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Polymorphisms of 5,10-methylenetetralydrofolate reductase (MTHFR), fruit and vegetable intake, and the risk of stomach cancer

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Abstract

Stomach cancer is a serious public health problem in China. 5,10-Methylenetetralydrofolate reductase (MTHFR) may be involved in both DNA methylation and DNA synthesis. Folate deficiency is associated with cancer risk that may be modulated by a genetic variation in the MTHFR gene in folate metabolism. The main goal of this study was to evaluate the association between polymorphisms of the MTHFR gene and the risk of stomach cancer. This study also explored the modification effects of fruit and vegetable intake (one of the main constituents is folate) on the risk of this disease. A population-based case-control study was conducted in Taixing, China, consisting of 206 newly diagnosed cases with primary stomach cancer and 415 healthy population controls. Polymorphisms of MTHFR C677T and A1298C were assayed by polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) techniques. The data were analysed using the logistic regression model. No obvious association between the MTHFR A1298C polymorphism and the risk of stomach cancer was observed in this study. The frequencies of 677 C/C, C/T, and T/T were 34.5, 50.9, and 14.6%, respectively, in controls. The frequency of the MTHFR 677 wild homozygotic genotype was 25.8% in cases, which was lower than that in controls (34.5%). The adjusted odds ratio (OR) for the MTHFR 677 any T genotype was 2.05 (95% confidence interval (CI), 1.26–3.34) when compared with the C/C genotype. In the low fruit and vegetable intake group an increasing trend was observed with the T allele exposure, $p = 0.0056$. The adjusted ORs were 1.68 (95% CI = 0.86–3.29) for the C/T genotype and 3.58 (95% CI = 1.46–8.75) for the T/T genotype, respectively. The MTHFR 677 any T genotype was associated with an increased risk of primary stomach cancer among the Chinese population. Folate deficiency might modify the MTHFR gene polymorphism and influence the risk of stomach cancer.

Keywords: *5,10-Methylenetetralydrofolate reductase (MTHFR), genetic polymorphism, vegetable and fruit intake, primary stomach cancer.*

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Introduction

China is a high-risk country for stomach cancer. In fact, 42% of stomach cancer cases in the world are diagnosed there. Both the incident rates and mortality rates in China were much higher than most other countries in the world during the same period (Parker 2001). The estimated age-standardized incident rates were 21.5 per 100 000 for men and 10.4 per 100 000 for women. The age-standardized mortality rates were 15.6 per 100 000 for men and 7.8 per 100 000 for women (Yang et al. 2004).

The aetiology of stomach cancer is believed to be multi-step and multifactorial. Environmental risk factors, nutritional factors, microbial exposure, as well as inherited gene susceptibility are involved in the risk of developing stomach cancer. In the recent year, accumulating evidence suggests an association between folate deficiency and an increased risk of stomach cancers (Zheng et al. 1989, Heimburger 1992, Gonzalez et al. 1994, La Vecchia et al. 1994, Mason & Levesque 1996, Harrison et al. 1997, Zhang et al. 1997, Mayne et al. 2001, Chen et al. 2002, Nomura et al. 2003). A chemoprevention trial conducted in China also observed an obvious improvement of gastric mucosallesions with more patients displaying reversed lesions or stable atrophy and inflammation in the folate group (Zhu et al. 2003). Folate is a vitamin B found naturally in many food sources, particularly in dark green leafy vegetables and fruits. It is essential for regenerating methionine, the methyl donor for DNA methylation and for producing the purines and pyrimidine thymidylate required for DNA synthesis and repair. The crucial role of DNA methylation, synthesis and repair in the tumour process has led to great interest in evaluating enzymes involved in the folate metabolic pathway, which includes 5,10-methylenetetralydrofolate reductase (MTHFR), methionine synthase (MS), and cystathionine 13-synthase (CBS) (CS). The gene MTHFR, located at chromosome 1p36.3, is part of a complex metabolic entity involving both the generation of universal methyl-group donor *S*-adenosylmethionine and DNA synthesis. The single nucleotide polymorphism in the MTHFR gene C677T has been identified. The A C to T transition at nucleotide 677 (C677T) in exon 4 changes an alanine to valine and affects the catalytic domain of the enzyme (Goyette et al. 1994). This form of protein (677CT, 677TT) has a 30 or 65% reduced enzyme activity, respectively (Frosst et al. 1995). The CC wild-type and individuals who are homozygous and heterozygous for this mutation have an increased amount of homocysteine (Goyette et al. 1994, Frosst et al. 1995). Another common variant is an A to C transversion at position on 1298 (A1298C) in exon 7, which causes a glutamine to alanine at position 429. This polymorphism influences specific activity of the enzyme to a lesser extent than the C677T polymorphism (Van der Put et al. 1998). This amino acid, within the regulatory domain of protein, does not seem to affect the enzyme's function by itself. However, enzyme levels of MTHFR are reduced when the heterozygosity for both MTHFR mutations are combined (Van der Put et al. 1998, Weisberg et al. 1998).

