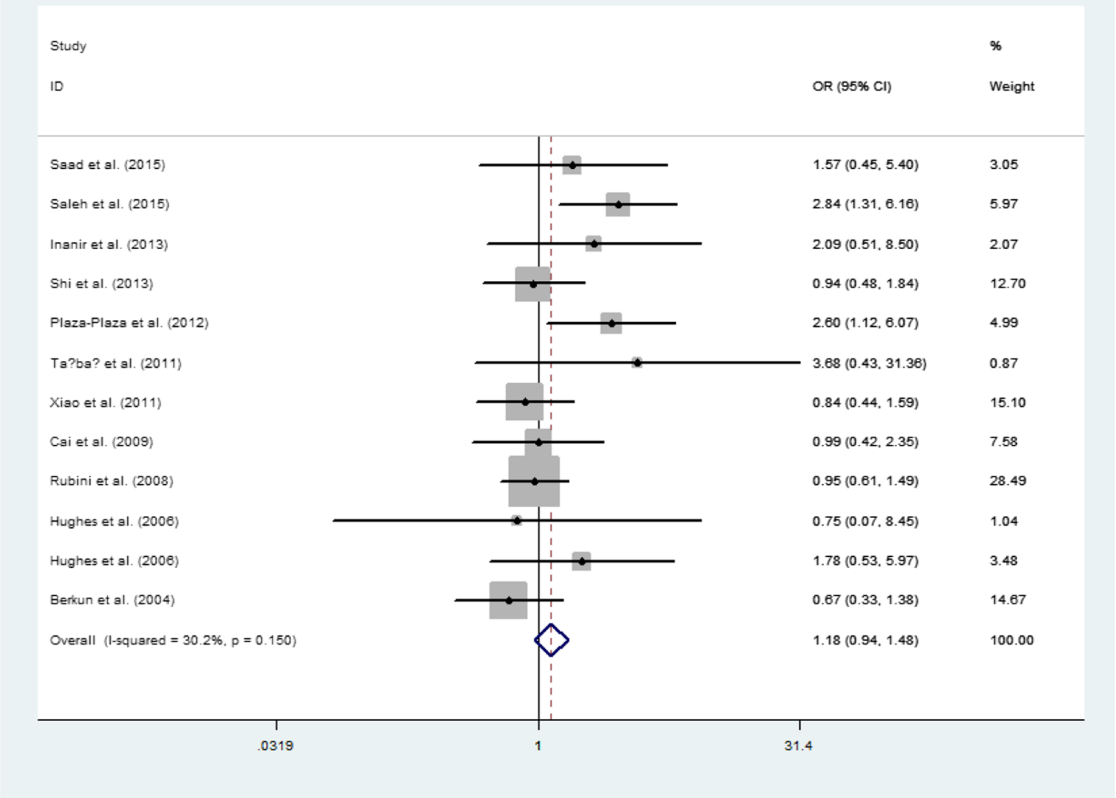
b


Study
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Saad et al. (2015) 1.57 (0.45, 5.40) 3.05
Saleh et al. (2015) 2.84 (1.31, 6.16) 5.97
Inanir et al. (2013) 2.09 (0.51, 8.50) 2.07
Shi et al. (2013) 0.94 (0.48, 1.84) 12.70
Plaza-Plaza et al. (2012) 2.60 (1.12, 6.07) 4.99
Ta?ba? et al. (2011) 3.68 (0.43, 31.36) 0.87
Xiao et al. (2011) 0.84 (0.44, 1.59) 15.10
Cai et al. (2009) 0.99 (0.42, 2.35) 7.58
Rubini et al. (2008) 0.95 (0.61, 1.49) 28.49
Hughes et al. (2006) 0.75 (0.07, 8.45) 1.04
Hughes et al. (2006) 1.78 (0.53, 5.97) 3.48
Berkun et al. (2004) 0.67 (0.33, 1.38) 14.67
Overall (I-squared = 30.2%, p = 0.150) 1.18 (0.94, 1.48) 100.00
.0319
1
31.4

