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Association between folate metabolism-related polymorphisms and colorectal cancer risk

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Abstract. Folate has essential roles in DNA synthesis, repair and methylation. Folate metabolism-related gene variants may modulate the levels of this vitamin and affect the cancer risk. Thus, whether these polymorphisms play an important role in carcinogenesis, particularly colorectal cancer (CRC) development, has been a subject interest. The present study investigated the association between polymorphisms in the methylenetetrahydrofolate reductase (*MTHFR*), thymidylate synthase (*TS*) and the reduced folate carrier 1 (*RFC1*) genes and CRC risk. Polymorphisms in *MTHFR* (677C>T and 1298A>C), *TS* [1494del6 and the *TS* enhancer region (TSER)] and *RFC1* (-43T>C, 80G>A and 696C>T) were characterized using polymerase chain reaction-restriction fragment length polymorphism in 477 CRC cases and 514 controls. Although no polymorphisms were significantly associated with the CRC risk in the overall sample, significant associations between folate metabolism-related polymorphisms and CRC risk were identified in the stratified analyses. The *MTHFR* 677CT/1298AC and *MTHFR* 1298AC+CC/*TSER* 2R3R genotypes in the presence of plasma folate levels ≤4.12 ng/ml were associated with significantly increased CRC risk. In addition, individuals with the *MTHFR* 677TT/*TSER* 3R3R or *MTHFR* 677/*TSER* 3R3R/*TS* 1494 0bp6bp+6bp6bp genotypes and diabetes mellitus (DM) were at an increased risk for CRC. Therefore, the data suggest that i) *MTHFR* polymorphisms combined with low plasma folate levels and ii) polymorphisms in folate metabolism-related genes combined with metabolic syndrome risk factors (hypertension and DM) increase the odds of developing CRC.

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Introduction

Folate is important for cell division and homeostasis due to its essential role in the synthesis of S-adenosyl-methionine, the methyl donor required for all methylation reactions in the cell. In addition to its function in cell homeostasis, folate has been hypothesized to play a role in carcinogenesis, particularly in colorectal cancer (CRC) development. Several mechanisms may underlie folate-deficiency mediated CRC, including DNA strand breaks, aberrant DNA methylation and impaired DNA repair. Thus, folate has been proposed as a possible candidate nutrient for CRC prevention. Genetic variants in folate metabolism-related genes may modulate levels of this vitamin and influence carcinogenesis risk. Thus far, several epidemiological studies have demonstrated that folate is one determinant of the CRC risk.

The effect of several polymorphic genes involved in folate metabolism, including methylenetetrahydrofolate reductase (*MTHFR*), thymidylate synthase (*TS*) and reduced folate carrier 1 (*RFC1*), on CRC risk has been investigated. MTHFR is a key enzyme in folate metabolism and DNA synthesis, as it catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate; the latter is the methyl donor for the conversion of homocysteine to methionine, while the former and its derivatives, are essential cofactors for thymidylate and *de novo* purine synthesis (1). The 2.2 kb gene encoding *MTHFR* is located on chromosome 1 and includes 11 exons. Despite the fact that several *MTHFR* polymorphisms have been identified, only two, 677C>T and 1298A>C, have been investigated in depth. The *MTHFR* C-to-T transition at nucleotide position 677 in exon 4 generates an alanine-to-valine substitution at amino acid 222. This substitution lies at the binding site for flavin adenine dinucleotide, an important cofactor for *MTHFR*. The A-to-C transversion at nucleotide 1298 (in exon 7) results in the substitution of glutamate for alanine at codon 429, which represents the S-adenosylmethionine regulatory domain of *MTHFR* (2). These two polymorphisms produce a thermolabile enzyme with reduced functional activity, which results in an altered intracellular distribution of folate substrates (3). A previous meta-analysis reported an association between the

677C>T polymorphism and decreased CRC risk, although the A1298C substitution was not associated with the CRC risk (4). However, the link between *MTHFR* polymorphisms and CRC risk has been inconsistent across populations and the statistical significance of such associations appears to be dependent on folate supplementation status (4,5).

TS is a key rate-limiting enzyme in folate metabolism, catalyzing the conversion of deoxyuridine monophosphate to deoxythymidine monophosphate (dTMP). This conversion is indispensable for the production of thymine, a nucleotide required for DNA synthesis and repair. Increased TS levels may lead to an accumulation of homocysteine and/or folate deficiency, while low TS levels impair dTMP synthesis. Two functionally important polymorphisms in the *TS* 5'- and 3'-untranslated regions (UTRs) have been well characterized. The first is a polymorphism that contains either triple (3R) or double (2R) repeats of a 28-base pair (bp) sequence in the *TS* enhancer region (*TSER*). The second is a 6-bp insertion/deletion polymorphism in the 3'UTR at position 1494. The *TSER* 3R and *TS* 1494 6-bp insertion alleles are associated with increased TS production (6,7). In addition to MTHFR and TS, RFC1 is also active in folate metabolism, aiding in the active transport of 5-methyltetrahydrofolate from the plasma to the cytosol. Several polymorphisms in the *RFC1* gene appear to influence its affinity for folate (8).

Thus, polymorphisms in the folate metabolism-related genes *MTHFR*, *TS* and *RFC1* may result in altered enzymatic activity, potentially affecting CRC risk. However, the associations detected between genetic variants in folate metabolism-related genes and CRC have been inconsistent across populations. This may be due to nutritional and clinical factors that contribute to the function of folate-related genes and CRC risk. Thus far, only small to moderate-sized studies have been conducted in Asian samples, limiting the conclusions that can be drawn. In the present study, polymorphisms in *MTHFR* [677C>T (rs1801133) and 1298A>C (rs1801131)], *TSER* (rs34743033), *TS* 1494del6 (rs16430) and *RFC1* [-43T>C (rs1131596), 80G>A (rs1051266) and 696C>T (rs12659)] were analyzed in patients with CRC and healthy controls. The aim was to clarify the role of these polymorphisms in CRC predisposition using stratified analyses.

