RESEARCH COMMUNICATION

Meta-analysis of the Relationship between the Metholenetetrahydrofolate Reductase C677T Genetic Polymorphism, Folate Intake and Esophageal Cancer

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Abstract

Objective: To evaluate the effect of the methylenetetrahydrofolate reductase C677T genetic polymorphism (*MTHFR C677T*) and folate intake on the risk of esophageal cancer. Methods: A total of 17 studies (3,277 cases and 4,661 controls) regarding *MTHFR C677T* and 6 studies (1,817 cases and 7,678 controls) regarding folate intake published between 2001 and 2011 were identified through researching MEDLINE, EMBASE and the Chinese Biomedical Database. The data of the last search was February 2011. A meta-analysis was performed to obtain summary estimated odd ratios and 95% confidence intervals of folate intake and *MTHFR C677T* for esophageal cancer. Results: A significant association was seen between *MTHFR 677 CT* [adjusted OR (95% CI)=1.55(1.28-1.88)] and *TT* [crude OR (95% CI)=1.63(1.24-2.15)] genotypes and esophageal cancer. Folate intake was seen to have a preventive effect on esophageal cancer [OR (95% CI)=0.60(0.50-0.70)]. Non-drinkers with *MTHFR 677 CT* and *TT* showed light esophageal cancer risk, and higher esophageal cancer risk was found among smokers. Also, the *MTHFR 677 CT* and *TT* genotypes were associated with light esophageal cancer risk in non-drinkers and a higher risk in drinkers. The meta-regression analysis showed the effect of *MTHFR 677 CT* and *TT* increased with the level of alcohol and tobacco consumption. The *MTHFR 677 TT* genotype showed a decreased risk of esophageal cancer in the high folate intake group. Conclusion: *MTHFR 677CT/TT* increase the risk of esophageal cancer, and the effects are greatly modified by alcohol, tobacco and folate intake. Folate intake was seen to have a preventive effect on developing esophageal cancer.

Keywords: Esophageal cancer - methylenetetrahydrofolate reductase C677T - folate intake - meta-analysis

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Introduction

Esophageal cancer is a global health problem, and ranked the eighth in incidence and sixth in mortality in 2002 (Blount et al., 2007). Its incidence and mortality rates showed a wide geographic variation at an international level, and there are marked differences between high-risk and low-risk areas (Choi and Mason, 2000; Kim, 2004; Blount et al., 2007), suggesting that genetic factors and environmental factors play a role in development (Choi and Mason, 2000). The main esophageal cancers are squamous worldwide. However, there has been a recent increase in esophageal adenocarcinoma in Western countries, accounting for about 27% of esophageal cancers in men and 16% in women(Vizcaino et al., 2002). Esophageal cancer is associated with exposure to tobacco and alcohol consumption, nitrosamines via food or environment, and a diet low in folate (Parkin et al., 2005).

Folate is a water-soluable B vitamin which is in citrus fruits, green leafy vegetables, cruciferous nvegetables, legumes and so on, and evidence is mounting for a role of folate in carcinogenesis. Folate deficiency increases the risk of gastric cancer 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).

Except for an inadequate folate intake, functional polymorphisms in folate-metabolizing genes may influence susceptibility to cancer. Metholenetetrahydrofolate reductase C677T (*MTHFR C677T*) is a central enzyme in folate metabolism which catalyzes the reduction of 5, 10-methylene-tetrahydrofolate to 5-methyltetrahydrofolate, and methionine synthase then catalyzing the reaction of 5-methyltetrahydrofolate and homocysteine to generate methionine and tetrahydrofolate.

