RESEARCH ARTICLE

Methylenetetrahydrofolate Reductase Polymorphisms and Susceptibility to Esophageal Cancer in Chinese Populations: a Meta-analysis

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Abstract

   Although many epidemiologic studies investigated the methylenetetrahydrofolate reductase (MTHFR) polymorphisms and their associations with esophageal cancer, definite conclusions could not be drawn. To clarify the effects of MTHFR polymorphisms on the risk of esophageal cancer, a meta-analysis was here performed in Chinese populations. A total of 16 studies including 3,040 cases and 4,127 controls were involved in this meta-analysis. Overall, significant associations were found between the MTHFR C677T polymorphism and esophageal cancer risk when all studies in Chinese populations were pooled into the meta-analysis (T vs. C, OR = 1.19, 95% CI = 1.06–1.34; TT vs. CC, OR = 1.35, 95% CI = 1.07–1.70; TT+ CT vs. CC, OR = 1.29, 95% CI = 1.08–1.54; TT vs. CC + CT, OR = 1.19, 95% CI = 1.03–1.37). In subgroup analyses stratified by ethnicity and source of controls, the same results were found in Kazakh (TT vs. CC, OR = 1.38, 95% CI = 1.02-1.87; TT + CT vs. CC, OR = 1.50, 95% CI = 1.03-2.18), in not stated populations (T vs. C, OR = 1.24, 95% CI = 1.08-1.42; TT vs. CC, OR = 1.47, 95% CI = 1.10-1.96; TT + CT vs. CC, OR = 1.30, 95% CI = 1.05-1.60; TT vs. CC + CT, OR = 1.32, 95% CI = 1.12-1.56), and in hospital-based studies (T vs. C, OR = 1.34, 95% CI = 1.19-1.51; TT vs. CC, OR = 1.81, 95% CI = 1.37-2.39; TT + CT vs. CC, OR = 1.51, 95% CI = 1.26-1.83; and TT vs. CC + CT, OR = 1.39, 95% CI = 1.13-1.70). In conclusion, this meta-analysis provides evidence that the MTHFR C677T polymorphism contributes to esophageal cancer development in Chinese populations.

Keywords: Meta-analysis - methylenetetrahydrofolate reductase - polymorphism - esophageal cancer

Introduction

   Esophageal cancer (EC) is a global health problem, and an estimated 482, 300 new esophageal cancer cases and 406, 800 deaths occurred in 2008 worldwide (Jemal et al., 2011). Its incidence rates vary internationally, and the highest rates found in Southern and Eastern Africa and Eastern Asia were nearly 16-fold, compared with lowest rates observed in Western and Middle Africa and Central America in both males and females (Jemal et al., 2011). Esophageal cancer is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer death in China, with an estimated 259, 235 new cases and 211, 084 deaths in 2008 (Lin et al., 2013). The estimated age-adjusted incidence rate of esophageal cancer in China in 2008 was 16.7 per 100, 000 population (Lin et al., 2013). Epidemiological studies have indicated that low folate, a constituent of vegetables and fruits, is associated with an increased risk of cancer, including esophageal cancer (Chang-Claude et al., 1990; Hu et al., 1994; Zhang et al., 1997). Methylenetetrahydrofolate reductase (MTHFR) is a central regulatory enzyme in folate metabolism that

catalyses the reduction of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulating form of folate. Thus, MTHFR acts as a critical juncture in folate metabolism by directing folate metabolites toward the DNA methylation pathway and away from the DNA synthesis pathway. Two common functional polymorphisms of the MTHFR gene, C677T and A1298C, have been identified, and the variant genotypes are associated with low plasma folate levels and significantly diminished the MTHFR activity of individuals (Frosst et al., 1995; Weisberg et al., 1998; Friedman et al., 1999), so polymorphisms in the MTHFR gene may contribute to genetic susceptibility to esophageal and other cancers (Kim, 2000). In this study, we assess the relationships of MTHFR C677T and A1298C polymorphisms with risk of esophageal cancer in Chinese populations by conducting meta-analyses of available case-control and cohort studies.

