Original Article

Effects of MTHFR A1298C polymorphism on peripheral blood folate concentration in healthy populations: a meta-analysis of observational studies

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Background and Objectives: Methylenetetrahydrofolate reductase (MTHFR) irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the main form of folate used in the body. Previous studies suggest that MTHFR polymorphism influences folate metabolism, but conflicting results are reported. We performed a meta-analysis to accurately characterize the association between MTHFR A1298C polymorphism and peripheral blood folate concentration in healthy populations. **Methods and Study Design:** Studies focusing on MTHFR A1298C polymorphism and folate concentrations were identified and subjected to a meta-analysis using Review Manager 5.1. Standard mean differences (SMD) with 95% confidence intervals (95% CI) were used to assess the association between these variables. **Results:** A total of 14 studies with 5616 healthy individuals were included in this meta-analysis. Significant differences in folate concentration were found in the MTHFR homozygote model (SMD=0.12, 95% CI=0.00-0.24, I^2=17%, p=0.04) and the dominant model (SMD=0.07, 95% CI=0.01-0.14, I^2=22%, p=0.02) in the general population excluding the elderly. While abnormal folate concentrations are more common in elderly, no association between MTHFR A1298C polymorphism and peripheral blood folate concentration was found in the meta-analysis when elderly were included. **Conclusions:** This meta-analysis indicates that, in the general population excluding the elderly, the C allele of MTHFR 1298 polymorphism is associated with the risk for an increased folate concentration.

Key Words: methylenetetrahydrofolate reductase, A1298C, polymorphism, folate concentration, meta-analysis

INTRODUCTION

Folate is an essential water-soluble vitamin (vitamin B9) that is found in green leafy vegetables, cereals, legumes, and fruit.[1] As a major methyl group donor, it plays an important role in one-carbon metabolism and is involved in DNA, RNA, and protein synthesis, in addition to its significant role in energy production and normal cell division.[2] Research indicates that low blood folate concentrations are associated with an increased risk for neural tube defects during pregnancy.[3-5] Epidemiological and experimental studies have reported that an abnormal folate intake or status correlates with an increased risk for several types of cancer,[6] such as breast cancer.[7] 5,10-Methylenetetrahydrofolate reductase (MTHFR) is involved in folate metabolism and numerous studies have reported that mutations in the gene that encodes this enzyme increase the risk for congenital anomalies,[8] such as occlusive vascular disease, colorectal cancer, breast cancer, prostate cancer, neural tube defects, acute leukemia, and schizophrenia.[1,9-13] Because of the serious consequences associated with abnormalities in MTHFR, it is important to determine how MTHFR polymorphism affects blood folate concentrations

The *MTHFR* gene is located on chromosome 1p36.3 and includes 11 exons, and spans 2.2 kb (GenBank accession number: U09806). MTHFR is a key enzyme in the folate metabolism pathway, which regulates intracellular folate concentrations. *In vivo*, it irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate,[14,15] the circulating and physiologically active form of folate, which serves as a methyl donor for homocysteine remethylation to methionine, at the expense of DNA and RNA biosynthesis.[6] Normal MTHFR activity may contribute to maintaining the pool of circulating folate

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and methionine and possibly prevent a buildup of homocysteine.[8] Studies have indicated that specific mutations in the *MTHFR* gene could change the activity of this enzyme. At least 23 polymorphisms in the *MTHFR* gene have been identified; MTHFR C677T and A1298C are the two most common functional polymorphisms,[16] although the latter has been studied less.

Studies have reported that the frequency of A1298C homozygosity among Canadian and Dutch control populations is approximately 9%,[8,17,18] while in a healthy Chinese population, it was found to be 5%.[19] This substitution of an adenine (A) to a cytosine (C) at position 1298 (in exon 7) results in the conversion of a glutamic acid (E) codon to an alanine (A) codon.[20,21] The enzyme activity of those homozygous for MTHFR A1298C (CC genotype) is reduced by approximately 30-40% compared with that of the wild type (AA genotype).[18,22]

In the last decade, a number of studies have focused on the association between MTHFR A1298C polymorphism and peripheral blood folate concentration, but conflicting results have been reported.[23,24] The inconsistencies in results may be because of differences in sample sizes, study designs, ethnicities, and random errors. It is important to evaluate the magnitude of the impact that the MTHFR A1298C polymorphism may have on folate concentrations as it is significant for determining the population-level risk for some diseases, such as neural tube defects.

The aim of this study was to determine the association between the MTHFR A1928C genotype and peripheral blood folate concentration among normal populations. To this end, we conducted a meta-analysis to develop summary estimates of differences between blood folate concentrations by genotype with data from observational studies.

