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Impact of Methylenetetrahydrofolate Reductase C677T Polymorphism on the Risk of Gastric Cancer and Its Interaction with *Helicobacter pylori* Infection

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ABSTRACT

Background: Attempts for early detection of gastric cancer have recently focused on host's genetic susceptibility factors and gene-environment interactions. We have, herein, studied the association of MTHFR C677T single nucleotide polymorphism (SNP) and its interaction with *Helicobacter pylori* infection, smoking, age and gender on the risk of gastric cancer among an Iranian population. **Methods:** Gastric cancer patients (n = 450) and cancer-free controls (n = 780) were studied for serum *H. pylori*-specific IgG antibodies by ELISA and MTHFR C677T polymorphism (SNP) by PCR-RFLP. Demographic and life style data were collected through patient interviews. Unconditional logistic regression model estimated odds ratio (OR) and the corresponding 95% confidence intervals (CI). **Results**: The interactions of MTHFR genotype with *H. pylori* infection ($P = 0.03$), age ($P = 0.049$) and gender ($P = 0.007$) were statistically significant. Accordingly, MTHFR C677T carriers who were also positive for *H. pylori* infection exhibited 80% (OR = 1.8, 95% CI = 1.0-2.9) significant excess risk of non-cardia gastric cancer. Furthermore, subjects over the age of 50 or female subjects carrying MTHFR C677T SNP showed 40 (OR = 1.4, 95% CI = 1.0-2.0) and 100 (OR = 2.0, 95% CI = 1.2-3.2) percent increased risk of gastric cancer, respectively. **Conclusion:** MTHFR *C677T* SNP seems to increase the risk of gastric cancer and the effect is significantly inflated by interactions with *H. pylori* infection, age and gender. *Iran. Biomed. J. 16 (4): 179-184, 2012*

Keywords: *Helicobacter pylori*, Smoking, Gender identity, Age group, Methylenetetrahydrofolate reductase

INTRODUCTION

Gastric cancer is the fourth most frequent cancer [1] and the second cause of cancer-related death [2] worldwide. *Helicobacter pylori* infection is the main established risk factor for gastric cancer with variable strengths of associations [3]. Nevertheless, gastric cancer has a multifactorial etiology and is co-modulated by different factors including host factors, such as age, gender and genetic predisposition in addition to environmental factors such as smoking, socioeconomic status and consumption of fruits and vegetables [3]. The question regarding what combination of risk factors predisposes affected individuals to gastric cancer remains the topic of investigations worldwide [4].

Recently, the influence of genetic factors such as single nucleotide polymorphisms (SNP), one of the largest types of inherited genetic variations, in regulatory genes, which may affect the individual's susceptibility to cancer, has come to the center of attention [5, 6].

MTHFR gene located on chromosome 1p36.3 encodes for a key enzyme in folate metabolism [7]. MTHFR C677T and A1298C are the two common functional polymorphisms [8]. The MTHFR C677T SNP results in the substitution of the amino acid alanine for valine, which results in a less active form of

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the enzyme. Consequently, the heterozygote genotype (CT) and homozygote mutant genotype (TT) respectively retain 60% and 30% of the original enzymatic activity of the wild type (CC) form [8]. The role of MTHFR in the metabolism of folate is to catalyze the reduction of 5, 10-methylene-tetra-hydrofolate to 5-methyltetrahydrofolate, the dominant form of folate in the circulation, which donates the methyl group for remethylation of homocysteine to methionine [9] and finally to S-adenosyl-L-methionine. A reduced enzyme activity may result in lower levels of S-adenosyl-L-methionine and an increased risk of cancers as a consequence of gene hypomethylation [10]. It may also potentiate cancer development by increasing the ratio of deoxyuridylate monophosphate to deoxythymidylate monophosphate, thereby increasing the incorporation of uracil into DNA instead of thymine, leading to point mutations and DNA/chromosome damage [11]. Association of *MTHFR*C677T polymorphism with various types of cancers, including leukemia [12], colorectal [13], breast [14], esophageal [15] and gastric [16] cancers have been reported.

