MTHFR *C677T* and *A1298C* Polymorphisms: Diet, Estrogen, and Risk of Colon Cancer

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Abstract

5,10-methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism, diverting metabolites toward methylation reactions or nucleotide synthesis. Using data from an incident case-control study (1608 cases and 1972 controls) we investigated two polymorphisms in the *MTHFR* gene, *C677T* and *A1298C*, and their associations with risk of colon cancer. All of the combined genotypes were evaluated separately, and the *1298AA/677CC* (wild-type/wild-type) group was considered the reference group. Among both men and women, the *677TT/1298AA* (variant/wild-type) genotype was associated with a small reduction in risk [men: odds ratio (OR), 0.7, 95% confidence interval (CI), 0.5–1.0; women: OR, 0.8, 95% CI, 0.5–1.2]. However, the *677CC/1298CC* (wild-type/variant) genotype was associated with a statistically significant lower risk among women (OR, 0.6; 95% CI, 0.4–0.9) but not men. When the polymorphisms were considered individually, for *A1298C* a significant risk reduction associated with the homozygous variant *CC* genotype was seen among women only (OR, 0.6; 95% CI, 0.5–0.9), and nonstatistically significant reduced risks were observed for the variant *677 TT* genotypes among both men and women. Stratification by nutrient intakes showed inverse associations with higher intakes of folate, vitamin B_2, B_6, B_{12}, and methionine among women with the *MTHFR 677CC/1298AA* genotypes, but not those with *677TT/1298AA*. We observed opposite risk trends for both *MTHFR* variants, depending on whether women used hormone-replacement therapy or not (*P* for interaction = <.01).

In summary, this study supports recent findings that the *MTHFR A1298C* polymorphism may be a predictor of colon cancer risk and have functional relevance. The possible interaction with hormone-replacement therapy warrants additional investigation.

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Introduction

Folate acts as a one-carbon donor and is an essential nutrient for the synthesis of nucleotides (*e.g.*, thymidine and purines) and the provision of methyl groups (1). Dietary folate intakes have been found associated with a reduced risk of colorectal neoplasia in most, although not all, studies (2–9). Recent studies on genetic polymorphisms in folate-metabolizing enzymes lend support to a causal relationship between folate and colorectal carcinogenesis (9–13). Potential mechanisms for the observed associations include an altered provision of *S*-adenosylmethionine for methylation reactions, including DNA methylation, and changes in the availability of nucleotides for DNA synthesis and repair (14, 15).

MTHFR (5,10-methylenetetrahydrofolate reductase) plays a central role in folate metabolism. This enzyme catalyzes the irreversible reaction of 5,10-methylene-tetrahydrofolate to 5-methyl tetrahydrofolate, which serves as a substrate for the remethylation of homocysteine to methionine, with the subsequent synthesis of *S*-adenosylmethionine. However, the substrate of MTHFR, 5,10-methylene-tetrahydrofolate, is also required for thymidine synthesis via thymidylate synthase, and indirectly for purine synthesis. Two common polymorphisms have been described in the *MTHFR* gene, both single nucleotide substitutions resulting in amino acid changes (*C677T* → *Ala222Val* and *A1298C* → *Glu429Ala*; Refs. 16, 17). Whereas *C677T* unequivocally affects enzyme function and has been associated with increased plasma homocysteine concentrations and an altered balance of folate metabolites (16, 18), the *in vivo* functional relevance of the *A1298C* variant is less well defined. *A1298C* affects *in vitro* enzyme function to a lesser degree, and individuals carrying the variant have frequently normal homocysteine and plasma folate concentrations (19, 20). However, some studies have noted a substantially decreased risk of acute lymphocytic or hyperdiploid pediatric leukemia associated with the variant *MTHFR 1298* allele (21, 22), and Keku *et al.* (23) reported recently a significantly decreased risk of colon cancer among whites with the *1298CC* genotype. *Glu429Ala* is located in the regulatory domain of the human enzyme (17, 24). It is unclear whether the substitution affects folate metabolism under specific physiological conditions, *e.g.*, under low nutrient intakes.

We have reported previously on the association between the *MTHFR C677T* polymorphism and risk of colon cancer (9). Here we extend these findings to the *A1298C* polymorphism, combined genotypes, dietary associations, and colon cancer risk.

Materials and Methods

Participants were African-American, Caucasian, or Hispanic subjects from the Kaiser Permanente Medical Care Program of Northern California, an eight county area in Utah, and the metropolitan Twin Cities area of Minnesota. Eligibility criteria for cases included diagnosis with first-primary incident colon

cancer (ICD-O 2nd edition codes 18.0 and 18.2–18.9) between October 1, 1991 and September 30, 1994; between 30 and 79 years of age at time of diagnosis; and mentally competent to complete the interview. Proximal tumors were defined as cecum through transverse colon; tumors in the splenic flexure, and descending and sigmoid colon were categorized as distal. Cases with adenocarcinoma or carcinoma of the rectosigmoid junction or rectum (defined as the first 15 cm from the anal opening), with known familial adenomatous polyposis, ulcerative colitis, or Crohn's disease were not eligible. Of all of the cases identified, 65% of those contacted consented to participate in the study. Controls who had never had a previous colorectal tumor were selected from Kaiser Permanente Medical Care Program membership lists in California, driver's license lists, random-digit-dialing, or Centers for Medicare & Medicaid Services lists (formerly known as the Health Care Finance Administration), for Utah, and driver's license or state identification lists in Minnesota. These methods have been described in detail (25). Of all of the controls selected, 64% participated.

