G&I Genomics & Informatics

pISSN 1598-866X eISSN 2234-0742
Genomics Inform 2012;10(3):184-193
http://dx.doi.org/10.5808/GI.2012.10.3.184

ORIGINAL ARTICLE

Association Study between Folate Pathway Gene Single Nucleotide Polymorphisms and Gastric Cancer in Koreans

Jae-Young Yoo[1†], Sook-Young Kim[1†], Jung-Ah Hwang[1], Seung-Hyun Hong[1], Aesun Shin[2], Il Ju Choi[3]*, Yeon-Su Lee[1]**

[1]Cancer Genomics Branch, National Cancer Center, Goyang 410-769, Korea, [2]Molecular Epidemiology Branch, National Cancer Center, Goyang 410-769, Korea, [3]Gastric Cancer Branch, Research Institute, National Cancer Center, Goyang 410-769, Korea

Gastric cancer is ranked as the most common cancer in Koreans. A recent molecular biological study about the folate pathway gene revealed the correlation with a couple of cancer types. In the folate pathway, several genes are involved, including methylenetetrahydrofolate reductase (*MTHFR*), methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*), and methyltetrahydrofolate-homocysteine methyltransferase (*MTR*). The *MTHFR* gene has been reported several times for the correlation with gastric cancer risk. However, the association of the MTRR or *MTR* gene has not been reported to date. In this study, we investigated the association between the single nucleotide polymorphisms (SNPs) of the *MTHFR*, *MTRR*, and *MTR* genes and the risk of gastric cancer in Koreans. To identify the genetic association with gastric cancer, we selected 17 SNPs sites in folate pathway-associated genes of *MTHFR*, *MTR*, and *MTRR* and tested in 1,261 gastric cancer patients and 375 healthy controls. By genotype analysis, estimating odds ratios and 95% confidence intervals (CI), rs1801394 in the *MTRR* gene showed increased risk for gastric cacner, with statistical significance both in the codominant model (odds ratio [OR], 1.39; 95% CI, 1.04 to 1.85) and dominant model (OR, 1.34; 95% CI, 1.02 to 1.75). Especially, in the obese group (body mass index $\geq$ 25 kg/m^2), the codominant (OR, 9.08; 95% CI, 1.01 to 94.59) and recessive model (OR, 3.72; 95% CI, 0.92 to 16.59) showed dramatically increased risk ($p < 0.05$). In conclusion, rs1801394 in the *MTRR* gene is associated with gastric cancer risk, and its functional significance need to be validated.

Keywords: 5-methyltetrahydrofolate-homocysteine S-methyltransferase, folate pathway, genetic olymorphism, methionine synthase reductase, methylenetetrahydrofolate reductase (NADPH2), stomach neoplasms

Introduction

According to the Korea Central Cancer Registry data, gastric cancer is ranked as the most common cancer in Korean and men and accounted for about 20.1% of all cancers in Koreans. In Korea, 29,727 cases of gastric cancer were newly diagnosed, and the crude incidence rate of gastric cancer in 2009 was 59.9 per 100,000. In males, the number of newly diagnosed cases and incidence rates of stomach cancer in 2009 were 19,953 cases and 80.2 per 100,000, respectively. The 5-year relative survival rates of gastric cancers were increased by 22.5% from 42.8% in 1993-1995 to 65.3% in 2005-2009 [1].

Evidence from pathology and epidemiology studies has provided a human model of gastric carcinogenesis with the following sequential stages: chronic gastritis; atrophic gastritis; intestinal metaplasia; and dysplasia [2]. Also, an environmental element of gastric cancer occurrence exists plentifully. The best well-known risk factors for gastric cancer are *Helicobacter pylori* infection, by far the strongest established risk factor for gastric cancer; a family history; and smoking. Several factors related to nutrition and food

Received July 31, 2012; Revised August 23, 2012; Accepted August 24, 2012

***Corresponding author 1:** Tel: +82-31-920-2282, Fax: +82-31-920-2542, E-mail: cij1224@ncc.re.kr

****Corresponding author 2:** Tel: +82-31-920-2551, Fax: +82-31-920-2542, E-mail: yslee2@ncc.re.kr

†These authors contributed equally to this work.

preservation, such as high intake of salt-preserved foods and dietary nitrite or low intake of fruit and vegetables, are likely to increase the risk of gastric cancer [3].

Cancerogenesis, which is the loss of cellular differentiation that leads the digestive tract to cancer, is inhibited by nutrition factors, such as retinoid, vitamins B-complex (including folate), vitamin C, D3, and E, polyphenol, fiber, calcium, selenium, and polyunsaturated fatty acids (e.g., Omega-3) [4]. Especially, the environmental factors in cancer and a high intake of vitamin B-complex play important roles in DNA synthesis, repair, and methylation [5]. There have been many epidemiological studies that gastric cancer is associated with high-risk dietary profiles (low folate, vitamin B6 intake, and high alcohol), smoking, and low blood folate concentration [6-8].

Folate is the water-soluble form of vitamin B9 in foods [9]. Leafy vegetables, such as spinach, turnip greens, lettuces, dried beans, peas, fortified cereal products, sunflower seeds, and certain fruits, are rich sources of folate. The recommended dietary allowance for adults is 400 μg of food folate a day, which is equivalent to about 240 μg synthetic folic acid in supplements or fortified food. Women of child-bearing age planning a pregnancy should take 400 μg synthetic folic acid daily in addition to their normal dietary intake [10, 11]. There is now substantial data to support an important role for folate in the prevention of neural tube defects (NTDs), Down syndrome, vascular disease, various cancers, Alzheimer's disease, cognitive function, and affective disorders [12]. Cumulative evidences suggest that food containing folate decreases the risk of colorectal, pancreatic, and esophageal cancers [13]. Also, a western lifestyle, which is associated with high total caloric or fat intake (include red meat), and inactive life pattern, has been considered one of the main reasons for increasing trends of cancer in Koreans [14].

