RESEARCH ARTICLE

The Methylenetetrahydrofolate Reductase C677T Polymorphism Influences Risk of Esophageal Cancer in Chinese

Hong-Hong Qu[1,2&], Li-Hong Cui[4&], Ke Wang[1,2], Peng Wang[1,2,3], Chun-Hua Song[1,2,3], Kai-Juan Wang[1,2,3], Jian-Ying Zhang[1,2,3], Li-Ping Dai[1,2,3*]

Abstract

Methylenetetrahydrofolate reductase (*MTHFR*) plays a central role in folate metabolism. This study with 381 esophageal cancer patients and 432 healthy controls was conducted to examine the association of *MTHFR* C677T and A1298C polymorphisms with susceptibility to esophageal cancer (EC) in a Chinese population. Compared with the CC genotype of *MTHFR* C677T, subjects carrying homozygote TT and variant genotypes (CT+TT) demonstrated reduced risk of EC with adjusted ORs (95% CI) of 0.44 (0.28-0.71) and 0.57 (0.37-0.88), respectively. However, no association was found between the *MTHFR* A1298C polymorphism and the risk of EC. Comparing to haplotype CA, haplotypes TA and TC could reduce the susceptibility to EC with adjusted ORs (95% CI) of 0.61(0.47-0.79) and 0.06 (0.01-0.43), respectively. In conclusion, the present study suggested that the *MTHFR* C677T polymorphism can markedly influence the risk of EC in Chinese.

Keywords: Esophageal cancer - polymorphism - *MTHFR* - susceptibility - interaction

Asian Pacific J Cancer Prev, **14 (5)**, 3163-3168

Introduction

Esophageal cancer (EC) is a global health problem, and an estimated 482,300 new esophageal cancer cases and 406,800 deaths occurred in 2008 worldwide (Jemal et al., 2011). Its incidence rates vary internationally, and the highest rates found in Southern and Eastern Africa and Eastern Asia were nearly 16-fold, compared with lowest rates observed in Western and Middle Africa and Central America in both males and females (Jemal et al., 2011). In China, there are about 250,000 cases diagnosed yearly, contributing to about half of the world's cases (Yang et al., 2003).

Many studies demonstrated that the risk factors of EC included poor nutritional status, low intake of fruits and vegetables, cigarette smoking, alcohol drinking, and drinking beverages at high temperatures (Gao et al., 1994; Islami et al., 2009; Wu et al., 2009; Wu et al., 2009). Folate deficiency caused by low consumption of vegetables and fruits increase the risk of several cancers, including esophageal cancer (Jaskiewicz et al., 1988; Prasad et al., 1992; Giovannucci et al., 1995; Tseng et al., 1996). Providing methyl groups required for intracellular methylation reactions and de novo deoxynucleoside triphosphate synthesis is an important biological function of folate; therefore, folate deficiency is thought to be carcinogenic through disruption of DNA methylation and synthesis or impaired DNA repair (Choi et al., 2000). Besides inadequate folate intake, functional polymorphisms in folate-metabolizing genes may influence susceptibility to cancer.

Methylenetetrahydrofolate reductase (*MTHFR*), a key enzyme in folate metabolism, catalyzes the reduction of 5,10-methylene-tetrahydrofolate to 5-methyltetrahydrofolate (Bailey et al., 1999), the dominant circulating form of folate, which provides the methyl group with the ability to convert homocysteine to methionine (Banerjee et al., 1990). Two common functional polymorphisms of *MTHFR* gene, C677T and A1298C, have been identified, and the variant genotypes are associated with a significant reduction of enzyme activity (Frosst et al., 1995; Weisberg et al., 1998). Individuals with the *MTHFR* 677TT genotype have significantly lower plasma folate levels and higher plasma homocysteine levels than those with 677CC genotype (Larsson et al., 2006; Larsson et al., 2006). It also has been indicated that genomic DNA methylation is diminished in subjects with the *MTHFR* 677TT genotype, especially when folate status is low (Stern et al., 2000; Friso et al., 2002).