A number of studies have reported associations between the MTHFR T allele carrier and increased risk of stomach cancer (Sarbia et al. 2005, Shen et al. 2005, Si et al. 2005, Wang et al. 2005, Graziano et al. 2006, Lacasana-Navarro et al. 2006), oesophageal cancer (Song et al. 2001), bladder cancer (Lin et al. 2004), and cervical cancer (Goodman 2001). Until now, nine studies have examined the effect of MTHFR 677 variant genotypes on the risk of stomach cancer and have produced inconsistent results (Gao et al. 2002, Miao et al. 2002, Kim et al. 2004, Sarbia et al. 2005, Shen et al. 2005, Si et al. 2005, Wang et al. 2005, Graziano et al. 2006,

Lacasana-Navarro et al. 2006). Among all nine studies, three of the five studies conducted in China (Gao et al. 2002, Miao et al. 2002, Wang et al. 2005) and one of the three studies conducted in Caucasian (Graziano et al. 2006) suggested a positive relationship between the MTHFR C677T polymorphism and increased risk of stomach cancer. Results on the MTHFR 1298 polymorphism have been conflicting. A significant association was observed in a meta-analysis for gastric cancer adenocarcinoma and the MTHFR A1298C polymorphism among Eastern Asian populations. However, most studies found a negative relationship between the two. In previous studies, fruits and vegetables have been established as a major source of folate intake. The potential effect of the gene polymorphism might depend on the folate intake level. However, the possible modifying effect of fruits and vegetables on MTHFR function has not been explored among these studies.

We hypothesize that MTHFR 677, 1298 polymorphisms might be associated with increased risk of stomach cancer due to their important role in folate metabolism. To test this hypothesis, we conducted a population-based case-control study in a low folate intake area to assess the associations between MTHFR C677T and A1298C polymorphisms and the risk of stomach cancer. We also examined the possible modifying effect of fruit and vegetable intake on gene polymorphisms for the risk of this disease.

Material and methods

Background

Taixing City (formerly Taixing County before 1995) is located on the east bank of the Yangtze River in Jiangsu province, Southeast China. The population-based tumour registry is within the Division of Chronic Disease Prevention, Taixing Center for Diseases Prevention and Control (CDC). The incidence for the top three cancers combined (oesophagus, liver and stomach) was 176/100 000. Stomach cancer is considered the third most fatal disease among people diagnosed with cancer, following oesophageal cancer and liver cancer. The incidence rate was 55/100 000 for stomach cancer in 2000.

Study population

A population-based case-control study was conducted in Taixing City of Jiangsu province, China. The data collected included questionnaires and blood samples for assaying molecular markers. Although the original study included three cancer sites (oesophagus, stomach and liver) and one common population-control group, this analysis only included cases with newly diagnosed stomach cancer and population controls. The healthy population control group was a random sample from the local population from which the cases were derived.

Cases

Patients diagnosed with stomach cancer from 1 June–30 December 2000 with pathologically or clinically confirmed diagnoses reported to Taixing Tumor Registry at Taixing Anti-Epidemic Station (CDC) were eligible cases. During the study period, we intended to interview all incident cases with primary stomach cancer with the

following restrictions: patients must be newly diagnosed, aged 20 years or older, in a stable medical condition as determined by their physicians, and willing to participate. The study was also restricted to people living in Taixing for 10 or more years. In the study period, we recruited 206 patients with primary stomach cancer, which represents 65% of all new cases ($n = 316$) diagnosed within 6 months of the study period. Among these cases, all 206 patients completed the questionnaires, and 196 DNA samples were isolated. Five per cent of cases ($n = 10$) had inadequate blood samples for DNA extraction.

