Associations of the *MTHFR* rs1801133 polymorphism with gastric cancer risk in the Chinese Han population

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Abstract. In recent years, increasing evidence has implicated the importance of mutations in the *MTHFR* gene in the risk of gastric cancer risk. A single nucleotide polymorphism (SNP) in the *MTHFR* gene (rs1801133) may serve a critical role in gastric cancer. A hospital-based case-controlled study was performed to assess the risk of the rs1801133 polymorphism on gastric cancer. A total of 307 patients with gastric cancer and 560 patients in the control group were recruited. Genomic DNA was extracted from peripheral blood and genotyped for rs1801133 using the ligase detection reaction. The relationship between rs1801133 and gastric cancer risk was evaluated by unconditional logistical regression analysis. The rs1801133-TT genotype was associated with a borderline significantly decreased risk of gastric cancer [(TT vs. CC, adjusted odds ratio (OR)=0.54, 95% confidence intervals (CI)=0.35-0.83; P=0.006; CT vs. CC, adjusted OR=0.59, 95% CI=0.44-0.79, P<0.001; and TT/CT vs. CC, adjusted OR=0.61, 95% CI=0.44-0.83, P=0.001), and further analysis showed the relationship was evident amongst older patients and patients who never drank alcohol. The C>T mutation at rs1801133 of the *MTHFR* gene was associated with a decreased risk of gastric cancer in older individuals and those who never drink.

Introduction

Gastric cancer is one of the most common types of cancer. Worldwide, gastric cancer has the fourth highest morbidity and the second highest mortality rate (1). However, the incidence and mortality of different types of gastric cancer vary; for example, there is a decrease in the incidence of non-cardia gastric cancer, concurrent with an increase in the morbidity and mortality rate of gastric cardia adenocarcinoma (2-5).

According to the statistics of Global Cancer in 2012, ~952,000 cases of stomach cancer were diagnosed, and there were >700,000 deaths, almost half of which came from China. Additionally, there were 405,000 new cases and 325,000 deaths in China (6). Developing countries have a higher proportion of gastric cancer cases, accounting for 70% of the worldwide total. Among these cases, East Asia accounts for ~50% of cases, and China is the worst affected, accounting for 45% of all gastric cancer-related deaths. Additionally, 42.6% of the incidence is attributed to China (7,8). Several studies have shown the importance of various environmental factors on the risk of gastric cancer (9). In addition to environmental factors, the risk of gastric cancer is also influenced by internal factors, for example, age, genetic factors, lifestyle and sex. In particular, genetic factors may impact gastric cancer.

5,10-methylene 4 hydrogen folate is a methylene 4 hydrogen folic acid reductase Methylenetetrahydrofolate reductase (MTHFR) catalyst, converts irreversibly into 5-methyl folate four hydrogen, which directs the distribution of folic acid derivatives to homocysteine remethylation and DNA methylation, or to biosynthesis of DNA and RNA, at a key metabolic branching point (10). Chromosome 1p36.3 contains 11 exons that encode *MTHFR*. More importantly, 5-MTHF is the only single carbon donor that produces homocysteine remethylation in the methionine cycle (11). The *MTHFR* gene contains >20 SNPs, some of which are non-synonymous and some of which are associated with cancer risk. In particular, genetic polymorphisms in the *MTHFR* gene are associated with colorectal cancer risk (12,13). A previous study found that rs4846048 increased the risk of colorectal cancer through its association with miR-522, and further regulated the survival and apoptosis of HeLa cells (14). Additionally, rs1801131 is associated with an increased risk of gastrointestinal toxicity (15), and the rs1801131-CC genotype is associated with sporadic breast cancer (16). The most frequently studied

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SNP is rs1801133, which is less common in gastric cancer; however, its role and prevalence in breast cancer and ovarian cancer have been studied (16-18).