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Under the condition of folate deficiency, the *MTHFR C677T* may play a role in cancer risk in the folate metabolic pathway by facilitating the conversion of 5,10-methylene THF to 5-methyl THF, and result in point mutations and/or chromosomal breaks. Also, it may cause decline of 5-methyl THF to induce a decrease of the conversion of homocysteine to methionine, and which could result in a carcinogenesis process of DNA hypomethylation. Heterozygotes (*CT*) and homozygotes (*TT*) for the *C677T* polymorphism of *MTHFR* respectively have about 65% and 30%, respectively, of the *MTHFR* activity of those with the 677CC genotype (Bailey and Gregory, 1999). Individuals with the *TT* genotype also have significantly lower plasma folate levels and higher plasma homocysteine levels than those with the *CC* genotype (Bailey and Gregory, 1999). *TT* homozygotes have been associated with lower serum folate levels and higher homocysteine levels than CC genotype. Also, the alcohol and smoking which damage the folate metabolic pathway may interact with folate and the *MTHFR C677T* polymorphisms and the risk of esophageal cancer(Yang et al., 2005).

Previous meta-analysis indicated inverse associations of dietary folate intake and the *MTHFR C677T* polymorphisms with the risk of esophageal cancer only revealed that association of *MTHFR C677T* polymorphisms and esophageal cancer, but did not explore the gene-environment interaction of folate intake and *MTHFR C677T* polymorphisms with alcohol drinking and smoking and esophageal cancer (Larsson et al., 2006). In the present study, we conducted a comprehensive meta-analysis to clarify the effect of folate intake and *MTHFR C677T* polymorphisms with risk of esophageal cancer, whether the association was modified by drinking and smoking.

Materials and Methods

Study selection

Case-control studies that investigated the association of folate and/or *MTHFR C677T* polymorphisms with esophageal cancer were included in the meta-analysis. Tri¬als had to be original data from case-control studies regarding folate and/or *MTHFR C677T* with esophageal cancer; Trials had to provide odds ratios(ORs) or rate ratios with their confidence intervals (CLs) for the association of dietary folate intake, total folate intake, blood folate levels, or *MTHFR C677T* genotype frequencies with esophageal cancer risk.

Searching strategy for identification of studies

We searched MEDLINE, EMBASE and the Chinese Biomedical Database. The data of the last search was February 2011. We designed a comprehensive and exhaustive search strategy for MEDLINE, EMBASE and the Chinese Biomedical Database databases to identify all relevant studies.

Two reviewers (Liu YX and Li CW) independently extracted the following data from each publication: the first author's last name, year of publication, country where the study was conducted, sample size, measure of exposure and prevalence of the variant genotype in the study population. A total of 86 articles were identified, 63 studies were excluded because 13 studies were reviews, 47 studies focused on the cellular level or animal models, and 3 studies were duplicate publications. Finally, we yielded 17 case-control studies on the relationship of MTHFR C677T polymorphism and esophageal cancer, and 6 case-control studies on foalte intake and esophageal cancer risk. By contracting the related authors of four studies to supplement the missing data, data from one article were obtained, and others were not response or failure to contact. Two independent reviewers (Liu YX and Wang B) independently extract data.

Statistical analysis

The Stata SE version 9.0 software package(version 9, STATA, College Station, TX) was used for all of the statistical analysis. The lowest category of dietary folate intake was regarded as reference for the highest category, and the *MTHFR 677 CC* genotypes served as the reference for *MTHFR 677 CT* and *TT* genotypes. Statistical analysis was performed for the case-control studies. We used both the adjusted data (adjusted OR with 95% CI) and crude data (unadjusted). The heterogeneity was tested with a Q-statistics with p-values < 0.05, and its possible sources were assessed by subgroup analysis as described below. Fixed-effect model was applied to obtain the summaried ORs and their 95% confidence interval if there is no heterogeneity between studies, otherwise random-effect model was used. The Hardy-Weinberg equilibrium in the controls in each study was assessed by using the $\chi 2$ test, and Egger's regression asymmetry test was taken to evaluate publication bias ($p<0.1$ was considered representative of statistically significant publication bias). A subgroup analysis was taken regarding tobacco, alcohol consumption and folate intake as well as ethnicity. A random-effects meta-regression was conducted to investigate sources of heterogeneity and to provide an estimate of unexplained heterogeneity (Stern et al., 2001). In addition, a sensitivity analysis was performed to explore robustness of the results.

