RESEARCH COMMUNICATION

Folate Intake, Methylenetetrahydrofolate Reductase Polymorphisms, and Risk of Esophageal Cancer

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Abstract

Aim: Genetic and environmental factors may play roles in the pathogenesis of esophageal cancer and susceptibility may be modified by functional polymorphisms in folate metabolic genes, such as methylenetetrahydrofolate reductase (MTHFR). We here evaluated associations of the MTHFR C677T polymorphism and folate intake with esophageal cancer. Methods: A matched hospital-based case-control study with 155 esophageal cancer and 310 non-cancer controls was conducted in a Chinese population with gene-environment interactions evaluated between the MTHFR C667T polymorphism and drinking and smoking, as well as folate intake. Results: Individuals carrying MTHFR 667CT [adjusted odds ratio (OR), 1.95; 95% confidence interval (CI), 1.23-2.62] and TT [adjusted odds ratio (OR), 3.36; 95% confidence interval (CI), 1.46-8.74] had significantly increased esophageal cancer risk compared with those with MTHFR 667CC genotype. Folate intake was seen to have non-significant preventive effect. In former, moderate and heavy drinkers, a high esophageal cancer risk was observed for those with an MTHFR 677T allele genotype [ORs: 5.0(1.29-18.88), 3.70(1.83-7.66) and 5.77(2.11-15.72), respectively]. Significant interaction was found for moderate-heavy drinking and the MTHFR 677T allele genotype for esophageal cancer risk ($p<0.05$). Significant increased risk was also found in moderate and heavy smokers with the two genotypes [ORs: 3.58(1.64-7.80) and 4.51(1.15-17.78), respectively]. High folate intake and MTHFR 677TT was associated with a non-significant tendency for decreased esophageal cancer risk. Conclusion: Our finding supports the hypothesis that MTHFR C667T polymorphisms play a role in pathogenesis of esophageal cancer in the Chinese population.

Keywords: Esophageal cancer risk - folate intake - methylenetetrahydrofolate reductase polymorphism

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Introduction

Esophageal cancer is the sixth most common cancer worldwide in 2002(Blount et al., 2007). Its rates showed a wide international geographic variation in the incidence and mortality of esophageal cancer(Choi and Mason, 2000; YI 2004; Blount et al., 2007), suggesting that the role of genetic and environmental factors in the pathogenesis of this cancer(Choi and Mason, 2000).

As we know, vegetable and fruit are full of folate, vitamin and trace element, and these factors are proved to be the protective factor in carcinogenesis of many types of cancer. Deficiency of them is associated with increased risk of several cancers, including esophageal cancer. Folate is a water-solution B vitamin, and folate deficiency can influence the carcinogenesis of esophageal cancer through two ways: one is inducing misincorportion of uracil into DNA to result in chromosomal breaks and mutations. Another is leading the alteration in DNA methylation and thus to alter the expression of tumor suppressor genes (Choi and Mason, 2000; Yi 2004; Blount et al., 2007). Previous epidemiologic studies have shown that folate deficiency could increase the carcinogenesis of esophageal cancer(CS 2000; Mayne et al., 2001; Chen et al., 2002).

Except for an inadequate folate intake, Methylenetetrahydrofolate reductase (MTHFR), functional polymorphisms in folate metabolism, may also play a role in the susceptibility of esophageal cancer risk. It facilitates the conversion of 5,10-methylene THF to 5-methyl THF, and leads point mutations and/or chromosomal breaks. Also, it may cause decline of 5-methyl THF to induce a decrease of the conversion of homocysteine to methionine, which could result in a carcinogenesis process of DNA hypomethylation (Blount et al., 1997; Fang and Xiao, 2003; Stern et al., 2000). Approximately 60 polymorphisms have been described in the MTHFR gene (Leclerc and Rozen., 2007). 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

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exon 4 at nucleotide 677, resulting in the conversion of alanine to valine at position 222 of the MTHFR amino acid sequence (Frosst et al., 1995; Choi and Mason., 2000). 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)(Bailey and Gregory, 1999). As a result, TT homozygotes have been associated with lower serum folate levels and higher homocysteine levels than their wild type homozygous counterparts. An impact of this polymorphism on several types of cancer including colorectal cancer, acute lymphocytic leukemia, lung, prostrate, gallbladder and esophageal cancers has been conducted (Boccia et al., 2007; Suzuki, 2007; Cao, 2008; Yu, 2008; Bai, 2009). However, the association between the MTHFR C677T polymorphism and esophageal cancer is conflicting (Stolzenberg-Solomon et al., 2003; Yang et al., 2005; He et al., 2007; Umar et al., 2010).