Materials and methods

Study population. A case-control study was conducted of 991 individuals. Four hundred and seventy-seven patients diagnosed with CRC at CHA Bundang Medical Center (Seongnam, Korea) were enrolled between June 1996 and January 2009. The study only included CRC patients who had undergone surgical resection with a curative intent and who had histologically-proven adenocarcinoma. Tumor staging of CRCs was performed according to the sixth edition of the American Joint Committee on Cancer staging manual. The control group consisted of 514 randomly selected individuals, following a health screening at CHA Bundang Medical Center. The screening excluded patients with a history of thrombotic diseases or cancer. Patients with a high baseline blood pressure (systolic ≥140 mmHg or diastolic ≥90 mmHg) on more than one occasion or a history of antihypertensive medication were classified as having hypertension (HTN). Patients with high fasting plasma glucose (≥126 mg/dl), who took oral hypoglycemic agents, or with a history of insulin treatment were classified as having diabetes mellitus (DM). All the study subjects were Korean and provided written informed consent. The study protocol was approved by the Institutional Review Board of CHA Bundang Medical Center.

Genetic analysis. DNA was extracted from leukocytes using a G-DEX™ II Genomic DNA Extraction kit (Intron Biotechnology, Seongnam, Korea) according to the manufacturer's instructions. Nucleotide changes were determined by polymerase chain reaction (PCR)-restriction fragment length polymorphism analyses. Polymorphisms were characterized following the digestion of amplified DNA with the endonucleases *Hin*fI and *Fnu*4HI for the *MTHFR* 677C>T and 1298A>C polymorphisms. For the 677C>T polymorphism, an undigested PCR product (203 bp) indicated the homozygous wild-type genotype, while three bands of 203, 173 and 30 bp indicated the heterozygous genotype and two bands of 173 and 30 bp indicated the homozygous minor variant genotype. For the 1298A>C polymorphism, a single band of 138 bp indicated the homozygous wild-type genotype and two fragments of 119 and 19 bp indicated the homozygous minor variant genotype. The *TSER* polymorphism was genotyped according to amplified fragment size; 2R corresponded to a 220 bp amplicon and 3R corresponded to a 248 bp amplicon. The *TS* 1494del6 polymorphism was characterized using *Dra*I (New England BioLabs, Ipswich, MA, USA) digestion. The 6-bp insertion-containing 158-bp fragment was digested into 70 and 88 bp fragments following *Dra*I digestion, while the 152-bp fragment without the insertion was not digested. The *RFC1* -43T>C polymorphism was characterized by digesting the PCR product with *Tsp*RI (New England Biolabs). The C allele remained uncut (194 bp), whereas the T allele was cut into two fragments of 57 and 137 bp. The *RFC1* 80G>A polymorphism was analyzed by digesting the PCR product with *Hae*II (New England Biolabs). The A allele remained uncut (230 bp), whereas the G allele was cut into two fragments of 126 and 104 bp. The *RFC1* 696C>T polymorphism was analyzed by digesting the PCR products with *Eco*NI (New England Biolabs). The C allele remained uncut (341 bp), whereas the T allele was cut into two fragments of 163 and 178 bp. Primers and PCR conditions for each polymorphism analysis were as described previously (9-12). Genotyping of the seven sites was confirmed by sequencing 20% of the samples, chosen randomly. The concordance rate was 100%.

Measurement of plasma homocysteine and folate levels. Levels of plasma homocysteine and folate were measured in study participants after 12 hours of fasting. Homocysteine levels were determined using a fluorescence polarization immunoassay performed with the Abbott IMx analyzer (Abbott Laboratories, Abbott Park, IL, USA). Folate levels were determined using competitive immunoassays performed on the ACS 180 Chemiluminesence System (Bayer Diagnostics, Tarrytown, NY, USA).

Statistical analysis. χ^2 tests were used to assess differences between cases and controls with regard to categorical data, while Student's t-tests were employed for continuous data. The

Table I. Characteristics of colorectal cancer cases and controls.

Characteristics	Cases	Controls	P-value[a]
N	477	514	
Age, years[b]	62.34±11.86	62.01±11.91	0.661
Male gender, n (%)	276 (57.9)	266 (51.8)	0.054
Hypertension, n (%)	295 (61.8)	241 (46.9)	<0.001
Diabetes mellitus, n (%)	163 (34.2)	77 (15.0)	<0.001
Homocysteine[b], μmol/l	10.41±5.61 (n=442)	10.06±4.21 (n=508)	0.284
Folate[b], ng/ml	7.70±6.72 (n=440)	9.25±8.33 (n=414)	0.003
Tumor size ≥5cm, n (%)	278 (58.3)	-	
Lymph node invasion, n (%)	226 (47.4)	-	
Tumor site, n (%)			
Proximal colon	156 (32.7)	-	
Distal colon	112 (23.5)	-	
Mixed colon	7 (1.5)	-	
Rectum	197 (41.3)	-	
Unclassified	5 (1.0)		
TNM stage, n (%)			
I	49 (10.3)	-	
II	200 (41.9)	-	
III	191 (40.0)	-	
IV	37 (7.8)	-	

[a]χ^2 used for categorical data, two-sided t-test used for continuous data; [b]data are mean ± standard deviation. TNM, tumor-node-metastasis.

association between each genetic polymorphism and CRC risk was estimated using crude odds ratios, adjusted odds ratios (AORs) and a 95% confidence interval (CI) obtained from multivariate logistic regression models adjusted for age, gender, and HTN and DM status. Analyses were performed using GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA, USA), HAPSTAT 3.0 (University of North Carolina, Chapel Hill, NC, USA) and Medcalc version 12.1.1.0 (Medcalc Software, Mariakerke, Belgium). P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. Baseline characteristics of the study population are described in Table I. The proportion of males and females was 51.8 and 48.2% in the control group and 57.9 and 42.1% in the CRC case group, respectively. The mean age of the control and case groups was 62.01 and 62.34 years, respectively. There were no differences in the gender distribution and age between the two groups (P=0.054 and 0.661, respectively). Compared to the control group, CRC cases had a more frequent HTN and DM history (P<0.001). Among cases, 275 patients (57.7%) had colon cancer and 197 patients (41.3%) had rectal cancer. The type of cancer for 5 patients was unclassified due to mixed colon-rectal cancer. Pathological staging following curative resection was as follows: 49 (10.3%) stage I, 200 (41.9%) stage II, 191 (40.0%) stage III and 37 (7.8%) stage IV cancers.

Genotype frequency and association with CRC risk. Hardy-Weinberg test results confirmed that all the genotypes were in equilibrium (P>0.05). The distribution of genotypes and the AOR for each genetic polymorphism and CRC are shown in Table II. Overall, the distribution of the *MTHFR* gene polymorphisms between CRC cases and controls was not different. The AOR associated with the *MTHFR* 1298CC genotype was elevated but did not reach statistical significance (AOR=2.36; 95% CI, 0.94-5.97; P=0.069). No difference in the distribution of *TS* (*TSER* and 1494del6) and *RFC1* (-43T>C, 80G>A and 696C>T) genotypes between cases and controls was detected.

