

## RESEARCH ARTICLE

# No Association Between MTHFR A1298C Gene Polymorphism and Head and Neck Cancer Risk: A Meta-analysis Based on 9,952 Subjects

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### Abstract

**Objective:** Findings for associations between the methylenetetrahydrofolate reductase (MTHFR) A1298C gene polymorphism and head and neck cancer risk have been conflicting. We therefore performed a meta-analysis to derive a more precise relationship. **Methods:** Ten published case-control studies were collected and odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the association between MTHFR A1298C polymorphism and head and neck cancer risk. Sensitivity analysis and publication bias assessment also were performed to guarantee the statistical power. **Results:** Overall, no significant association between MTHFR A1298C polymorphism and head and neck cancer risk was found in this meta-analysis (C vs. A: OR=1.04, 95% CI=0.87-1.25, P=0.668, P<sub>heterogeneity</sub><0.001; CC vs. AA: OR=1.07, 95% CI=0.70-1.65, P=0.748, P<sub>heterogeneity</sub><0.001; AC vs. AA: OR=1.06, 95% CI=0.88-1.27, P=0.565, P<sub>heterogeneity</sub><0.001; CC+AC vs. AA: OR=1.06, 95% CI=0.86-1.30, P=0.571, P<sub>heterogeneity</sub><0.001; CC vs. AA+AC: OR=1.02, 95% CI=0.69-1.52, P=0.910, P<sub>heterogeneity</sub><0.001). Similar results were also been found in succeeding analysis of HWE and stratified analysis of ethnicity. **Conclusions:** In conclusion, our meta-analysis demonstrates that MTHFR A1298C polymorphism may not be a risk factor for developing head and neck cancer.

**Keywords:** MTHFR - polymorphism - head and neck cancer - meta-analysis

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### Introduction

Head and neck cancer is one of the most prevalence malignant diseases worldwide, there were 633,000 new cases and 355,000 deaths in 2008, especially in South-Central Asia, Central and Eastern Europe and the lowest in Africa (Jemal et al., 2011). The treatment measures of head and neck cancer will always lead to the decreased quality of life in patients with function disability and physiognomy abnormalities. Head and neck cancer is a multifactorial disease (Ragin et al., 2007). Smoking and alcohol consumption were considered the major risk factors, some other genetic factors may potentially alter individual susceptibility to head and neck cancer (Argiris et al., 2008).

MTHFR is a key enzyme in folate metabolism, irreversibly catalyzing the 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the cosubstrate for transmethylation from homocysteine to methionine. Methionine is the precursor for S-adenosyl-L-methionine, which is the primary methyl donor in the DNA methylation process (Stern et al., 2000; Kawakami et al., 2003). On the other metabolism circulation,

the 5, 10-methylenetetrahydrofolate involves in the conversion of deoxyuridylate monophosphate to deoxythymidylate monophosphate. The low levels of 5,10-methylenetetrahydrofolate would lead to increased incorporation of uracil into DNA in place of thymine and led to the increased ratio of point mutations and DNA breakage (Blount et al., 1997). All these factors will play an important role during the cancer development.

The common MTHFR A1298C polymorphism may be involved in the cancer development, through alteration of MTHFR enzyme activity. The previous research had reported that the carriers of the A1298C variant allele could increase the serum folate levels, possibly influencing cancer risk susceptibility (Lievers et al., 2001; Parle-McDermott et al., 2006).

From 2005, the first study on the head and neck cancer association with MTHFR A1298C polymorphism was conducted by Neumann et al. Decreased risk was found between the MTHFR A1298C polymorphism and head and neck cancer in the Caucasians (Neumann et al., 2005). Since then, a lot of studies on MTHFR A1298C polymorphism and head and neck cancer risk were conducted, but the results are controversial. So, we

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performed the meta-analysis with all relative studies to derive a more precise estimation of the associations of MTHFR A1298C head and neck cancer risk.

## Materials and Methods

### Search strategy

Three online bibliographic databases (PubMed and Embase and CNKI) were searched with the following search strategy “head and neck cancer”, “head and neck cancer”, “oropharyngeal cancer”, “MTHFR”, “methylenetetrahydrofolate reductase”, “polymorphism”, “variant” in English and Chinese, the relevant studies were identified by a hand search from the references of original studies and review articles on the association between MTHFR A1298C polymorphism and head and neck cancer risk from 2005, when the first study on the association between MTHFR A1298C polymorphism and head and neck cancer risk was reported, to July 2012. All the eligible articles and relevant reviews were checked carefully. All selected studies complied with the following three criteria: (a) case–control study about MTHFR A1298C polymorphism and head and neck cancer risk; (b) sufficient published data for estimating the odds ratio (OR) and 95% confidence interval (CI); (c) Only the largest or most recent publication was selected when multiple studies reported a same or overlapping data(Little et al., 2002).

