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Review and pooled analysis of studies on MTHFR C677T polymorphism and esophageal cancer

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Abstract

Esophageal cancer has been associated with tobacco and alcohol consumption, gastric reflux, exposure to nitrosamines from food or other environmental sources, and diets lacking folate. Susceptibility to esophageal cancer may be modified by functional polymorphisms in genes along the folate metabolic pathway, such as methylenetetrahydrofolate reductase (*MTHFR*). The *C677T* polymorphism is the most common functional variant, leading to a reduction in enzyme activity. We report a pooled analysis of 5 studies on the association of *MTHFR C677T* polymorphism and esophageal cancer, including 725 cases and 1531 controls. A significant association between the *MTHFR 677 TT* genotype and esophageal cancer was observed (OR = 2.63, 95% CI: 1.75–3.94), although there was significant heterogeneity between studies. A sensitivity analysis excluded one study; the association between *TT* genotype and esophageal cancer was still present, although of reduced magnitude (OR = 1.57, 95% CI: 0.96–2.56). A significant interaction between smoking and *TT* genotype on esophageal cancer risk was observed, while no interaction was observed between alcohol consumption and genotype.

Keywords

Methylenetetrahydrofolate reductase; Esophagus; Genetic epidemiology; case control studies

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POTENTIAL CONFLICTS OF INTEREST

None declared

GENE AND GENE FUNCTION

The 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene contains a 2.0 kb coding region with 11 exons, and is located on chromosome 1p36.3^{1,2}. The gene product of *MTHFR* is a 77-kd protein of the same name (EC 1.5.1.20). Methylenetetrahydrofolate reductase is a key enzyme in folate metabolism that catalyzes the conversion of intracellular 5,10-methylenetetrahydrofolate (5,10-methylene THF) to 5-methyltetrahydrofolate (5-methyl THF), the predominant circulatory form of folate and primary methyl donor for the remethylation of homocysteine to methionine²⁻⁵ (figure 1). Methionine is a substrate for S-adenosylmethionine (SAM), which is the principal biological methyl group donor for the DNA methylation process⁵.

MTHFR, FOLATE METABOLISM, AND CANCER RISK

The involvement of MTHFR in the folate metabolic pathway may affect cancer risk according to two primary mechanisms. Increased activity level of MTHFR enzyme facilitates the conversion of 5,10-methylene THF to 5-methyl THF. Low cytosolic levels of 5,10-methylene THF reduce the synthesis of deoxythymidylate monophosphate, and increase the deoxyuridylate monophosphate / deoxythymidylate monophosphate (dUMP/dTMP) ratio, thus inducing uracil misincorporation into DNA. This may result in point mutations and/or chromosomal breaks⁶. The second mechanism involves a decrease in available 5-methyl THF, which causes a decline in the conversion of homocysteine to methionine. This diminishes the availability of SAM, resulting in DNA hypomethylation, a process associated with carcinogenesis^{7,8}. Folate deficiency leads to a reduction of both intra- and extracellular folate, and therefore is believed to increase cancer risk through both mechanisms described above⁹.

GENE VARIANT AND FREQUENCY

Approximately 60 polymorphisms, as well as 41 rare but deleterious mutations have been described in the *MTHFR* gene¹⁰. The most common functional variant and most studied to date is the thermolabile *MTHFR C677T* polymorphism (rs1801133). The *C677T* variant is a C to T transition in exon 4 at nucleotide 677, resulting in the conversion of alanine to valine at position 222 of the MTHFR amino acid sequence^{4,5}. Heterozygotes (*CT*) and homozygotes (*TT*) for the *C677T* variant have respectively 65% and 30% of the MTHFR enzyme activity observed in homozygous wild type subjects (*CC*)¹¹. As a result, *TT* homozygotes have been associated with lower serum folate levels and higher homocysteine levels than their wild type homozygous counterparts¹²⁻¹⁴.

The frequency of *MTHFR C677T* varies by ethnicity. The *T* allele frequency ranges from 0.25–0.44 in European Caucasians, 0.18–0.42 in Asians, and 0.12–0.24 in African Americans¹⁵. The prevalence of the *TT* genotype ranges from 8–18% in Caucasians, 11–16% in Asians, 21–25% in Latinos, and 0–6% for those of African descent¹⁶.

DISEASE

It is estimated that there were 15,650 new cases of esophageal cancer in the US in 2007, with 13,940 attributable deaths¹⁷, and a 16% 5-year survival rate. Esophageal cancer is the eighth most common cancer in the world, with approximately 462,000 cases and 386,000 deaths worldwide in 2002, making it the 6th most common cause of cancer-related deaths¹⁸.

There is substantial geographic variation in the occurrence of esophageal cancer, with nearly 20-fold difference in male incidence between areas at high-risk such as Eastern Asia and

low-risk Western Africa¹⁹. Other regions with relatively high incidence and mortality include Eastern Africa, Southern Africa, South Central Asia, Northern Europe, and South America (males). Age-adjusted incidence rates in less-developed geographic regions are approximately double compared with those observed in more-developed regions, and age-adjusted mortality rates are 5 to 6-fold higher¹⁹. (Figure 2) The estimated age-adjusted 5-year survival rate in developed countries is 15% for males and 8% for females, while it is 17% and 16% respectively in developing countries¹⁸. This cancer occurs more frequently in men than women¹⁸. Globally, most esophageal cancers are squamous, and originate in the middle to lower third of the esophagus. 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 women²⁰. Esophageal cancer is associated with exposure to tobacco and alcohol consumption, gastric reflux or other chronic trauma, exposure to nitrosamines via food or environment, and a diet low in folate^{18,21}.