Controls

Eligible controls were randomly selected among healthy individuals from the general population in Taixing. Since the original study included three upper-gastrointestinal cancers (stomach, liver, and oesophagus), we used a common control group for all three cancer sites. The control group was selected according to the frequency distribution of the sex and age of all three cancer cases interviewed from each village or resident block in the city where cancer cases originated. For each village or resident block, a list of residents with the same gender within the same age group was generated. Random numbers were used to select healthy controls according to the control-to-case ratio of 2:3. When the control did not fit the criteria, or if the subject refused to interview, we recorded his/her basic demographic data and used the same selection process to choose another control. On average, 18–20 healthy controls were selected for each township (centre of town). A total of 464 controls were finally selected from the entire population of 1280 000 residents in the Taixing area. Due to the method of control selection, the age and sex distribution of controls were correspondent to all three cancer sites and might not have completely matched the distribution of stomach cancer cases. A higher proportion of younger cases for liver cancer resulted in a high proportion of younger controls.

We interviewed eligible controls during the study period with the following criteria: subjects must be aged 20 years or older, in a stable medical condition, and willing to participate. They must also have lived in Taixing for 10 or more years. Following the criteria above, the interviewer located the controls, explained the study, interviewed them at their homes, and collected approximately 8 ml of blood. A total of 464 potential healthy controls were approached and 415 controls completed interviews (89.9%). Among these interviewed controls, a total of 397 DNA samples were isolated from blood samples. Four percent of interviewed controls did not have DNA samples for analysis because of limited blood samples.

Epidemiologic data collection

Our trained interviewers interviewed cases and controls using a standard questionnaire. Interviews were monitored frequently by the professional staff in the Division of Chronic Disease Prevention of the Taixing CDC. For cases, the interviews took place either in the hospital or at the study subjects' homes. All healthy controls were interviewed in their village or in the county doctor's office.

Using a standard questionnaire, we attempted to include all possible risk or protective factors that were considered important in the Chinese population. The questionnaire included (1) demographic factors, such as the subject's age, gender, residence, place of birth, education, annual income, blood type, and disease

diagnostic information; (2) residence and drinking water history; (3) detailed dietary history including main foods, meat, eggs and milk, fried foods, beans, salted or smoked foods, vegetables, fruits, as well as dry fruit. A total of 27 items of vegetable categories and 13 items of fruit categories included almost all common foods in this area; (4) detailed smoking history; (5) alcohol drinking habits; (6) tea drinking habits; (7) detailed information on disease history; (8) occupational history and related exposures; (9) family history of stomach cancer and other cancers; and (10) physical activities.

Laboratory assays

DNA isolation. Genomic DNA was isolated from blood clots using a modified phenol–chloroform protocol.

Polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) analysis for the MTHFR polymorphism. The PCR-RFLP analysis was modified from methods described previously (Skibola et al. 1999). The primers for MTHFR 677 were 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ and 5′-AGGACGGTGCGGTGAGAGTG-3′; primers for MTHFR 1298 were 5′-CTTTGGGGAGCTGAAGGACTACTAC-3′ and 5′-CACTTTGTGACCATTCCGGTTTG-3′. PCR products of MTHFR 677 were then digested overnight with 5 units of restriction enzyme Hinf I (Promega, Madison, WI, USA), which distinguishes between the C allele and the T allele. Polymorphic bands of MTHFR C677T were 198 bp (C/C), 175 bp and 23 bp (T/T), 198 bp, 175 bp and 23 bp (C/T). PCR products of MTHFR 1298 were then digested overnight with 5 units of restriction enzyme Mbo II (New England Biolabs, Ipswich, MA, USA), which distinguishes between the A allele and the C allele. The 1298 A/A wild-type homozygotes was identified by the presence of five fragments of 56, 31, 30, 28, and 18 bp; 1298A/C heterozygotes by six fragments of 84, 56, 31, 30, 28, and 18 bp; and 1298C/C homozygotes by four fragments of 84, 31, 30, and 18 bp.

Measurement of Helobacter pylori. *H. pylori* was measured by assaying antibodies for (CagA– *H. pylori*) IgG. The presence of anti-(CagA– *H. pylori*) IgG in the serum was measured by indirect enzyme immunoassay (EIA) using kits from the Reagent Company of the Shanghai Biotechnology Industry Park (Pudong, Shanghai, China). The procedure was performed according to the manufacturer's instructions. If efficient results ($OD_{PC} > OD_{NC} + 0.5$) were not reached, measurements were repeated. OD_{PC} represents the average OD value of positive controls and OD_{NC} represents the average OD value of negative controls. Samples were considered positive when: $OD > OD_{NC} + 0.30$.