The impact of combination of MTHFR C677T polymorphism and folate intake on esophageal cancer risk was not explored in Chinese population, and the interaction among the MTHFR C677T polymorphism and alcohol and tobacco consumption was not studied in China. Therefore, we conducted the present matched case-control study to clarify the impact of folate intake and MTHFR C677T on esophageal cancer risk in Chinese population, and its gene-environment interaction with drinking and smoking, as well as folate intake was explored.

Materials and Methods

Study population

A hospital-based case-control study has been carried out in the General Hospital of Chengdu Military Area. The study included 155 patients aged 37-75 years who had histological confirmed diagnosis of esophageal squamous cell cancer in the China-Japan Union Hospital of Jilin University and First Affiliate Hospital of Shantou University from Jan. 2008 to Dec. 2010. Hosptial-based controls were individually matched to cases by gender and age (±5 years). Controls were first-visit outpatients who visited the same two institutes during the same period and were confirmed to have no malignancy, digestive diseases, chronic diseases and also no prior history of malignancy. Ratio of cases to controls was 1:2. Totally, we had 310 controls who were non-cancer or cancer-free subjects. Informed consents were obtained from patients and controls.

A self-administered structured questionnaire was used in our study, consisting 65 items. Information were collected about demographic (age, sex and family history of cancer) and clinical characteristics (histopathology, tumor location, and lymph modes status), tobacco usage, smoking, alcohol-drinking habits and dietary habits (including 45 foods/food groups). A face to face interview was performed for all of the subjects by trained interviewers. Completed questionnaires were obtained from 155 cases and 310 controls. Cancer patients were asked to refer about habits a year before the disease diagnosed.

Sample collection

A total of 5 ml venous blood was collected from each study and kept frozen at -20°C until DNA extraction. Genomic DNA was extracted, using standard methods(Miller SA 1988), from peripheral blood of the controls or patients with esophageal cancer. The MTHFR genotypes at the C677T site were analyzed by PCR-based RFLP methods. The DNA was amplified with the forward primers 5'-TTTGAGGCTGACCTG A A G C A C T T G A A G G A G - 3 ' a n d 5'-GAGTGGTAGCCCTGGATGGGAAAGATCCCG-3'(Xing D 2003; Qin JM 2008). Each 25.0 μL reaction mixture contained 2.5 μL 10 × PCR buffer, 1.5 μL MgCl2 (25 mmol/L), 0.5 μL dNTP (10 mmol/L), 0.5 μL forprimer (20 μmol/L), 0.5 μL revprimer (20 μmol/L), 0.2 μL Taq DNA polymerase (5 U/μL), 1.5 μL template DNA, and 17.8 μL nuclease free water. The PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 65 s, at 60°C for 65 s, at 72°C for 90 s, and a final extension at 72°C for 5 min. After transient centrifugation, agarose electrophoresis was conducted. The PCR products included 173-bp fragments of 677C/C wild-type homozygotes; 173-, 125-, and 48-bp fragments of 677C/T heterozygotes, and 125- and 48-bp fragments of 677T/T homozygotes.

Assessment of folate intake

The folate intake was assessed according to our self-administered structured questionnaire. The folate intake was computed by multiplying the food intake (in grams) and the folate content (per gram) of food in our questionnaire, and then the sum of all folate intake from various foods/food groups was calculated.

Statistical analysis

Statistical analyses were performed by using Stata version 8 (Stata, College Station, TX). Conditional logistic regression was employed to calculate odds ratios (ORs) and their 95% confidence intervals (CIs). The chi-square goodness-of-fit test was used for any deviation in controls from Hardy-Weinberg Equilibrium. Alcohol exposure was categorized into four levels, former drinkers, non-drinkers (never drinker), moderate drinkers and heavy drinkers. Individuals who quit drinking more than one year were considered as former drinkers, individuals who drank alcoholic beverages 5 days or more per week with an amount of 50 g or more ethanol on each occasion while moderate drinkers were defined as drinkers consuming less frequently and/or lower amounts.

