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ORIGINAL PAPER

Genetic variations in *MTHFR* and esophageal squamous cell carcinoma susceptibility in Chinese Han population

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Abstract Esophageal cancer is the sixth most common cancer worldwide. Esophageal squamous cell carcinoma (ESCC) is a fatal malignancy associated with low 5-year survival rate. The aim of this study was to assess the association between methylenetetrahydrofolate reductase (*MTHFR*) tagging single nucleotide polymorphisms (SNPs) rs1801133 C>T, rs3753584 A>G, rs4845882 G>A, rs4846048 A>G and rs9651118 T>C genotypes and ESCC susceptibility in a hospital-based case–control study. We conducted genotyping analyses for these five SNPs with 629 ESCC cases and 686 controls in a Chinese Han population. Ligation detection reaction method was used to identify genotypes of these *MTHFR* SNPs. Our results demonstrated that *MTHFR* rs1801133 C>T was associated with the risk of ESCC; however, *MTHFR* rs4845882 G>A and rs4846048 A>G SNPs were associated with the decreased risk of ESCC, and *MTHFR* rs3753584 A>G and rs9651118 T>C SNPs were not associated with ESCC risk. Our findings suggests that *MTHFR* rs1801133 C>T, rs4845882 G>A and rs4846048 A>G SNPs may be genetic modifiers for developing ESCC in Chinese Han population.

Keywords *MTHFR* · Polymorphism · Esophageal cancer · Cancer susceptibility

Abbreviations

CI	Confidence interval
OR	Odds ratio
MTHFR	Methylenetetrahydrofolate reductase
HWE	Hardy–Weinberg equilibrium
ESCC	Esophageal squamous cell carcinoma
PCR-LDR	Polymerase chain reaction–ligase detection reaction
SNP	Single nucleotide polymorphism

Introduction

Esophageal cancer (EC) is the sixth most common cancer with an estimated 482,300 new cases and 406,800 deaths occurred in 2008 worldwide [1]. Incidence rates of EC vary internationally by almost 16-fold, with 22.14 per 10,000 in China in 2009 [1, 2]. Every year there are about 250,000 EC cases diagnosed in China, accounting for half of the global cases [3, 4]. The mortality rate for EC patients is very high, and 5-year survival rate accounts only 12.3 % [5]. Esophageal squamous cell carcinoma (ESCC) is the most frequent subtype of EC and a lethal malignancy associated with low survival rates. In the highest risk area such as Iran and China, 90 % of cases are ESCC histologically [6].

Many epidemiological studies indicated that the risk factors for ESCC involved poor nutritional status, low fruits and

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vegetables consumption, smoking, heavy alcohol use and drinking hot beverages [7–11]. However, only a fraction of individuals who are exposed in risk factors ultimately develop EC, suggesting that genetic factors such as single nucleotide polymorphisms (SNPs) may play an important role in developing EC [12–14]. Methylenetetrahydrofolate reductase (MTHFR), a key enzyme of methylation, is located on 1p36.3. It is a methyl donor and catalyzes reduction of 5,10-methylenetetrahydrofolate to 5-methyltetra hydrofolate [15]. Functional polymorphisms of *MTHFR* may lead to attenuate of 5-methyl tetrahydrofolic acid (THFA) to induce a decrease in the conversion of homocysteine to methionine, which could result in a carcinogenesis [16]. There were more than 20 kinds of genetic polymorphisms of *MTHFR*; some non-synonymous SNPs were the most studies genetic polymorphisms. An impact of these polymorphisms on ECs has been performed. However, the association between the *MTHFR* SNPs and EC was inconsistence. According to the biological significance of *MTHFR*, it is possible that functional SNPs in the gene may contribute to the development of ESCC. The aim of this study was to assess the association between *MTHFR* rs1801133 C>T, rs3753584 A>G, rs4845882 G>A, rs4846048 A>G and rs9651118 T>C genotypes and ESCC susceptibility. In a hospital-based case–control study, we conducted genotyping analyses for the five SNPs with 629 ESCC cases and 686 controls in Chinese Han population.