Variations in levels of serum total homocysteine (tHcy) can result from genetic or nutrient-related disturbances in the folate pathway. In this mechanism, fasting levels of tHcy mainly reflect the remethylation pathway. In the remethylation pathway, the primary methyl donor for the vitamin B12-dependent conversion of Hcy to methionine is 5-methlytetrahydrofolate, which in turn forms 5, 10-methlytetrahydrofolate by means of the enzyme methylenetetrahydrofolate reductase (MTHFR).

Recent reports suggested that individual genetic variation or single nucleotide polymorphisms (SNPs) in various genes involved in cellular folate metabolism or transport may also be implicated in cancer risk. In the folate metabolism pathway, cellular folate act as donors and receptors of methyl groups in the biosynthesis of nucleotide precursors used for DNA synthesis and provide methyl groups for methylation of DNA, RNA, and proteins [15]. *MTHFR*, methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*), and methyltetrahydrofolate-homocysteine methyltransferase (*MTR*) genes are well known for their association with the folate pathway [16, 17].

MTHFR maps to chromosome 1p36.3 and is 2.2-kb long and contains 11 exons. The gene product is a 77-kD protein, although a smaller isoform of approximately 70 kD has been observed in some tissues, such as liver [18]. SNPs in *MTHFR*, such as C677T (rs1801133), A1298C (rs1801131), and G1793A (rs2274976), have been suggested to be associated with several cancers (colon, gastric, and breast), cardiovascular disease, NTDs, and pregnancy complications [6, 9, 19-21]. In cancer patients, higher sensitivity to 5-fluorouracil (5-FU) among patients carrying the *MTHFR* 677TT and 1298AA genotypes compared to the others was reported, demonstrating a strong predictive ability of these polymorphisms in response to 5-FU-based chemotherapy in gastric cancer [22, 23].

The *MTRR* gene was mapped to human chromosome 5p15.3-p15.2, is 34 kb long, comprises 15 exons, has 22 codon SNP (cSNP) variation sites, and is thought to produce cytosolic and mitochondrial mRNA isoforms [24, 25]. Polymorphisms in *MTRR*—rs1801394 (A66G), rs1532268 (S175L), and rs10380 (H595Y)—have been associated with the risk of cancers (breast, colon, prostate, pancreatic, and acute lymphoblastic leukemia), Down syndrome, and Alzheimer disease [26-31]. The *MTR* gene maps to human chromosome 1q42, is 105 kb, comprises 33 exons, and has 116 SNP variation sites. One polymorphism in *MTR* (rs1805087) has been associated with colorectal cancer and non-Hodgkin lymphoma risk [8, 32].

For gastric cancer, a genetic variation in *MTHFR* has been recently reported [33, 34]. Also, case-control studies with specific results on folate intake (or blood concentration) and gastric cancer risk suggest a protective role in a couple of reports [35, 36]. The *MTR* or *MTRR* gene variations were associated with colorectal and pancreatic cancer risk [8, 37], but there has been no report on gastric cancer. In this study, we investigated the association between polymorphisms in *MTHFR*, *MTRR*, and *MTR* and the risk of gastric cancer in Koreans.

Methods

Clinical samples

Buffy coat samples of 1,261 patients (69.4%) who had undergone surgery at the Gastric Cancer Center, National Cancer Center (NCC) of Korea, between September 2001 and December 2005 were included as cases. Most of the

selected gastric cancer patients possessed distal stomach tumors. Archival 375 (30.6%) normal buffy coat samples who had joined cancer screening examinees from the NCC of Korea between August 2002 and December 2005 were also included as controls in this study. Specification of group is listed in Table 1. This study was approved by the Institutional Review Board (IRB) of the NCC of Korea (NCCNSH03-024).

Table 1. Description of the case and control group

Variables	Controls (n = 375)	Cases (n = 1,261)
Age (y)	55.33 ± 7.67	55.91 ± 13.22
Height (cm)	163.00 ± 8.04	162.85 ± 8.17
Weight (kg)	63.16 ± 9.79	62.97 ± 10.12
BMI	23.71 ± 2.82	23.68 ± 3.03
Family history for gastric cancer (yes)	9.6	40.6
Smoking (none)	57.8	39.6
Alcohol drinking (none)	39.0	32.8
Helicobacter pylori (infection)	65.3	84.1
Lauren Classification (intestinal)		48.1
Stage, pathologic grade (≤II)		52.7
T stage (≤2)		85.5
N stage (≤1)		86.0
M stage (0)		95.6

Values are presented as mean ± SD or percentage.

DNA extraction and sample preparation

DNA was extracted from 350 μL of whole blood using the MagAttract DNA Blood Midi M48 Kit (Qiagen, Valencia, CA, USA) using a Qiagen BioRobot M48 workstation, according to the manufacturer's protocols automatically. The purity and concentration of isolated DNA were determined by a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). We needed more detailed quantity of each sample for genotyping reaction; so, we measured the quantity of DNA using the Quant-iT PicoGreen dsDNA assay kit (Invitrogen, Inc., Carlsbad, CA, USA) and made a dry plate for genotyping reactions with 10 ng per well of 384 plates.