METHODS

Literature search

We searched the PubMed and Embase databases for all articles on the association between the MTHFR A1298C polymorphism and folate concentration published up until June 2016. We used the following key words: "methylenetetrahydrofolate reductase/ MTHFR/ A1298C/ 1298A>C/ rs1801131," "blood folic acid/ blood folate/ plasma folic acid/ plasma folate/ serum folic acid/serum folate," and "folic acid concentration/ folate concentration/ folic acid level/ folate level," limiting the results to those for humans, regardless of sample size. References in the identified reports were also checked and relevant additional literature was included. Two authors independently conducted literature searches and selected studies for inclusion. Any differences in opinion were resolved by discussion among all of the authors.

Inclusion criteria and exclusion criteria

All studies included in this meta-analysis had to meet the following criteria: (1) cohort or case-control; (2) populations satisfied randomization or had healthy controls and ignored gender or age differences (studies with mixed genders and ages would be included if the pregnancy or lactation status of their population was not stated explicitly); (3) study outcomes including folate concentration (plasma, serum, or red blood cell folate), folate assay method, and MTHFR A1298C genotype frequency; and (4) sufficient folate concentration data available to calculate mean concentrations with standard deviations. Studies were excluded if they failed to meet any of the inclusion criteria or if sufficient data were not provided.

Data extraction

All available data were extracted from the articles included in this meta-analysis by two independent authors according to the inclusion criteria. Data collected included first author's name, publication year, country of origin, study design, participant characteristics (ethnicity, gender, and age), number of samples grouped by MTHFR A1298C genotype, tissue sampled, and analysis method. Studies that presented folate concentration results without the mean concentration or without the standard deviation or variance were excluded. Data with only sample sizes and 95% confidence intervals were converted to the mean and standard deviations using the calculator tools in Review Manager (ver. 5.1; Cochrane Collaboration, Oxford, UK). Findings were grouped using a specific data extraction template.

Statistical analysis

Extracted data on folate concentrations presented as mean±standard deviations were pooled and analyzed using Review Manager (ver. 5.1). Folate concentrations were converted to nmol/L if provided originally as ng/mL using the equation: 1 ng/mL=2.266 nmol/L. Continuous outcomes are presented as the standard mean difference (SMD) with the 95% confidence interval (95% CI). A p-value <0.05 was considered statistically significant. Heterogeneity among studies was assessed using the chi-square test of heterogeneity and the I^2 measure of inconsistency, and $p>0.1$ with $I^2<50\%$ was considered to indicate that there was little heterogeneity among studies. Fixed effects models were used when there was no evidence of heterogeneity; otherwise, a random effects model was used. Stratified analyses were also performed. Funnel plots were used to evaluate publication bias. To explore the source of heterogeneity among studies, meta-regression was performed. Sensitivity analysis was performed to determine the influence of each individual study on the pooled results. Both the meta-regression and sensitivity analyses were conducted using Stata Statistical Software (ver. 12.0; StataCorp, College Station, TX).

RESULTS

Characteristics of the studies

Through the systematic computer-based search, we identified a total of 264 references. After a review of the titles and the removal of duplicate studies, 54 studies were retrieved for full text analysis. Of these, two articles were excluded because they did not focus on the A1298C polymorphism; six articles were excluded because of a lack of randomization or healthy controls; 21 articles were excluded because they lacked data on the folate concentration by genotype; six articles were excluded because they lacked data on the population size by genotype; one article was excluded because it lacked standard deviation data. Two additional articles were included after a refer-

ence review.

Overall, 14 publications were finally considered to be eligible for this meta-analysis (seven case-control and seven cohort studies), which included 5616 individuals. The process for selecting studies and reasons for exclusion are presented in Figure 1. Selected characteristics of these studies and their associations with folate concentrations are summarized in Table 1. In the seven case-control studies, information on the control group regarding MTHFR A1298C polymorphism distributions and folate concentrations was extracted. In the study by Hiraoka et al 2004,[21] data from two groups were extracted, called Hiraoka-Y (young) and Hiraoka-E (elderly). Among the 14 studies, there were six studies on Caucasians, six on Asians, and two on a mixture of ethnicities. Participants in four studies were all female, two studies were all male, and eight studies were both male and female. For age, mean ages of individuals from four studies were all above 55 years old, and the others were below 55 years. The sample sizes of nine studies were fewer than 500 people per study, while the other five studies had more than 500 people per study. For types of samples tested, there were two studies on plasma and 12 on serum. For the folate assay method, eight studies used chemiluminescent immunoassay, two used microbial assay, two used radioimmunoassay, and the remaining two used enzyme immunoassay.