Several studies have indicated a substantial impact of the two polymorphisms, especially *MTHFR* C677T, on several types of cancer including colorectal cancer (Ma et al., 1997; Ma et al., 1997; Slattery et al., 1999), acute lymphocytic leukemia (Skibola et al., 1999), malignant lymphoma (Matsuo et al., 2001; Matsuo et al., 2004), and

*[1]Department of Epidemiology and Biostatistics, College of Public Health, [3]Proteomics Research Center, Zhengzhou University, [2]Henan Key Laboratory of Tumor Epidemiology, Zhengzhou, China, [4]Digestive System Department, PLA Navy General Hospital, Beijing, China [&]Equal contributors *For correspondence: lpdai@zzu.edu.cn*

gastric cancer (Gao et al., 2013) .

Currently, there are some researches concerning of the polymorphisms of *MTHFR* and the risk of esophageal cancer. However, the conclusions of these researches were inconsistent due to either small sample size or populations with mixed genetic backgrounds. Therefore, the present study was carried out to further investigate the association of *MTHFR* C677T and A1298C polymorphisms with esophageal cancer susceptibility in Henan Province, the highest incidence area of EC in China.

Materials and Methods

Study population

This study population included 381 EC patients and 432 healthy controls. All subjects were unrelated Chinese with Han ethnicity in Henan Province. Patients with newly diagnosed and histologically confirmed primary EC before undergoing any treatment were recruited at the First Affiliated Hospital of Zhengzhou University between March 2008 and January 2010. Healthy controls were recruited from a census of digestion diseases carried out in Xinxiang County and Xin'an County of Henan Province. The controls matched to cases on gender and age were required to be free of any digestion diseases, having no cancer history and related clinical signs.

Uniform trained interviewers used a special questionnaire to collect data from the study subjects on age, gender, tobacco smoking, alcohol drinking, family history of cancer, etc. The venous blood (5 ml) obtained from each study subject was collected in EDTA and stored at -70°C for extraction of genomic DNA. Smokers were defined as former or current individuals smoking at least one cigarette per day for at least one year. Alcohol intake was defined as drinking at least once a week with more than 100 gram every time for at least six months. The study was approved by the Ethics Committee of Zhengzhou University and the written informed consent was obtained from all study subjects.

Genotyping analysis

Genomic DNA was extracted from buffy coats using the DNA Blood Mini kit (TIANGEN BIOTECH (BEIJING) CO. , LTD) according to the manufacture's instructions.

MTHFR C677T genotyping was determined using the PCR-restriction fragment length polymorphism (PCR-RFLP) assay. The primers used for PCR amplification were 5'-ATCCCTCGCCTTGAACAG-3' (forward), and 5'-CAAACCCCTCAACAGACACT-3' (reverse). The PCR reaction was carried out in a 15 μl PCR mixture which contained 7.5 μl 2×Tap PCR MasterMix, 0.8 μM each primer, 50 ng DNA, 5.7 μl deionized water. The PCR reaction consisted of an initial denaturing step at 95°C for 5 min, 35 cycles of denaturing at 94°C for 30s, annealing at 58°C for 30s and elongation at 72°C for 45s, and a final extension step at 72°C for 5 min. The 5 μl PCR products were digested for 5-14 hours at 37°C in a 10 μl reaction volumn containing 1 μl 10×T buffer, 10 U HinfI restriction enzyme (Fermentas) and 3.5 μl deionized water. The digested products were separated by 3% agarose gel electrophoresis with ethidium bromide. The *MTHFR* 677 CC genotype (wild type) produced one 469bp fragment; the CT genotype (heterozygote) produced 469bp, 334bp and 135bp fragments; TT genotype (variant type) produced 334 and 135 bp fragments (Figure 1).

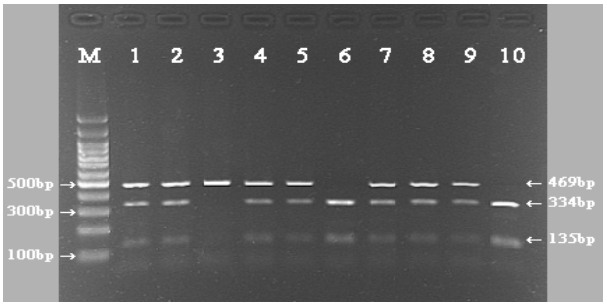

Figure 1. MTHFR C677T Genotypes. Lane M: 100bp DNA ladder; lane 6, 10: TT genotype; lane 1, 2, 4, 5 ,7, 8, 9: CT genotype; lane 3: CC genotype

