

RESEARCH COMMUNICATION

Systematic Review on the Relationship between Genetic Polymorphisms of Methylenetetrahydrofolate Reductase and Esophageal Squamous Cell Carcinoma

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Abstract

Background: Both genetic and environmental factors play roles in pathogenesis of esophageal squamous cell carcinoma (ESCC) and susceptibility may be modified by functional polymorphisms in folate metabolic genes, such as methylenetetrahydrofolate reductase (MTHFR). We here aimed to evaluate associations of MTHFR C677T and A1298C polymorphisms with ESCC. **Methods:** We searched MEDLINE, EMBASE and the Chinese Biomedical Database and 2 evaluators independently reviewed all the articles identified according to predetermined criteria. **Results:** A total of 15 case-control studies published between 2001 and 2010 were included. When all the studies were pooled, the crude odds ratio (95% CI) of ESCC for individuals carrying MTHFR 677 CT and TT genotypes, as compared to CC, were 1.39 (1.11-1.75) and 1.79 (1.31-2.43), respectively. Individuals with MTHFR 1298CC showed non-significantly increased risk of ESCC, with an OR (95% CI) of 3.31(0.90-12.17). In smokers, a significantly increased risk of ESCC was observed for those with the MTHFR 677T allele (OR (95% CI)=2.2 (1.31-2.41)). Chinese carrying MTHFR 677T and MTHFR 1298C alleles had a greater increase in ESCC risk than other ethnicities. **Conclusions:** The present meta-analysis provided evidence that MTHFR 677CT/TT plays a carcinogenic role in ESCC, and its effect is modified by tobacco and ethnicity. The small number of subjects with the MTHFR 1299C allele genotype in published studies limits conclusions regarding this polymorphism.

Keywords: Methylenetetrahydrofolate reductase - polymorphisms - esophageal cancer - meta-analysis

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Introduction

Esophageal cancer (EC) is the sixth most common cancer worldwide in 2002(Parkin et al., 2005). Its incidence and mortality rates show a wide geographic variation at an international levels, with remarkable differences between high-risk and low-risk areas (Corley and Buffler, 2001; Ferlay et al., 2004; Parkin et al., 2005), which suggested the role of genetic and environmental factors in the pathogenesis of this cancer(Chen et al.,1996; Choi et al., 2000).

To our knowledge, folate deficiency resulting from low consumption of vegetables and fruits is related to increased risk of several cancers, including EC(Prasad et al., 1992), Giovannucci et al., 1995; Gallus and La, 2007). The carcinogenesis of EC by folate deficiency is through two mechanisms ways: one is inducing misincorporation of uracil into DNA to lead to disruption of DNA integrity and DNA repair. Another is causing the alteration in DNA methylation, which could induce the altering expression of critical tumor suppressor genes and proto-oncogenes(Choi and Mason, 2000; Kim 2004; Blount et al., 2007).

MTHFR is a central enzyme in folate metabolism which catalyzes the reduction of 5, 10-methylene-tetrahydrofolate to 5-methyltetrahydrofolate, and then methionine synthase catalyzed the reaction of 5-methyltetrahydrofolate and homocysteine to generate methionine and tetrahydrofolate. Under the condition of folate deficiency, MTHFR may result in point mutations and/or chromosomal breaks, facilitate the conversion of 5,10-methylene THF to 5-methyl THF, and cause decline of 5-methyl THF to decrease the conversion of homocysteine to methionine. Ultimately, MTHFR plays a role in the carcinogenesis process of DNA hypomethylation.

There were about 20 kinds of genetic polymorphisms of MTHFR, and non-synonymous C677T and A1298C variants are the most studied genetic polymorphisms. The C677T variant (Ala 222 Val, rs 1801133) has been associated with a decreased activity of MTHFR, an increased level of homocysteine and an altered distribution of folate(Frosst et al., 1995; Bagley and Selhub, 1998; Brattstrom et al., 1998). The MTHFR A1298C variant (Glu 429Ala, rs1801131) has also been related to a reduced MTHFR activity, but at a lower degree compared

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to C677T(Weisberg et al., 1998). The MTHFR A1298C have about 60% of normal MTHFR activity. Several studies have explored the impact of MTHFR C677T and A1298C polymorphism in various cancers, but the association with EC is conflicting (Song et al., 2001; Stolzenberg-Solomon et al., 2003; Yang et al., 2005). Tobacco and alcohol consumption are two important risk factors for EC, and their association with MTHFR C677T and A1298C polymorphism is inconsistency(Yang et al., 2005; He et al., 2007).