GENE-ENVIRONMENT AND GENE-GENE INTERACTIONS

An interaction between folate status and the *MTHFR C677T* polymorphism has been described. Subjects who are homozygous for the *MTHFR C677T* variant and have a low folate status have been reported to have relative DNA hypomethylation compared with wild-type subjects, while no such effect is observed in subjects with high folate status²². Nutrients such as B₆, B₁₂, riboflavin, and methionine are involved in the folate metabolic pathway (figure 1). Flavin adenine dinucleotide (FAD), a phosphorylated form of riboflavin, acts as a cofactor for MTHFR. Increased levels of riboflavin significantly decrease serum homocysteine in subjects carrying the *MTHFR 677 TT* genotype but not in subjects with other *MTHFR 677* genotypes^{23,24}.

Tobacco smoke has been shown to transform folate and B₁₂ co-enzymes into biologically inactive compounds^{25,26}. Alcohol acts as a folate antagonist, resulting in malabsorption, increased folate excretion, or abnormalities in folate metabolism through the inhibition of methionine synthase (MTR)^{27,28}. These effects are illustrated in figure 1. An interaction between *MTHFR C677T* and alcohol consumption has been reported in gastric and colon cancers; alcohol consumption has been associated with increased risk of gastric cancer in subjects with the *MTHFR 677 TT* genotype^{29,30}; in heavy drinkers, *TT* genotype has been associated with higher risk of colon cancer compared with the wild type (*CC*), whereas *TT* genotype appears to have a protective effect in non-heavy drinkers^{31,32}. However, the epidemiological data on *MTHFR C677T* and esophageal cancer do not seem to follow the prediction derived from experimental studies or from epidemiological findings from studies conducted on other gastrointestinal cancers. Yang³³ reported a significant inverse trend for esophageal cancer across *MTHFR 677* genotypes in heavy drinkers (*CC* > *CT* > *TT*; *p* = 0.024), while this was not observed in non-heavy drinkers (*p* = 0.46). Stolzenberg-Solomon²⁹ reported no interaction between *MTHFR C677T* and esophageal cancer and alcohol use during the previous 12 months. Yang³³ found no significant interaction between the *MTHFR C677T* polymorphism and smoking on esophageal cancer risk.

Few gene-gene interactions have been reported in the literature regarding *MTHFR C677T* and esophageal cancer. The interaction between the *MTHFR C677T* and *A1298C* polymorphisms was evaluated in a study on incident and prevalent esophageal cancer cases³⁴, and reported a joint effect of 677 *CT* and 1298 *CC* genotypes on esophageal cancer risk, although no significant interaction was found. In a recent case-control study, Wang³⁵ reported an interaction between *MTHFR C677T* and *SHMT₁ C1420T* polymorphisms on esophageal squamous cell carcinoma; an increased cancer risk in individuals with wild-type *SHMT* and variant *MTHFR TT* genotypes was found.

OBJECTIVE

There have been mixed reports in the literature regarding the association of *MTHFR C677T* polymorphism with esophageal cancer^{29,33–39} (table 1). A recent meta-analysis of 7 publications on the association of *MTHFR C677T* and esophageal cancer³ observed significant heterogeneity among the studies, and therefore no summary estimate was reported. We conducted a pooled analysis to assess the overall effect of *MTHFR C677T* polymorphism on esophageal cancer risk and to test for possible gene-environment interactions with smoking and alcohol consumption.

DATA COLLECTION

The pooled-analysis on the association between *MTHFR C677T* and esophageal cancer was conducted using data available in the GSEC database⁴⁰. Briefly, GSEC is an international collaborative initiative aimed at collection of published and unpublished data sets on metabolic polymorphisms and cancer risk.

A MEDLINE literature search for case-control studies published between 1966 and July 1, 2007 on the association of *MTHFR C677T* polymorphism and esophageal cancer was conducted using the search term: (*mthfr* OR *methylenetetrahydrofolate reductase*) AND (*esophagus* OR *esophageal*) AND (*cancer* OR *carcinoma*), yielding 24 results. Of those, 14 were excluded; two publications because they were a review⁴¹ and a meta-analysis³; six because they reported the effect of *MTHFR C677T* polymorphism on treatment outcomes^{42–47}; two because they evaluated the association of *MTHFR C677T* polymorphism and gastric cancer^{48,49}; one because it reported on *MTHFR A1298C* polymorphism⁵⁰; and one because it was an update on the status of the HERPACC-II project⁵¹ and did not report case-control data. Two studies evaluated *MTHFR* promoter methylation, but did not report case-control *MTHFR 677* genotype data^{52,53}. After exclusions, 8 studies with unique case-control data sets were identified^{29,33–39}. Authors of all relevant publications were contacted and their data was requested for inclusion in the GSEC database. Data sets were required to have case-control data for *MTHFR C677T* and esophageal cancer with *MTHFR 677* genotypes in Hardy-Weinberg equilibrium to be included in the analysis⁵⁴. We received data from 4 of the 8 case-control studies^{29,33,34,36} and from 1 unpublished case-control data set (Vasavi et al), for a total of 725 cases and 1,621 controls. A description of the pooled data set is provided in table 2.