Materials and Methods

Materials
   A computerized literature search was carried out

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

Reference	Source of controls	Area	Ethnicity	Genotype	C677T						A1298C					
					Cases			Controls			Cases			Controls		
					CC	CT	TT	CC	CT	TT	AA	AC	CC	AA	AC	CC
Zhang 2006	PB	Xinjiang	Kazakh	C677T	53	34	7	57	29	12						
Zhang 2006	PB	Xinjiang	Han	C677T	27	34	23	13	30	14						
Qin 2008	PB,HB	Xinjiang	Kazakh	C677T	60	53	7	170	59	11						
Wang 2007	PB	Hebei	Han	C677T	73	263	248	119	234	187						
Gao 2004	PB	Jiangsu	Han	A1298C							90	48	3	164	64	0
Wu 2002	PB	Jiangsu	Han	C677T	31	47	15	63	99	38						
Song 2001	PB	Beijing	Not stated	C677T, A 1298C	29	118	93	126	172	62	179	54	7	242	113	5
Wang 2005	PB	Henan	Not stated	C677T	51	105	119	74	143	98						
Li 2011	PB	Feicheng	Not stated	C677T	77	85	64	91	97	58						
Stolzenberg-Solomon 2003 *	PB	Linxian	Not stated	C677T	23	58	48	65	209	124						
Cai 2011	PB, HB	Xinjiang	Kazakh	C677T	32	66	27	114	95	41						
Yang 2012	PB	Sichuan	Not stated	C677T	37	45	18	40	41	16						
Wang 2009	HB	Shandong	Not stated	C677T	39	47	16	58	36	14						
Zhang 2008	PB	Xinjiang	Kazakh	A1298C							56	30	2	52	20	0
Qu 2013	PB	Henan	Han	C677T, A 1298C	69	181	128	47	185	193	273	99	9	310	104	13
Zhao 2011	HB	Chengdu	Not stated	C677T	68	74	13	179	120	11						
Chen 2009	HB	Xinjiang	Han	C677T, A 1298C	11	49	43	45	85	51	69	33**		141	56**	

PB Population-based, HB hospital-based; *Study excluded from the meta-analysis of MTHFR A1298C; not in Hardy-Weinberg equilibrium; **Number of (AC+CC)

in PubMed, Springer Link, OvidSP, CBM (Chinese biomedical database), CNKI (Chinese national knowledge infrastructure), VIP (Chinese) database, and Wanfang (Chinese) Database to collect articles of case-control studies or cohort studies on associations between MTHFR polymorphisms and susceptibility to esophageal cancer before 25 November 2013. We also reviewed the reference lists of the relevant articles and performed searching based on Google scholar and Baidu scholar to identify additional studies. The PubMed search was run using the Mesh terms: (mthfr OR methylenetetrahydrofolate reductase) AND (esophagus OR esophageal) AND (cancer OR carcinoma). In Chinese Databases, the following words were used: (MTHFR OR Chinese technical term of MTHFR) and (esophageal cancer OR esophageal tumor OR relevant Chinese technical terms).

Inclusion/exclusion criteria

Studies included in our meta-analysis have to meet the following criteria: (1) the study used case-control study or cohort study design; (2) sufficient published data about the size of the sample, odds ratio (OR), and their 95 % confidence interval (CI); (3) published in English or Chinese language; (4) the gene distributions of control groups were in agreement with Hardy-Weinberg equilibrium; (5) all participants were Chinese. Studies were excluded when they were: (1) not case-control study or cohort study; (2) duplicate of previous publication; (3) based on incomplete data; (4) meta-analyses, letters, reviews, or editorial articles.

Data extraction

Data was independently extracted by two reviewers using a standardized data extraction form. Discrepancies were resolved by discussion and if consensus was not achieved the decision was made by the third reviewer. The title and abstract of all potentially relevant articles were screened to determine their relevance. Full articles were also scrutinized if the title and abstract were ambiguous. We extracted standardized data sets from studies of MTHFR polymorphism and esophageal cancer. The following information was sought from each publication: authors, journal and year of publication, study design, sample size, geographical location, ethnicity of subjects, numbers of cases and controls, genotype frequencies of MTHFR C677T and A1298C.

Statistical analysis

Statistical analysis was conducted by using STATA statistical package (version 9, STATA, College Station, TX). The distributions of genotypes in controls were tested by Hardy-Weinberg equilibrium using the Chi-square test. The association of polymorphisms of MTHFR and esophageal cancer risk was estimated by odds ratio (ORs) with 95% confidence intervals (CIs). The heterogeneity was tested by the Q-statistics with P-values < 0.1, and its possible sources of heterogeneity were assessed by subgroup analysis. If there was heterogeneity, the random effect model would be used. Otherwise, a fixed-effect model was applied to obtain the summary OR and their 95% CI. All the P-values were two sided. A P-value less than 0.05 was considered statistically significant.

