Short Communication

Methionine Synthase D919G Polymorphism, Folate Metabolism, and Colorectal Adenoma Risk

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Abstract

Methionine synthase [5-methyltetrahydrofolate-homocysteine *S*-methyltransferase (MTR)] is involved in folate-mediated one-carbon metabolism, a pathway known to play a role in colorectal carcinogenesis. We investigated whether the *MTR* D919G polymorphism was associated with risk of colorectal adenoma in a colonoscopy-based study of 513 cases and 609 controls from Minneapolis, MN. Adenoma risk appeared nonsignificantly increased among women with DG or GG genotype [adjusted odds ratio (OR) *versus* DD, 1.4; 95% confidence interval (CI), 0.9–2.1] but not men (OR, 1.0; 95% CI, 0.7–1.5). An interaction with methionine intake was observed among women, such that low *versus* high intake was associated with a 2.3-fold increased risk only among those with DG or GG genotype (95% CI, 1.1–4.9; *P* for interaction = 0.05). Similarly, risk associated with alcohol intake was not elevated among women with the DD genotype; however, consumption of >7 g of alcohol/day *versus* none was associated with an increased risk among women with DG or GG genotype (adjusted OR, 2.5; 95% CI, 1.4–4.4; *P* for interaction = 0.03). An interaction between *MTR* D919G and the thymidylate synthase (*TS* or *TYMS*) 3′-untranslated region polymorphism 1494del6 was also observed among women (*P* for interaction = 0.007). No evidence of interaction with intake of folate, vitamin B_{12}, or vitamin B_6 or with genotype at *MTHFR* C677T or the *TS* enhancer region 28-bp repeat polymorphism was seen. These findings add to what is known about the complexities of genetic variations in one-carbon-metabolizing enzymes in relation to colorectal carcinogenesis.

Introduction

Substantial evidence suggests that folate-mediated one-carbon metabolism plays a role in colorectal carcinogenesis (1, 2). Low-folate diets have been found to increase the risk of colorectal cancer and adenomatous polyps (a cancer precursor; Refs. 3, 4). Increased risks observed with low intake of vitamin B_{12}, vitamin B_6, and methionine and with high intake of alcohol, which acts as a folate antagonist, may be attributable to their involvement in this metabolic pathway [for reviews, see Choi and Mason (1) and Giovannucci (5)]. In addition, polymorphisms in the folate-metabolizing enzymes methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthase (TS or TYMS) have been associated with colorectal cancer and adenoma risk in several studies, possibly via interaction with relevant dietary factors (6–10). Potential mechanisms for these associations involve both DNA methylation and nucleotide synthesis (1, 11).

Methionine synthase [5-methyltetrahydrofolate-homocysteine *S*-methyltransferase (*MTR*)], on chromosome 1q43, encodes one of several key enzymes involved in the folate-mediated one-carbon metabolism. It catalyzes the methylation of homocysteine to methionine with simultaneous conversion of 5-methyl-tetrahydrofolate (5-methyl-THF) to tetrahydrofolate (THF). MTR is essential for the provision of *S*-adenosylmethionine, the universal donor of methyl groups, as well as the provision of THF for use in nucleotide synthesis.

A common *MTR* variant consists of an A-to-G transition at base-pair 2756 and leads to a change from aspartic acid to glycine at codon 919 (D919G; Ref. 12). Although the direct functional impact of this polymorphism has not been established, there is some evidence that this may be an activating polymorphism; in some studies, individuals with GG genotype have lower homocysteine concentrations (13–16) and higher serum folate concentrations (14). Other studies, however, suggest no functional differences (17–20). In a clinic-based case-control study, we sought to assess associations between the *MTR* D919G polymorphism and risk of colorectal adenoma. We also examined possible interactions with dietary intakes of vitamin B_{12}, vitamin B_6, methionine, folate, and alcohol and with polymorphisms in *MTHFR* (C677T) and in *TS* [*TS* enhancer region (TSER) 28-bp tandem repeats and 1494del6].

Materials and Methods

Study Population. Subject recruitment for the Minnesota Cancer Prevention Research Unit case-control study has been described previously (21). Briefly, cases and controls were recruited through a multiclinic private gastroenterology practice in metropolitan Minneapolis, Digestive Healthcare. Patients who were scheduled for colonoscopy between April 1991 and April 1994 were screened for eligibility and recruited before colonoscopy. Recruitment at Digestive Healthcare sites was initiated at the time of scheduling so that both patient and recruiter would be blind to the final diagnosis. The study protocol was approved by the internal review boards of the University of Minnesota and each Digestive Healthcare site, and written informed consent was given by each study participant.

