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Meta-analysis on the relationship between genetic polymorphism of methylenetetrahydrofolate reductase genetic polymorphisms and esophageal squamous cell carcinoma in Chinese population

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We aimed to evaluate the association of Methylenetetrahydrofolate reductase C677T polymorphism with ESCC. We searched MEDLINE, EMBASE and the Chinese Biomedical Database. All the articles searched were independently reviewed and selected by 2 evaluators according to the predetermined criteria. A total of 10 case-control studies published between 2001 and 2011 were included. When all the studies were pooled, the crude odds ratio (95% CI) of ESCC for individuals carrying MTHFR 677 CT and TT genotype were 1.70 (1.32 to 2.20) and 2.10 (1.58 to 2.79), respectively. An Egger's test provided that the effect estimates were not related to study size (p values for MTHFR 677 CT and TT were 0.16 and 0.17, respectively). The meta-analysis provides evidence that MTHFR 677CT/TT plays a carcinogenic role in ESCC, and its effect is modified by alcohol, tobacco and ethnicity.

Key words: Methylenetetrahydrofolate reductase C677T, polymorphism, esophageal cancer, meta-analysis.

INTRODUCTION

Esophageal cancer is the eighth most common cancer worldwide with 481 000 new cases (3.8% of the total) estimated in 2008 and the sixth most common cause of death from cancer with 406 000 deaths (5.4% of the total). These figures encompass both adenocarcinoma and squamous cell carcinoma (SCC) types. More than 80% of the cases and of the deaths occur in developing countries (IARC, 2011). The incidence rates of oesophageal cancer vary internationally more than 15-fold in men, and almost 20-fold in women which suggested the role of genetic and environmental factors in the pathogenesis of this cancer (Chen et al., 1996; Choi and Mason, 2000). 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. 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 Selhub, 1999).

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Table 1. Characteristics of studies of MTHFR C677T polymorphism and ESCC.

| Study ID | County | Control source | Case | Control | Adjusted OR (95%CI) | | Case/control | | |
					CT vs CC	TT vs CC	CC	CT	TT
Cheng Y 2009	China	Hospital	103	181	2.21(1.05-4.69)	3.45(1.59-7.48)	11/45	49/85	43/51
Li DQ et al., 2008[1]	China	Population	126	169	NA	NA	22/41	52/62	52/66
He YT 2007[1]	China	Population	584	540	1.76(1.22-2.52)	2.36(1.62-3.42)	73/119	263/234	248/187
Feng CW et al., 2006	China	Population	275	315	0.96	1.58	51/74	105/143	119/98
Song C 2001	China	Population	240	360	3.14 (1.94-5.08)	6.18 (3.32-11.51)	29/126	118/172	93/62
Wang LD 2005	China	Population	275	315	0.96(0.61-1.50)	1.58(0.99-2.50)	51/74	105/143	119/98
Wang 2007	China	Population	92	184	NA	NA	48/130	40/45	4/9
Zhang J 2004	China	Population	189	141	1.81 (1.28-2.54)	2.13 (1.50-3.02)	16/25	93/54	89/62
Zhang JH 2003[1]	China	Population	198	141	NA	NA	16/25	93/54	89/62
Stolzenberg RZ 2003	China	Population	129	398	0.86 (0.48-1.54)	1.24 (0.68-2.26)	23/65	58/209	48/124
Miao XP et al., 2002	China	Population	217	468	NA	NA	47/151	107/217	63/100
Pooled results (random effect model)			2984	4054	1.37(0.79-1.84)	1.72(1.12-2.43)	387/875	1083/1418	958/919

[1]The genotype frequencies among the controls differed significantly from the Hardy-Weinberg equilibrium ($P < 0.05$). MTHFR 677T: methylenetetrahydrofolate reductase C677T. OR: odds ratio. CI: confidence interval.