Vegetable and fruit index. We classified study subjects into three categories according to their fruit and vegetable intake frequency. Subjects with a fruit and vegetable intake frequency lower than 50% were placed in the low intake group and given a score of 0. Subjects with a fruit and vegetable intake frequency higher than 50%, but lower than 75% were placed in the middle intake group and given a score of 1. Subjects with a fruit and vegetable intake frequency higher than 75% were placed in the high intake group and given a score of 2. The index is the sum of scores for fruit and vegetable intake frequency. We assume that there are additive interactions among fruit and vegetable intake and the contribution to the carcinogenesis is similar to each other.

According to the sum of scores calculated from fruit and vegetable intake as mentioned in the methods section, we separated all subjects into two groups. The low fruit and vegetable intake group had less than 4 points while the high fruit and vegetable intake group had more than 4 points.

Statistical analysis

All analyses were performed using SAS 8.0 software. We evaluated relationships between stomach cancer and MTHFR gene polymorphisms by using adjusted odds ratios (ORs). Their 95% confidence intervals (CIs) were derived from an unconditional logistic regression model. Adjusted potential confounding factors include age (continuous), gender (male = 1, female = 0), education (continuous), income (continuous variable), body mass index (BMI, continuous variable), pack-years of smoking (continuous), alcohol drinking, consumption of very hot foods (yes or no), *H. pylori* infection (CagA + or −), stomach disease history (any one of the following stomach-related diseases: chronic atrophic gastritis, other chronic gastritis, acute gastritis, gastric ulcer, gastric polypus, or pernicious anaemia) and a family history of stomach cancer. Among these adjusted factors, tobacco smoking, consumption of very hot foods, stomach disease history and family history of stomach cancer were observed as possible risk factors of stomach cancer by the univariate analysis. Others including age, gender, education, income, BMI, alcohol drinking and *H. pylori* infection were considered as potential confounding factors and were adjusted with risk factors. In the data analysis, dummy variables were used in a logistic regression model to estimate the ORs for each category of exposure.

Results

Table I shows the distribution of potential confounders in cases and controls. The proportion of males in cases (66.99%) was similar to that in controls (69.16%). More cases were distributed in the age groups older than 60 years old than in controls, $p < 0.05$. Compared with cases, controls received more years of education. Obvious differences between cases and controls were also observed for the average income and body mass index. It seems that a higher proportion of the lower social economic class had stomach cancer. Cases reported a lower proportion (45.77%) of non-smokers, and a higher proportion (12.44%) of heavy smokers (pack-year < 40) relative to controls. No significant difference was found for alcohol drinking and *H. pylori* infection between cases and controls. Compared with the control group, a significant high prevalence of family history of stomach cancer, a history of stomach disease, as well as habits of consuming very hot foods were found in the case group.

The associations between MTHFR genetic susceptibility and stomach cancer are shown in Table II. The frequencies of the C/C, C/T, and T/T genotypes of MTHFR C677T were 25.8, 54.6, and 19.6% in cases and 34.5, 50.9, and 14.6% in controls, respectively. Using the C/C genotype as the referent, adjusted ORs were 1.87 (95% CI = 1.13–3.12) for the C/T genotype and 2.80 (95% CI = 1.41–5.56) for the T/T genotype, respectively. The p-value for trend was 0.0018. The adjusted OR for the any T genotype was 2.05 (95% CI = 1.26–3.34) when compared with the C/C genotype. T allele frequencies were 47% in cases and 40% in controls. A total of 75% of cases and 70% of controls carried MTHFR 1298 A/A homozygotes. The 1298 A/C

Table I. Distribution of the main characteristics among cases and controls.

