RESEARCH ARTICLE

Genetic Polymorphism of MTHFR A1298C and Esophageal Cancer Susceptibility: A Meta-analysis

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Abstract

Background: Associations between the methylenetetrahydrofolate reductase (MTHFR) A1298C polymorphism and esophageal cancer risk have been reported in many articles recently, but results were controversial. Therefore the present meta-analysis was conducted to to provide a more precise estimation. Methods: Odds ratios (ORs) with 95% confidence intervals (CIs) were used to evaluate the strength of associations. Results: Finally, six case-control studies involving a total of 1,302 cases and 2,391controls for the A1298C polymorphism were included. The meta-analysis showed that significantly increased risk for Asians (CC versus AA, OR=3.799, 95%CI=1.541-9.365, *P*=0.004; CCversusCA+AA, OR=3.997, 95%CI=1.614-9.900, *P*=0.003) and Caucasians (CC versus AA, OR=1.797, 95%CI=1.335-2.418, *P*=0.000; CC+CA versus AA,OR=1.240, 95%CI=1.031-1.492, *P*=0.022; CCversusCA+AA, OR=1.693, 95%CI=1.280-2.240, *P*=0.000). In addition, there was an association with risk for both ESCC (CC versus AA, OR=2.529, 95%CI=1.688-3.788, *P*=0.000; CCversusCA+AA, OR=2.572, 95%CI=1.761-3.758, *P*=0.000) and esophageal adenocarcinoma (EAC) (CC versus AA, OR=1.592, 95%CI=1.139-2.227, *P*=0.007; CC+CA versus AA,OR=1.247, 95%CI=1.016-1.530, *P*=0.035; CCversusCA+AA, OR=1.466, 95%CI=1.069-2.011, *P*=0.018). Conclusion: This meta-analysis suggested associations of the A1298C polymorphism with increased risk of esophageal cancer in both Asians and Caucasians. In addition, we found that the MTHFR A1298C polymorphism might influence risk ofESCC and EAC in the overall studies.

Keywords: MTHFR - A1298C - polymorphism - esophageal cancer - meta-analysis

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Introduction

In all human cancers, esophageal cancer (EC), with the five- year survival rate less than 20%, is regarded as one of the most common lethal malignancies worldwide. Because of highly incidence and mortality, even in Southern and Eastern Africa and Eastern Asia, EC has been listed as eighth and sixth in all cancers respectively in the world (Jemal et al., 2011). Squamous cell carcinoma (ESCC) and adenocarcinoma (EAC) are the main histological types, In the "esophageal cancer belt" of the ESCC even has reached 90% (Wheeler et al., 2012). It is a multi-factor and multi-step process to develop EC, such as poor nutritional status, drinking of alcohol, smoking, ethnic group, hot beverage and high-temperature cooking methods, serious shortage of vegetable and fruit intake (Yu et al., 1988; Farrow et al., 2000; Lin et al., 2011). However, not all exposed factors develop EC, it may be shown that genetic factors play an important role for developing EC.

Methylenetetrahydrofolate reductase (MTHFR) located in 1p36.3, acting as a critical enzyme, which catalyzed reduction of 5, 10-methylene-tetrahydrofolate to 5-methyltetra hydrofolate, as a methyl donor for the remethylation of homocysteine to methionine. MTHFR Glu 429 Ala (A→C)variants have been identified in protein coding sequences. The MTHFR mutation led to 5 - methyltetrahydrofolate reduction and homocysteine accumulation in the blood, Which made the methyl donor of the methionine dyssynthesis, eventually caused hypomethylation of DNA and decreased the activity of the enzyme, leading to increase the cancer susceptibility (Chen et al., 1996; Ekiz et al., 2012). Besides, it has been suggested that a significant function of folate is to provide the required methyl groups for intracellular methylation reaction and denovo deoxynucleotide triphosphate synthesis, folate deficienc might cause carcinogenic by disruption of DNA methylation (Umar et al., 2010).