Eligibility criteria for both cases and controls were (*a*)

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resident of the Twin Cities metropolitan area; (*b*) age 30–74 years; (*c*) English speaking; (*d*) no known genetic syndrome associated with colonic neoplasia; and (*e*) no history of cancer (except nonmelanoma skin cancer), adenomatous polyps, or inflammatory bowel disease. Indications for colonoscopy have been published previously (9); 68% of all colonoscoped patients participated.

Colonoscopy findings were recorded at the colonoscopy visit, and only participants with a complete colonoscopy reaching the cecum were eligible. All polyps were removed and evaluated histologically. Patients with polyps showing invasive carcinoma were not included. Adenomatous polyp cases were those found to have at least one adenomatous polyp; controls were polyp free at colonoscopy. To reduce the possibility of population admixture, this analysis was restricted to Caucasians (97.7% of the study sample).

Data Collection. At the colonoscopy visit, questionnaires were administered and blood was drawn. Data collected included information on dietary intake, physical activity, smoking habits, anthropometric measurements, medical information, demographic information, and reproductive history. When data were incomplete, study staff followed up by telephone. The dietary questionnaire used was an adaptation of the Willett food-frequency questionnaire, which has been studied previously for validity and repeatability (22). Folate values derived from this questionnaire correlated ($r = 0.56$) with RBC folate levels (an indicator of long-term folate status; Ref. 4).

Genotyping. Genomic DNA was extracted from peripheral WBCs with a Puregene kit (Gentra Systems, Minneapolis, MN). The *MTR* D919G polymorphism was genotyped on a 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) with a fluorescent allelic discrimination assay. Amplification was performed in 20-μl reactions using the TaqMan core reagent kit (Applied Biosystems) with 200 nM each of the amplification primers 5′-TGTTCCCAGCTGTTAGATGAAAATC-3′ and 5′-AATCTGTTTCTACCACTTACCTTGAGAGA-3′, 100 nM each of the MGB probes (Applied Biosystems) 5′VIC-AGACAGGACCATTATG3′-NFQ (D allele) and 5′-6-FAM-AGACAGGGCCATTAT3′-NFQ (G allele), and 10 ng of genomic DNA. Cycling conditions were 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. Each plate contained negative and positive controls for all genotypes. For quality control purposes, genotyping was repeated for 94 randomly selected samples, and there were no discrepancies between results. Genotype frequencies did not deviate from those expected under Hardy–Weinberg equilibrium. Genotyping methods for *MTHFR* C677T, the TSER 28-bp repeat polymorphism, and *TS* 1494del6 [deletion of TTAAAG in the 3′-untranslated region (UTR)] are described elsewhere (8, 9).

Statistical Analysis. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for associations between *MTR* genotypes and risk of colorectal adenoma. The following confounders or adjustment variables were identified as those whose inclusion modified *MTR* ORs: age, sex, hormone therapy (women only; ever/never), body mass index, pack-years of smoking, regular use of nonsteroidal antiinflammatory drugs (≥1/week), daily energy intake (kilocalories), dietary fiber, percentage of energy from fat, and daily dietary intakes of folate, vitamin B_{12}, vitamin B_6, methionine, and alcohol. In general, the impact of confounding was minimal; however, for consistency of reporting, multivariable-adjusted ORs are presented. Regular use of aspirin (≥1/week), hours of physical activity, daily dietary intake of protein, and percentage of energy from protein did not confound any association studied.

Gene-Diet Interactions. Examination of gene-diet interactions (*MTR* D919G with daily dietary intakes of folate, vitamin B_{12}, vitamin B_6, methionine, and alcohol) consisted of OR estimation using a common referent group of individuals with DD genotype and the highest nutrient intake level (or lowest alcohol intake level). In this manner, patterns of genotype-specific risks within dietary intakes and dietary-specific risks within genotype were observed. Tests of trends for risks associated with dietary intakes within genotype groups consisted of likelihood ratio comparisons of grouped linear *versus* quantitative parameterizations of dietary variables. Because trends by dietary intakes were expected, significance testing of gene-diet interactions consisted of comparisons across *MTR* genotype of the within-genotype trends in risk associated with dietary intakes.