Variables	Case, *n* (%)	Control, *n* (%)	Total, *n* (%)	*p*⋆
Gender				
Male	138 (66.99)	287 (69.16)	425 (68.44)	0.5844
Female	68 (33.01)	128 (30.84)	196 (31.56)	
Age (years)				
<40	5 (2.43)	31 (7.47)	36 (5.80)	0.0019
40–49	19 (9.22)	69 (16.63)	88 (14.17)	
50–59	65 (31.55)	136 (32.77)	201 (32.37)	
60–69	73 (35.44)	116 (27.95)	189 (30.43)	
≥70	44 (21.36)	63 (15.18)	107 (17.23)	
Education				
Illiteracy	66 (32.04)	73 (17.59)	139 (22.38)	<0.0001⋆⋆
Primary	107 (51.94)	142 (34.22)	249 (40.10)	
Middle	30 (14.56)	124 (29.88)	154 (24.80)	
High	2 (0.97)	66 (15.90)	68 (10.95)	
College	1 (0.49)	10 (2.41)	11 (1.77)	
Income⋆⋆⋆				
<60	59 (28.64)	88 (21.21)	147 (23.67)	0.0472
60–100	41 (19.90)	74 (17.83)	115 (18.52)	
101–160	66 (32.04)	135 (32.53)	201 (32.37)	
≥160	40 (19.42)	118 (28.43)	158 (25.44)	
BMI				
≤22	122 (59.22)	180 (43.37)	302 (48.63)	0.0002
>22	84 (40.78)	235 (56.63)	319 (51.37)	
Pack-years				
0	92 (45.77)	217 (52.42)	309 (50.24)	0.0606
0–20	42 (20.90)	85 (20.53)	127 (20.65)	
21–40	42 (20.90)	86 (20.77)	128 (20.81)	
≥40	25 (12.44)	26 (6.28)	51 (8.29)	
Alcohol				
Never	111 (55.22)	207 (50.24)	318 (51.88)	0.6987
Seldom	31 (15.42)	72 (17.48)	103 (16.80)	
Often	32 (15.92)	75 (18.20)	107 (17.46)	
Everyday	27 (13.43)	58 (14.08)	85 (13.87)	
Family history†				
No	168 (81.95)	392 (94.69)	560 (90.47)	<0.0001
Yes	37 (18.05)	22 (5.31)	59 (9.53)	
H. pylori				
CagA+	130 (64.68)	251 (68.77)	381 (67.31)	0.3208
CagA−	71 (35.32)	114 (31.32)	185 (32.69)	
Stomach disease‡				
No	110 (53.40)	362 (87.23)	472 (76.01)	<0.0001
Yes	96 (46.60)	53 (12.77)	149 (23.99)	
Very hot foods				
No	169 (84.92)	379 (92.67)	548 (90.13)	0.0027
Yes	30 (15.08)	30 (7.33)	60 (9.87)	
Total	206	415	621	

⋆Based on a chi-square test.
⋆⋆Fisher's exact test.
⋆⋆⋆Income per capita (RMB, Yuan).
†Family history of stomach cancer.
‡Stomach disease history.

Table II. MTHFR 677 and 1298 polymorphisms and the risk of stomach cancer.

MTHFR		Case, *n* (%)	Control, *n* (%)	Adjusted OR and 95% CI[1]
MTHFR677				
C/C		50 (25.77)	135 (34.53)	1.00
C/T		106 (54.64)	199 (50.90)	1.87 (1.13–3.12)
T/T		38 (19.59)	57 (14.58)	2.80 (1.41–5.56)
				p trend = 0.0018
C/T or T/T		144 (74.23)	256 (65.47)	2.05 (1.26–3.34)
T allele		182 (46.9)	313 (40)	
Total		194	391	
MTHFR1298				
A/A		147 (75.00)	275 (69.80)	1.00
A/C		49 (25.00)	112 (28.43)	0.98 (0.60–1.59)
C/C		0 (0)	7 (1.78)	–
A/C or C/C		49 (25.0)	119 (30.2)	0.94 (0.58–1.51)
C allele		49 (12.5)	126 (16.0)	
Total		196	394	
MTHFR677	MTHFR1298			
C/C	A/A	29 (14.95)	71 (18.16)	
C/C	A/C or C/C	21 (10.82)	64 (18.37)	1.15 (0.51–2.60)
C/T or T/T	A/A	116 (59.79)	202 (51.66)	2.12 (1.13–3.99)
C/T or T/T	A/C or C/C	28 (14.43)	54 (13.81)	2.49 (1.12–5.55)

