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REVIEW ARTICLE

MTHFR gene polymorphisms and susceptibility to rheumatoid arthritis: a meta-analysis based on 16 studies

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Abstract

Rheumatoid arthritis (RA) is the most common autoimmune rheumatic disease, in which an epigenetic implication in the disease etiopathogenesis has been noted. Here in this meta-analysis, we attempted to investigate the pooled association of methylenetetrahydrofolate reductase (MTHFR) gene C677T and A1298C polymorphisms and susceptibility to RA risk. A systematic search was performed in the main databases, including MEDLINE and Scopus to search for studies assessing the association between *MTHFR* gene C677T and A1298C polymorphisms and the risk of RA prior to December 2019. In this meta-analysis, 15 studies with 2165 patients and 1751 healthy controls for C677T SNP and 14 studies containing 2021 patients and 1760 healthy controls for A1298C SNP were included. A significant positive association between C677T SNP and RA risk was recognized in the dominant, recessive, and allelic model, but not TT and CT genotypes. The results indicated that the risk of RA in African population was increased under all genotype models while these results were repeated in Asian population just for recessive model, allelic model, and TT genotype. Moreover, the analysis of A1298C SNP demonstrated a significant association in overall population according to only the recessive model and CC genotype. Subgroup analysis according to the genotyping method indicated that RFLP-PCR method could impress the results of association between *MTHFR* gene A1298C and C677T SNPs and RA risk. The outcome of this meta-analysis indicated that *MTHFR* gene C677T SNP was much possibly be associated with RA risk.

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

Introduction

Rheumatoid arthritis (RA) is a most common systemic and autoimmune disorder characterized by synovial inflammation, erosive articular degradation, and joint destruction, leading joint deformity and movement limitation [1]. Although the main etiology of RA is yet to be elucidated, studies demonstrated that both environmental and genetic risk factors may play a major role in the onset and progression of the disease [2]. The prevalence of the disease is almost 0.5–1% among the world population and is more frequently occurred in women than in men (sex ratio 3:1) [3, 4]. According to the most recent studies, genetic factors may contribute to approximately 60% of the total risk in susceptibility to RA [5]. The class II Human leukocyte antigen (HLA) has been identified as the most powerful genetic factors in susceptibility to RA [6, 7]. On the other hand, a number of non-HLA genes, such as *Cytotoxic T lymphocyte-associated protein 4* (*CTLA4*), *TNF receptor associated factor 1* (*TRAF1*), *Protein tyrosine phosphatase non-receptor type 22* (*PTPN22*), *Peptidyl arginine deiminase 4* (*PADI4*), *TNF alpha induced protein 3* (*TNFAIP3*), and *Methylenetetrahydrofolate reductase* (*MTHFR)* have been consistently associated with RA predisposition [8–13].

The folate biological function is to provide methyl groups required for metabolic processes, such as DNA methylation, synthesis, and repair. Therefore, folate deficiency can disrupt these processes [14]. MTHFR, an essential enzyme in folate

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homeostasis and metabolic pathway, catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, which serves a methylation group toward the conversion of homocysteine to methionine with the precursor of S-adenosylmethionine (SAM) [15]. Reduced function of MTHFR results in hypomethylation of DNA and enhanced levels of homocysteine, which leads to increased secretion of proinflammatory cytokines that are implicated in the pathogenesis of RA [16]. Therefore, genetic variations of MTHFR could impress the susceptibility to RA. On the other hand, genetic variants of the MTHFR gene have been associated with hyperhomocysteinemia, which has been considered as risk factor for cardiovascular diseases in RA patients [17].

MTHFR gene is located on the chromosomal region 1p36.3, and several single nucleotide polymorphisms (SNPs) within this gene have been recognized in susceptibility to diverse autoimmune disorders [18]. Two common polymorphisms C677T (rs1801133) and A1298C (rs1801131) have been recognized. These polymorphisms are correlated with a reduction in MTHFR enzyme activity. The C677T mutation in the MTHFR gene causes alanine (A) to valine (V) amino acid substitution. This situation is an important cause of reduced enzyme activity and leads to a higher level of homocysteine levels [19]. Besides, the A to C change at position 1298 leads to glutamine to alanine substitution, resulting in decreased enzyme activity [20].

Several studies evaluated the associations between *MTHFR* polymorphisms (C677T and A1298C) and susceptibility to RA in different populations with variable frequency and conflicting results. This discrepancy might be owing to different sample sizes, ethnicity, clinical heterogeneity, and publication bias. To compensate these limitations, we conducted this most updated meta-analysis to investigate whether *MTHFR* gene polymorphisms play a role in RA proneness.

Methods

This meta-analysis follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [21]. The current study is registered in the International Prospective Register of Systematic Reviews (PROSPERO).

Literature search

A comprehensive search was conducted through MEDLINE and Scopus databases to retrieve all potential publications considered the association between MTHFR (C677T and A1298C) gene polymorphism and susceptibility to RA. The following combinations of key words were searched: ("MTHFR" OR "methylenetetrahydrofolate reductase") AND ("Rheumatoid Arthritis" OR "Arthritis" OR "RA") AND ("single nucleotide polymorphism" OR "SNP" OR "polymorphisms" OR "mutation" OR "variation"). The reference list of all studies was cross-checked to find other potential studies which might miss during initial search.

Inclusion and exclusion criteria

We included studies in quantitative analysis if they met the following criteria: (i) all observational studies (cohort or case-control design) considered the association between MTHFR (C677T and A1298C) gene polymorphism and susceptibility to RA; (ii) studies reporting sufficient data to extract or calculate risk estimates with 95% CI; (iii) studies with sufficient information regarding numbers or genotype frequencies in cases and healthy controls. Duplicates, book chapters, letters to editor, animal study, case reports, review articles, and studies with repetitive subjects all were excluded. The application of these criteria recognized 16 and 14 eligible studies for C677T and A1298C SNPs, respectively.

Study selection criteria

The results of initial search were exported to Endnote software. Then two reviewers independently assessed titles and abstracts of all studies. Articles which not follow the eligibility criteria were excluded according to a hierarchical approach. The full-text examination was examined if we could not decide to include or exclude studies based on title and abstract. In particular conditions, if an author has published more than one study by the same case series, the most recently published study was included. Any disagreements were discussed and resolved by consensus.

Data extraction

The detailed data of all eligible studies were extracted according to a standardized extraction form including: the author's name, journal and year of publication, country of origin, ethnicity, mean or range of age, genotyping method, allele frequency of cases and controls, total sample size of cases and controls, and the number of cases and controls for each genotype. Any discrepancy between two reviewers was solved by mutual discussion.

Quality assessment

Methodological quality of eligible studies was evaluated by Newcastle–Ottawa Scale (NOS), a validated scale for non-randomized studies in meta-analysis [22]. Accordingly, studies were categorized to high quality (7–9), intermediate quality (4–6), and low quality (1–3). Furthermore, chi-square tests were calculated to disclose potential deviation from Hardy–Weinberg equilibrium (HWE) for distribution of the allele frequencies in the control group.