Given know the Enzyme activity of the MTHFR A1298C, individuals mutational homozygous compared for the common, had been reduced approximately60% of enzyme activity (Ekiz et al., 2012). And the variations of MTHFR A1298C, it has been shown that these polymorphisms may strongly affect cancer risk, such as lung cancer, colorectal cancer, gastric cancer, breast cancer (Dong et al., 2008; Li et al., 2011; de et al., 2012; Zhang et al., 2012). However, recently a meta-analysis has also suggested that MTHFR A1298C polymorphism might correlate with ESCC by alcohol status (Fang et al.,

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Table 1. Studies Summary of MTHFR A1298C Polymorphism with Esophageal Cancer

Investigator et al.	Year	Race	Country	Case			Control			Control source	methods
				CC	CA	AA	CC	CA	AA		
Ekiz et al.	2012	Caucasian	Turkey	3	13	10	2	17	11	Hosptial-based	PCR
Ibiebele et al.	2011	Caucasian	Australia	100	298	280	121	572	610	Population-based	Sequenom
zhang et al.	2008	Asian	China	2	30	56	0	20	52	Population-based	PCR-RFLP
Gao et al.	2004	Asian	China	3	48	90	0	60	164	Population-based	PCR-RFLP
Stolzenberg et al.	2003	Asian	China	3	32	94	0	104	294	Hosptial-based	PCR
Song et al.	2001	Asian	China	7	54	179	5	113	242	Population-based	PCR

2011), but the results were conflicting and inconclusive. Therefore, a meta-analysis was conducted to investigate the relationship between both A1298C polymorphism and susceptibility to EC.

Materials and Methods

Search strategy and selection criteria

We search from Pubmed, Embase and Chinese Biomedical Database for all medical publications until January, 2013 with the following keywords :

Methylenetetrahydrofolate reductase, MTHFR, Glu 429 Ala, A1298C, polymorphism, variant; and 'esophagus"or"esophageal"combined with"carcinoma", "cancer", "squamous cell", or "adenocarcinoma". the following criteria must be fulfill: (1) full-text and human articles, (2)using case-control design, (3) the association between MTHFR A1298C polymorphism and esophageal cancer, (4) odds ratio (OR) and 95% confidence interval(95% CI) are obtained for each study, (5) sufficient genotype data can be obtained.

Data extraction

Two investigators (X.Tan and Y.Y.Wang) were carefully to extract data independently from the above selection criteria. Disagreement must be discussed and decided by third investigators (M.W.Chen). The data items included: First author, publication date, country,race, source of controls, genotyping method, and different genotype numbers in all studies.

Statistical analysis

The association between MTHFR A1298C polymorphism and EC risk was calculated by ORs with 95% CIs. In order to avoid the use of a specific genetic model and lead to the outcome bias, at least three possible genotypes was performed comparing in the meta-analysis. We estimated the OR of a cancer associated with Co-dominant model (CCversusAA, CA versusAA), Dominant model (CC+CAversus AA)and Recessive model (CC versus CA+AA), respectively.

Chi-square based Q statistic test and p value was used to assess the between-study heterogeneity (Higgins et al., 2003). The studies heterogeneity did not exist when *P* -value > 0.1and I^2 < 25%. If there was no heterogeneity, The fixed-effects model was performed using the Mantel–Haenszel's method (Mantel et al., 1959). The heterogeneity was considered to be of statistical significance when I^2 > 50% or *P* -value <0.1, sensitivity analysis and subgroup analysis were used to explore the source of the heterogeneity , so the random effects model