Association between MTHFR, TS and RFC1 polymorphisms and CRC risk in stratified analyses. Analyses were conducted that were stratified by age, gender, HTN, DM, tumor site, tumor size and lymph node (L/N) invasion status to evaluate the association between *MTHFR*, *TS* and *RFC1* polymorphisms and CRC risk under various conditions. The genotype frequencies of *MTHFR*, *TS* and *RFC1* polymorphisms in these strata are presented in Table III. The *MTHFR* 1298CC genotype was associated with an increased risk of CRC compared to the AA+AC genotypes in subjects >62 years (AOR=3.65; 95% CI, 1.04-12.73; P=0.043), females (AOR=4.62; 95% CI, 1.20-17.80; P = 0.026), patients with rectal cancer (AOR=3.27; 95% CI, 1.13-9.47; P=0.029) and patients with large tumors (≥5 cm) (AOR=3.63; 95% CI, 1.38-9.53; P=0.009) (Table III). Similarly, the *TSER* 2R3R+2R2R genotypes were associated with an increased risk of CRC compared to the 3R3R genotype in patients >62 years (AOR=1.63; 95% CI, 1.09-2.43; P=0.017). The 2R2R genotype was also associated with an increased CRC risk in patients without HTN (AOR=7.36; 95% CI, 1.44-37.58; P=0.016) or L/N invasion (AOR=2.81;

Table II. Prevalence of *MTHFR*, *TS* and *RFC1* polymorphisms in colorectal cancer cases and controls.

Genotypes	Controls (n=514)	Cases (n=477)	AOR (95% CI)[a]	P-value
MTHFR 677C>T				
CC	172 (33.5)	159 (33.3)	1.00 (reference)	
CT	265 (51.6)	248 (52.0)	0.99 (0.75-1.33)	0.971
TT	77 (15.0)	70 (14.7)	0.93 (0.62-1.40)	0.736
Dominant (CC vs. CT+TT)			0.98 (0.74-1.29)	0.882
Recessive (CC+CT vs. TT)			0.93 (0.65-1.34)	0.708
HWE P-value	0.125	0.091		
MTHFR 1298A>C				
AA	364 (70.8)	336 (70.4)	1.00 (reference)	
AC	143 (27.8)	125 (26.2)	0.96 (0.72-1.29)	0.784
CC	7 (1.4)	16 (3.4)	2.36 (0.94-5.97)	0.069
Dominant (AA vs. AC+CC)			1.03 (0.77-1.37)	0.846
Recessive (AA+AC vs. CC)			2.46 (0.98-6.20)	0.056
HWE P-value	0.089	0.305		
TSER 2R/3R				
3R3R	357 (69.5)	316 (66.2)	1.00 (reference)	
2R3R	147 (28.6)	148 (31.0)	1.08 (0.81-1.44)	0.579
2R2R	10 (1.9)	13 (2.7)	1.63 (0.69-3.85)	0.270
Dominant (3R3R vs. 2R3R+2R2R)			1.11 (0.84-1.47)	0.443
Recessive (3R3R+2R3R vs. 2R2R)			1.56 (0.66-3.66)	0.310
HWE P-value	0.248	0.379		
TS 1494 0bp/6bp				
0bp0bp	245 (47.7)	250 (52.4)	1.00 (reference)	
0bp6bp	225 (43.8)	183 (38.4)	0.80 (0.61-1.06)	0.115
6bp6bp	44 (8.6)	44 (9.2)	1.05 (0.66-1.68)	0.840
Dominant (0bp0bp vs. 0bp6bp+6bp6bp)			0.84 (0.65-1.09)	0.196
Recessive (0bp0bp+0bp6bp vs. 6bp6bp)			1.16 (0.74-1.82)	0.516
HWE P-value	0.447	0.215		
RFC1 -43T>C				
TT	108 (21.0)	102 (21.4)	1.00 (reference)	
TC	266 (51.8)	219 (45.9)	0.88 (0.63-1.25)	0.485
CC	140 (27.2)	156 (32.7)	1.18 (0.82-1.70)	0.368
Dominant (TT vs. TC+CC)			0.99 (0.72-1.36)	0.946
Recessive (TT+TC vs. CC)			1.28 (0.97-1.70)	0.086
HWE P-value	0.376	0.127		
RFC1 80G>A				
GG	103 (20.0)	96 (20.1)	1.00 (reference)	
GA	242 (47.1)	218 (45.7)	0.98 (0.69-1.39)	0.904
AA	169 (32.9)	163 (34.2)	1.05 (0.73-1.51)	0.775
Dominant (AA vs. AG+GG)			1.01 (0.73-1.39)	0.966
Recessive (AA+AG vs. GG)			1.05 (0.80-1.39)	0.709
HWE P-value	0.334	0.140		
RFC1 696C>T				
CC	110 (21.4)	105 (22.0)	1.00 (reference)	
CT	262 (51.0)	229 (48.0)	0.97 (0.69-1.36)	0.867
TT	142 (27.6)	143 (30.0)	1.07 (0.74-1.53)	0.729
Dominant (CC vs. CT+TT)			1.00 (0.73-1.37)	0.996
Recessive (CC+CT vs. TT)			1.09 (0.82-1.45)	0.562
HWE P-value	0.595	0.462		

[a]Models adjusted for age, gender, hypertension and diabetes mellitus status. HWE, Hardy-Weinberg equilibrium; *MTHFR*, methylenetetrahydrofolate reductase; *TS*, thymidylate synthase; *TSER*, *TS* enhancer region; *RFC1*, reduced folate carrier 1; AOR, adjusted odds ratio; CI, confidence interval.

95% CI, 1.17-6.76; P=0.021) (Table III). The *RFC1* -43CC genotype was associated with a significantly increased risk of CRC compared to the TT + TC genotypes in patients without DM (AOR=1.46; 95% CI, 1.06-2.02; P=0.020), with large

Table III. Stratified analysis of the association between folate-related gene polymorphisms and colorectal cancer risk.