◀ **Fig. 3** ORs and 95 % CIs of individual studies and pooled data for the association between rheumatoid arthritis (RA) and *MTHFR* C677T polymorphism. **a** TT + CT versus CC; **b** TT versus CT + CC

mechanism of MTX used to treat RA has not been completely elucidated, available evidence suggests that MTX could act through inhibiting several enzymes implicated in the folate metabolism pathway. MTHFR is not inhibited by MTX directly but in view of the effect of MTHFR in folate pool, so the function of MTHFR might affect the outcomes (efficacy and toxicity) of patients with RA treated by MTX. Within the MTHFR gene, the two most commonly studied SNPs (C677T and A1298C) could cause the alteration of enzyme activity [13–17]. Up to now, multiple studies have been performed to test whether the *MTHFR* C677T and A1298C polymorphisms are associated with the outcomes of MTX among RA patients and related meta-analyses have been reported [43, 44].

Apart from the associations of the *MTHFR* C677T and A1298C polymorphisms with the outcomes of MTX in RA patients, associations between the C677T and A1298C polymorphisms of *MTHFR* and genetic susceptibility to RA have also been extensively investigated [18–35]. The *MTHFR* C677T polymorphism T allele and A1298C polymorphism C allele have both been reported to be associated with reduced enzyme activity [13–17]. The rationale of investigating the associations of the *MTHFR* C677T and A1298C polymorphisms with genetic susceptibility to RA is as follows: First, MTHFR could catalyze the conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, which is indispensable for nucleic acid methylation, thus impaired MTHFR activity might lead to hypomethylation of genomic DNA, and altered methylation patterns have been detected in several cell types in RA [5]. Second, MTHFR could act as the primary methylation donor for remethylation of homocysteine into methionine, so impaired MTHFR function might result in been increased level of homocysteine, and this possibility has been documented by multiple studies [7, 13–15, 17]. Several studies have shown that the level of homocysteine in RA patients is elevated compared with health controls [6, 7, 45–47], and homocysteine could cause the elevation of inflammation in RA through activating the proinflammatory transcription factor NF-κB [6]. Third, the chromosomal region containing *MTHFR* has been shown to be associated with genetic susceptibility to RA by genome-wide linkage studies [9–11].

Although the associations between the C677T and A1298C polymorphisms of *MTHFR* and RA susceptibility have been examined in different populations, the results are controversial. To our knowledge, so far, no meta-analysis has been reported regarding the associations of RA susceptibility with the *MTHFR* C677T and A1298C polymorphisms. Thus, we performed this meta-

Table 3 Meta-analysis of the *MTHFR* A1298C polymorphism with RA susceptibility

Polymorphism	Comparison	Population	Sample size		No. of studies	Test of association			Model	Test of heterogeneity		
			Case	Control		OR	95 % CI	*p* value		*Q*	*p* value	I^2 (%)
MTHFR A1298C	A vs. C	Overall	1094	1026	8	1.117	0.955–1.306	0.167	F	8.10	0.324	13.6
		Asian	403	480	4	1.174	0.943–1.463	0.152	F	3.66	0.301	18.0
		European	67	67	1	1.612	0.944–2.752	0.080	NA	NA	NA	NA
		African-American	138	53	1	1.321	0.715–2.441	0.374	NA	NA	NA	NA
		Caucasian-American	393	50	1	0.883	0.564–1.384	0.588	NA	NA	NA	NA
		Jewish	93	376	1	0.933	0.667–1.306	0.685	NA	NA	NA	NA
	AA + AC vs. CC	Overall	1094	1026	8	0.973	0.572–1.657	0.921	R	13.82	0.054	49.4
		Asian	403	480	4	0.997	0.589–1.685	0.990	F	5.12	0.163	41.4
		European	67	67	1	2.175	0.701–6.749	0.178	NA	NA	NA	NA
		African-American	138	53	1	2.635	0.162–42.900	0.496	NA	NA	NA	NA
		Caucasian-American	393	50	1	0.935	0.379–2.308	0.885	NA	NA	NA	NA
		Jewish	93	376	1	0.445	0.254–0.780	0.005	NA	NA	NA	NA
	AA vs. AC + CC	Overall	1094	1026	8	1.281	1.048–1.565	0.016	F	6.71	0.460	0
		Asian	403	480	4	1.296	0.980–1.714	0.069	F	3.92	0.270	23.5
		European	67	67	1	1.620	0.817–3.212	0.167	NA	NA	NA	NA
		African-American	138	53	1	1.338	0.671–2.669	0.409	NA	NA	NA	NA
		Caucasian-American	393	50	1	0.828	0.459–1.492	0.530	NA	NA	NA	NA
		Jewish	93	376	1	1.424	0.903–2.246	0.128	NA	NA	NA	NA