Statistical analysis

The data analyses were carried out using STATA (version 14.0; Stata Corporation, College Station, TX) and SPSS (version 23.0; SPSS, Inc. Chicago, IL). In this study, crud OR and its 95% CI was calculated to estimate the association between MTHFR gene polymorphism and the risk of RA. Genotype models were defined as follows: MTHFR C677T, [dominant model (TT+CT vs. CC), recessive model (TT vs. CT+CC), allelic model (T vs. C), homozygote (TT vs. CC), heterozygote (CT vs. CC)] and MTHFR A1298C, [dominant model (CC+AC vs. AA), recessive model (CC vs. AC+AA), allelic model (C vs. A), homozygote (CC vs. AA), heterozygote (AC vs. AA)]. In consideration of the possible heterogeneity (between-study variability) across included studies, chi-square Q-test was used [23]. Additionally, to show heterogeneity quantitatively, the other index (I^2) was calculated. There was significant heterogeneity if I^2 values exceeded 50% or the Q statistic had a P value less than 0.1. In the presence of significant heterogeneity, the random-effects model (REM) (DerSimonian–Laird approach) was performed. Otherwise, the fixed-effects model (FEM) (Mantel–Haenszel approach) was performed for combination of data [24, 25]. We used sensitivity analysis to assess the stability of our results. Additionally, the Egger's test and Begg's test were applied for publication bias [26, 27].

Results

Study characteristics

The four-phase search and screening process based on the PRISMA statement is presented in Fig. 1. After the removal

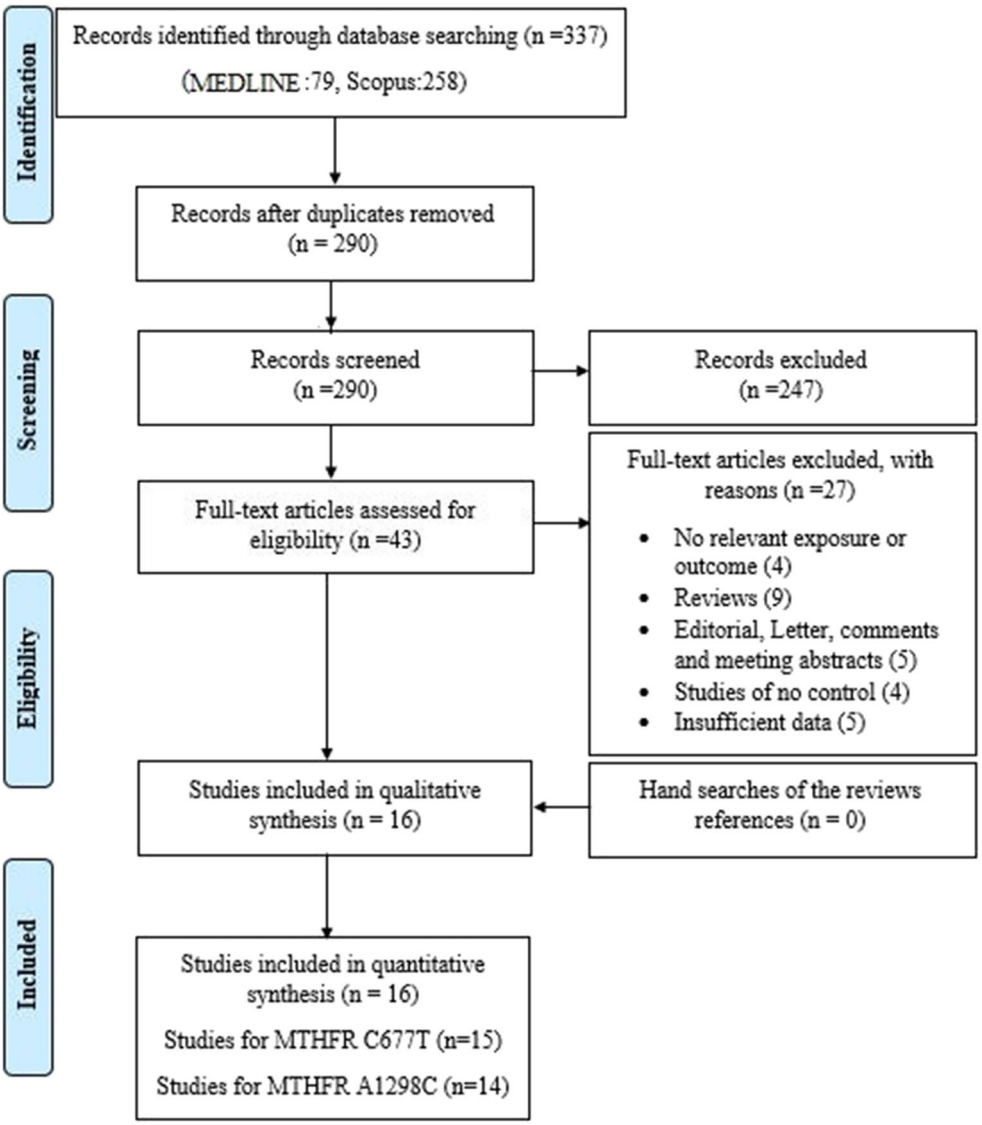

Fig. 1 Flow diagram of study selection process

Table 1 Characteristics of studies included in meta-analysis of MTHFR gene C677T polymorphism

Study author	Year	Country	Ethnicity	Total cases/controls	Age case/control (mean)	Genotyping method	Quality score
MTHFR C677T							
Berkun et al.	2004	Israel	Jewish	93/377	58.7/NR	PCR-RFLP	6
Hughes et al.	2006	USA	African-Americans	138/52	NR/NR	Taq man	6
Hughes et al.	2006	USA	Caucasian	393/50	NR/NR	Taq man	6
Rubini et al.	2008	Italy	Caucasian	217/251	NR/NR	PCR-RFLP	7
Cai et al.	2009	China	Asian	86/101	NR/NR	PCR-RFLP	5
Tasbas et al.	2011	Turkey	Caucasian	64/31	48.7 + 12.5/46.2 + 13.4	PCR-RFLP	5
Plaza-Plaza et al.	2012	Spain	Caucasian	67/67	49.11 ± 14.6/38.12 ± 13.01	PCR-RFLP	5
Inanir et al.	2013	Turkey	Caucasian	147/150	52.7 ± 13.72/52.1 ± 15.66	PCR-RFLP	6
Boughrara et al.	2015	Algeria	African	110/89	48.8 ± 13.4/47.3 ± 15.3	Taq man	7
Saad et al.	2015	Egypt	African	105/70	NR/NR	PCR-RFLP	6
Saleh et al.	2015	Jordan	Asian	159/170	NR/NR	PCR-RFLP	7
Shaker et al.	2016	Egypt	African	62/21	42.7 ± 12.7/NR	PCR-RFLP	5
Gonzalez-Mercado et al.	2017	Mexico	Mexican	68/82	NR/NR	Taq man	5
Abd El-Aziz et al.	2017	Egypt	African	160/120	34.3 ± 4.2/33.4 ± 4.5	PCR-RFLP	7
Wang et al.	2019	China	Asian	296/120	54.6 ± 11.6/35.7 ± 2.6	PCR-RFLP	7
MTHFR A1298T							
Berkun et al.	2004	Israel	Jewish	93/377	58.74/NR	PCR-RFLP	6
Hughes et al.	2006	USA	African-Americans	138/53	NR/NR	Taq man	6
Hughes et al.	2006	USA	Caucasian	393/50	NR/NR	Taq man	6
Rubini et al.	2008	Italy	Caucasian	217/251	NR/NR	PCR-RFLP	7
Cai et al.	2009	China	Asian	86/101	NR/NR	PCR-RFLP	5
Tasbas et al.	2011	Turkey	Caucasian	64/31	48.7 + 12.5/46.2 + 13.4	PCR-RFLP	5
Plaza-Plaza et al.	2012	Spain	Caucasian	67/67	52.7 ± 13.71/52.1 ± 15.65	PCR-RFLP	5
Boughrara et al.	2015	Algeria	African	86/97	48.8 ± 13.4/47.3 ± 15.3	Taq man	7
Saad et al.	2015	Egypt	African	105/80	NR/NR	PCR-RFLP	6
Saleh et al.	2015	Jordan	Asian	159/170	NR/NR	PCR-RFLP	7
Hashiguchi et al.	2016	Japan	Asian	159/299	NR/NR	Taq man	8
Shaker et al.	2016	Egypt	African	62/21	42.7 ± 12.7/NR	PCR-RFLP	5
Premkumar et al.	2018	India	Asian	96/43	43.3 ± 8.98/44.4 ± 10.19	PCR-RFLP	6
Wang et al.	2019	China	Asian	296/120	54.6 ± 11.6/35.7 ± 2.6	PCR-RFLP	7