Variables	Models	*MTHFR* 677C>T AOR (95% CI)[a]	P-value	*MTHFR* 1298A>C AOR (95% CI)[a]	P-value	*TSER* 2R/3R AOR (95% CI)[a]	P-value	*TS* 1494 0bp/6bp AOR (95% CI)[a]	P-value	*RFC1* -43T>C AOR (95% CI)[a]	P-value	*RFC1* 80G>A AOR (95% CI)[a]	P-value	*RFC1* 696C>T AOR (95% CI)[a]	P-value
Age, years															
≤62	D	0.88 (0.59-1.32)	0.545	1.05 (0.69-1.61)	0.809	0.76 (0.51-1.14)	0.187	0.69 (0.47-1.00)	0.052	0.75 (0.47-1.21)	0.239	0.73 (0.45-1.17)	0.191	0.74 (0.46-1.18)	0.204
	R	1.15 (0.67-1.97)	0.623	1.67 (0.39-7.21)	0.495	1.43 (0.31-6.58)	0.647	0.72 (0.37-1.43)	0.349	1.27 (0.85-1.92)	0.249	1.19 (0.80-1.77)	0.397	1.07 (0.71-1.62)	0.734
>62	D	1.32 (0.89-1.96)	0.170	1.05 (0.70-1.58)	0.802	1.63 (1.09-2.43)	0.017	1.04 (0.72-1.50)	0.837	1.41 (0.89-2.21)	0.141	1.55 (0.97-2.47)	0.066	1.50 (0.96-2.35)	0.076
	R	0.83 (0.50-1.38)	0.482	3.65 (1.04-12.73)	0.043	1.34 (0.46-3.96)	0.591	1.71 (0.90-3.25)	0.101	1.30 (0.86-1.96)	0.207	0.97 (0.65-1.44)	0.876	1.14 (0.76-1.73)	0.527
Gender															
Male	D	1.02 (0.70-1.48)	0.929	1.21 (0.81-1.80)	0.347	1.03 (0.70-1.49)	0.895	0.84 (0.59-1.19)	0.321	1.01 (0.67-1.52)	0.976	0.99 (0.65-1.50)	0.946	0.97 (0.65-1.46)	0.888
	R	0.78 (0.47-1.29)	0.334	1.39 (0.37-5.20)	0.624	0.98 (0.32-3.04)	0.972	1.26 (0.69-2.32)	0.449	1.35 (0.92-1.98)	0.129	1.14 (0.78-1.65)	0.499	1.16 (0.78-1.71)	0.460
Female	D	1.03 (0.68-1.55)	0.900	0.89 (0.58-.36)	0.588	1.21 (0.80-1.85)	0.369	0.83 (0.56-1.23)	0.358	1.05 (0.63-1.75)	0.861	1.12 (0.66-1.90)	0.677	1.16 (0.70-1.93)	0.560
	R	1.18 (0.69-2.02)	0.545	4.62 (1.20-17.80)	0.026	2.18 (0.59-8.08)	0.242	1.02 (0.51-2.07)	0.949	1.26 (0.82-1.94)	0.287	1.03 (0.68-1.56)	0.886	1.07 (0.69-1.64)	0.772
HTN															
No	D	0.90 (0.60-1.36)	0.623	0.76 (0.49-1.17)	0.208	1.19 (0.78-1.81)	0.424	1.01 (0.68-1.50)	0.972	0.88 (0.55-1.41)	0.603	0.79 (0.49-1.27)	0.329	0.90 (0.56-1.43)	0.653
	R	0.67 (0.37-1.23)	0.201	1.67 (0.48-5.80)	0.416	7.36 (1.44-37.58)	0.016	1.65 (0.84-3.28)	0.149	1.49 (0.96-2.30)	0.076	1.28 (0.84-1.95)	0.260	1.26 (0.80-1.97)	0.320
Yes	D	1.09 (0.75-1.59)	0.660	1.30 (0.88-1.94)	0.190	1.04 (0.72-1.52)	0.821	0.76 (0.53-1.07)	0.116	1.05 (0.68-1.63)	0.815	1.24 (0.79-1.93)	0.347	1.15 (0.75-1.77)	0.524
	R	1.07 (0.66-1.72)	0.787	3.63 (0.76-17.36)	0.106	0.65 (0.22-1.96)	0.444	0.88 (0.48-1.63)	0.691	1.10 (0.76-1.60)	0.616	0.88 (0.61-1.27)	0.504	0.97 (0.67-1.41)	0.878
DM															
No	D	0.93 (0.68-1.27)	0.627	1.03 (0.74-1.42)	0.867	1.21 (0.88-1.66)	0.248	0.81 (0.60-1.09)	0.156	0.94 (0.65-1.35)	0.730	0.94 (0.65-1.36)	0.738	0.96 (0.66-1.37)	0.804
	R	0.92 (0.60-1.40)	0.687	2.00 (0.68-5.92)	0.211	1.65 (0.67-4.11)	0.279	1.02 (0.62-1.69)	0.943	1.46 (1.06-2.02)	0.020	1.14 (0.84-1.56)	0.406	1.24 (0.90-1.72)	0.188
Yes	D	1.19 (0.68-2.11)	0.540	1.01 (0.56-1.82)	0.976	0.90 (0.51-1.57)	0.699	1.05 (0.61-1.82)	0.863	1.23 (0.65-2.34)	0.530	1.24 (0.64-2.39)	0.525	1.27 (0.68-2.38)	0.452
	R	0.93 (0.4-1.93)	0.841	3.76 (0.46-30.92)	0.218	0.84 (0.07-9.67)	0.891	2.34 (0.65-8.43)	0.194	0.84 (0.47-1.50)	0.548	0.84 (0.47-1.49)	0.555	0.75 (0.41-1.35)	0.330
Tumor site															
Colon	D	0.98 (0.71-1.36)	0.915	0.90 (0.64-1.26)	0.526	1.05 (0.76-1.46)	0.757	0.78 (0.58-1.06)	0.117	1.21 (0.82-1.78)	0.331	1.17 (0.79-1.73)	0.432	1.19 (0.82-1.75)	0.361
	R	0.90 (0.58-1.39)	0.631	2.01 (0.69-5.82)	0.198	0.95 (0.31-2.92)	0.932	1.21 (0.72-2.04)	0.479	1.36 (0.98-1.89)	0.065	1.08 (0.79-1.50)	0.620	1.17 (0.84-1.63)	0.359
Rectum	D	1.02 (0.71-1.46)	0.922	1.25 (0.86-1.80)	0.238	1.15 (0.80-1.65)	0.440	0.97 (0.69-1.36)	0.876	0.80 (0.54-1.20)	0.297	0.86 (0.57-1.30)	0.476	0.87 (0.58-1.29)	0.489
	R	1.01 (0.63-1.60)	0.982	3.27 (1.13-9.47)	0.029	1.97 (0.72-5.38)	0.186	1.14 (0.63-2.05)	0.665	1.11 (0.77-1.61)	0.578	0.97 (0.68-1.39)	0.879	0.93 (0.64-1.36)	0.702
Tumor size															
< 5cm	D	1.20 (0.83-1.73)	0.338	0.76 (0.51-1.12)	0.163	1.01 (0.70-1.45)	0.978	0.83 (0.59-1.17)	0.296	0.99 (0.65-1.49)	0.947	0.95 (0.63-1.44)	0.804	1.04 (0.69-1.56)	0.864
	R	1.25 (0.80-1.95)	0.337	1.17 (0.32-4.29)	0.816	1.59 (0.55-4.59)	0.393	0.66 (0.33-1.33)	0.247	1.00 (0.69-1.46)	0.989	0.89 (0.62-1.29)	0.544	0.90 (0.62-1.33)	0.609
≥ 5cm	D	0.86 (0.63-1.19)	0.370	1.28 (0.92-1.77)	0.141	1.17 (0.85-1.61)	0.348	0.86 (0.63-1.16)	0.311	1.00 (0.69-1.46)	0.984	1.08 (0.73-1.58)	0.709	0.99 (0.69-1.42)	0.948
	R	0.74 (0.47-1.16)	0.189	3.63 (1.38-9.53)	0.009	1.40 (0.52-3.81)	0.509	1.57 (0.96-2.56)	0.072	1.44 (1.05-1.99)	0.026	1.14 (0.83-1.57)	0.412	1.18 (0.85-1.64)	0.315
L/N invasion															
No	D	0.82 (0.59-1.14)	0.248	1.08 (0.77-1.52)	0.665	1.14 (0.81-1.58)	0.457	0.88 (0.65-1.21)	0.436	0.77 (0.53-1.11)	0.162	0.88 (0.60-1.28)	0.496	0.77 (0.53-1.11)	0.161
	R	1.00 (0.65-1.54)	0.994	2.32 (0.80-6.77)	0.123	2.81 (1.17-6.76)	0.021	1.00 (0.57-1.77)	0.994	1.04 (0.73-1.47)	0.838	0.81 (0.58-1.14)	0.237	0.88 (0.61-1.25)	0.466
Yes	D	1.23 (0.87-1.75)	0.249	1.00 (0.70-1.43)	1.000	1.05 (0.75-1.49)	0.766	0.80 (0.58-1.10)	0.173	1.39 (0.91-2.11)	0.129	1.22 (0.80-1.84)	0.354	1.42 (0.94-2.16)	0.100
	R	0.89 (0.56-1.40)	0.606	2.71 (0.94-7.76)	0.064	0.21 (0.03-1.65)	0.137	1.35 (0.79-2.32)	0.271	1.52 (1.08-2.14)	0.017	1.33 (0.95-1.85)	0.097	1.30 (0.92-1.84)	0.141

