

Association of *MTHFR* C677T and A1298C polymorphisms with oral cancer susceptibility: evidence from a meta-analysis

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Abstract: Methylenetetrahydrofolate reductase (*MTHFR*) is a central enzyme involved in folate metabolism and plays an important role in DNA synthesis and methylation. Several studies have been conducted to illustrate the associations between *MTHFR* C677T and A1298C polymorphisms with oral cancer susceptibility; however, the results are inconsistent. Therefore, we conducted an updated meta-analysis to obtain a more reliable estimation of the associations. We retrieved eligible studies from PubMed, EMBASE, and CBM databases through September 2016. Ultimately, pooled analyses involved 10 studies with 1443 cases and 1640 controls for the C677T polymorphism, as well as five studies with 973 cases and 1024 controls for the A1298C polymorphism. Risk estimates were presented as odds ratios (ORs) and 95% confidence intervals (95% CIs). Pooled results indicated that neither C677T nor A1298C polymorphism was associated with oral cancer susceptibility. However, a borderline significant association was detected between *MTHFR* C677T polymorphism and a decreased oral cancer risk (homozygous model: OR=0.71, 95% CI=0.50–1.00) in hospital-based studies. Our results suggested that *MTHFR* C677T and A1298C polymorphisms might not be associated with oral cancer risk. However, more evidence is needed to further confirm these findings in the future.

Keywords: *MTHFR*, polymorphisms, oral cancer, susceptibility, meta-analysis

Introduction

Cancer is a major public health burden. An estimated 14.1 million new cancer cases and 8.2 million cases of cancer deaths arose worldwide, according to the data from GLOBOCAN 2012.¹ Oral cancer, a subtype of head and neck cancer, is one of the most frequently diagnosed human malignancies in the world. Lines of evidence suggest that smoking,² alcohol drinking,³ insufficient fruits and vegetables intake,⁴ and oral HPV infection⁵ are well-established risk factors for oral cancer. However, only a small part of exposed people eventually develop oral cancer. One acceptable explanation for this phenomenon is that genetic variations also modify individuals' risk of developing cancer, beside health-risk environmental exposures and lifestyles.⁶

Previous studies demonstrated that folic acid deficiency resulting from low intake of fruits and vegetables increases the risk of developing cancer.^{7,8} In humans, folate provides the majority of methyl groups for de novo deoxynucleoside synthesis and intracellular methylation reactions.⁹ Folate deficiency may lead to uracil misincorporation during DNA synthesis, thus impairing the stability of DNA.¹⁰ Folate-metabolizing enzyme, methylenetetrahydrofolate reductase (*MTHFR*), is a key component in folate acid cycle and DNA synthesis. *MTHFR* catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, donating a methyl group for the

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methylation of homocysteine to methionine.¹¹ Dysfunction of MTHFR may disrupt the dynamic equilibrium of methyl pool and consequently influence the deoxynucleoside synthesis and intracellular methylation reactions.

The human *MTHFR* gene is located on chromosome 1p36.3. By now, two common nonsynonymous *MTHFR* single-nucleotide polymorphisms (SNPs), C677T (Ala222-Val, rs1801133) and A1298C (Glu429Ala, rs1801131), have been widely investigated.^{12,13} It has been shown that C677T and A1298C polymorphisms are associated with the risk of several types of cancer, including breast cancer,¹⁴ colorectal cancer,¹⁵ non-Hodgkin's lymphoma,¹⁶ and cervical cancer.¹⁷ Different research groups have extensively assessed the association of C677T and A1298C polymorphisms with oral cancer risk, yet produce discrepant results.^{18,19} Four meta-analyses regarding such association have been conducted previously but somehow fail to establish reliable relationship between the two *MTHFR* SNPs and oral cancer risk.^{20–23} Therefore, we performed this evidence-based quantitative meta-analysis to provide a precise evaluation of the associations by including more eligible investigations.