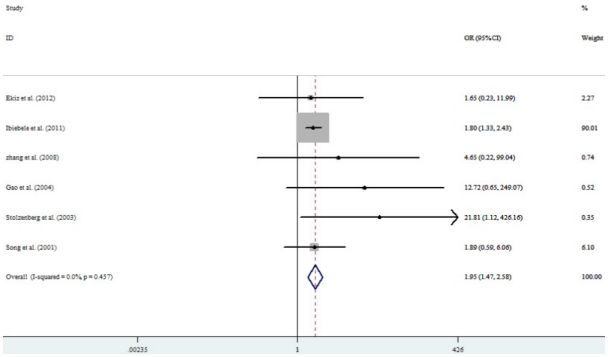

Figure 1. The Forest Plot Describing the Meta- analysis with a Fix-effect Model (CCvs.AA) for the Association of MTHFR A1298C Polymorphism and Esophageal Cancer Risk in Overall Studies. Each study is depicted with size inversely proportional to its variance, accompanied by the respective 95% confidence intervals

was performed using DerSimonian and Laird method when the heterogeneity still existed (Lau et al., 1997).

Begg's funnel plot was test for Potential publication bias,and funnel plot unweighted was by applied regression test (Peters et al., 2006). Due to different geographic, ethnic, and pathological type of esophageal cancer, in order to assess the effects of covariance, we performed subgroup analyses by ethnic subgroup (Caucasian and Asian) and pathological type subgroup (ESCC and EAC). all analyses were carried out by the Stata software version 11.1 (Stata Corporation. USA). All of *P* values were two-sided, less than 0.05 was considered statistically significant.

Results

Characteristics of relevant studies

According to our search strategy and inclusion criteria,six studies with full-text articles were remained the relationship of MTHFR A1298C polymorphism with EC (Song et al., 2001; Stolzenberg et al., 2003; Gao et al., 2004; Zhang et al., 2008; Ibiebele et al., 2011; Ekiz et al., 2012). In the ethnic subgroup analysis, there were four studies on Asian (Song et al., 2001; Stolzenberg et al., 2003; Gao et al., 2004; Zhang et al., 2008) and two studies on Caucasian (Ibiebele et al., 2011; Ekiz et al., 2012). For one study (Ibiebele et al., 2011) including two pathologic types, which were treated as two separate studies. so there were four studies for ESCC (Song et al., 2001; Stolzenberg et al., 2003; Zhang et al., 2008; Ibiebele et al., 2011) and two studies for EAC (Ibiebele et al., 2011; Ekiz et al., 2012). Source of controls all came from hospital-based and population-based were all shown in the Table 1.

Table 2. Main Results of the Meta-analysis MTHFRA1298Cpolymorphism Relation with Esophageal Cancer

Variable	n	CC vs. AA			CA vs. AA			Dominant model			Recessive model		
		OR(95%CI)	p^a	P^b	OR(95%CI)	p^a	P^b	OR(95%CI)	p^a	P^b	OR(95%CI)	p^a	P^b
Total	6	1.951(1.475-2.581)	0	0.457	1.044(0.900-1.211)	0.573	0.102	1.790(1.312-2.444)	0	0.003	1.843(1.414-2.402)	0	0.435
Ethnicity													
Asian	4	3.799(1.541-9.365)	0.004	0.34	1.002(0.684-1.468)	0.615	0.992	1.011(0.808-1.264)	0.924	0.052	3.997(1.614-9.900)	0.003	0.409
Caucasian	2	1.797(1.335-2.418)	0	0.932	1.125(0.924-1.368)	0.24	0.606	1.240(1.031-1.492)	0.022	0.592	1.693(1.280-2.240)	0	0.936
Histopathology													
ESCC[c]	4	2.529(1.688-3.788)	0	0.48	0.883(0.715-1.091)	0.25	0.184	1.033(0.753-1.417)	0.839	0.085	2.572(1.761-3.758)	0	0.511
EAC[d]	2	1.592(1.139-2.227)	0.007	0.972	1.175(0.947-1.458)	0.142	0.551	1.247(1.016-1.530)	0.035	0.584	1.466(1.069-2.011)	0.018	0.085

n, number of studies in each analysis; Dominant model, CC+CA vs. AA; Recessive model, CC vs. CA+AA; OR, odds ratio; CI, confidence interval; [a]the pool *p* value; [b]*p* value for heterogeneity test; [c]esophageal squamous cell carcinoma; [d]esophageal adenocarcinoma