Results

Our study included 17 studies (3,277 cases and 4,661 controls) regarding *MTHFR C677T* and 6 studies (1,817 cases and 7,678 controls) regarding folate intake published between 2001 and 2011. As shown in Table 1, a significantly association was seen between *MTHFR 677 CT* [crude OR (95% CI)=1.55(1.28-1.88)] and TT [crude OR (95% CI)=1.63(1.24-2.15)] genotypes and esophageal cancer in a random-effect analysis. The adjusted OR also presented a significant association between the two genotypes and esophageal cancer (OR (95% CI) for MTHFR 677 CT and TT were 1.41(1.03-1.79) and 1.57(1.02-2.12), respectively). There was a statistically significant heterogeneity across studies regarding *MTHFR 677 CT* (p=0.003) and *TT* (p<0.001).

As shown in Table 2, folate intake was seen to have a preventive effect on esophageal cancer by using the adjusted data [OR (95% CI)=0.60(0.50-0.70)]. A

Table 1. Characteristics of Studies of *MTHFR C677T* Polymorphism and Risk of Esophageal Cancer

Study ID	County	Control source	Case	Control	Adjusted OR(95%CI)		Crude OR(95%CI)	
					CT vs CC	TT vs CC	CT vs CC	TT vs CC
Cheng 2009	China	Hospital	103	181	2.21 (1.05-4.69)	3.45 (1.59-7.48)	2.36 (1.12-4.98)	3.45 (1.59-7.48)
Wang 2009[1]	China	Hospital	102	108	NA	NA	1.94 (1.07-3.52)	1.70 (0.75-3.88)
Qin 2008	China	Population	120	240	2.69 (1.63-4.44)	2.15 (0.77-5.98)	2.55 (1.59-4.09)	1.80 (0.67-4.86)
Li 2008[1]	China	Population	126	169	NA	NA	1.56 (0.83-2.95)	1.47 (0.78-2.77)
He 2007[1]	China	Population	584	540	1.76 (1.22-2.52)	2.36 (1.62-3.42)	1.83 (1.30-2.58)	2.16 (1.53-3.06)
Zhang 2006[1]	China	Hospital	94	98	NA	NA	1.26 (0.68-2.35)	0.63 (0.23-1.71)
Feng 2006	China	Population	275	315	0.96	1.58	1.07 (0.69-1.65)	1.76 (1.13-2.75)
Song 2001	China	Population	240	360	3.14 (1.94-5.08)	6.18 (3.32-11.5)	2.98 (1.87-4.75)	6.52 (3.89-10.9)
Wang 2005	China	Population	275	315	0.96 (0.61-1.50)	1.58 (0.99-2.50)	1.07 (0.69-1.65)	1.76 (1.13-2.75)
Yang 2005	Japan	Hospital	165	493	0.97 (0.63-1.49)	0.66 (0.35-1.25)	1.07 (0.73-1.56)	0.74 (0.42-1.30)
Zhang 2004	German	Population	241	256	NA	NA	1.15 (0.79-1.68)	1.04 (0.59-1.82)
Zhang 2004	China	Population	189	141	1.81 (1.28-2.54)	2.13 (1.50-3.02)	2.69 (1.32-5.48)	2.02 (0.99-4.10)
Kureshi 2004	Pakistan	Population	34	54	NA	NA	0.97 (0.39-2.41)	0.16 (0.01-3.13)
Zhang 2003[1]	China	Population	198	141	NA	NA	2.69 (1.32-5.48)	2.24 (1.11-4.55)
Stolzenberg 2003	China	Population	129	398	0.86 (0.48-1.54)	1.24 (0.68-2.26)	0.78 (0.45-1.37)	1.09 (0.61-1.96)
Miao 2002	China	Population	217	468	NA	NA	1.58 (1.06-2.37)	2.02 (1.29-3.19)
Wu 2002	China	Population	93	200	1.0 (0.57-1.75)	0.86 (0.59-1.25)	0.96 (0.54-1.74)	0.80 (0.36-1.78)
Wang 2007	China	Population/Hospital	92	184	NA	NA	2.41 (1.40-4.13)	1.20 (0.35-4.09)
Pooled results (Random effect model)			3,064	4,221	1.41 (1.03-1.79)	1.57 (1.02-2.12)	1.55 (1.28-1.88)	1.63 (1.24-2.15)

