Genetic Polymorphisms in Folate Metabolism and the Risk of Stomach Cancer

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Abstract

Folate deficiency has been implicated in the etiology of stomach cancer through abnormal DNA methylation and disrupted DNA synthesis and repair. Enzyme-coding genes involved in folate metabolism are often polymorphic. In a population-based study of 305 cases and 427 controls in Warsaw, Poland, we evaluated the risk of stomach cancer in relation to polymorphisms in folate-metabolizing genes, including *MTHFR* (Ex5+79C>T and Ex8−62A>C), *MTR* (Ex26−20A>G), and *MTRR* (Ex2−64A>G, Ex5+123C>T, Ex15+572C>T, Ex15−405A>T, Ex9−85C>T, Ex15−526G>A, and Ex14+14C>T). Polymorphisms in the *MTHFR* gene were not associated with stomach cancer risk. No notable effect was found for polymorphisms in *MTR* or *MTRR* either, although *MTR* Ex26−20 A>G and *MTRR* Ex5+123C>T polymorphisms were associated with a borderline increased risk of stomach cancer (*MTR* Ex26−20A>G, AG/GG versus AA: odds ratio, 1.35; 95% confidence interval, 0.96-1.90; *MTRR* Ex5+123C>T, CT/TT versus CC: odds ratio, 1.30; 95% confidence interval, 0.93-1.82). We did not find significant interactions between polymorphisms in *MTHFR*, *MTR*, and *MTRR* genes and dietary folate and alcohol consumption. Our study did not identify strong genetic determinants in the folate metabolism pathway for stomach cancer risk. (Cancer Epidemiol Biomarkers Prev 2007;16(1):115–21)

Introduction

Vegetable and fruit intake has been long associated with a decreased risk of stomach cancer (1-3). Folate is an important constituent of vegetables and fruits. It functions as the coenzyme in the biosynthesis of thymidylate and purines (4-6). Insufficient folate status could increase DNA damage and instability by uracil misincorporation into DNA (5, 7, 8). Sufficient folate is also required to maintain an adequate cellular pool of *S*-adenosylmethionine, the universal methyl donor for DNA methylation (5, 6, 9). Folate deficiency may result in abnormal DNA methylation and uncontrolled gene expression leading to malignant transformation (5, 8, 10). Epidemiologic studies have suggested that diminished folate status increased the risk of cancer in various sites, including the stomach (6, 11-15).

Most enzyme-coding genes involved in folate metabolism are polymorphic. Methylenetetrahydrofolate reductase (*MTHFR*) irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (16, 17). 5-Methyltetrahydrofolate is the main circulating form of folate, which donates a methyl group to homocysteine to form methionine, a precursor of *S*-adenosylmethionine. Two polymorphisms identified in the *MTHFR* gene (Ex5+79C>T and Ex8−62A>C) are associated with reduced enzyme activities (16, 18). The remethylation of homocysteine to methionine is catalyzed by methionine synthase (*MTR*). A polymorphism causing an *A*-to-*G* change in the protein-binding region of *MTR* (Ex26−20A>G) results in the substitution of aspartic acid with glycine (18, 19). The change to nonpolar amino acid (glycine) may perturb the three-dimensional structure of the protein and affect the enzyme function (19). *MTR* is maintained in its active form by methionine synthesis reductase (*MTRR*). A reported polymorphism in the *MTRR* gene (Ex2−64A>G) is an *A*-to-*G* substitution that leads to a change of isoleucine to methionine (18, 19).

Existing studies evaluating the effect of polymorphisms in folate-metabolizing genes on stomach cancer risk have yielded inconsistent results (20-26). Most studies have assessed only a limited number of polymorphisms. Few have investigated the genetic polymorphisms in conjunction with dietary risk factors that may modify the genetic effect. We conducted a population-based case-control study in Poland, which has one of the highest stomach cancer incidences in the world (27). The typical Polish diet before 1999 is characterized by a low intake of fresh vegetables and fruits (28) and high per capita consumption of alcohol (29), which may have contributed to the high stomach cancer rates in Poland. These characteristics render this population particularly important for evaluating the etiology of stomach cancer. In the present study, we examined the association between 10 genetic polymorphisms in three folate-metabolizing genes (*MTHFR*, *MTR*, and *MTRR*) and stomach cancer risk and explored the potential effect modification by dietary folate intake and alcohol consumption.