[1]Adjusted on age (continuous variable), gender (male or female), education (continuous variable), income (continuous variable), body mass index (continuous variable), pack-years of smoking (continuous variable), alcohol drinking (1 = never, 2 = seldom, 3 = often, 4 = everyday), habit of eating very hot foods, *H. pylori* infection (CagA + or −), stomach disease history and a family history of stomach cancer.
The observed genotype frequency among the control subjects was in agreement with the Hardy–Weinberg equilibrium ($\Sigma x^2 = 1.3353$, $p > 0.05$ for the MTHFR C677T, $\Sigma x^2 = 1.3515$, $p > 0.05$ for the MTHFR A1298C).

heterozygote was more distributed in the control group, with a proportion of 28.4%. 1.78% of controls had the C/C homozygote genotype, whereas this genotype was not found in the case group. When we compared the A/C with the A/A genotype, the adjusted OR = 0.98 (95% CI = 0.60–1.59). Individuals with the A/C or C/C genotype had a risk of 0.94 (95% CI = 0.58–1.51) for developing stomach cancer. We further evaluated the combined effect of two SNPs. Subjects with both the MTHFR 677 and 1298 wild genotype were used as the referent. The OR for an individual with the MTHFR 677 Any T and 1298 Any C genotype was 2.49 (95% CI = 1.12–5.55).

This study also examined the relationship between fruit and vegetable intake frequency and risk of stomach cancer. The results are present in Table III. Compared with the low consumption subgroup, individuals with high consumption of fruit and vegetable have decreased cancer risk, with the crude OR = 0.57 (95% CI = 0.36–0.89). After adjusting for potential confounders, the OR = 0.77 (95% CI = 0.44–1.35). We observed decreased cancer risks for moderate and high consumption groups with the crude OR = 0.59 (95% CI = 0.39–0.90) and 0.47 (95% CI = 0.30–0.74). Although results suggested a dose-response relationship with stomach cancer risk, once potential confounders were adjusted, the ORs were no longer statistically significant.

We then evaluated the possible variation of the effect of MTHFR 677 and 1298 polymorphisms on the risk of stomach cancer. The results are shown in Table IV.

Table III. Fruit and vegetable intake and the risk of stomach cancer.

Variables	Case, *n* (%)	Control, *n* (%)	Adjusted OR and 95% CI[1]
Vegetable intake (%)			
<50	114 (55.3)	208 (50.1)	
50–75	59 (28.6)	101 (24.3)	1.08 (0.65–1.80)
>75	33 (16.0)	106 (25.5)	0.77 (0.44–1.35)
			p trend = 0.4427
Fruit intake (%)			
<50	134 (65.05)	206 (49.64)	
50–75	40 (19.42)	104 (25.06)	0.68 (0.40–1.15)
75	32 (15.53)	105 (25.30)	1.01 (0.57–1.80)
			p trend = 0.7068
Vegetable and fruit index			
0	142 (68.93)	228 (54.94)	
1	64 (31.07)	187 (45.06)	0.85 (0.54–1.34)
Total	206	415	

[1]Adjusted on age (continuous variable), gender (male or female), education (continuous variable), income (continuous variable), body mass index (continuous variable), pack-years of smoking (continuous variable), alcohol drinking (1 = never, 2 = seldom, 3 = often, 4 = everyday), habit of eating very hot food, *H. pylori* infection (CagA + or −), stomach disease history and a family history of stomach cancer.

In the high fruit and vegetable intake group, the adjusted ORs were 1.82 (95% CI = 0.79–4.21) and 1.54 (95% CI = 0.46–5.15) for the C/T and T/T genotype, respectively. In the low fruit and vegetable intake group, an increasing trend was observed with the T allele exposure, $p = 0.0056$. The adjusted ORs were 1.68 (95% CI = 0.86–3.29) for the C/T genotype and 3.58 (95% CI = 1.46–8.75) for the T/T genotype, respectively. Adjusted ORs for the Any T genotype were 1.77 (95% CI = 0.79–3.91) and 2.02 (95% CI = 1.07–3.83) for both high and low fruit and vegetable intake groups. However, no significant association was found for MTHFR 1298 genotypes and stomach cancer risk. Few high risks for variant genotypes among the

Table IV. MTHFR polymorphism and fruit and vegetable intake and the risk of stomach cancer.