Ultimately, MTHFR plays a role in the carcinogenesis process of DNA hypomethylation. Several studies have explored the impact of MTHFR C677T polymorphism in various cancers, but the association with EC is conflicting (Song et al., 2001; Stolzenberg-Solomon et al., 2003; Yang et al., 2005). We conducted a comprehensive meta-analysis to clarify the effect of folate intake and MTHFR C677T polymorphisms with risk of esophageal cancer.

MATERIALS AND METHODS

Searching strategy

We searched and reviewed MEDLINE (from January 1966 to May 2011), EMBASE (from January 1988 to May 2011), and the Chinese Biomedical Database (CBM; from January 1980 to May 2011) related to MTHFR C677T polymorphisms and ESCC risk in case-control studies. We designed a comprehensive and exhaustive search strategy for MEDLINE, EMBASE and the Chinese Biomedical Database databases to identify all relevant studies.

Data extraction

Two reviewers 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. 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 10 case-control studies on the relationship of MTHFR C677T polymorphism and ESCC.

Statistical analysis

Statistical analysis was performed for the case-control studies by STATA statistical package (version 9, STATA, College Station, TX). 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 MTHFR 677 CC genotypes served as the reference for MTHFR 677 CT and TT genotypes. In carrying out the meta-analysis, random effect models were used to take into account the possibility of heterogeneity between studies and its possible sources were assessed by subgroup analysis. The publication bias was identified by Egger's test and we estimated that there was a publication bias if the p-value for Egger's test was less than 0.05. 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.

RESULTS

Our study included a total of 10 case-control studies (2428 cases and 3212 controls) published between 2001 and 2010. Table 1 showed the main characteristics of the studies included in the analysis. Of the 13 case-control studies, 9 studies were conducted in China, one in China and German, one in Japan, one in Pakistan and one in India. A significant association was seen between individuals with MTHFR 677 CT [crude OR (95%)
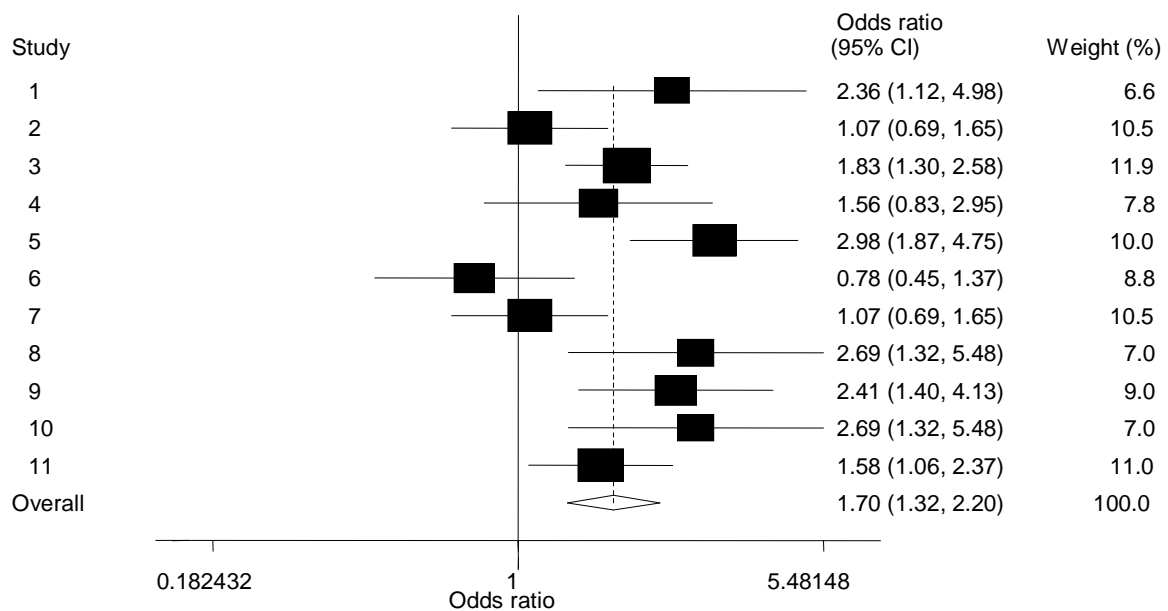
	Odds ratio (95% CI)	Weight (%)
Study		
1	2.36 (1.12, 4.98)	6.6
2	1.07 (0.69, 1.65)	10.5
3	1.83 (1.30, 2.58)	11.9
4	1.56 (0.83, 2.95)	7.8
5	2.98 (1.87, 4.75)	10.0
6	0.78 (0.45, 1.37)	8.8
7	1.07 (0.69, 1.65)	10.5
8	2.69 (1.32, 5.48)	7.0
9	2.41 (1.40, 4.13)	9.0
10	2.69 (1.32, 5.48)	7.0
11	1.58 (1.06, 2.37)	11.0
Overall	1.70 (1.32, 2.20)	100.0