NR, not reported; *M*, male; *F*, female

of duplicates (47 studies), 290 studies were remained. Of these, 247 studies were excluded based on title and abstract and 43 studies excluded by full-text evaluation. Ultimately, 16 studies were qualified and included in quantitative analysis (Fig. 1) [28–43]. All included studies were conducted between 2004 and 2019 and had good methodological score. RFLP-PCR and Taq-man genotyping methods were used by most of included studies. Tables 1 and 2 summarized the characteristics and genotype frequency of the included studies.

Quantitative analysis

Meta-analysis of C677T and RA risk

Overall, 15 studies with 2165 patients and 1751 healthy controls were included in quantitative analysis of *MTHFR* gene C677T polymorphism and RA risk. The analysis of overall population revealed a significant positive association between C677T SNP and RA risk across dominant model (OR = 1.28, 95% CI, 1.01–1.65, $P = 0.04$), recessive model (OR = 1.47, 95% CI, 1.15–1.89, $P < 0.001$), allelic model (OR = 1.28, 95% CI, 1.06–1.56, $P = 0.01$), but not TT vs. CC model (OR = 1.31, 95% CI, 0.98–1.75, $P = 0.07$), and CT vs. CC model (OR = 1.12, 95% CI, 0.96–1.32, $P = 0.15$) (Fig. 2). Additionally, subgroup analysis was performed to evaluate ethnicity-specific effect on the association of C677T SNP and RA risk. Since there was only one study for Jewish, African-American and Mexican ethnicity these studies were excluded from subgroup analysis. The results indicated that the risk of RA in African population increases under all genotype models while these results were repeated in Asian population just for recessive model (OR = 2.18, 95% CI, 1.40–3.41, $P < 0.001$), allelic model (OR = 1.32, 95% CI, 1.06–1.64, $P = 0.01$), and TT vs. CC model (OR = 2.07, 95% CI, 1.16–3.69, $P = 0.01$). No significant association was detected in causations (Fig. 3). Furthermore, we stratified studies according to genotyping method and found that utilization of RFLP-

Table 2 Distribution of genotype and allele among RA patients and controls

Study author	RA patients					Healthy control					P-HWE	MAF
	CC	CT	TT	C	T	CC	CT	TT	C	T		
MTHFR C677T												
Berkun et al.	45	38	10	128	58	153	166	58	472	282	0/246	0/374
Hughes et al.	109	27	2	245	31	39	12	1	90	14	0/945	0/134
Hughes et al.	196	157	40	549	237	25	22	3	72	28	0/518	0/28
Rubini et al.	73	100	44	246	188	65	133	53	263	239	0/324	0/476
Cai et al.	31	44	11	106	66	43	45	13	131	71	0/819	0/351
Tasbas et al.	29	28	7	86	42	20	10	1	50	12	0/852	0/193
Plaza-Plaza et al.	20	26	21	66	68	26	31	10	83	51	0/878	0/380
Inanir et al.	102	39	6	243	51	121	26	3	268	32	0/267	0/106
Boughrara et al.	41	63	6	145	75	48	38	3	134	44	0/164	0/247
Saad et al.	46	51	8	143	67	50	19	1	119	21	0/589	0/15
Saleh et al.	73	62	24	208	110	94	66	10	254	86	0/722	0/252
Shaker et al.	26	30	6	82	42	15	5	1	35	7	0/512	0/166
Gonzalez-Mercado et al.	23	32	13	78	58	23	42	17	88	76	0/786	0/463
Abd El-Aziz et al.	79	57	24	215	105	68	45	7	181	59	0/901	0/245
Wang et al.	160	110	26	430	162	68	47	5	183	57	0/372	0/237
	AA	AC	CC	A	C	AA	AC	CC	A	C		
MTHFR A1298T												
Berkun et al.	50	20	23	120	66	169	159	49	63	257	0/233	0/340
Hughes et al.	102	35	1	239	37	36	16	1	71	18	0/606	0/169
Hughes et al.	178	165	50	521	265	25	19	6	380	31	0/429	0/31
Rubini et al.	105	86	26	296	138	127	112	12	198	136	0/04	0/270
Cai et al.	52	28	6	132	40	50	47	4	62	55	0/079	0/272
Tasbas et al.	24	31	9	79	49	6	16	9	71	34	0/815	0/548
Plaza-Plaza et al.	40	22	5	102	32	32	25	10	49	45	0/18	0/335
Boughrara et al.	25	50	11	100	72	50	42	5	111	52	0/308	0/268
Saad et al.	46	35	24	127	83	19	51	10	94	71	0/009	0/443
Saleh et al.	82	60	17	224	94	76	81	13	137	107	0/172	0/314
Hashiguchi et al.	109	42	8	260	58	206	84	9	92	102	0/902	0/170
Shaker et al.	26	22	14	74	50	5	13	3	58	19	0/253	0/452
Premkumar et al.	30	45	21	105	87	14	22	7	111	36	0/737	0/418
Wang et al.	178	90	28	446	146	70	41	9	208	59	0/389	0/245

P-HWE, *P* value for Hardy–Weinberg equilibrium; *MAF*, minor allele frequency of control group

PCR method can affect the results of association between C677T SNP and RA risk (Fig. 4).