Materials and Methods

Study Design. The study design has been described elsewhere (28, 29). Briefly, a population-based case-control study was conducted in the city of Warsaw to determine risk factors contributing to the high stomach cancer incidence in Poland. Newly diagnosed stomach cancer cases were

Received 6/28/06; revised 9/25/06; accepted 10/13/06.

Grant support: Intramural Research Program of the NIH, Division of Cancer Epidemiology and Genetics.

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doi:10.1158/1055-9965.EPI-06-0513

identified among Warsaw residents ages 21 to 79 years between March 1, 1994 and April 30, 1996 by collaborating physicians in each of the 22 hospitals serving the study area. A total of 72 clinics and endoscopic departments within the hospitals and 8 private endoscopic units were covered. Diagnostic information was abstracted in a standardized manner from hospital records and endoscopy, surgical, and pathology reports by a collaborating physician or by the study physician. All pathologic slides were reviewed for confirmation of the diagnosis by two pathologists. Controls were randomly selected between March 1, 1994 and April 30, 1996 among Warsaw residents from a computerized registry of all legal residents in Poland (the Polish Electronic System of Residence Evidence) and frequency matched to cases by gender and age in 5-year strata.

Among 515 eligible cases and 549 eligible controls identified, in-person interviews were conducted with 324 (62.9%) cases and 480 controls (87.4%) to elicit detailed information on demographic background and selected exposures including lifetime tobacco and alcohol use 2 years before cancer diagnosis or interview. Proxy interviews were conducted with next of kin of 140 deceased cases (27.2%). The median length between cancer diagnosis and interview was 42 days for directly interviewed cases and 247 days for interviews with next of kin. An ever smoker was defined as a smoker of at least one cigarette per day for ≥6 months. An ever drinker was defined as a drinker of at least one serving of beer, wine, or liquor per month for ≥6 months. Dietary intake was assessed using a modified version of the Block food frequency questionnaire. Usual frequency of intake before 1990, a year of political and economic changes in Poland resulting in significant increases in food selection and availability, was assessed for 118 food and beverage items. Nutrient intake was estimated from the weekly consumption of food items, the average portion size, and the nutrient composition of each food item. Total intake of each nutrient was then summed across all food items. Nutrient content of each food was estimated from Polish food tables if available, or from the National Cancer Institute's DietSys database derived from the U.S. Department of Agriculture food composition data. For several unique Polish complex dishes, original recipes were used to calculate food components and nutrients (28).

Genotyping. A 30-mL blood sample was collected from 345 cases (74.4%) and 442 controls (92.1%) who completed the interview. We examined 10 single nucleotide polymorphisms, both synonymous and nonsynonymous, that were located in the coding region of the *MTHFR, MTR*, and *MTRR* genes. Of these, two commonly reported polymorphism in the *MTHFR* gene, including Ex5+79C>T (*MTHFR* C677T; rs1801133) and Ex8−62A>C (*MTHFR* A1298C; rs1801132), and one polymorphism in the *MTR* gene, Ex26−20A>G (*MTR* A2756G; rs1805087), were analyzed. We also examined seven polymorphisms in the *MTRR* gene, including one previously reported polymorphism, Ex2−64A>G (*MTRR* A66G; rs1801394), and six polymorphisms that have not been reported in prior literature, including Ex5+123C>T (rs1532268), Ex15+572C>T (rs9282787), Ex15−405A>T (rs8659), Ex9−85C>T (rs2287780), Ex15−526G>A (rs9332), and Ex14+14C>T (rs10380). The 10 single nucleotide polymorphisms in these genes were selected based on evidence reported in the literature regarding the function and disease associations of each single nucleotide polymorphism and the characteristics of the polymorphisms. Synonymous single nucleotide polymorphisms in coding region were included to improve the coverage of the candidate genes.