Meta-analysis results

For genotypes, the 14 studies included 2728 individuals with MTHFR 1298AA, 2281 with MTHFR 1298AC, and 607 with MTHFR 1298CC, who were pooled for the meta-analysis to evaluate the association between MTHFR1298 polymorphism and folate concentration. We used the AA genotype as a reference category. Comparisons were performed using three models: heterozygote (MTHFR 1298: AC vs AA), homozygote (MTHFR 1298: CC vs AA), and dominant (MTHFR 1298: AC + CC vs AA). There was clear heterogeneity under all models (I^2>50%, p<0.1), so a random effects model was conducted to pool the results. Among these genetic models, no statistically significant evidence of an association between MTHRF A1298C polymorphism and folate concentration was found in heterozygote (SMD=0.03, 95% CI=−0.07-0.13, I^2=60%, p=0.58), homozygote (SMD=0.35, 95% CI=−0.15-0.85, I^2=95%, p=0.17), and dominant models (SMD=0.07, 95% CI=−0.03-0.16, I^2=60%, p=0.16) (Figure 2). Publication bias of the selected studies was explored using funnel plots, the results of which identified the existence of bias in these comparisons (Figure S10a-c).

Considering the high heterogeneity in these analysis models, we performed subgroup analysis by ethnicity, gender, age of participants, study design, sample size, tissue sampled, and folate assay method for these models. Stratified analyses are shown in Table 2. When stratified by gender, there was a significant difference in folate concentrations between MTHFR 1298 AC and AA in the male subgroup (SMD=−0.25, 95% CI=−0.45-−0.05, I^2=0%, p=0.01) (Figure S2a). There was a significant difference in folate concentrations between MTHFR 1298 AC + CC and AA both in the male and female subgroup and in the male subgroup (SMD=0.13, 95% CI=0.03-0.23, I^2=37%, p=0.008 and SMD=−0.29, 95% CI=−0.53-−0.05, I^2=27%, p=0.02, respectively) (Figure S2c). When stratified by age, there was a significant difference in folate concentrations between MTHFR 1298 AC + CC and AA in the younger than 55 years subgroup (SMD=0.08, 95% CI=0.01-0.16, I^2=22%, p=0.03) (Figure S3c). For subgroups stratified by study design, there was a signifi-