Smoking status was also divided into four categories considering cumulative exposure to tobacco: former smokers, non-smokers (never smokers), moderate smokers and heavy smokers. Individuals who quit smoking more than one year were considered as former smokers, individuals with pack-years (PYs) ≤40 were regarded as moderate smokers, and smokers with PYs>40 were regarded as heavy smokers. Because the proportion of MTHFR 677TT genotype is rare both in cases and controls, we analyzed the interaction gene-environment interactions by combining MTHFR 677TT and CT genotype together.

Table 1. Characteristics of Esophageal Cancer Patients and Matched Controls

		Cases	Controls	OR (95% CI)	P value
Mean age (years)		51.4±5.6	51.6±4.9	-	0.35
Sex	Male	86(55.5)	172(55.5)	-	-
	Female	69(44.5)	138(44.5)	-	-
Alcohol drinking status					
	Never	42(27.1)	142(45.8)	1.0	
	Former	16(10.3)	20(6.45)	2.70(1.19-6.03)	<0.05
	Moderate	69(44.5)	116(37.4)	2.01(1.25-3.26)	<0.05
	Heavy	28(18.1)	32(10.3)	2.96(1.53-5.70)	<0.05
Smoking status					
	Never	73(47.1)	178(57.4)	1.0	
	Former	11(7.11)	12(3.87)	2.23(0.85-5.79)	0.06
	Moderate	52(33.6)	102(32.9)	1.24(0.79-1.95)	0.32
	Heavy	19(12.3)	18(5.81)	2.57(1.20-5.51)	<0.05
Daily folate consumption (ug/day)					
	Mean (SE)	275(21.7)	298(32.5)	-	<0.05
	<230	42(27.1)	61(19.7)	1.0	
	230-300	61(39.4)	127(41.0)	0.70(0.41-1.19)	0.20
	>300	52(33.6)	122(39.4)	0.61(0.36-1.07)	0.10

Table 2. Relationship between the MTHFR C677T Polymorphism and Esophageal Cancer Risk

	Cases	Controls	OR (95% CI)	OR[1](95% CI)
CC	68(43.8)	179(57.7)	1.0	1.0
CT	74(47.7)	120(38.7)	1.62(1.06-2.48)	1.95(1.23-2.62)
TT	13(8.39)	11(3.55)	3.11(1.21-8.04)	3.36(1.46-8.74)
CT/TT	87(56.1)	131(42.3)	1.75(1.16-2.63)	1.93(1.29-2.97)

OR, Odds ratio; [1]adjusted for age, sex, smoking and drinking

Results

Demographical characteristics of patients are shown in Table 1. The mean age of cases and controls was 51.4±5.6 and 51.6±4.9 years, respectively. There was no significant difference in age between cases and controls (p=0.35). The distributions of smoking and drinking habits were seemed a large extent between cases and controls. Former, moderate and heavy drinkers were seemed to increase esophageal cancer risk than non-drinkers, with ORs (95% CI) of 2.70 (1.19-6.03), 2.01 (1.25-3.26) and 2.96 (1.53-5.70), respectively. Heavy smokers showed an increased esophageal cancer risk than non-smokers with the ORs (95% CI) of 2.57 (1.20-5.51). The mean adjusted daily folate consumption was 275.2±21.7 ug/day in cases and 297.9±32.5 ug/day in controls, a higher folate concentration showed a non-significant decreased risk of esophageal cancer (Table 1).

The genotype distributions of MTHFR C677T in cases and controls were summarized in Table 2. The frequencies of MTHFR 677 CC, CT and TT were 43.9%, 47.74% and 8.39% among cases, and were 57.7%, 38.71% and 3.55% among controls, respectively. The frequencies of the MTHFR C677T genotypes in controls were according to the Hardy-Weinberg equilibrium (p=0.09). Individuals carrying the MTHFR 677TT and CC genotypes showed a significantly increased risk of esophageal cancer compared with those with CC genotype, with the adjusted ORs and 95%CI of 1.95(1.23-2.62) and 3.36(1.46-8.74), respectively. Similarly, those carrying MTHFR 677T allele had a 1.93-fold risk(95% CI: 1.29-2.97) of developing esophageal cancer compared with those with CC genotype. The impact of combination of MTHFR C677T polymorphisms and folate intake on esophageal cancer risk is shown in Table 3. Among the high folate intake group, the MTHFR 677TT genotype showed a non-significant decreased risk of esophageal cancer. No significant interaction was found between folate consumption and MTHFR genotype (p=0.32).