Materials and methods

Literature search

We performed a comprehensive literature search in the PubMed and EMBASE, as well as Chinese databases (China National Knowledge infrastructure and WANFANG). No language limitation was applied in the literature search. The last research was updated in September 2016. The following search terms were adopted: “MTHFR or methylenetetrahydrofolate reductase”, “polymorphism or polymorphisms or SNP or single nucleotide polymorphism or variant”, and “oral cancer or oral carcinoma”. We also manually searched references listed in the retrieved articles after carefully reviewing. The latest or the largest study was included in the final meta-analysis, if there exist two or more articles containing overlapping data.

Inclusion/exclusion criteria

We used the following standards to select eligible articles: 1) studies evaluating the association of *MTHFR* C677T and A1298C polymorphisms with oral cancer risk; 2) case-control design; 3) sufficient data provided to compute odds ratios (ORs) and 95% confidence intervals (95% CIs); and 4) in the controls, genotype frequency of *MTHFR* C677T, A1298C, or other SNPs in agreement with Hardy–Weinberg equilibrium (HWE). Exclusion criteria were as follows: 1) case-only studies or case reports; 2) reviews or

meta-analyses; 3) studies without detailed genotyping data; and 4) duplicate publications.

Data extraction

Two investigators (Zhu-Ming Hua and Zhen-Jian Zhuo) independently extracted data from the eligible studies. For conflicting results, a consensus was achieved after thorough discussion. The following characteristics were selectively extracted from each study: first author's surname, year of publication, country, ethnicity, the source of controls, genotyping methods, and genetic distribution of cases and controls. The stratification analysis was conducted by ethnicity (Asians and Caucasians) and control source (hospital based and population based).

Statistical methods

Chi-square test was used to check the genotype frequency of SNPs among the controls, with $P < 0.05$ suggesting a departure from HWE. The strength of association between *MTHFR* C677T and A1298C polymorphisms and oral cancer risk was assessed by calculating pooled ORs and 95% CIs. The following five genetic models were applied: homozygote model (C677T: TT vs CC; A1298C: CC vs AA), heterozygote model (C677T: CT vs CC; A1298C: AC vs AA), recessive model (C677T: TT vs CT/CC; A1298C: CC vs AC/AA), dominant model (C677T: CT/TT vs CC; A1298C: AC/CC vs AA), and allele contrast (C677T: T vs C; A1298C: C vs A). Stratification analyses were also performed by ethnicity (Asians and Caucasians) and source of control (hospital based and population based). We performed chi-square-based Q statistic test and inconsistency index statistics (I^2) to calculate between-study heterogeneity. If the studies were found to be homogeneous (with $P_{\text{het}} > 0.10$ or $I^2 < 50\%$), ORs were estimated using the fixed-effects model (the Mantel–Haenszel method). Otherwise, the random-effects model (the DerSimonian and Laird method) was adopted. Sensitivity analysis was performed by sequentially removing one study at a time and reanalyzing the pooled estimates, in order to exclude single studies that have significant influences on the overall ORs. Furthermore, potential publication bias was estimated by Begg's funnel plot as well as Egger's linear regression test. An asymmetric plot and a P -value < 0.05 indicated the existence of publication bias. All the data management and statistical analysis were performed using STATA 11.0 software (Stata Corporation, College Station, TX, USA). All statistical tests were two-sided. A P -value of < 0.05 was set as a significant threshold.

Trial sequential analysis (TSA)

To avoid the random errors caused by dispersed data and repeated significance testing, we performed the TSA to obtain a more reliable conclusion.²⁴ We only applied TSA to *MTHFR* C677T polymorphism, but not A1298C polymorphism, as the latter involves less than six studies. We calculated the required information size and estimated the sample size needed to make a robust conclusion. The required information size was evaluated by considering an overall type I error (α) of 5% and type II error (β) of 20%. Based on required information size as well as risk for type I and type II errors, TSA monitoring boundaries were constructed. If the cumulative Z-curve (blue line) crosses a TSA monitoring boundary (red lines sloping inward) before the required information size is reached, the robustness of evidence may be confirmed and no further trials are necessary. Otherwise, the evidence is inadequate to get a robust conclusion and it is necessary to continue to do trials. TSA software version 0.9 beta was used.