Data Collection. Trained interviewers collected diet and lifestyle data using laptop computers. Study quality control methods have been described (26, 27). The referent period for the study was the calendar year ~2 years before date of diagnosis (cases) or date of selection (controls).

Dietary intake data were ascertained using an adaptation of the validated CARDIA diet history questionnaire (27, 28). Participants were asked to determine which foods were eaten and the frequency with which foods were eaten. Nutrients were calculated using the Minnesota Nutrition Coordinating Center's nutrient database version 19. Study participants were asked if they took multivitamins and other vitamin or mineral supplements on a regular basis. Multivitamin and supplement use was categorized as described previously (29). One third of participants reported taking a multivitamin regularly. Less than 1% reported taking individual supplements of folate, vitamin B6, and vitamin B12. About 1% reported taking a B complex supplement.

MTHFR Genotyping. Of 4403 cases and controls with valid study data, 3680 (84%) had blood collected. Genomic DNA was extracted using methods described previously (30). Of individuals with DNA, originally 3283 had *MTHFR 677* genotype data. For this analysis, results for an additional 297 samples not available previously for *MTHFR 677* genotyping were assayed from DNA stored at the Fred Hutchinson Cancer Research Center, and subsequently all of the samples were genotyped for *MTHFR 1298* resulting in a total of 3580 cases and controls with genotype information for both *MTHFR 677* and *MTHFR 1298*.

The *C677T* and *A1298C* polymorphisms were detected by allelic discrimination using the 5′ nuclease assay on a 7900HT sequence detection system (Applied Biosystems, Foster City, CA). The 5′ nuclease genotyping assays were validated by genotyping 100 individuals by both 5′ nuclease assay and RFLP (16, 24). There were no discrepancies. Genotyping of the *C677T* polymorphism was performed in 20 μl reactions containing 1× Taqman PCR core reagents (Applied Biosystems), 5 mM $MgCl_2$, 200 nM each PCR primer (forward primer 5′CCGAAGCAGGGAGCTTTG 3′ and reverse primer 5′CGGTGCATGCCTTCACAA 3′), 100 nM MGB-probes (Applied Biosystems; C-allele 5′VIC-AAATCGgCTCCCGCAG3′, T-allele 5′FAM-TGAAATCGaCTCCCGCA3′), 0.5 units AmpliTaq Gold, 0.2 units AmpErase UNG, and 5 ng genomic DNA. Genotyping of the A1298C polymorphism was performed in 20 μl reactions containing 1× Taqman PCR core reagents (Applied Biosystems), 3 mM $MgCl_2$, 200 nM each PCR primer (forward primer 5′AGAGCAAGTCCCCCAAGGA 3′ and reverse primer 5′CTTTGTGACCATTCCGGTTTG3′), 100 nM MGB probes (A-allele 5′VIC-AGTGAAGaAAGTGTCTTT3′ and C-allele 5′ 6-FAM-AGTGAAGcAAGTGTCTT3′) 0.5 units AmpliTaq Gold, 0.2 units AmpErase UNG, and 5 ng genomic DNA. The amplification cycles were 50°C for 5 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s, and 60°C for 1 min. Positive controls for all of the genotypes as well as four negative controls were included in each plate. For quality control purposes, genotyping for 94 randomly selected samples was repeated. There were no discrepancies. Genotype frequencies among cases and controls for both *MTHFR* 677 and *MTHFR* 1298 were compatible with Hardy-Weinberg equilibrium (χ^2 test).

Statistical Methods. Logistic regression models were used to estimate associations in various ways. We stratified the data by combined *MTHFR 677* and *1298* genotype and estimated the risk of colon cancer given a certain genotype and population characteristics (*e.g.*, proximal or distal tumor site). The combined effects of *MTHFR 677* and *1298* were calculated using individuals who were homozygous wild-type for enzyme activity at both loci as the referent group. We assessed the joint interaction between genotype and specific level of nutrient intake by using those at the highest risk as a common referent point (low nutrient intake and wild-type for both *MTHFR 677* and *1298*). Similarly, the interaction between genotype and recent estrogen status in postmenopausal women was assessed using those at the highest risk as the referent group [no hormone replacement therapy (HRT) use] and wild-type for both *MTHFR 677* and *1298*.