Meta-analysis of A1298C and RA risk

In the quantitative analysis of MTHFR A1298C polymorphism and RA risk, 14 studies containing 2021 patients and 1760 healthy controls were included. The pooled results revealed a significant association in overall population across recessive model (OR = 1.58, 95% CI, 1.21–2.06, $P < 0.001$) and CC vs. AA model (OR = 1.34, 95% CI, 1–1.78, $P = 0.04$) (Fig. 2). The results of subgroup analysis by ethnicity rejected potential association between A1298C SNP and RA risk in all three populations (Africans, Asians, Causations) (Fig. 3). Moreover, subgroup analysis based on genotyping method highlighted the influence of RFLP-PCR on the association between A1298C SNP and RA risk (Fig. 4).

Evaluation of heterogeneity and publication bias

During the meta-analysis of *MTHFR* gene polymorphism, evidence of moderate heterogeneity was detected in some models. However, partial heterogeneity was resolved while

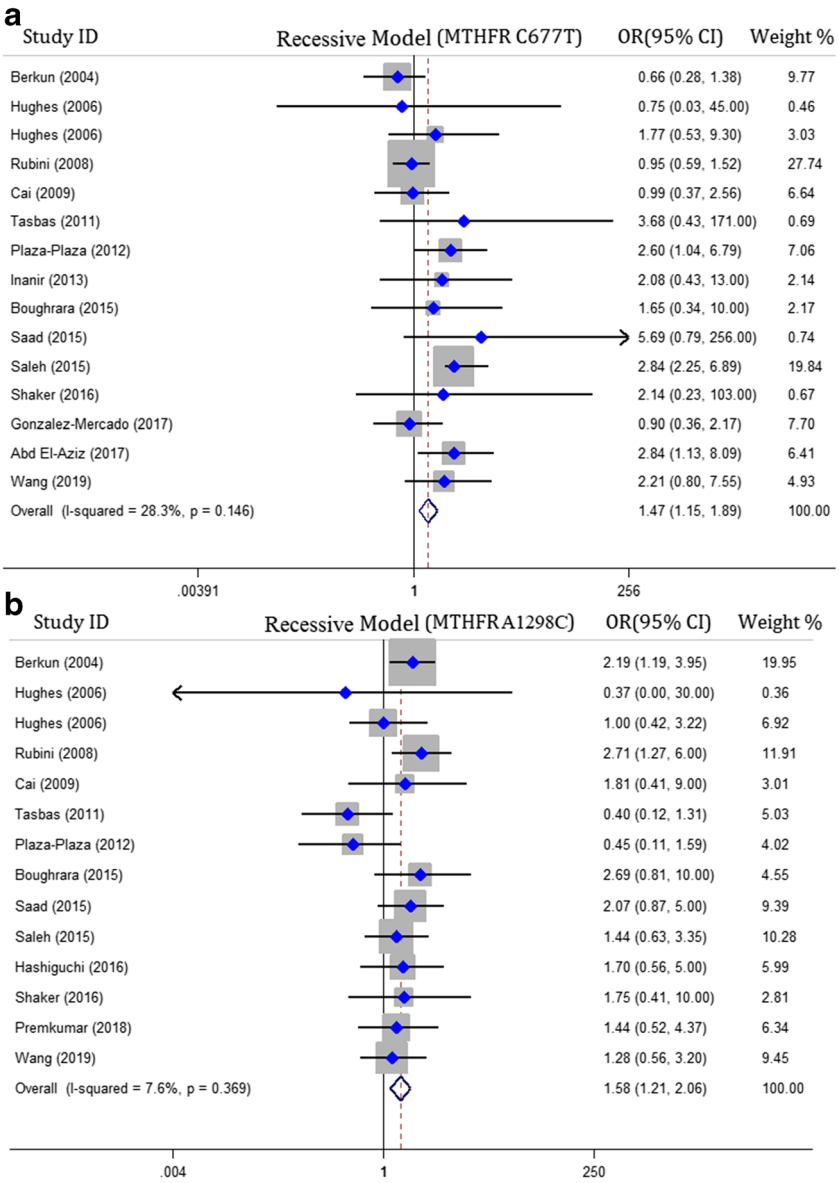

Fig. 2 Pooled OR and 95% CI of individual studies and pooled data for the association between *MTHFR* gene polymorphism and RA risk in recessive model (**a** C677T and **b** A1298C)

the data were stratified by genotyping method and ethnicity. Publication bias was evaluated by funnel plot, Begg's test, and Egger's test. Subsequently, there was no obvious evidence of asymmetry according to the funnel plots (Fig. 5), and all *P* values of Begg's test and Egger's test were > 0.05, indicating no evidences of publication biases.

Sensitivity analysis

The leave-one-out method was used in the sensitivity analysis to explore the effect of individual data on the pooled ORs (Table 3). The significance of ORs was not altered through omitting any single study in the dominant model for C677T and A1298C SNPs, indicating that our results were statistically robust (Fig. 6).

Discussion

Dysregulated function of MTHFR may lead to hypomethylation of DNA and hyperhomocysteinemia, both have been implicated in RA etiopathogenesis [44], conferring *MTHFR* as a candidate RA predisposing gene. Several *MTHFR* gene SNPs have been identified [18], and genome-wide association studies (GWASs) have identified this gene to be associated with genetic susceptibility to RA [45]. In addition, numerous studies have reported that *MTHFR* gene C677T and A1298C polymorphisms might be contributing genetic factor to RA risk. Although several studies in different ethnic groups have tried to divulge the plausible association between *MTHFR* gene C677T and A1298C SNPs and RA risk, the observations are still controversial and an apprehensive meta-analysis seems to be indispensable to disclose the bona fide association of these

