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Polymorphisms of 5,10-Methylenetetrahydrofolate Reductase and Risk of Stomach Cancer in a Korean Population

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Abstract. *Background: Methylentetrahydrofolate reductase plays a central role in converting folate to methyl donor for DNA methylation. Genetic variations in folate metabolism are believed to contribute to the risk of acute lymphoblastic leukemia, colon, esophageal and stomach cancer, as well as cardiovascular and cerebrovascular diseases. MTHFR C677T and A1298C polymorphisms are known to be risk factors for gastric cancer in the Chinese population. Therefore, we hypothesized that the MTHFR polymorphisms are associated with the risk of stomach cancer in Korean subjects. Patients and Methods: We conducted a Korean population-based case-control study to examine the relationship between genetic polymorphisms in MTHFR and risk of stomach cancer. The study subjects were 133 patients with stomach cancer and 445 population controls, matched according to sex and age. Genomic DNA was extracted from blood samples of the controls and from surgically resected "normal" tissues adjacent to the tumor of stomach cancer patients. MTHFR genotypes at the C677T and A1298C sites were analyzed by PCR-based RFLP methods. Results: We found no evidence for an association between the MTHFR C677T and A1298C polymorphisms and stomach cancer in any of the subjects. The adjusted odds ratios and 95% confidence intervals for MTHFR C677T were 0.924 (0.581-1.469) for 677CT versus 677CC wild-type and 1.147 (0.850-1.549) for 677TT versus 677CC, and for MTHFR A1298C, they were 1.114 (0.695-1.783) for 1298AC versus 1298AA wild-type and 0.834 (0.284-2.450) for 1298CC versus 1298AA. Conclusion: These results suggest that the MTHFR C677T and A1298C polymorphisms by themselves do not play an important role in the etiology of stomach cancer in the Korean population.*

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Recent results from the Netherlands Cohort Study on Diet and Cancer show an association between vegetables and fruit and a reduced risk of cancer (1). Folate is one of the important constituents of vegetables and fruits that may provide protection against cancer. An important function of folate is to provide methyl groups required for intracellular methylation reactions and *de novo* deoxynucleoside synthesis. Therefore, folate deficiency is thought to be carcinogenic through the disruption of DNA methylation, synthesis and repair (2, 3). A highly significant inverse association between folate intake and lung cancer was also reported in the New York State Cohort Study (4). MTHFR is responsible for the circulatory form of folate, 5-methyltetrahydrofolate, which converts methionine to *S*-adenosylmethionine, the universal methyl donor for various intracellular methylation reactions, particularly DNA methylation (5, 6). Two germ-line mutations have been identified in the MTHFR locus at nucleotides 677 (C677T) and 1298 (A1298C), and the variant genotypes are associated with an increased thermolability and significantly diminished specific activity of the enzyme (7, 8). The variant 677TT homozygotes reportedly have about 30% of the *in vitro* MTHFR activity of the 677CC wild-type homozygotes, whereas the heterozygotes (677CT) have about 65% of normal enzyme activity (7). The frequency of the homozygous 677TT genotype is up to 15% in the general population, and this variant genotype is associated with higher plasma homocysteine levels and significantly reduced MTHFR activity (9), leading to a greater pool of methylene-THF. The second common MTHFR polymorphism, a glutamate-to-alanine (A to C) change at nucleotide 1298 of the gene, also influences the specific activity of the enzyme and homocysteine levels, but to a lesser extent than the C677T polymorphism (10). Previous studies of colorectal

Table I. *MTHFR C677T and A1298C genotype distributions and allele frequencies in stomach cancer patients and healthy controls.*

		C677T (Ala/Val)			Allele frequency	
		CC (%)	CT (%)	TT (%)	C	T
Cases (n=133)						
A1298C (Glu/Ala)					0.556	0.444
AA (%)		27(20.30)	44(33.08)	27(20.30)		
AC (%)		14(10.53)	20(15.08)	-		
CC (%)		1(0.75)	-	-		
Allele frequency						
A	0.865					
C	0.135					
Controls (n=445)						
A1298C (Glu/Ala)					0.59	0.41
AA (%)		83(18.65)	162(36.40)	63(14.16)		
AC (%)		52(11.69)	77(17.30)	-		
CC (%)		8(1.80)	-	-		
Allele frequency						
A	0.837					
C	0.163					

Table II. *Association between MTHFR genotypes and stomach cancer.*

Genotype	Cases (n=133)	Control (n=445)	Adjusted OR* (95% CI)
C677T			
CC	42 (31.58)	143 (32.13)	1
CT	64 (48.12)	239 (53.71)	0.924 (0.581-1.469)
TT	27 (20.30)	63 (14.16)	1.147 (0.850-1.549)
A1298C			
AA	98 (73.68)	308 (69.21)	1
AC	34 (25.53)	129 (28.99)	1.114 (0.695-1.783)
CC	1 (0.75)	8 (1.80)	0.834 (0.284-2.450)

*Adjusted for age and gender.

cancer reported a significantly decreased risk of colorectal cancer associated with the 677TT genotype that was not observed among those with low folate intakes or serum levels (11). Another study reported that individuals with the MTHFR 677TT, 1298AC and 1298CC genotypes have reduced risk of adult acute leukemia (12). In China, Shen *et al.* (13) and Song *et al.* (14) reported that MTHFR C677T variants contributed to gastric cancer and increased the risk of esophageal squamous cell carcinoma, respectively. Recently, Miao *et al.* (15) and Stolzenberg-Solomon *et al.* (16) reported that the MTHFR genotype may be a determinant of gastric cardia adenocarcinoma among this at-risk Chinese population. In the present case-control study, we examined whether the MTHFR polymorphisms are associated with the risk of stomach cancer in Korean subjects. Our data show that the MTHFR C677T and A1298C polymorphisms do not play an important role in the etiology of stomach cancer in the Korean population.