STATISTICAL ANALYSIS

Adjusted odds ratios and 95% confidence intervals for *MTHFR C677T* were estimated using unconditional logistic regression, including study, age, gender, and smoking status as covariates in the model. Interactions were assessed between *MTHFR 677* genotype and smoking status, and between *MTHFR 677* genotype and alcohol use. For all included studies, smoking and alcohol status was derived from direct interview or self administered questionnaire data. No validation of smoking status by cotinine was available. Age and gender data was 100% complete; smoking data was complete in 99.5% of the subjects (11 missing); and alcohol data was available for 73.1% of subjects (607 missing, study by Song et al³⁴). Heterogeneity was tested using a Q-statistic⁵⁵ with p-values < 0.05, calculated using crude ORs from the individual studies. Egger's⁵⁶ and Begg's⁵⁷ tests were performed to evaluate publication bias among the pooled studies. Funnel plots were generated for a visual representation of potential publication bias (not shown). When heterogeneity between studies was observed, sensitivity analysis was performed by restricting the pooled analysis to those studies without significant heterogeneity, testing the robustness of the results.

Hardy-Weinberg equilibrium was verified on all pooled studies⁵⁸ with exact methods using REAP v. 4.0 (University of Maine, Orono, ME). All other analyses were performed using Intercooled Stata 10 (Stata Corp, College Station, TX).

RESULTS

The results of the pooled analysis are summarized in table 3. There was a significant overall association between *MTHFR* 677 *TT* genotype (OR = 2.63, 95% CI: 1.75 – 3.94), but not *MTHFR* 677 *CT* genotype (OR = 1.22, 95% CI: 0.84–1.77) and esophageal cancer, with a significant trend across genotypes ($CC < CT < TT$; $p < 0.01$). However, significant heterogeneity was observed (*CT*: $p < 0.01$; *TT*: $p < 0.01$). No evidence of publication bias was found for the *CT* genotype ($p = 0.57$) nor for the *TT* genotype ($p = 0.15$).

The sensitivity analysis indicated that removal of one study³⁴ from the analysis reduced the evidence of heterogeneity (*CT*: $p = 0.47$; *TT*: $p = 0.60$). The direction of the overall association of *TT* genotype with esophageal cancer was similar, although the magnitude of the association decreased (OR = 1.57, 95% CI: 0.96–2.56). No association was observed for *CT* genotype and esophageal cancer (OR = 0.88, 95% CI: 0.57–1.36). After performing sensitivity analysis there was still no evidence of publication bias for *TT* genotype ($p = 0.96$), but there was evidence of publication bias for *CT* genotype ($p = 0.05$).

The analysis was then restricted to squamous cell carcinoma ($n = 579$); similar results were observed for the *TT* genotype (OR = 2.00, 95% CI: 1.49–2.69), and the *CT* genotype (OR = 1.36, 95% CI: 1.05–1.76). There were insufficient cases to analyze the association of *MTHFR* C677T and esophageal adenocarcinoma.

A significant positive association between the *MTHFR* 677 *TT* genotype and esophageal cancer was observed in never smokers (OR = 2.57, 95% CI: 1.68–3.93), but not for the *CT* genotype (OR = 1.23, 95% CI: 0.83–1.81), with a positive trend across genotypes ($CC < CT < TT$; $p < 0.01$). Among ever-smokers, there was no association between the genotype and esophageal cancer (*TT*: OR = 1.26, 95% CI: 0.89–1.78; *CT*: OR = 1.25, 95% CI: 0.94–1.65; trend: $p = 0.16$). A significant interaction was identified between smoking and the *MTHFR* 677 genotype in both the full pooled analysis and in the analysis restricted to the homogeneous studies.

We further evaluated the effect of cumulative smoking dose, measured in average packs of cigarettes smoked per year (pack-years), on esophageal cancer risk according to the *MTHFR* 677 genotype (table 4). There was no effect of the genotype on the risk of the esophageal cancer associated with smoking dose.

No significant interaction was found between alcohol consumption and the *MTHFR* 677 genotype. No significant three-way interaction between alcohol, smoking, and *MTHFR* 677 genotype was observed.

DISCUSSION

This pooled analysis, containing 725 cases and 1,531 controls from 5 studies, suggests an overall association between *MTHFR* 677 *TT* genotype and esophageal cancer, although with some heterogeneity between studies, and a non significant association between *CT* genotype and esophageal cancer. A recent meta-analysis by Larsson (2006)³ also examined the association between *MTHFR* C677T and esophageal cancer, but did not report a summary estimate due to significant heterogeneity between published studies. Meta-analyses and/or pooled-analyses examining the role of *MTHFR* C677T and other cancers have reported significant positive associations with gastric cancer, multiple myeloma, and breast cancer

(postmenopausal women)^{15,59–61}; while significant inverse associations have been reported for leukemia (adults) and colorectal cancer⁶⁰. However, a recent meta-analysis reported no significant association for *MTHFR C677T* and lung cancer⁶².

Additionally, we report a significant negative interaction between smoking and *MTHFR 677* genotype. The direction of the interaction is contrary to the expected effect, given that tobacco smoke has been reported to impair folate status through biological activation of folate and B₁₂. Since low folate status has been shown to interact with *MTHFR C677T*²², it would be expected that *TT* genotype would be associated with increased risk compared with *CC* genotype in smokers. We did not observe an interaction between *MTHFR 677* genotype and alcohol. In a previous study, Yang et al³³ observed no interaction between smoking and *MTHFR C677T* polymorphism on esophageal cancer risk, but reported an interaction between alcohol consumption and genotype, observing an inverse trend across genotypes (*CC* > *CT* > *TT*) in heavy drinkers. As with tobacco smoke, alcohol has been shown to act as a folate antagonist (figure 1). It is possible that individual folate levels may play a role in the interaction between alcohol, smoking of the *MTHFR C677T* polymorphism; unfortunately we did not have sufficient individual folate data for use as a covariate in the pooled analysis.