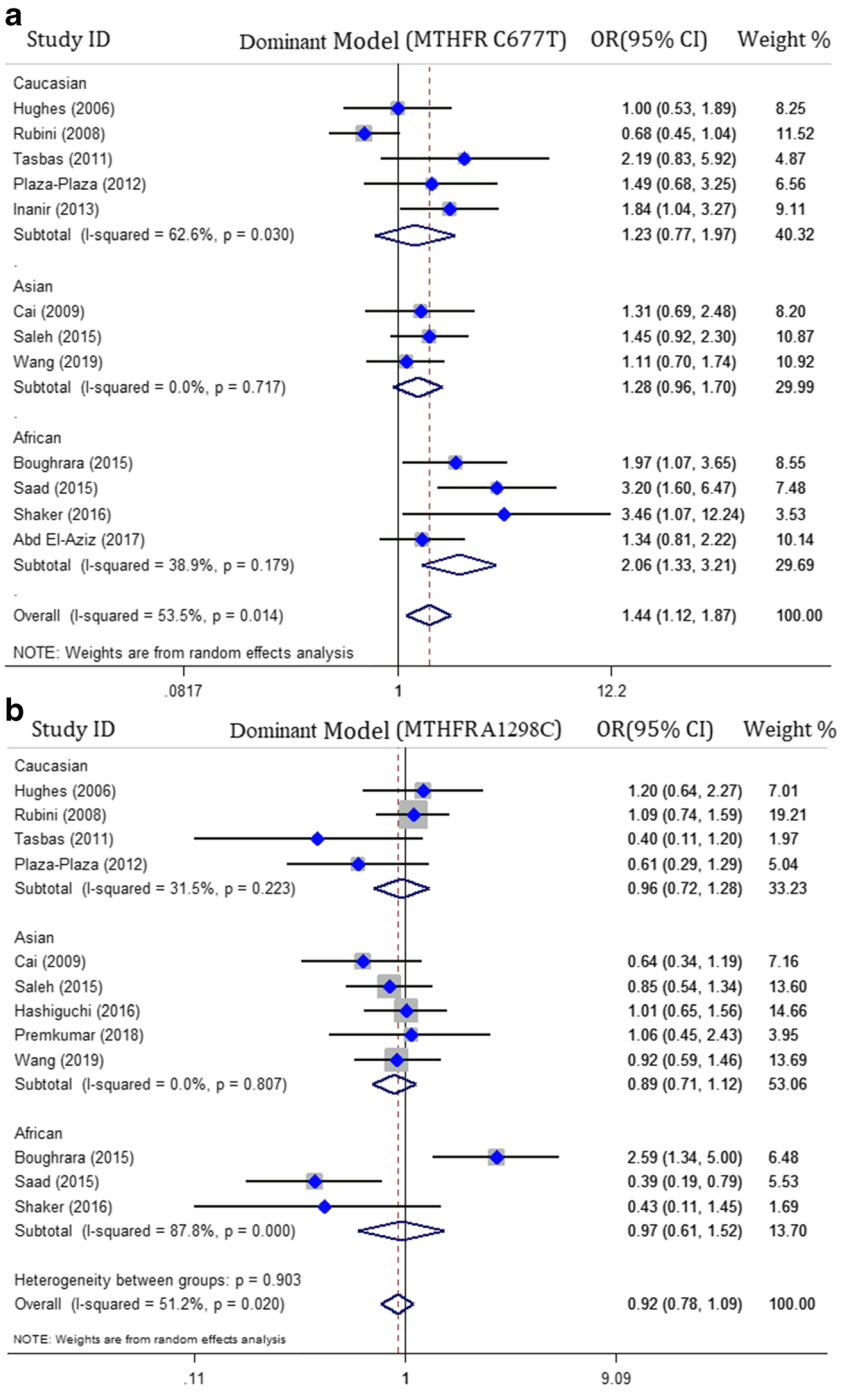

Fig. 3 Pooled OR and 95% CI of individual studies and pooled data for the association between *MTHFR* gene polymorphism and RA risk in different ethnicity subgroups and overall populations for dominant model (**a** C677T and **b** A1298C)

variations with RA disease. Hence, this meta-analysis was performed to reveal the approximation of *MTHFR* gene C677T and A1298C SNPs and RA risk. According to the pooled analysis, both polymorphisms increased the risk of RA.

MTHFR plays a vital role in the folate metabolism and is involved in the conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate [46]. As a structural analog of folic acid, methotrexate (MTX) and sulphasalazine (SSZ) has been prescribed for the treatment of RA according to its well-known efficacy and less toxicity [47]. These drugs competitively inhibit dihydrofolate reductase (DHFR), an enzyme that participates in the tetrahydrofolate synthesis and leads to increased plasma homocysteine level. However, polymorphisms in the genes of the folate pathway are potentially responsible for interpatient variation in MTX and sulphasalazine efficacy. Studies demonstrated patients homozygous for the mutation in the *MTHFR* gene had significantly higher baseline homocysteine and heterozygous MTHFR genotype induced significantly higher plasma homocysteine

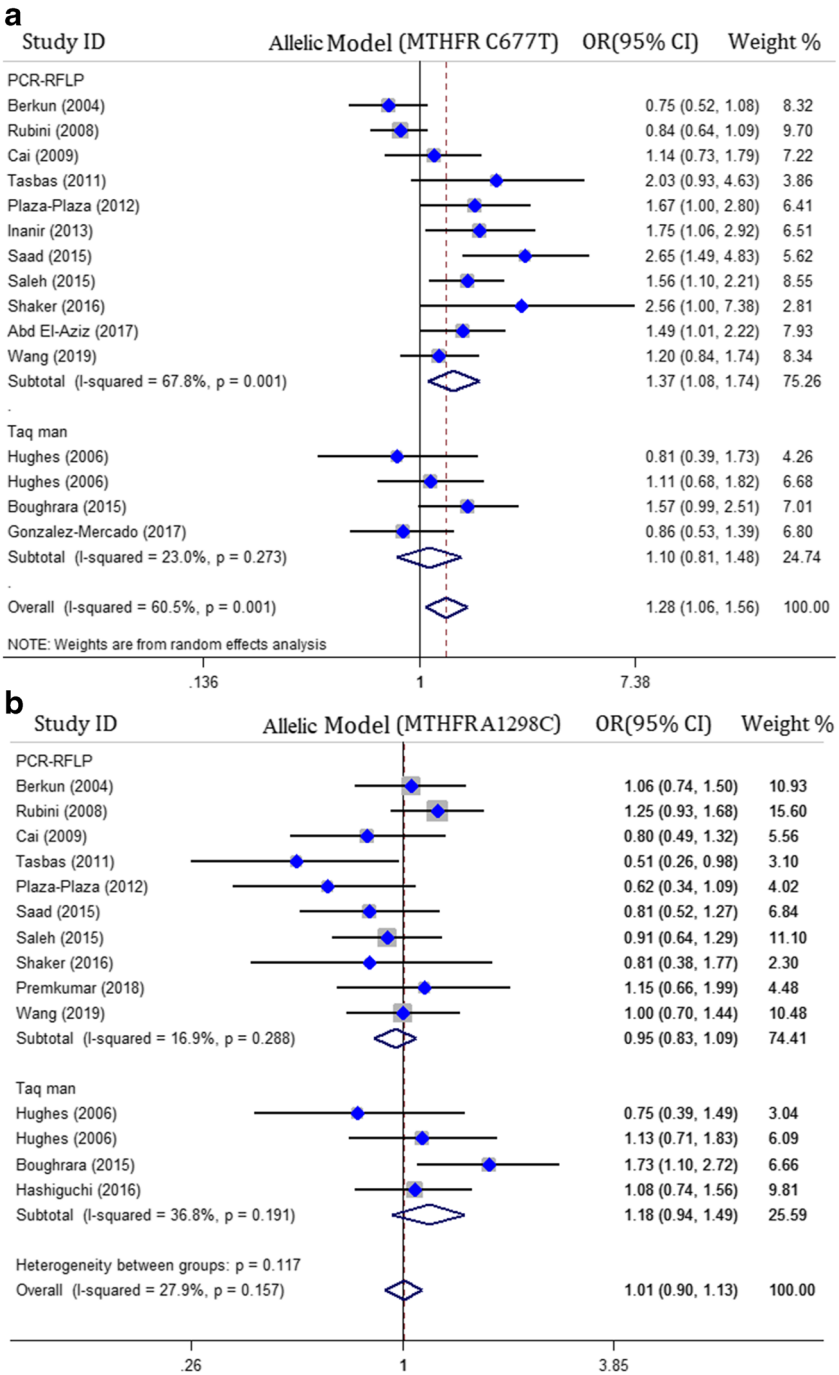

Fig. 4 Pooled odds ratio (OR) and 95% confidence interval of individual studies and pooled data in different genotyping method subgroups for dominant model (**a** C677T and **b** A1298C)

compared with no mutation. Taken together, these findings illustrate the critical role of MTHFR polymorphisms in increasing homocysteine levels [48, 49].