Gene-Gene Interactions. Examination of gene-gene interactions (*MTR* D919G with *MTHFR* C677T, the TSER repeat polymorphism, and *TS* 1494del6) consisted of OR estimation using a common referent group of individuals with wild-type genotypes. In this manner, patterns of *MTR* genotype-specific risk within other genotype groups and other genotype-specific risks within the *MTR* genotype were observed. Significance of gene-gene interactions was assessed with likelihood ratio testing of models with relevant multiplicative interaction terms.

Results

A total of 513 individuals with adenomatous polyps (190 women and 323 men) and 609 controls (373 women and 236 men) were genotyped at the D919G polymorphism of *MTR*. The G allele frequency was 17% among women controls and 19% among men controls. *MTR* genotype frequencies and selected characteristics of study participants are shown in Table 1; other characteristics have been described previously (9, 21). Adjusted ORs for individuals with DG ($n = 344$) and GG ($n = 42$) genotypes were 1.1 (95% CI, 0.8–1.5) and 1.6 (95% CI, 0.8–3.2), respectively, compared with individuals with DD genotype ($n = 736$), suggesting only a modest, statistically nonsignificant increase in adenoma risk with D919G. These results contradict the inverse association predicted by some functional studies (13–15), but are consistent with a previous study of colorectal cancer (23). No statistically significant interaction between genotype and sex was seen; we observed adjusted ORs of 1.4 (DG *versus* DD; 95% CI, 0.9–2.1) and 1.7 (GG *versus* DD; 95% CI, 0.7–4.3) for women and 1.0 (DG *versus* DD; 95% CI, 0.6–1.4) and 1.6 (GG *versus* DD; 95% CI, 0.5–4.6) for men. These estimates did not vary when stratified by polyp size, as another study of colorectal adenoma in women suggested (24), or age, number of polyps, or location of largest polyp (data not shown).

Gene-Diet Interactions. Results of analyses stratified by daily dietary intake of folate, vitamin B_{12}, vitamin B_6, methionine, and alcohol are shown in Table 2. We hypothesized that associations between *MTR* D919G genotype and adenoma may be modified by the amount of substrate (folate), product and inhibitor (methionine), relevant cofactors (vitamins B_{12} and B_6), or factors affecting folate absorption (alcohol); however, no interactions with intake of these nutrients were seen (Table 2).

Among women, an interaction was suggested between daily methionine intake and *MTR* D919G ($P = 0.05$), such that the expected increased adenoma risk with lower methionine intake was seen only among women with a *MTR* DG or GG genotype (Table 2). No association with methionine intake was

Table 1 Characteristics of study participants

	Women (n = 563)[a]			Men (n = 559)		
	Cases (n = 190)	Controls (n = 373)	P^b	Cases (n = 323)	Controls (n = 236)	P^b
MTR D919G genotype, n (%)						
DD	114 (60)	256 (69)		214 (66)	152 (64)	
DG	64 (34)	105 (28)		97 (30)	78 (33)	
GG	12 (6)	12 (3)	0.25	12 (4)	6 (3)	0.59
Age, n (%)						
<40 years	8 (4)	52 (14)		14 (4)	28 (12)	
40–49 years	32 (17)	91 (24)		47 (15)	68 (29)	
50–59 years	57 (30)	115 (31)		107 (33)	77 (33)	
60–69 years	69 (36)	91 (24)		120 (37)	47 (20)	
70+ years	24 (13)	24 (6)	<0.001	35 (11)	16 (7)	<0.001
Other characteristics,[c] mean (range)						
Folate intake, μg/day	404 (70–1623)	411 (84–1396)	0.75	396 (79–1952)	423 (101–1457)	0.16
Vitamin B_{12} intake, μg/day	7.9 (0.4–62.5)	8.6 (0.4–102.5)	0.31	9.5 (1.1–221.8)	10.6 (0.9–105.3)	0.29
Vitamin B_6 intake, mg/day	5.9 (0.8–102.2)	7.8 (0.7–103.7)	0.24	4.2 (0.7–106.1)	5.1 (0.6–101.8)	0.39
Methionine intake, g/day	1.8 (0.4–5.6)	1.9 (0.3–5.0)	0.12	2.2 (0.5–5.6)	2.1 (0.7–6.3)	0.44
Alcohol intake, g/day	4.9 (0.0–91.7)	3.6 (0.0–68.0)	0.12	13.5 (0.0–118.7)	10.9 (0.0–96.3)	0.11
Pack-years of smoking	12.5 (0.0–130.5)	10.1 (0.0–135.0)	0.15	24.2 (0.0–138.8)	16.9 (0.0–157.3)	<0.001
Body mass index, kg/m^2	26.3 (17.3–44.9)	26.8 (16.3–46.2)	0.36	27.8 (14.4–44.6)	27.0 (18.6–40.1)	0.02