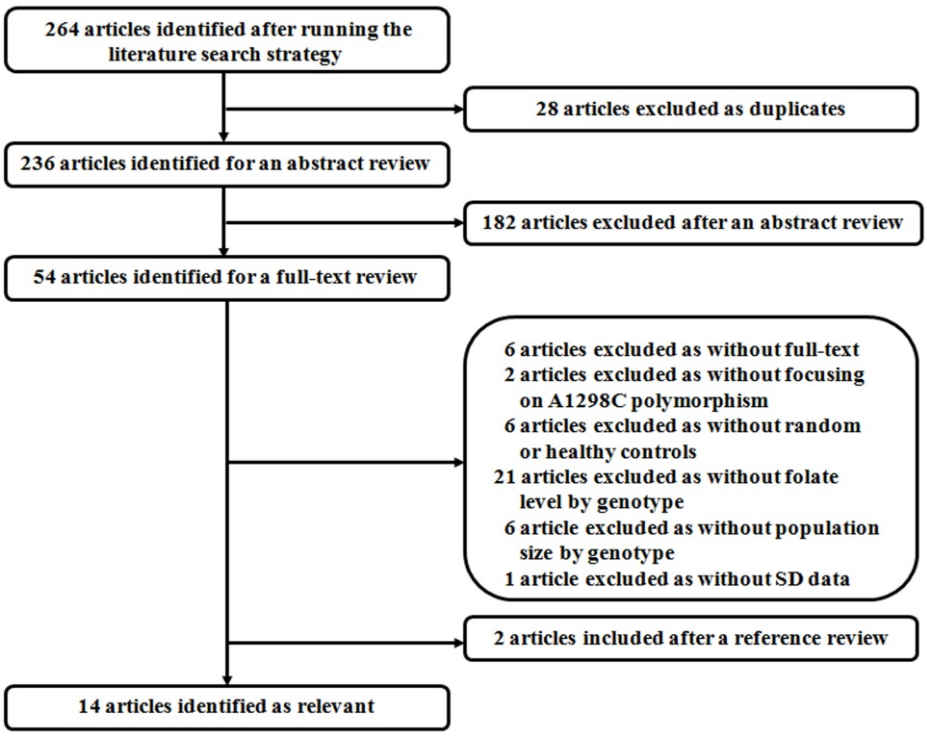

Figure 1. Flow diagram of included and excluded studies

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

Study	Year	Country	Ethnicity	Study design	Gender	Age	Population size n (%)			Tissue sampled	Folate assay method	Genotyping method
							AA	AC	CC			
Chango[6]	2000	France	Caucasian	Cohort	M/F	27-47[†]	31 (46.9)	27 (41.0)	8 (12.1)	Serum	Chemiluminescent immunoassay	PCR-RFLP
Esfahani[4]	2003	USA	mixed	Cohort	F	33±15	132 (61.4)	76 (35.3)	7 (3.3)	Serum	Microbial assay	PCR-RFLP
Hiraoka-Y[21]	2004	Japan	Asian	Cohort	F	21±1.6	172 (68.8)	74 (29.6)	4 (1.6)	Serum	Chemiluminescent immunoassay	PCR-RFLP
Hiraoka-E[21]	2004	Japan	Asian	Cohort	F	66±10	34 (68.0)	16 (32.0)	0 (0%)	Serum	Chemiluminescent immunoassay	PCR-RFLP
Devlin[7]	2006	UK	Caucasian	Cohort	M/F	77.9±0.2	478 (45.9)	466 (44.8)	97 (9.3)	Serum	Microbial assay	PCR-RFLP
Lee[25]	2006	Korea	Asian	Case-control	M/F	---[‡]	145 (61.9)	75 (32.1)	14 (6.0)	Plasma	Radio immunoassay	PCR-RFLP
Angeline[26]	2007	India	Asian	Case-control	M	---[‡]	48 (48.0)	38 (38.0)	14 (14.0)	Serum	Chemiluminescent immunoassay	PCR-RFLP
Ozarda[27]	2009	Turkey	Caucasian	Cohort	M/F	18-45	182 (45.3)	168 (41.8)	52 (12.9)	Serum	Chemiluminescent immunoassay	CVD Strip Assay
Safarinejad[28]	2010	Iran	Caucasian	Case-control	M	62.5±14.2	158 (45.4)	150 (43.1)	40 (11.5)	Serum	Chemiluminescent immunoassay	PCR-RFLP
Biselli[29]	2010	Brazil	Caucasian	Case-control	M/F	57.8±12.3	54 (50)	49 (45.4)	5 (4.6)	Plasma	Competitive immunoassay	Allele-specific amplification
Tripathi[30]	2010	India	Asian	Case-control	M/F	35.8±11.1	347 (60.9)	204 (35.9)	18 (3.2)	Serum	Multiparticular enzyme immunoassay	PCR-RFLP
Yakub[31]	2012	Pakistan	Asian	Cohort	M/F	18-60[*]	181 (20.8)	425 (48.7)	266 (30.5)	Serum	Radioassay	PCR-RFLP
Akilzhanova[1]	2013	Kazakhstan	Asian/ Caucasian	Case-control	F	40.5±13.4	318 (52.6)	242 (40.1)	44 (7.3)	Serum	Chemiluminescent immunoassay	TaqMan
Zappacosta[32]	2014	Italy	Caucasian	Cohort	M/F	40.6±10.6	96 (48.7)	86 (43.7)	15 (7.6)	Serum	Chemiluminescent immunoassay	PCR-RFLP
Lu[33]	2015	China	Asian	Case-control	F	47.3±8.9	352 (62.9)	185 (33.0)	23 (4.1)	Serum	Chemiluminescent immunoassay	TaqMan

PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism.
[†]Min-max age.
[‡]Data not shown.