To better understand these issues, we conducted a comprehensive meta-analysis to clarify the quantitative association between the MTHFR C677T and A1298C polymorphisms and risk of esophageal squamous cell carcinoma (ESCC), and evaluate its modification by alcohol, tobacco and ethnicity.

Materials and Methods

Searching strategy

We searched and reviewed MEDLINE (from Jan. 1966 to April. 2010), EMBASE (from January 1988 to April. 2010), and the Chinese Biomedical Database (CBM; from January 1980 to April. 2010) by using selected common key words related to MTHFR C677T and A1298C polymorphisms and ESCC risk in case-control studies. We also scanned bibliographies of relevant articles to identify additional studies. As the key words for the literature search, we selected 'esophageal squamous cell carcinoma', 'esophagus', 'carcinoma or cancer or neoplasm or tumour or tumor', 'Methylenetetrahydrofolate reductase', or 'MTHFR' for the outcome factors.

Selection criteria

Only case-control studies reporting an association between MTHFR C677T and A1298C polymorphisms and ESCC were included in the meta-analysis. Trials had to be original and presented the MTHFR C677T and A1298C genotype frequencies in cases and controls. When the results of a study were published more than once, only the study that contained the most complete data was included in the analysis. Besides, we only selected articles written in English and excluded those studies with no available data for outcome measures.

Data extraction

All of the studies retrieved from the databases were independently evaluated by 2 evaluators. When there were disagreements between the evaluators concerning the selected studies, these differences of opinion were resolved by discussion. In instances where the data were insufficient or missing, we attempted to contact the authors of the articles in order to request the relevant data. From those studies finally selected, we extracted the following data: author names, year of publication, country, design, population size and adjusted OR with 95%CI. Finally, we yielded 15 case-control studies on the relationship of MTHFR C677T and A1298C polymorphism and ESCC.

Statistical analysis

Statistical analysis was performed for the case-1862 *Asian Pacific Journal of Cancer Prevention, Vol 12, 2011*

control studies by STATA statistical package (version 9, STATA, College Station, TX). We used both crude data (unadjusted) and adjusted data (adjusted OR with 95% CI) for meta-analysis. The ORs for ESCC associated with the MTHFR 677 CT and TT genotypes were estimated by using the MTHFR 677 CC genotype as reference group, and MTHFR 1298 AA genotype was used to be the reference group for MTHFR 1298 CC and AC. In carrying out the meta-analysis, random effect models were used to take into account the possibility of heterogeneity between studies, which was tested by the Q-statistics with p-values < 0.1, and its possible sources were assessed by subgroup analysis. Otherwise, a fixed-effect model was applied to obtain the summary OR and their 95% CI if there was no heterogeneity between studies. The Hardy-Weinberg equilibrium(HWE) in controls in each study was assessed using the χ^2 test. A funnel plot was also used to present the publication bias. A subgroup analysis in regard to smoking and drinking as well as ethnicity was performed. A sensitivity analysis was performed to explore robustness of the results by excluding the large sample study and studies which did not have controls in HWE.

Results

Our study included a total of 15 case-control studies (3213 cases and 4354 controls) published between 2001 and 2010. Table 1 shows the main characteristics of the studies in the analysis. Among 15 case-control studies, 13 studies were regarded on MTHFR C677T, and 5 studies were regarded on MTHFR A1298C. A significant association was seen between MTHFR 677 CT [crude OR(95%)=1.39(1.11-1.75)] and TT [crude OR(95%)=1.79(1.31-2.43)] genotypes and ESCC risk (p<0.05). There was significant heterogeneity across studies regarding MTHFR 677 CT (p<0.001) and TT (p<0.001), but it reduced after conducting subgroup analysis of alcohol and tobacco consumption. MTHFR 1298CC showed non-significantly increased risk of ESCC, with OR (95%CI) of 3.31(0.90-12.17). No significant heterogeneity was found across studies on MTHFR 1298AC and CC genotypes.