[a] Controls were 61% female; cases were 63% male ($P < 0.001$).
[b] Significance of case-control differences; χ^2 test for categorical traits and t test for continuous traits.
[c] Dietary intakes were available from 554 women and 547 men and included intakes from dietary supplements.

found among women with the DD genotype. When women with DG or GG genotypes were analyzed as one group, increasing ORs of 0.6 (95% CI, 0.3–1.4), 1.2 (95% CI, 0.5–2.7), and 1.6 (95% CI, 0.7–3.7) were seen with high (>2.18 g), medium (1.54–2.18 g), and low (<1.54 g) daily methionine intake, respectively, compared with women with DD genotype and in the high tertile of methionine intake (P for interaction = 0.01). Among men, patterns of risk associated with methionine intake did not seem to vary by *MTR* genotype (Table 2). Similar results were seen when sex-specific methionine tertile cut-points were used (women, 1.44 and 2.01 g/day; men, 1.68 and 2.37 g/day).

A statistically significant interaction between *MTR* D919G genotype and current alcohol intake was observed among women (P = 0.03). No association with alcohol intake was observed among women with the DD genotype; however, an increased risk of adenoma was seen with intake of alcohol among women with DG or GG genotypes (Table 2). When women with DG or GG genotypes were analyzed jointly, increasing ORs of 0.8 (95% CI, 0.4–1.4), 1.9 (95% CI, 1.0–3.6), and 3.3 (95% CI, 1.4–8.1) were seen with intake of no alcohol, less than median intake among alcohol consumers (≤7 g/day), and more than median intake (>7 g/day), respectively, compared with women with DD genotype and no alcohol intake (P for interaction = 0.01). When the same referent group was used, women with DG or GG genotypes who drank >11 g/day had a somewhat greater increase in risk (OR = 4.8; 95% CI, 1.5–15.7). A similar pattern of interaction was suggested among men (Table 2). Similar trends were seen when sex-specific median alcohol intake cut-points were used (women, 3.9 g/day; men, 12.0 g/day).

Gene-Gene Interactions. We hypothesized that associations between *MTR* D919G genotype and colorectal adenoma may be modified by variation in genes encoding other folate-metabolizing enzymes. *MTHFR* C677T and the TSER 28-bp repeat polymorphism have been shown to be functionally relevant (giving decreased MTHFR activity and TS expression, respectively). The *MTHFR* and TSER genotypes did not appear to interact with the *MTR* genotype in relation to adenoma risk among women or men (Table 3).

A statistically significant interaction (P = 0.007) was seen between *MTR* D919G and *TS* 1494del6 among women, suggesting that they may have a joint role in adenoma risk. Women who were either homozygous variant at one polymorphism and homozygous wild type at the other or were doubly heterozygous appeared to have an increased risk compared with those with wild-type genotypes at both polymorphisms [ORs (95% CIs) *versus MTR* DD and *TS* 3′ UTR wild type (wt)/wt were as follows: DD and deletion (del)/del, 2.8 (1.2–6.5); GG and wt/wt, 5.0 (1.3–19.5); DG and wt/del, 2.6 (1.4–4.9)]. The functional significance of the *TS* 3′-UTR polymorphism has not yet been reported. We found only 22 women with the GG genotype, and Bonferroni adjustment for the number of strata and gene-gene interactions assessed reduced the significance of the interaction to P = 0.04.

Discussion

In summary, our analysis of colorectal adenoma cases and colonoscopy-screened controls suggests that the *MTR* D919G polymorphism may have a modest role in colorectal adenoma development, particularly among women. Our data additionally suggested that the role of *MTR* D919G in women may become more apparent in the presence of higher alcohol intake or lower methionine intake. We observed a statistically significant interaction between this *MTR* polymorphism and the *TS* 3′-UTR polymorphism 1494del6 among women. A strength of this study is the use of fully colonoscoped subjects, which led to a clear distinction between cases and polyp-free controls.