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from unconditional logistic regression models. In these models, age at diagnosis or selection, body mass index reported for the referent period (kg/m^2), long-term vigorous leisure-time physical activity, total energy intake, dietary fiber, dietary calcium, and usual number of cigarettes smoked per day on a regular basis were included as covariates to adjust for potential confounding. In models of risk of colon cancer, given genotype and nutrient intake, other B vitamins (dietary folate, B_2, B_6, and B_{12}), methionine, and current alcohol consumption were also included as covariates where appropriate. Categories of exposure for nutrients were based on the distribution of the control population for men and women separately. Tertile cut-points were used to designate low (lowest tertile), intermediate (middle tertile), or high intake (upper tertile). Long-term alcohol exposure was based on gram amount from reported wine, beer, and liquor consumption averaged for 10 and 20 years ago. The cut-point for high exposure was ≥20 g/day for men and ≥10 g/day for women, corresponding approximately with the upper tertile.

Separate analyses were performed for men and women to determine whether differences existed by sex, as most of the literature has focused on either men or women. Assessment of interactions among genotypes, diet, HRT, and the risk of colon cancer were based on departure from additive risks using the relative excess risk due to interaction formulation of Hosmer and Lemeshow (31) extended to more than two allelic combinations and/or environmental exposures. In addition to relative risk due to interaction, interactions between genotypes and hormone replacement therapy were assessed using a Wald χ^2 test of the difference between slopes from the (assumed linear) change in ORs holding either *MTHFR 677* or *1298* constant

(wild-type) and varying the other *MTHFR* genotype from wild-type to heterozygous to homozygous variant.

Results

Selected characteristics of the study population and *MTHFR* genotype frequencies by case and control status are presented in Table 1. The study participants were predominantly self-identified as Caucasian (92%), with the remainder Hispanic (4%) and African-American (4%). For African-Americans in the study population ($n = 130$ with *MTHFR* data) variant genotypes *677 TT* or *1298 CC* were rarely observed (frequencies of 2% and 5%, respectively).

The distribution of cases and controls for *MTHFR* genotypes shows that *C677T* and *A1298C* are in complete linkage disequilibrium (χ^2 test, $P < 0.0001$), with no evidence of the existence of an allele that carries both variants [inferred *MTHFR* alleles: *677C/1298A* (wild-type), *677T/1298A*, and *677C/1298C*]. When polymorphisms were evaluated separately, with respect to the *C677T* polymorphism the homozygous variant genotype was associated with a slightly reduced risk in men (*CT versus CC* wild-type: OR, 1.1, 95% CI, 0.9–1.3; *TT versus CC:* OR, 0.8, 95% CI, 0.5–1.1) and women (*CT versus CC:* OR, 0.9, 95% CI, 0.7–1.1; *TT versus CC:* OR, 0.8, 95% CI, 0.5–1.1). For *A1298C* no risk reduction was observed in men (*AC versus AA* wild-type: OR, 1.0, 95% CI, 0.8–1.2; *CC versus AA:* OR, 1.0, 95% CI, 0.7–1.4), but a reduced risk was seen for women (*AC versus AA* wild-type: OR, 1.0, 95% CI, 0.8–1.2; *CC versus AA*: OR, 0.6, 95% CI, 0.5–0.9). These risk estimates included the respective other polymorphism in the multivariate adjustment. Subsequently, we evaluated associations of *MTHFR 677/1298* genotypes compared with a reference group of individuals carrying two wild-type alleles (*MTHFR 677 CC/1298 AA*). Overall colon cancer risk estimates, stratified by sex, are shown in Table 2. Men who were homozygous variant *677 TT* and wild-type *1298 AA* showed the lowest risk in relation to the reference group (OR, 0.7; 95% CI, 0.5–1.0). When stratified by age, this association was observed only among men ≥65 years of age (OR, 0.6; 95% CI, 0.3–0.9). In men, the *A1298C* variant allele was not associated with colon cancer risk. Conversely in women, the *1298 CC* variant genotype (always occurring with wild-type *677 CC* genotype) was associated with a decreased risk (OR, 0.6; 95% CI, 0.4–0.9), and this association did not differ by age.

No appreciable difference in risk was seen for tumor site and either the *MTHFR 677* or *1298* genotypes (data not shown). Although allele frequencies for African-Americans were significantly different from those of Caucasians or Hispanics, stratification by race was not useful in determining associations for African-American participants due to small sample size and imprecise estimates (data not shown). The distribution of *MTHFR 677* and *MTHFR 1298* genotypes among African-Americans or Hispanics did not differ between cases and controls.

Associations between several dietary factors relevant to folate metabolism (folate, vitamin B_{12}, and alcohol) and combined *MTHFR* genotype in men and women are shown in Table 3. Due to the imprecise measure of intakes from supplements, this stratified analysis was restricted to individuals who indicated no regular intakes of vitamin supplements (1088 cases and 1315 controls), and, thus, reflects total dietary intakes.