No. number, *OR* odds ratio, *CI* confidence interval, *F* fixed effects model, *R* random effects model, *NA* not applicable

analysis to assess whether the C677T and A1298C polymorphisms of *MTHFR* are associated with genetic predisposition of RA and to summarize the effect sizes of these polymorphisms.

Our meta-analysis results revealed that non-significant association of the *MTHFR* C677T polymorphism T allele with RA susceptibility was detected. Since heterogeneity was found in the overall meta-analysis of the *MTHFR* C677T polymorphism T allele with RA susceptibility, we performed stratified meta-analysis by ethnicity, and the heterogeneity disappeared in the subgroup analysis of Asians but remained significant in Europeans, and significant association between RA susceptibility and the *MTHFR* C677T polymorphism T allele was observed in Asians but not in Europeans. Overall meta-analysis revealed non-significant association between RA susceptibility and the *MTHFR* C677T polymorphism T allele under dominant model and recessive model. Heterogeneity was also detected for the overall meta-analysis of the association between RA susceptibility and the *MTHFR* C677T polymorphism T allele under dominant model, so subgroup meta-analysis by ethnicity was conducted, and the results showed that significant association was found in Asians but not in Europeans. Although no heterogeneity was detected for the overall meta-analysis of the association between RA susceptibility and the *MTHFR* C677T polymorphism T allele under recessive model, we also performed stratified meta-analysis according to the ethnicity. However, non-significant association was found in Asians or Europeans. Since the number of studies in non-Asians is small, further studies with larger sample sizes are required to examine the association between *MTHFR* C677T polymorphism and RA susceptibility in non-Asian populations. Our meta-analysis indicated that the *MTHFR* C677T polymorphism is involved in the genetic background of RA in Asians. The overall meta-analysis revealed that significant association between RA susceptibility and the *MTHFR* A1298C polymorphism A allele under recessive model was found, but non-significant association was detected for the association between RA susceptibility and the *MTHFR* A1298C polymorphism A allele under allelic contrast and dominant model. Only studies performed in Asians were eligible for subgroup meta-analysis, but non-significant association was found between RA susceptibility and the *MTHFR* A1298C polymorphism in Asians, and this might be due to the small number of eligible study and the relatively low power of each study caused by small sample size.

Besides the relationships between *MTHFR* polymorphisms and RA genetic susceptibility, a few studies have been performed to assess the associations of *MTHFR* C677T and A1298C polymorphisms with cardiovascular events (CVs) and osteoporosis among RA patients [27, 48–50]. Although the results are controversial, these two polymorphisms within *MTHFR* might be associated with CVs and osteoporosis, so comorbidities might