In the interaction of MTHFR C677T polymorphisms with environmental risk factors such as tobacco and alcohol consumption, we found the MTHFR 677T allele genotype had a positive association with tobacco and alcohol habit, and the OR raised with the increased consumption of tobacco and alcohol. Significant interaction was found in moderate-heavy drinking and MTHFR 677T allele genotype for esophageal cancer risk (p<0.05), but no significant interaction was not found between smoking and MTHFR 677T allele genotype (p=0.08).

Discussion

In our study, we observed the MTHFR C677T polymorphisms were associated with susceptibility to esophageal cancer, and high folate consumption is associated with a non-significant decreased risk of esophageal cancer. A gene-environment association was found between MTHFR polymorphism and tobacco or alcohol consumption, and high the tobacco or alcohol

Table 2. MTHFR C677T Polymorphisms and Esophageal Cancer Risk According to Drinking, Smoking and Folate Intake

Variable		Genotype CC		Genotype CT/TT		
		Cases/Controls	OR (95% CI)	Cases/Controls	OR (95% CI)	P for interaction
Alcohol drinking status	Never	17/85	1.0	25/57	2.19(1.03-4.73)	-
	Former	9/13	3.46(1.11-10.56)	7/7	5.00(1.29-18.9)	0.13
	Moderate	29/62	2.34(1.12-4.95)	40/54	3.70(1.83-7.66)	<0.05
	Heavy	13/19	3.42(1.28-8.93)	15/13	5.77(2.11-15.7)	<0.05
Tobacco status	Never	22/85	1.0	53/93	2.20(1.19-4.13)	-
	Former	7/8	3.38(0.92-11.88)	4/5	3.09(0.56-15.6)	0.08
	Moderate	27/75	1.39(0.70-2.79)	25/27	3.58(1.64-7.80)	0.31
	Heavy	12/12	3.86(1.37-10.78)	7/6	4.51(1.15-17.8)	0.07
Daily folate consumption	<230 ug/day	21/37	1.0	21/26	1.42(0.60-3.36)	0.32
	230-300 ug/day	28/63	0.78(0.37-1.67)	33/64	0.91(0.43-1.91)	0.51
	>300 ug/day	19/63	0.53(0.24-1.19)	33/59	0.98(0.47-2.08)	0.09

consumption contributed synergy effect with the MTHFR 677T allele genotype on esophageal cancer susceptibility. Moreover, a significant interaction was found between the MTHFR 677T allele genotype and alcohol consumption.

We found significant association with MTHFR 677TT/CT genotypes for developing esophageal cancer. In previous published literatures, the role of MTHFR 677TT/CT genotypes for esophageal cancer risk was inconsistent (Sarbia et al., 2006; Wu et al., 2006; Langevin et al., 2009). A study conducted in Japan reported the MTHFR 677TT genotype showed a tendency for lowering esophageal cancer risk. Another study conducted in German was not observed a significant association between them. However, studies conducted in China reported the MTHFR 677TT genotype had 1.76 to 3.14 fold risk of developing esophageal cancer comparing with those with CC genotype (Song et al., 2001; He et al., 2007). There are two possible explanations: firstly, The prevalence of variant genotypes of MTHFR C677T polymorphisms varies to a great extent among different human population (Wilcken et al., 2003). The frequency of MTHFR 677TT in German is 13%(Zhang et al., 2004), whereas 1% in Africans, 2.2% in India and 15% in Japan(Yang et al., 2005; Umar et al., 2010). In our study, the frequency of MTHFR 677TT genotype in control of our study is 3.55%, which is in line with the previous studies from Chinese population (Wang et al., 2005; Wang et al., 2007). The variation of frequency of MTHFR C677T decides the difference in the association between MTHFR C677T polymorphism and esophageal cancer risk. Secondly, the difference in folate consumption among populations is related to the function of the MTHFR 677TT genotype. Most of the esophageal cancer patients in China are lived in poor rural areas, the nutrition of those people are usually lower than those in developed countries. Correspondingly, the insufficient folate consumption in Chinese esophageal cancer patients may alter DNA methylation, the inactive MTHFR 677T alllele may lower the 5-methyl THF to intensify the DNA hypomethylation, and initiate carcinogenesis.