Genotyping was done by Taqman essay (Applied Biosystems, Foster City, CA) using a 384-well plate and analyzed on an ABI 7900HT sequence detection system, plotted with SDS software. Assays were validated and optimized as described in the SNP500 Cancer web site.[7] Assay-specific primer/probe concentrations and thermocycling conditions are also available on the web site. Internal laboratory quality controls included four of each of the Coriell DNA samples containing homozygous major allele, heterozygous and homozygous minor allele genotypes for each polymorphism, and four no-template controls in every 384 samples. Approximately 8% blind quality control samples from two individuals were interspersed with the study samples, showing >99% concordance. The successful genotyping rates for *MTHFR* Ex5+79C>T, *MTHFR* Ex8−62A>C, *MTR* Ex26−20A>G, *MTRR* Ex2−64A>G, *MTRR* Ex5+123C>T, *MTRR* Ex15+572C>T, *MTRR* Ex15−405A>T, *MTRR* Ex9−85C>T, *MTRR* Ex15−526G>A, and *MTRR* Ex14+14C>T were 94.8%, 94.4%, 96.4%, 96.7%, 96.4%, 95.8%, 95.4%, 96.4%, 95.1%, and 96.6%, respectively.

Statistical Analysis. We applied SAS/Genetics (SAS 9.1, SAS Institute, Cary, NC) to investigate the potential deviations from the Hardy-Weinberg equilibrium of each polymorphism and assess the pairwise linkage disequilibrium between markers within each gene. Unconditional logistic regression was employed to calculate the odds ratios (OR) and 95% confidence intervals (95% CI) associated with each genotype (30). For all genotypes, the homozygote of the common allele was used as the reference.

We used Phase v.2.1 (31, 32) and SAS/Genetics to estimate haplotype and diplotype frequencies in controls. The ORs and 95% CIs associated with haplotypes/diplotypes were estimated using unconditional logistic regression. The haplotype comprising common alleles was treated as the reference. The diplotype that has no minor alleles in both chromosomes was treated as the reference. Polytomous logistic regression model was applied to comparing cases by Lauren histologic classification (intestinal versus diffuse type), anatomic subsite (cardia versus noncardia), and tumor stage (localized or regional metastasized tumors versus displaced metastasized tumors) for each polymorphism (33).

When assessing potential effect modification by dietary folate intake, the reference group include individuals who did not carry the ''risk'' allele and consumed more than the median level of dietary folate (108.6 μg per day per 1,000 kcal). When evaluating potential effect modification by alcohol drinking, the reference was individuals who did not carry the ''risk'' allele and were nondrinkers. Drinkers were defined as individuals who drank alcohol ≥1 drink per month for ≥6 months. In addition, the sensitivity of the interaction results was evaluated with drinkers defined as individuals consuming ≥1 drink per week or ≥3 drinks per week for ≥6 months. The likelihood ratio test was used to test for multiplicative interaction (34). A prior list of confounders, including education, cigarette smoking, alcohol drinking, and consumption of raw vegetables and fruits, were tested and included in final models if they changed the estimates on effect of polymorphisms of >10%. Density variables (intake per 1,000 kcal) were created to adjust for caloric intake (35), which correspond to the effect of increasing the percentage of nutrient intake while keeping total energy intake constant.

Results

In 464 cases and 480 controls, those who did (345 cases and 442 controls) and did not donate a blood sample (119 cases and 38 controls) were comparable with regard to age, gender, education, family history of stomach cancer, alcohol drinking, intake of fresh vegetables and fruits intake, and body mass index (data not shown). However, cases with blood samples

[7] http://snp500cancer.nic.nih.gov.

were less likely to have distant metastasis (57%) than patients who did not donate a blood sample (70%; $P < 0.0001$), most of whom died before they could be contacted by a phlebotomist. Cases who donated a blood sample were more likely to be smokers, whereas controls with blood samples tended to be nonsmokers.

Among 345 cases and 442 controls who donated a blood sample, genotyping results were available in 305 cases (88.4%) and 427 controls (96.6%). In this latter group, case and controls did not differ in age, gender, dietary folate intake, total caloric intake, and body mass index (Table 1). However, cases tended to have a lower education, positive family history of stomach cancer, higher proportion of smokers and drinkers, and lower consumption of raw vegetables and fruits. These results were similar to those previously reported for the complete sets of cases and controls in this study, regardless of genotyping data (28, 29). The majority of stomach cancer cases was intestinal type (67.5%) and located in noncardia stomach (73.1%).