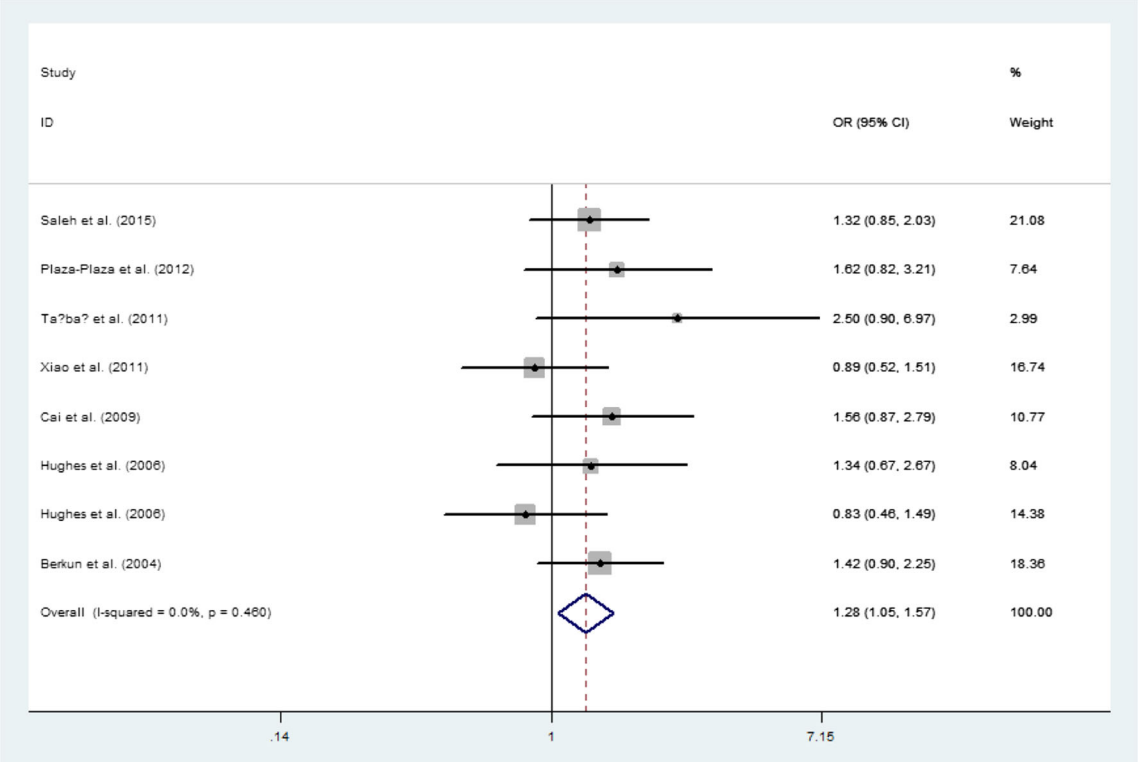
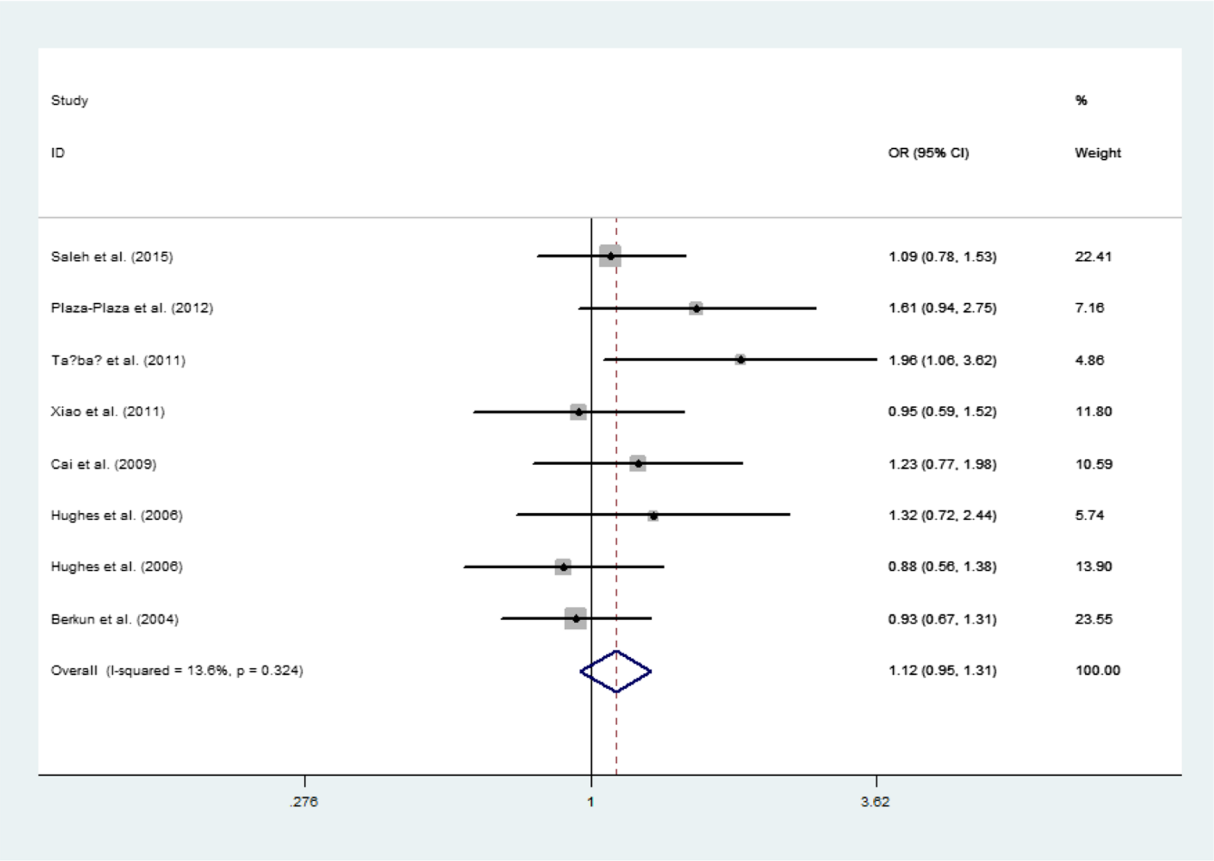