Primer selection and assay design

Seventeen SNPs in *MTHFR*, *MTRR*, and *MTR* were selected, covering previously studied SNPs, such as C677T (rs1801133) and A1298C (rs1801131) in *MTHFR* [29] and A66G (rs1801394) in *MTR* [27]. The SNP information, including nucleic acid sequences, was collected from dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/; Build 136). SNPs in coding regions and promoters were chosen at first. Regulatory SNPs with validated allele frequency and non-synonymous cSNPs were finally included. Multiplexed PCR primers were designed for the best PCR reaction and designed for evading the biophysical hurdle − secondary structure, self-ligation, competition of primers, etc. − using

Table 2. Primers used for the genotyping of polymorphisms in folate metabolic pathway genes

SNPs	Forward (5' to 3')	Reverse (5' to 3')	Extension (5' to 3')	Gene
rs12404124	TACCTCACGGATGTTTTCCC	GCAGGATGGAGAATTAAAAG	TTTCCCATTATGAATGCTGAC	*MTHFR*
rs1476413	TCAATGTGAAGGTAGGCCAG	TAGGTGCTGGGTGTTTGCTC	CCAGGGTTCCCACAGAGTACCA	*MTHFR*
rs1801131	AGGAGCTGCTGAAGATGTGG	TCTCCCGAGAGGTAAAGAAC	GGAGCTGACCAGTGAAG	*MTHFR*
rs1801133	CTTCACAAAGCGGAAGAATG	CTTGAAGGAGAAGGTGTCTG	AAAAGCTGCGTGATGATGAAATCG	*MTHFR*
rs2066470	TGTCACCAGATTCCAATCGC	TAGTTCGAGATGTTCCACCC	TCTACCGGAGTCTCTCATGCCGCTC	*MTHFR*
rs2274976	ATGTACTGGATGATGGTGCG	TATGTGTGTGTAGGACGAGG	GGCATACAGCTTTCCCCAC	*MTHFR*
rs3737964	TCAAATAGGAACCAGCCCTC	TGATGGCTGTAGATCCTCAC	GAAACAGCCCTCAAAAAAAACCTTTC	*MTHFR*
rs4846048	CTTGCTAGGCTATCAACCTC	TCTCTCTACCCAAAGGCATC	CCCTTCTATCAACCTCTTATCACCA	*MTHFR*
rs7533315	AGCCCTTCCCTACTTCTACC	AAAATTCTCCCAGGAGGCAG	CCCCTCCTACTTCTACCTGGGCA	*MTHFR*
rs1805087	TCTACCACTTACCTTGAGAG	CTTTGAGGAAATCATGGAAG	GGCTGACCTTGAGAGACTCATAATGG	*MTR*
rs1801394	GCAGAAAATCCATGTACCAC	CTATATGCTACACAGCAGGG	TGTACCACAGCTTGCTCACA	*MTRR*
rs1532268	ACAAGAGGAGATAAGTGGCG	TGTAGCAGCTCTGACTTCAC	CCCCGGCATCACCTGCATCCT	*MTRR*
rs2303080	GAAAAACTTCCTTACCTGGC	GAATATTCCTGGTTTACCCC	TCCTTACCTGGCCAAGAG	*MTRR*
rs162036	TAAAAGAGAGCACTGCGTCC	CACAGCATCAGGGCTGTTAC	GAAAATAAAGGCAGACACAA	*MTRR*
rs2287780	GGAGCTGTGCAGTAAACAAG	GGAGGAGATCCAACAAGCAG	GGGGCAGCCGATTATAGC	*MTRR*
rs16879334	TTTTTCTAGAACATCTTCC	ATAGTAGTACCTTGCACACG	CTAACATCTTCCTAAACTTCAAC	*MTRR*
rs10380	GATGAGTTAAGATCCCATGC	TGACAACCTTTTAGTGATCC	TTAATATCCCATGCTTAAGGAAAT	*MTRR*

SNP, single nucleotide polymorphism; *MTHFR*, methylenetetrahydrofolate reductase; *MTR*, methyltetrahydrofolate-homocysteine methyltransferase; *MTRR*, methyltetrahydrofolate-homocysteine methyltransferase reductase.

Table 3. Association between the folate pathway polymorphisms and gastric cancer patient risk, OR[a] value (95% confidence intervals)