Table 1. Demographical Characteristics of the Study Population

Variables	Case (%) n=381	Control (%) n=432	χ^2	*P*
Age				
<40	1.3	2.6		
40-	36.5	42.1		
≥60	62.2	55.3	4.51	0.11
Gender				
Male	67.2	64.4		
Female	32.8	35.6	0.72	0.39
Smoking status				
No-smokers	59.6	60.5		
Smokers	40.4	39.5	0.17	0.68
Drinking status				
Non-drinkers	80.7	81.9		
Drinkers	19.3	18.1	0.18	0.68
Family history of cancer				
Without	73.7	85.2		
With	26.3	14.8	14.96	0.00

The genotypes of *MTHFR* A1298C were analyzed by PCR-confronting two-pair primers (PCR-CTPP) as described in our previous report (Wang et al., 2011).

For quality control, genotyping was performed with blinding to case/control status, and a 10% random sample of subjects (n=82) was genotyped again by different persons, and the consistency was 100%. Each type of PCR products was randomly sequenced to ascertain the genotype.

Statistical analysis

χ^2 test was used to compare the frequency distributions of demographic variables, smoking status, drinking status, family history of cancer and *MTHFR* genotypes in the study groups. Unconditional logistic regression models were applied to calculate odds ratios (OR) and 95% confidence intervals (95%CI), which were adjusted by age, gender, smoking, drinking and family history of cancer. The Hardy–Weinberg equilibrium of the genotype frequency was assessed by χ^2 test in the control group. D' linkage disequilibrium (LD) value was calculated using Haploview4.0 software. Haplotypes were inferred by using the SNPHAP2.0 software. Gene–environment

Table 2. Frequency Distribution of *MTHFR* Polymorphisms and Association with Esophageal Cancer

Genotypes	Case N (%)	Control N (%)	*P*	OR (95%CI)	*P**	OR (95%CI)*
MTHFR 677						
CC	69(18.2)	47(11.1)		1.00		1.00
CT	181(47.9)	185(43.5)	0.06	0.67(0.44-1.02)	0.13	0.71(0.45-1.11)
TT	128(33.9)	193(45.4)	0.00	0.45(0.29-0.70)	0.00	0.44(0.28-0.71)
CC+TT	309(81.8)	378(89.9)	0.00	0.56(0.37-0.83)	0.01	0.57(0.37-0.88)
C	319(42.2)	279(32.8)		1.00		1.00
T	437(57.8)	571(67.2)	0.00	0.67(0.55-0.82)	0.00	0.66(0.53-0.82)
MTHFR 1298						
AA	273(71.6)	310(72.6)		1.00		1.00
AC	99(26.0)	104(24.4)	0.63	1.08(0.79-1.49)	0.89	1.03(0.72-1.46)
CC	9(2.4)	13(3.0)	0.58	0.79(0.33-1.87)	0.84	0.91(0.37-2.26)
AA+CC	108(28.4)	117(27.4)	0.76	1.05(0.77-1.43)	0.94	1.01(0.72-1.42)
A	645(84.6)	724(84.8)		1.00		1.00
C	117(15.4)	130(15.2)	0.94	1.01(0.77-1.33)	1.00	1.00(0.74-1.34)

*Adjusted for age, gender, smoking, drinking and family history of cancer

Table 3. Haplotype Analysis of *MTHFR* C677T and A1298C

Haplotypes	Case N (%)	Control N (%)	OR (95%CI)*	*P**
CA	206(27.0)	168(19.4)	1.00	
CC	116(15.2)	112(13.0)	0.81(0.56-1.15)	0.24
TA	439(57.6)	565(65.4)	0.61(0.47-0.79)	0.00
TC	1(0.1)	19(2.2)	0.06(0.01-0.43)	0.01

*Adjusted for age, gender, smoking, drinking and family history of cancer

interactions were evaluated by using Multifactor dimensionality reduction (MDR, V2.0 Beta 2). A detailed explanation about MDR has been provided elsewhere (Ritchie et al., 2001; Hahn et al., 2003). All analyses were performed using SPSS12.0 software. All tests were two sided and *P* values<0.05 were considered as statistically significant.