Results

The characteristics of included studies

According to the inclusion criteria, 15 case-control studies (Song et al., 2001; Wu et al., 2002; Gao et al., 2004; Wang et al., 2005; Zhang et al., 2006; Wang et al., 2007; Qin et al., 2008; Zhang et al., 2008; Chen et al., 2009; Wang et al., 2009; Cai et al., 2011; Li et al., 2011; Zhao et al., 2011; Yang et al., 2012; Qu et al., 2013) and one case-cohort study (Stolzenberg-Solomon et al., 2003) were included and 56 articles were excluded. The publication year of involved studies ranged from 2001 to 2013. The flow chart of study selection is shown in Figure 1. In total,
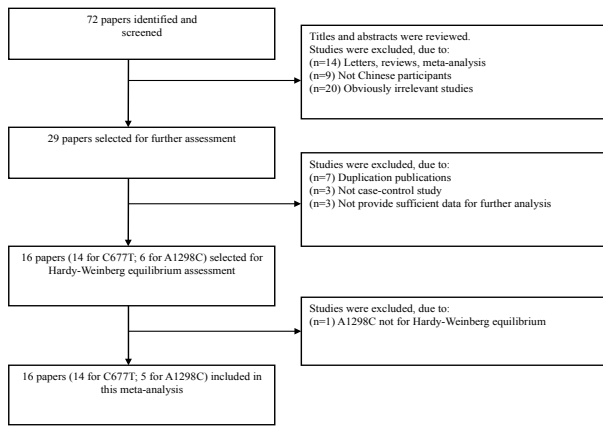
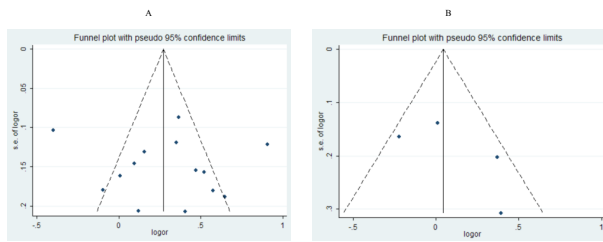**Figure 1. Paper Identification and Exclusion**

Figure 2. Funnel Plot Analysis to Detect Publication Bias. Each point represents a separate study for the indicated association. a Funnel plot for allele contrast (T vs. C) of C677T polymorphism in overall analysis; b Funnel plot for allele contrast (C vs. A) of A1298C polymorphism in overall analysis
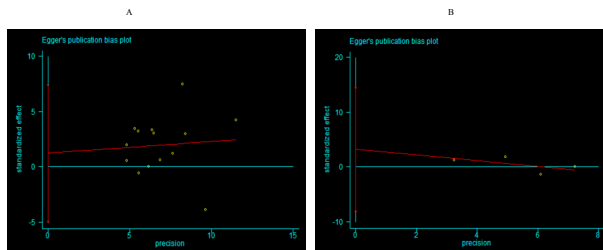

Figure 3. Egger's Linear Regression Test for Publication Bias. a: For allele contrast (T vs. C) of C677T polymorphism in overall analysis; b: For allele contrast (C vs. A) of A1298C polymorphism in overall analysis

3,040 esophageal cancer cases and 4,127 healthy controls were involved in this meta-analysis, which evaluated the relationship between MTHFR polymorphism and esophageal cancer risk. The source of controls was mainly based on a healthy population. Fourteen of these studies conducted for MTHFR C677T polymorphisms, five studies for MTHFR A1298C polymorphisms. The characteristics of the included studies are summarized in Table 1.