In this study, we did not observe statistically significant alterations in colon-cancer risk associated with folate intake or *MTHFR* genotype in men. Trends toward a reduction in risk with high folate intake were observed only among those with *MTHFR 677CC/1298AA* (wild-type/wild-type) genotypes or those who carried a heterozygous/wild-type combination. Among women, any possible risk reduction associated with high-*versus*-low folate intakes was seen only among individuals with the *MTHFR 677 CC* genotype [note for example, among *MTHFR 677CC/1298AA*: low folate intake OR, 1.0 (ref); high folate intake OR, 0.5 and 95% CI, 0.2–1.3]. The lowest risk of colon cancer was seen among women with *677 CC* and *1298 CC* (wild-type/variant) genotypes who consumed a diet high in folate (OR, 0.2; 95% CI, 0.1–0.7; *P* interaction = 0.09).

With respect to stratification by vitamin B_{12} and *MTHFR* genotypes, no significant differences in colon cancer risk were seen for men. Among women, patterns were similar to those observed for folate intake, with a risk reduction suggested among those with the *MTHFR 1298 CC* variant genotype, in combination with a *677 CC* wild-type genotype (low vitamin B_{12} intake: OR, 0.8 and 95% CI, 0.3–2.1; high vitamin B_{12} intake OR, 0.2 and 95% CI, 0.1–0.7).

Table 1 Characteristics of the study population ($n = 3580$)

	Cases ($n = 1608$)	Controls ($n = 1972$)	P^a
Tumor site			
Proximal	794 (49%)		
Distal	775 (48%)		
Unknown site	39 (3%)		
Age at diagnosis or selection (range, 30–79)			
Years[b]	64.4 +/− 9.8	64.5 +/− 10.1	0.77
Sex			
Men	900 (56%)	1042 (53%)	
Women	708 (44%)	930 (47%)	0.06
Recent hormone-replacement therapy use, postmenopausal women[c]			
No	470 (77%)	551 (69%)	
Yes	144 (23%)	243 (31%)	<0.01
Kilocalories[b]			
Men	2773 +/− 1217	2637 +/− 1160	0.01
Women	2048 +/− 876	1972 +/− 831	0.07
Folate (mcg/day)[d]			
Men	387 (215)	393 (214)	0.80
Women	320 (177)	321 (174)	0.68
Vitamin B_{12} (mcg/day)[d]			
Men	6.5 (4.9)	6.3 (4.7)	0.18
Women	4.5 (3.4)	4.5 (3.4)	0.61
Long-term alcohol intake (gm/day)[d]			
Men	14.7 (27.0)	12.0 (24.8)	0.08
Women	3.7 (9.2)	3.7 (9.0)	0.43
MTHFR 677 and 1298 genotypes[e]			
677 CC (wt) and 1298 AA (wt)	223 (14%)	247 (13%)	
677 CT (het) and 1298 AA (wt)	384 (24%)	455 (23%)	
677 TT (var) and 1298 AA (wt)	150 (9%)	227 (12%)	
677 CC (wt) and 1298 AC (het)	358 (22%)	424 (21%)	
677 CT (het) and 1298 AC (het)	340 (21%)	403 (20%)	
677 TT (var) and 1298 AC (het)	—	—	
677 CC (wt) and 1298 CC (var)	153 (10%)	216 (11%)	
677 CT (het) and 1298 CC (var)	—	—	
677 TT (var) and 1298 CC (var)	—	—	0.17

[a] Based on χ^2 or *t* test.
[b] Mean +/− SD.
[c] Hormone replacement therapy within 2 years of diagnosis/selection.
[d] Median (interquartile range) for dietary intake. Long-term alcohol intake based on consumption of wine, beer, and liquor for drinkers reported 10 and 20 years ago, averaged.
[e] Some genotypes of MTHFR 677 and 1298 were not observed, presumably due to complete linkage disequilibrium.

Table 2 Association between MTHFR genotype and colon cancer risk stratified by sex and age[a,b]

MTHFR 677	AA				MTHFR 1298 AC				CC			
	Cases (*n*)	Controls (*n*)	OR[c]	95% CI	Cases (*n*)	Controls (*n*)	OR	95% CI	Cases (*n*)	Controls (*n*)	OR	95% CI
Men												
CC	131	145	1.0	—	178	217	0.9	(0.6–1.2)	93	103	1.0	(0.7–1.4)
CT	211	236	1.0	(0.7–1.3)	201	215	1.0	(0.7–1.4)				
TT	78	123	0.7	(0.5–1.0)								
Age of diagnosis/selection <65 years												
CC	52	66	1.0	—	70	89	1.0	(0.6–1.6)	38	34	1.3	(0.7–2.4)
CT	82	107	0.9	(0.5–1.4)	102	92	1.3	(0.8–2.1)				
TT	32	37	1.1	(0.6–2.0)								
Age of diagnosis/selection 65–79 years												
CC	79	79	1.0	—	108	128	0.8	(0.5–1.2)	55	69	0.8	(0.5–1.3)
CT	129	129	1.1	(0.7–1.6)	99	123	0.9	(0.6–1.3)				
TT	46	86	0.6	(0.3–0.9)								
P interaction, age category and *MTHFR* genotype = 0.34												
Women												
CC	89	102	1.0	—	178	206	1.0	(0.7–1.4)	60	113	0.6	(0.4–0.9)
CT	171	217	0.9	(0.6–1.3)	134	184	0.9	(0.6–1.2)				
TT	71	103	0.8	(0.5–1.2)								
Age of diagnosis/selection <65 years												
CC	38	47	1.0	—	78	72	1.4	(0.8–2.4)	21	39	0.7	(0.3–1.4)
CT	72	88	1.1	(0.6–1.8)	52	81	0.9	(0.5–1.7)				
TT	27	38	1.0	(0.5–1.9)								
Age of diagnosis/selection 65–79 years												
CC	51	55	1.0	—	100	134	0.8	(0.5–1.3)	39	74	0.6	(0.3–1.0)
CT	99	129	0.8	(0.5–1.3)	82	103	0.8	(0.5–1.4)				
TT	44	65	0.7	(0.4–1.2)								
P interaction, age category and *MTHFR* genotype = 0.86												