	High folate intake subjects		Low folate intake subjects	
Gene	Case/control	Adjusted OR and 95% CI[1]	Case/control	Adjusted OR and 95% CI[1]
MTHFR677[2]				
C/C	17/55	1.00	33/80	1.00
C/T	37/91	1.82 (0.79–4.21)	69/108	1.68 (0.86–3.29)
T/T	8/29	1.54 (0.46–5.15)	30/28	3.58 (1.46–8.75)
		p trend = 0.3010		*p* trend = 0.0056
C/T or T/T	45/120	1.77 (0.79–3.91)	99/136	2.02 (1.07–3.83)

[1]Adjusted on age (continuous variable), gender (male or female), education (continuous variable), income (continuous variable), body mass index (continuous variable), pack-years of smoking (continuous variable), alcohol drinking (1 = never, 2 = seldom, 3 = often, 4 = everyday), habit of eating very hot food, *H. pylori* infection (CagA + or −), stomach disease history and a family history of stomach cancer.
[2]*P* is based on Mantel–Haenszel statistics = 0.0184.
[3]*P* is based on Mantel–Haenszel statistics = 0.0949.
[4]*P* for gene and diet interaction = 0.6784 (adjusted).

low fruit and vegetable intake group were observed, compared with the high fruit and vegetable intake group.

Discussion

In the present study, the frequencies of the MTHFR 677 genotype in healthy controls were 34.5, 50.9, and 14.6% for the C/C, C/T and T/T genotypes, respectively. These results are similar to most previous studies of Chinese populations (Song et al. 2001, Gao et al. 2002, Miao et al. 2002, Shen et al. 2005), but lower than two recent published papers that reported a higher frequency of the C/T and T/T genotypes (Stolzenberg-Solomon et al. 2003, Wang et al. 2005). The proportion of the T allele, which was 40% in healthy controls, was higher than that in Caucasians and Pakistanis (Ulrich et al. 2000, Kureshi et al. 2004, Lin et al. 2004). An association between the MTHFR C/T or T/T genotype and increased risk of stomach cancer was shown in this study. This significant trend suggests that having more T alleles results in a higher risk of stomach cancer, which is consistent with the results of previously published studies (Gao et al. 2002, Miao et al. 2002, Graziano et al. 2006). Another study used C/C and C/T genotypes as referents and found a slightly increased risk of stomach cancer for the MTHFR T/T genotype (Stolzenberg-Solomon et al. 2003). A frequency of 13–18% for the MTHFR 1298 C allele was reported in healthy Chinese controls (Miao et al. 2002, Stolzenberg-Solomon et al. 2003, Shen et al. 2005). The present study found a frequency of 16%. This frequency was lower than that of 30–50% of the C allele in Caucasians and African-Americans (Ergul et al. 2003, Gerhard et al. 2003, Lin et al. 2004). Up until now, no significant association was found between the MTHFR 1298 polymorphism and whole stomach cancer risk. Only a possible relationship with the risk of GCA was reported.

MTHFR is a critical enzyme in both DNA synthesis and methylation. It affects DNA stability and gene expression and plays an important role in tumour progression. However, inconsistent results among various cancer sites are hard to explain. Besides stomach cancer, elevated risk of oesophageal cancer (Song et al. 2001), ovarian cancer (Gershoni-Baruch et al. 2000), cervical cancer (Goodman 2001), bladder cancer (Lin et al. 2004), as well as lung cancer (Siemianowicz et al. 2003) for the T allele carrier were reported. However, the T allele has been found to be related producing a protective effect on colon/rectum cancer (Chen et al. 1996, Ma et al. 1997, Slattery et al. 1999, Ulvik et al. 2004, Yin et al. 2004), acute lymphocytic leukaemia (Skibola et al. 1999, Franco et al. 2001, Wiemels et al. 2001), and breast cancer (Sharp et al. 2002). These conflicting results might be explained by the modifying effect of the MTHFR polymorphism on the balance between DNA methylation and DNA synthesis.