Our observation of a modest increase in colorectal adenoma risk with the *MTR* D919G variant genotypes, particularly among women [for DG/GG *versus* DD, OR = 1.4 (95% CI, 0.9–2.1)] contributes to a varied literature. Previous case-control analyses of *MTR* D919G have suggested increased risk for adenoma (25) and colorectal cancer (23) and no reported sex differences, and one analysis found no suggestion of colorectal

Table 2 Association between *MTR* genotype, dietary intakes, and risk of adenomatous polyps

	Women							Men						
	DD		DG		GG		*P*, interaction[d]	DD		DG		GG		*P*, interaction[d]
	n[a]	OR[b,c] (95% CI)	*n*[a]	OR[b] (95% CI)	*n*[a]	OR[b] (95% CI)		*n*[a]	OR[b] (95% CI)	*n*[a]	OR[b] (95% CI)	*n*[a]	OR[b] (95% CI)	
Unstratified	106/234	1.0 (reference)	60/98	1.4 (0.9–2.1)	11/11	1.7 (0.7–4.3)		201/143	1.0 (reference)	96/74	1.0 (0.6–1.4)	11/6	1.6 (0.5–4.6)	
Folate[e]														
High	37/84	1.0 (reference)	19/31	1.6 (0.8–3.4)	3/2	3.1 (0.4–22.1)		62/49	1.0 (reference)	32/31	0.9 (0.4–1.7)	2/1	1.5 (0.1–17.2)	
Medium	34/66	1.2 (0.6–2.2)	23/35	1.3 (0.6–2.7)	4/6	1.2 (0.3–4.8)		71/50	1.1 (0.6–1.9)	41/26	1.1 (0.6–2.3)	5/1	6.4 (0.6–65.0)	
Low	35/84	0.9 (0.4–1.8)	18/32	1.2 (0.5–2.9)	4/3	1.9 (0.3–10.6)		68/44	1.3 (0.7–2.7)	23/17	1.3 (0.6–3.2)	4/4	1.0 (0.2–4.6)	
P, trend[f]		1.00		0.61		1.00	0.94		0.58		0.61		0.26	0.64
Vitamin B_{12}[e]														
High	31/79	1.0 (reference)	15/24	1.2 (0.5–2.8)	3/4	1.8 (0.3–9.5)		63/62	1.0 (reference)	27/25	1.4 (0.7–2.7)	5/2	2.9 (0.5–17.1)	
Medium	31/75	1.1 (0.6–2.3)	19/32	1.7 (0.7–3.7)	2/3	1.0 (0.1–7.2)		76/44	2.0 (1.1–3.6)	46/27	2.1 (1.1–4.2)	2/2	1.5 (0.2–11.6)	
Low	44/80	1.5 (0.7–3.2)	26/42	2.1 (0.9–4.7)	6/4	3.5 (0.8–15.8)		62/37	2.7 (1.3–5.5)	23/22	1.4 (0.6–3.2)	4/2	4.3 (0.7 (0.7–27.8)	
P, trend[f]		0.34		0.20		0.31	0.95		0.009		0.22		0.43	0.21
Vitamin B_6[e]														
High	33/81	1.0 (reference)	18/30	1.4 (0.7–3.1)	3/3	1.7 (0.3–10.0)		56/53	1.0 (reference)	27/29	0.9 (0.5–1.9)	3/2	1.2 (0.2–8.5)	
Medium	36/72	1.2 (0.6–2.5)	23/35	1.5 (0.7–3.4)	2/3	1.9 (0.3–14.3)		80/44	1.9 (1.0–3.7)	42/25	1.9 (0.9–4.0)	4/3	2.3 (0.4–12.1)	
Low	37/81	1.1 (0.5–2.5)	19/33	1.6 (0.6–3.9)	6/5	2.0 (0.5–8.5)		65/46	2.1 (1.0–4.7)	27/20	1.9 (0.8–4.8)	4/1	7.4 (0.7–77.5)	
P, trend[f]		1.00		0.76		1.00	1.00		0.04		0.16		0.16	0.78
Methionine[e]														
High	29/65	1.0 (reference)	12/36	0.6 (0.3–1.5)	0/3	NA		89/69	1.0 (reference)	39/28	1.2 (0.6–2.2)	4/2	1.7 (0.3–11.1)	
Medium	36/79	0.9 (0.5–1.9)	17/28	1.2 (0.5–2.7)	4/4	1.5 (0.3–7.0)		65/38	1.7 (0.9–3.2)	38/25	1.6 (0.8–3.3)	3/2	2.2 (0.3–14.4)	
Low	41/90	0.7 (0.3–1.6)	31/34	1.5 (0.6–3.7)	7/4	2.1 (0.5–9.5)		47/36	1.7 (0.8–3.7)	19/21	1.2 (0.5–3.0)	4/2	2.8 (0.4–18.2)	
P, trend[f]		1.00		0.27		0.46	0.05		0.38		0.64		0.82	0.63
Alcohol[g]														
None	55/112	1.0 (reference)	28/62	0.8 (0.5–1.5)	0/4	NA		66/47	1.0 (reference)	26/22	0.9 (0.4–1.8)	6/3	1.4 (0.3–6.6)	
Low	29/78	0.9 (0.5–1.5)	18/28	1.6 (0.8–3.3)	7/4	3.7 (0.9–14.8)		47/34	1.0 (0.5–1.9)	19/24	0.6 (0.3–1.3)	1/1	1.4 (0.1–23.6)	
High	22/44	1.0 (0.5–2.0)	14/8	4.0 (1.5–11.1)	4/3	1.9 (0.4–9.3)		88/62	0.9 (0.6–1.6)	51/28	1.3 (0.7–2.5)	4/2	1.7 (0.3–10.1)	
P, trend[f]		0.60		0.62		0.08	0.03		1.00		0.19		1.00	0.55