Gastric cancer is the most common cancer in Iran with an incidence rate of about 20 per 100,000 [17]. However, the interactions between gene and environmental factors on the risk of gastric cancer have not been sufficiently investigated. In the current study, we have evaluated the impact of MTHFR C677T polymorphism on the risk of gastric cancer as well as its potential interaction with other established risk factors such as *H. pylori* infection in an Iranian case-control study.

MATERIALS AND METHODS

Cases and controls. Cases included patients with histologically confirmed gastric cancer (n = 405), who had undergone gastrectomy at Cancer Institute of Iran between 2002 and 2011. These patients were further subdivided based on anatomical subsites (cardia and non-cardia), and histopathological subtype (intestinal and diffuse) of their tumors according to the updated Lauren's classification system [18]. Patients with tumors involving the entire stomach, of mixed histological subtype or undocumented subsite or subtype were excluded from the stratified analyses. Controls included 780 subjects over the age of 35 and comprised of 381 unselected healthy individuals having referred for routine laboratory check-ups and 399 ulcer/cancer-free subjects gastroscopied at Amiralam Hospital (Tehran, Iran) during the same time period. Due to similar distribution of the demographic data and established risk factors, including age, sex, and smoking among the two control groups, they were pooled together and used as a single control group for all statistical analyses. Each participant provided a written informed consent prior to the interview and collection of the exposure information and biological samples. This study was approved by the Iranian National Ethical Committee for Medical Research.

H. pylori infection. Sera were isolated from fasting blood samples and kept at -70°C for further studies. *H. pylori*-specific IgG antibodies were detected through an ELISA assay developed at Pasteur Institute of Iran according to the previously described protocol [19]. Sera with borderline titers (n = 50) were excluded from the statistical analyses.

Blood sampling and DNA extraction. Genomic DNA was extracted from white blood cells according to sodium salting out extraction method [20]. The DNA purity and concentrations were determined by spectrophotometric measurement of absorbance at 260/280 nm.

MTHFR genotyping. The C677T SNP (rs#1801133) of MTHFR gene was studied by PCR-RFLP method as previously described [21]. Briefly, we used the forward (5'-CGA AGC AGG GAG CTT TGA GGC TG-3') and reverse (5'-AGG ACG GTG CGG TGA GAG TG-3') PCR primers to amplify a 233-bp product, which was digested by HinfI at 37°C overnight. Visualization of PCR products on 2% agarose gel revealed a 233-bp fragment for the wild-type (CC), 233, 176 and 57 bp fragments for heterozygotes (CT), and 176 and 57 bp fragments for homozygotes (TT). Due to the limited power and low prevalence of TT genotype (7.2%) among our study population, we compared the T carrier (including CT and TT) genotype with the wild type (CC) as the reference group.

Statistical analysis. Research subjects were classified into ever/never smokers, *H. pylori* positive/negative, younger/older than the age of 50 in all statistical analyses. Unconditional logistic regression model was used to estimate the crude and adjusted odds ratios (OR) and the corresponding 95% confidence intervals (CI) measuring the association between MTHFR C677T SNP and the risk of gastric cancer. We further evaluated the interactions of MTHFR C677T SNP with age, gender, *H. pylori* and smoking status on the risk of gastric cancer. *P* values of the interaction term in the logistic regression model were used for the homogeneity test. Every model was adjusted for age and gender unless stated as crude. The statistical package STATA version 10 was used to perform the statistical analyses.

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Table 1. Distribution of age, gender, *H. pylori* and smoking status among cases and controls.