Materials and methods

Subjects

In total, 629 from unrelated Chinese Han ESCC patients and 686 cancer-free subjects were consecutively recruited from the Affiliated People's Hospital of Jiangsu University and Affiliated Hospital of Jiangsu University (Jiangsu Province, China) between October 2008 and December 2010. Diagnoses of all ESCC cases were confirmed by postoperative pathologic means. The ESCC patients who formerly had a history of cancer or autoimmune diseases, or had undergone radiotherapy or chemotherapy were removed. Ethnicity (Chinese), frequency of sex and average age (±5 years) of the 686 controls were group matched to cases. The majority of the control individuals were admitted to the two hospitals for the cure of trauma. At recruitment, this study was approved by the Institutional Review Board of Jiangsu University (Zhenjiang, China) and written informed consent was obtained from each subject. Two experienced research doctors were assigned to administer a structured questionnaire to each subject. The information collected included demographic data (e.g., age and gender) and related risk factors (including tobacco use and alcohol consumption). After completed the in-person interview, each participant donated 2-ml sample of peripheral venous blood. Subjects who smoked at least one cigarette per day over 1 year were defined as "smokers," and those who drinked no less than three times a week for >6 months were considered to be "alcohol drinkers".

DNA extraction, SNP selection and genotyping

Ethylenediamine tetraacetic acid (EDTA)-anticoagulated peripheral venous blood sample was collected from each subject, and genomic DNA was isolated using the QIAamp DNA Blood Mini Kit (Qiagen, Berlin, Germany). *MTHFR* tagging SNPs were chosen based on the HapMap Project (http://www.hapmap.org/, phase II Nov08, on NCBI B36 assembly, dbSNP b126; population: Chinese Han population, CHB; minor allele frequency (MAF) ≥0.05, Hardy–Weinberg equilibrium (HWE) $P \geq 0.05$ and call rate ≥95 %) on the basis of pairwise linkage disequilibrium (LD) r^2 threshold of 0.8. with Haploview 4.2 software [17]. Genotypes of *MTHFR* at the rs1801133 C>T, rs3753584 A>G, rs4845882 G>A, rs4846048 A>G and rs9651118 T>C sites were analyzed by using the ligation detection reaction (LDR) method [18]. Technical support was come from the Shanghai Biowing Applied Biotechnology Company. For quality control, 160 (12.17 %) randomly selected samples were repeated analysis by LDR method with high DNA quality and the accordance rates were 100 %.

Statistical analysis

Chi-square test (χ^2) was used to examine the differences in the distributions of demographic characteristics, selected variables and genotypes between cases and controls. The associations between *MTHFR* rs1801133 C>T, rs3753584 A>G, rs4845882 G>A, rs4846048 A>G and rs9651118 T>C genotypes and the risk of ESCC were evaluated by odds ratios (ORs) and their 95 % confidence intervals (CIs) using unconditional logistic regression analyses for crude ORs and adjusted ORs when appropriate. An internet-based HWE calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) was used to assess the HWE among the control subjects. Statistical analysis was performed by SAS 9.1.3 software (SAS Institute, Cary, NC). Statistical significance was defined as $P < 0.05$ (two-tailed) for all statistical analyses.