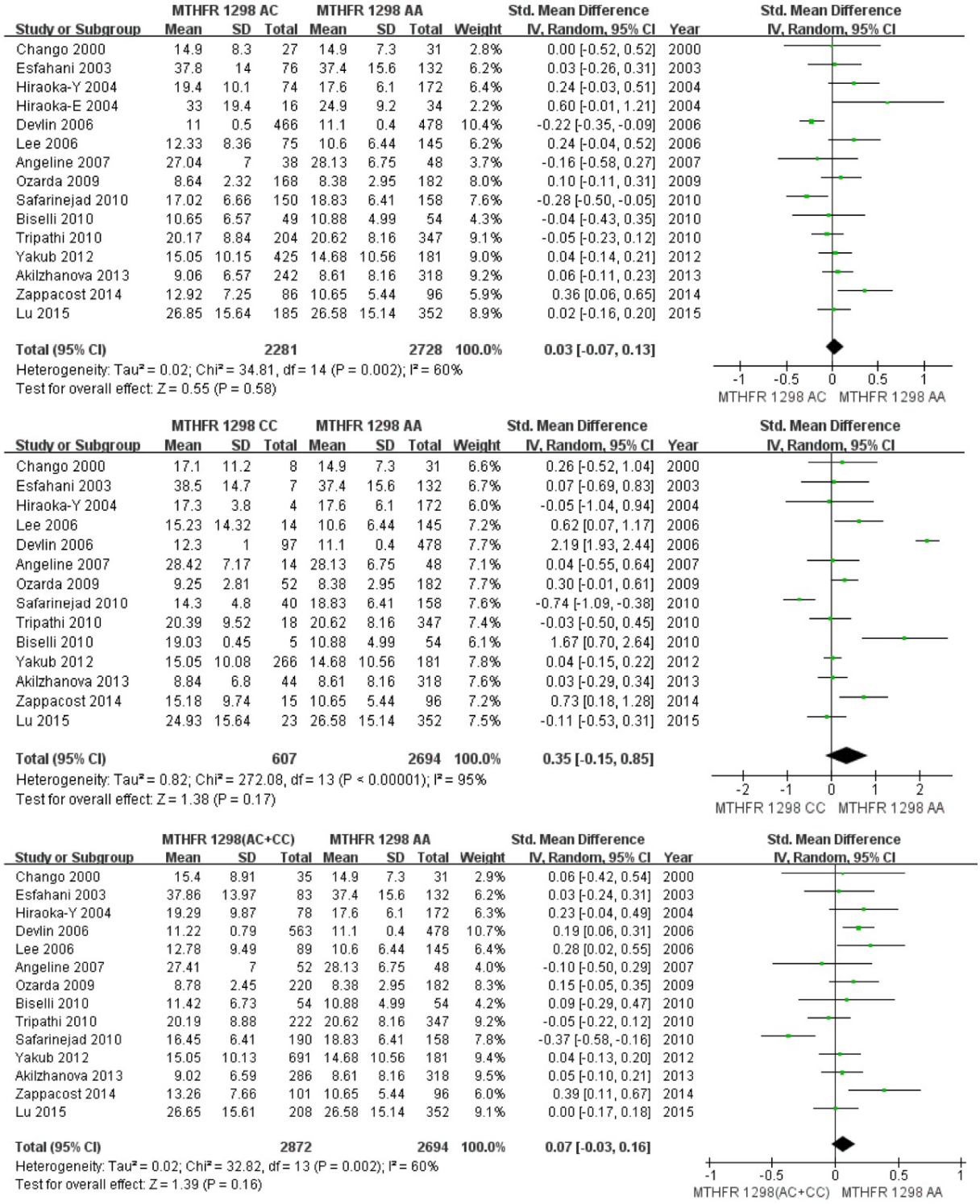

Figure 2. Forest plot analyses for MTHFR A1298C polymorphism and folate concentration in three genetic models: heterozygote (AC vs AA), homozygote (CC vs AA), and dominant (AC + CC vs AA).

cant difference in folate concentrations between MTHFR 1298 AC + CC and AA in the cohort subgroup (SMD=0.15, 95% CI=0.07-0.23, I^2=2%, p=0.0001) (Figure S4c). For subgroups stratified by the tissue sampled, there was a significant difference in folate concentrations between MTHFR 1298 AC + CC and AA in the serum subgroup (SMD=0.09, 95% CI=0.02-0.16, I^2=26%, p=0.01) (Figure S6c). We also identified a significant difference in folate concentrations between MTHFR 1298 AC + CC and AA in the microbial assay subgroup stratified by folate assay method (SMD=0.16, 95% CI=0.04-0.28, I^2=4%, p=0.008) (Figure S7c). No other significant associations were found in any subgroup under the genetic models described above. Forest plots for the subgroup analyses are shown in Figures S1-7.

There was heterogeneity among studies in overall comparisons and also subgroup analyses. To explore the sources of heterogeneity, we evaluated the following variables using meta-regression: ethnicity, gender, age, study design, sample size, tissue sampled, and folate assay method. We did not observe any source of heterogeneity (all p>0.05) (Figure S8).

Table 2. Stratified analyses of MTHFR A1298C polymorphism on blood folate concentrations in three genetic models