	Cases		Controls		Co-dominant	Dominant	Recessive
	No.	%	No.	%			
rs12404124	(n = 1,239)		(n = 372)				
CC	192	15.5	75	20.2	1		
CA	233	18.8	57	15.3	1.25 (0.78-1.99)	1.12 (0.80-1.58)	
AA	814	65.7	240	64.5	1.08 (0.76-1.55)		0.96 (0.722-1.29)
rs1476413	(n = 1,245)		(n = 373)				
GG	849	68.2	250	67.0	1		
GA	355	28.5	115	30.8	1.02 (0.76-1.38)	1.08 (0.81-1.45)	
AA	41	3.3	8	2.2	1.86 (9.83-4.14)		1.85 (0.85-4.02)
rs1801131	(n = 1,251)		(n = 374)				
AA	848	67.8	248	66.3	1		
AC	360	28.8	119	31.8	0.98 (0.72-1.31)	1.05 (0.79-1.40)	
CC	43	3.4	7	1.9	1.97 (0.86-4.49)		2.05 (0.92-4.58)
rs1801133	(n = 1,248)		(n = 373)				
CC	426	34.1	109	29.2	1		
CT	595	47.7	185	49.6	0.75 (0.55-1.02)	0.77 (0.58-1.03)	
TT	227	18.2	79	21.2	0.78 (0.52-1.17)		0.91 (0.64-1.29)
rs2066470	(n = 1,239)		(n = 372)				
CC	1,011	81.6	299	80.4	1		
CT	215	17.4	71	19.1	0.86 (0.61-1.23)	0.92 (0.65-1.30)	
TT	13	1.0	2	0.5	2.66 (0.54-13.01)		2.96 (0.60-14.67)
rs2274976	(n = 1,246)		(n = 374)				
GG	1,036	83.1	305	81.6	1		
GA	200	16.1	67	17.9	0.87 (0.61-1.26)	0.92 (0.65-1.30)	
AA	10	0.8	2	0.5	2.07 (0.39-10.91)		2.25 (0.43-11.86)
rs3737964	(n = 1,251)		(n = 365)				
GG	1,050	83.9	305	83.6	1		
GA	190	15.2	58	15.9	1.14 (0.79-1.64)	1.15 (0.80-1.66)	
AA	11	0.9	2	0.5	1.35 (0.36-5.12)		1.49 (0.49-5.67)
rs4846048	(n = 1,237)		(n = 372)				
AA	1,036	83.8	310	83.3	1		
AG	189	15.3	61	16.4	1.08 (0.75-1.55)	1.12 (0.78-1.62)	
GG	12	1.0	1	0.3	2.99 (0.52-17.30)		3.26 (0.55-19.28)
rs7533315	(n = 1,225)		(n = 351)				
CC	1,038	84.7	296	84.3	1		
CT	176	14.4	53	15.1	1.08 (0.74-1.58)	1.10 (0.76-1.59)	
TT	11	0.9	2	0.6	1.25 (0.33-4.68)		1.38 (0.36-5.24)
rs1805087	(n = 1,250)		(n = 368)				
AA	888	71.0	264	71.7	1		
AG	330	26.4	98	26.6	1.02 (0.74-1.39)	0.98 (0.73-1.32)	
GG	32	2.6	6	1.6	1.05 (0.43-2.51)		1.02 (0.75-1.38)
rs1801394	(n = 1,249)		(n = 369)				
AA	655	52.4	212	57.5	1		
AG	513	41.1	135	36.6	1.39 (1.04-1.85)[b]	1.34 (1.02-1.75)[b]	
GG	81	6.5	22	6.0	1.03 (0.58-1.81)		0.98 (0.558-1.73)
rs1532268	(n = 1,252)		(n = 369)				
CC	963	76.9	291	78.9	1		
CT	276	24.4	74	20.1	1.09 (0.77-1.54)	1.10 (0.79-1.52)	
TT	13	1.1	4	1.1	1.14 (0.40-3.29)		1.12 (0.40-3.10)
rs2303080	(n = 1,251)		(n = 374)				
TT	1,021	81.6	313	83.7	1		
TA	214	17.1	59	15.8	1.07 (0.74-1.54)	1.08 (0.76-1.55)	
AA	16	1.3	2	0.5	1.67 (0.36-7.68)		1.51 (0.30-7.45)

Table 3. Continued

	Cases		Controls		Co-dominant	Dominant	Recessive
	No.	%	No.	%			
rs162036	(n = 1,246)		(n = 368)				
AA	837	67.2	258	70.1	1		
AG	373	29.9	98	26.6	1.32 (0.97-1.79)	1.27 (0.94-1.70)	
GG	36	2.9	12	3.3	0.94 (0.41-2.11)		0.89 (0.40-1.98)
rs2287780	(n = 1,253)		(n = 374)				
CC	836	66.7	244	65.2	1		
CT	368	29.4	117	31.3	0.88 (0.67-1.18)	0.88 (0.67-1.16)	
TT	49	3.9	13	3.5	0.91 (0.44-1.86)		0.84 (0.40-1.75)
rs16879334	(n = 1,247)		(n = 367)				
CC	829	66.5	237	64.6	1		
CG	369	29.6	117	31.9	0.87 (0.65-1.16)	0.86 (0.65-1.23)	
GG	49	3.9	13	3.5	0.89 (0.44-1.84)		0.83 (0.40-1.74)
rs10380	(n = 1,243)		(n = 362)				
CC	909	73.1	282	77.9	1		
CT	312	25.1	72	19.9	1.38 (0.99-1.93)	1.32 (0.96-1.83)	
TT	22	1.8	8	2.2	0.78 (0.28-2.24)		0.74 (0.26-2.11)

[a]OR: odds ratio, adjusted for sex, family history, smoking, drinking, and *Helicobacter pylori* infection; [b]$p < 0.05$.

MassARRAY Assay Designer version 3.0 (Sequenom, Inc., San Diego, CA, USA) (Table 2).

PCR amplification

PCR reactions were performed in a total volume of 5 μL with 10 ng of genomic DNA, 1.625 mM $MgCl_2$, 0.1 units of HotStarTaq polymerase (Qiagen), 0.5 mM dNTPs (Invitrogen, Inc.), and 100 nM primers. The PCR reactions started at 94°C for 15 min, followed by 45 cycles of 94°C for 20 s, 50°C for 30 s, and 72°C for 1 min, with a final extension of 72°C for 3 min.

Genotyping

Genotyping was carried out using the iPLEX assay on the MassARRAY Platform (Sequenom, Inc.). The iPLEX extension was performed in a total volume of 9 μL with 50 μM dNTP/dideoxynucleotide phosphate (ddNTP) each, 0.063 unit/μL Thermo Sequenase (Sequenom, Inc.), and 625 nM to 1.25 μM extension primers. iPLEX extension was performed using 2-step 200 short-cycle programs. The sample was denatured at 94°C for 5 s, and strands were annealed at 52°C for 5 s and extended at 80°C for 5 s. The annealing and extension cycle was repeated 4 more times for a total of 5 cycles, looped back to a 94°C denaturing step for 5 s, and then entered the 5-cycle annealing and extension loop again. The 5 annealing and extension steps with the single denaturing step were repeated an additional 39 times for a total of 40 cycles. A final extension was done at 72°C for 3 min. iPLEX extension products were desalted by adding 6 mg resin (SpectroCLEAN; Sequenom, Inc.) and 16 μL water. After full rotation in room temperature, the reaction mixture was centrifuged at 3,500 *g*, 5 min. After desalting, products were transferred to SpectroCHIP using a Nanodispenser (SpectroPOINT; Sequenom, Inc.) and then read through matrix-assisted laser desorption/ionization time-of-flight (SpectroReader; Sequenom, Inc.). The resulting genotype data were collected by MassArray Typer software version 4.0 (Sequenom, Inc.).

