Association of Methylenetetrahydrofolate reductase genetic polymorphisms and folate intake with susceptibility of esophageal squamous cell carcinoma: A meta-analysis

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ABSTRACT

Objectives: We conducted an update meta-analysis to investigate the association of MTHFR C677T and folate intake with esophageal cancer risk.

Methodology: PubMed, EMBASE and the CNKI were searched in our study. The pooled Odds ratio (ORs) and 95% confidence intervals (CIs) of MTHFR C677T and folate intake and ESCC risk from each study were pooled using meta-analysis.

Results: Twenty studies (4271 cases and 5559 controls) were included for meta-analysis. MTHFR 677 CT and TT genotypes were associated with an increased risk of ESCC, with OR(95%CI) of 1.48(1.25-1.77) and 1.65(1.24-2.20). By subgroup analysis, we found individuals carrying CT+TT genotype and low intake of folate had an increased risk of ESCC risk when compared with CC genotype [OR(95CI%)=1.55(1.02-2.57)]. There was a significant interaction between folate intake and ESCC. No publication bias was found among studies regarding MTHFR 677 CT and TT by Egger’s test.

Conclusions: Our meta-analysis indicated MTHFR 677CT and/or TT genotypes are associated with the risk of ESCC, and folate could modify their association.

KEY WORDS: Methylenetetrahydrofolate reductase C677T, Folate intake, Esophageal squamous cell carcinoma, Meta-analysis.

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INTRODUCTION

Esophageal cancer has been ranked as one of the most common malignancies worldwide.¹ Comparing with esophageal adenocarcinoma which is one of the most prevalent cancer in Western Countries, and the major phenotype in Asian countries, especially in China, is esophageal squamous cell carcinoma(ESCC). In China, and it is estimated there are new diagnosed 250,000 cases every year. Possible risk factors for ESCC include smoking and drinking habits, high intake of hot-temperature food, salted food and pickled vegetables, low intake of vegetable, chronic mucosal irritation.² It is also reported that deficiency of nutrients, such as vitamins and microelements, is association with an enhanced risk for ESCC.³

It is well known folate is a water-soluble vitamin element from green leafy vegetables, cereals, legumes and fruits.⁴ Lack of folate may damage the function of DNA repair and chromosomal fragile site expression, and induce the breaks of chromosome and formation of micronucleus.⁵ Folate is also function as a major methyl group donor and had a key role in DNA methylation.⁶ Previous studies have shown the aberrant DNA methylation

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is associated with cancer risk. The common polymorphism for Methylene tetrahydrofolate reductase (MTHFR) gene is a C to T substitution at nucleotide 677 (MTHFR C677T), and this variation leads to a reduced activity of this enzyme. Thus the variation of folate metabolic enzyme activity altered by the genetic polymorphisms may have a role in the susceptibility to methylation-related carcinogenesis. However, the role of dietary folate in esophageal cancer and its relationship between MTHFR C677T were controversial. The inconsistency of these results may be explained by differences in source of selected subjects, sample size, study design and random error. We therefore performed a systematic review to investigate potential genetic associations of MTHFR C677T and folate intake with ESCC risk by reducing random error and obtaining precise estimates.

**METHODOLOGY**

**Searching strategy:** We searched PubMed (from Jan. 1966 to Oct. 2012), EMBASE (from January 1988 to Oct. 2012), and the Chinese Journal Net (CNKI; from January 1980 to Oct. 2012) by using the following key words for searching published papers: ‘esophageal squamous cell carcinoma’, ‘esophagus’ or ‘oesophageus’, ‘carcinoma or cancer or neoplasm or tumour or tumor’, ‘Methylenetetrahydrofolate reductase’, or ‘MTHFR’. We only extract published paper in English and Chinese. All references cited in studies and published review articles were extracted for additional eligible studies. The criteria of including data were as follows: (1) a case-control study design; (2) reporting the association between MTHFR C677T polymorphisms and ESCC; (3) original data about the genotype distributions of MTHFR C677T genotypes. (4) If the data were published for more than twice, we only extracted the most complete data. Two reviewers independently evaluated and extracted the potential selected articles, and the disagreements were resolved by discussion. If the data were missing, we attempted to contact the authors of the articles by email or phone so as to request the relevant data. All the data were organized as first author’s name, year of publication, study site, numbers of cases and controls, genotype frequencies of MTHFR C677T.

The quality of studies was evaluated by a previous defined scale reported by Jiang (Table-I). The total quality score was ranged from 0 to 15. Score<10 was regarded as low quality of study, and score >10 was regarded as high quality of study.