Among controls, frequencies of the T allele in *MTHFR* Ex5+79C>T polymorphism, the *C* allele in *MTHFR* Ex8−62A>C polymorphism, and the *G* allele in *MTR* Ex26−20A>G polymorphism were 31%, 33%, and 20%, respectively, and were comparable with previously reported allele frequencies among Caucasians (ref. 18; Table 2). The frequency of the G allele in *MTRR* Ex2−64A>G polymorphism was 58%, slightly higher than that reported in other Caucasian populations (50-53%; refs. 18, 36, 37). Among six polymorphisms in *MTRR* that have not been reported previously, the Ex5+123C>T and Ex15−405A>T polymorphisms were common (*T* alleles: 32% and 38%, respectively) followed by Ex15+572C>T (*T* allele: 20%). Polymorphisms of Ex9−85C>T, Ex15−526G>A, and Ex14+14C>T were less frequent (minor alleles: 6-9%). The genotype distributions of each of the 10 assessed polymorphisms in controls were in agreement with the Hardy-Weinberg equilibrium. The two polymorphisms in the *MTHFR* gene were in linkage disequilibrium ($R^2 = 0.0720$). Linkage disequilibrium measures of polymorphisms in the *MTRR* gene were generally high, suggesting a departure from interlocus equilibrium between these polymorphisms (Table 3).

As shown in Table 2, *MTHFR* Ex5+79C>T and Ex8−62A>C polymorphisms were not associated with stomach cancer risk. No notable effect was found for polymorphisms in the *MTR* or *MTRR* gene either, although carrying the *G* allele in *MTR* Ex26−20A>G (OR, 1.35; 95% CI, 0.96-1.90 for AG/GG versus AA), or the *T* allele in *MTRR* Ex5+123C>T (OR, 1.30; 95% CI, 0.93-1.82 for CT/TT versus CC) was each related to a borderline increased risk of stomach cancer after adjusting for age, gender, education, dietary folate intake, alcohol drinking, cigarette smoking, and total caloric intake.

Table 1. Distribution of demographic and other characteristics in cases and controls, Warsaw, Poland, 1994-1996

	Cases (n = 305)	Controls (n = 427)	P
Age, mean ± SD	63.0 ± 10.5	63.7 ± 10.6	0.36
Gender, *n* (%)			
Male	202 (66.2)	276 (64.6)	
Female	103 (33.8)	151 (35.4)	0.66
Education, *n* (%)			
≤High school	144 (47.2)	160 (37.5)	
Some college	104 (34.1)	144 (33.7)	
≥College graduate	57 (18.7)	123 (28.8)	<0.01
Family history of stomach cancer, *n* (%)			
No	257 (87.7)	402 (95.7)	
Yes	36 (12.3)	18 (4.3)	<0.0001
Alcohol drinking, *n* (%)			
Nondrinkers	107 (35.9)	141 (33.0)	
Former drinkers	97 (32.6)	62 (14.5)	
Current drinkers	94 (31.5)	224 (52.5)	<0.0001
Frequency of alcohol drinking, *n* (%)			
Nondrinkers	107 (35.4)	141 (33.2)	
<7 drinks per week	124 (41.1)	224 (52.7)	
≥7 drinks per week	71 (23.5)	60 (14.1)	0.001
Drinks per week, mean ± SD	5.92 ± 14.86	3.30 ± 6.00	0.001
Dietary folate intake (μg/d), mean ± SD	317.65 ± 90.25	310.37 ± 90.62	0.31
Total caloric intake (kcal), mean ± SD	2861.67 ± 817.80	2820.93 ± 788.51	0.52
Smoking, *n* (%)			
Nonsmokers	87 (33.9)	170 (39.8)	
Former smokers	90 (39.5)	138 (32.3)	
Current smokers	126 (51.4)	119 (27.9)	<0.001
Weekly frequency of raw vegetable and fruit intake, *n* (%)			
Q1 (<5.5)	62 (24.1)	84 (20.3)	
Q2 (5.6-9.7)	87 (33.9)	112 (27.1)	
Q3 (9.8-13.9)	57 (22.2)	109 (26.4)	
Q4 (≥14.0)	51 (19.8)	108 (26.2)	0.06
Body mass index (kg/m^2), *n* (%)			
<25	129 (42.3)	194 (45.4)	
≥25	176 (57.7)	233 (54.6)	0.40
Lauren classification, *n* (%)			
Intestinal	206 (67.5)		
Diffuse	50 (16.4)		
Indeterminate	32 (10.5)		
Unknown/missing	17 (5.6)		
Location within stomach, *n* (%)			
Cardia	35 (11.5)		
Distal stomach	223 (73.1)		
Combined	36 (11.8)		
Unknown/missing	11 (3.6)		

Table 2. Effect of polymorphisms in *MTHFR, MTR*, and *MTRR* on stomach cancer risk, Warsaw, Poland, 1994-1996