[a] Adjusted for age, body mass index, lifetime vigorous activity, energy intake, dietary fiber, dietary calcium, and usual number of cigarettes smoked. Participants with missing data for any of these variables were excluded (11 men and 10 women).
[b] *MTHFR 677/1298* genotypes *CT/CC*, *TT/AC*, and *TT/CC* were not observed in the study population.
[c] OR, odds ratio; CI, confidence interval.

A significant interaction was seen in men for long-term alcohol intake and *MTHFR* genotypes ($P = 0.03$). Men with the *677 TT/1298 AA* genotype and alcohol intake of <20 g/day experienced a reduced risk (OR, 0.3; 95% CI, 0.1–0.7) compared with *677 CC/1298AA* (wild-type/wild-type) and no alcohol intake. In women, alcohol intake of at least 10 g/day was associated with reduced risk among those with the *677 CC/1298 CC* genotype (OR, 0.2; 95% CI, 0–0.8); the *P* for interaction was not significant.

Associations among vitamins B_2, B_6, and methionine, and *MTHFR* genotype were similar to folate results in both men and women (data not shown); among women, apparent risk reductions with higher intakes were limited to those with *677 CC* genotypes, predominantly those with the combined *677CC/1298CC* (wild-type/variant) genotype (data not shown).

Analyses using nutrients based on quartile categories or collapsed quintile categories (extreme quintiles and the middle three groups combined) were not different from those using categories based on tertile cutoff points. When nutrient densities (per 1000 kcal) were used to stratify intake category, results were also not materially different.

Due to the observed differences in *MTHFR* genotype risk among men and women, and because recent studies suggest a relationship between HRT and homocysteine concentrations (32–36), we investigated associations between *MTHFR* genotype and HRT use within the past 2 years in postmenopausal women (Table 4). For *MTHFR C677T*, any risk reduction found with use of HRT was limited to individuals with both wild-type *MTHFR 677 CC/1298 AA* genotypes (no HRT use: OR, 1.0, ref *versus* HRT use: OR, 0.3; 95% CI, 0.1–0.6), but not seen among those homozygous for the variant *677 T* allele (no HRT use: OR, 0.6, 95% CI, 0.4–1.1 *versus* HRT use: OR, 0.6, 95% CI, 0.3–1.2). The same relationship was observed for the *MTHFR A1298C* polymorphism: HRT was associated with a decreased risk among those with the wild-type *677CC/1298AA* (no HRT use: OR, 1.0, ref *versus* HRT use: OR, 0.3, 95% CI, 0.1–0.6) but not among those with the *677CC/1298CC* (wild-type/homozygous variant) genotype (no HRT use: OR, 0.4, 95% CI, 0.2–0.7 *versus* HRT use: OR, 0.5, 95% CI, 0.2–1.1). Concurrently, we observed opposite trends associated with both variant *MTHFR* alleles compared with joint wild-type among postmenopausal women who used HRT within the past 2 years *versus* those who did not. The interaction between combined *MTHFR* genotypes and HRT use was statistically significant calculated as relative excess risk of interaction ($P < 0.01$). A test for interaction based on comparison of slopes was also statistically significant for the *A1298C*/HRT use relationship (two-sided $P = 0.04$ for Wald χ^2 test of slopes). Although these analyses are based on relatively small numbers of participants, their consistency across both *MTHFR* variants may be suggestive of a true interaction between hormone replacement therapy and *MTHFR* genotype.