The validity of the present pooled analysis may be limited because the available data only includes Asian and a small proportion of Caucasian subjects. However, this is representative of the published literature, which contains 8 studies; 6 of which report on Asian populations^{29,33–36,38}, 1 on Caucasians³⁷, and 1 on both Asian and Caucasian subjects³⁹. Currently, there is no literature evaluating the association between the *MTHFR C677T* polymorphism and esophageal cancer in races other than Asian and Caucasian, such as Latinos, Native Americans, Aborigines, or people of African descent.

A strength of our analysis, and pooled analyses in general, is that the aggregation of data from multiple studies may afford better statistical power necessary to detect an association if one exists. The pooled analysis has advantages over meta-analysis⁶³, allowing for adjustment for potential confounders, testing of interactions, and stratification. Thus, we were able to adjust for age, gender, race and smoking status; test for interactions between alcohol consumption, smoking, and *MTHFR C677T*; and stratify our results by smoking habit, which would not be possible with meta-analysis given the aggregate nature of the data available in the published studies.

In conclusion, we report an association of *MTHFR 677 TT* genotype and esophageal cancer, and an interaction between smoking and genotype. However, the folate metabolic pathway is very complex, and is mediated by a variety of gene products, folate, and nutrient cofactors. Future research on *MTHFR C677T* and esophageal cancer should be further targeted at the interactive effects of dietary and environmental factors, and gene-gene interactions. Large-scale studies are required to whether an interaction exists between *MTHFR C677T* and folate levels on esophageal cancer, and if this modifies the risk of esophageal cancer associated with both smoking and alcohol.

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REFERENCES

1. Goyette P, Sumner JS, Milos R, Duncan AMV, Rosenblatt DS, Matthews RG, Rozen R. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. *Nat Genet.* 1994 Jun.7:195–200. [PubMed: 7920641]
2. Fodinger M, Horl WH, Sunder-Plassmann G. Molecular biology of 5,10-methylenetetrahydrofolate reductase. *J Nephrol.* 2000; 13:20–33. [PubMed: 10720211]
3. Larsson SC, Giovannucci E, Wolk A. Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis. *Gastroenterology.* 2006; 131:1271–1283. [PubMed: 17030196]
4. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, Rozen R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet.* 1995 May.10:111–113. [PubMed: 7647779]
5. Friso S, Choi SW. Gene-nutrient interactions in one-carbon metabolism. *Curr Drug Metab.* 2005; 6:37–46. [PubMed: 15720206]
6. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB, Ames BN. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc Nat Acad Sci USA.* 1997 Apr.94:3290–3295. [PubMed: 9096386]
7. Fang JY, Xiao SD. Folic acid, polymorphism of methyl-group metabolism genes, and DNA methylation in relation to GI carcinogenesis. *J Gastroenterol.* 2003; 38:821–829. [PubMed: 14564626]
8. Stern LL, Mason JB, Selhub J, Choi SW. Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase gene. *Cancer Epidemiol Biomarkers Prev.* 2000 Aug.9:849–853. [PubMed: 10952104]
9. McCullough ML, Giovannucci EL. Diet and cancer prevention. *Oncogene.* 2004; 23:6349–6364. [PubMed: 15322510]
10. Leclerc D, Rozen R. Molecular genetics of MTHFR: polymorphisms are not all benign. *Med Sci (Paris).* 2007 Mar; 23(3):297–302. [PubMed: 17349292]
11. Bailey LB, Gregory JF Jr. Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: metabolic significance, risks and impact on folate requirement. *J Nutr.* 1999; 129:919–922. [PubMed: 10222379]
12. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J, Rozen R. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation.* 1996; 93:7–9. [PubMed: 8616944]
13. Ashfield-Watt PA, Pullin CH, Whiting JM, Clark ZE, Moat SJ, Newcombe RG, Burr ML, Lewis MJ, Powers HJ, McDowell IF. Methylenetetrahydrofolate reductase 677 C>T genotype modulates homocysteine responses to a folate-rich diet or low dose folic acid supplement: a randomized controlled trial. *Am J Clin Nutr.* 2002; 76:180–186. [PubMed: 12081832]
14. Ulvik A, Ueland PM, Fredriksen A, Meyer K, Vollset SE, Hoff G, Schneede J. Functional inference of the methylenetetrahydrofolate reductase 677 C>T and 1298 A>C polymorphisms from a large-scale epidemiological study. *Hum Genet.* 2007; 121:57–64. [PubMed: 17115185]
15. Boccia S, Hung R, Ricciardi G, Gianfagna F, Ebert MP, Fang JY, Gao CM, Götze T, Graziano F, Lacasaña-Navarro M, Lin D, López-Carrillo L, Qiao YL, Shen H, Stolzenberg-Solomon R, Takezaki T, Weng YR, Zhang FF, van Duijn CM, Boffetta P, Taioli E. Meta- and Pooled Analyses of the Methylenetetrahydrofolate Reductase C677T and A1298C Polymorphisms and Gastric Cancer Risk: A Huge-GSEC Review. *Am J Epidemiol.* 2008; 167(5):505–516. [PubMed: 18162478]
16. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate Reductase Gene Variants and Congenital Anomalies: A HuGE Review. *Am J Epidemiol.* 2000; 151(9):862–877. [PubMed: 10791559]
17. [Accessed Feb 26, 2007] National Cancer Institute. Esophageal Cancer. <http://www.cancer.gov/cancertopics/types/esophageal/>.