### Data extraction

Two investigators (Niu and Shen) independently extracted the following data from each included publication: the first author’s name, publication data, country, ethnicity (categorized as either Asian or Caucasian), number of cases and controls, and Hardy-Weinberg equilibrium (HWE). The informations from all included studies were compared to accuracy and the discrepancies were discussed to draw an inconsistent conclusion by two reviews. A third reviewer was then introduced to judge the still arguments during the review.

### Statistical analysis

All ORs with 95% CIs were calculated to assess the strength of the correlation between the MTHFR A1298C polymorphism and head and neck cancer risk. The pooled ORs were performed for allele contrast (C

vs. A), codominant model (CC vs. AA, AC vs. AA), dominant model (CC+AC vs. AA), and recessive model (CC vs. AA+ AC), respectively. In the subgroup analysis, statistical analysis was conducted on ethnicity of Asians and Caucasians. Heterogeneity assumption was assessed and considered significant at  $P < 0.10$  by the chi-square based Q-test statistic (Lau et al., 1997). The pooled OR estimation of each study was calculated by the fixed-effects model (the Mantel–Haenszel method) when  $P > 0.10$  (Mantel et al., 1959). Otherwise, the random-effects model (the DerSimonian and Laird method) was used (DerSimonian et al., 1986). The potential publication bias was estimated by the modified Egger’s linear regression test(Harbord et al., 2006). Statistical analysis was performed using STATA version 11.0 (Stata Corporation, College Station, TX, USA), two-sided P-values was used,  $P < 0.05$  was considered statistically significant.

## Results

### Study characteristic

Ten related case-control studies involved 3534 cases and 6418 controls on the relationship between MTHFR A1298C polymorphism and head and neck cancer risk were included in this meta-analysis (Capaccio et al., 2005; Neumann et al., 2005; Galbiatti et al., 2012; Hung et al., 2007; Suzuki et al., 2007; Ni et al., 2008; Cao et al., 2010; Kruszyna et al., 2010; Sailasree et al., 2011; Tsai et al., 2011). All the main characteristics of these studies are shown in Table 1. Five studies involved Asian populations, whereas four studies involved Caucasian populations, one studies focus on mixed populations. All diverse genotyping method was PCR-RFLP and Taqman, three studies deviated from HWE of the genotypic distribution in the controls (Neumann et al., 2005; Kruszyna et al., 2010; Galbiatti et al., 2012).

### Meta-analysis

The main results of this meta-analysis and the heterogeneity test are shown in Table 2. Overall, no significant relationship was observed between MTHFR A1298C polymorphism and head and neck cancer risk in the total populations (C vs. A: OR=1.04, 95%CI=0.87-1.25,  $P=0.668$ ,  $P_{heterogeneity} < 0.001$ ; CC vs. AA: OR=1.07, 95%CI=0.70-1.65,  $P=0.748$ ,  $P_{heterogeneity} < 0.001$ ; AC vs. AA: OR=1.06, 95%CI=0.88-1.27,  $P=0.565$ ,  $P_{heterogeneity} < 0.001$ ;

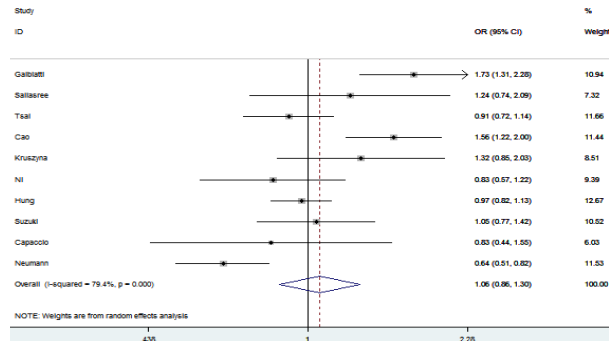
**Table 1. Characteristics of Case-control Studies Included in the Meta-analysis**