It was found that rs1801133 was associated with the risk of colorectal cancer (19,20), ovarian cancer (17), gastric cancer (21) and liver cancer (22). To further verify whether rs1801133 is associated with the risk of gastric cancer, a case-controlled study was performed using individuals recruited from Zhenjiang (Jiangsu, China). The aim of the present study was to explore the relationship between rs1801133, environmental factors and the risk of gastric cancer.

Materials and methods

Ethics statement. The present hospital-based case-controlled study protocol was approved by the Ethics Committee for Human Subject study of Jiangsu University (Zhenjiang, China), and the study conformed to the guidelines of the Helsinki Declaration (23) on Ethical Behavior in Human Research.

Study population. All subjects in the present study, including the cases and controls, were of Chinese Han origin. A total of 307 patients with gastric cancer from the Affiliated People's Hospital of Jiangsu University and the Affiliated Hospital of Jiangsu University were collected between October 2014 and June 2016. Each patient was diagnosed with gastric cancer pathologically. The exclusion criteria were: Pre-existing cancer; the patient had undergone radiation or received chemotherapy; evidence of metastasis; or had an autoimmune disease. A total of 560 individuals with no cancer were recruited from the above two hospitals at the same time. Each of these individuals were interviewed to gather demographic data (such as age) and related risk factors (involving tobacco smoking and alcohol drinking). Participants who smoked at least 1 cigarette a day for 1 year were defined as 'smokers'. Individuals who had more than 3 drinks a week for 6 months were considered 'alcohol drinkers'. The median age was 63 years (age range, 40-90) in the case group and 62 years (age range, 40-88) in the control group. There were 223 males and 84 females amongst the case group, and 373 males and 187 females in the control group.

Genotyping. From each individual, 3-5 ml venous blood was collected. The sample was stored in a test tube lined with EDTA at 4˚C. Genomic DNA was extracted from whole blood samples using a commercially available DNA blood mini-kit (Qiagen, Inc.). Genotyping quality was determined using a procedure that included success rates of >95%, repeated genotyping, internal positive control samples, and Hardy-Weinberg Equilibrium (HWE) tests (24). The *MTHFR* tag SNP rs1801133 was assessed according to the HapMap Project and Haploview version 4.2 (ihg.gsf.de/cgi-bin/hw/hwa1.pl). The ligase detection reaction method was used to genotype rs1801133, for which Shanghai Biowing Applied Biotechnology Company provided technical support (25). For quality control, 104 samples (12.00%) were randomly reanalyzed, and the accuracy rate was 100% (data not shown). To analyze the regulome DB score, regulomedb.org/ was used, and to analyze the minor allele frequency in Chinese individuals, snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm was used.

Statistical analysis. A t-test was used to compare the age of the cases and controls. A χ^2 test was used to detect differences in demographic variables, variations between smoking status, history of alcohol consumption, and frequency of the rs1801133 genotype between the cases and controls. A HWE test determined the expected frequency in controls using the standard χ^2 test. Deviations from HWE in the control group were assessed using an internet-based HWE tool (ihg.gsf.de/cgi-bin/hw/hwa1.pl) (26).

To assess the relationship between the genetic polymorphism and risk of gastric cancer, the odds ratio (OR) and 95% confidence intervals (CIs) were calculated using unconditional logistical regression analysis, and adjusted for age, sex, smoking status and drinking history. Furthermore, genetic polymorphisms were stratified by age, sex, smoking status and drinking history. All statistical analyses was performed using SPSS version 21.0 (IBM Corp.). The data are expressed as the mean ± standard deviation. All P-values are bilateral and $P<0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics of the study population. As shown in Table I, there were 307 cases and 560 controls included in the present study. There was no statistical difference in age and sex between the cases and the control groups (P=0.218 and 0.067, respectively). The case group contained 185 smokers and 122 nonsmokers. Meanwhile, in the controls, 373 individuals were smokers and 187 were non-smokers. There was a statistically significant difference between the case and the control group ($P<0.001$). However, there was no statistically significant difference in alcohol consumption (P=0.223).