Results

Selection of studies

The identification and selection of studies are graphically presented in Figure 1. A total of 41 potentially relevant published records were initially retrieved from PubMed and EMBASE, and only one article was identified from Chinese

databases. Among them, 25 articles were excluded after screening the titles and abstracts, and 17 remaining studies were further subjected to full-text assessment. Among them, seven studies were removed for the following reasons: five were meta-analyses or reviews,^{6,20–23} one was duplication publication,²⁵ and one lacked description of oral cancer.²⁶ Finally, 10 publications were included in the meta-analysis, of which 10 studies involved C677T polymorphism^{18,19,27–34} and five involved A1298C polymorphism.^{18,19,28,29,32}

Study characteristics

The study characteristics of the final selected studies are shown in Table 1. Ten studies with 1443 cases and 1640 controls for the C677T polymorphism were ultimately included in the meta-analysis. Among them, seven studies were conducted in Caucasians and three in Asians. As to the source of control, four were population-based studies and six were hospital based. Moreover, there were five studies for the A1298C polymorphism with 973 cases and 1024 controls. Of these studies, three were conducted in Caucasians and two in Asians. All of them were hospital-based design.

Meta-analysis results

The main results of the current meta-analysis are listed in Table 2 and Figure 2. As for C677T polymorphism, no

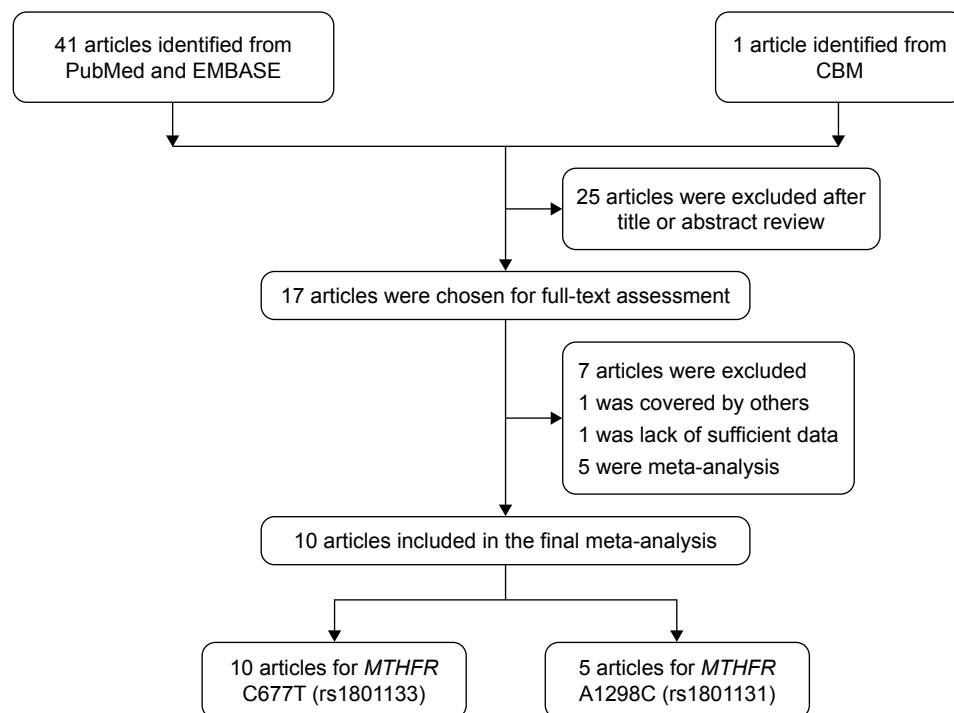


Figure 1 Flow diagram of the included studies for the associations between *MTHFR* C677T and A1298C polymorphisms and oral cancer susceptibility.
Abbreviation: MTHFR, methylenetetrahydrofolate reductase.