First author	Year	Country	Racial descent	Source of controls	Case	Control	Genotype distribution						Genotyping type	Location	P for HWE
							Case			Control					
							AA	AC	CC	AA	AC	CC			
Galbiatti	2012	Brazil	Mixed	Population control	322	531	148	117	57	316	168	47	PCR-RFLP	HNC	<0.05
Sailasree	2011	India	Asian	Hospital control	130	139	37	74	19	46	59	34	PCR-RFLP	Oral	0.09
Tsai	2011	China	Asian	Hospital control	620	620	407	192	21	393	198	29	PCR-RFLP	Oral	0.53
Cao	2010	China	Asian	Population control	484	542	238	221	25	326	194	22	PCR-RFLP	Nasopharyngeal	0.30
Kruszyna	2010	Poland	Caucasian	Population control	131	250	48	65	18	108	126	16	PCR-RFLP	laryngeal	0.01
Ni	2008	China	Asian	Population control	207	400	153	48	6	281	113	6	PCR-RFLP	Larynx	0.15
Hung	2007	Europe	Caucasian	Hospital control	802	2585	363	363	76	1148	1160	277	TaqMan	HNC	0.53
Suzuki	2007	Japan	Asian	Hospital control	236	706	149	79	8	453	222	31	TaqMan	HNC	0.57
Capaccio	2005	Italy	Caucasian	Population control	65	100	31	28	6	43	51	6	PCR-RFLP	HNC	0.07
Neumann	2005	USA	Caucasian	Population control	537	545	328	199	10	274	240	31	PCR-RFLP	HNC	0.02

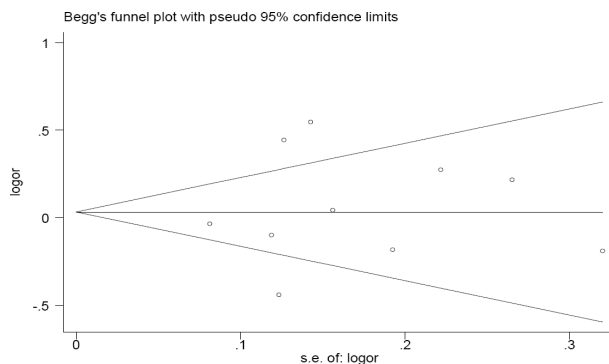
**Table 2. Summary ORs and 95% CI of MTHFR A1298C Polymorphism and Head and Neck Cancer Risk**

	C vs. A				CC vs. AA				AC vs. AA				CC+AC vs. AA				CC vs. AA+AC			
	OR	95% CI	P	P <sup>a</sup>	OR	95% CI	P	P <sup>a</sup>	OR	95% CI	P	P <sup>a</sup>	OR	95% CI	P	P <sup>a</sup>	OR	95% CI	P	P <sup>a</sup>
Total	1.04	0.87-1.25	0.668	<0.001	1.07	0.70-1.65	0.748	<0.001	1.06	0.88-1.27	0.565	<0.001	1.06	0.86-1.30	0.571	<0.001	1.02	0.69-1.52	0.91	<0.001
HWE	1.00	0.88-1.14	0.949	0.052	0.92	0.71-1.13	0.405	0.360 <sup>b</sup>	1.07	0.88-1.29	0.509	0.015	1.04	0.87-1.25	0.633	0.022	0.88	0.68-1.14	0.339	0.262 <sup>b</sup>
Ethnicity																				
Asian	1.02	0.84-1.24	0.848	0.024	0.95	0.70-1.31	0.772	0.204 <sup>b</sup>	1.13	0.86-1.47	0.384	0.01	1.09	0.85-1.40	0.502	0.013	0.84	0.62-1.14	0.271	0.156 <sup>b</sup>
Caucasian	0.94	0.71-1.24	0.649	0.001	0.92	0.40-2.10	0.844	<0.001	0.88	0.69-1.13	0.315	0.071	0.89	0.66-1.20	0.445	0.011	0.97	0.46-2.04	0.926	0.001

<sup>a</sup>Test for heterogeneity; <sup>b</sup>Estimates for fixed effects model



**Figure 1. OR of Head and Neck Cancer Associated with MTHFR A1298C Polymorphism for the CC+AC vs. AA Model in Total**



**Figure 2. Funnel Plot Analysis to Detect Publication Bias for CC+AC vs. AA Model.** Each point represents a separate study

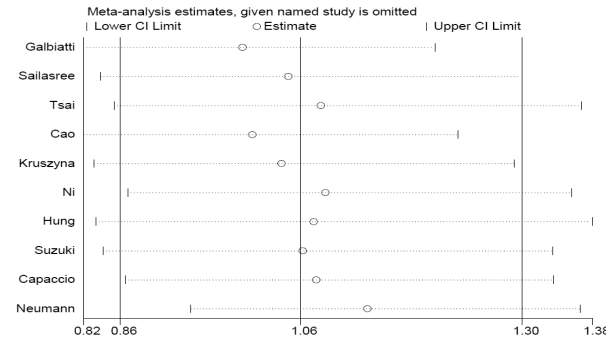
CC+AC vs. AA: OR=1.06, 95%CI=0.86-1.30, P=0.571, P<sub>heterogeneity</sub><0.001 (Figure 1); CC vs. AA+AC: OR=1.02, 95%CI=0.69-1.52, P=0.910, P<sub>heterogeneity</sub><0.001). Similarly, in the succeeding analysis of HWE and stratified analysis of ethnicity, we also did not find any significant association between MTHFR A1298C polymorphism and head and neck cancer.