The genotyping call rate was 100%. In Table II, the genotype distribution of rs1801133 C>T (P=0.112) is shown, which conforms with the HWE ($P>0.05$). The minor allele frequency (MAF) in the controls is similar to the Chinese MAF in the SNP rs1801133 database (27).

rs1801133 and gastric cancer risk. The frequency of alleles and genotypes, and the associated ORs (95% CI) between cases and controls are shown in Table III. The observed frequency of the rs1801133-T allele in the control group was 43.9%, which was similar to the HapMap frequency of the Han Chinese in Beijing (44.4%). Compared with the control group (44.37%), the frequency of the T allele was significantly reduced (35.83%). The frequency of rs1801133 genotypes was 29.3% (CC), 52.7% (CT), and 18% (TT) in controls, and 41.4% (CC), 45.6% (CT) and 13% (TT) in cases, respectively.

When the rs1801133-CC homozygous genotype was used as the reference group, the CT genotype was associated with risk of gastric cancer (CT vs. CC, OR=0.61, 95% CI=0.45-0.83, P=0.002). The TT genotype was significantly associated with a reduced risk of gastric cancer (TT vs. CC, OR=0.51, 95% CI=0.33-0.79, P=0.002). In the dominant model, rs1801133-TT compared with rs1801133-CC genotype (CT/TT vs. CC, OR=0.59, 95% CI=0.44-0.79, $P<0.001$) was also associated with a reduced risk of gastric cancer risk. In the recessive model, the TT homozygous genotype was not related to the risk of gastric cancer (CC/CT vs. TT, OR=0.68,

Table I. Clinicopathological characteristics and risk factors of the patients with in gastric cancer and control group.

	Cases, n=307		Controls, n=560		
Variable	n	%	n	%	P-value
Age, years					0.218
≥63	162	52.8	271	48.4	
<63	145	47.2	289	51.6	
Sex					0.067
Male	223	72.6	373	66.6	
Female	84	27.4	187	33.4	
Smoking status					<0.001[a]
Never	185	60.3	402	71.8	
Smoker	122	39.7	158	28.2	
Alcohol use					0.223
Never	222	72.3	426	76.1	
Drinker	85	27.7	134	23.9	

[a]P<0.05.

Table II. Primary information of the MTHFR rs1801133 C>T polymorphisms.

Item	Information
Genotyped SNPs	MTHFR rs1801133 C>T
Chromosome	1
Gene Official Symbol	MTHFR
Function	Missense
Chromosome position[a]	11778965
Regulome DB Score[b]	4
nsSNP	Y
MAF for Chinese in database[c]	0.439
MAF in our controls, n=560	0.444
P-value for HWE test in our controls	0.112
Genotyping method	LDR
Genotyping value	100.00%

[a]Based on Genome Build version 36.3. [b]Based on regulomedb.org/. [c]Based on snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm. MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; LDR, ligation detection reaction; SNP, single nucleotide polymorphism; nsSNP, nonsynonymous single nucleotide polymorphism.

95% CI=0.46-1.01, P=0.681) using the rs1801133-CC/CT genotypes as the reference group (Table III).

After adjustment for age, sex, smoking and drinking status, the TT genotype was associated with a significantly reduced risk of gastric cancer (TT vs. CC, adjusted OR=0.54, 95% CI=0.35-0.83, P=0.006; CT vs. CC, adjusted OR=0.59, 95% CI=0.44-0.79, P<0.001; and TT/CT vs. CC, adjusted OR=0.61, 95% CI=0.44-0.83, P=0.001). Using the rs1801133 CC/CT genotype as the reference group, the TT homozygous genotype was not associated with gastric cancer risk (CC/CT vs. TT, OR=0.71, 95% CI=0.48-1.07, P=0.098).