Variables	Controls (n = 780)	All GC (n = 405)	Subtype		Subsite	
			Intestinal (n = 142)	Diffuse (n = 80)	Cardia (n = 152)	Non-cardia (n = 210)
Average age (SD)	47.61 (14.1)	62.6 (11.8)	65.6 (10.3)	58.4 (11.2)	64.3 (11.9)	61.0 (12.3)
Male/female ratio	0.70	3.0	3.6	2.6	3.0	2.9
Positive *H. pylori*	71.50%	70.1%	74.1%	79.7%	74.0%	65.8%
Ever smoker	18.20%	38.6%	40.9%	46.8%	39.9%	35.6%
MTHFR variants no. (%)						
CC	422 (54.1%)	198 (48.9%)	69 (48.6%)	39 (48.8%)	77 (50.7%)	99 (47.1%)
CT	308 (39.5%)	172 (42.5%)	59 (41.5%)	34 (42.5%)	60 (39.5%)	98 (46.7%)
TT	50 (6.4%)	35 (8.6%)	14 (9.9%)	7 (8.8%)	15 (9.9%)	13 (6.2%)
T Carriers (CT or TT)	358 (45.9%)	207 (51.1%)	73 (51.4%)	41 (51.3)	75 (49.3%)	111 (52.9%)

RESULTS

Demographic data. The average age was higher in the cases (62.6 ± 11.8) than in the control group (47.61 ± 14.1) (Table 1) and the male to female ratio was 3.0 and 0.7, respectively. The majority of cases and controls were *H. pylori* positive with a similar distribution (70.1% vs. 71.5%). Smoking habit was nearly twice as prevalent in the cases as compared to the controls (38.6% vs.18.2%). The most frequent subsite and subtype of gastric tumor in the cases were non-cardia and intestinal, respectively.

MTHFR C677T polymorphism and the risk of gastric cancer. The T allele frequency amongst the control group was 26% and the genotype distribution confirmed no deviation from the Hardy-Weinberg Equilibrium (P>0.05). The associations between the MTHFR 677 genotypes and risk of gastric cancer are shown in Table 2. We found 20% increased risk of gastric cancer (OR = 1.2, 95% CI = 1.0-1.6) and 30 percent increased risk of tumors of the non-cardia subsite (OR = 1.3, 95% CI = 1.0-1.8) among MTHFR C677T carriers compared to the wild type. However, after adjustment for age and gender, the excess risk did not remain statistically significant (Table 2). We found no differences in the risk of diffuse (OR = 1.2, 95% CI = 0.9-1.8) and intestinal (OR = 1.2, 95% CI = 0.8-2.0) subtypes of gastric cancer in MTHFR C677T carriers.

Interaction of MTHFR C677T polymorphism with age, gender, H. pylori infection and smoking. The interactions of *MTHFR* genotypes with age (P = 0.01) and gender (P = 0.007) were statistically significant (Table 3). Subjects over the age of 50 showed 40% higher risk of gastric cancer (OR = 1.4, 95% CI = 1.0-2.0). In addition, female carriers of *MTHFR C677T* SNP demonstrated 2 folds increased risk of gastric cancer compared to the wild type (OR = 2.0, 95% CI = 1.2-3.2). The prevalence of *H. pylori* infection was similar and approximately 70% in both cases and controls. The interaction term between *H. pylori* infection and *MTHFR677* genotype distribution in the regression model was statistically significant (P = 0.03, Table 3). Accordingly, among the *H. pylori*-positive stratum, the risk of gastric cancer was 1.5 folds higher in the *MTHFR C677T* carrier group compared to the wild type (OR = 1.5, 95% CI = 1.0-2.2) and the odds inflated to 1.8 for the non-cardia gastric cancer in this group (OR = 1.8, 95% CI = 1.0-

Table 2. Association of *MTHFR C677T* polymorphism and gastric cancer stratified by the tumor anatomic subsite and histological subtype.