Our study indicated there was significant positive interaction of MTHFR 677T allele with alcohol drinking, which is in line with studies conducted in China (Li et al., 2008; Qin et al., 2008). But another study in Japan showed MTHFR 677 variant genotype was significant inverse associated with esophageal cancer in moderate-heavy drinkers (Yang et al., 2005). Alcohol is recognized as a major risk factor for many cancers, including esophageal cancer, and it may induce DNA damage and resultant modification of nucleotides. When individuals consuming large amounts of alcohol, the inactive MTHFR 677T allele is expected to have low 5-methyl THF level, would make the DNA synthesis harder, and results in an increased risk of DNA damage and carcinogenesis.

Earlier studies showed interaction of MTHFR 677T allele and heavy tobacco usage (Weinstein et al., 2002; Boccia et al., 2007). Li et al. and Zhang et al. reported a significant association of MTHFR 677 variant genotype with heavy smokers (Zhang et al., 2003; Li et al., 2008). A report by Boccia et al. suggested the role of MTHFR C677T polymorphism in modulation of risk in gastric cancer patients with a smoking habit (Boccia et al., 2007), which is in line with our findings. Thus it is biologically plausible that smoking may be involved in the folate metabolism, and have a synergy role for esophageal cancer susceptibility by MTHFR 677TT genotype. A study in India found a significant but inverse association of MTHFR 677CT genotypes with a tobacco habit in development of esophageal cancer (Umar et al., 2010), which is inconsistence with our study. The reason might be the difference in the incidence of esophageal cancer in Northern India, which is much lower than that in China. Thus there may be genuine population-specific difference in the risk of esophageal cancer due to MTHFR C677T polymorphism.

Potential limitations of the present study should be considered. The present study may have recall bias to the questionnaire because all data were collected after diagnosis. But the recall bias could not be avoided in every case-control study, so we used method of reminding them habits refer to a year prior to diagnosis. Another methodological issue is selection of the population for controls. We selected non-cancer patients in hospital, which may induce selection bias. The notable point of our control population is its similarity to the general population. The frequencies of our controls are according to the Hardy-Weinberg Equilibrium. The small sample size is not enough to evaluate the interaction between gene-environment factors in our study, so further studies in a larger scale appear warranted.

In conclusion, our study indicates that the MTHFR C677T polymorphism is associated with the risk of esophageal cancer. Gene-environment interaction is found between alcohol consumption and MTHFR 677TT genotype. This study provides more information on the gene biomarker for prevention of esophageal cancer in Chinese population.

References

Bai JL, Zheng MH, Xia X, et al (2009). MTHFR C677T polymorphism contributes to prostate cancer risk among Caucasians: a meta-analysis of 3511 cases and 2762 controls. *Eur J Cancer*, **46**, 1443-9.

Bailey L B, Gregory JF (1999). 3rd Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: metabolic significance, risks and impact on folate requirement. *J Nutr*, **129**, 919-22.

Blount BC, Mack MM, Wehr CM, et al (2007). Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc Natl Acad Sci USA*, **94**, 3290-5.

Boccia S, Gianfagna F, Persiani R, et al (2007). Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and susceptibility to gastric adenocarcinoma in an Italian population. *Biomarkers*, **12**, 635-44.

Cao HX, Gao CM, Takezaki T, et al (2008). Genetic polymorphisms of methylenetetrahydrofolate reductase and susceptibility to colorectal cancer. *Asian Pac J Cancer Prev*, **9**, 203-8.

Chen HTK, Graubard BI, Heineman EF, et al (2002). Nutrient intakes and adenocarcinoma of the esophagus and distal stomach. *Nutr Cancer*, **42**, 33-40.

Choi SW, Mason JB (2000). Folate and carcinogenesis: an integrated scheme. *J Nutr*, **130**, 129-32.

Fang JY, Xiao SD (2003). Folic acid, polymorphism of methyl-group metabolism genes, and DNA methylation in relation to GI carcinogenesis. *J Gastroenterol*, **38**, 821-9.

Frosst P, Blom HJ, Milos R, et al (1995). A candidate genetic risk factor for vascular disease: a commonmutation inmethylenetetrahydrofolate reductase. *Nat Genet*, **10**, 111-3.

He Y, Wang Y, Zhang J, et al (2007). Correlation between a polymorphism in the methylene tetrahydrofolate reductase gene and susceptibility to carcinoma of the esophagus and gastric cardia. *Chinese J Clin Oncol*, **34**, 194-7.

Kim YI (2004). Folate and DNA methylation: a mechanistic link between folate deficiency and colorectal cancer? *Cancer Epidemiol Biomarkers Prev*, **1**3, 511-9.