**Statistical analysis:** All statistical analyses were performed using STATA statistical package (Version 9.1, STATA, College Station, TX). Variation of genotype frequency distribution in controls from the expected under Hardy-Weinberg equilibrium (HWE) were calculated using Chi-square test. The pooled Odds ratio (ORs) and 95% confidence intervals (CIs) were used to assess the association of polymorphisms in MTHFR C677T and folate intake with ESCC risk. The heterogeneity between studies was assessed by the Q-statistics, and P values<0.1 was defined as statistical significance. Subgroup analysis was conducted to explore the possible sources of heterogeneity. If there was heterogeneity between studies, a random effect model would be used to obtain the pooled OR and its 95% CI. Otherwise, a fixed-effect model would be applied. Funnel plot an Egger’s test were used to assess the publication bias. Statistical significance was defined as a two-sided P value of less than 0.05.

**RESULTS**

60 studies were achieved by literature search from PubMed, EMBASE and CNKI database, using different combinations of key terms. However, 39 studies were excluded from analysis, mainly due...
Esophageal squamous cell carcinoma to reviews, overlapping data and being without meeting the criteria. Finally, a total of 20 studies were selected for meta-analysis, including 4271 cases and 5559 controls (Table-II). Among the 20 studies, frequencies of genotypes in controls of eight studies were in line with Hardy-Weinberg equilibrium, which suggested there might be population stratification and sample bias. Among 20 countries, we found only six studies had high quality, and the remained quality scores were ranged from 7 to 10. Of 20 case-control studies, 17 studies were conducted in China, one in India, one in Japan, one in German and one in Pakistan. MTHFR 677 CT and TT genotypes were associated with an increased risk of ESCC, with OR(95% CI) of 1.48(1.25-1.77) (Figure-1) and 1.65(1.24-2.20) (Figure-2). Significant heterogeneity was found among studies regarding MTHFR 677 CT and TT genotypes (P<0.05).

Subgroup analysis was taken in terms of folate intake and different quality of studies. We found individuals carrying CT+TT genotype and low intake of folate had a light increased risk of ESCC risk when compared with CC genotype [OR(95CI%)=1.55(1.02-2.57)] (Table-III), and a significant heterogeneity was found. However, a non-significant increased risk was found in individuals carrying CT+TT genotype. There was a significant interaction between folate intake and ESCC (P<0.05, data not shown). We also found significant heterogeneity after conducting stratification of quality of studies.

Egger's test was used to assess the publication bias, and it provided evidence that there was no publication bias among studies regarding MTHFR 677 CT and TT(P=0.46 and P=0.08, respectively).

**DISCUSSION**

Although previous epidemiologic studies investigated the role of MTHFR C677T for ESCC risk, the results were inconsistent. Most of these epidemiologic studies have limited sample size, and more importantly, are not power enough to give a precise estimated effect of gene variation on the ESCC risk. Therefore, meta-analysis could play a key role to obtain a more precise effect on the genetic polymorphisms on risk of disease. Three previous studies investigate the role of MTHFR C677T for ESCC risk. The three meta-analyses showed individuals carrying variation of MTHFR C677T had an increased risk of ESCC, with an OR(95% CI) ranged from 1.58 to 1.78. However, the previous three meta-analyses only showed the association of MTHFR C677T on ESCC, and did not indicate the interaction between MTHFR C677T
Therefore, we conducted an updated meta-analysis by critically reviewing 20 individual case-control studies on MTHFR C677T and folate intake with esophageal cancer risk. Compared with previous three meta-analyses, this updated meta-analysis included another seven new case-control studies, and we have shown folate modify the association between polymorphism in MTHFR C677T and ESCC risk.

It is reported that folate mediates the DNA methylation reactions and function of DNA synthesis, replication, and repair, which may enhance the susceptibility of cancer for individuals with low intake of folate. Previous studies have reported a high intake of food full of folate can prevent the development of various cancers. Ours study has shown individuals carrying MTHFR 677TT genotype who have high intake of folate are associated with a non-significant increased risk of ESCC, and the results suggest folate intake modify the function of polymorphism in MTHFR C677T with ESCC risk.