18. Parkin DM, Bray F, Ferlay J, Pisani P. Global Cancer Statistics, 2002. *CA Cancer J Clin.* 2005; 55:74–108. [PubMed: 15761078]
19. Parkin, DM.; Whelan, SL.; Ferlay, J.; Storm, H. IARC CancerBase No.7. Lyon: 2005. Cancer Incidence in Five Continents, Vol. I to VIII.
20. Vizcaino AP, Moreno V, Lambert R, Parkin DM. Time trends incidence of both major histologic types of esophageal carcinomas in selected countries, 1973–1995. *Int J Cancer.* 2002; 99:860–868. [PubMed: 12115489]
21. Mayne ST, Risch HA, Dubrow R, Chow WH, Gammon MD, Vaughan TL, Farrow DC, Schoenberg JB, Stanford JL, Ahsan H, West AB, Rotterdam H, Blot WJ, Fraumeni JF jr. Nutrient Intake and Risk of Subtypes of Esophageal and Gastric Cancer. *Cancer Epidemiol Biomarkers Prev.* 2001; 10:1055–1062. [PubMed: 11588131]
22. Friso S, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, Olivieri O, Jacques PF, Rosenberg IH, Corrocher R, Selhub J. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *Proc Natl Acad Sci USA.* 2002; 99(8):5606–5611. [PubMed: 11929966]
23. McNulty H, Dowey LC, Strain JJ, Dunne A, Ward M, Molloy A, McAnena LB, Hughes JP, Hannon-Fletcher M, Scott JM. Riboflavin lowers homocysteine in individuals homozygous for the MTHFR 677 C>T polymorphism. *Circulation.* 2006; 113:74–80. [PubMed: 16380544]
24. Jacques PF, Kalmbach R, Bagley PJ, Russo GT, Rogers G, Wilson PWF, Rosenberg IH, Selhub J. The Relationship between Riboflavin and Plasma Total Homocysteine in the Framingham Offspring Cohort Is Influenced by Folate Status and the C677T Transition in the Methylenetetrahydrofolate Reductase Gene. *J Nutr.* 2002; 132:283–288. [PubMed: 11823591]
25. Khaled MA, Watkins CL, Krumdieck CL. Inactivation of B12 and folate coenzymes by butyl nitrite as observed by NMR: implications on one-carbon transfer mechanism. *Biochem Biophys Res Commun.* 1986 Feb; 135(1):201–207. [PubMed: 3954771]
26. Khaled MA, Krumdieck CL. Association of Folate Molecules as Determined by Proton NMR: Implications on Enzyme Binding. *Biochem Biophys Res Commun.* 1985; 130(3):1273–1280. [PubMed: 3839676]
27. Halsted CH, Villanueva JA, Devlin AM, Chandler CJ. Metabolic Interactions of Alcohol and Folate. *J Nutr.* 2002; 132:2367S–2372S. [PubMed: 12163694]
28. Barak AJ, Beckenhauer HC, Tuma DJ, Badakhsh S. Effects of prolonged ethanol feeding on methionine metabolism in rat liver. *Biochem Cell Biol.* 1987 Mar; 65(3):230–233. [PubMed: 3580171]
29. Stolzenberg-Solomon RZ, Qiao YL, Abnet CC, Ratnasinghe DL, Dawsey SM, Dong ZW, Taylor PR, Mark SD. Esophageal and gastric cardia cancer risk and folate- and vitamin B(12)-related polymorphisms in Linxian, China. *Cancer Epidemiol Biomarkers Prev.* 2003 Nov; 12:1222–1226. [PubMed: 14652285]
30. Graziano F, Kawakami K, Ruzzo A, Watanabe G, Santini D, Pizzagalli F, Bissoni R, Mari D, Floriani I, Catalano V, Silva R, Tonini G, Torri V, Giustini L, Magnani M. Methylenetetrahydrofolate reductase 677C/T gene polymorphism, gastric cancer susceptibility and genomic DNA hypomethylation in an at-risk Italian population. *Int J Cancer.* 2006 Feb; 118(3): 628–632. [PubMed: 16094648]
31. Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, Willett WC, Selhub J, Hennekens CH, Rozen R. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Res.* 1997 Mar; 57(6):1098–1102. [PubMed: 9067278]
32. Chen J, Giovannucci E, Kelsey K, Rimm EB, Stampfer MJ, Colditz GA, Spiegelman D, Willett WC, Hunter DJ. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res.* 1996 Nov; 56(21):4862–4864. [PubMed: 8895734]
33. Yang CX, Matsuo K, Ito H, Shinoda M, Hatooka S, Hirose K, Wakai K, Saito T, Suzuki T, Maeda T, Tajima K. Gene-environment interactions between alcohol drinking and the MTHFR C677T polymorphism impact on esophageal cancer risk: results of a case-control study in Japan. *Carcinogenesis.* 2005; 26:1285–1290. [PubMed: 15790587]