[a] Number of cases/number of controls.
[b] Adjusted for age, hormone therapy (women only; ever/never), body mass index, pack-years of smoking, regular use of nonsteroidal anti-inflammatory drugs (≥1/week), daily energy intake, dietary fiber, percentage of daily kilocalories from fat, and daily dietary intakes of folate, vitamin B_{12}, vitamin B_6, methionine, or alcohol (excluding the nutrient used to stratify).
[c] OR, odds ratio; CI, confidence interval; NA, not calculated.
[d] *P* for interaction from comparison across genotype groups of OR slopes by dietary component within genotype group.
[e] Tertile cutpoints: folate, 267 and 434 μg; vitamin B_{12}, 5.09 and 9.35 μg; vitamin B_6, 1.94 and 3.06 mg; methionine, 1.54 and 2.18 g. Includes intakes from dietary supplements.
[f] *P* for intragenotype trend in ORs by dietary component from likelihood ratio test of grouped linear *vs.* continuous models.
[g] Median cut-point for alcohol users, 7 g/day.

cancer association (25). Some functional studies have indicated that the variant G allele is associated with lower homocysteine (13–16) and higher folate concentrations (14); because these levels have been consistently associated with decreased risk of adenoma, we hypothesized that the G allele would be inversely associated with adenoma risk. In addition, nested case-control analyses from the Nurses Health Study and the Physician's Health and Health Professional Follow-up Studies found decreased adenoma risks for women (24) and decreased colorectal cancer risks for men (26), respectively.

We examined whether differences in results among studies may be attributable to differing baseline folate-related nutrient levels and found that women in one study showing an inverse association had slightly higher folate intake than the women in the present study [tertiles of 310 and 508 μg/day (24) *versus* 267 and 434 μg/day]. Exclusion of the participants with lower folate levels (<280 or <300 μg/day) in the current analysis did not change the results, and an inverse association was not suggested. It is important to note that, in this and other studies, 95% CIs for variant genotype OR estimates included 1.0, and that the true relationship between *MTR* D919G and colorectal cancer or adenoma may thus be no association. Our observation that *MTR* D919G may play a more important role in colorectal carcinogenesis among women than men was not reported in other colorectal cancer or adenoma studies.

One difficulty in interpreting the observed associations arises from the lack of data regarding the functional impact of the *MTR* D919G polymorphism on protein function. Studies of homocysteine levels that suggested this might be an activating polymorphism examined only male study participants (13–15) or consisted of more than 75% men (16). Other studies of both men and women have not observed this association (17–19, 27), and the authors of one study reported higher homocysteine levels only among men with the variant allele and no difference among women (28). It is possible that the impact of the variant allele differs between men and women and that additional studies stratified by sex may help discern any differences. It is also possible that discrepancies in the results from functional studies may be attributable to differences in the studied populations in folate or vitamin B_{12} intakes, which were often not reported.