Variables	Heterozygote model AC vs AA				Homozygote model CC vs AA				Dominant model AC + CC vs AA			
	n†	Sample size	SMD (95% CI)	*p*‡	n†	Sample size	SMD (95% CI)	*p*‡	n†	Sample size	SMD (95% CI)	*p*‡
MTHFR A1298C	15	2281/2728	0.03 (-0.07, 0.13)	0.58	14	607/2694	0.35 (-0.15, 0.85)	0.17	14	2872/2694	0.07 (-0.03, 0.16)	0.16
Ethnicity												
Asia	7	1017/1279	0.07 (-0.05, 0.19)	0.24	6	339/1245	0.05 (-0.10, 0.20)	0.49	6	1340/1245	0.05 (-0.05, 0.15)	0.32
Caucasian	6	946/999	-0.03 (-0.23, 0.17)	0.76	6	217/999	0.73 (-0.36, 1.81)	0.19	6	1163/999	0.08 (-0.14, 0.30)	0.46
Mixed	2	318/450	0.05 (-0.09, 0.20)	0.49	2	51/450	0.03 (-0.26, 0.33)	0.81	2	369/450	0.05 (-0.09, 0.19)	0.49
Gender												
Male and female	8	1500/1514	0.03 (-0.11, 0.18)	0.63	8	475/1514	0.71 (-0.01, 1.43)	0.05	8	1975/1514	0.13 (0.03, 0.23)	<0.01**
Male	2	188/206	-0.25 (-0.45, -0.05)	0.01*	2	54/206	-0.39 (-1.15, 0.37)	0.32	2	242/206	-0.29 (-0.53, -0.05)	0.02*
Female	5	593/1008	0.09 (-0.03, 0.21)	0.13	4	78/974	-0.01 (-0.25, 0.22)	0.91	4	655/974	0.06 (-0.04, 0.16)	0.25
Age												
<55 years old	11	1600/2004	0.07 (-0.00, 0.14)	0.06	11	465/2004	0.14 (-0.00, 0.28)	0.06	11	2065/2004	0.08 (0.01, 0.16)	0.03*
>55 years old	4	681/724	-0.12 (-0.34, 0.11)	0.30	3	142/690	1.03 (-1.17, 3.23)	0.36	3	807/690	-0.03 (-0.43, 0.36)	0.87
Study Design												
Case-control	7	943/1422	-0.02 (-0.13, 0.09)	0.71	7	158/1422	0.11 (-0.31, 0.52)	0.60	7	1101/1422	-0.02 (-0.16, 0.12)	0.78
Cohort	8	1338/1306	0.09 (-0.08, 0.26)	0.28	7	449/1272	0.53 (-0.29, 1.35)	0.21	7	1771/1272	0.15 (0.07, 0.23)	<0.001***
Sample size												
Less than 500	10	759/1052	0.09 (-0.06, 0.24)	0.25	9	159/1018	0.28 (-0.15, 0.71)	0.20	9	902/1018	0.08 (-0.09, 0.26)	0.34
More than 500	5	1522/1676	-0.04 (-0.16, 0.07)	0.49	5	448/1676	0.43 (-0.55, 1.41)	0.39	5	1970/1676	0.06 (-0.03, 0.15)	0.19
Tissue Sampled												
Plasma	3	274/357	-0.03 (-0.37, 0.30)	0.85	3	59/357	0.46 (-0.86, 1.78)	0.49	3	333/357	-0.01 (-0.45, 0.43)	0.97
Serum	12	2007/2371	0.04 (-0.06, 0.14)	0.44	11	548/2337	0.33 (-0.22, 0.88)	0.24	11	2539/2337	0.09 (0.02, 0.16)	0.01*
Folate assay method												
Chemiluminescent immunoassay	9	986/1391	0.07 (-0.07, 0.21)	0.32	8	200/1357	0.04 (-0.29, 0.37)	0.83	8	1170/1357	0.05 (-0.11, 0.20)	0.55
Microbial assay	2	542/610	-0.13 (-0.36, 0.10)	0.27	2	104/610	1.16 (-0.91, 3.23)	0.27	2	646/610	0.16 (0.04, 0.28)	<0.01**
Radio immunoassay	2	500/326	0.11 (-0.08, 0.30)	0.27	2	280/326	0.27 (-0.29, 0.83)	0.35	2	780/326	0.14 (-0.10, 0.37)	0.26
Enzyme immunoassay	2	253/401	-0.05 (-0.21, 0.11)	0.53	2	23/401	0.77 (-0.89, 2.43)	0.37	2	276/401	-0.03 (-0.18, 0.13)	0.73

SMD: standard mean difference; CI: confidence interval.
†Number of comparisons.
‡*p*-value of Z test for pooled SMD. SMD values that reached statistical significance are shown in bold ($p<0.05$).
*$p<0.05$; **: $p<0.01$; ***: $p<0.001$.

To determine the stability of the results, sensitivity analyses were performed to assess the influence of each individual study on the pooled results. When Safarinejad et al (2010)[28] was excluded, there was a significant difference in the dominant model (Figure S9c).

From stratified analysis by age, there was a significant difference in folate concentrations in the dominant model in populations younger than 55 years old. As sensitivity analysis suggested that the study by Safarinejad et al (2010)[28] had a high sensitivity, we explored the potential sources of heterogeneity. To this end, we removed four studies (Safarinejad et al 2010[28], Devlin et al 2006[7], Biselli et al 2010[29], and Hiraoka-E et al 2004[21]) in which mean ages of the studied individuals were all above 55 years. After exclusion, we reassessed the meta-analyses. The heterogeneity disappeared and significant differences in the homozygote (SMD=0.12, 95% CI=0.00-0.24, I^2=17%, p=0.04) and dominant models (SMD=0.07, 95% CI=0.01-0.14, I^2=22%, p=0.02) were found. No significant difference in folate concentrations was found in MTHFR 1298 (AC vs. AA) (SMD=0.07, 95% CI=−0.00-0.13, I^2=3%, p=0.06) (Figure 3). Publication bias of the selected studies was explored using funnel plots; with the results showing no bias in these comparisons (Figure S10d-f).