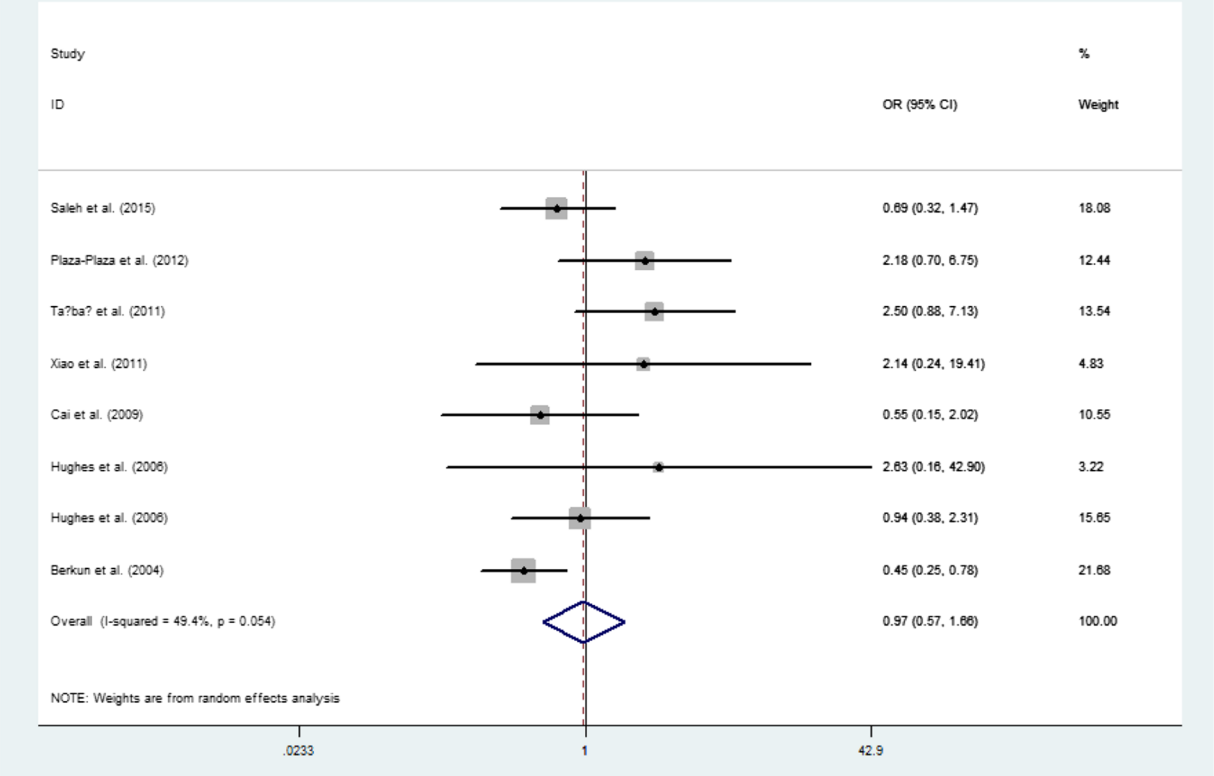
Fig. 4 ORs and 95 % CIs of individual studies and pooled data for the association between rheumatoid arthritis (RA) and *MTHFR* A1298C polymorphism A allele under recessive model