0.182432   1   5.48148
Odds ratio

Figure 1. Relationship between MTHFR 677 CT vs. CC and ESCC.

	Odds ratio (95% CI)	Weight (%)
Study		
1	3.45 (1.59, 7.48)	7.1
2	1.76 (1.13, 2.75)	11.0
3	2.16 (1.53, 3.06)	12.3
4	1.47 (0.78, 2.76)	8.6
5	6.52 (3.89, 10.92)	10.1
6	1.09 (0.61, 1.96)	9.3
7	1.76 (1.13, 2.75)	11.0
8	2.02 (0.99, 4.10)	7.8
9	1.20 (0.35, 4.09)	4.0
10	2.24 (1.11, 4.55)	7.8
11	2.02 (1.28, 3.19)	10.9
Overall	2.10 (1.58, 2.79)	100.0

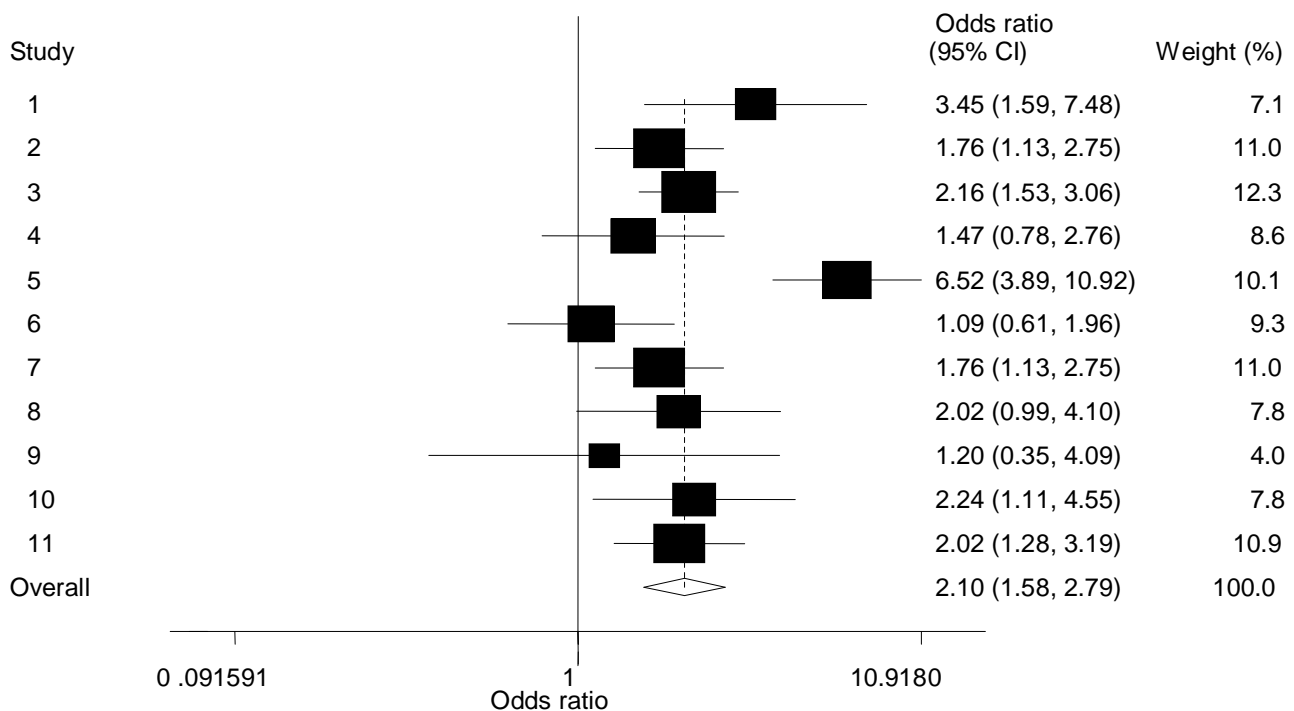0 .091591   1   10.9180
Odds ratio

Figure 2. Relationship between MTHFR 677TT vs. CC and ESCC.