There are two meta-analyses about these hypothesis; Fisher et al. [50] showed that the C677T polymorphism (not A1298C polymorphism) is associated with increased toxicity (OR 1.71, 95% CI 1.32–2.21, $P < 0.001$). In consistent with them, Song et al. revealed a significant association between the MTHFR C677T and A1298C polymorphisms and MTX toxicity. They suggested that modulation in the activity of MTHFR enzyme as the result of SNPs in the *MTHFR* gene might be expected reason [51–53].

Studies have disclosed the role of *MTHFR* gene C677T and A1298C polymorphisms in reduced activity of the enzyme [54], which may impress the RA pathogenesis through three major pathways. First, dysfunction of MTHFR enzyme may cause DNA hypomethylation, which has been implicated to be involved in the pathogenesis of RA [55]. Second, MTHFR plays a role in donation of methyl group for methylation of homocysteine to generate methionine; hence, dysregulation of

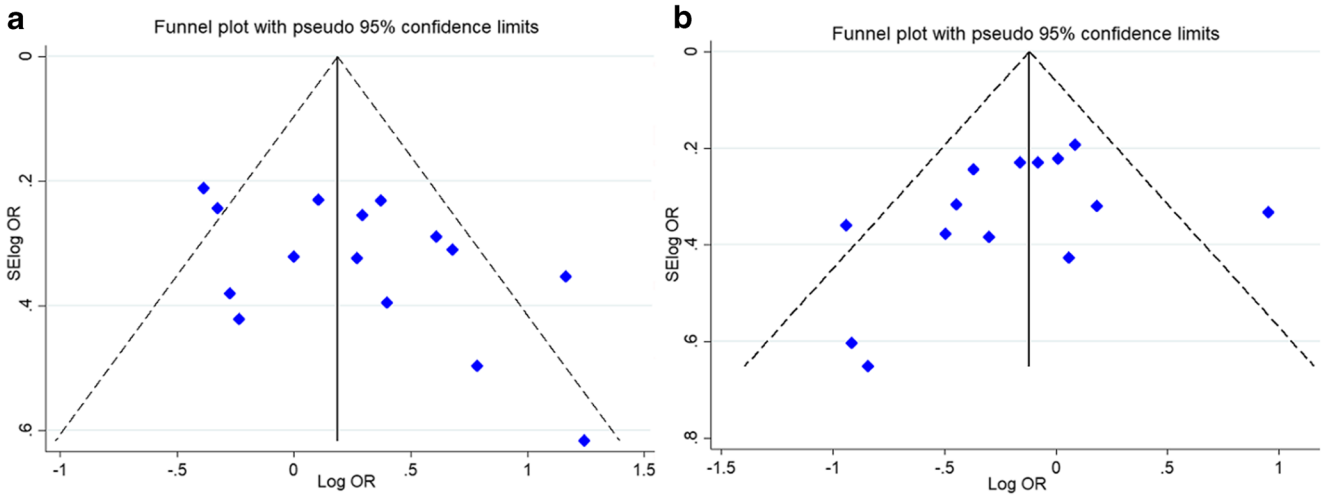

Fig. 5 Begg's funnel plot for publication bias test (**a** C677T and **b** A1298C)

MTHFR might lead to high homocysteine level [16], which has been observed in RA patients compared with healthy subjects [56]. In addition, homocysteine has been shown to trigger the proinflammatory transcription factor of nuclear factor (NF)-κB, which has been shown to be involved in inflammatory settings in RA [44]. Third, GWASs has reported that the chromosomal region harboring the *MTHFR* gene is associated with genetic susceptibility to RA risk [57, 58]. Hence, it is rational to study the RA pathogenesis with respect to genetic diversity and impaired function of MTHFR.

In this most recent systematic review and meta-analysis, 15 studies with 2165 patients and 1751 healthy controls were included in quantitative analysis of *MTHFR* gene C677T polymorphism and RA risk as well as 14 studies containing 2021 patients and 1760 healthy controls were included for evaluation of *MTHFR* gene A1298C polymorphism and RA risk. In addition, we performed a subgroup analysis with respect to the technique applied for genotyping (RFLP-PCR), which had not been performed in the previous meta-analyses. Our analysis indicated a significant positive association between C677T SNP and RA risk in the dominant model (OR = 1.28), recessive model (OR = 1.47), allelic model (OR = 1.28), but not TT and CT genotypes. The results indicated that the risk of RA in African population was increased under all genotype models while these results were repeated in Asian population just for recessive model (OR = 2.18), allelic model (OR = 1.32), and TT genotype (OR = 2.07). However, no significant association was observed in the causations. The analysis of another SNP, namely A1298C, revealed a significant association in overall population according to the recessive model (OR = 1.58) and CC genotype (OR = 1.34). However, the results of subgroup analysis by ethnicity did not show association between A1298C SNP and RA risk in all three populations, including Africans, Asians, and Causations. That notwithstanding, the previous meta-analysis, applying less studies and including less patients and controls, indicated that the recessive model of *MTHFR* gene C677T polymorphism was not associated with RA risk [59], unlike our meta-analysis that indicated a statistically significant association of this polymorphism with RA risk. Moreover, only the recessive model of *MTHFR* gene A1298C polymorphism was associated with RA risk in the late meta-analysis that was associated with RA risk in recessive model and CC genotype in the current meta-analysis. This difference originates from the number of studies and subjects included in each meta-analysis.

In this meta-analysis, the subgroup analysis was also conducted based on genotyping technique to further explore the influence of genotyping method on the associations. Subgroup analysis according to the genotyping method indicated that RFLP-PCR method could impress the results of association between *MTHFR* gene A1298C and C677T SNPs and RA risk. Therefore, the differences in the genotyping method may have significant impressions on the overall observations of the association between *MTHFR* gene A1298C and C677T SNPs and RA susceptibility.

Despite our attempt to carry out the best possible meta-analysis and conclusion of the available information, a number of limitations can be raised toward this meta-analysis. First, it was not possible to analyze the role of gender, age, lifestyle, drugs, and other genetic variations on the adjusted association of *MTHFR* gene polymorphisms and RA risk. Therefore, further research in respective of the gene–gene and gene–environment interactions is still required to come up with a more apprehensive estimation of MTHFR gene polymorphisms with RA risk. Second, we only searched the publications with English language. Third, the number of studies available for the subgroup analysis based on the ethnicity was fairly small. This issue did not allow to perform comprehensive subgroup analysis across all populations.