Statistical analysis

The chi-square test for association was used to test differences of genotype frequencies between normal and gastric cancer patients. Odds ratios (OR) and their 95% confidence intervals (CI) in relation to *MTHFR*, *MTTRR*, and *MTR* genotypes were calculated. Also, after adjustment for sex, family history, smoking, drinking, and *H. pylori* infection, global chi-square test was also employed to calculate OR and their 95% CIs for individuals. Statistical analyses were performed using SAS version 9.0 (SAS Institute Inc., Cary, NC, USA).

Results

Association between folate pathway genes and gastric cancer risk

One thousand two hundred sixty-one gastric cancer patients and 375 control groups were included in the present study. From 17 SNPs, rs1476413, rs2066470, rs2274976, rs3737964, rs4846048, rs7533315, rs1805087, rs1532268, rs2303080, rs162036, rs2287780, rs16879334, and rs10380

Table 4. Association between the folate pathway polymorphisms for gastric cancer risk by obesity, OR[a] value (95% confidence intervals)

	Obesity group (BMI ≥ 25)						
	Case		Control		Co-dominant	Dominant	Recessive
	No.	%	No.	%			
rs12404124	(n = 232)		(n = 97)				
CC	39	16.8	19	19.6	1		
CA	48	20.7	13	13.4	1.66 (0.65-4.21)	1.22 (0.60-2.49)	
AA	145	62.5	65	67.0	1.08 (0.51-2.31)		0.81 (0.45-1.45)
rs1476413	(n = 237)		(n = 99)				
GG	148	62.4	68	68.7	1		
GA	78	32.9	28	28.3	1.29 (0.71-2.36)	1.31 (0.73-2.34)	
AA	11	4.6	3	3.0	1.37 (0.25-7.46)		1.35 (0.26-6.89)
rs1801131	(n = 237)		(n = 98)				
AA	150	63.3	67	68.4	1		
AC	74	31.2	28	28.6	1.26 (0.28-5.62)	1.27 (0.70-2.30)	
CC	13	5.5	3	3.1	1.25 (0.68-2.33)		1.29 (0.31-5.31)
rs1801133	(n = 237)		(n = 98)				
CC	86	36.3	30	30.6	1		
CT	112	47.3	45	45.9	0.77 (0.43-1.39)	0.79 (0.45-1.37)	
TT	39	16.5	23	23.5	0.66 (0.31-1.42)		0.78 (0.40-1.49)
rs2066470	(n = 231)		(n = 98)				
CC	188	81.4	82	83.7	1		
CT	38	16.5	15	15.3	0.99 (0.46-2.17)	1.05 (0.50-2.21)	
TT	5	2.2	1	1.0	1.66 (0.15-18.22)		1.83 (0.17-20.01)
rs2274976	(n = 235)		(n = 98)				
GG	193	82.1	79	80.6	1		
GA	37	15.7	18	18.4	0.85 (0.40-1.78)	0.90 (0.44-1.83)	
AA	5	2.1	1	1.0	1.68 (0.15-18.52)		1.83 (0.17-18.92)
rs3737964	(n = 237)		(n = 96)				
GG	190	80.2	82	85.4	1		
GA	45	19.0	14	14.6	1.31 (0.63-2.69)	1.36 (0.66-2.79)	
AA	2	0.8	0	0.0	-		-
rs4846048	(n = 235)		(n = 99)				
AA	188	80.0	83	83.8	1		
AG	44	18.7	16	16.2	1.03 (0.51-2.08)	1.13 (0.57-2.24)	
GG	3	1.3	0	0.0	-		-
rs7533315	(n = 226)		(n = 92)				
CC	183	81.0	78	84.8	1		
CT	41	18.1	14	15.2	1.11 (0.54-2.28)	1.16 (0.57-2.37)	
TT	2	0.9	0	0.0	-		-
rs1805087	(n = 237)		(n = 96)				
AA	166	70.0	69	71.9	1		
AG	66	27.8	23	24.0	1.22 (0.65-2.30)	1.02 (0.57-1.84)	
GG	5	2.1	4	4.2	0.34 (0.08-1.39)		0.32 (0.08-1.32)
rs1801394	(n = 236)		(n = 96)				
AA	124	52.5	69	71.9	1		
AG	91	38.6	23	24.0	1.61 (0.88-2.95)	1.93 (1.06-3.49)[b]	
GG	21	8.9	4	4.2	9.08 (1.01-94.59)[b]		3.72 (0.92-16.59)
rs1532268	(n = 237)		(n = 96)				
CC	182	76.8	82	85.4	1		
CT	53	22.4	14	14.6	1.40 (0.68-2.87)	1.52 (0.75-3.07)	
TT	2	0.8	0	0.0	-		-
rs2303080	(n = 237)		(n = 98)				
TT	197	83.1	78	79.6	1		
TA	38	16.0	19	19.4	0.49 (0.24-1.00)[b]	0.46 (0.23-0.93)[b]	
AA	2	0.8	1	1.0	-		-