= 1.70 (1.32-2.20)] and TT [crude OR (95%) = 2.10 (1.58 to 2.79)] genotypes and ESCC risk (p<0.05) (Figures 1 and 2). The crude OR was similar to the adjusted OR of 1.37 (0.79 to 1.84) for MTHFR 677 CT and 1.72 (1.12 to 2.43) for MTHFR 677 TT genotype. There was significant heterogeneity across studies regarding MTHFR 677 CT (p = 0.003) and TT (p<0.001). An Egger's test provided that the effect estimates were not related to study size (p
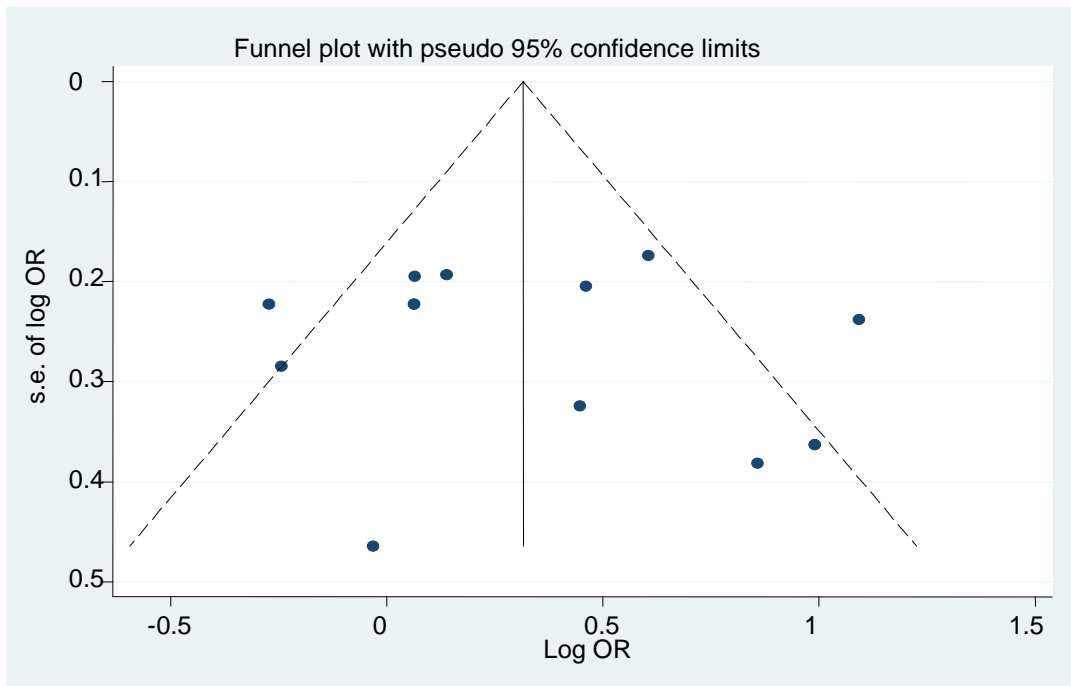
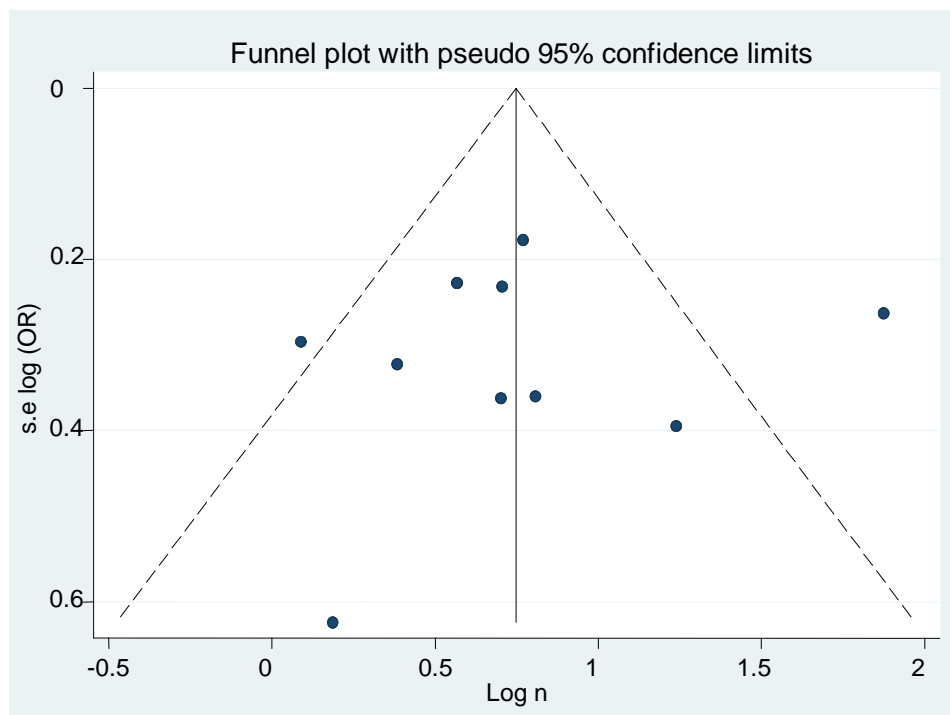**Figure 3.** Publication bias on studies of MTHFR 677CT vs. CC.