34. Song C, Xing D, Tan W, Wei Q, Lin D. Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. *Cancer Res.* 2001; 61:3272–3275. [PubMed: 11309278]
35. Wang Y, Guo W, He Y, Chen Z, Wen D, Zhang X, Wang N, Li Y, Ge H, Zhang J. Association of MTHFR C677T and SHMT(1) C1420T with susceptibility to ESCC and GCA in a high incident region of Northern China. *Cancer Causes Control.* 2007 Mar;18:143–152. [PubMed: 17206530]
36. Gao CM, Takezaki T, Wu JZ, Liu YT, Ding JH, Li SP, Su P, Hu X, Kai HT, Li ZY, Matsuo K, Hamajima N, Sugimura H, Tajima K. Polymorphisms in thymidylate synthase and methylenetetrahydrofolate reductase genes and the susceptibility to esophageal and stomach cancer with smoking. *Asian Pac J Cancer Prev.* 2004; 5:133–138. [PubMed: 15244514]
37. Kureshi N, Ghaffar S, Siddiqui S, Salahuddin I, Frossard PM. Head and neck cancer susceptibility: a genetic marker in the methylenetetrahydrofolate reductase gene. *ORL J Otorhinolaryngol Relat Spec.* 2004; 66:241–245. [PubMed: 15583437]
38. Wang LD, Guo RF, Fan ZM, He X, Gao SS, Guo HQ, Matsuo K, Yin LM, Li JL. Association of methylenetetrahydrofolate reductase and thymidylate synthase promoter polymorphisms with genetic susceptibility to esophageal and cardia cancer in a Chinese high-risk population. *Dis Esophagus.* 2005; 18:177–184. [PubMed: 16045580]
39. Zhang J, Zotz RB, Li Y, Wang R, Kiel S, Schulz WA, Wen D, Chen Z, Zhang L, Wang S, Gabbert HE, Sarbia M. Methylenetetrahydrofolate reductase C677T polymorphism and predisposition towards esophageal squamous cell carcinoma in a German Caucasian and a northern Chinese population. *J Cancer Res Clin.Oncol.* 2004; 130:574–580. [PubMed: 15449187]
40. Gaspari L, Marinelli D, Taioli E. Collaborative Group on Genetic Susceptibility to Environmental Carcinogens. International collaborative study on genetic susceptibility to environmental carcinogens (GSEC): an update. *Int J Hyg Environ Health.* 2001 Oct; 204(1):39–42. [PubMed: 11725343]
41. Xing D, Tan W, Lin D. Genetic polymorphisms and susceptibility to esophageal cancer among Chinese population (review). *Oncol Rep.* 2003 Sep-Oct;10(5):1615–1623. [PubMed: 12883749]
42. Hofler H, Langer R, Ott K, Keller G. Prediction of response to neoadjuvant chemotherapy in carcinomas of the upper gastrointestinal tract. *Adv Exp Med Biol.* 2006; 587:115–120. [PubMed: 17163161]
43. Langer R, Specht K, Becker K, Ewald P, Sarbia M, Busch R, Feith M, Stein HJ, Siewert JR, Hofler H. Prediction of response to neoadjuvant chemotherapy in Barrett's carcinoma by quantitative gene expression analysis. *Verh Dtsch Ges Pathol.* 2004; 88:207–213. [PubMed: 16892554]
44. Wu X, Gu J, Wu TT, Swisher SG, Liao Z, Correa AM, Liu J, Etzel CJ, Amos CI, Huang M, Chiang SS, Milas L, Hittelman WN, Ajani JA. Genetic variations in radiation and chemotherapy drug action pathways predict clinical outcomes in esophageal cancer. *J Clin Oncol.* 2006 Aug 10; 24(23):3789–3798. [PubMed: 16785472]
45. Wu X, Lu C, Chiang SS, Ajani JA. Pharmacogenetics in esophageal cancer. *Semin Oncol.* 2005 Dec; 32(6 Suppl 9):S87–S89. [PubMed: 16399440]
46. Sarbia M, Stahl M, von Weyhern C, Weirich G, Puhlinger-Oppermann F. The prognostic significance of genetic polymorphisms (Methylenetetrahydrofolate Reductase C677T, Methionine Synthase A2756G, Thymidilate Synthase tandem repeat polymorphism) in multimodally treated oesophageal squamous cell carcinoma. *Br J Cancer.* 2006 Jan 30; 94(2):203–207. [PubMed: 16333305]
47. Langer R, Specht K, Becker K, Ewald P, Bekesch M, Sarbia M, Busch R, Feith M, Stein HJ, Siewert JR, Hofler H. Association of pretherapeutic expression of chemotherapy-related genes with response to neoadjuvant chemotherapy in Barrett carcinoma. *Clin Cancer Res.* 2005 Oct 15; 11(20):7462–7469. [PubMed: 16243820]
48. Kim JK, Kim S, Han JH, Kim HJ, Chong SY, Hong SP, Hwang SG, Ahn JY, Cha KY, Oh D, Kim NK. Polymorphisms of 5,10-methylenetetrahydrofolate reductase and risk of stomach cancer in a Korean population. *Anticancer Res.* 2005 May-Jun;25(3B):2249–2252. [PubMed: 16158971]
49. Miao X, Xing D, Tan W, Qi J, Lu W, Lin D. Susceptibility to gastric cardia adenocarcinoma and genetic polymorphisms in methylenetetrahydrofolate reductase in an at-risk Chinese population. *Cancer Epidemiol Biomarkers Prev.* 2002 Nov; 11(11):1454–1458. [PubMed: 12433726]