Results

Subjects characteristics

The demographical characteristics of the study population were showed in Table 1. There were no significant differences in the distribution of gender, age, smoking and alcohol drinking between case group and control group. However, the family history of cancer (*P*=0.00) had significant difference between case and control groups.

The observed genotype distributions of the *MTHFR* C677T and A1298C were consistent with Hardy–Weinberg equilibrium (χ^2=0.07, *P*=0.79 and χ^2=1.36, *P*=0.24) in the control group.

The relationship between MTHFR polymorphisms and esophageal cancer

For *MTHFR* C677T polymorphism, the frequencies of CC, CT and TT were 18.2%, 47.9% and 33.9% in cases, and 11.1%, 43.5% and 45.4% in controls, respectively (Table 2). There was statistically significant difference in frequency between cases and controls (χ^2=14.68, *P*<0.05). Compared with the CC genotype, the risk of esophageal cancer was reduced in individuals carrying homozygote TT and variant genotypes (CT+TT), the OR (95%CI) were 0.45 (0.29-0.70) and 0.56 (0.37-0.83), and the adjusted (for age, gender, smoking, drinking and family history of cancer) OR (95% CI) were 0.44 (0.28-0.71) and 0.57 (0.37-0.88). The frequencies of the C and T allele were 42.2%, 32.8% in cases, and 32.8%, 67.2% in controls. There was also statistically significant difference for allele frequency between the cases and controls (χ^2=15.04, *P*<0.05). And compared with the wild allele C, the variant allele T could reduce the susceptibility to esophageal cancer (adjusted OR: 0.66, 95% CI: 0.53-0.82).

For *MTHFR* A1298C, genotype frequencies for AA, AC and CC were 71.6%, 26.0% and 2.4% in cases and 72.6%, 24.4%, and 3.0% in controls (Table 2).The distributions of genotypes were not statistically significant different between the case and control group (χ^2=0.58, *P*=0.75). Compared with the genotype AA, the risk of esophageal cancer was not increased for individuals carrying AC, CC and variant genotypes (AC+CC) with adjusted OR (95% CI): 1.03(0.72-1.46), 0.91(0.37-2.26) and 1.01(0.72-1.42), respectively.

Haplotype analysis

The linkage disequilibrium and haplotype of *MTHFR* C677T and A1298C polymorphisms were examined, given their close relation in a chromosome location. The analysis indicated strong linkage disequilibrium between the two loci (D'=0.88). Table 3 showed totally four possible haplotypes (CA, CC, TA, TC). Comparing to haplotype CA with both wild allele, haplotypes TA and TC which contain the variant T allele of polymorphism C677T could reduce the susceptibility to esophageal cancer. The adjusted OR (95% CI) were 0.61(0.47-0.79) and 0.06(0.01-0.43), respectively.

Combined genotypes analysis of MTHFR C677T and A1298C

There were totally nine combined genotypes of *MTHFR* C677T and A1298C, which were shown in Table 4. The combined wild genotype CC/AA was taken as the reference, there were significant differences in the distribution of frequencies for CT/AA, CT/AC and TT/AA, which contain minor allele T of C677T, between case and control groups, and the adjusted OR (95% CI) were 0.48 (0.24-0.98), 0.46 (0.22-0.98) and 0.31 (0.15-0.62),

Table 4. Combination Analysis of *MTHFR* C677T and A1298C Polymorphisms

Combined genotypes	Case N (%)	Control N (%)	*P*	OR (95%CI)	*P**	OR (95%CI)*
CC/AA	34(9.0)	15(3.5)		1.00		1.00
CC/AC	28(7.4)	24(5.7)	0.11	0.51(0.23-1.16)	0.14	0.52(0.22-1.25)
CC/CC	7(1.9)	7(1.7)	0.18	0.44(0.13-1.48)	0.27	0.49(0.14-1.76)
CT/AA	110(29.1)	113(26.7)	0.01	0.43(0.22-0.83)	0.04	0.48(0.24-0.98)
CT/AC	70(18.5)	66(15.6)	0.03	0.47(0.23-0.94)	0.04	0.46(0.22-0.98)
CT/CC	1(0.3)	6(1.4)	0.02	0.07(0.01-0.67)	0.05	0.11(0.01-0.97)
TT/AA	128(33.9)	180(42.5)	0.00	0.31(0.16-0.60)	0.00	0.31(0.15-0.62)
TT/AC	0(0.0)	13(3.1)	0.53	-	-	-
TT/CC	0(0.0)	0(0.0)	-	-	-	-