Table 4. Continued

	Obesity group (BMI ≥ 25)						
	Case		Control		Co-dominant	Dominant	Recessive
	No.	%	No.	%			
rs162036	(n = 235)		(n = 96)				
AA	156	66.4	60	62.5	1		
AG	69	29.4	33	34.4	1.07 (0.58-1.97)	1.05 (0.58-1.89)	
GG	10	4.3	3	3.1	0.87 (0.17-4.38)		1.32 (0.32-5.44)
rs2287780	(n = 237)		(n = 98)				
CC	156	65.8	61	62.2	1		
CT	70	29.5	31	31.6	0.66 (0.37-1.17)	0.63 (0.36-1.08)	
TT	11	4.6	6	6.1	0.52 (0.14-1.88)		0.48 (0.13-1.64)
rs16879334	(n = 236)		(n = 95)				
CC	155	65.7	58	61.1	1		
CG	70	29.7	31	32.6	0.60 (0.34-1.08)	0.58 (0.32-1.01)	
GG	11	4.7	6	6.3	0.52 (0.13-1.95)		0.48 (0.13-1.72)
rs10380	(n = 235)		(n = 94)				
CC	169	71.9	68	72.3	1		
CT	61	26.0	23	24.5	1.46 (0.76-2.79)	1.30 (0.70-2.42)	
TT	5	2.1	3	3.2	0.58 (0.10-3.23)		0.58 (0.11-2.98)

[a]OR: odds ratio, adjusted for sex, family history, smoking, drinking, and *Helicobacter pylori* infection; [b]$p < 0.05$.

had a minor allele frequency less than 5%. For each SNP, the p-value of χ^2-test and OR were calculated (Table 3).

Significant associations between genotypes of folate pathway SNPs and the risk of gastric cancer were only observed for rs1801394 ($p < 0.05$). In rs1801394, the frequencies of the AG heterozygote genotype were 0.411 and 0.366 in patients and control groups, respectively. The risk of gastric cancer in patients with the risk allele was increased as OR, 1.39 (codominant model; 95% CI, 1.04 to 1.85) or OR, 1.34 (dominant model; 95% CI, 1.02 to 1.75) with statistical significance ($p < 0.05$).

Association between folate pathway genes and obese gastric cancer patients

Further, the association between the 17 SNPs of folate pathway genes and gastric cancer risk was analyzed and stratified by obesity categories (BMI, <25 vs. ≥25) (Table 4).

Interestingly, the risk of rs1801394 was dramatically increased for the codominant model (OR, 9.08; 95% CI, 1.01 to 94.59; $p < 0.05$) only among obese subjects. For the dominant model, the OR was also increased with statistical significance (OR, 1.98; 95% CI, 1.06 to 3.49; $p < 0.05$). Also, the recessive model showed significantly higher risk (OR, 3.72; 95% CI, 0.92 to 16.59). Additionally, we analyzed the correlation among family history, *H. pylori* infection, and folate gene SNPs but did not find any significant association (data not shown).

Discussion

For early detection and diagnosis of cancer, the discovery of new biomarkers is very important, and the interest of researchers is growing rapidly. Also, genetic factors, including polymorphisms of genes involved in tumorigenesis, may partly explain the difference in individual susceptibility to cancer [38]. In the present study, we studied the impact of folate pathway gene polymorphisms on the risk of gastric cancer in a Korean population. Since folic acid was a critical cofactor in one-carbon metabolism involving in the biological methylation and nucleotide synthesis pathways, our study may find clues for the possible effect to dietary effects on gastric cancer.

In the report of previous studies, the frequency of genotypes for *MTHFR* was similar with our results-less than 5% for many SNPs [39, 40]. For *MTHFR*, one study found the association of rs1801133 with gastric cancer risk but no association of rs1801131 [34]. However, different results have been reported in other studies, showing no association for either SNP with gastric cancer [33]. We also found no association of either SNP with gastric cancer risk, which is concordant with the results of Kim's group [33]. For *MTR*, although a couple of reports have shown a significant association between the polymorphism of *MTR* and the risk

of certain cancers [32], we could not find any association in our study population of gastric cancer.

In our study, a significant association of rs1801394 (A66G) in *MTRR* was found, especially with high OR among the obese gastric cancer group. MTRR regenerates a functional methionine synthase via reductive methylation so that methionine synthase can catalyze methionine synthesis, which is an essential amino acid required for protein synthesis and one-carbon metabolism. The A66G polymorphism is reported to be functional, so that the variant enzyme has a lower affinity for MTR [41]. A large number of studies have been conducted to evaluate the role of rs1801394 in different kinds of cancers; the results are still plausible. One meta-analysis reported that the A66G polymorphism should contribute to tumor susceptibility, showing significantly increased risk among Asians with the G allele, which is also in concordance with our result [42].

Our result is biologically plausible, since the polymorphisms or gene-environment interactions, rather than folate intake alone, would have an impact on the risk for digestive track cancer, because functional SNPs in folate-related genes were known to contribute to the alteration of folate metabolism [30]. The SNPs in folate pathway genes (such as MTHFR) were reported to influence to the decrease of the activity of the enzyme, leading to hyperhomocysteinemia, particularly in folate-deficient states [43]. Homocysteine was related to cancer formation, like tumor necrosis factor, obesity, and the folate pathway, and is known to be one of the main risk factors for distal gastric cancer, including *H. pylori* infection and dietary factors [44]. Although obesity (BMI > 25) was more prevalent in patients with cardia cancer compared to patients with gastric distal cancer in Koreans [45], a previous study reported that obesity is a major risk factor for several types of cancer, including gastric cancer [46]. Also, many epidemiological studies have shown that obesity is a risk factor for breast cancer, colon and kidney cancer, and malignant adenomas of the esophagus. Obesity subjects have an approximately 1.5-3.5-fold increased risk of developing these cancers compared with normal-weight subjects [47].

Here, we report the association of genetic variations in *MTRR* with the risk of gastric cancer for the first time. In further studies, we need to validate our finding in a larger population, considering detailed clinical information, and study the functional relevance of polymorphisms with cancer development more. Also, we need to consider other genes in the folate pathway and investigate gastric cancer susceptibility with epidemiological and environmental factors (e.g., nutrition intake, 5-FU drug interference, blood folate concentration, etc.).