Discussion

In this large multicenter case-control study of colon cancer, we were able to investigate both common *MTHFR* polymorphisms (*C677T* and *A1298C*) by stratifying on the combined genotypes, and to undertake subgroup analyses by nutrients and hormone-replacement therapy. Overall, we showed similar risk estimates for the *C677T* variant (see also Ref. 9) as other groups, indicating a slight inverse association with colon cancer

Table 3 Associations between MTHFR genotypes and colon cancer risk, stratified by dietary nutrient intakes (nonsupplement users)[a,b]

MTHFR 677	MTHFR 1298 AA				AC				CC			
	Cases (*n*)	Controls (*n*)	OR[c]	95% CI	Cases (*n*)	Controls (*n*)	OR	95% CI	Cases (*n*)	Controls (*n*)	OR	95% CI
Men												
Folate[d] *P* interaction = 0.90[e]												
CC												
Low	32	33	1.0	—	38	50	0.8	(0.4–1.6)	20	23	1.0	(0.4–2.1)
Int	35	30	1.3	(0.6–2.5)	53	55	1.0	(0.5–1.9)	27	21	1.3	(0.6–2.9)
High	28	39	0.7	(0.3–1.5)	37	53	0.6	(0.3–1.3)	24	26	1.0	(0.4–2.3)
CT												
Low	54	54	1.1	(0.6–2.1)	45	48	1.1	(0.6–2.0)				
Int	56	64	0.9	(0.5–1.7)	51	56	1.0	(0.5–1.9)				
High	37	53	0.7	(0.3–1.4)	52	51	1.1	(0.5–2.2)				
TT												
Low	18	23	0.8	(0.4–1.9)								
Int	18	36	0.6	(0.3–1.2)								
High	20	26	0.8	(0.4–1.8)								
Vitamin B_{12}[d]—*P* interaction = 0.90[e]												
CC												
Low	25	33	1.0	—	32	61	0.7	(0.4–1.4)	19	19	1.5	(0.6–3.4)
Int	33	37	1.3	(0.6–2.6)	54	44	1.7	(0.9–3.3)	29	31	1.4	(0.7–2.9)
High	37	32	1.6	(0.7–3.4)	42	53	1.0	(0.5–2.2)	23	20	1.4	(0.6–3.4)
CT												
Low	35	51	1.0	(0.5–2.1)	41	44	1.4	(0.7–2.8)				
Int	55	54	1.4	(0.7–2.7)	59	62	1.4	(0.7–2.7)				
High	57	66	1.1	(0.6–2.2)	48	49	1.3	(0.8–2.7)				
TT												
Low	16	27	0.9	(0.5–1.9)								
Int	19	34	0.8	(0.4–1.8)								
High	21	24	1.1	(0.5–2.6)								
Long-term alcohol intake[f] *P* interaction = 0.03[e]												
CC												
None	31	25	1.0	—	28	40	0.5	(0.3–1.1)	14	21	0.5	(0.2–1.3)
Mod	31	44	0.6	(0.3–1.1)	59	67	0.6	(0.3–1.2)	32	30	0.8	(0.4–1.7)
High	33	33	0.6	(0.3–1.3)	41	51	0.5	(0.3–1.0)	25	19	0.8	(0.4–1.9)
CT												
None	38	41	0.8	(0.4–1.5)	38	48	0.7	(0.3–1.3)				
Mod	63	70	0.7	(0.4–1.3)	64	74	0.7	(0.3–1.2)				
High	46	60	0.5	(0.2–0.9)	46	33	0.9	(0.4–1.9)				
TT												
None	21	26	0.6	(0.3–1.4)								
Mod	17	43	0.3	(0.1–0.7)								
High	18	16	0.7	(0.3–1.8)								
Women												
Folate[d]—*P* interaction = 0.09[e]												
CC												
Low	21	14	1.0	—	35	46	0.5	(0.2–1.1)	16	18	0.6	(0.2–1.7)
Int	19	29	0.5	(0.2–0.8)	36	42	0.6	(0.3–1.4)	12	26	0.3	(0.2–0.8)
High	16	20	0.5	(0.2–1.3)	37	37	0.7	(0.3–1.7)	8	20	0.2	(0.1–0.7)
CT												
Low	44	58	0.5	(0.3–1.1)	28	37	0.5	(0.2–1.2)				
Int	28	43	0.4	(0.2–0.9)	28	42	0.5	(0.2–1.1)				
High	34	35	0.7	(0.3–1.1)	26	37	0.5	(0.2–1.4)				
TT												
Low	16	27	0.4	(0.2–0.9)								
Int	13	17	0.5	(0.3–1.3)								
High	15	23	0.4	(0.2–1.0)								
Vitamin B_{12}[d]—*P* interaction = 0.90[e]												
CC												
Low	22	19	1.0	—	37	45	0.7	(0.3–1.6)	14	17	0.8	(0.3–2.1)
Int	16	25	0.6	(0.2–1.4)	27	50	0.4	(0.2–1.0)	15	25	0.4	(0.2–1.1)
High	18	19	0.7	(0.3–1.3)	44	30	1.2	(0.5–2.8)	7	22	0.2	(0.1–0.7)
CT												
Low	32	44	0.6	(0.3–1.4)	28	41	0.6	(0.3–1.3)				
Int	40	41	0.8	(0.4–1.8)	30	37	0.7	(0.3–1.6)				
High	34	51	0.5	(0.2–1.2)	24	38	0.6	(0.2–1.4)				
TT												
Low	19	26	0.7	(0.3–1.5)								
Int	11	25	0.4	(0.1–1.0)								
High	14	16	0.7	(0.3–2.1)								