	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)*	OR (95% CI)†
MTHFR Ex5+79C>T *rs1801133*				
CC	146 (49.5)	185 (46.4)	1.00	1.00
CT	116 (39.3)	178 (44.6)	0.82 (0.60-1.13)	0.79 (0.56-1.13)
TT	33 (11.2)	36 (9.0)	1.17 (0.70-1.97)	1.13 (0.64-1.99)
CT/TT	149 (50.5)	214 (53.6)	0.88 (0.65-1.19)	0.85 (0.61-1.18)
MTHFR Ex8−62A>C *rs1801132*				
AA	135 (46.4)	180 (45.0)	1.00	1.00
AC	125 (43.0)	179 (44.8)	0.93 (0.68-1.28)	1.00 (0.70-1.42)
CC	31 (10.7)	41 (10.3)	1.02 (0.61-1.71)	1.08 (0.62-1.89)
AC/CC	156 (53.6)	220 (55.0)	0.95 (0.70-1.28)	1.01 (0.73-1.41)
MTR Ex26−20A>G *rs1805087*				
AA	182 (62.1)	270 (65.4)	1.00	1.00
AG	96 (32.8)	123 (29.8)	1.17 (0.84-1.62)	1.38 (0.96-1.98)
GG	15 (5.1)	20 (4.8)	1.10 (0.55-2.21)	1.18 (0.56-2.48)
AG/GG	111 (37.9)	143 (34.6)	1.16 (0.85-1.58)	1.35 (0.96-1.90)
MTRR Ex2−64A>G *rs1801394*				
GG	106 (35.9)	147 (35.6)	1.00	1.00
AG	133 (45.1)	188 (45.5)	0.98 (0.70-1.37)	0.94 (0.65-1.35)
AA	56 (19.0)	78 (18.9)	0.99 (0.64-1.51)	1.10 (0.69-1.76)
AG/AA	189 (64.1)	266 (64.4)	0.98 (0.72-1.34)	0.98 (0.70-1.38)
MTRR Ex5+123C>T *rs1532268*				
CC	118 (39.9)	190 (46.3)	1.00	1.00
CT	146 (49.3)	176 (42.9)	1.35 (0.98-1.85)	1.34 (0.94-1.90)
TT	32 (10.8)	44 (10.7)	1.20 (0.72-1.99)	1.18 (0.67-2.06)
CT/TT	178 (60.1)	220 (53.7)	1.32 (0.97-1.79)	1.30 (0.93-1.82)
MTRR Ex9−85C>T *rs2287780*				
CC	260 (87.8)	364 (88.8)	1.00	1.00
CT	36 (12.2)	45 (11.0)	1.12 (0.70-1.79)	1.07 (0.64-1.79)
TT	0	1 (0.2)	—	—
CT/TT	36 (12.2)	46 (11.2)	1.10 (0.69-1.74)	1.04 (0.62-1.74)
MTRR Ex14+14C>T *rs10380*				
CC	271 (91.3)	361 (88.1)	1.00	1.00
CT	25 (8.4)	47 (11.5)	0.70 (0.42-1.16)	0.68 (0.38-1.21)
TT	1 (0.3)	2 (0.5)	0.67 (0.06-7.42)	0.97 (0.08-11.67)
CT/TT	26 (8.8)	49 (12.0)	0.70 (0.42-1.15)	0.69 (0.39-1.21)
MTRR Ex15-526G>A *rs9332*				
GG	255 (87.6)	351 (86.7)	1.00	1.00
AG	36 (12.4)	50 (12.4)	0.98 (0.62-1.56)	0.98 (0.59-1.64)
AA	0	4 (1.0)	—	—
AG/AA	36 (12.4)	54 (13.3)	0.91 (0.58-1.43)	0.93 (0.56-1.54)
MTRR Ex15−405A>T *rs8659*				
AA	107 (36.8)	160 (39.3)	1.00	1.00
AT	145 (49.8)	181 (44.5)	1.20 (0.86-1.67)	1.14 (0.79-1.64)
TT	39 (13.4)	66 (16.2)	0.86 (0.54-1.38)	0.93 (0.57-1.54)
AT/TT	184 (63.2)	247 (60.7)	1.11 (0.81-1.51)	1.08 (0.77-1.52)
MTRR Ex15+572C>T *rs9282787*				
TT	182 (62.54)	264 (64.39)	1.00	1.00
TC	99 (34.02)	129 (31.46)	1.13 (0.82-1.56)	1.14 (0.80-1.63)
CC	10 (3.4)	17 (4.15)	0.86 (0.38-1.92)	0.98 (0.43-2.24)
TC/CC	109 (37.5)	146 (35.6)	1.10 (0.80-1.50)	1.12 (0.80-1.58)

*Adjusted for age and gender.
†Adjusted for age, gender, education, dietary folate intake, alcohol drinking, cigarette smoking, and total caloric intake.