Table 1 Characteristics of studies included in this meta-analysis

Surname	Year	Country	Ethnicity	Control source	Genotype method	Case			Control				MAF	HWE	
C677T polymorphism						CC	CT	TT	All	CC	CT	TT	All		
Weinstein ³³	2002	Puerto Rico	Caucasian	PB	PCR-RFLP	67	53	15	135	69	62	15	146	0.32	0.846
Capaccio ²⁸	2005	Italy	Caucasian	HB	PCR	18	33	14	65	36	46	18	100	0.41	0.623
Solomon ³⁰	2008	India	Asian	PB	PCR-RFLP	48	55	23	126	48	42	10	100	0.31	0.855
Sailasree ²⁹	2011	India	Asian	HB	PCR-RFLP	92	8	1	101	108	29	1	138	0.11	0.527
Supic ³¹	2011	Serbia	Caucasian	PB	PCR-RFLP	50	32	14	96	80	66	16	162	0.30	0.661
Tsai ³²	2011	China	Asian	HB	PCR	391	186	43	620	322	236	62	620	0.29	0.058
Vylliotis ³⁴	2013	Greek/Germany	Caucasian	PB	PCR-RFLP	28	76	6	110	45	65	10	120	0.35	0.044
Bezerra ²⁷	2014	Brazil	Caucasian	HB	PCR-RFLP	19	12	1	32	48	41	1	90	0.24	0.016
Barbosa ¹⁹	2015	Brazil	Caucasian	HB	PCR-RFLP	50	45	6	101	50	41	11	102	0.31	0.555
Miri-Moghaddam ¹⁸	2016	Iran	Caucasian	HB	PCR	34	21	2	57	47	14	1	62	0.13	0.971
A1298C polymorphism						AA	AC	CC	All	AA	AC	CC	All		
Capaccio ²⁸	2005	Italy	Caucasian	HB	PCR	31	28	6	65	44	51	6	101	0.31	0.076
Sailasree ²⁹	2011	India	Asian	HB	PCR-RFLP	37	74	19	130	46	59	34	139	0.46	0.088
Tsai ³²	2011	China	Asian	HB	PCR	407	192	21	620	393	198	29	620	0.21	0.528
Barbosa ¹⁹	2015	Brazil	Caucasian	HB	PCR-RFLP	60	36	5	101	53	44	5	102	0.26	0.275
Miri-Moghaddam ¹⁸	2016	Iran	Caucasian	HB	PCR	30	26	1	57	34	26	2	62	0.24	0.259

Abbreviations: MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; PB, population based; HB, hospital based; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism.

significant association with overall oral cancer risk was observed under all the five genetic models: homozygote model (TT vs CC: OR=0.92, 95% CI=0.71–1.20), heterozygous model (CT vs CC: OR=0.98, 95% CI=0.72–1.33), recessive model (TT vs CT/CC: OR=0.95, 95% CI=0.74–1.23), dominant model (CT/TT vs CC: OR=1.00, 95% CI=0.74–1.37), and allele model (T vs C: OR=1.00, 95% CI=0.80–1.27). Stratified analysis by ethnicity did not revealed any significant association between C677T polymorphism and oral cancer risk among either ethnic group. In terms of source of controls, only a borderline significant association was detected between C677T polymorphism and a decreased oral cancer risk under the homozygous model (OR=0.71, 95% CI=0.50–1.00) in hospital-based studies.