Acknowledgments

This work was supported by a National Cancer Center Grant in Korea (NCC-1110270-1 and 1010190).

References

1. Jung KW, Park S, Kong HJ, Won YJ, Lee JY, Seo HG, *et al*. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2009. *Cancer Res Treat* 2012;44:11-24.
2. Kim HY. What is the most important factor for gastric carcinogenesis in Koreans: *Helicobacter pylori*, host factor or environmental factor? *Korean J Gastroenterol* 2007;49:60-71.
3. Brenner H, Rothenbacher D, Arndt V. Epidemiology of stomach cancer. *Methods Mol Biol* 2009;472:467-477.
4. Divisi D, Di Tommaso S, Salvemini S, Garramone M, Crisci R. Diet and cancer. *Acta Biomed* 2006;77:118-123.
5. Ma E, Iwasaki M, Junko I, Hamada GS, Nishimoto IN, Carvalho SM, *et al*. Dietary intake of folate, vitamin B6, and vitamin B12, genetic polymorphism of related enzymes, and risk of breast cancer: a case-control study in Brazilian women. *BMC Cancer* 2009;9:122.
6. Götze T, Röcken C, Röhl FW, Wex T, Hoffmann J, Westphal S, *et al*. Gene polymorphisms of folate metabolizing enzymes and the risk of gastric cancer. *Cancer Lett* 2007;251:228-236.
7. Tao MH, Shields PG, Nie J, Marian C, Ambrosone CB, McCann SE, *et al*. DNA promoter methylation in breast tumors: no association with genetic polymorphisms in *MTHFR* and *MTR*. *Cancer Epidemiol Biomarkers Prev* 2009;18:998-1002.
8. Theodoratou E, Farrington SM, Tenesa A, McNeill G, Cetnarskyj R, Barnetson RA, *et al*. Dietary vitamin B6 intake and the risk of colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 2008;17:171-182.
9. Gianfagna F, De Feo E, van Duijn CM, Ricciardi G, Boccia S. A systematic review of meta-analyses on gene polymorphisms and gastric cancer risk. *Curr Genomics* 2008;9:361-374.
10. Food labeling: health claims and labeling statements; dietary fiber and cancer; antioxidant vitamins and cancer; omega-3 fatty acids and coronary heart disease; folate and neural tube defects; revocation. Food and Drug Administration, HHS. Final rule. *Fed Regist* 2000;65:58917-58918.
11. Osterhues A, Holzgreve W, Michels KB. Shall we put the world on folate? *Lancet* 2009;374:959-961.
12. Neuhouser ML, Beresford SA. Folic acid: are current fortification levels adequate? *Nutrition* 2001;17:868-872.
13. World Cancer Research Fund/American Institute for Cancer Research. *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective*. Washington, DC: American Institute for Cancer Research, 2007.
14. Sauvaget C, Nagano J, Hayashi M, Spencer E, Shimizu Y, Allen N. Vegetables and fruit intake and cancer mortality in the Hiroshima/Nagasaki Life Span Study. *Br J Cancer* 2003;88:689-694.
15. Hubner RA, Houlston RS. Folate and colorectal cancer prevention. *Br J Cancer* 2009;100:233-239.
16. Boccia S, Hung R, Ricciardi G, Gianfagna F, Ebert MP, Fang JY,