We had hypothesized that any association between *MTR* D919G genotype and risk of adenoma could be modified by intake of folate (which interacts with other folate-related polymorphisms and adenoma risk), vitamin B_{12} (a required MTR cofactor), vitamin B_6 (a required cofactor for conversion of THF to 5,10-methylene-THF), methionine (the product of and inhibitor of MTR), or alcohol (an inhibitor of folate absorption). No suggestions of interaction with folate, vitamin B_{12}, or vitamin B_6 were seen among women or men, consistent with

Table 3 Association between *MTR* D919G genotype, polymorphisms in folate metabolism, and risk of adenomatous polyps

	Women							Men						
	DD		DG		GG		*P*, interaction[d]	DD		DG		GG		*P*, interaction[d]
	n^a	OR[b,c] (95% CI)	n^a	OR[b] (95% CI)	n^a	OR[b] (95% CI)		n^a	OR[b] (95% CI)	n^a	OR[b] (95% CI)	n^a	OR[b] (95% CI)	
Unstratified	106/234	1.0 (reference)	60/98	1.4 (0.9–2.1)	11/11	1.7 (0.7–4.3)		201/143	1.0 (reference)	96/74	1.0 (0.6–1.4)	11/6	1.6 (0.5–4.6)	
MTHFR C677T														
CC	52/101	1.0 (reference)	32/51	1.3 (0.7–2.4)	9/6	2.1 (0.7–6.8)		90/66	1.0 (reference)	47/31	0.9 (0.5–1.6)	6/4	1.3 (0.3–5.2)	
CT	37/103	0.7 (0.4–1.1)	19/35	1.0 (0.5–1.9)	2/5	0.6 (0.1–3.6)		94/57	1.1 (0.7–1.8)	39/37	0.9 (0.5–1.6)	5/1	4.1 (0.4–39.8)	
TT	16/29	1.1 (0.5–2.4)	8/12	1.1 (0.4–3)	0/0	NA	0.80	15/19	0.4 (0.2–1.0)	10/6	1.4 (0.5–4.4)	0/1	NA	0.21
TSER[e]														
3 rpt/3 rpt	30/61	1.0 (reference)	16/23	1.3 (0.6–3.1)	3/2	2.3 (0.3–18.1)		62/41	1.0 (reference)	32/16	1.6 (0.8–3.5)	2/1	1.5 (0.1–21.3)	
3 rpt/2 rpt	50/120	0.8 (0.5–1.5)	32/56	1.1 (0.5–2.1)	7/6	1.7 (0.5–6.1)		93/73	0.9 (0.5–1.5)	43/41	0.8 (0.4–1.5)	9/2	4.0 (0.8–21.0)	
2 rpt/2 rpt	24/53	0.9 (0.5–1.9)	11/17	1.8 (0.7–4.8)	1/3	0.7 (0.1–7.4)	0.84	44/28	1.1 (0.5–2.1)	21/15	0.7 (0.3–1.6)	0/3	NA	0.33
TS 3′UTR[f]														
wt/wt	41/122	1.0 (reference)	22/47	1.6 (0.8–3.1)	7/4	5.0 (1.3–19.5)		97/77	1.0 (reference)	49/26	1.5 (0.8–2.7)	5/6	0.8 (0.2–3.0)	
wt/del	51/92	1.7 (1.0–2.8)	36/39	2.6 (1.4–4.9)	4/6	1.1 (0.3–4.4)		78/52	1.2 (0.7–1.9)	40/40	0.9 (0.5–1.5)	3/0	NA	
del/del	14/20	2.8 (1.2–6.5)	2/12	0.4 (0.1–2.2)	0/1	NA	0.007	25/14	1.6 (0.8–3.4)	7/8	0.8 (0.3–2.5)	3/0	NA	0.15