*Adjusted for age, gender, smoking, drinking and family history of cancer

Table 5. MDR Models of Selected Polymorphisms and Environmental Factors

Models	TBA	CVC	OR (95%CI)	*P*
FHC	0.6074	10/10	3.02(2.19-4.18)	<0.0001
FHC, C677T	0.6126	8/10	3.06(2.27-4.12)	<0.0001
FHC, smoking, C677T	0.6086	7/10	3.74(2.74-5.10)	<0.0001
FHC, smoking, C677T, A1298C	0.6104	10/10	4.18(3.08-5.69)	<0.0001

TBA, testing balance accuracy; CVC, cross-validation consistency, FHC: familial history of cancer

suggesting that these combined genotypes could reduce the risk of esophageal cancer.

Gene-environment interaction analysis

MDR acts by reducing multiple variables, attributes into a single, binary attribute with ''low risk'' and ''high risk'' categories. The specifics of the algorithm used have been described elsewhere (Ritchie et al., 2001; Hahn et al., 2003). Analysis was performed with *MTHFR* polymorphisms (C677T and A1298C), smoking, drinking and family history of cancer. The results of MDR analysis were shown in Table 5. The best model consisted of four factors (family history of cancer, smoking, C677T and A1298C) with testing balance accuracy (TBA): 0.6104 and cross validation consistency (CVC): 10/10, which could increase the EC risk in the "high risk group" with 4.18-fold (OR: 4.18, *P*<0.0001, 95% CI: 3.08-5.69), compared to the "low risk group".

Discussion

There have been several studies showing that the *MTHFR* 677T allele could increase the esophageal cancer risk (Song et al., 2001; Zhang et al., 2004; Wang, 2005; Wang, 2007), while no risk change has been observed among the other studies (Wu et al., 2002; Wu et al., 2002; Stolzenberg-Solomon et al., 2003). However, the present study was the first report suggesting the *MTHFR* 677T allele could decrease the risk of EC, which was consistent with the result of Nelofar Kureshi et al. (Kureshi et al., 2004; Kureshi et al., 2004), who have shown that C allele was associated with susceptibility to head and neck cancer, although their results did not reach statistical significance. Yang et al. (2005) found high folate consumption and the *MTHFR* 677TT genotype to be associated with a non-significant decrease in the risk of esophageal cancer. The association of *MTHFR* 677T allele with malignancy was best studied in colon cancer. Huang et al. (2007) performed a meta-analysis, which has suggested the 677T allele showed a small but significant protective effect against colorectal cancer compared to the 677C allele for a worldwide population. There may be a possible explanation, the cancer risk associated with *MTHFR* polymorphisms depending on the level of folate intake, which has been suggested in the studies on colorectal cancer (Chen et al., 1996; Ma et al., 1997). Therefore, based on this hypothesis, when folate intake is sufficient, individuals with the *MTHFR* CT or TT genotypes may have a reduced risk of cancer, since decrease in *MTHFR* activity due to 677T allele might lead to an elevation in 5,10-methylene-tetrahydrofolate, which would facilitate DNA synthesis and diminish DNA damage, while adequate provision of methyl donors could still be ensured. However, under the condition of low folate intake, both impaired DNA methylation and DNA synthesis/repair may become the primary mechanisms of carcinogenesis. Although, there were not data for folate intake in the present study, the result might be consistent with this hypothesis.

For the *MTHFR* A1298C polymorphism, the present study was consisted with Stolzenberg et al. (2003), who did not find it associated with susceptibility to esophageal cancer, while Song et al. (2001) reported a significant 4.43-fold increased EC risk with the *MTHFR* 1298CC genotype. There was a small number of subjects with the CC genotype in each study, which limited the conclusions of any results. The variant *MTHFR* A1298C genotypes clearly reduce *MTHFR* activity, albeit to a lesser extent than the C677T variant genotypes. Consequently its effect on homocysteine levels is also attenuated and, in fact, may only be significant when an individual carries both variants and/or has poor nutrient status (Weisberg et al., 1998).