Table 3 Continued

MTHFR 677	MTHFR 1298 AA				AC				CC			
	Cases (*n*)	Controls (*n*)	OR[c]	95% CI	Cases (*n*)	Controls (*n*)	OR	95% CI	Cases (*n*)	Controls (*n*)	OR	95% CI
Long-term alcohol intake[f] − *P* interaction = 0.62												
CC												
None	33	34	1.0	—	51	67	0.8	(0.4–1.5)	25	32	0.8	(0.4–1.6)
Mod	15	21	0.7	(0.3–1.6)	39	42	0.9	(0.5–1.7)	9	20	0.4	(0.2–1.0)
High	8	8	1.1	(0.3–3.2)	18	16	1.1	(0.5–2.6)	2	12	0.2	(0.0–0.8)
CT												
None	49	58	0.8	(0.4–1.5)	64	62	0.7	(0.4–1.4)				
Mod	36	60	0.6	(0.3–1.2)	50	42	0.9	(0.4–1.7)				
High	21	18	1.2	(0.5–2.7)	20	12	0.6	(0.2–1.8)				
TT												
None	26	37	0.7	(0.4–1.5)								
Mod	15	21	0.8	(0.3–1.8)								
High	3	9	0.3	(0.1–1.3)								

[a] Adjusted for age, body mass index, lifetime vigorous activity, energy intake, dietary fiber, calcium, usual number of cigarettes and folate, vitamin B_2, vitamin B_6, vitamin B_{12}, methionine, and alcohol, where appropriate.
[b] MTHFR 677/1298 genotypes CT/CC, TT/AC, and TT/CC were not observed in the study population.
[c] OR, odds ratio; CI, confidence interval.
[d] Tertile cut-off points for men were: folate, 318/459 mcg/day; vitamin B_{12}, 4.8/7.8 mcg/day. Tertile cut-off points for women were: folate, 273/388 mcg/day; vitamin B_{12}, 3.5, 5.7 mcg/day.
[e] Relative excess risk due to interaction (31).
[f] High alcohol intake from consumption of beer, wine, and liquor averaged for 10 and 20 years ago: men, ≥20 gm/day; women, ≥10 gm/day.

risk (10, 37, 38). Surprisingly, the *1298 CC* variant genotype was associated with a significantly decreased risk among women (overall *CC versus AA* OR, 0.6; 95% CI, 0.4–0.9), and reduced risk estimates more strongly than the *C677T* polymorphism (*TT versus CC* OR, 0.8; 95% CI, 0.5–1.1). The biochemical relevance of the *A1298C* polymorphism is not well defined; although the variant allele has been shown to reduce MTHFR activity modestly *in vivo* and *in vitro* in lymphocyte extracts, a thorough biochemical evaluation of the human recombinant MTHFR protein by Yamada *et al.* (19) did not show a difference in its biochemical phenotype. *In vivo*, its effects on enzyme function appear less pronounced than those of the *C677T* polymorphism, as evidenced by little alteration of homocysteine or serum folate concentrations (17, 24). However, in studies of adult and childhood acute leukemia (21, 22) the *A1298C* variant has been found to be associated with reduced risk, at least of some subtypes. Keku *et al.* (23) reported recently a statistically significantly reduced risk of colon cancer among whites with the *677 CC/1298 CC* genotype (OR, 0.5; 95% CI, 0.2–0.8); these findings are consistent with the risk reduction seen in our study among women (OR, 0.6) but not men (OR, 1.0). Within the Physician's Health Study, risk for this subgroup of individuals was reported as OR, 0.78 (95% CI, 0.36–1.72; Ref. 39); these confidence intervals were wide because the risk estimate was based on 15 cases and 28 controls. Nonetheless, the findings are certainly consistent with the larger case-control study by Keku *et al.* (23) and the one presented here. Interestingly, within the Physician's Health Study, individuals with the genotype combination of wild-type *677 CC* and variant *1298 CC* had marginally elevated homocysteine concentrations (12.3 *versus* 10.8 nmol/ml for w/w), which lends support to an *in vivo* phenotype for the *A1298C* polymorphism (39).

The amino acid affected by this single nucleotide substitution, Glu429, is located near the binding site for the allosteric MTHFR inhibitor *S*-adenosyl-methionine, and, thus, may possibly affect feedback inhibition. Although current experimental data do not show differences of *S*-adenosyl-methionine binding, studies of the impact of *A1298C* under different nutritional status have not yet been undertaken. It has been suggested that the decreased levels of activity stem from differences in protein stability rather than from the properties of the purified enzyme *per se* (19).

For both *MTHFR* polymorphisms, risk estimates associated with the variant alleles were lower among older men and women (Table 2). These findings suggest that other factors associated with aging interact with *MTHFR.* However, the relationships observed are inconsistent with our study on colorectal adenoma (12).