In conclusion, the results of pooled analysis supported significant association between *MTHFR* gene C677T SNP and

Table 3 Main results of pooled ORs in meta-analysis of *MTHFR* gene polymorphisms

Subgroup		Sample size	Test of association		Test of heterogeneity		Test of publication bias (Begg's test)		Test of publication bias (Egger's test)	
	Genetic model	Case/control	OR	95% CI (*P* value)	I^2 (%)	*P*	*Z*	*P*	*T*	*P*
MTHFR C677T										
Overall	Dominant model	2165/1751	*1.28*	*1.01–1.63 (0.04)*	56.2	≤0.001	1.53	0.12	1.91	0.07
	Recessive model	2165/1751	*1.47*	*1.15–1.89 (≤ 0.001)*	28.3	0.14	0.45	0.65	0.87	0.39
	Allelic model	2165/1751	*1.28*	*1.06–1.56 (0.01)*	60.5	≤0.001	1.73	0.08	2.21	0.04
	TT vs. CC	2165/1751	1.31	0.98–1.75 (0.07)	32.5	0.10	0.45	0.65	2.37	0.03
	CT vs.CC	2165/1751	1.12	0.96–1.32 (0.15)	45.9	0.02	1.53	0.12	1.88	0.08
Subgroups										
Caucasian	Dominant model	888/549	1.23	0.77–1.97 (0.38)	62.6	0.03	0.98	0.32	2.10	0.12
	Recessive model	888/549	1.26	0.85–1.87 (0.24)	14.9	0.32	0.98	0.32	2.16	0.12
	Allelic model	888/549	1.32	0.91–1.91 (0.14)	66.4	0.01	0.98	0.32	3.73	0.03
	TT vs. CC	888/549	1.07	0.68–1.70 (0.76)	34.6	0.19	0.98	0.32	4.38	0.02
	CT vs. CC	888/549	1	0.75–1.33 (0.97)	51.9	0.08	0.98	0.32	1.37	0.26
Asian	Dominant model	541/391	1.28	0.96–1.70 (0.09)	0	0.7	0.52	0.60	0.13	0.91
	Recessive model	541/391	*2.18*	*1.40–3.41 (≤ 0.001)*	41.5	0.18	−0.52	0.60	−0.95	0.51
	Allelic model	541/391	*1.32*	*1.06–1.64 (≤ 0.001)*	0	0.46	−1.57	0.11	−0.99	0.50
	TT vs. CC	541/391	*2.07*	*1.16–3.69 (0.01)*	0	0.39	−0.52	0.60	−0.62	0.64
	CT vs. CC	541/391	1.14	0.84–1.54 (0.41)	0	0.73	1.57	0.11	1.27	0.42
African	Dominant model	437/300	*2.06*	*1.33–3.21 (≤ 0.001)*	38.9	0.17	1.36	0.17	1.71	0.22
	Recessive model	437/300	*2.61*	*1.19–5.74 (0.01)*	0	0.89	0	1	0.09	0.94
	Allelic model	437/300	*1.77*	*1.34–2.34 (≤ 0.001)*	9..6	0.34	1.36	0.17	1.71	0.22
	TT vs. CC	437/300	*3.08*	*1.37–6.92 (≤ 0.001)*	0	0.90	0.68	0.49	0.97	0.43
	CT vs. CC	437/300	*1.76*	*1.25–2.49 (≤ 0.001)*	5.03	0.11	1.36	0.17	1.49	0.27
RFLP- PCR	Dominant model	1456/1478	*1.36*	*1.02–1.82 (0.03)*	62.5	≤0.001	2.41	0.01	2.87	0.01
	Recessive model	1456/1478	*1.53*	*1.17–2 (≤ 0.001)*	44.7	0.05	0.8	0.93	1.03	0.32
	Allelic model	1456/1478	*1.37*	*1.08–1.74 (≤ 0.001)*	67.8	≤0.001	2.10	0.03	3.17	0.01
	TT vs. CC	1456/1478	1.34	0.97–1.84 (0.07)	47.1	0.04	0.39	0.69	2.61	0.02
	CT vs. CC	1456/1478	1.13	0.94–1.35 (0.19)	52.4	0.02	2.41	0.01	2.76	0.02
Taq-Man	Dominant model	709/273	1.09	0.69–1.72 (0.71)	40.9	0.16	−1.36	0.17	−1.69	0.22
	Recessive model	709/273	1.15	0.58–2.27 (0.68)	0	0.83	0	1	0.48	0.68
	Allelic model	709/273	1.10	0.81–1.48 (0.55)	23	0.27	−0.68	0.49	−0.85	0.48
	TT vs. CC	709/273	1.16	0.55–.2.44 (0.69)	0	0.63	0.68	0.49	0.48	0.67
	CT vs. CC	709/273	1.10	0.77–1.58 (0.59)	38.3	0.18	−1.36	0.17	−1.69	0.23
MTHFR A1298C										
Overall	Dominant model	2021/1760	0.89	0.76–1.04 (0.13)	45.9	0.03	−1.70	0.09	−1.32	0.21
	Recessive model	2021/1760	*1.58*	*1.21–2.06 (≤ 0.001)*	7.6	0.36	−1.26	0.20	−1.77	0.10
	Allelic model	2021/1760	1.01	0.90–1.13 (0.89)	27.9	0.15	−1.37	0.17	−2.18	0.05
	CC vs. AA	2021/1760	0.89	0.76–1.04 (0.13)	45.9	0.03	−1.70	0.09	−1.59	0.13
	AC vs. AA	2021/1760	0.74	0.57–0.97 (0.03)	57.5	≤0.001	−1.48	0.13	−1.11	0.28
Subgroups										
Caucasian	Dominant model	741/399	0.96	0.72–1.28 (0.78)	31.5	0.22	−2.04	0.04	−1.98	0.18
	Recessive model	741/399	1.16	0.70–1.92 (0.57)	68.9	0.02	−1.36	0.17	−5.95	0.02
	Allelic model	741/399	1.01	0.81–1.25 (0.94)	66	0.03	−2.04	0.04	−3.26	0.08
	CC vs. AA	741/399	1.18	0.69–2.04 (0.54)	70.2	0.01	−2.04	0.04	−43.8	0.001
	AC vs. AA	741/399	0.90	0.66–1.22 (0.49)	0	0.55	−1.36	0.17	−0.95	0.45
Asian	Dominant model	796/733	0.89	0.71–1.12 (0.32)	0	0.80	−0.98	0.32	−0.33	0.76
	Recessive model	796/733	1.46	0.93–2.30 (0.09)	0	0.93	1.47	0.14	2.16	0.11
	Allelic model	796/733	0.98	0.82–1.17 (0.81)	0	0.84	0.98	0.32	0.04	0.96
	CC vs. AA	796/733	1.27	0.80–2.04 (0.31)	0	0.98	0.49	0.62	0.51	0.64
	AC vs. AA	796/733	0.79	0.62–1.01 (0.06)	0	0.71	−0.49	0.62	−0.31	0.77
African	Dominant model	253/198	0.97	0.61–1.52 (0.88)	87.8	≤0.001	−0.52	0.60	−0.49	0.70
	Recessive model	253/198	*2.16*	*1.12–4.16 (0.02)*	0	0.90	−0.52	0.60	−0.07	0.94
	Allelic model	253/198	1.12	0.83–1.50 (0.46)	68	0.75	0.52	0.60	−0.35	0.78
	CC vs. AA	253/198	1.56	0.74–3.29 (0.24)	41.1	0.18	0.52	0.60	0.12	0.92
	AC vs. AA	253/198	0.57	0.10–3.17 (0.52)	90.8	≤0.001	−0.52	0.60	−0.53	0.69
RFLP-PCR	Dominant model	1245 /1261	*0.79*	*0.66–0.95 (0.01)*	16.6	0.29	−2.41	0.01	−2.68	0.02
	Recessive model	1245 /1261	*1.59*	*1.19–2.14 (≤ 0.001)*	26.1	0.20	−1.16	0.24	−1.92	0.09
	Allelic model	1245 /1261	0.95	0.83–1.09 (0.49)	16.9	0.28	−1.88	0.06	−3.02	0.01
	CC vs. AA	1245 /1261	1.25	0.91–1.72 (0.16)	18.7	0.27	−1.88	0.06	−2.42	0.04
	AC vs. AA	1245 /1261	*0.62*	*0.47–0.82 (≤ 0.001)*	42.3	0.07	−1.52	0.12	−1.90	0.09
Taq-Man	Dominant model	776/499	1.20	0.90–1.61 (0.21)	59.4	0.06	0	1	0.29	0.80
	Recessive model	776/499	1.51	0.80–2.84 (0.20)	0	0.60	0.68	0.49	−0.41	0.72
	Allelic model	776/499	1.18	0.94–1.49 (0.15)	36.8	0.19	−0.68	0.49	−0.61	0.60