Regarding the A1298C polymorphism, no association with overall oral cancer susceptibility was found (CC vs AA: OR=0.76, 95% CI=0.52–1.13; AC vs AA: OR=0.97, 95% CI=0.80–1.17; CC vs AC/AA: OR=0.71, 95% CI=0.49–1.03; AC/CC vs AA: OR=0.93, 95% CI=0.77–1.11; and C vs A: OR=0.90, 95% CI=0.78–1.05). Similarly, we could not observe any association between A1298C polymorphism and oral cancer risk in the stratified analysis by ethnicity.

Heterogeneity and sensitivity analysis

As shown in Table 2, substantial heterogeneities existed among the investigations for the *MTHFR* C677T polymorphism and oral cancer risk under the heterozygous ($P=0.001$) and dominant ($P<0.001$) models, as well as allele contrast ($P=0.001$). Thus, the random-effects model was applied to

calculate the pooled ORs and 95% CIs for these genetic models. The fixed-effect model was adopted for other two genetic models without heterogeneity.

The sequential leave-one-out sensitivity analysis was applied to investigate the influence of a single study on the pooled ORs. The similar recalculated ORs were obtained after the omission of each individual study, indicating the reliability and credibility of our results (data not shown).

Publication bias

We performed Begg's funnel plot and quantitative Egger's linear regression test to evaluate the publication bias. The shape of the Begg's funnel plots appeared to be symmetrical for C677T and A1298C polymorphisms. Moreover, we did not observe significant publication bias as suggested by Egger's test.

TSA

In this meta-analysis, 10 studies with 3083 individuals were included to investigate the association of C677T polymorphism with oral cancer susceptibility. The TSA for C677T polymorphism showed that the cumulative Z-curve did not cross the trial monitoring boundary before reaching the required information size, suggesting that additional trials were needed to further verify the conclusion (Figure 3). TSA was not applicable for A1298C polymorphism, since the number of eligible studies was too small.

Discussion

In this meta-analysis, we analyzed the association of *MTHFR* C677T and A1298C polymorphisms with oral cancer risk.

Table 2 Meta-analysis of the association between *MTHFR* C677T and A1298C polymorphisms and oral cancer risk

Variables	No of studies	Sample size	Homozygous		Heterozygous		Recessive		Dominant		Allele	
			OR (95% CI)	P _{het}	OR (95% CI)	P _{het}	OR (95% CI)	P _{het}	OR (95% CI)	P _{het}	OR (95% CI)	P _{het}
C677T												
All	10	1,443/1,640	TT vs CC 0.92 (0.71–1.20)	0.114	CT vs CC 0.98 (0.72–1.33)	0.001	TT vs (CT + CC) 0.95 (0.74–1.23)	0.247	(CT + TT) vs CC 1.00 (0.74–1.37)	<0.001	T vs C 1.00 (0.80–1.27)	0.001
Ethnicity												
Caucasian	7	596/782	1.14 (0.77–1.68)	0.744	1.15 (0.85–1.54)	0.155	1.06 (0.74–1.54)	0.583	1.15 (0.89–1.49)	0.236	1.09 (0.92–1.28)	0.536
Asian	3	847/858	0.77 (0.54–1.10)	0.014	0.69 (0.37–1.27)	0.015	0.86 (0.61–1.22)	0.051	0.72 (0.37–1.43)	0.003	0.79 (0.42–1.47)	0.001
Source of control												
HB	6	976/1,112	0.71 (0.50–1.00)	0.278	0.88 (0.56–1.38)	0.006	0.77 (0.55–1.07)	0.520	0.88 (0.57–1.37)	0.005	0.89 (0.64–1.26)	0.407
PB	4	467/528	1.39 (0.91–2.12)	0.506	1.12 (0.76–1.65)	0.112	1.31 (0.88–1.96)	0.337	1.18 (0.85–1.65)	0.173	1.15 (0.96–1.39)	0.006
A1298C												
All	5	973/1,024	CC vs AA 0.76 (0.52–1.13)	0.869	AC vs AA 0.97 (0.80–1.17)	0.338	CC vs (AC + AA) 0.71 (0.49–1.03)	0.546	(AC + CC) vs AA 0.93 (0.77–1.11)	0.702	C vs A 0.90 (0.78–1.05)	0.977
Ethnicity												
Caucasian	3	223/265	1.04 (0.45–2.39)	0.761	0.83 (0.57–1.21)	0.621	1.16 (0.52–2.61)	0.694	0.85 (0.60–1.22)	0.701	0.92 (0.69–1.23)	0.803
Asian	2	750/759	0.70 (0.45–1.09)	0.989	1.02 (0.82–1.27)	0.097	0.62 (0.41–0.95)	0.485	0.96 (0.77–1.18)	0.276	0.90 (0.76–1.06)	0.975