et al. Meta- and pooled analyses of the methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and gastric cancer risk: a huge-GSEC review. *Am J Epidemiol* 2008;167:505-516.
17. Collin SM, Metcalfe C, Zuccolo L, Lewis SJ, Chen L, Cox A, *et al*. Association of folate-pathway gene polymorphisms with the risk of prostate cancer: a population-based nested case-control study, systematic review, and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2009;18:2528-2539.
18. Rozen R. Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). *Thromb Haemost* 1997;78:523-526.
19. Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG, *et al*. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. *Nat Genet* 1994;7:195-200.
20. Justenhoven C, Hamann U, Pierl CB, Rabstein S, Pesch B, Harth V, *et al*. One-carbon metabolism and breast cancer risk: no association of *MTHFR*, *MTR*, and *TYMS* polymorphisms in the GENICA study from Germany. *Cancer Epidemiol Biomarkers Prev* 2005;14:3015-3018.
21. Rosenberg N, Murata M, Ikeda Y, Opare-Sem O, Zivelin A, Geffen E, *et al*. The frequent 5,10-methylenetetrahydrofolate reductase C677T polymorphism is associated with a common haplotype in whites, Japanese, and Africans. *Am J Hum Genet* 2002;70:758-762.
22. De Mattia E, Toffoli G. C677T and A1298C *MTHFR* polymorphisms, a challenge for antifolate and fluoropyrimidine-based therapy personalisation. *Eur J Cancer* 2009;45:1333-1351.
23. Gao CM, Lu JW, Toshiro T, Wu JZ, Cao HX, Chen HQ, *et al*. Polymorphism of methylenetetrahydrofolate reductase and sensitivity of stomach cancer to fluoropyrimidine-based chemotherapy. *Zhonghua Liu Xing Bing Xue Za Zhi* 2004; 25:1054-1058.
24. Leclerc D, Wilson A, Dumas R, Gafuik C, Song D, Watkins D, *et al*. Cloning and mapping of a cDNA for methionine synthase reductase, a flavoprotein defective in patients with homocystinuria. *Proc Natl Acad Sci U S A* 1998;95:3059-3064.
25. Zavadáková P, Fowler B, Suormala T, Novotna Z, Mueller P, Hennermann JB, *et al*. cblE type of homocystinuria due to methionine synthase reductase deficiency: functional correction by minigene expression. *Hum Mutat* 2005;25:239-247.
26. Beyer K, Lao JI, Latorre P, Riutort N, Matute B, Fernández-Figueras, *et al*. Methionine synthase polymorphism is a risk factor for Alzheimer disease. *Neuroreport* 2003;14: 1391-1394.
27. Bosco P, Guéant-Rodriguez RM, Anello G, Barone C, Namour F, Caraci F, *et al*. Methionine synthase (MTR) 2756 (A --> G) polymorphism, double heterozygosity methionine synthase 2756 AG/methionine synthase reductase (MTRR) 66 AG, and elevated homocysteinemia are three risk factors for having a child with Down syndrome. *Am J Med Genet A* 2003;121A: 219-224.
28. Gast A, Bermejo JL, Flohr T, Stanulla M, Burwinkel B, Schrappe M, *et al*. Folate metabolic gene polymorphisms and childhood acute lymphoblastic leukemia: a case-control study. *Leukemia* 2007;21:320-325.
29. Oh D, Kim NK, Jang MJ, Kim HC, Lee JH, Lee JA, *et al*. Association of the 5,10-methylenetetrahydrofolate reductase (*MTHFR* C677T and A1298C) polymorphisms in Korean patients with adult acute lymphoblastic leukemia. *Anticancer Res* 2007;27:3419-3424.
30. Pardini B, Kumar R, Naccarati A, Prasad RB, Forsti A, Polakova V, *et al*. *MTHFR* and *MTRR* genotype and haplotype analysis and colorectal cancer susceptibility in a case-control study from the Czech Republic. *Mutat Res* 2011;721:74-80.
31. Shrubsole MJ, Gao YT, Cai Q, Shu XO, Dai Q, Jin F, *et al*. *MTR* and *MTRR* polymorphisms, dietary intake, and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2006;15:586-588.
32. Lim U, Wang SS, Hartge P, Cozen W, Kelemen LE, Chanock S, *et al*. Gene-nutrient interactions among determinants of folate and one-carbon metabolism on the risk of non-Hodgkin lymphoma: NCI-SEER case-control study. *Blood* 2007;109: 3050-3059.
33. Kim JK, Kim S, Han JH, Kim HJ, Chong SY, Hong SP, *et al*. Polymorphisms of 5,10-methylenetetrahydrofolate reductase and risk of stomach cancer in a Korean population. *Anticancer Res* 2005;25:2249-2252.
34. Sun L, Sun YH, Wang B, Cao HY, Yu C. Methylenetetrahydrofolate reductase polymorphisms and susceptibility to gastric cancer in Chinese populations: a meta-analysis. *Eur J Cancer Prev* 2008;17:446-452.
35. Lissowska J, Gail MH, Pee D, Groves FD, Sobin LH, Nasierowska-Guttmejer A, *et al*. Diet and stomach cancer risk in Warsaw, Poland. *Nutr Cancer* 2004;48:149-159.
36. Vollset SE, Igland J, Jenab M, Fredriksen A, Meyer K, Eussen S, *et al*. The association of gastric cancer risk with plasma folate, cobalamin, and methylenetetrahydrofolate reductase polymorphisms in the European Prospective Investigation into Cancer and Nutrition. *Cancer Epidemiol Biomarkers Prev* 2007;16:2416-2424.
37. Ohnami S, Sato Y, Yoshimura K, Ohnami S, Sakamoto H, Aoki K, *et al*. His595Tyr polymorphism in the methionine synthase reductase (*MTRR*) gene is associated with pancreatic cancer risk. *Gastroenterology* 2008;135:477-488.
38. Cho YG, Choi BJ, Song JH, Kim CJ, Cao Z, Nam SW, *et al*. No association of *MDM2* T309G polymorphism with susceptibility to Korean gastric cancer patients. *Neoplasma* 2008;55: 256-260.
39. Liu H, Jin G, Wang H, Wu W, Liu Y, Qian J, *et al*. Association of polymorphisms in one-carbon metabolizing genes and lung cancer risk: a case-control study in Chinese population. *Lung Cancer* 2008;61:21-29.
40. Martinez CA, Northrup H, Lin JI, Morrison AC, Fletcher JM, Tyerman GH, *et al*. Genetic association study of putative functional single nucleotide polymorphisms of genes in folate metabolism and spina bifida. *Am J Obstet Gynecol* 2009;201: 394.e1-394.e11.
41. Olteanu H, Munson T, Banerjee R. Differences in the efficiency of reductive activation of methionine synthase and exogenous electron acceptors between the common polymorphic variants of human methionine synthase reductase. *Biochemistry* 2002;41:13378-13385.
42. Han D, Shen C, Meng X, Bai J, Chen F, Yu Y, *et al*. Methionine

synthase reductase A66G polymorphism contributes to tumor susceptibility: evidence from 35 case-control studies. *Mol Biol Rep* 2012;39:805-816.
43. Huang Y, Han S, Li Y, Mao Y, Xie Y. Different roles of *MTHFR* C677T and A1298C polymorphisms in colorectal adenoma and colorectal cancer: a meta-analysis. *J Hum Genet* 2007; 52:73-85.
44. Crew KD, Neugut AI. Epidemiology of gastric cancer. *World J Gastroenterol* 2006;12:354-362.
45. Cho Y, Lee DH, Oh HS, Seo JY, Kim N, Jeong SH, *et al*. Higher Prevalence of Obesity in Gastric Cardia Adenocarcinoma Compared to Gastric Non-Cardia Adenocarcinoma. *Dig Dis Sci* 2012 Apr 7 [Epub]. http://dx.doi.org/10.1007/s10620- 012-2095-6.
46. Li Q, Zhang J, Zhou Y, Qiao L. Obesity and gastric cancer. *Front Biosci* 2012;17:2383-2390.
47. Pischon T, Nöthlings U, Boeing H. Obesity and cancer. *Proc Nutr Soc* 2008;67:128-145.