[1]The genotype frequencies among the controls differed significantly from the Hardy-Weinberg equilibrium ($P < 0.05$); MTHFR 677T, Metholenetetrahydrofolate reductase C677T; OR, Odds ratio; CI, confidence interval; NA, not available

Table 2. Characteristics of Studies of Folate Intake and Risk of Esophageal Cancer

Study ID	Country	Control source	Range of Intake	Cancer	Cases	Control	Adjusted OR(95%CI)
Chen 2002	USA	Population	Highest vs lowest	SCC	124	449	0.80 (0.40-1.40)
Aune 2011	Uruguay	Hospital	275.31-123.83ug /day	SCC	234	2,032	0.40 (0.19-0.85)
Ibiebele 2011	Austria	Population	Highest vs lowest	SCC	245	1,507	0.78 (0.51-1.19)
Ibiebele 2011	Austria	Population	Highest vs lowest	AC	636	1,507	0.72 (0.53-0.98)
Galeone 2006	Italy/Swiss	Hospital	Highest vs lowest	SCC	90	314	0.68 (9.46-1.00)
Mayne 2001	USA	Population	Highest vs lowest	SCC	206	687	0.48 (0.36-0.66)
Mayne 2001	USA	Population	Highest vs lowest	AC	282	687	0.58 (0.39-0.86)
Yang 2005	Japan	Hospital	300-400ug/day	SCC	165	495	0.77 (0.45-1.31)
Pooled results (Random effect model)					1,817	7,678	0.60 (0.50-0.70)

SCC, squamous cancer; AC, adenocarcinoma; OR, Odds ratio; CI, confidence interval

significantly heterogeneity was found across studies (p=0.009). High intake of folate intake was also found preventive effect of esophageal cancer among smokers [OR (95% CI)= 0.75(0.56-0.94)].

Figures 1 and 2 show that the *MTHFR 677 CT* and *TT* genotypes were seen to significantly increase the esophageal cancer risk either in non-smokers (*CT* genotype: OR (95% CI)=1.41 (1.08-1.86); *CC* genotype: OR (95% CI)=1.26 (0.78-2.04)], or drinkers (*CT* genotype: OR (95% CI)=2.36 (1.13-4.94); *CC* genotype: OR (95% CI)=2.43(1.31-4.51)] (Figures 1 and 2). The *MTHFR 677 CT* and *TT* genotypes also was seen light esophageal cancer risk in non-drinkers [*CT* genotype: OR (95% CI)=1.59(1.11-2.27); *CC* genotype: OR (95% CI)=1.68(1.14-2.49)], and a higher risk in drinkers [*CT* genotype: OR (95% CI)=2.15(1.47-3.15); *CC* genotype: OR (95% CI)=2.69(1.56-4.64)] (Figures 3 and 4). The meta-regression analysis showed the more alcohol or tobacco consumed, the great OR for *MTHFR 677 CT* and *TT* genotypes was observed (p<0.05), suggesting a clear gene-environment interaction for esophageal cancer.

We further evaluated the modification of ethnicity and folate status on the effect of *MTHFR 677 CT* and *TT* genotypes for esophageal cancer (Table 3). The *MTHFR 677 CT* and *TT* genotypes were associated with esophageal cancer risk in Chinese, and light risk in German. But preventive effect was found of the two genotypes for esophageal cancer in Japan and Pakistan. The *MTHFR 677 TT* genotype showed a decreased risk of esophageal cancer only in the high folate intake group[OR (95% CI)=