The analysis of haplotype and combined genotypes supplied a greater amount of information than a single SNP. The association between the haplotype of *MTHFR* C677T and A1298C polymorphisms and the susceptibility to EC was not reported previously. The present study found the TA and TC haplotypes decreased the risk of esophageal cancer, with adjusted OR (95%CI) of 0.61 (0.47-0.79) and 0.06 (0.01-0.43), respectively. Song et al. (Song et al., 2001) reported a joint effect between *MTHFR* 677CT and 1298CC variant genotypes on rising risk of ESCC. However, in this study, the combined genotypes CT/AA, CT/AC, and TT/AA diminished the risk of EC as compared with *MTHFR* 677CC/1298AA genotype, which was in line with Mastuo et al. (2004), who determined that possible

hypomethylation inducible alleles (*MTHFR* 677T, 1298C) were protective for malignant lymphoma. Although the *MTHFR* C677T polymorphism was associated with significant reduced risk and the A1298C polymorphism showed no effect, when they were separately analyzed. For this inconsistency, there was a possible explanation that phenotypic effect of A1298C may be limited compared with the C677T polymorphism. Yamada et al. (2001) previously verified that the recombinant human *MTHFR* activity in vitro to be more strongly influenced by C677T than A1298C.

Esophageal cancer is a complex disease likely resulting from multiple interacting genetic polymorphisms and gene-environment interactions. Some earlier studies have shown an interaction of *MTHFR* C677T polymorphism with environmental risk factors such as smoking and alcohol (Weinstein et al., 2002; Yang et al., 2005; Boccia et al., 2007). However, this study performed the analysis of gene–environment interaction by MDR method, and found a best model including familial history of cancer, smoking and *MTHFR* polymorphisms (C677T and A1298C). High- and low-risk groups were generated by MDR and the OR value was 4.18 (95% CI: 3.08-5.69).

The limitation of this study was nonavailability of folate levels in study subjects. Because in the presence of low folate intake, both impaired DNA methylation and DNA synthesis/repair may become the primary mechanism of carcinogenesis in those who had the variant *MTHFR* genotypes. However, it has been suggested that in those with the variant *MTHFR* 677T allele, decreased risk of colorectal neoplasia was observed among subjects with adequate folate levels (Chen et al., 1996; Ma et al., 1997). Therefor the result of the present study was reliable.

In conclusion, the current study suggested that *MTHFR* C677T polymorphism could reduce the risk of EC, and *MTHFR* A1298C was not associated with the risk of EC. However, the haplotype and combined genotypes of *MTHFR* C677T and A1298C could reduce the risk of esophageal cancer. In addition, this study showed an interaction in *MTHFR* polymorphisms, smoking and familial history of cancer.

Acknowledgements

This study was supported by grants from National Natural Science Foundation of China (No: 30872181, 81072360) and Program for Science & Technology Innovation Talents in Universities of Henan Province (No:2010HASTIT027). We acknowledge the blood samples of cases given by the First Affiliated Hospital of Zhengzhou University. We also wish to acknowledge the residents in Xinxiang County and Xin'an County of Henan Province support our epidemiological study on the scene. The author(s) declare that they have no competing interests.

References

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

Banerjee RV, Matthews RG (1990). Cobalamin-dependent methionine synthase. *FASEB J*, **4**, 1450-9.

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.

Chen J, Giovannucci E, Kelsey K, et al (1996). A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res*, **56**, 4862-4.

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

Friso S, Choi SW, Girelli D, et al (2002). A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *P Natl Acad Sci USA*, **99**, 5606-56.

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

Gao S, Ding LH, Wang JW, et al (2013). Diet folate, DNA methylation and polymorphisms in methylenetetrahydrofolate reductase in association with the susceptibility to gastric cancer. *Asian Pac J Cancer Prev*, **14**, 299-302.