Non-smokers having the MTHFR 677T allele were seemed to increase ESCC risk, with the OR(95% CI) of 1.50(1.06-1.94) (p for heterogeneity=0.12), and smokers with MTHFR 677T allele were observed a moderate increased risk, with the OR (95% CI) of 2.2 (1.31-2.41) (p for heterogeneity =0.11) (Figure 1). For alcohol status, there was significantly decreased risk of ESCC for non-drinkers carrying MTHFR 677T allele [OR (95% CI) =0.80(0.64-0.96), p for heterogeneity =0.57], and non-significant light increased risk for drinkers with MTHFR 677T allele [OR=1.14(0.81-1.47), p for heterogeneity =0.11] (Figure 2). Non-smokers and smokers with MTHFR 1298C allele did not show significant increased risk of ESCC, and no significant increased risk of ESCC was seemed for non-drinkers and drinkers with MTHFR 1298C allele (Figures 3 and 4). Subgroup analysis was taken according to the ethnicity, which showed that Chinese carrying MTHFR 677T allele and MTHFR 1298C allele had a increased ESCC risk than other ethnicities.

Table 1. Characteristics of studies of MTHFR C677T and A1298C Polymorphism and ESCC

Study ID	Country	Controls Source	Genotypes	Cases	Controls	MTHFR C677T Adjusted OR (95%CI)		MTHFR A1298C Crude OR (95%CI)
						CT vs CC	TT vs CC	AC/CC vs AA
Cheng 2009	China	Hospital	C677T, A1298C	103	181	2.36(1.12-4.98)	3.45(1.590-7.48)	2.36(1.12-4.98)
Li 20081	China	Population	C677T	126	169	1.56(0.83-2.95)	1.47(0.78-2.77)	
He 20071	China	Population	C677T	584	540	1.83(1.30-2.58)	2.16(1.53-3.06)	
Feng 2006	China	Population	C677T	275	315	1.07(0.69-1.65)	1.76(1.13-2.75)	
Song 2001	China	Population	C677T, A1298C	240	360	2.98(1.87-4.75)	6.52(3.89-10.92)	0.65(0.44-0.94) 1.89(0.59-6.06)
Wang 2005	China	Population	C677T	275	315	1.07(0.69-1.65)	1.76(1.13-2.75)	
Yang 2005	Japan	Hospital	C677T	165	493	1.07(0.73-1.56)	0.74(0.42-1.30)	
Zhang 2004	China	Population	C677T	430	397	2.69(1.32-5.48)	2.02(0.99-4.10)	
Kureshi 2004	Pakistan	Population	C677T	34	54	0.97(0.34-2.41)	0.16(0.01-3.13)	
Zhang 2003	China	Population	C677T	198	141	2.69(1.32-5.48)	2.24(1.11-4.55)	
Stolzenberg 2003	China	Population	C677T, A1298C	129	398	0.78(0.45-1.37)	1.09(0.61-1.96)	0.96(0.61-1.52) 21.8(1.12-426)
Miao 2002	China	Population	C677T	217	468	1.58(1.06-2.37)	2.02(1.29-3.19)	
Umar 2010	India	Hospital	C677T	208	223	0.76(0.49-1.18)	0.99(0.28-3.53)	
Gao 2004	China		A1298C	141	228	-	-	1.45(0.93-2.27)
Zhang 2008	China		A1298C	88	72	-	-	1.39(0.71-2.75) 4.65(0.22-5.49)
Pooled results(Random effect model)				3213	4354	1.39(1.11-1.75)	1.79(1.31-2.43)	0.89(0.59-1.35) 3.31(0.90-12.1)
P for heterogeneity						<0.001	<0.001	0.116 0.297

The genotype frequencies among the controls differed significantly from the Hardy-Weinberg equilibrium(P<0.05)

Symmetry funnel plots also showed no existence of publication bias (Figure 5 and Figure 6). A sensitivity analysis indicated the overall crude ORs did not greatly

changed after excluding three studies which did not have controls in HWE[MTHFR 677 CT and MTHFR 677 TT were 1.27(1.01-1.66) and 1.72(1.14-2.60), respectively].