In order to evaluate the effects of the rs1801133 genotype on the risk of gastric cancer based on sex, smoking, age and alcohol consumption, a hierarchical analysis was performed (Table IV). Compared with the CC genotype, gastric cancer risk in the CT (adjusted OR=0.546, 95% CI=0.377-0.791, P=0.001) and TT (adjusted OR=0.447, 95% CI=0.276-0.823, P=0.008) and CT/TT (adjusted OR=0.525, 95% CI=0.369-0.748, P<0.001) genotype carriers was significantly decreased for male patients. Compared with the CC and CT genotypes, gastric cancer risk in the CT (adjusted OR=0.590, 95% CI=0.378-0.920, P=0.020), TT (adjusted OR=0.369, 95% CI=0.181-0.753, P=0.006), CT/TT (adjusted OR=0.534, 95% CI=0.348-0.818, P=0.004), and CT+CC vs. TT (adjusted OR=0.500, 95% CI=0.259-0.967, P=0.040) genotype carriers was significantly decreased in the younger patients. For older patients, compared with the CC genotype, the risk of gastric cancer was reduced in the CT (adjusted OR=0.601, 95% CI=0.386-0.935, P=0.024) and CT/TT (adjusted OR=0.618, 95% CI=0.408-0.936, P=0.023) genotype carriers. In addition to internal factors, the CT genotype may decrease the risk of gastric cancer (CT vs. CC, adjusted OR=0.411, 95% CI=0.236-0.717, P=0.002; and TT/CT vs. CC, adjusted OR=0.421, 95% CI=0.247-0.718, P=0.001) for those patients who have smoked. Based on alcohol consumption, the CT and TT genotypes may reduce gastric cancer risk (TT/CT vs. CC, adjusted OR=0.673, 95% CI=0.471-0.962, P=0.030) for individuals who drink. The CT and TT genotype may decrease the risk of gastric cancer (CC vs. CT, adjusted OR=0.427, 95% CI=0.226-0.809, P=0.002, and TT/CT vs. CC, adjusted OR=0.437, 95% CI=0.237-0.804, P=0.008) for individuals who never drink.

Discussion

In the present hospital-based case-controlled study on gastric cancer, whether the functional SNP rs1801133 genotypes in the *MTHFR* gene affected the susceptibility of the Chinese Han population to gastric cancer was assessed in the present study. There was no evidence that rs1801133 TT, CT and TT/CT genotypes were associated with decreased risk of gastric cancer. The rs1801133 C>T polymorphism in the *MTHFR* gene was negatively associated with the risk of gastric cancer, as well as with age, smoking status and drinking history after correction.

Several studies have not been able to confirm a relationship between the *MTHFR* C677T genotype and gastric cancer susceptibility. The results of this study are consistent with those of Lin *et al* (28) and Chen *et al* (29), although the present study included more cases than the study by Lin *et al* (28). In the case group, more patients aged 55-63 years, male patients, and patients with a history of smoking and drinking were also enrolled in the present study. The study by Lin *et al* (28) recruited individuals predominantly from southern China, whereas the present study recruited individuals from eastern China primarily. Therefore, the present study is more relevant

Table III. Logistical regression analyses of the associations between MTHFR rs1801133 C>T polymorphisms and the risk of gastric cancer.

MTHFR rs1801133 C>T genotype	Cases, n (%)	Controls, n (%)	Crude OR (95% CI)	P-value	Adjusted OR[c] (95% CI)	Adjusted P-value[c]
C	394 (64.2)	623 (55.3)				
T	220 (35.3)	497 (44.4)				
CC	127 (41.4)	164 (29.3)	1			
TT	40 (13)	101 (18)	0.51 (0.33-0.79)	0.002	0.54 (0.35-0.83)	0.006[a]
CT	140 (45.6)	295 (52.7)	0.61 (0.45-0.83)	0.002	0.59 (0.44-0.79)	<0.001[b]
CT+TT	180 (58.6)	396 (70.7)	0.59 (0.44-0.79)	<0.001	0.61 (0.44-0.83)	0.001[b]
CC+CT	167 (54.4)	265 (47.3)	1			
TT	40 (13)	295 (52.7)	0.68 (0.46-1.01)	0.681	0.71 (0.48-1.07)	0.098

[a]P<0.01, [b]P<0.001. [c]Adjusted for age, sex, smoking status and alcohol consumption. OR, odds ratio; CI, confidence interval.

for gastric cancer susceptibility in eastern China. However, as the results of the present study and Lin *et al* (28) were consistent, this highlights the potential generalizability of the results at least regarding the Chinese population.