Quantitative synthesis

The main results of this meta-analysis and the heterogeneity test were shown in Table 2. With respect to C677T polymorphism, significantly elevated esophageal cancer risk was found in overall analysis (T vs. C, OR = 1.19, 95% CI = 1.06-1.34; TT vs. CC, OR = 1.35, 95% CI = 1.07-1.70; TT + CT vs. CC, OR = 1.29, 95% CI = 1.08-1.54; TT vs. CC + CT, OR = 1.19, 95% CI = 1.03-1.37). In the subgroup analysis by ethnicity, significantly increased risk was found in Kazakh (TT vs. CC, OR =

Table 2. Summary ORs and 95% CI of MTHFR C667T Polymorphism and Esophageal Cancer Risk

Analysis model	Ethnicity	OR	95% CI (*P* value)	*P*[a]
T vs. C	Overall	1.19[b]	1.06-1.34 (0.003)	<0.001
	Han	1.04[b]	0.84-1.29 (0.712)	<0.001
	Kazakh	1.27[b]	0.98-1.65 (0.069)	0.014
	Not stated	1.24[b]	1.08-1.42 (0.003)	<0.001
TT vs. CC	Overall	1.35[b]	1.07-1.70 (0.010)	<0.001
	Han	1.10[b]	0.71-1.72 (0.660)	<0.001
	Kazakh	1.38	1.02-1.87 (0.035)	0.077
	Not stated	1.47[b]	1.10-1.96 (0.008)	<0.001
TT + CT vs. CC	Overall	1.29[b]	1.08-1.54 (0.005)	<0.001
	Han	1.08[b]	0.76-1.54 (0.662)	<0.001
	Kazakh	1.50[b]	1.03-2.18 (0.036)	0.011
	Not stated	1.30[b]	1.05-1.60 (0.015)	<0.001
TT vs. CC + CT	Overall	1.19[b]	1.03-1.37 (0.017)	<0.001
	Han	1.05[b]	0.87-1.26 (0.614)	0.001
	Kazakh	1.09	0.84-1.42 (0.500)	0.317
	Not stated	1.32[b]	1.12-1.56 (0.001)	0.008
Source of controls				
Population-based				
T vs. C	-	1.10[b]	0.95-1.28 (0.189)	<0.001
TT vs. CC	-	1.20[b]	0.90-1.59 (0.213)	<0.001
TT + CT vs. CC	-	1.14[b]	0.92-1.40 (0.238)	<0.001
TT vs. CC + CT	-	1.13[b]	0.95-1.36 (0.172)	<0.001
Hospital-based				
T vs. C	-	1.34	1.19-1.51 (<0.001)	0.525
TT vs. CC	-	1.81	1.37-2.39 (<0.001)	0.215
TT + CT vs. CC	-	1.51	1.26-1.83 (<0.001)	0.444
TT vs. CC + CT	-	1.39	1.13-1.70 (0.002)	0.309

[a]*P* value for heterogeneity; [b]Estimates for random effects model

1.38, 95% CI = 1.02-1.87; TT + CT vs. CC, OR = 1.50, 95% CI = 1.03-2.18), in not stated populations (T vs. C, OR = 1.24, 95% CI = 1.08-1.42; TT vs. CC, OR = 1.47, 95% CI = 1.10-1.96; TT + CT vs. CC, OR = 1.30, 95% CI = 1.05-1.60; TT vs. CC + CT, OR = 1.32, 95% CI = 1.12-1.56), but not in Han population (T vs. C, OR = 1.04, 95% CI =0.84-1.29). In the subgroup analysis by source of controls, significant association was found in hospital-based studies (T vs. C, OR = 1.34, 95% CI = 1.19-1.51; TT vs. CC, OR = 1.81, 95% CI = 1.37-2.39; TT + CT vs. CC, OR = 1.51, 95% CI = 1.26-1.83; and TT vs. CC + CT, OR = 1.39, 95% CI = 1.13-1.70).

With respect to A1298C polymorphism, no significant association with esophageal cancer risk was demonstrated in overall analysis (C vs. A, OR = 1.02, 95%CI = 0.93-1.13; CC vs. AA, OR = 1.62, 95%CI = 0.68-3.85; CC + AC vs. AA, OR = 1.02, 95%CI = 0.92-1.13; CC vs. AC + AA, OR = 1.60, 95%CI = 0.70-3.66). Due to the limited number of studies about MTHFR A1298C polymorphism in this meta-analysis, subgroup analyses stratified by the ethnicity and source of controls were not conducted.