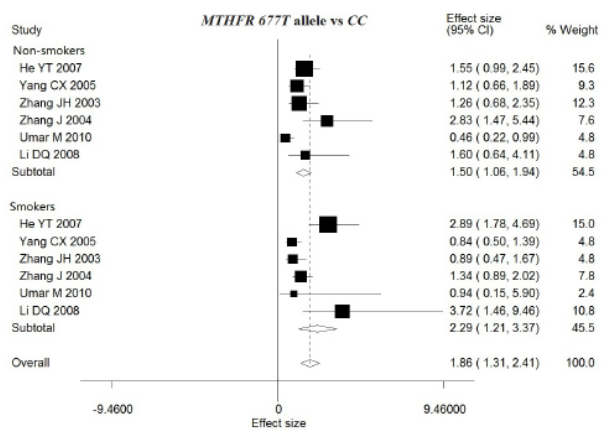


Figure 1. MTHFR 677T Allele versus CC With Regard to Smoking

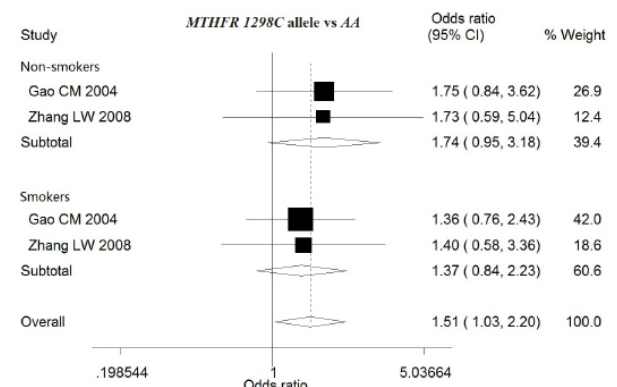


Figure 3. MTHFR 1298C Allele versus AA With Regard to Smoking

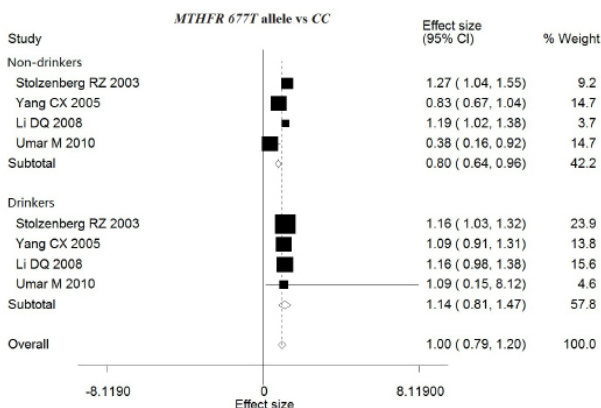


Figure 2. MTHFR 677T Allele versus CC With Regard to Alcohol Drinking

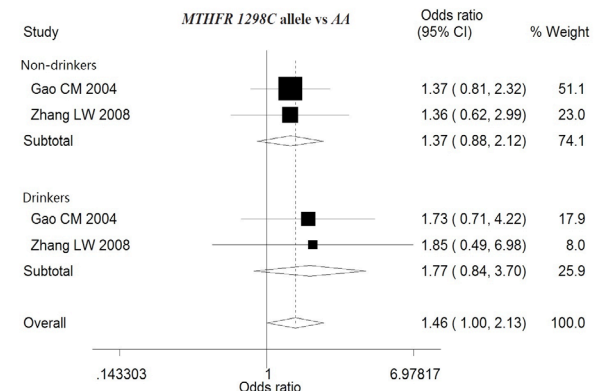


Figure 4. Relationship between MTHFR A1298C polymorphism and ESCC stratified by alcohol status