	MTHFR variants	Case/ Control	Crude OR (95%CI)	Adjusted OR (95%CI)
All GC	CC	198/422	Ref.	Ref.
	T carriers	208/358	1.2 (1.0-1.6)	1.1 (0.9-1.5)
Carida GC	CC	77/422	Ref.	Ref.
	T carriers	75/358	1.1 (0.8-1.6)	1.1 (0.7-1.6)
Non-cardia GC	CC	99/422	Ref.	Ref.
	T carriers	112/358	1.3 (1.0-1.8)	1.2 (0.8-1.7)
Diffuse GC	CC	39/422	Ref.	Ref.
	T carriers	41/358	1.2 (0.9-1.8)	1.1 (0.7-1.7)
Intestinal GC	CC	69/422	Ref.	Ref.
	T carriers	73/358	1.2 (0.8-2.0)	1.2 (0.7-1.9)

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Table 3. Association of *MTHFR C677T* polymorphism with the risk of gastric cancer and its subsite categories among different age, gender, *H. pylori* and smoking strata.

	≤50 y		>50 y		*P* values for interaction
	Case/ Control	Adjusted OR* (95%CI)	Case/ Control	Adjusted OR* (95%CI)	
All GC	58/454	0.6 (0.3-1.0)	341/307	1.4 (1.0-2.0)	0.01
Cardia GC	18/454	0.5 (0.2-1.5)	133/307	1.2 (0.8-1.8)	0.2
Non-cardia GC	32/454	0.6 (0.3-1.2)	174/307	1.5 (1.0-2.2)	0.049
	Female		**Male**		
All GC	100/454	2.0 (1.2-3.2)	303/322	0.8 (0.6-1.2)	0.007
Cardia GC	38/454	2.0 (1.0-4.0)	114/322	0.8 (0.5-1.3)	0.03
Non-cardia GC	53/454	1.6 (1.0-3.0)	156/322	1.0 (0.7-1.6)	0.3
	***H. pylori*-negative**		***H. pylori*-positive**		
All GC	96/152	0.9 (0.5-1.5)	274/453	1.5 (1.0-2.0)	0.08
Cardia GC	30/152	1.1 (0.5-2.5)	108/453	1.1 (0.7-1.7)	0.9
Non-cardia GC	56/152	0.8 (0.4-1.6)	114/453	1.8 (1.0-2.9)	0.03
	Never smoker		**Ever smoker**		
All GC	225/404	1.1 (0.7-1.6)	150/95	1.2 (0.7-2.1)	0.9
Cardia GC	84/404	1.1 (0.6-1.8)	59/95	1.2 (0.6-2.6)	0.8
Non-cardia GC	123/404	1.1 (0.7-1.8)	72/95	1.2 (0.6-2.4)	0.9

*odds ratios were adjusted for age and gender

2.9, Table 3). We found no interaction between the *MTHFR 677* genotypes and smoking habits on the risk of gastric cancer (P = 0.9) or its subcategories. Stratification for the histological subtype did not change the relative risk for any of the above interaction analyses (data not shown).

DISCUSSION

A recent meta-analysis has investigated the controversies regarding the impact of *MTHFR*C677T polymorphism and gastric cancer by analyzing 22 qualified case-control studies and concluded that MTHFR C677T polymorphism increases the risk of gastric cancer by about 17% (OR = 1.17, 95% CI = 1.05-1.27) [16].

In our case control study, we have demonstrated that although the overall effect of *MTHFR C677T* polymorphism on the risk of gastric cancer was modest with a borderline significance, the risk was inflated by interaction with *H. pylori* infection, age and gender.