Abbreviations: het, heterogeneity; OR, odds ratio; CI, confidence interval; HB, hospital based; PB, population based.

No significant association with oral cancer was observed for the two SNPs under all the five genetic models. In addition, stratified analyses by ethnicity revealed null association in Caucasians and Asians. However, we detect a borderline significant association of C677T polymorphism with a decreased oral cancer risk under the homozygous model in studies with hospital-based design. To the best of our knowledge, this is the largest meta-analysis on this topic, including all the eligible epidemiological studies in English and Chinese.

The *MTHFR* C677T polymorphism, a C-to-T transition at position 677, induces the substitution of valine for alanine in codon 222.³⁵ It is a functional genetic variation. Studies elucidated that the variant T alleles of this *MTHFR* polymorphism reduced the enzyme activity to nearly 50%, compared to the wild-type C allele.³⁶ It was reported that C677T polymorphism was associated with a decreased risk in prostate cancer,³⁷ colorectal carcinoma,³⁸ leukemia,³⁹ and malignant lymphoma.⁴⁰ However, studies on breast cancer,⁴¹ gastric cancer,⁴² hepatocellular cancer,⁴³ and cervical cancer⁴⁴ have found an increased cancer risk associated with the variant T allele. Although numerous studies have been performed, the associations of *MTHFR* C677T and A1298C polymorphisms with oral cancer susceptibility remain contradictory, in part, due to the limited sample size. By now, four meta-analyses were published regarding association between these two *MTHFR* polymorphisms and oral cancer risk, with three articles written in English and one in Chinese.

Zhuo et al²⁰ conducted the first meta-analysis including six studies on C677T polymorphism and two studies on A1298C polymorphism in 2012. They found no impact of C677T polymorphism on oral cancer susceptibility in the pooled analysis. However, the same study observed the significant association of A1298C polymorphism with decreased oral cancer susceptibility under the recessive model. In 2013, Xu et al²² performed an updated meta-analysis in Chinese to identify the role of C677T polymorphism in oral cancer risks with seven studies. They failed to find the association between C677T polymorphism and oral cancer risk. Subgroup analysis based on the source of control revealed decreased susceptibility to cancer among hospital-based population. After that, Jia et al²¹ performed another meta-analysis including 1318 cases and 1817 controls from seven studies. They found a borderline association between C677T polymorphism and oral cancer risk under the heterogeneous model. Moreover, stratified analysis demonstrated ethnic- and control design-specific associations with C677T polymorphism, ie, T allele was identified as a protective allele against oral cancer in Asians and in hospital-based studies. In a recent study conducted