MTHFR catalyses the reduction of 5,10-methlenetetrahydrolate to 5-methyltetrahydrofolate, the major circulatory form of folate in the body and a carbon donor for the conversion of homocysteine to methionine. As a precursor of *S*-adenosylmethionine (SAM), methionine is the universal methyl donor for DNA methylation (Goyette et al. 1994, Bailey & Gregory 1999). On the one hand, the low enzyme activity of MTHFR C677T variant genotypes are associated with DNA hypomethylation, which may induce genomic instability and thereby affect the expression of oncogenes or tumour suppressor genes (Solomon et al. 1991, Laird & Jaenisch 1996, Blount et al. 1997, Chen et al. 1998, Siegfried et al. 1999). On the other hand, individuals with low

MTHFR enzyme activity will have higher plasma homocysteine levels, leading to a great pool of methylene-THF. Enhanced availability of methylene-THF in the DNA synthesis pathway reduces misincorporation of uracil in DNA. This balance depends on environmental factors, particularly on dietary folate intake, which not only influences the availability of the pool of 5,10-methylene-THF, the substrate for thymidylate synthesis, but also the availability of the pool of *S*-adenosylmethionine, the universal methyl donor for methyltransferases. Thus, for subjects who are carrying the MTHFR 677T allele and, have low folate intake, both DNA methylation and DNA synthesis might be impaired (Kim et al. 2000), increasing the risk of cancer. However, when the dietary folate level is adequate, the T allele is a protective factor for cancer due to the sufficient methyl donor and beneficial DNA synthesis.

There is a big difference in the dietary styles between Chinese and Western populations, which may result in a big gap on folate status. The USA and Canada carried out mandatory folic acid fortification for food many years ago, which resulted in a significant increase of total folate intake thereafter. Additionally, consumption of citrus fruits, juice, strawberries, dark green leafy vegetables, asparagus, as well as peanuts, which are naturally high in folate, were much higher in Western countries, compared with China, especially in more rural areas (Bailey et al. 2003). Previous studies have reported that folate deficiency is common in most areas of China, including Beijing (Zhang & Ge 1986, Zheng et al. 1989, Ronnenberg et al. 2000). The phenomena may explain why more positive associations between increased risks of cancers and the MTHFR T allele in Chinese population were reported, but these associations were found less in Western populations. Taixing, the field area of the present study, is located north of the Jiangsu province, where economic levels are relatively low. Most residents in this area have a very low consumption of fresh fruits and vegetables. Based on the subjects' folate deficiency status, increased risk of stomach cancer for the T allele carrier could be explained by DNA hypomethylation due to reduced levels of SAM, which is caused by low MTHFR enzyme activity.

Plant foods are the major source for folate (88%) and the daily intake of fruits and vegetables is moderately correlated with total folate ($r = 0.56$) (Shrubsole et al. 2001). Other studies have also suggested that plasma folate concentration might be a useful biomarker for the intake of fruits and vegetables (Brevik et al. 2005). Based on this fact, we tried to explore possible differences in cancer risk patterns for variant MTHFR 677 genotype carriers between high and low fruit and vegetable intake groups. An increasing trend of stomach cancer risk was observed for more MTHFR 677 T allele carriers in the low fruit and vegetable intake group, while a decreased risk was found for the MTHFR T/T genotype when compared with the MTHFR C/T genotype in the high fruit and vegetable intake group. The present results give epidemiological support for the previous hypothesis that folate might play an important role on modulating the balance of DNA methylation and DNA synthesis.

MTHFR A1298C was not been observed to have any significant associations with stomach cancer and no combined effect of the MTHFR A1298C with the C677T genotype was suggested in this study. These results might imply that the effects of MTHFR 1298 AC and CC genotypes do not profoundly impair MTHFR enzyme activity like the MTHFR 677 CT or TT genotype do (Van der Put et al. 1998, Skibola et al. 1999, Shen et al. 2001).

In addition, methodological limitations should be addressed. Questionnaire-based information on vegetable and fruits intake history gave us a good opportunity to

analyse the possible modification of folate status on the effect of MTHFR polymorphism. However, we were unable to make any conclusions based on these results since fruits and vegetables do not make up the entire source of folate intake and cannot be used as surrogates. In addition, the similar dietary habits and economic situation in the study population does not offer a big enough difference on folate levels to make this variation clear. Nonetheless, our results might prompt further epidemiological studies on this interesting topic. Another limitation in the present study is our limited sample size. This study has a relatively large sample size, which enables us to detect moderate associations for the gene polymorphism under study. However, our limited sample size may compromise the precision of measurements when evaluating potential interactions. Thus, a study with a larger sample size is needed to estimate the possible interaction between the gene polymorphism and environmental factors.

In summary, this study suggested the role of the MTHFR 677 variant genotype as an independent risk factor for stomach cancer and also implied that the effect of the MTHFR 677 variant genotype on stomach cancer will be more obvious in individuals with low fruit and vegetable intake.

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