Results

Characteristics of the study population

Characteristic of all subjects is presented in Table 1. There were no significant differences between patients and controls in terms of age distributions and sex distributions

Table 1 Distribution of selected demographic variables and risk factors in ESCC case subjects and control subjects

Variable	Cases ($n = 629$)		Controls ($n = 686$)		P[a]
	n	%	*n*	%	
Age (years) mean ± SD	62.85 (±8.13)		62.58 (±7.89)		0.541
Age (years)					0.155
<63	310	49.28	365	53.21	
≥63	319	50.72	321	46.79	
Sex					0.185
Male	444	70.59	461	67.20	
Female	185	29.41	225	32.80	
Tobacco use					**<0.001**
Never	355	56.44	499	72.74	
Ever	274	43.56	187	27.26	
Alcohol use					**<0.001**
Never	428	68.04	526	76.68	
Ever	201	31.96	160	23.32	

[a] Two-sided χ^2 test and Student's *t* test; bold values are statistically significant ($P < 0.05$)

($P = 0.155$ and $P = 0.185$, respectively), which indicated the matching was adequate. However, significant difference was detected on drinking status and smoking rate between patients and controls ($P < 0.001$). The primary information of five tagging SNPs of *MTHFR* was included in Table 2. For these five SNPs, the genotyping success rate ranged from 96.43 to 98.48 % in all 1,315 samples. In this study, MAF of control subjects was similar to that for Chinese in database for all these five SNPs (Table 2). The genotypic frequencies for *MTHFR* rs3753584 A>G, rs4846048 A>G and rs9651118 T>C polymorphisms among controls were in HWE ($P = 0.648$, $P = 0.312$ and $P = 0.344$) except *MTHFR* rs1801133 C>T and rs4845882 G>A ($P = 0.045$ and $P = 0.029$) (Table 2).

MTHFR polymorphisms and the risk of ESCC

The genotype distributions of *MTHFR* rs1801133 C>T, rs3753584 A>G, rs4845882 G>A, rs4846048 A>G and rs9651118 T>C in cases and controls were presented in Table 3. In the single locus analyses, the genotype frequencies of *MTHFR* rs1801133 C>T were 30.57 % (CC),

Table 2 Primary information for *MTHFR* rs1801133 C>T, rs3753584 A>G, rs4845882 G>A, rs4846048 A>G and rs9651118 T>C polymorphisms

Genotyped SNPs	*MTHFR* rs1801133 C>T	*MTHFR* rs3753584 A>G	*MTHFR* rs4845882 G>A	*MTHFR* rs4846048 A>G	*MTHFR* rs9651118 T>C
Chromosome	1	1	1	1	1
Function	Missense	Neargene-5	Intron	Intron	Intron
Chr Pos (genome build 36.3)	11778965	11787173	11765754	11768839	11784801
Regulome DB score[a]	4	4	1f	3a	5
TFBS	–	Y	–	–	Y
Splicing (ESE or ESS)	–	Y	–	–	–
miRNA (miRanda)	–	–	–	Y	–
nsSNP	Y	–	–	–	–
MAF for Chinese in database	0.439	0.093	0.198	0.105	0.382
MAF in our controls ($n = 686$)	0.446	0.064	0.179	0.105	0.350
P value for HWE					
Test in our controls	0.045	0.648	0.029	0.312	0.344
Genotyping method	LDR	LDR	LDR	LDR	LDR
% Genotyping value	96.43	96.43	98.48	98.17	96.43

TFBS transcription factor binding site (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm); *MAF* minor allele frequency; *HWE* Hardy–Weinberg equilibrium; *LDR* ligation detection reaction

[a] http://www.regulomedb.org/

Table 3 Logistic regression analyses of associations between *MTHFR* polymorphisms and the risk of ESCC