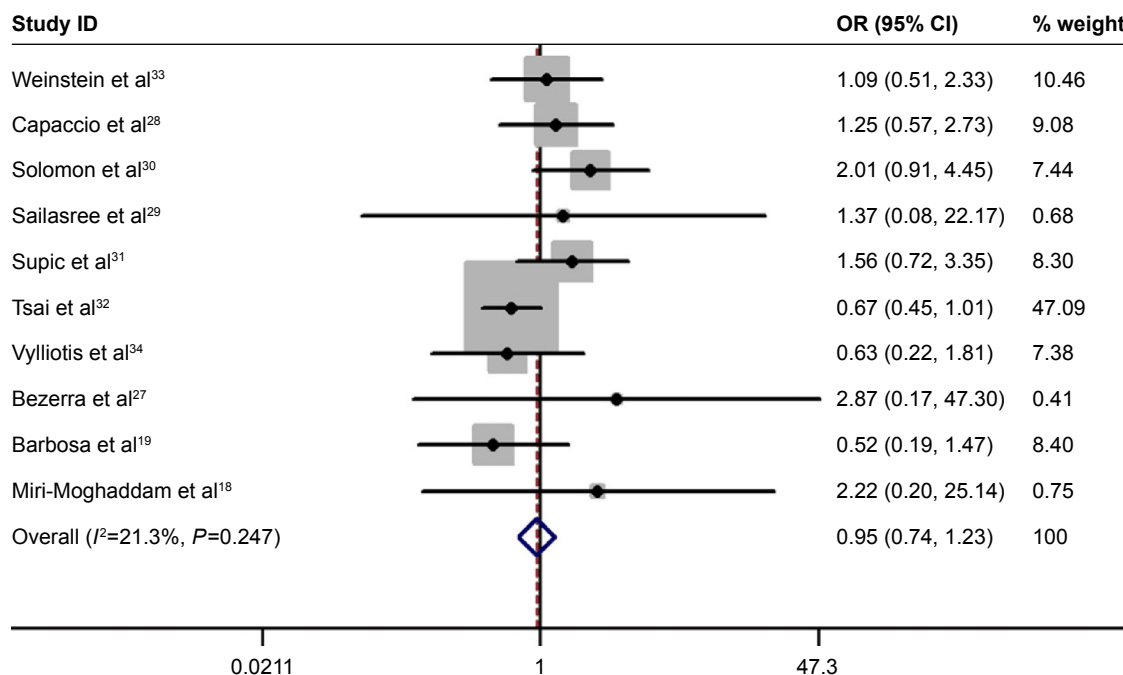


Figure 2 Forest plot for the association between *MTHFR* C677T polymorphism and oral cancer risk under recessive model.

Notes: For each study, the estimated OR and its 95% CI are plotted with a box and a horizontal line. ◇, pooled ORs and its 95% CIs.

Abbreviations: *MTHFR*, methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval.

by Zhu et al²³ in 2016, they suggested that *MTHFR* A1298C polymorphism was associated with decreased oral cancer risk. However, this association may not be convincing enough due to the relatively small sample; thus, it is necessary for us to update this research.

It should be noted that our stratified analyses revealed a borderline significant association of C677T polymorphism with a decreased oral cancer risk under the homozygous model in studies with hospital-based design. As the variant T alleles of *MTHFR* C677T polymorphism could reduce the