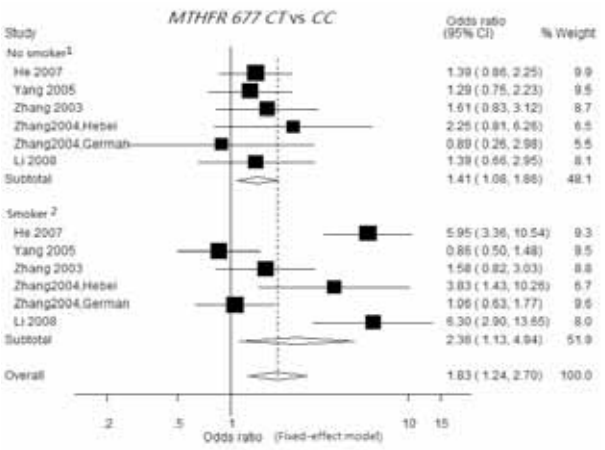

Figure 1. Relationship between MTHFR 677CT and Esophageal Cancer Stratified by Tobacco Consumption. 1, never smoker; 2, ever and current smokers

Table 3. Risk of Esophageal Cancer Associated with MTHFR 677 CT and TT Regarding Ethnicity and Folate Intake

Items	MTHFR 677 CT vs CC Pooled OR (95% CI)	MTHFR 677 TT vs CC Pooled OR (95% CI)
Ethnicity		
Chinese (Song et al., 2001; Wu et al., 2002; Miao et al., 2002; Stolzenberg-Solomon et al., 2003; Zhang et al., 2003; Zhang et al., 2004; Wang et al., 2005; Feng et al., 2006; Zhang et al., 2006; He et al., 2007; Wang et al., 2007; Li et al., 2008; Chen et al., 2009; Wang et al., 2009)	1.62 (1.30-2.02)	1.84 (1.39-2.44)
Pakistani (Kureshi et al., 2004)	0.97 (0.39-2.41)	0.16 (0.01-3.13)
Japanese (Yang et al., 2005)	1.07 (0.73-1.56)	0.74 (0.42-1.30)
German (Zhang et al., 2004)	0.89 (0.26-2.98)	1.18 (0.21-6.70)
Folate intake		
≤300 μg/day (Stolzenberg-Solomon et al., 2003; Li et al., 2008)	1.10 (0.58-2.09)	1.38 (0.43-4.47)
≥300 μg/day (Yang et al., 2005)	1.00 (0.61-1.64)	0.57 (0.28-1.18)

MTHFR 677T, Metholenetetrahydrofolate reductase C677T; OR, Odds ratio; CI, confidence interval

Table 4. Risk of Esophageal Cancer Associated with Folate Intake Regarding SAmoking and Drinking

Items	Highest folate intake vs lowest Pooled OR (95% CI)
Smoking status	
Non-smoker*	0.82 (0.48-1.16)
Smoker*	0.75 (0.56-0.94)
Drinking status	
Non-drinker*	0.72(0.37-1.06)
Drinker*	1.01(0.71-1.31)

*Yang et al., 2005; Graziano et al., 2006

0.57 (0.28-1.18)], suggesting high intake of folate could decreasing the esophageal cancer risk of people with *MTHFR 677 TT* genotype. With smoking and drinking there was only a protective interaction with smokers (Table 4)

Sensitivity analysis and publication bias

The Egger's test showed no significant publication bias in the *MTHFR C677T* polymorphism studies, and the P values for *MTHFR 677 CT* and *TT* were 0.07 and 0.23, respectively. The sensitivity analysis showed the overall ORs of the *MTHFR 677 CT* genotype did not reverse after excluding one large-sample studies (He et al., 2007), and overall ORs of the *MTHFR 677 TT* did not change either after excluding two large-sample study (Feng et al., 2006;

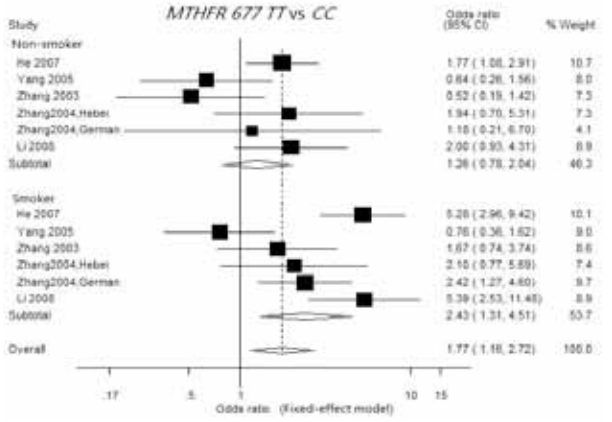

Figure 2. Relationship between MTHFR 677TT and Esophageal Cancer Stratified by Tobacco Consumption