Figure 4. Publication bias on studies of MTHFR 677TT vs. CC.

values for MTHFR 677 CT and TT were 0.16 and 0.17, respectively), providing some reassurance that small study bias, such as publication bias has not distorted the findings. A symmetry funnel plot also showed no existence of publication bias (Figures 3 and 4). A sensitivity analysis indicated the overall crude ORs did

not greatly changed after excluding six studies which did not have controls in HWE.

Similarly, the overall ORs of individuals with MTHFR 677 CT 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 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 CT and TT genotypes increased ESCC risk. This finding supports the hypothesis that polymorphism of MTHFR C677T plays a critical role in the development of ESCC. We found Chinese carrying MTHFR 677 CT and TT genotypes were associated with a higher ESCC risk than other ethnicities. Our study showed subjects with MTHFR 677 TT genotype had high risk of esophageal cancer compared with CC 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 1. 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). There are several limitations in our studies. Firstly, most of the studies are from China, so the evidence to distinguish the difference in ethnicities is not too strong. Secondly, it is important to explore the interaction between folate and MTHFR C677T, but only one study considering their interaction; therefore, we did not do this analysis. Thirdly, five 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.

In conclusion, the results of our meta-analysis indicate that the MTHFR 677CT/TT increase the risk of esophageal cancer. The finding provides more information on screening the high risk group of esophageal cancer and new strategy to prevention of esophageal cancer.

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