[a]Models adjusted for age, gender, hypertension and diabetes mellitus status. *MTHFR*, methylenetetrahydrofolate reductase; *TS*, thymidylate synthase; *TSER*, *TS* enhancer region; *RFC1*, reduced folate carrier 1; AOR, adjusted odds ratio; CI, confidence interval; D, dominant; R, recessive; HTN; hypertension; DM, diabetes mellitus; L/N, lymph node.

Table IV. Haplotype analysis of *MTHFR*, *TS* and *RFC1* genotypes and colorectal cancer risk.

Haplotypes	Controls (2n=1028)	Cases (2n=954)	OR (95% CI)	P-value[a]
MTHFR 677/1298				
C-A	452 (44.0)	409 (42.9)	0.956 (0.801-1.142)	0.650
C-C	157 (15.3)	157 (16.5)	1.093 (0.859-1.391)	0.498
T-A	419 (40.8)	388 (40.7)	0.996 (0.833-1.192)	1.000
TSER/TS 1494				
3R-0bp	660 (64.2)	620 (65.0)	1.035 (0.861-1.245)	0.742
3R-6bp	201 (19.6)	160 (16.8)	0.829 (0.659-1.043)	0.116
2R-0bp	55 (5.4)	63 (6.6)	1.251 (0.861-1.816)	0.255
2R-6bp	112 (10.9)	111 (11.6)	1.077 (0.815-1.423)	0.619
RFC1 -43/80/696				
T-G-C	429 (41.7)	392 (41.1)	0.974 (0.814-1.165)	0.784
T-G-T	9 (0.9)	2 (0.2)	0.238 (0.051-1.104)	0.067
T-A-C	35 (3.4)	28 (2.9)	0.858 (0.518-1.422)	0.609
T-A-T	9 (0.9)	1 (0.1)	0.119 (0.015-0.940)	0.022
C-G-C	6 (0.6)	10 (1.0)	1.804 (0.653-4.985)	0.317
C-G-T	4 (0.4)	5 (0.5)	1.349 (0.361-5.039)	0.746
C-A-C	12 (1.2)	9 (0.9)	0.806 (0.338-1.923)	0.667
C-A-T	524 (51.0)	507 (53.1)	1.091 (0.914-1.301)	0.345

[a]Models adjusted for age, gender, hypertension and diabetes mellitus status. *MTHFR*, methylenetetrahydrofolate reductase; *TS*, thymidylate synthase; ; *TSER*, *TS* enhancer region; *RFC1,* reduced folate carrier 1; OR, odds ratio; CI, confidence interval.

tumors (AOR=1.44; 95% CI, 1.05-1.99; P=0.026) or with L/N invasion (AOR=1.52; 95% CI, 1.08-2.14; P=0.017) (Table III).

Haplotype analysis. Haplotype analyses were conducted to evaluate the combined effect of polymorphisms on CRC risk (Table IV). Three haplotypes were constructed using the *MTHFR* 677C>T and 1298A>C polymorphisms, four were constructed using the *TSER* and *TS* 1494del6 polymorphisms and eight were constructed using the *RFC1* -43T>C, 80G>A and 696C>T polymorphisms. Haplotype analysis revealed no significant differences among the CRC cases and controls with regard to *MTHFR* and *TS* polymorphisms. However, the T-A-T *RFC1* -43/80/696 haplotype was associated with a decreased odds ratio of CRC (OR=0.119; 95% CI, 0.015-0.940; P=0.022).