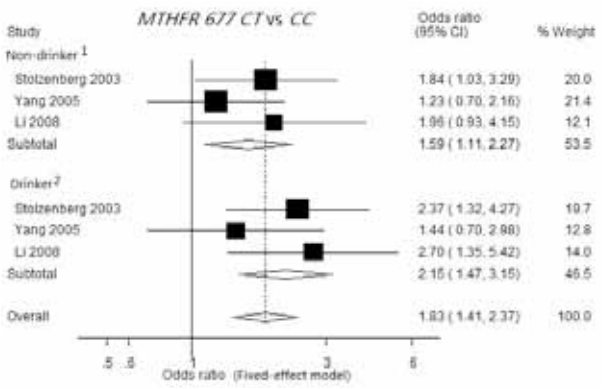

Figure 3. Relationship between MTHFR 677CT and Esophageal Cancer Stratified by Alcohol Consumption. 1, never drinker; 2, ever and current drinker

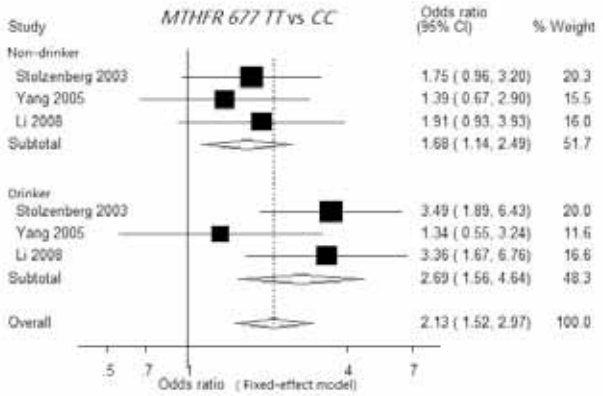

Figure 4. Relationship between MTHFR 677TT and Esophageal Cancer Stratified by Alcohol Consumption

He et al., 2007), which suggested the robust nature of our findings.

Discussion

Our results from the 17 cases-control studies regarding *MTHFR C677T* and 6 case-control studies regarding folate intake in the meta-analysis support that *MTHFR 677 CT* and TT are associated with an increased risk of esophageal cancer, and a dietary folate intake is related to a significant inverse esophageal cancer risk. Summary results show that individuals with *MTHFR 677 CT* and *TT* genotypes are about 70% to 80% more likely to develop esophageal

cancer compared with CC genotypes among smokers and drinkers. A high dietary folate intake is about 30% to 60% higher risk of developing esophageal cancer.

Our study showed subjects with *MTHFR 677 TT* genotype had high risk of esophageal cancer compared with *CC* genotype, and also seemed in drinker and smokers, mainly due to the active of *MTHFR 677 CT* is about two times than *TT* genotype (Bailey and Gregory, 1999). Previous study proved the *MTHFR 677 TT* genotype had the OR of 2.13 to 6.18 for esophageal cancer, and CT genotype only had the OR of 1.81 to 3.14 compared with *MTHFR 677 CC* genotype (Song et al., 2001; Zhang et al., 2004; Wang et al., 2007). But it is controversy to studies conducted in Japan, German and Pakistani, which presents the *MTHFR 677 TT* genotype is significant inverse associations with esophageal cancer in Table 3. The main reason may be the risk of MTHFR C677T polymorphisms for esophageal cancer depends on the level of folate intake (Chen et al., 1996; Ma et al., 1997), and inactive *MTHFR* may elevate the 5,10-methylene-tetrahydrofolate and facilitate DNA synthesis, while adequate provision of methyl donors could still be ensured, therefore, subjects with *MTHFR 677 TT/CT* genotypes may have a decreased risk of cancer when folate intake is sufficient (Yang et al., 2005). While in the high risk area of esophageal cancer in China, most patients are living in urban areas and often lack of folate intake (Stolzenberg-Solomon et al., 2003), and this induces inactive *MTHFR 677CT/TT* genotype carrying a higher risk for development of esophageal cancer in China than developed countries.