Because risk of colorectal neoplasia associated with MTHFR activity has been shown previously to differ by nutritional status (10–12, 40, 41), we stratified the analyses of genotype combinations by dietary intakes of folate and nutrients involved in folate metabolism (vitamin B_{12}, vitamin B_6, vitamin B_2, alcohol, and methionine). Among individuals who reported never having taken multivitamins or other supplements regularly (67%), associations were similar to reports by others (10–12), indicating that a reduced risk associated with higher folate intakes was observed only among those with *MTHFR 677 CC* (wild-type) or *CT* genotype, but not *TT* with reduced enzyme activity. The study population was recruited before any fortification of food sources, and the nutritional assessment included a detailed in-person dietary interview with food models; results include only individuals with a complete diet history. Our study supports a reduced risk associated with higher vitamin B_{12} intakes among women with wild-type *677CC* genotype, particularly when combined with a *1298CC* variant genotype. However, no such associations were seen among men, and the risk estimates were fairly unstable due to the few individuals in each cell.

Among women, high alcohol intake (≥10g/day) was associated with a decreased risk for carriers of the homozygous variant *MTHFR* genotypes (*677 CC/1298 CC:* OR, 0.2, 95% CI, 0.0–0.8; *677TT/1298AA:* OR, 0.3, 95% CI, 0.1–1.3, both compared with *677CC/1298AA* reference group). For men, a significant risk reduction with moderate alcohol consumption (<20g/day) was seen among individuals with the variant *677TT/1298AA* genotype, but not for the variant *677CC/*

Table 4 Association between MTHFR genotypes and colon cancer risk, stratified by hormone replacement therapy (postmenopausal women)[a]

Recent hormone replacement therapy	MTHFR 677	MTHFR 1298											
		AA				AC				CC			
		Cases (*n*)	Ctrls (*n*)	Odds ratio	95% confidence interval	Cases (*n*)	Ctrls (*n*)	Odds ratio	95% confidence interval	Cases (*n*)	Ctrls (*n*)	Odds ratio	95% confidence interval
No	CC	70	61	1.0	—	107	120	0.8	(0.5–1.2)	35	76	0.4	(0.2–0.7)
	CT	115	127	0.8	(0.5–1.2)	92	100	0.8	(0.5–1.3)				
	TT	46	64	0.6	(0.4–1.1)								
Yes	CC	10	31	0.3	(0.1–0.6)	41	61	0.6	(0.3–1.0)	12	23	0.5	(0.2–1.1)
	CT	33	58	0.5	(0.3–0.9)	31	43	0.6	(0.4–1.1)				
	TT	17	26	0.6	(0.3–1.2)								

[a] Adjusted for age, body mass index, lifetime vigorous activity, energy intake, diet fiber, calcium, usual number of cigarettes smoked. Relative excess risk due to interaction $P < 0.01$. Wald χ^2 test of slopes, *A1298C*/HRT use relationship $P = 0.04$. Wald χ^2 test of slopes, *C677T*/HRT use relationship $P = 0.09$.

1298CC genotype. These results are consistent with our findings for colorectal adenomas, showing a reduced risk among moderate drinkers with the *MTHFR 677TT* genotypes (12). However, other groups, particularly those involving prospective data collection, have reported opposite trends (*e.g.*, increased risks with higher alcohol consumption and *677 TT* genotypes; Refs. 10, 37, 41). The underlying biological mechanisms with respect to an interaction between folate and moderate alcohol consumption are unclear; most studies on the effects of alcohol consumption on folate absorption or metabolism have focused on very high, chronic alcohol intakes (42–44).

Our study showed some evidence for an interaction between estrogen status and *MTHFR* genotypes. Inverse associations between HRT and colon cancer incidence were only evident among women carrying both *MTHFR* wild-type alleles. Similarly, reduced risks associated with both *MTHFR* variants were limited to women who did not report current or recent use of hormone replacement therapy. Several recent studies show reductions of homocysteine concentrations under HRT (32–34, 36), or variations in homocysteine concentrations among women of different ages and lifestyles that are consistent with effects attributable to estrogen status (35). One study has investigated modifying effects of the *MTHFR C677T* polymorphism on the HRT-homocysteine relationship and observed no discernable differences, yet did not appear to have sufficient statistical power to test for such an interaction (34).

MTHFR genotypes can affect methylation capacity, as illustrated by studies on overall methylation patterns in lymphocytes (45, 46). It is also known that methylation of the estrogen receptor is an early event in colorectal carcinogenesis, which may be less likely to occur in the presence of HRT or endogenous estrogens (47–49). However, there is currently no clear biological rationale linking overall methylation capacity (based on the availability of the methyl-donor *S*-adenosylmethionine) to hypermethylation of a specific gene. Our study provides support for a possible link between MTHFR or folate and estrogen status in colorectal carcinogenesis, which should be pursued in further studies.

In summary, the *MTHFR A1298C* polymorphism may be as relevant in predicting colon cancer risk as the *C677T* variant, at least among women. This finding raises questions about the possible impact of the A1298C polymorphism on the function of MTHFR or its regulation; the interaction with use of HRT warrants additional investigation.

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MTHFR *C677T* and *A1298C* Polymorphisms: Diet, Estrogen, and Risk of Colon Cancer

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