Genotype	Cases ($n = 629$)		Controls ($n = 686$)		Crude OR (95 % CI)	*P*	Adjusted OR[a] (95 % CI)	*P*
	n	%	*n*	%				
MTHFR rs1801133 C>T								
CC	188	30.57	188	28.79	1.00		1.00	
CT	288	46.83	348	53.29	0.83 (0.64–1.07)	0.147	0.79 (0.61–1.03)	0.082
TT	139	22.60	117	17.92	1.19 (0.86–1.63)	0.289	1.21 (0.87–1.67)	0.259
CT + TT	427	69.43	465	71.21	0.92 (0.72–1.17)	0.488	0.90 (0.70–1.15)	0.382
CC + CT	476	77.40	536	82.08	1.00		1.00	
TT	139	22.60	117	17.92	**1.34 (1.02–1.76)**	**0.038**	**1.39 (1.05–1.84)**	**0.021**
T allele	566	46.02	582	44.56				
MTHFR rs3753584 A>G								
AA	538	87.48	571	87.44	1.00		1.00	
AG	74	12.03	80	12.25	0.98 (0.70–1.38)	0.915	0.94 (0.66–1.32)	0.704
GG	3	0.49	2	0.31	1.59 (0.27–9.56)	0.611	1.79 (0.29–10.95)	0.529
AG + GG	77	12.52	82	12.56	1.00 (0.72–1.39)	0.984	0.96 (0.68–1.34)	0.791
AA + AG	612	99.51	651	99.69	1.00		1.00	
GG	3	0.49	2	0.31	1.60 (0.27–9.58)	0.609	1.80 (0.30–11.03)	0.524
G allele	80	6.50	84	6.43				
MTHFR rs4845882 G>A								
GG	440	71.08	464	68.64	1.00		1.00	
GA	167	26.98	182	26.92	0.97 (0.76–1.24)	0.794	0.96 (0.75–1.24)	0.748
AA	12	1.94	30	4.44	**0.42 (0.21–0.84)**	**0.013**	**0.41 (0.20–0.82)**	**0.011**
GA + AA	179	28.92	212	31.36	0.89 (0.70–1.13)	0.340	0.88 (0.69–1.12)	0.304
GG + GA	607	98.06	646	95.56	1.00		1.00	
AA	12	1.94	30	4.44	**0.43 (0.22–0.84)**	**0.014**	**0.41 (0.21–0.82)**	**0.012**
A allele	191	15.43	242	17.90				
MTHFR rs4846048 A>G								
AA	514	84.12	547	80.44	1.00		1.00	
AG	94	15.38	123	18.09	0.81 (0.61–1.09)	0.169	0.85 (0.63–1.14)	0.271
GG	3	0.49	10	1.47	0.32 (0.09–1.17)	0.084	**0.26 (0.07–0.96)**	**0.044**
AG + GG	97	15.88	133	19.56	0.78 (0.58–1.04)	0.085	0.79 (0.59–1.07)	0.125
AA + AG	608	99.51	670	98.53	1.00		1.00	
GG	3	0.49	10	1.47	0.33 (0.09–1.21)	0.094	**0.27 (0.07–0.99)**	**0.048**
G allele	100	8.18	143	10.51				
MTHFR rs9651118 T>C								
TT	256	41.90	272	41.40	1.00		1.00	
TC	277	45.34	310	47.18	0.95 (0.75–1.20)	0.666	0.92 (0.73–1.17)	0.517
CC	78	12.77	75	11.42	1.11 (0.77–1.58)	0.586	1.09 (0.76–1.58)	0.632
TC + CC	355	58.10	385	58.60	0.98 (0.78–1.23)	0.857	0.96 (0.76–1.20)	0.704
TT + TC	533	87.23	582	88.58	1.00		1.00	
CC	78	12.77	75	11.42	1.14 (0.81–1.59)	0.461	1.14 (0.81–1.61)	0.454
C allele	433	35.43	460	35.01				

[a] Adjusted for age, sex, smoking and drinking status; bold values are statistically significant ($P < 0.05$)