When two polymorphisms in *MTHFR* gene were evaluated jointly, no individuals were found to carry two copies of minor alleles in both *MTHFR* polymorphisms. Our results did not support a significant interaction between two *MTHFR* polymorphisms ($P_{\text{multiplicative interaction}} = 0.17$). The estimated *MTRR* haplotype frequencies in controls suggested that the most common haplotype (29.5%) is the one that carried no minor allele in all seven polymorphisms in the *MTRR* gene (GCGCCAT; Table 4). *MTRR* haplotypes harboring ≥1 minor alleles did not differ significantly from the reference haplotype that carried no minor allele with regard to stomach cancer risk.

We evaluated genetic associations separately in males and females and found no meaningful difference by gender (data not shown). The genetic associations were also comparable between intestinal-type and diffuse-type cases, between localized/regional metastasis and displaced metastasis cases, and between cardia and noncardia gastric adenocarcinomas, except that the increased risk associated with the *MTRR* Ex5+123C>T (CT/TT versus CC) polymorphism was mainly confined to cases with intestinal-type tumors (for intestinal type: OR, 1.57; 95% CI, 1.11-2.23; for diffuse type: OR, 0.82; 95% CI, 0.45-1.51).

When evaluated by status of dietary folate and alcohol intake, our results suggested a significant increased risk of stomach cancer associated with the *MTR* Ex26−20A>G polymorphism among individuals whose dietary folate intake was above the median (AG/GG versus AA: OR, 1.73; 95% CI, 1.08-2.09), but the excess risk was confined mainly among heterozygotes (data not shown). A significant inverse association was found between *MTRR* Ex14+14C>T polymorphism and stomach cancer among nondrinkers of alcohol (CT/TT versus CC: OR, 0.32; 95% CI, 0.10-1.00). However, both interactions were not statistically significant at the multiplicative scale. In addition, no significant interaction with folate intake or alcohol drinking was observed for the other *MTRR* polymorphisms or the *MTHFR* polymorphisms in this study. The results were similar when the threshold of a regular drinker was increased to ≥1 drink per week or ≥3 drinks per week for ≥6 months. Additional adjustment for family history of stomach cancer and *Helicobacter pylori* seropositivity did not

Table 3. R^2 between polymorphisms in *MTRR* among controls, Warsaw, Poland, 1994-1996

	Ex2−64 A>G	Ex9−85C>T	Ex15−526G>A	Ex14+14C>T	Ex5+123C>T	Ex15+572C>T	Ex15+405A>T
Ex2−64 A>G	1						
Ex9−85C>T	0.0590	1					
Ex15−526G>A	0.0761	0.0012	1				
Ex14+14C>T	0.0648	0.0029	0.7649	1			
Ex5+123C>T	0.0179	0.0208	0.0459	0.0289	1		
Ex15+572C>T	0.1132	0.0210	0.0135	0.0081	0.4414	1	
Ex15+405A>T	0.2842	0.0833	0.0854	0.0722	0.1590	0.1094	1

affect the estimates meaningfully (data not shown). Small numbers after stratification prevented us from examining the effect modification by the joint status of dietary folate and alcohol intake.

Discussion

The decline in stomach cancer incidence in western countries has been described as ''epidemiology of unplanned triumph''(38). A wealth of evidence has suggested that this decline can be largely explained by a decreased prevalence of *H. pylori* infection; improved living conditions, including the use of refrigeration; and increased consumption of fresh vegetables and fruits (1, 3). Among nutrients that have been proposed to explain the protective effect of vegetables and fruits, folate is an important candidate. However, epidemiologic studies evaluating the effect of dietary folate on stomach cancer are inconsistent. Genetic variations in folate metabolism may affect normal patterns of DNA methylation and synthesis and therefore determine the susceptibility of stomach cancer. We evaluated the effect of 10 genetic polymorphisms in folate-metabolizing genes jointly with dietary folate and alcohol intake in a Polish population, which has experienced a restricted supply of fresh vegetables and fruits and high per capita alcohol consumption before 1990 (28, 29).