Patients and Methods

Study subjects. This case-control study consisted of 133 patients (mean age ± SD, 58.08±12.81; age range, 28 to 79 years) with stomach cancer and 445 population controls (mean age ± SD, 48.16±16.46; age range, 23 to 91 years). All subjects were Korean. All patients with historically confirmed cases of stomach cancer were enrolled. Control subjects were cancer-free individuals. The selection criteria included no individual history of cancer and frequency matching with cases by sex and age. Each control subject provided a 2-ml blood sample. Personal data from each participant regarding demographic characteristics such as sex and age were collected.

MTHFR genotyping. Genomic DNA was extracted from blood samples of the controls and from surgically resected "normal" tissues adjacent to the tumor of stomach cancer patients. MTHFR genotypes at the C677T and A1298C sites were analyzed by PCR-based RFLP methods, as described previously (7). 677CC wild-type homozygotes were identified by the presence of only a 198-bp fragment, 677CT heterozygotes were identified by 198-, 175- and 23-bp fragments, while 677TT homozygotes were identified by 175- and 23-bp fragments. 1298AA wild-type homozygotes produce only a fragment of 138 bp. The 1298AC heterozygotes produce three fragments of 138 , 119 and 19 bp and the 1298CC homozygous variants produce two fragments of 119 and 19 bp.

Statistical analysis. The associations between stomach cancer and MTHFR genotypes were estimated by computing the ORs and their 95% CIs from both univariate and multivariate logistic regression analyses. Stratification analysis was used to study subgroups of age and sex. All of the statistical analyses were performed with Statistical Analysis System Software for Windows (Version 8.2, NC, USA).

Results

MTHFR C677T and A1298C genotype distributions and allele frequencies in stomach cancer patients and healthy controls. The MTHFR C677T and A1298C genotype distributions and allele frequencies in cases and controls are summarized in Table I. The distributions of the genotypes among the controls were in Hardy-Weinberg equilibrium. We observed 677TT genotype and 677T allele frequencies of 0.2030 and 0.4436, respectively, in the case and 0.1416 and 0.4122 in the control groups (Table I). The frequencies of the 1298CC genotype and 1298C allele were 0.0180 and 0.1630, respectively, in controls, which were also very similar to the values in cases (0.0075 and 0.1353, respectively). There were no statistically significant differences in genotype frequencies between the cases and controls.

Association between MTHFR genotypes and stomach cancer. On logistic regression analysis, the variant MTHFR genotypes 677TT and 677CT were not significantly associated with risk of stomach cancer when compared with the 677CC genotype (adjusted OR, 0.924; 95% CI, 0.581-1.469 for 677CT and adjusted OR, 1.147; 95% CI, 0.850-1.549 for 677TT; Table II). Similarly, there was no significant association between the MTHFR A1298C genotype and risk of stomach cancer (adjusted OR, 1.114; 95% CI, 0.695-1.783 for 1298AC and adjusted OR, 0.834; 95% CI, 0.284-2.450 for 1298CC; Table II).

Overall, there was no evidence of any association between the MTHFR genotype and risk of stomach cancer among the different subgroups for either the C677T or the A1298C polymorphisms.

Discussion

We found no evidence of an association between the MTHFR C677T and A1298C polymorphisms and risk of stomach cancer in the Korean population. Therefore, the data do not support the hypothesis that MTHFR C677T and A1298C genotypes are associated with the risk of stomach cancer.

However, previous reports suggested that the MTHFR 677TT genotype appeared to protect against colorectal cancer and acute leukemia (11, 12). Shen *et al.* (17) reported that MTHFR C677T variants contribute to gastric carcinogenesis, particularly in gastric cardia in the Chinese population. In 2002, Miao *et al.* (15) also reported that the MTHFR genotype may be a determinant of gastric cardia adenocarcinoma among this at-risk Chinese population, and that the MTHFR A1298C polymorphism had no effect on risk of gastric cardia adenocarcinoma. Choi *et al.* (18) reported that the homozygous C677T mutation in the MTHFR gene is a risk predictor in the subtype of ischemic stroke, such as small-artery disease.

In our study, the frequencies of the variant MTHFR 677TT genotype and 677T allele were 0.1416 and 0.444, respectively, which were consistent with those reported for the control subjects in a previous study in Indonesia (19). Sadeva *et al.* (19) reported the genotype and allele frequencies of 0.13 and 0.37 for the MTHFR 677TT genotype and 677T allele, respectively in the Japanese population. In addition, the frequency of the MTHFR 677T allele in our data of 0.41 compared to 0.39 reported by Kim *et al.* (20) is very similar. This frequencies is also similar to the frequencies found in the Japanese (0.41) and Chinese (0.40), though higher than Caucasians (0.27 ~ 0.37) (20). However, we cannot rule out the possibility that MTHFR alterations in genes influence the risk of developing stomach cancer.

On the other hand, the MTHFR mutant is known as a protectant against colon cancer, its mutation being related to the stage of cancer patients and also reported as a predictor of clinical response to chemotherapeutic drugs (21, 22). Unfortunately, it is still not clear what the influence of MTHFR gene mutation would be to stomach cancer treatments. Recently, many researchers have investigated the relationship between MTHFR and TSER mutation and the influence of the anticancer drugs 5-FU, MTX (23-26). This study will also contribute to diagnosis, prognosis and prediction of stomach cancer.

In conclusion, our study does not provide evidence for associations between the MTHFR C677T and A1298C variant genotypes and risk of stomach cancer in this study population. A large prospective study is needed to verify our findings. We are currently testing this hypothesis and investigating the associations among MTHFR gene polymorphisms, cancer risk and dietary folate intake.

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