46.83 % (CT) and 22.60 % (TT) in the case subjects and 28.79 % (CC), 53.29 % (CT) and 17.92 % (TT) in the control subjects, and the difference was statistically significant ($P = 0.04$). When the *MTHFR* rs1801133 CC homozygote genotype was used as the reference group, the CT genotype was not associated with the risk of ESCC (CT vs. CC: OR 0.83, 95 % CI 0.64–1.07, $P = 0.147$) and the TT genotype was not associated with the risk of ESCC (TT vs. CC: OR 1.19, 95 % CI 0.86–1.63, $P = 0.289$). In the recessive model, when the *MTHFR* rs1801133 CC/CT genotypes were

used as the reference group, the TT homozygote genotype was associated with the risk of ESCC (TT vs. CC/CT: OR 1.34, 95 % CI 1.02–1.76, $P = 0.038$). In the dominant model, the *MTHFR* rs1801133 CT/TT variants were not associated with the risk of ESCC, compared with the *MTHFR* rs1801133 CC genotype (CT/TT vs. CC: OR 0.92, 95 % CI 0.72–1.17, $P = 0.488$) (Table 3). After adjusting for age, gender, smoking and drinking status, a statistically increased risk of ESCC was also observed in the recessive model (TT vs. CC/CT: adjusted OR 1.39, 95 % CI 1.05–1.84, $P = 0.021$) (Table 3).

The genotype frequencies of *MTHFR* rs4845882 G>A were 71.08 % (GG), 26.98 % (GA) and 1.94 % (AA) in the case subjects and 68.64 % (GG), 26.92 % (GA) and 4.44 % (AA) in the controls, and the difference was statistically significant ($P = 0.039$). When the *MTHFR* rs4845882 GG homozygote genotype was used as the reference group, the GA genotype was not associated with the risk of ESCC (GA vs. GG: OR 0.97, 95 % CI 0.76–1.24, $P = 0.974$). The AA genotype was associated with the decreased risk of ESCC (AA vs. GG: OR 0.42, 95 % CI 0.21–0.84, $P = 0.013$). In the recessive model, when the *MTHFR* rs4845882 GG/GA genotypes were used as the reference group, the AA homozygote genotype was associated with the decreased risk of ESCC (AA vs. GG/GA: OR 0.43, 95 % CI 0.22–0.84, $P = 0.014$). In the dominant model, the *MTHFR* rs4845882 GA/AA variants were not associated with the risk of ESCC, compared with the *MTHFR* rs1801133 GG genotype (GA/AA vs. GG: OR 0.89, 95 % CI 0.70–1.13, $P = 0.340$) (Table 3). After adjusting for age, gender, smoking and drinking status, a statistically decreased risk of ESCC was observed both in the homozygote comparing model (AA vs. GG: adjusted OR 0.41, 95 % CI 0.20–0.82, $P = 0.011$) and in recessive model (AA vs. GG/GA: adjusted OR 0.41, 95 % CI 0.21–0.82, $P = 0.012$) (Table 3).

The genotype frequencies of *MTHFR* rs4846048 A>G were 84.12 % (AA), 15.38 % (AG) and 0.49 % (GG) in the cases and 80.44 % (AA), 18.09 % (AG) and 1.47 % (GG) in the controls, and the difference was not statistically significant ($P = 0.082$). Logistic regression analyses revealed that the *MTHFR* rs4846048 A>G polymorphisms was not associated with the risk of ESCC. After adjusting for age, gender, smoking and drinking status, the results showed that the *MTHFR* rs4846048 A>G polymorphisms were associated with the decreased risk of ESCC in homozygote comparing model (GG vs. AA: adjusted OR 0.26, 95 % CI 0.07–0.96, $P = 0.044$) and in recessive model (GG vs. AA/AG: adjusted OR 0.27, 95 % CI 0.07–0.99, $P = 0.048$) (Table 3).

MTHFR rs3753584 A>G and *MTHFR* rs9651118 T>C SNPs did not achieved significant differences in the genotype distributions between patients and controls ($P = 0.871$ and $P = 0.694$) (Table 3). Logistic regression analyses revealed that the *MTHFR* rs3753584 A>G and *MTHFR* rs9651118 T>C polymorphisms were not associated with the risk of ESCC (Table 3).