The present study showed no apparent association between two commonly reported polymorphisms in *MTHFR* and stomach cancer risk in a Polish population. Existing studies evaluating *MTHFR* polymorphisms on stomach cancer risk were mostly conducted in Chinese populations, reporting a 40% to 90% excess risk associated with the *T* allele in the *MTHFR* Ex5+79C>T polymorphism (20-22, 24-26). A recent study in central Italy reported a >2-fold increased risk of stomach cancer associated with the *T* allele, although the sample size was relatively small (39). Our findings of null associations were in line with results from two case-control studies in Korean and German populations (23, 40).

Consumption of liver, liverwurst, and organ meats provided an important source of dietary folate in our controls who had a mean level of dietary folate intake of 331.5 μg/d in men and 270.6 μg/d in women, comparable with other populations no using supplement (41-43). We previously found no significant effect of dietary folate intake on stomach cancer in this population (28). Diet-gene interaction could explain inconsistent results for folate-metabolizing genes reported across ethnic groups. For example, the genotype TT of *MTHFR* Ex5+79C>T reduced the risk of colorectal cancer risk only among individuals with adequate dietary folate and/or no or little alcohol intake (18, 36, 44-47). Thus, *MTHFR* Ex5+79C>T may impair DNA synthesis/repair capacity only when folate intake is inadequate (43). We found, however, no evidence of effect modification in this first study evaluating the potential interaction between *MTHFR* polymorphisms and dietary folate intake on stomach cancer.

Alcohol consumption may increase the requirement for folate intake by reducing its intestinal absorption and increasing its renal excretion. One case-control study nested within a large intervention trial in Linxian, China (26) reported a significant increased risk of gastric cardia adenocarcinomas associated with the TT genotype of the *MTHFR* Ex5+79C>T polymorphism among individuals who drank alcohol in the past 12 months, whereas a hospital-based case-control study in Mexico reported no interaction between the *MTHFR* Ex5+79C>T polymorphism and alcohol consumption (48). Definition of drinkers was not described in the Mexican study. Our study ascertained alcohol intake >2 years before diagnosis as the drinking behavior of cases may be affected by recent

Table 4. Stomach cancer risk by *MTRR* haplotypes and diplotypes, Warsaw, Poland, 1994-1996

		Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)*	OR (95% CI)†
MTRR haplotypes‡ (no. minor alleles)					
0	GCGCCAT	148 (26.5)	231 (29.5)	1.00	1.00
1	GCGCCTT	48 (8.6)	57 (7.3)	1.32 (0.85-2.04)	1.21 (0.75-1.96)
2	GCGCTAC	114 (20.4)	153 (19.5)	1.19 (0.86-1.63)	1.24 (0.88-1.76)
	ACGCCTT	93 (16.7)	145 (18.5)	0.99 (0.71-1.38)	1.04 (0.73-1.49)
	ACGCTAT	70 (12.5)	82 (10.5)	1.35 (0.92-1.98)	1.36 (0.89-2.07)
3	ATGCCTT	34 (6.1)	45 (5.7)	1.18 (0.72-1.93)	1.13 (0.66-1.94)
4	ACATCTT	24 (4.3)	42 (5.4)	0.89 (0.52-1.54)	0.94 (0.51-1.71)
	Others§	27 (4.8)	29 (3.7)	1.47 (0.84-2.59)	1.48 (0.78-2.82)
MTRR diplotypes‡ (no. minor alleles in each chromosome)					
0-0	GCGCCAT-GCGCCAT	18 (6.1)	32 (7.8)	1.00	1.00
0-2	GCGCCAT-GCGCTAC	37 (12.5)	49 (12.0)	1.36 (0.66-2.78)	1.33 (0.61-2.91)
	GCGCCAT-ACGCCTT	25 (8.5)	45 (11.0)	0.99 (0.46-2.11)	0.94 (0.42-2.12)
	GCGCCAT-ACGCTAT	16 (5.4)	24 (5.9)	1.20 (0.51-2.84)	0.97 (0.38-2.48)
2-2	GCGCTAC-ACGCCTT	18 (6.1)	28 (6.9)	1.15 (0.50-2.63)	0.93 (0.37-2.31)
	Others§	181 (61.4)	231 (56.5)	1.40 (0.76-2.58)	1.30 (0.67-2.51)