Langevin SM, Lin D, Matsuo K, et al (2009). Review and pooled analysis of studies on MTHFR C677T polymorphism and esophageal cancer. *Toxicol Lett*, **184**, 73-80.

Leclerc D, Rozen R (2007). Molecular genetics of MTHFR: polymorphisms are not all benign. *Med Sci*, **23**, 297-302.

Li DQ, Diao YT, Li H, Fang XQ, Li HQ (2008). Association of the polymorphisms of MTHFR C677T, VDR C352T, and MPO G463A with risk for esophageal squamous cell dysplasia and carcinoma. *Arch Med Res*, **39**, 594-600.

Mayne ST, Risch HA, Dubrow R, et al (2001). Nutrient intake and risk of subtypes of esophageal and gastric cancer. *Cancer Epidemiol Biomarkers Prev*, **10**, 1055-62.

Miller SA, Dykes DD, Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*, **16**, 1215.

Qin JM, Yang L, Chen B, et al (2008). Interaction of methylenetetrahydrofolate reductase C677T, cytochrome P4502E1 polymorphism and environment factors in esophageal cancer in Kazakh population. *World J Gastroenterol*, **14**, 6986-6992.

Sarbia M, Stahl M, vonWeyhern C, Weirich G, Puhringer-Oppermann F (2006). 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*, **94**, 203-7.

Song C, Xing D, Tan W, Wei Q, Lin D (2001). Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. *Cancer Res*, **61**, 3272-5.

Stern LL, Mason JB, Selhub J, Choi SW (2000). 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*, **9**, 849-53.

Stolzenberg-Solomon RZ, Qiao YL, Abnet CC, et al (2003). Esophageal and gastric cardia cancer risk and folate- and vitamin B(12)-related polymorphisms in Linxian, China. *Cancer Epidemiol Biomarkers Prev*, **12**, 1222-6.

Suzuki T, Matsuo K, Hasegawa Y, et al (2007). One-carbon metabolism-related gene polymorphisms and risk of head and neck squamous cell carcinoma: case-control study. *Cancer Sci*, **98**, 1439-46.

Umar M, Upadhyay R, Khurana R, et al (2010). Evaluation of MTHFR677C>T polymorphism in prediction and prognosis of esophageal squamous cell carcinoma: a case-control study in a northern Indian population. *Nutr Cancer*, **62**, 743-9.

Wang LD, Guo RF, Fan ZM, et al (2005). 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*, **18**, 177-84.

Wang Y, Guo W, He Y, et al (2007). 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*, **18**, 143-52.

Weinstein SJ, Gridley G, Harty LC, et al (2002). Folate intake, serum homocysteine and methylenetetrahydrofolate reductase (MTHFR)C677T genotype are not associated with oral cancer risk in Puerto Rico. *J Nutr*, **132**, 762-7.

Wilcken B, Bamforth F, Li Z, et al (2003). Geographical and ethnic variation of the 677C>T allele of 5,10 methylenetetrahydrofolate reductase (MTHFR): findings from over 7000 newborns from 16 areas world wide. *J Med Genet*, **40**, 619-25.

Wu X, Gu J, Wu TT, et al (2006). Genetic variations in radiation and chemotherapy drug action pathways predict clinical outcomes in esophageal cancer. *J Clin Oncol*, **24**, 3789-3798.

Xing D, Tan W, Lin D (2003). Genetic polymorphisms and susceptibility to esophageal cancer among Chinese population (review). *Oncol Rep*, **10**, 1615-23.

Yang CS (2000). Vitamin nutrition and gastroesophageal cancer. *J Nutr*, **130**, 338S-9S.

Yang CX, Matsuo K, Ito H, et al (2005). 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*, **26**, 1285-90.

Yu MC, Yuan JM, Lu SC (2008). Alcohol, cofactors, and the genetics of hepatocellular carcinoma. *J Gastroenterol Hepatol*, **23**, S92–S97.

Zhang J, Zotz RB, Li Y, et al (2004). Methylenetetrahydrofolate reductase C677T polymorphism and predisposition towards esophageal squamouscell carcinoma in a German Caucasian and a northern Chinese population. *J Cancer Res Clin Oncol*, **130**, 574-80.

Zhang JH, Li Y, Guo Y, et al (2003). The association of methylenetetrahydrofolate reductaseC677T polymorphism and esophageal squamous cell carcinoma analyzed by LightCycler. *Prog Biochem Biophys*, **30**, 555-9.