DISCUSSION

This study was a comprehensive meta-analysis focusing on the correlation between MTHFR A1298C polymorphism and peripheral blood folate concentration. The results suggest that, in the general population excluding the elderly, C-allele carriers might be at risk for an increased

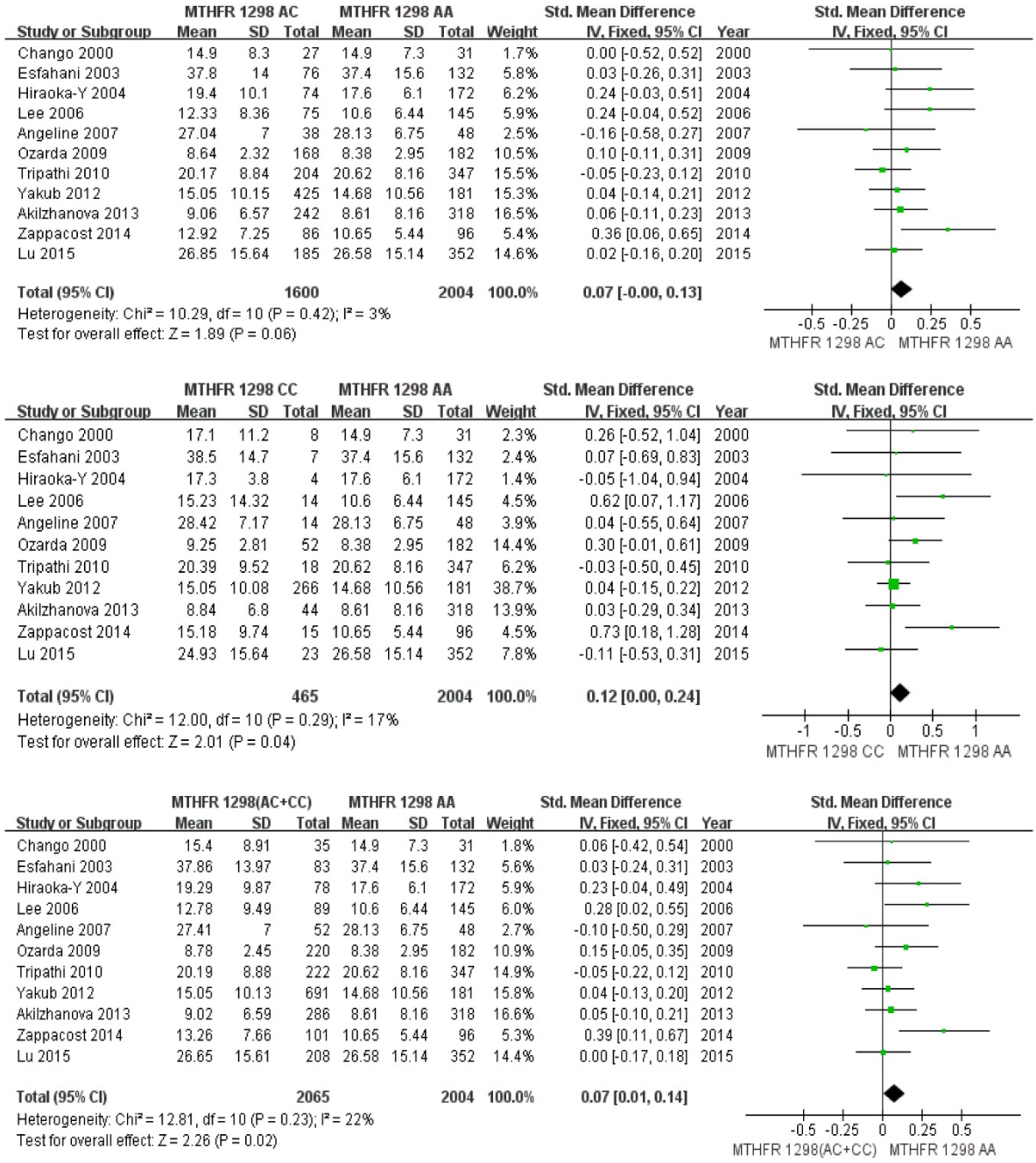

Figure 3. Forest plot analyses for MTHFR A1298C polymorphism and folate concentration in three genetic models: heterozygote (AC vs AA), homozygote (CC vs AA), and dominant (AC + CC vs AA) with four studies removed.

folate concentration compared with AA homozygotes.