Sensitive analysis

In order to compare the difference and evaluate the sensitivity of the meta-analysis, we used both models (the fixed effect model and random effect model) to evaluate the stability of the meta-analysis. All the results were not materially altered except the (T vs. C), (TT vs. CC) of C677T in Kazakh population, and (T vs. C), (TT vs. CC), (TT + CT vs. CC) of C677T in population-based studies (data not shown). Hence, results of the sensitivity analysis suggest that the data in this meta-analysis are relatively stable and credible.

Bias diagnosis

The Begg's funnel plot and Egger's test were performed to assess the publication bias of literatures. As showed in Figure 2, the shape of the funnel plot did not reveal obvious asymmetry. Then, the Egger's test (Figure 3) was used to provide statistical evidence of funnel plot symmetry. The Egger's test showed no publication bias for T vs. C of C677T (t=0.44, *p*=0.667), and C vs. A of A1298C (t= 1.22, *p*=0.347).

Discussion

Although many epidemiologic studies investigated the MTHFR polymorphisms and their associations with esophageal cancer, definite conclusions can not be drawn. Most of those studies involved few cases, and these few sample size limited the genetic effect reliably. A recent meta-analysis had indicated that almost 80% of case-control studies for the association between MTHFR polymorphisms and esophageal cancer were conducted in China (Wen et al., 2013). And this study (Wen et al., 2013) included some duplication publications. Therefore, we conducted an updated meta-analysis by critically reviewing 16 individual studies on MTHFR polymorphisms with esophageal cancer risk in Chinese populations only, to lessen the impact of different genetic background. In the meta-analysis, we found that the variant genotypes of the MTHFR C677T polymorphisms were significantly associated with esophageal cancer risk. Our results were consistent with previously published meta-analyses (Langevin et al., 2009; Fang et al., 2011; Liu et al., 2011; Zacho et al., 2011; Wen et al., 2013), which showed an increased risk of esophageal cancer associated with only the MTHFR C677T genotype; inconsistent with Tan et al.' meta-analysis (Tan et al., 2013) which suggested associations of the A1298C polymorphism with increased risk of esophageal cancer. However, these previously published meta-analyses included a smaller number of studies which were conducted in Chinese populations than ours did. And they did not calculate pooled Ors for all studies in Chinese population.

When we performed the subgroup analyses by ethnicity and source of controls, significant association with susceptibility for the development of esophageal was found among Kazakh, not stated populations and hospital-based studies. There might be some reasons could be explained that. First, the relationship between genes and genes might be susceptible in different ethnicity. In addition, gene-environmental interaction might play an important role in susceptibility to esophageal cancer. Since the hospital-based studies may have some biases because such controls are not representative of the general population. And the low sample size or some other potentially suspected factors such as smoking status, alcohol consumption, occupational and lifestyle might influence our research. There were only three hospital-based studies in this meta-analysis, so considering this kind of selection bias, our subgroup results by source of controls should be interpreted with caution.

Our meta-analysis has several strengths. First, we obeyed the inclusion and exclusion criteria strictly to reduce selection bias. Second, a funnel plot and Egger's linear regression test were used to assess publication bias. Third, our inclusion of non-English language reports, were important in minimizing a major potential threat to the validity of any meta-analysis-publication bias and the related threat of a language bias. At last, the sensitivity analysis had been performed to confirm the reliability and stability of this meta-analysis. Therefore, the 16 studies would appear to be comparable in all respects relevant to our meta-analysis.

Some limitations of this meta-analysis should be discussed. Firstly, observational studies are susceptible to various biases (e.g., recall bias in case-control studies) because of their retrospective nature. Therefore, recall bias could invalidate the results from this meta-analysis. Another potential limitation was that our results were based on unadjusted estimates. More precise analyses can be conducted if individual data was available, which would allow for the adjustment by other covariates including sex, age, location, race and other factors. Thirdly, because some relevant published and unpublished studies which were likely to have null results were not included, a possible bias, especially the outcome-reporting bias, could not be ruled out, although the result for publication bias was not statistically significant. Fourthly, the conclusions drawn from subgroup analyses might be limited because of the small sample size.

In summary, this meta-analysis supports that MTHFR C677T polymorphism most likely contributes to individual susceptibility to esophageal cancer in Chinese populations. Future research on MTHFR and esophageal cancer should be further targeted at the interactive effects of dietary and environmental factors, and gene-gene interactions. Such studies taking these factors into account may eventually lead to our better, comprehensive understanding of the association between the MTHFR polymorphism and esophageal cancer risk.

Acknowledgements

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