Associations between folate metabolism-related polymorphisms and CRC risk, stratified by plasma folate levels, HTN and diabetes status. The combined effects of genotype and environmental factors (HTN, DM and plasma folate levels) on CRC risk were examined. HTN, DM and lower plasma folate levels were associated with increased CRC odds ratios in the present study population. In addition, several synergistic effects between genetic and environmental factors were observed for the CRC risk (Tables V and VI). Individuals with *MTHFR* 677CT/1298AC (OR=8.99; 95% CI, 1.76-45.93) or *MTHFR* 1298AC+CC/*TSER* 2R3R (OR=10.80; 95% CI, 1.39-83.76) genotypes and plasma folate levels ≤4.12 ng/ml had significantly higher odds of developing CRC (Table V). In addition, individuals with *MTHFR* 677TT/*TSER* 3R3R (OR=5.99; 95% CI, 1.94-18.45) or *MTHFR* 677/*TSER* 3R3R/*TS* 1494 0bp6bp+6bp6bp (OR=20.13; 95% CI, 1.76-230.92) genotypes, as well as DM had significantly higher odds of CRC (Table VI).

Discussion

In the present large hospital-based, case-control study, whether the *MTHFR*, *TS* and *RFC1* polymorphisms were associated with CRC susceptibility was investigated in the Korean population. Overall, the *MTHFR*, *TS* and *RFC1* polymorphisms were not associated with increased CRC risk. However, these polymorphisms were associated with heightened risk in the presence of various clinical or environmental conditions, such as low folate status, HTN or DM.

Numerous studies have investigated the association between the *MTHFR* gene polymorphisms and CRC susceptibility, but the results have been inconsistent. Previously, a comprehensive meta-analysis demonstrated that the *MTHFR* 677T allele is associated with reduced risk of CRC (13). In addition, Yang *et al* (14) conducted a meta-analysis incorporating 21 studies of the Asian population and obtained similar results. Although the mechanism responsible for the association between the *MTHFR* 677C>T polymorphism and CRC risk remains unclear, it is possible that *MTHFR* variants increase the level of 5,10-methylenetetrahydrofolate available for DNA synthesis, thereby protecting against CRC. In contrast to these meta-analysis results, a protective effect associated with the *MTHFR* 677C>T polymorphism was not identified. However, among the 61 studies considered in the comprehensive meta-analysis cited, only 4 showed a protective effect, while 53 did not show any significant association between *MTHFR* 677C>T status and CRC risk. A few studies have also demonstrated an association between *MTHFR* 1298A>C status and CRC (5). Collectively, the available research suggests that the *MTHFR* 677C>T and 1298A>C polymorphisms have a weak impact on CRC risk, absent consideration of interplay with nutritional or environmental factors. Previously,

Table V. Adjusted odds ratios for polymorphisms in folate-related genes and colorectal cancer, stratified by plasma folate concentrations[a].

	Plasma folate quintiles, ng/ml				
Genotypes	>11	>7.72 to ≤11	>5.76 to ≤7.72	>4.12 to ≤5.76	≤4.12
N	170	170	172	170	172
Total	1.00 (reference)	1.10 (0.69-1.73)	1.34 (0.85-2.11)	1.54 (0.98-2.42)	3.34 (2.06-5.43)
MTHFR 677C>T					
CC	1.00 (reference)	0.62 (0.29-1.32)	1.09 (0.50-2.35)	1.12 (0.51-2.47)	3.23 (1.24-8.39)
CT+TT	1.00 (reference)	1.53 (0.85-2.78)	1.48 (0.83-2.62)	1.85 (1.06-3.25)	3.68 (2.04-6.63)
MTHFR 1298A>C					
AA	1.00 (reference)	1.25 (0.72-2.18)	1.31 (0.76-2.25)	1.38 (0.81-2.38)	2.69 (1.52-4.77)
AC+CC	1.00 (reference)	0.67 (0.28-1.62)	1.41 (0.61-3.28)	1.85 (0.77-4.44)	6.10 (2.27-16.44)
TSER 2R/3R					
3R3R	1.00 (reference)	1.19 (0.68-2.09)	1.11 (0.63-1.95)	1.62 (0.93-2.83)	3.09 (1.72-5.55)
2R3R+2R2R	1.00 (reference)	0.96 (0.43-2.16)	1.96 (0.90-4.27)	1.38 (0.62-3.04)	3.99 (1.63-9.74)
TS 1494 0bp/6bp					
0bp0bp	1.00 (reference)	1.32 (0.66-2.65)	1.40 (0.72-2.69)	1.35 (0.72-2.54)	3.57 (1.71-7.47)
0bp6bp+6bp6bp	1.00 (reference)	1.01 (0.54-1.90)	1.33 (0.70-2.51)	1.65 (0.85-3.21)	3.45 (1.77-6.73)
MTHFR 677/1298					
CC/AA	1.00 (reference)	0.50 (0.16-1.55)	0.47 (0.13-1.66)	0.35 (0.09-1.27)	2.59 (0.51-13.12)
CC/AC+CC	1.00 (reference)	0.59 (0.19-1.87)	1.90 (0.61-5.90)	2.69 (0.85-8.56)	4.79 (1.34-17.13)
CT+TT/AA	1.00 (reference)	1.70 (0.87-3.33)	1.56 (0.82-2.98)	1.95 (1.04-3.64)	3.13 (1.64-5.98)
CT/AC	1.00 (reference)	0.92 (0.22-3.92)	1.08 (0.27-4.34)	1.14 (0.27-4.82)	8.99 (1.76-45.93)
MTHFR 677/*TSER*					
CC/3R3R	1.00 (reference)	0.88 (0.35-2.18)	0.65 (0.24-1.78)	1.91 (0.70-5.19)	2.19 (0.73-6.62)
CC/2R3R+2R2R	1.00 (reference)	0.22 (0.03-1.66)	2.50 (0.60-10.49)	0.40 (0.09-1.81)	10.35 (0.83-129.72)
CT+TT/3R3R	1.00 (reference)	1.49 (0.71-3.12)	1.34 (0.66-2.74)	1.60 (0.81-3.20)	3.66 (1.78-7.50)
CT+TT/2R3R+2R2R	1.00 (reference)	1.67 (0.59-4.70)	1.83 (0.67-4.99)	2.71 (0.97-7.61)	4.96 (1.51-16.28)
MTHFR 1298/*TSER*					
AA/3R3R	1.00 (reference)	1.64 (0.82-3.27)	1.24 (0.62-2.49)	1.48 (0.75-2.89)	2.86 (1.42-5.73)
AA/2R3R+2R2R	1.00 (reference)	0.82 (0.30-2.25)	1.60 (0.64-3.97)	1.19 (0.47-3.04)	2.54 (0.87-7.39)
AC+CC/3R3R	1.00 (reference)	0.55 (0.18-1.67)	1.02 (0.36-2.84)	2.03 (0.70-5.86)	4.48 (1.37-14.70)
AC+CC/2R3R	1.00 (reference)	0.89 (0.17-4.65)	6.97 (0.89-54.71)	1.57 (0.28-8.86)	10.80 (1.39-83.76)
MTHFR 1298/*TS* 1494					
AA/0bp0bp	1.00 (reference)	1.47 (0.65-3.33)	1.35 (0.62-2.95)	1.35 (0.63-2.88)	3.47 (1.44-8.34)
AA/0bp6bp+6bp6bp	1.00 (reference)	1.13 (0.52-2.46)	1.30 (0.59-2.87)	1.32 (0.60-2.93)	2.23 (0.99-5.04)
AC+CC/0bp0bp	1.00 (reference)	0.81 (0.17-3.91)	1.30 (0.36-4.68)	1.52 (0.44-5.28)	4.24 (0.93-19.38)
AC+CC/ 0bp6bp+6bp6bp	1.00 (reference)	0.69 (0.21-2.25)	1.45 (0.45-4.62)	2.48 (0.61-10.04)	8.85 (2.23-35.16)
TSER/*TS* 1494					
3R3R/0bp0bp	1.00 (reference)	1.79 (0.79-4.07)	1.43 (0.66-3.07)	1.76 (0.85-3.65)	4.07 (1.75-9.47)
3R3R/0bp6bp+6bp6bp	1.00 (reference)	0.99 (0.42-2.38)	0.82 (0.34-1.98)	1.22 (0.47-3.18)	2.70 (1.07-6.79)
2R3R+2R2R/0bp0bp	1.00 (reference)	0.63 (0.11-3.69)	2.79 (0.53-14.67)	0.42 (0.08-2.11)	2.79 (0.40-19.67)
2R3R+2R2R/ 0bp6bp+6bp6bp	1.00 (reference)	1.15 (0.44-3.04)	2.38 (0.90-6.29)	2.39 (0.88-6.47)	4.77 (1.64-13.86)