50. Gao CM, Toshiro T, Wu JZ, Cao HX, Liu YT, Ding JH, Li SP, Su P, Hu X, Kai HT, Kazuo T. A case-control study on the polymorphisms of methylenetetrahydrofolate reductase 1298A-->C and susceptibility of esophageal cancer. *Zhonghua Liu Xing Bing Xue Za Zhi*. 2004 Apr; 25(4):341–345. [PubMed: 15231206]
51. Hamajima N, Matsuo K, Saito T, Hirose K, Inoue M, Takezaki T, Kuroishi T, Tajima K. Gene-environment Interactions and Polymorphism Studies of Cancer Risk in the Hospital-based Epidemiologic Research Program at Aichi Cancer Center II (HERPACC-II). *Asian Pac J Cancer Prev*. 2001; 2(2):99–107. [PubMed: 12718640]
52. Vasavi M, Ponnala S, Gujjari K, Boddu P, Bharatula RS, Prasad R, Ahuja YR, Hasan Q. DNA methylation in esophageal diseases including cancer: special reference to hMLH1 gene promoter status. *Tumori*. 2006 Mar-Apr;92(2):155–162. [PubMed: 16724696]
53. Eads CA, Lord RV, Wickramasinghe K, Long TI, Kurumboor SK, Bernstein L, Peters JH, DeMeester SR, DeMeester TR, Skinner KA, Laird PW. Epigenetic patterns in the progression of esophageal adenocarcinoma. *Cancer Res*. 2001 Apr 15; 61(8):3410–3418. [PubMed: 11309301]
54. Taioli E, Pedotti P, Garte S. Importance of allele frequency estimates in epidemiological studies. *Mut Res*. 2004; 567:63–70. [PubMed: 15341902]
55. Takkouche B, Cadarso-Suárez C, Spiegelman D. Evaluation of old and new tests of heterogeneity in epidemiologic meta-analysis. *Am J Epidemiol*. 1999 Jul 15; 150(2):206–215. [PubMed: 10412966]
56. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 1997 Sep 13; 315(7109):629–634. [PubMed: 9310563]
57. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics*. 1994 Dec; 50(4):1088–1101. [PubMed: 7786990]
58. Guo SW, Thompson EA. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics*. 1992 Jun; 48(2):361–372. [PubMed: 1637966]
59. Macis D, Maisonneuve P, Johansson H, Bonanni B, Botteri E, Iodice S, Santillo B, Penco S, Gucciardo G, D'Aiuto G, del Turco R, Amodori M, Costa A, Decensi A. Methylenetetrahydrofolate reductase (MTHFR) and breast cancer risk: a nested-case-control study and a pooled meta-analysis. *Breast Cancer Res Treat*. 2007; 106:263–271. [PubMed: 17260091]
60. Dong LM, Potter JD, White E, Ulrich CM, Cardon LR, Peters U. Genetic susceptibility to cancer: the role of polymorphisms in candidate genes. *JAMA*. 2008 May; 299(20):2423–2436. [PubMed: 18505952]
61. Zintzaras E, Giannouli S, Rodopoulou P, Voulgarelis M. The role of MTHFR gene in multiple myeloma. *J Hum Genet*. 2008; 53:499–507. [PubMed: 18350248]
62. Mao R, Fan Y, Jin Y, Bai J, Fu S. Methylenetetrahydrofolate reductase gene polymorphisms and lung cancer: a meta-analysis. *J Hum Genet*. 2008; 53:340–348. [PubMed: 18340404]
63. Blettner M, Sauerbrei W, Schlehofer B, Scheuchnpflug T, Friedenreich C. Traditional reviews, meta-analyses and pooled analyses in epidemiology. *Int J Epidemiol*. 1999 Feb; 28(1):1–9. Review. [PubMed: 10195657]
64. Ferlay, J.; Bray, F.; Pisani, P.; Parkin, DM. IARC Cancer Base No. 5. v2.0. Lyon: IARC press; 2004. GLOBOCAN 2002: Cancer Incidence, Mortality, and Prevalence Worldwide. Available at: <http://www-dep.iarc.fr>;

Figure 1.
Simplified folate metabolic pathway showing the effects of alcohol and tobacco

Figure 2.
Worldwide age-standardized mortality rates for esophageal cancer by gender⁶⁴

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Table 1
Summary of published studies on the association between *MTHFR* C677T and esophageal cancer

Study	Cases	Controls	Country	Ethnicity	Control Source	Crude OR _{CT} (95% CI)	Crude OR _{TT} (95% CI)	Crude OR _{CT+TT} (95% CI)	Adj OR _{CT} (95% CI)	Adj OR _{TT} (95% CI)	Adj OR _{CT+TT} (95% CI)
Song ³⁴ *	240	360	China	Asian	Population	2.98 (1.87–4.75)	6.52 (3.89–10.92)	3.92 (2.51–6.11)	3.14 (1.94–5.08) ^a	6.18 (3.32–11.51) ^a	Not Reported
Stolzenberg-Solomon ²⁹ *	129	398	China	Asian	Population	0.78 (0.45–1.37)	1.09 (0.61–1.96)	0.90 (0.53–1.52)	0.86 (0.48–1.54) ^b	1.24 (0.68–2.26) ^b	Not Reported
Gao ³⁶ *	138	222	China	Asian	Population	Not Reported	Not Reported	1.25 (0.81–1.93)	Not Reported	Not Reported	Not Reported
Zhang (Caucasian) ³⁹	241	256	Germany	Caucasian	Population	1.15 (0.79–1.68)	1.04 (0.59–1.82)	1.12 (0.78–1.61)	Not Reported	Not Reported	1.03 (0.46–2.33) ^{a,e}
Kureshi ³⁷	34	54	Pakistan	Caucasian	Population	0.97 (0.39–2.41)	----	0.79 (0.33–1.93)	Not Reported	Not Reported	Not Reported
Wang LD ³⁸	275	315	China	Asian	Population	1.07 (0.69–1.65)	1.76 (1.13–2.75)	1.35 (0.90–2.01)	0.96 (0.61–1.50) ^c	1.58 (0.99–2.50) ^c	Not Reported
Yang ³³ *	165	493	Japan	Asian	Hospital	1.07 (0.73–1.56)	0.74 (0.42–1.30)	0.98 (0.68–1.41)	0.97 (0.63–1.49) ^d	0.66 (0.35–1.25) ^d	Not Reported
Wang Y ³⁵ , Zhang (Asian) ³⁹ ^	584	540	China	Asian	Population	1.83 (1.30–2.58)	2.16 (1.53–3.06)	1.98 (1.44–2.72)	1.81 (1.28–2.54) ^c	2.13 (1.50–3.02) ^c	Not Reported