*Adjusted for age and gender.
†Adjusted for age, gender, education, dietary folate intake, alcohol drinking, cigarette smoking, and total caloric intake.
‡The order of single nucleotide polymorphismss in *MTRR* are *MTRR* Ex2−64A>G (A66G), Ex9−85C>T, Ex15−526G>A, Ex14+14C>T, Ex5+123C>T, Ex15−405A>T, and Ex15+572C>T.
§Others are consisted of haplotypes/diplotypes with frequencies <5%.

cancer-related symptoms. In contrast to the gastric cardia adenocarcinomas included in the Linxian study, the majority of the cases in our study were noncardia gastric adenomas. We did not observe a significant interaction between alcohol intake and *MTHFR* polymorphisms.

The effect of *MTR* Ex26−20A>G has not been previously investigated in relation to stomach cancer risk. Studies investigating the *MTR* Ex26−20A>G polymorphism with colorectal cancer and leukemia have yielded inconsistent results (36, 37, 49). A reduced homocysteine level was linked to the GG genotype in some studies (49-52), leading to the hypothesis that this polymorphism may have an activating effect on the enzyme that increases the conversion of homocysteine to methionine. In our study, we found no clear evidence of a link between the *MTR* Ex26−20A>G polymorphism and stomach cancer, with a significant excess risk only among heterozygotes who consumed above-median level of folate.

Consistent with the null association reported in the nested case-control study in Linxian, China, our study observed no association between *MTRR* Ex26−20A>G polymorphism and stomach cancer risk. However, a borderline increased risk was associated with the *MTRR* Ex5+123C>T polymorphism. Compared with the two common polymorphisms in the *MTHFR* gene that substantially reduce enzymatic activities and lead to abnormal DNA methylation and disrupted DNA synthesis/repair patterns (18), the functionality of polymorphisms in *MTRR* is largely unknown. Two studies identified an elevated level of homocysteine among individuals carrying the AA genotype of the *MTRR* Ex26−20A>G polymorphism, whereas others failed to observe an association (18). An excessive homocysteine level could promote tumor cell growth in experimental models (39). An improved understanding of the biological functions of these polymorphisms is needed in helping interpret the observed effect of *MTRR* polymorphisms on stomach cancer risk.

To our knowledge, our study is the first to examine the frequency of genetic variations in folate metabolism and their associations with stomach cancer in a high-risk Polish population. We selected two functional polymorphisms in the *MTHFR* gene. When the functionality of polymorphisms in the *MTR* and *MTRR* genes was not well known, we examined synonymous or nonsynonymous polymorphisms located in the coding region of the gene. However, our inclusion of polymorphisms is still incomplete. Linkage disequilibrium with other genetic variants that were not included in our study may contribute to the observed borderline effect of two *MTR* and *MTRR* polymorphisms. On the other hand, the apparent borderline effects may be due to chance because multiple comparisons were made in our study (53). Our study is one of the largest studies evaluating both environmental and genetic contributors of stomach cancer and had a relatively high proportion of study participants who completed the interview and donated a blood sample. Nevertheless, the study sample size is still limited for assessment of gene-environment and gene-gene interactions and haplotype/diplotype associations. Therefore, cautions need to be exercised in interpreting these results. Despite the efforts to recruit cases immediately after diagnosis, a substantial proportion of them died before they could be contacted. To the extent that any of the studied polymorphisms might be related to survival, our results might not be generalizable to patients with advanced stomach cancer. However, we observed no consistent pattern when we stratified the results by tumor stage, suggesting that survival bias in our study was likely minimal. Although two important dietary factors (e.g., dietary folate and alcohol intake) were investigated with regard to their potential modifying effects on folate-metabolizing genes, the lack of data on other nutrients, such as other B vitamins and methionine, relating to one-carbon metabolism, which are reactions involving compounds that contain a single carbon atom, also limits our capacity of providing a more comprehensive view on the one-carbon metabolism pathways.

In summary, our results suggested that genetic polymorphisms involved in folate metabolism generally are not related to stomach cancer risk in this Polish study population. The significant associations with a few polymorphisms in the *MTR* and *MTRR* genes need replication in future studies.

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Cancer Epidemiol Biomarkers Prev 2007;16:115-121.