#### Publication bias

Funnel plot and Egger's test were performed to estimate the publication bias of literature. The shapes of the funnel plots in all genetic models did not reveal any evidence of obvious asymmetry. Figure 2 showed the shapes of the funnel plots of dominant model (CC+AC vs. AA) in all populations. The result was further supported by analysis via Egger's tests. No significant publication bias was found in this meta-analysis (P=0.745 for C vs. A; P=0.985 for CC vs. AA; P=0.728 for AC vs. AA; P=0.692 for CC+AC vs. AA; P=0.928 for CC vs. AA+AC).

#### Sensitivity analysis

One single study involved in the meta-analysis was deleted each time to reflect the influence of the individual dataset to the pooled ORs, the results were persistent



**Figure 3. Sensitivity Analysis Through Deleting Each Study to Reflect the Influence of the Individual Dataset to the Pooled ORs in CC+AC vs. AA Model**

(Figure 3 for dominant model), the analysis results demonstrate no materially change and statistically robust.

## Discussion

Genetic susceptibility has been demonstrating one of the important risks for cancer development, especially interactive with other dangerous environment factors. Lots of epidemiological studies have been carrying out to investigate the effect of gene polymorphisms during the cancer development. MTHFR is a key enzyme to participate nucleotide synthesis and methylation reactions. 5,10-methylenetetrahydrofolate (5,10-methyleneTHF) as the important substrate of MTHFR, involving in the thymidylate synthesis and DNA construction during the life cycle. Furthermore, the 5-methyltetrahydrofolate (5-methylTHF) is the main product of MTHFR activity, which can provides methyl groups for methionine synthesis and DNA methylation (Kim, 1999; Sailasree et al., 2011).

The A1298C polymorphism leads to an amino acid change from glutamate (Glu) to alanine (Ala) at codon 429 in exon 7, which may alter the protein activity and influence the genetic stability to increase the risk for cancer development (Weisberg et al., 1998; Kim, 1999; Weisberg et al., 2001). In 1998, van der Put et al. first described that the A1298C mutation could decrease the MTHFR enzyme activity (van der Put et al., 1998).

The correlations between A1298C mutation and head and neck cancer risk have been studied, but the results remain controversial. In 2005, the first study revealed that the C allele maybe a protective role during the head and neck cancer development (for CC+AC vs. AA: OR, 0.65; 95% CI: 0.51-0.82) (Neumann et al., 2005). To date, no consensus has been reached on the correlation between MTHFR A1298C polymorphism and head and neck cancer risk. The borderline protective effect between MTHFR A1298C polymorphism and head and neck cancer risk also been observed when CC genotype compared to

AA+AC genotype (RR=0.55, p=0.062) by Sailasree et al (2011). On the contrary, Cao et al. reported that a 1.57 fold increased risk in nasopharyngeal carcinoma when AC genotype compared to subjects with AA genotype (95%CI=1.21–2.03) (Cao et al., 2010). In the study of Galbiatti et al. in Brazilian, the dominant model also indicated increased risk for head and neck cancer (OR=2.42, 95%CI=1.21–2.73) with age over 49 years, tobacco and alcohol habits, especially in oral cancer (Galbiatti et al., 2012). But the other studies, included Tsai et al. (2011), Kruszyna et al. (2010), Ni et al. (2008), Hung et al. (2007), Suzuki et al. (2007), Capaccio et al. (2005), didn't find any significant association between MTHFR A1298C polymorphism and head and neck cancer risk.

The present meta-analysis, including 3534 cases and 6418 controls, was performed to assess the relationship between MTHFR A1298C polymorphism and head and neck cancer risk with ten published studies, but no significant association was found in the total population. Furthermore, no significant association was detected in all genetic models of the satisfied analysis by HWE and ethnicity.

Otherwise, some limitations should be addressed. First, these results are based on unadjusted estimates that lack the original data from the eligible studies, which limits the evaluation of the effects of interactions between the gene-gene and gene-environment. Second, the sample size is still relatively small to find a precise association between MTHFR A1298C polymorphism and head and neck cancer risk. Finally, in this meta-analysis, linkage disequilibrium (LD) and haplotype analysis with other mutation locus were not performed. Despite of these limitations, no publication bias was observed, and the large number of subjects still significantly guarantees the statistical power of the analysis.

In conclusion, our meta-analysis suggests that MTHFR A1298C polymorphism may not be associated with head and neck cancer development. In the future, large-scale case-control studies are necessary to validate the risks and to investigate the potential gene-gene and gene-environment interactions between MTHFR A1298C polymorphism and head and neck cancer cancer.

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