Kureshi 2004 had 0 esophageal cancer cases with *MTHFR* 677 TT genotypes

^a Odds Ratio (OR) adjusted for age, gender, and smoking status

^b Reports Relative Risk (RR) adjusted for age, gender, smoking status, BMI, and alcohol status

^c Odds Ratio (OR) adjusted for age and gender

^d Odds Ratio (OR) adjusted for smoking status, alcohol status, and folate status

^e This study reported OR for CC using CT+TT as baseline for comparison, therefore the inverse of the OR was calculated

* Included in pooled analysis. Pooled analysis also includes 1 unpublished case-control data set

[^] Overlaps Asian subjects in Zhang³⁹ study

Table 2

Description of the data available in the GSEC pooled data set

	Cases (n)	Controls (n)	
Age			p = 0.56
<i>Mean (years)</i>	57.3 (725)	57.6 (1531)	
Gender			p = 0.05
<i>Male</i>	68.0% (493)	72.0% (1103)	
<i>Female</i>	32.0% (232)	28.0% (428)	
Race			p < 0.01
<i>Asian</i>	92.8% (673)	96.7% (1481)	
<i>Caucasian</i>	7.2% (52)	3.3% (50)	
Smoking Status			p < 0.01
<i>Never-Smoker</i>	38.2% (276)	45.2% (688)	
<i>Ever-Smoker</i>	61.8% (446)	54.8% (835)	
Alcohol Use [^]			p < 0.001
<i>Never-Drinker</i>	30.9% (149)	41.1% (479)	
<i>Ever-Drinker</i>	69.2% (334)	58.9% (687)	
Histology [*]			
<i>Squamous Cell</i>	98.1% (579)	-----	
<i>Adenocarcinoma</i>	1.9% (11)	-----	

[^] Alcohol data was unavailable for one of the five studies (Song et al³⁴)^{*} Histology data was unavailable for one of the five studies (Gao et al³⁶)

Table 3Association between *MTHFR* 677 genotype and esophageal cancer in the GSEC pooled analysis

Genotype	Cases	Controls	Adj OR (95% CI)	Trend
Overall *				p < 0.01
CC	209	486	1.00 (<i>Ref</i>)	
CT	328	731	1.22 (0.84–1.77)	
TT	188	314	2.63 (1.75–3.94)	
Never-Smokers #				p < 0.01
CC	77	221	1.00 (<i>Ref</i>)	
CT	106	325	1.23 (0.83–1.81)	
TT	93	142	2.57 (1.68–3.93)	
Ever-Smokers #				p = 0.16
CC	130	264	1.00 (<i>Ref</i>)	
CT	221	400	1.25 (0.94–1.65)	
TT	95	171	1.26 (0.89–1.78)	
Never-Drinkers *				p = 0.65
CC	58	142	1.00 (<i>Ref</i>)	
CT	51	216	0.81 (0.47–1.38)	
TT	40	121	1.10 (0.61–1.98)	
Ever-Drinkers *				p = 0.37
CC	122	219	1.00 (<i>Ref</i>)	
CT	158	339	0.92 (0.67–1.25)	
TT	54	129	0.83 (0.55–1.25)	
Squamous Cell Carcinoma *				P < 0.01
CC	157	486	1.00 (<i>Ref</i>)	
CT	260	731	1.36 (1.05–1.76)	
TT	162	314	2.00 (1.49–2.69)	

* ORs are adjusted for study, age, race, gender, and smoking habit.

ORs are adjusted for study, age, race, and gender

Table 4

Association between *MTHFR* 677 genotype and esophageal cancer by smoking dose in the GSEC pooled analysis

Genotype	Cases	Controls	Adj OR (95% CI)
1 st Tertile (20 pack-years)			
<i>CC</i>	19	63	1.00 (<i>Ref</i>)
<i>CT</i>	61	99	2.39 (1.21–4.72)
<i>TT</i>	33	72	1.37 (0.65–2.90)
2 nd Tertile (21–39 pack-years)			
<i>CC</i>	33	71	1.00 (<i>Ref</i>)
<i>CT</i>	63	114	1.19 (0.70–2.01)
<i>TT</i>	23	40	1.24 (0.63–2.45)
3 rd Tertile (40 pack-years)			
<i>CC</i>	30	84	1.00 (<i>Ref</i>)
<i>CT</i>	54	122	1.27 (0.74–2.16)
<i>TT</i>	22	33	1.81 (0.91–3.58)