Previous study suggested the cancer risk associated with *MTHFR* polymorphisms depends on the level of folate intake(Ma et al., 1997). There might be a gene-nutrient interaction between folate consumption and the direction of impact of the *MTHFR 677T* allele. It means individuals with *MTHFR 677CT/TT* genotypes may have a decreased risk of cancer if folate intake is sufficient, because decrease in *MTHFR* activity associated with the *677TT* polymorphism might lead to an elevation in 5,10-methylene-tetrahydrofolate, facilitating DNA synthesis, while adequate provision of methyl donors could still be ensured. In contrast, in the presence of low folate intake, both impaired DNA methylation and DNA synthesis/repair may become the primary mechanisms of carcinogenesis. Therefore, under the condition of low folate intake, the impaired DNA methylation and DNA synthesis/repair may become the mechanisms of carcinogenesis. Our study is in line with the above hypothesis. Individuals with *TT* genotype and high folate intake showed decreased risk of esophageal cancer, but not found significant relationship in the *CT* genotype. Further more studies should be taken to explore the association between *TT* genotype and folate intake.

Interaction between the *MTHFR 677 CT/TT* genotypes and tobacco and alcohol consumption was found in our meta-analysis(Figures 1-4). Previous meta-analysis did not examine this issue in detail (Larsson et al., 2006), Previous study reported smokers with the *CT/TT* genotypes had 7.7 times higher risk of gastric cancer compared with non-smokers with the CC genotype (Gao et al., 2002), which is in line with our study and mainly due to the impair effect of smoke for folate status. Heavy drinking is recognized as a major risk factor for esophageal cancer and alcohol is considered to induce DNA damage and resultant modification of nucleotides (Wu and Cederbaum, 2003). Individuals with *677TT* genotype, who are expected to have high 5,10-methylene-tetrahydrofolate concentrations, would have lower esophageal cancer risk when exposed to high amounts of alcohol. Previous study showed individuals with *MTHFR 677 TT* presented a significant 5.3 times higher risk of gastric cardia adenocarcinoma (Stolzenberg-Solomon et al., 2003) and 5.4 times higher risk of gastric cancer (Graziano et al., 2006) compared non-drinkers with *CC* and *CT* genotypes. Our study proved the alcohol and smoking modified the *MTHFR C677T* association with esophageal cancer.

There are several limitations in our studies. Firstly, as a observational studies, there is potential for recall bias from case-control studies. Secondly, a potential source of bias in studies of genotypes might be the inclusion of individuals from different ethnic backgrounds. But our study performed subgroup analysis regarding ethnicity. Thirdly, the category criteria of tobacco, alcohol consumption and folate intake is different in studies, which could bring measure bias in our study. Fourthly, five studies are not in Hardy-Weinberg equilibrium, which indicated the samples could not better represent the expected distribution of the genotypes and may distort our findings. Finally, as with any meta-analysis, publication bias could be of concern. Tests for publication bias in the literature on dietary folate intake and *MTHFR 677CT/TT* and risk of esophageal were not statistically significance. However, there are many studies on the folate-rich foods and *MTHFR 677CT/TT* gene-related factors, such as fruits and vegetables in relation to esophageal cancer that were not included in this meta-analysis because they did not present results in terms of folate and *MTHFR 677CT/TT*.

In conclusion, the results of our meta-analysis indicate the *MTHFR 677CT/TT* increase the risk of esophageal cancer at all levels of exposure to alcohol and tobacco, and its effect is greatly modified by alcohol and tobacco. Folate intake presents preventive effect on the esophageal cancer, and individuals with *MTHFR 677CT/TT* genotypes may have a decreased risk of cancer if folate intake is sufficient. The finding provides more information on screening the high risk group of esophageal cancer, and new strategy to prevention of esophageal cancer.

Acknowledgments

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