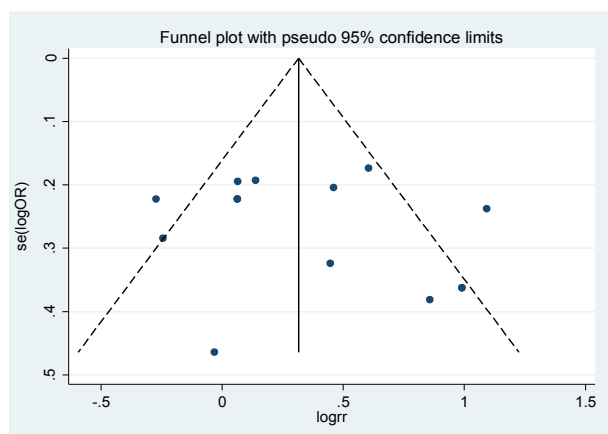


Figure 5. Publication bias on studies of MTHFR C677T and ESCC

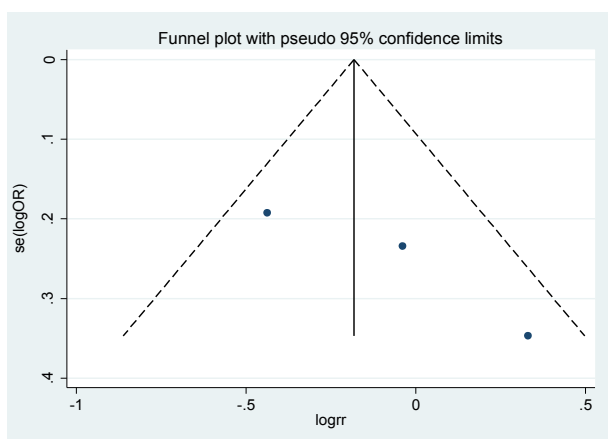


Figure 6. Publication bias on studies of MTHFR A1298C and ESCC

Similarly, the overall ORs of individuals with MTHFR 677 CT and TT genotype did not appear to have changed greatly after excluding one large-sample study (He et al., 2007).

Discussion

Although many experimental studies have indicated a role for MTHFR C677T and A1298C for EC risk, epidemiological evidence for the effect of the gene polymorphism on cancer risk is conflicting. In order to address this discrepancy, we carried out this meta-analysis. The results showed individuals with MTHFR 677CT and TT genotypes increased ESCC risk, and smokers carrying MTHFR 677T allele genotype greatly increased the ESCC risk. Individuals with MTHFR 1298CC were seemed non-significant increased risk for ESCC. The small number of subjects with the MTHFR 1299C allele genotype in published studies limits the conclusion on this polymorphism.

We found significant association with MTHFR 677TT and CT genotypes for developing esophageal cancer. Previous studies reported the heterozygotes (CT) and homozygotes (TT) for the MTHFR C677T polymorphism respectively had about 65% and 30%, respectively, of the MTHFR activity of those with the 677CC genotype (Bailey and Gregory, 1999). As a result, TT homozygotes have been associated with lower serum folate levels and higher

Table 2. Relationship between MTHFR C677T Polymorphism and ESCC Stratified by Ethnicity

Ethnicity	677T vs CC	P value	1298C vs AA	P value
	OR* (95% CI)		OR* (95% CI)	
Chinese	1.65 (1.25-2.16)	0.002	2.15 (1.60-2.88)	0.001
Japanese	1.07 (0.73-1.56)	-	0.74 (0.42-1.30)	-
German	0.76 (0.49-1.18)	-	1.00 (0.28-3.52)	-
Pakistan	0.97 (0.39-2.41)	-	0.16 (0.01-3.13)	-
India	1.15 (0.79-1.68)	<0.001	1.04 (0.59-1.82)	<0.001

*pooled

homocysteine levels than their wild type homozygous counterparts. The lower serum folate level leads to DNA hypomethylation, which is commonly observed in many cancers and in the early stages of carcinogenesis, may result in chromosomal instability, increased mutation rates, and activation of proto-oncogenes.