Fig. 5 ORs and 95 % CIs of individual studies and pooled data for the association between rheumatoid arthritis (RA) and *MTHFR* A1298C polymorphism. **a** A versus C; **b** AA + AC versus CC

a


Study
ID
OR (95% CI)
%
Weight
Saleh et al. (2015)
1.09 (0.78, 1.53)
22.41
Plaza-Plaza et al. (2012)
1.61 (0.94, 2.75)
7.16
Ta?ba? et al. (2011)
1.96 (1.06, 3.62)
4.86
Xiao et al. (2011)
0.95 (0.59, 1.52)
11.80
Cai et al. (2009)
1.23 (0.77, 1.98)
10.59
Hughes et al. (2006)
1.32 (0.72, 2.44)
5.74
Hughes et al. (2006)
0.88 (0.56, 1.38)
13.90
Berkun et al. (2004)
0.93 (0.67, 1.31)
23.55
Overall (I-squared = 13.6%, p = 0.324)
1.12 (0.95, 1.31)
100.00
.276
1
3.62

b


Study
ID
OR (95% CI)
%
Weight
Saleh et al. (2015)
0.69 (0.32, 1.47)
18.08
Plaza-Plaza et al. (2012)
2.18 (0.70, 6.75)
12.44
Ta?ba? et al. (2011)
2.50 (0.88, 7.13)
13.54
Xiao et al. (2011)
2.14 (0.24, 19.41)
4.83
Cai et al. (2009)
0.55 (0.15, 2.02)
10.55
Hughes et al. (2006)
2.63 (0.16, 42.90)
3.22
Hughes et al. (2006)
0.94 (0.38, 2.31)
15.65
Berkun et al. (2004)
0.45 (0.25, 0.78)
21.68
Overall (I-squared = 49.4%, p = 0.054)
0.97 (0.57, 1.66)
100.00
NOTE: Weights are from random effects analysis
.0233
1
42.9

contribute to the heterogeneity among eligible studies. However, information about comorbidity among eligible studies is unavailable; we could not determine whether comorbidity is an influencing factor of heterogeneity. In addition, it is also valuable to examine the relationships between *MTHFR* polymorphisms and clinical features of RA patients. However, among all the eligible studies, only one study has analyzed the association of *MTHFR* C677T polymorphism with ocular involvement and rheumatoid factor and the results are non-significant [22]. Studies are needed to clarify the relationships between *MTHFR* polymorphisms and clinical features of RA in the future.

Several limitations of the present meta-analysis should be mentioned. First, significant heterogeneity was detected among several comparisons, which might distort the meta-analysis. Second, the number of studies among subgroup analysis by ethnicity was small. Only two studies from European were available for the meta-analysis of the *MTHFR* C667T polymorphism with RA. In the subgroup meta-analysis for the *MTHFR* A1298C polymorphism, only four studies from Asian population were available and the number of study from non-Asian population was only one. Third, publication bias might exist, although the Egger's test provided non-significant result. Fourth, this meta-analysis was based on uncorrected estimate, so a more precise analysis could be conducted if the potential confounding factors, such as age, sex, clinical characteristic, and environmental factors were available. Finally, due to the lack of related data, the associations of *MTHFR* polymorphisms with clinical features of RA could not be evaluated by meta-analysis.

In conclusion, our meta-analysis results demonstrate that the *MTHFR* C677T polymorphism is involved in the genetic susceptibility of RA in Asians, and the *MTHFR* A1298C polymorphism is associated with genetic susceptibility to RA in the overall population. Given the paucity of studies, especially in non-Asian populations, further well-designed studies with larger sample sizes are required to elucidate the role of *MTHFR* polymorphisms in the genetic basis of RA in different ethnic populations.

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Compliance with ethical standards

Disclosures None.

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