[a]Models adjusted for age, gender, hypertension, diabetes mellitus and plasma folate concentrations. Results for genetic combinations associated with ORs lower than that found for the total sample in the lowest quintile (folate ≤4.12 ng/ml) are not shown. *MTHFR*, methylenetetrahydrofolate reductase; *TS*, thymidylate synthase; *TSER*, *TS* enhancer region; ORs, odds ratios.

Ma *et al* (15) demonstrated that the association of the *MTHFR* 677C>T polymorphism with the CRC risk is modulated by diet-supplied methyl levels. This study identified that the protective effect of the polymorphism was absent in subjects with plasma folate levels ≥3 ng/ml or low alcohol consumption (15). Keku *et al* (5) also reported interactions between folate intake and two *MTHFR* polymorphisms. In their study, in individuals who have low dietary folate intake, the *MTHFR* 677CT+TT genotype was associated with an increased CRC risk. Similarly, the *MTHFR* 1298AA genotype in individuals with low dietary folate intake was associated with significantly increased CRC risk, due to linkage disequilibrium between the *MTHFR* 677T and 1298A alleles (5). As the present study did not consider the individual participant dietary habits, the impact of folate intake on CRC risk could not be assessed. However, the interplay between *MTHFR* polymorphisms and

Table VI. Adjusted odds ratios for polymorphisms in folate-related genes and colorectal cancer, stratified by hypertension and diabetes mellitus status[a].

	Hypertension		Diabetes mellitus	
Genotypes	No	Yes	No	Yes
N	455	536	751	240
Total	1.00 (reference)	1.72 (1.31-2.26)	1.00 (reference)	2.73 (2.00-3.73)
MTHFR 677C>T				
CC+CT	1.00 (reference)	1.59 (1.19-2.14)	1.00 (reference)	2.73 (1.94-3.83)
TT	1.00 (reference)	2.74 (1.30-5.79)	1.00 (reference)	3.16 (1.41-7.12)
MTHFR 1298A>C				
AA	1.00 (reference)	1.52 (1.10-2.09)	1.00 (reference)	2.77 (1.91-4.04)
AC+CC	1.00 (reference)	2.52 (1.49-4.26)	1.00 (reference)	2.67 (1.51-4.73)
TSER 2R/3R				
3R3R	1.00 (reference)	1.90 (1.36-2.65)	1.00 (reference)	3.07 (2.07-4.56)
2R3R+2R2R	1.00 (reference)	1.41 (0.88-2.27)	1.00 (reference)	2.26 (1.35-3.80)
TS 1494 0bp/6bp				
0bp0bp	1.00 (reference)	2.08 (1.41-3.06)	1.00 (reference)	2.50 (1.62-3.87)
0bp6bp+6bp6bp	1.00 (reference)	1.41 (0.96-2.08)	1.00 (reference)	3.10 (1.97-4.88)
MTHFR 677/1298				
CC+CT/AA	1.00 (reference)	1.30 (0.90-1.86)	1.00 (reference)	2.80 (1.82-4.30)
CC+CT/AC+CC	1.00 (reference)	2.52 (1.49-4.26)	1.00 (reference)	2.67 (1.51-4.73)
TT/AA	1.00 (reference)	2.74 (1.30-5.79)	1.00 (reference)	3.16 (1.41-7.12)
MTHFR 677/*TSER*				
CC+CT/3R3R	1.00 (reference)	1.85 (1.30-2.65)	1.00 (reference)	2.84 (1.85-4.35)
CC+CT/2R3R+2R2R	1.00 (reference)	1.12 (0.67-1.89)	1.00 (reference)	2.57 (1.44-4.58)
TT/3R3R	1.00 (reference)	2.04 (0.78-5.33)	1.00 (reference)	5.99 (1.94-18.45)
TT/2R3R+2R2R	1.00 (reference)	4.63 (1.26-16.96)	1.00 (reference)	1.60 (0.41-6.24)
MTHFR 677/*TS* 1494				
CC+CT/0bp0bp	1.00 (reference)	2.19 (1.44-3.33)	1.00 (reference)	2.51 (1.56-4.04)
CC+CT/0bp6bp+6bp6bp	1.00 (reference)	1.15 (0.76-1.75)	1.00 (reference)	3.07 (1.86-5.07)
TT/0bp0bp	1.00 (reference)	1.18 (0.39-3.50)	1.00 (reference)	2.60 (0.78-8.65)
TT/0bp6bp+6bp6bp	1.00 (reference)	5.22 (1.72-15.84)	1.00 (reference)	3.81 (1.19-12.22)
MTHFR 677/*TSER*/*TS* 1494				
CC+CT/3R3R/0bp0bp	1.00 (reference)	2.56 (1.61-4.09)	1.00 (reference)	3.03 (1.74-5.25)
CC+CT/3R3R/0bp6bp+6bp6bp	1.00 (reference)	1.16 (0.65-2.07)	1.00 (reference)	2.61 (1.30-5.26)
CC+CT/2R3R+2R2R/0bp0bp	1.00 (reference)	0.92 (0.32-2.61)	1.00 (reference)	1.29 (0.46-3.68)
CC+CT/2R3R+2R2R/0bp6bp+6bp6bp	1.00 (reference)	1.14 (0.61-2.10)	1.00 (reference)	3.55 (1.71-7.37)
TT/3R3R/0bp0bp	1.00 (reference)	0.97 (0.28-3.40)	1.00 (reference)	3.97 (0.95-16.58)
TT/3R3R/0bp6bp+6bp6bp	1.00 (reference)	5.21 (0.87-31.18)	1.00 (reference)	20.13 (1.76-230.92)
TT/2R3R+2R2R/0bp0bp	1.00 (reference)	0.41 (0.01-17.12)	1.00 (reference)	0.36 (0.01-9.49)
TT/2R3R+2R2R/0bp6bp+6bp6bp	1.00 (reference)	12.05 (1.78-81.53)	1.00 (reference)	1.71 (0.28-10.42)