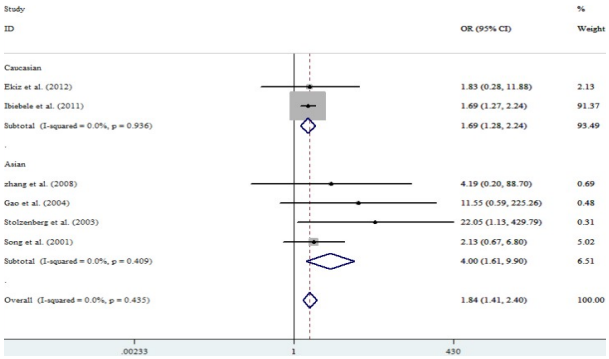

Figure 2. The Forest Plot Describing the Meta- analysis with a Fix-effect Recessive Model (CCvs.CA+AA) for the Association of MTHFR A1298C Polymorphism and Esophageal Cancer in Ethnicity. Each Study is Depicted with size inversely proportional to its variance, accompanied by the respective 95% confidence intervals

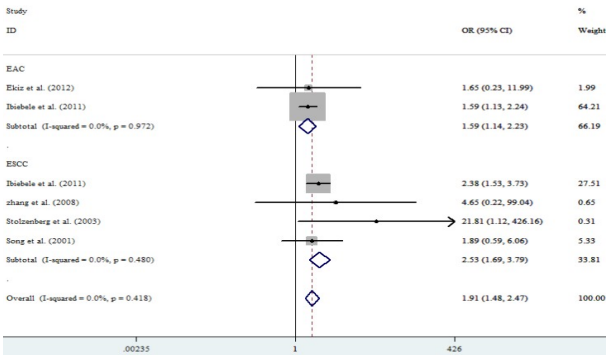

Figure 3. The Forest Plot Describing the Meta- analysis with a Fix-effect Model (CCvs.AA) for the Association of MTHFR A1298C Polymorphism and Esophageal Cancer Risk in ESCC and EAC. Each study is depicted with size inversely proportional to its variance, accompanied by the respective 95% confidence intervals

Meta-analysis results

The main results of the meta-analysis on the association between MTHFR A1298C polymorphism and EC risk was shown in Table 2. When all studies were pooled, we performed analysis using fixed-effects models if the Q -test of heterogeneity was considered no significant, otherwise, using random-effects models. In the A1298C polymorphism, we found that a stronger risk for EC (CCversus AA, OR=1.951, 95%CI=1.475-2.581, *P*=0.000; Dominant model CC+CA versus AA, OR=1.790, 95%CI=1,312-2.444, *P*=0.000; Recessive model CCversusCA+AA, OR=1.843, 95%CI=1.414-2.402, *P*=0.000) (Figure 1).

To assess the covariance effects, ethnicity and histological type were performed by subgroup analyses. In the ethnic subgroup analysis, significant risk association was found between A1298C polymorphism and Asian population (CC versus AA, OR=3.799, 95%CI=1.541-9.365, *P*=0.004; CCversusCA+AA, OR=3.997, 95%CI=1.614-9.900, P=0.003)and Caucasian population (CC versus AA, OR=1.797, 95%CI=1.335-2.418, *P*=0.000; CC+CA versus AA,OR=1.240, 95%CI=1.031-1.492, *P*=0.022; CCversusCA+AA, OR=1.693, 95%CI=1.280-2.240, *P*=0.000) (Figure 2).

Similarly,when in subgroup analyses for histological type, there was associated with risk for ESCC (CC versus AA, OR=2.529, 95%CI=1.688-3.788, *P*=0.000; CCversusCA+AA, OR=2.572, 95%CI=1.761-3.758, *P*=0.000) and EAC(CC versus AA, OR=1.592, 95%CI=1.139-2.227, *P*=0.007; CC+CA versus AA, OR=1.247, 95%CI=1.016-1.530, *P*=0.035; CCversusCA+AA, OR=1.466, 95%CI=1.069-2.011, *P*=0.018) (Figure 3)

Test for heterogeneity, sensitive analysis and test for publication bias

When we overall pooled analysis the heterogeneity was existence. To explore the sources of heterogeneity, ethnicity and histological type were performed by subgroup analyses. No heterogeneity was reduced. Sensitivity analysis was performed in our meta-analysis. When we omitted every study at each time, the results of reanalyse for A1298C polymorphism was stabily, which suggested that our meta-analysis results was reliable (data not show).