[a] Number of cases/number of controls; numbers differ slightly for some polymorphisms.
[b] Adjusted for age, hormone therapy (women only; ever/never), body mass index, pack-years of smoking, regular use of nonsteroidal anti-inflammatory drugs (≥1/week), daily energy intake, dietary fiber, percentage of daily kilocalories from fat, and daily dietary intakes of folate, vitamin B_{12}, vitamin B_6, methionine, and alcohol.
[c] OR, odds ratio; CI, confidence interval; NA, not calculated; TSER, *TS* enhancer region; rpt, repeats; UTR, untranslated region; wt, wild type; del, deletion.
[d] Significance of gene–gene interactions determined by likelihood ratio testing of models with and without a multiplicative interaction term.
[e] *TS* enhancer region, 28-bp repeat polymorphism.
[f] *TS* 3′ untranslated region, 1494del6.

other published studies of colorectal cancer and adenoma (23, 24, 26). *MTR* D919G may interact with a wider variation in intake levels of these nutrient than existed in the present study; however, no evidence for this was seen in the current clinic-based sample or other population-based samples.

Our results suggest that the *MTR* DG and GG genotypes are associated with increased risk of colorectal adenoma in the presence of low methionine intake among women; no interaction was seen among men. Because of limited information on methionine in most nutrient databases, methionine measurements from food frequency questionnaires may be error prone, leading to a bias of ORs toward the null. However, this would not be expected to occur preferentially among women with DD genotypes, in whom decreased risk with lower methionine intake was in fact suggested. Methionine synthase is subject to product inhibition through methionine (29), although to our knowledge, a potential impact of the *MTR* polymorphism on the product inhibition through methionine has not yet been investigated. Other colorectal cancer and adenoma studies have not reported any interaction between *MTR* genotype and methionine intake.

We also report that *MTR* DG and GG genotypes were associated with increased risk of colorectal adenoma in the presence of higher alcohol consumption (>7 g/day) among women and that additional increased risk was observed with higher intake (>11 g/day). A similar pattern was also seen among men, although tests of interaction did not meet statistical significance at the $\alpha = 0.05$ level. These observations are consistent with a previous study of colorectal cancer in men in which those with GG genotype who had higher alcohol intake (≥1 drink/day) had an increase in risk for colorectal cancer (OR = 2.6; 95% CI, 0.7–10.8) that was not seen in other genotype groups ($P = 0.04$; Ref. 26). Unfortunately, measurement of alcohol consumption may be unreliable, particularly among women, who underreport usage more often than men. High alcohol intake has been shown to decrease folate absorption (30, 31), and acetaldehyde can inhibit *MTR* activity (32). although the effects of moderate alcohol consumption are less well described and results based on alcoholic subjects, animals, or *in vitro* analyses may be less relevant to the population in the present study.

We observed a statistically significant interaction between *MTR* D919G and the *TS* 3′-UTR polymorphism 1494del6 among women ($P = 0.007$). Women who were either homozygous wild type at one polymorphism and homozygous variant at the other or doubly heterozygous appeared to have an increased risk compared with those with wild-type genotypes at both polymorphisms. Although the functional relevance of the *TS* deletion polymorphism is unknown at present, these results are consistent with the two polymorphisms having opposite associations with adenoma risk that become obvious for each only in the presence of the other. The relationship between these two polymorphisms and adenoma risk may be attributable to differential provision of metabolites to various "products" of one-carbon metabolism, particularly *S*-adenosylmethionine production or pyrimidine synthesis. No interactions were suggested with *MTHFR* C677T or the TSER 28-bp repeat polymorphism. Both of these polymorphisms have previously been shown to yield a functional change and to be associated with colorectal cancer or adenoma risk in the presence of low folate levels (6, 8, 9, 23, 33). It is possible that additional higher-order interactions exist between intakes of relevant nutrients and polymorphisms in *MTR*, *MTHFR*, and *TS*; however, a dataset the size of our study population lacks the power to detect three-way interactions between these exposures and *MTR* genotypes with the stratification approach used at present.

Our results should be interpreted with caution because the functional impact of the *MTR* D919G polymorphism is not well established and small numbers of individuals in some strata increase the likelihood that variation in risk estimates by *MTR* D919G genotype may be attributable to chance. This study is also limited in not having data on homocysteine levels, which may be considered an intermediate phenotype. Considering the complexity of folate-mediated one-carbon metabolism and genetic variability in multiple enzymes, even relatively large molecular epidemiological studies may not capture these inter-

actions adequately, particularly as the number of possible comparisons continues to increase. Although the present study provides hypotheses for future research, a continued interdisciplinary approach integrating nutrition science, biochemistry, epidemiology, and mathematical modeling is needed to understand this increasingly important biological pathway.

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