Gao YT, McLaughlin JK, Blot WJ, et al (1994). Risk factors for esophageal cancer in Shanghai, China. I. Role of cigarette smoking and alcohol drinking. *Int J Cancer*, **58**, 192-6.

Giovannucci E, Rimm EB, Ascherio A, et al (1995). Alcohol, low-methionine--low-folate diets, and risk of colon cancer in men. *J Natl Cancer Inst*, **87**, 265-73.

Gao YT, McLaughlin JK, Gridley G, et al (1994). Risk factors for esophageal cancer in Shanghai, China. II. Role of diet and nutrients. *Int J Cancer*, **58**, 197-202.

Hahn LW, Ritchie MD, Moore JH (2003). Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. *Bioinformatics*, **19**, 376-82.

Huang Y, Han S, Li Y, et al (2007). Different roles of *MTHFR* C677T and A1298C polymorphisms in colorectal adenoma and colorectal cancer: a meta-analysis. *J Hum Genet*, **52**, 73-85.

Islami F, Boffetta P, Ren JS, et al (2009). High-temperature beverages and foods and esophageal cancer risk—A systematic review. *Int J Cancer,* **125**, 491-524.

Jaskiewicz K, Marasas WF, Lazarus C, et al (1988). Association of esophageal cytological abnormalities with vitamin and lipotrope deficiencies in populations at risk for esophageal cancer. *Anticancer Res*, **8**, 711-5.

Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.

Kureshi N, Ghaffar S, Siddiqui S, et al (2004). Head and neck cancer susceptibility: a genetic marker in the methylenetetrahydrofolate reductase gene. *ORL J Otorhinolaryngol Relat Spec*, **66**, 241-5.

Larsson SC, Giovannucci E, Wolk A (2006). Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis. *Gastroenterology*, **131**, 1271-83.

Ma J, Stampfer MJ, Giovannucci E, et al (1997). Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Res*, **57**, 1098-102.

Matsuo K, Hamajima N, Suzuki R, et al (2004). Methylenetetrahydrofolate reductase gene (*MTHFR*) polymorphisms and reduced risk of malignant lymphoma. *Am J Hematol*, **77**, 351-7.

Matsuo K, Suzuki R, Hamajima N, et al (2001). Association between polymorphisms of folate- and methionine-

metabolizing enzymes and susceptibility to malignant lymphoma. *Blood*, **97**, 3205-9.
Prasad MP, Krishna TP, Pasricha S, et al (1992). Esophageal cancer and diet-a case-control study. *Nutr Cancer*, **18**, 85-93.
Ritchie MD, Hahn LW, Roodi N, et al (2001). Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. *Am J Hum Genet*, **69**, 138-47.
Skibola CF, Smith MT, Kane E, et al (1999). Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. *P Natl Acad Sci USA*, **96**, 12810-5.
Slattery ML, Potter JD, Samowitz W, et al (1999). Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev*, **8**, 513-8.
Song C, Xing D, Tan W, et al (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, et al (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.
Tseng M, Murray SC, Kupper LL, et al (1996). Micronutrients and the risk of colorectal adenomas. *Am J Epidemiol*, **144**, 1005-14.
Weisberg I, Tran P, Christensen B, et al (1998). A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab*, **64**, 169-72.
Wang K, Zhang JT, Yun YX, et al (2011). Improvement on PCR-CTPP: a SNP genotyping approach based on mismatch technique. *HEREDITAS*, **33**, 182-8.
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.
Wu JZ, Gao CM, Ding JH, et al (2002). Polymorphisms of methylenetetrahydrofolate reductase C677T and the risk of esophageal cancer. *Tumor*, **22**, 268-70.
Wu M, Liu AM, Kampman E, et al (2009). Green tea drinking, high tea temperature and esophageal cancer in high- and low-risk areas of Jiangsu Province, China: A population-based case–control study. *Int J Cancer*, **124**, 1907-13.
Yamada K, Chen Z, Rozen R, et al (2001). Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. *P Natl Acad Sci USA*, **98**, 14853-8.
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.
Yang L, Parkin DM, Li L, et al (2003). Time trends in cancer mortality in China: 1987–1999. *Int J Cancer*, **106**, 771-83.
Zhang J, Zotz RB, Li Y, et al. (2004). 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*, **130**, 574-80.