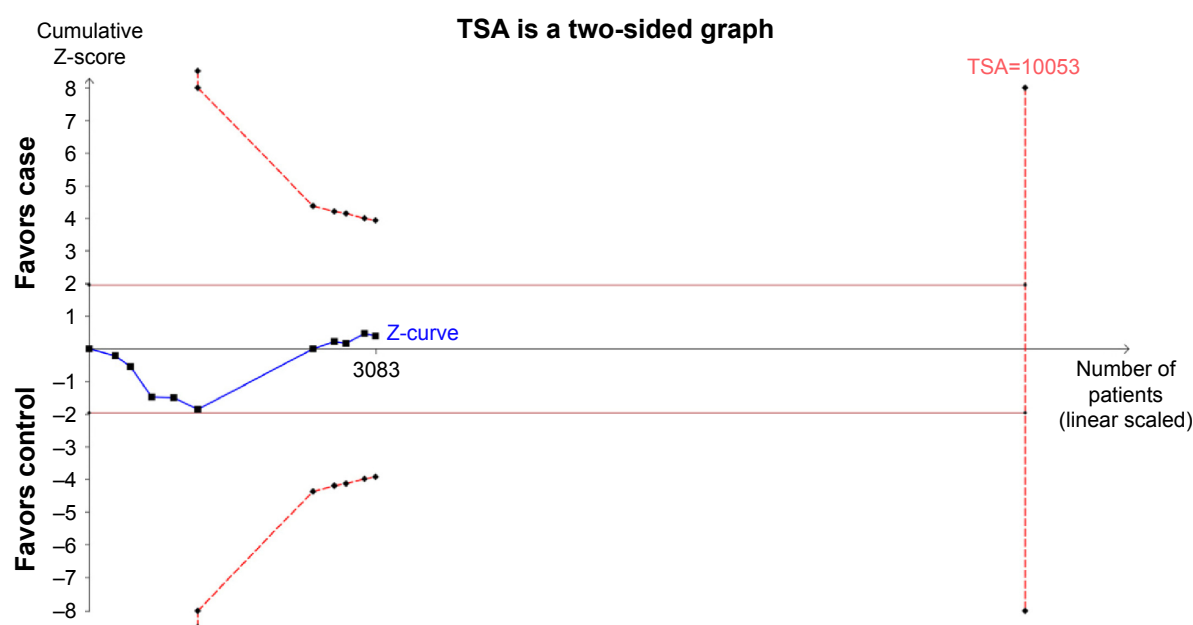


Figure 3 The required information size to demonstrate the association between *MTHFR* C677T polymorphism with oral cancer susceptibility.

Notes: The solid blue line is the cumulative Z-curve. The dashed inward-sloping line to the left represents the trial sequential monitoring boundaries.

Abbreviations: *MTHFR*, methylenetetrahydrofolate reductase; TSA, trial sequencing analysis.

enzyme activity to nearly 50% compared to the wild-type C allele, we hypothesize that the reduced MTHFR activity will cause intracellular accumulation of 5,10-methylenetetrahydrofolate and result in a reduced incidence of DNA double-strand breakage. The reduced incidence of DNA double-strand breakage will maintain genetic stability and prevent oral cancer development.⁴⁵ Besides, we also observed that there seem to be different results from Asian and Caucasian studies for the association between C677T polymorphism and oral cancer risk. The discrepant results may be explained by the fact that different races may modify the genetic effect on oral cancer risk.

Compared to the previous meta-analyses, we have undertaken some critical measurements to assure the credibility of our conclusion. The publication bias analysis and sensitivity analysis indicated a lack of publication bias and the stability of the results. We also employed TSA to test the conclusion reliability of this meta-analysis. However, the Z-curve failed to cross the trial monitoring boundary before reaching the required information size, which indicated that more samples were needed to clarify the correlation between MTHFR C677T polymorphism and oral cancer risk.

In spite of the merits, some weaknesses of the study should be pointed out. First, the statistical power of the meta-analysis was restricted because of relative small sample size. Second, the existence of between-study heterogeneity in some comparisons might compromise the reliability of conclusion. Third, only relevant studies in English and Chinese were included, some investigations in other languages might be omitted. Forth, due to the utilization of unadjusted ORs, we were only able to examine the association of interest by single-factor estimation from genetic perspective. The impacts of other risk factors for oral cancer, including life-styles, environment exposures, and gene–environment interactions, are also needed to be considered. Finally, there were some inherent limitations due to the retrospective nature of meta-analysis, such as inconsistent qualities of the included studies, misclassified genotypes, incomplete histological details, and unwell-matched sources of controls.

Conclusion

Despite some limitations, this meta-analysis indicated that there was no association observed between the two MTHFR polymorphisms (C677T and A1298C) and oral cancer risk. Further in-depth and well-designed investigations with larger sample sizes are warranted to explore the correlation, since this null correlation estimated by conventional meta-analysis failed to get the conformation of TSA.

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Disclosure

The authors report no conflicts of interest in this work.

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