[a]Models adjusted for age, gender, hypertension, diabetes mellitus and genotypes. The results of genetic factors and combinations containing lower OR values compared to total (hypertension or diabetes mellitus) were eliminated. *MTHFR*, methylenetetrahydrofolate reductase; *TS*, thymidylate synthase; *TSER*, *TS* enhancer region.

plasma folate concentrations was analyzed. Individuals with the *MTHFR* 677CT/1298AC genotypes and plasma folate levels ≤4.12 ng/ml were more likely to develop CRC. The enzyme activity of the *MTHFR* 677CT/AC and 677TT/AA variants is significantly lower than that of *MTHFR* encoded by other genotypes (16). These results suggest that *MTHFR* polymorphisms in the presence of low dietary folate can increase the CRC risk.

As the 3R allele of *TSER* and the 6-bp insertion allele of *TS* 1494 are believed to increase TS expression (7,17,18), numerous studies on *TS* polymorphisms and cancer susceptibility have been performed. However, the results of these studies are inconclusive. A recent meta-analysis did not show any association between these *TS* polymorphisms (*TSER* and *TS* 1494del6) and cancer risk (19). However, stratified analyses demonstrated that the *TSER* 2R2R genotype was associated with a significantly increased risk of cancer in the Asian population, but not in Caucasians. Similarly, the 2R2R and 2R3R genotypes were associated with reduced CRC risk in the Caucasian, but not Asian, population (19). As only two studies

have been performed in Asian populations, these results may not be representative of all Asian populations. Several factors may contribute to the finding that *TS* polymorphisms have differential associations with the CRC risk depending on ethnicity. First, genotype frequency may play an important role, as Caucasians have relatively high frequencies of the *TSER* 2R2R and *TS* 6bp6bp genotypes, while Asians have high frequencies of the 3R3R and 0bp0bp genotypes (11,20-23). Second, cultural factors that influence nutritional status may confound the effect of these polymorphisms on CRC susceptibility. In the present study, the 2R2R variant genotype, particularly in elderly patients, non-HTN patients and non-L/N invasion patients, was associated with a significantly increased risk of CRC. Although these results indicate the *TSER* polymorphism in CRC development, due to the low frequency of the 2R allele in Korea, replicating this finding in a larger Asian sample is necessary.

RFC1 is one of the key enzymes in folate metabolism. It is biologically plausible that *RFC1* polymorphisms play a role in the development of cancer by altering plasma folate and homocysteine levels (8). Several studies have demonstrated an association between the *RFC1* 80G>A polymorphism and cancer (24,25), although no association with CRC risk has been found (23,26,27). Consistent with these studies, a significant association between the *RFC1* 80G>A polymorphism and CRC risk or between the *RFC1* -43T>C and 696C>T polymorphisms and CRC were not identified. Although *RFC1* polymorphisms affect plasma folate levels, it is unlikely that they play a dominant role in folate metabolism in CRC cases. This hypothesis is supported by a previous study that RFC1 mediates the transport of naturally-occurring folate rather than folic acid, the latter representing the form of the vitamin found in supplements (28). Since folic acid is absorbed better by the body than the natural form and comprise a dominant proportion of the total folate intake, genetic variants of *RFC1* polymorphisms may have limited potential effect to the CRC risk. Additionally, interplay between *RFC1* polymorphisms and plasma folate levels did not influence CRC risk in the present study (data not shown). Thus, it is unlikely that RFC1 variants significantly affect the CRC risk.

Finally, a combined effect of environmental factors (HTN and DM) and folate metabolism-related polymorphisms were observed on the CRC risk. Of note, synergistic effects were identified between i) the *MTHFR* 677TT and *MTHFR* 677TT/*TSER* 3R3R genotypes and HTN, and ii) the *MTHFR* 677TT, *MTHFR* 677TT/*TSER* 3R3R and *MTHFR* 677TT/*TSER* 3R3R/*TS* 1494 0bp6bp+6bp6bp genotypes and DM. At present, the interactions between these genetic variants, HTN and DM are poorly understood. However, it is possible these polymorphisms could cause folate deficiency, a CRC risk factor (29), and the presence of HTN or DM, risk factors for metabolic syndrome, could accelerate CRC development (30). Thus, interactions between genetic factors causing folate deficiency and metabolic syndrome risk factors (HTN and DM) could result in increased CRC susceptibility. This hypothesis merits testing in a large, population-based study.

In conclusion, the association between seven *MTHFR*, *TS* and *RFC1* polymorphisms and CRC susceptibility was investigated in a Korean population. Although these polymorphisms were not independent risk factors for CRC, a number of significant associations were identified in subsets of the population. The data suggest that the CRC risk is increased by i) *MTHFR* polymorphisms in the presence of low plasma folate levels and ii) genetic risk factors for folate deficiency in the presence of metabolic syndrome risk factors, such as HTN and DM.

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