Table 3 (continued)

Subgroup		Sample size	Test of association		Test of heterogeneity		Test of publication bias (Begg's test)		Test of publication bias (Egger's test)	
	Genetic model	Case/control	OR	95% CI (*P* value)	I^2 (%)	*P*	*Z*	*P*	*T*	*P*
	CC vs. AA	776/499	1.79	0.92–3.46 (0.08)	0	0.41	0.68	0.49	0.33	0.77
	AC vs. AA	776/499	1.19	0.75–1.88 (0.45)	51.1	0.10	0	1	0.44	0.70

The italicized items indicated are important

RA risk in the dominant, recessive, and allelic model, but not TT and CT genotypes. Nonetheless, the analysis of A1298C SNP revealed a significant association in overall population according to only the recessive model and CC genotype.

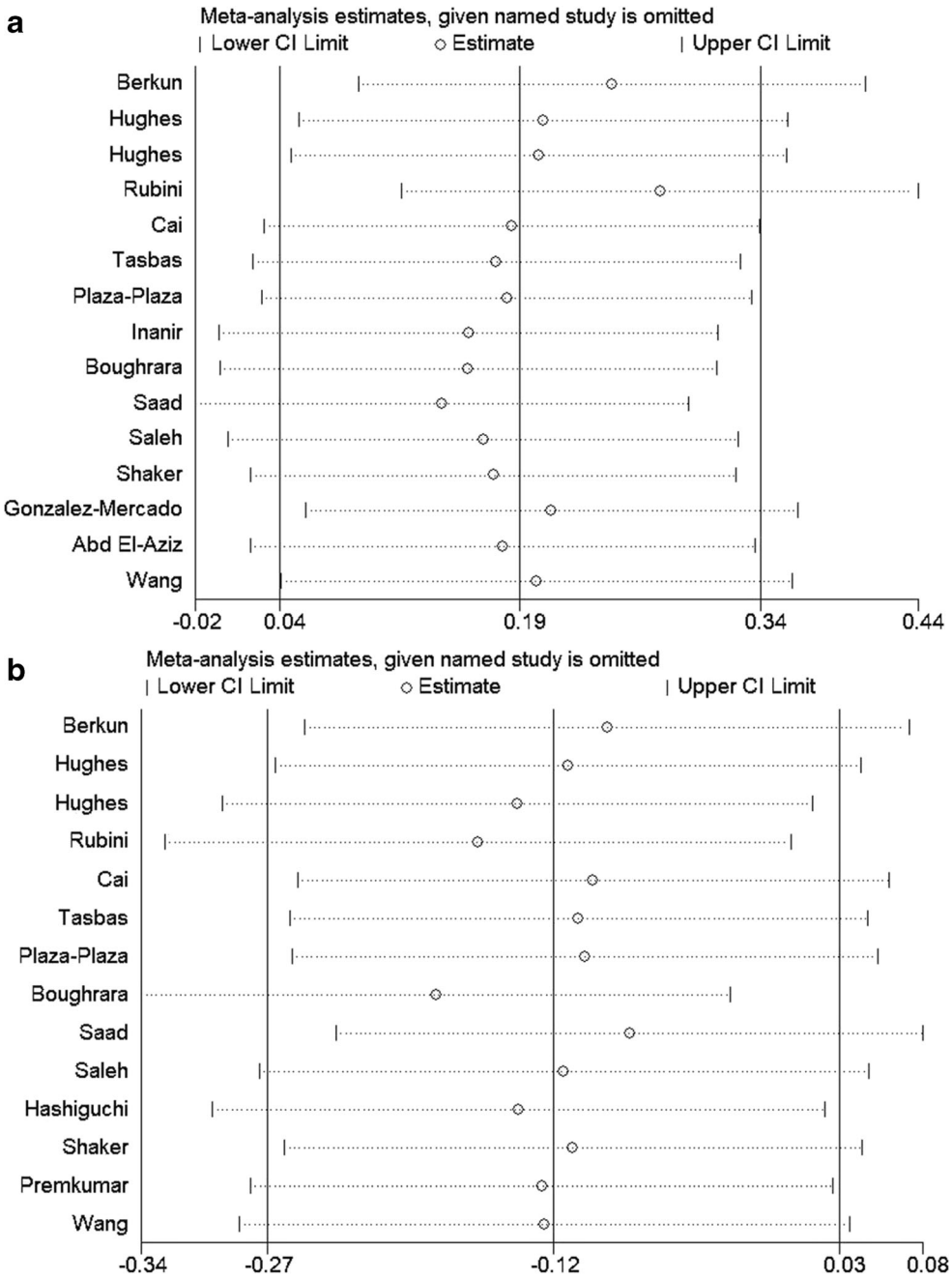

Fig. 6 Sensitivity analysis in present meta-analysis investigates the single-nucleotide polymorphisms of MTHFR contribute to risk for RA (**a** C677T and **b** A1298C)

However, the results of subgroup analysis by ethnicity did not show association between A1298C SNP and RA risk in all three populations, including Africans, Asians, and Causations. The major drawback of the valid estimation of the association of *MTHFR* gene SNPs with RA risk in this meta-analysis stems from the insufficient amount of original data, which requires further investigations in the future. Furthermore, the role of life style, age, and gender should be taken into consideration in the stratification analyses.

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Authors' contributions ZB generated the idea. DI analyzed and interpreted the data. ZB and HY prepared the original draft. ZB, HY, and MA critically revised the paper. MA supervised the project. All authors read and approved the final manuscript.

Compliance with ethical standards

Disclosures None.

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