Our meta-analysis showed smokers with MTHFR 677T allele had moderate increased risk of ESCC than those with the 677CC wide-type genotype. Previous study reported relationship between MTHFR C677T and tobacco was inconsistent (Weinstein et al., 2002; Yang et al., 2005; Boccia et al., 2007; Umar et al., 2010). Cigarette smoking could decrease folate in plasma and produce a localized deficiency of folic acid, and induce carcinogenesis process of DNA hypomethylation (Boccia et al., 2007). Meanwhile, the inactive of MTHFR 677 CT/TT is expected to have high 5,10-methylene-tetrahydrofolate concentrations, and would increase the ESCC risk when exposed to high amounts of tobacco use (Bailey and Gregory, 1999). Therefore, long term exposure to high tobacco consumption could increase the development of EC. Early study observed ever smokers carrying MTHFR 677T allele showed a significant increased risk of gastric cancer, which was in line with our study (Boccia et al., 2007). Our study suggested gene-environment association between high cigarette smoking and MTHFR 677TT genotype for elevated EC risk.

Our study indicated the MTHFR 677 CT/TT was related to ESCC risk in Chinese, but no association was found in Japanese, Indian and Pakistan, while an inverse association was observed in Japanese and German (Table 2). One reason might be the genuine population-specific differences of MTHFR C677T polymorphism in the risk of ESCC, and previous study indicated the genetic selection of the T allele had occurred in specific population (Mayor-Olea et al., 2008). The frequency of MTHFR 677TT in German is 13% (Zhang et al., 2004), whereas 1% in Africans, 2.2% in India and 15% in Japan (Yang et al., 2005; Umar et al., 2010), while the frequency of MTHFR 677TT genotype in China is about 3-5% (Wang et al., 2005; Wang et al., 2007). Secondly, the difference in folate consumption among populations is related to the function of the MTHFR 677TT genotype. Most of the esophageal cancer patients in China are lived in poor rural areas, the nutrition of those people are usually lower than those in developed countries. Correspondingly, the insufficient folate consumption in Chinese esophageal cancer patients may alter DNA methylation, the inactive MTHFR 677T allele may lower the 5-methyl THF to intensify the DNA hypomethylation, and initiate carcinogenesis.

A study conducted in Japan showed a reverse effect of MTHFR 677TT for ESCC in drinkers (Yang et al., 2005), which indicated the different population-specific folate intake played a role in the mechanism of MTHFR C677T polymorphism for ESCC risk, which proved our hypothesis.

This meta-analysis showed heterogeneity is existed in the MTHFR 677CT and TT studies, which suggests other risk factors are involved in the mechanism of MTHFR 677CT/TT for ESCC risk. After taking subgroup analysis in terms of alcohol and tobacco consumption, the heterogeneity between studies was reduced. This indicated alcohol and tobacco had an important role in the ESCC development.

There are several limitations in our studies. Firstly, most of the studies are from China, so the evidence to distinguish the different in ethnicities is not too strong. Secondly, it is important to explore the interaction between folate and MTHFR C677T, but only one study considered their interaction, therefore, we did not do this analysis. Thirdly, three studies were not in Hardy-Weinberg equilibrium, which suggested that the samples could not better represent the expected distribution of the genotypes and may distort our findings. But the sensitivity analysis showed the robust of this study. Finally, tobacco consumption measurement was defined by different criteria across studies, which would result in overestimation or underestimation of the ORs, but sensitivity analysis showed the robust of the results.

Overall, our meta-analysis supports the hypothesis that the MTHFR 677CT/TT increase the risk of ESCC, and its effect is greatly modified by tobacco and ethnicity. No significant increased risk of ESCC was found in individuals with MTHFR A1298C. This study supports evidence for a role of MTHFR 677CT/TT polymorphism in the ESCC pathogenesis, and supports the role of insufficient folate as a carcinogen for ESCC.

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