Begg's funnel plot and the Egger's test were conducted to estimate the publication bias of articles. Both the results of Begg's and Egger's test did not show any evidence of publication bias (CC vs.AA Begg's test, *P*=1.000,Egger's test, *P*=0.935; CA vs.AA Begg's test, *P*=0.707, Egger 's test, *P*=0.801; Dominant model, Begg's test, *P*=0.707, Egger 's test, *P*=0.740; Recessive model, Begg's test, *P*=1.000, Egger 's test, *P*=0.423)

Discussion

Methylenetetrahydrofolate reductase (MTHFR) is the limiting enzyme which catalyzed folate and methionine metabolism into methyl donor, played an important role in folate metabolism. Folate deficienc and metabolism disorders may cause DNA hypomethylation, and A to C substitution at nucleotide 1298 in MTHFR, which alters

enzyme activity, affecting DNA methylation or DNA synthesis, thereby increasing susceptibility to cancer(Umar et al., 2010;Ekiz et al., 2012. Our meta-analysis included six studies with a total of 1302 cases and 2391controls have investigated the A1298C polymorphism with EC. Our meta-analysis provided evidence that the A1298C polymorphism might play an important role in EC. No publication bias was found by the funnel plots which proved our meta-analysis conclusion more credible.

When we performed the subgroup analyses by ethnicity, significant association with susceptibility for the development of EC among Asian and Caucasian population. 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 EC. Each people stays with different lifestyle and environment. Inadequate folate intake might be also susceptible to EC (Zhao et al., 2011). Besides, it also seems to show association of polymorphism A1298C with increased risk of ESCC and EAC. And the exact mechanism for the incidence of ESCC and EAC is unclear. At last, it might be low sample size or some other potentially suspected factors such as smoking status, alcohol consumption, occupational and lifestyle to influence our research.There were only two studies for Caucasian population and two studies for EAC, so further study with large samples must to be performed.

Despite we have made great efforts to collect all possible data for investigating between MTHFR A1298C polymorphism and EC, but several potential limitations inherent in our meta-analysis should be known. First, present research associations between MTHFR A1298C polymorphism and EC risk was still fewer, there were only six studies with full-text articles relation of MTHFR A1298C polymorphism with EC, so the sample of participants included in our meta-analysis was comparatively small. results should be interpreted with caution. Second, it is well-known that publication bias is a problem for interpretating the results in meta-analysis. Although Begg's and Egger's test did not show any evidence of publication bias in our research, we only collected full-text articles in our research, excluded abstract and unpublished articles, and positive results may be easily accepted by journals, potential publication bias may occur. Besides, all of the studied objects may be not matched for the age, race and smoking status, living habits and environment was also different. As stated above, it might be sources of heterogeneity. At last, the gene-gene and gene–environmental interactions we did not make further research, there may be some predisposing factors effect on esophageal cancer.

Although these limitations, our meta-analysisof the association of MTHFR A1298C polymorphism with Esophageal Cancer risk is significantly more powerful than any single study. In all, our research suggeste that MTHFR A1298C polymorphism may increase risk for Esophageal Cancer in Asians and Caucasians. In addition, we also found that MTHFR A1298C polymorphism might be risk for ESCC and EAC. Folate intake, gene–gene and gene–environmental interactions on Esophageal Cancer risk, owing most data were insufficient, therefore, further studies with more comprehensive, larger sample, well-designed and high quality case-control studies are required to investigate the associated with MTHFR and Esophageal Cancer.

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