As a methyl group donor, folate plays a pivotal role in one-carbon metabolism, being involved in the synthesis, repair, and methylation of DNA. MTHFR is one of the main enzymes regulating folate metabolism. In humans, over 40 point mutations have been identified in the MTHFR gene, with A1298C (rs1801131) being one of the most studied. The C variant leads to alanine taking the place of glutamic acid, which affects the conversion of methyltetrahydrofolate to tetrahydrobiopterin.[34] MTHFR A1298C is believed to diminish enzyme activity,[17,18,34,35] which in turn affects folate metabolism in cells.[36,37] If the enzymatic activity of MTHFR is reduced, the capacity for processing folate to a usable form decreases, resulting in a decreased methyltetrahydrofolate concentration.[37] A mutation in the MTHFR gene can result in a highly reduced folic acid conversion ability.[6,17,38,39] Studies have reported that excess folic acid might increase the risk for cancer and mask some types of anemia.[40-42] As people with MTHFR gene mutations have difficulty converting folic acid to methylfolate, they might be at increased risk for cancer or other associated diseases resulting from the accumulation of folic acid. Although several hypotheses have been proposed to explain the influence of MTHFR A1298C polymorphism on peripheral blood folate concentration, as yet there is no clear evidence for a link between MTHFR polymorphism and folate concentration.[24,30] Inconsistent results are possibly because of the polymorphism having a small effect on the folate concentration or the relatively low statistical power of the reported studies.

In the current study, we performed a meta-analysis to evaluate the influence of MTHFR A1298C polymorphism on folate concentration. The meta-analysis included 14 studies with 5616 individuals. Results from sensitivity and stratified analyses suggested that the study by Safarinejad et al (2010)[28] had a high sensitivity and that there was a significant difference between MTHFR 1298 AC + CC and AA in blood folate concentrations in the younger than 55 years group. Studies have reported that low folate concentrations are more common in the elderly.[43] These results suggest that age may be a factor influencing the effect of MTHFR A1298C polymorphism on folate concentrations. Therefore, we removed four studies (Safarinejad et al 2010[28], Devlin et al 2006[7], Biselli et al 2010[29], and Hiraoka-E et al 2004[21]) in which the studied population was elderly; and found that their exclusion resulted in an absence of heterogeneity in the three models. Significant differences in folate concentrations were then found in homozygote (CC vs AA) and dominant (AC + CC vs AA) comparisons. Our meta-analysis indicated that, in the general population excluding the elderly, the C allele in MTHFR 1298 polymorphism is associated with the risk for an increased folate concentration. A previous study confirmed that, compared with the AA genotype, the MTHFR enzyme activity of those with the CC genotype is reduced by approximately 40%.[22]

In this meta-analysis, no association between MTHFR A1298C polymorphism with peripheral blood folate concentration was found in the MTHFR A1298C heterozygote (AC vs AA), homozygote (CC vs AA), and dominant models (AC + CC vs AA) when all studies were included. It is known that folate intake (dietary or supplementary) interacts with gene polymorphisms in the folate metabolism pathways. As potential sources of heterogeneity, individual characteristics (age, gender) could possibly complicate results. We found no association between MTHFR A1298C polymorphism and folate concentration in subgroup analysis by ethnicity, gender, age, study design, sample size, tissue sampled, and folate assay method, apart from for several sets of subgroups in heterozygote or dominant comparisons. These results suggest that gender, age, study design, tissue sampled or folate assay method might make substantial contributions to heterogeneity. The identification of heterogeneity among studies led us to explore its potential sources for all three models. However, we did not discover any source of heterogeneity with meta-regression. One reason for this is that the number of included studies was not large enough.

From stratified analyses, we found that the effect of MTHFR A1298C polymorphism on folate concentration may differ between genders or among tissues, although the sample sizes of the subgroups in this study were insufficient to obtain statistically significant results. Given that methods for genotype assays have changed over the years with the development of technology, all data included in these comparisons were obtained by different methods and the random effect was taken as a priority method for analysis. However, for the evaluation of a continuous variable, the incorporation of random effects increased the likelihood of accounting for inter-study heterogeneity and addressed the potential correlation among the reported results.

In the current study, screening and selection of eligible studies were conducted in accordance with a rigorous protocol. Asian and Caucasian populations were included in this meta-analysis. Upon the exclusion of three studies that featured a large number of elderly people, no evidence of publication bias was identified using funnel plot analysis. These findings support the reliability of our analysis.

This study did have some limitations. First, the sample size was small and insufficient for subgroup studies. Second, only published data were selected, with unpublished and ongoing studies not being included, which may have biased the results. Third, this meta-analysis was limited to only a single polymorphism, and other mutations of the *MTHFR* gene should be included in further analysis. Finally, certain factors, such as age, folate intake, and personal behaviour, were not considered here.

In conclusion, this meta-analysis indicates that, in the general population excluding the elderly, the C allele of MTHFR 1298 polymorphism is associated with the risk for an increased folate concentration. Further studies with larger sample sizes and high-quality, unified methods are required to identify and understand the mechanisms behind this association.

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AUTHOR DISCLOSURES

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