Single nucleotide polymorphisms of the MTHFR gene are associated with the risk of gastric cancer (30). Chen *et al* (29) found that MTHFR 677TT was associated with a reduced risk of gastric cancer, which has been confirmed by another study (31). In other studies, several groups reported that carriers of the TT genotype had higher response rates or improved survival rates than the CC or CT genotypes in gastric cancer patients receiving 5-fluorouracil (5-FU) chemotherapy (32,33). Of note, *MTHFR* polymorphisms have an impact on the efficacy of fluorouracil, as patients with different SNPs have been shown to have different reactivities (34,35). The homozygous genotypes rs2274976-GG and rs1801131-AA were more common in reactive patients, whereas the rs2274976-A allele (GA and AA) and rs1801131-C allele (AC and CC) were more common in unresponsive patients (6). *MTHFR* polymorphisms may serve an important role in regulating the clinical toxicity and efficacy of 5-FU (36). The C677T SNP may predict toxicity in patients with metastatic colorectal cancer and fluorouracil chemotherapy in Costa Rica (37). However, patients with gastric cancer with CC or CT genotypes tended to have less chemotoxicity than those with the TT genotype. The C677T polymorphism predicted severe chemotoxicity in GC patients receiving 5-FU based chemotherapy, but could not predict efficacy (21). Chen *et al* (38) found a significant increase in gastric cancer risk in the Asian population. Zintzaras (39) performed study with 1,584 cases and 2,785 controls, and showed that the evidence for association between MTHFR polymorphisms and GC was predominantly present in East Asians and was not significant in Caucasians (39). To further clarify the relationship between the rs1801133 variant of the *MTHFR* gene and gastric cancer risk, the present case-controlled study using a large sample was performed. Through the analysis of alcohol consumption, sex, age and smoking status, it was shown that male, younger, former smokers and drinkers with the rs1801133 C>T variant had a reduced risk of gastric cancer.

MTHFR polymorphisms are associated with the risk of neurological diseases, psoriasis, various types of cancer, infertility and vascular diseases (40). Folic acid levels are associated with the *MTHFR* C677T genotype binding and microRNA (miR)-21 expression. miR-21 is a noncoding small RNA that regulates gene expression and is often found in secreted microvesicles (41). Tumor-derived microbubbles induce skeletal muscle cell apoptosis, resulting in a decrease in skeletal muscle quality, which is a characteristic symptom of cancer cachexia (42).

The *MTHFR* gene produces methylene tetrahydrofolate reductase, a rate-limiting enzyme for folic acid metabolism and DNA methylation, and folic acid is essential to rescue a vulnerable state (43). It is an active 77 kDa protein that catalyzes the transfiguration of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (44). There is evidence that *MTHFR* gene mutations lead to decreased thymidylate synthase (TS) activity in cancer cells, as a consequence of decreased levels of 5, 10-methylenetetrahydrofolate (45); the latter of which supplies the methyl for the methylation of dUMP to dTMP (46). TS is a critical and rate-limiting enzyme for maintaining an appropriate supply of DNA to ensure accurate DNA synthesis and repair (47). Thus, SNPs in the *MTHFR* gene may contribute to genetic susceptibility to cancer (48).

According to one report, the C>T variant at nucleotide 677 (in exon 4 at the folate-binding site) leads to a valine substitution for alanine, which is functionally relevant, causing a reduction in the activity of MTHFR (19). Studies have shown that individuals who are heterozygous for the rs1801133 polymorphism had 70% of the normal enzyme activity, while carriers of the homozygous polymorphism had only 30% of the normal enzyme activity (47), affecting folate metabolism (49). With regards to the relationship between MTHFR and folate, it has been shown that compound heterozygosity for the 677T allele was associated with reduced plasma folic acid levels (50). Another study found that rs1801133 C>T was related to low plasma folate content and significantly decreased MTHFR activity (51).