Discussion

In this hospital-based case–control study, we investigated the associations of *MTHFR* rs1801133 C>T, rs3753584 A>G, rs4845882 G>A, rs4846048 A>G and rs9651118 T>C SNPs with the risk of ESCC in Chinese Han population. Our results revealed that *MTHFR* rs1801133 C>T was associated with the increased risk of ESCC, while *MTHFR* rs4845882 G>A and rs4846048 A>G were associated with the decreased risk of ESCC.

ESCC is one of the most general cancers worldwide. Of late, more and more evidence has demonstrated that genetic components, environmental factors, gene–gene and gene–environment interactions play pivotal roles in ESCC development and progression [19, 20]. Recent studies indicated that susceptibility of ESCC could be modulated by *MTHFR* SNPs; however, the results were inconsistent. In view of these investigations, we chose five tagging sites to evaluate their roles in ESCC. The metabolic pathway is considered to be very important in keeping normal DNA methylation, DNA synthesis and DNA repair [21]. The *MTHFR* genetic variation resulted in 5-methyltetrahydrofolate reduction and homocysteine amassing in the body, which made the methyl donor of the methionine dys-synthesis, eventually led to hypomethylation of DNA, decreasing the activity of the enzyme and increasing a number of cancers susceptibility [22].

MTHFR rs1801133 C>T (*MTHFR* C667T) mutation results in an alanine to valine substitution and a reduction in enzyme activity [23]. Recently, several investigations indicated that *MTHFR* rs1801133 C>T was associated with EC in Chinese population [24, 25]. In combination with our study, our outcomes showed that the mutation in *MTHFR* rs1801133, causing reduction in enzyme activity, DNA methylation and diminished DNA synthesis/repair, might dramatically increase the susceptibility of ESCC.

To the best of our knowledge, it was the first case–control study to assess the association between *MTHFR* rs4845882 G>A genotype and the susceptibility of cancer. Rs4845882 G>A and *MTHFR* tagging SNP rs1801131 (1298 A>C) are almost complete LD. A meta-analysis demonstrated that *MTHFR* rs1801131 acted as a protective role in the carcinogenesis of hepatocellular carcinoma [26]. Another study indicated that *MTHFR* rs1801131 A>C was not associated with cervical cancer risk [27]. In this study, we found *MTHFR* rs4845882 G>A played a protective role in the carcinogenesis of ESCC.

MTHFR rs4846048 is located at 463 bp upper stream of a polyadenylation signal position [28]. There is one polyadenylation signal sequence in most eukaryotic genes; however, sometimes multiple such sequences are existed, and the alternatively polyadenylated mRNAs are usually conditioned by translation efficiency and tissue-specific expression [29]. Therefore, the significant association of *MTHFR* rs4846048 with ESCC may indicate that the SNP of this polyadenylation signal site acts an crucial role in the occur and development progress of ESCC.

Several limitations should be acknowledged. First, all subjects were recruited from two hospitals and might not fully represent the general Chinese population. It might result in unavoidable selection bias. Second, the moderate sample sizes in our study restricted statistical power to indicate a more reliable effect. Third, *MTHFR* rs1801133 C>T and rs4845882 G>A genetic distribution of controls were deviated from HWE. Fourth, in current study, we do not have the data for the level of folate intake in individuals to further conduct examination of the gene–nutrient interaction. Further, better designed studies should be carried out to verify these results. Finally, since the detailed dataset on cancer metastasis and survival information of each subject was not available till now, the role of *MTHFR* polymorphisms in ESCC progression and prognosis could not be conducted further analyses.

In conclusion, this study indicates a significant association between the *MTHFR* rs1801133 C>T, rs4845882 G>A and rs4846048 A>G SNPs and risk of ESCC in Han Chinese population. Future, larger sample size studies on the role of the *MTHFR* SNPs—nutrient (the level of folate intake) interaction—are needed to verify these results.

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Conflict of interest None.

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