In our study, we found significant heterogeneity between studies. However, the heterogeneity decreased or disappeared after stratifying by the intake of folate, suggesting that the folate intake was an important factor of influencing the association between variation of MTHFR C677T and ESCC risk.

| Study ID   | County | Cases   | Controls | CC   | CT   | TT   | CC   | CT   | TT   | PHWE | Quality |
|------------|--------|---------|----------|------|------|------|------|------|------|------|---------|
| Yang 201213 | China  | 100     | 97       | 37   | 45   | 18   | 40   | 41   | 16   | 0.33 | 8       |
| Zhao 201114 | China  | 155     | 310      | 68   | 74   | 13   | 179  | 120  | 11   | 0.09 | 9       |
| Li 201115   | China  | 226     | 246      | 64   | 85   | 77   | 58   | 97   | 91   | <0.1 | 9       |
| Umar 201016 | India  | 208     | 223      | 155  | 48   | 5    | 155  | 63   | 5    | 0.63 | 13      |
| Wang 200917 | China  | 102     | 108      | 39   | 47   | 16   | 58   | 36   | 14   | 0.77 | 9       |
| Chen 200918 | China  | 103     | 181      | 11   | 49   | 43   | 45   | 85   | 51   | 0.42 | 10      |
| Qin 200819  | China  | 120     | 204      | 60   | 53   | 7    | 170  | 59   | 11   | 0.06 | 11      |
| Li 200820   | China  | 126     | 169      | 22   | 52   | 52   | 41   | 62   | 66   | <0.1 | 10      |
| Wang 200721 | China  | 584     | 540      | 73   | 263  | 248  | 119  | 234  | 187  | <0.1 | 11      |
| He 200722   | China  | 584     | 540      | 73   | 263  | 248  | 119  | 234  | 187  | <0.1 | 10      |
| Peng 200623 | China  | 275     | 315      | 51   | 105  | 119  | 74   | 143  | 98   | 0.12 | 8       |
| Wang 200524 | China  | 275     | 315      | 51   | 105  | 119  | 74   | 143  | 98   | 0.12 | 10      |
| Yang 200510 | Japan   | 165     | 493      | 63   | 82   | 20   | 186  | 227  | 80   | 0.45 | 9       |
| Zhang 200425 | Germany | 241     | 256      | 94   | 116  | 31   | 107  | 115  | 34   | 0.72 | 10      |
| Zhang 200425 | China   | 189     | 141      | 16   | 93   | 80   | 25   | 54   | 62   | <0.1 | 10      |
| Kureshi 200426 | Pakistan | 34     | 54       | 22   | 12   | 0    | 32   | 18   | 4    | 0.52 | 8       |
| Zhang 200327 | China   | 198     | 141      | 16   | 93   | 89   | 25   | 54   | 62   | <0.1 | 7       |
| Stolzenberg 20039 | China | 129   | 398      | 23   | 58   | 48   | 65   | 209  | 124  | 0.14 | 8       |
| Miao 200228 | China   | 217     | 468      | 47   | 107  | 63   | 151  | 217  | 100  | 0.18 | 12      |
| Song 20018  | China   | 240     | 360      | 29   | 118  | 93   | 126  | 172  | 62   | 0.8  | 11      |
| Total       |         | 4271    | 5559     | 1062 | 1896 | 1357 | 1886 | 2368 | 1357 |

**Table III: Subgroup analysis of MTHFR C677T polymorphism and ESCC**

| Items            | Cases | Control | OR(95% CI) | P for heterogeneity |
|------------------|-------|---------|------------|---------------------|
|                  | CC    | CT+TT   | CC         | CT+TT               |
| Folate intake    |       |         |            |                     |
| Low folate intake| 74    | 130     | 109        | 129                 | 1.55(1.02-2.57) | 0.41 |
| Moderate folate intake | 28  | 33      | 63         | 64                  | -                 | -   |
| High folate intake | 88   | 273     | 160        | 333                 | 1.62(0.80-3.23)  | 0.13 |
| Quality of study |       |         |            |                     |
| High quality     | 407   | 1170    | 667        | 1278                | 1.15(1.11-1.20)  | <0.05 |
| Low quality      | 659   | 2185    | 1107       | 2565                | 1.37(1.12-1.67)  | <0.05 |
they would induce between-study heterogeneity in the meta-analysis. Therefore, further studies should consider these potential risk factors.

Some possible limitations should be considered in our meta-analysis. Firstly, the eligibility criteria for inclusion of ESCC patients differed for each study, which might influence the obvious consistency of effects across the included studies and cause obvious between-study heterogeneity in this meta-analysis. Secondly, there might be misclassification during our study. Some controls in our study were selected from non-cancer inpatients, and some were selected from residents. Finally, owing to lack of other genetic and environmental data of ESCC risk, the gene-environment and gene-gene interaction for esophageal cancer could not be well assessed and the outcomes from this study might be affected by risk of selection biases. Further studies are warranted to interpret their interaction.

In conclusion, our meta-analysis indicated MTHFR 677CT and/or TT genotypes are associated with the risk of ESCC, and folate could modify their association. Further large sample size and well designed study are urgently needed to further identify the association of folate intake and polymorphism of MTHFR C677T with ESCC risk.

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