The present study has several limitations. Given that the case and control groups were recruited from the hospital, the study population may not represent the average Chinese Han population. Folate status may influence the relationship between the *MTHFR* SNP rs1801133 and gastric cancer

Table IV. Stratified analyses between MTHFR rs1801133 C>T polymorphism and gastric cancer risk based on sex, age, smoking status and alcohol consumption.

	MTHFR rs1801133 C>T, n, case/control[d]				Adjusted[e] odd ratio (95% confidence intervals), P-value				
Variable	CC	CT	TT	CT+TT	CC	CT	TT	CT+TT	TT vs. CT+CC
Sex									
Male	95/107	102/204	26/62	128/266	1	0.546 (0.377-0.791), P=0.001[b]	0.447 (0.276-0.823), P=0.008[b]	0.525 (0.369-0.748), P<0.001[c]	0.677 (0.412-1.114), P=0.125
Female	32/57	38/91	14/39	52/130	1	0.748 (0.420-1.335), P=0.326	0.639 (0.300-1.361), P=0.246	0.717 (0.417-1.234), P=0.230	0.745 (0.378-1.466), P=0.394
Age									
<63	64/86	68/152	13/51	81/203	1	0.590 (0.378-0.920), P=0.020[a]	0.369 (0.181-0.753), P=0.006[b]	0.534 (0.348-0.818), P=0.004[b]	0.500 (0.259-0.967), P=0.040[a]
≥63	63/78	72/143	27/50	99/193	1	0.601 (0.386-0.935), P=0.024[a]	0.681 (0.382-1.215), P=0.193	0.618 (0.408-0.936), P=0.023[a]	0.897 (0.534-1.510), P=0.683
Smoking status									
Never	72/121	86/201	27/80	113/281	1	0.763 (0.511-1.140), P=0.187	0.621 (0.360-1.072), P=0.087	0.720 (0.492-1.052), P=0.090	0.717 (0.441-1.168), P=0.182
Smoker	55/43	54/94	13/21	67/115	1	0.411 (0.236-0.717), P=0.002[b]	0.466 (0.195-1.109), P=0.084	0.421 (0.247-0.718), P=0.001[b]	0.803 (0.368-1.753), P=0.582
Alcohol consumption									
Never	85/126	105/217	32/83	137/300	1	0.704 (0.483-1.026), P=0.068	0.599 (0.355-1.009), P=0.054	0.673 (0.471-0.962), P=0.030[a]	0.732 (0.461-1.160), P=0.184
Drinker	42/38	35/78	08/18	43/96	1	0.427 (0.226-0.809), P=0.009[b]	0.486 (0.178-1.325), P=0.159	0.437 (0.237-0.804), P=0.008[b]	0.790 (0.312-1.997), P=0.618

[a]P<0.05, [b]P<0.01, [c]P<0.001. [d]Genotyping was successful in all cases and control. [e]Adjusted for age, sex, smoking status and alcohol consumption.

sensitivity. However, no data was available on the intake of folic acid in these subjects, for which a prospective study would be required. Finally, an even larger sample size is required to confirm the results. Further research is required to improve our understanding of the interactions between genes and the environment in the causation of gastric cancer.

In conclusion, the results of the present study showed that the functional *MTHFR* rs1801133 C>T polymorphism may contribute to the susceptibility of gastric cancer in Chinese Han individuals.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

ZH and HS designed the study. QG acquired the data. QG and ZH wrote the manuscript. ZH and HS performed the experiments. YF and XX analyzed the results. HS and QG edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The experimental protocol used in the present study was approved by the Ethics Committee for Human Subject study of Jiangsu University (Zhenjiang, China). All patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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