Our overall observation of 20% excess risk of gastric cancer among MTHFR C677T carriers did not retain statistical significance after adjustment of the analysis for age and gender. It was, however, in accordance with the summary OR reported by this meta-analysis [16]. Our study suggests that age and gender may interact with MTHFR C677T polymorphism in creating predisposition to gastric cancer. Consequently, stratification of our study population according to age and gender revealed 40 and 100 percent increased risk of gastric cancer for MTHFR C677T SNP carriers aged over 50 years and of the female gender, respectively. Similarly, De Re *et al.* [22] found an association between the 677TT genotype and gastric cancer in the female as well as older aged (>60 y) Italian population. The impact of age may be partly due to the increased methylation of CpG islands as a consequence of age [23]; older MTHFR 677T carriers may contain higher numbers of methylated CpG islands in comparison with the wild (CC) genotype [24]. Keeping in mind that our stratification for age has slightly dropped the case:control ratio below 1:1 in the stratum of less than 50 years of age, which reduces our strength of interpretation.

The higher susceptibility of female SNP carriers, with potentially defective folate metabolism, to gastric cancer development may be contributed by the low circulating folic acid during their reproductive age particularly in developing countries [25].

Infection with *H. pylori* is a strongly confirmed etiological factor for gastric cancer, which colonizes the gastric lumen of over 50% of the adult population worldwide [26]. *H. pylori* is a persistent mucosal pathogen that promotes gastric carcinogenesis through numerous mechanisms including induction of chronic inflammation, which is considered as a critical component in developing site-specific disease and tumor progression [27]. In addition, *H. pylori* infection eventually results in a decrease in folate absorption as a consequence of elevation of pH and/or reduction of vitamin C concentration in the gastric juice [28]. Hence, due to these and other outcomes, *H. pylori* infection may accentuate the existing deficiency in MTHFR enzyme activity. Accordingly, we have found

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a significant interaction between *MTHFR C677T* polymorphism and *H. pylori* in development of gastric cancer. In other words, *MTHFR C677T* polymorphism in *H. pylori*-positive subjects increased the risk of gastric cancer by 50%, which was inflated up to 80% for tumors of the non-cardia region. In addition, it has been demonstrated that *H. pylori* infection and chronic inflammation are factors which induce gene methylation in the stomach, which are mostly associated with non-cardia gastric cancer [29]. In addition, Tahara *et al.* [24] provided evidence that *MTHFR C677T* may affect DNA methylation of CpG island of p16 gene in *H. pylori*-infected gastric mucosa. Moreover, Neves Filho *et al.* [29] reported an association between infection with virulent strains of *H. pylori* and the methylation of CDKN2A in the distal stomach (non-cardia region) in patients with *MTHFR* 677TT genotype. Hence, it seems that *H. pylori*-infected mucosa with malfunctioning MTHFR enzyme due to gene polymorphism may influence gene methylation and create grounds for other carcinogenic activities leading to gastric cancer development particularly in the non-cardia location, where the *H. pylori* mostly resides.

In stratification analysis according to the histological subtype of the tumor, we found no differences in the risk of diffuse and intestinal gastric cancer in *MTHFR C677T* carriers. This finding is in agreement with data reported by hospital-based studies from Poland [30] and Germany [31]. These studies reported no significant added risk for GC histological subtypes for those carrying MTHFR 677T allele.

Previous studies have shown that smoking affects folate concentrations at plasma and tissue levels even after correction for folate intake [32, 33], which may cause disorders in DNA synthesis/repair and methylation. On the other hand, MTHFR has been shown to act as an essential component of the same pathway [34]. Contrary to our prior hypothesis, we observed no significant interaction between MTHFR C677T polymorphism and smoking for the risk of gastric cancer. There are, however, similar reports which have also failed to detect such an interaction [35].

In conclusion, although several studies from Iran have reported on the role of MTHFR SNP in various cancers, including breast [36], thyroid [37] and prostate cancers [38], to our knowledge, this is the first study reporting its impact on predisposition to gastric cancer. We, thereby, conclude that *MTHFR*C677T polymorphism is associated with the risk of gastric cancer of the non-cardia region, specifically in the *H. pylori* positive Iranian population. This finding may be an informative clue on the etiology of this deadly cancer in a high prevalence area for *H. pylori* infection.

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