# The Methylenetetrahydrofolate Reductase 677C→T Polymorphism and Distal Colorectal Adenoma Risk[1]

**A. Joan Levine,[2] Kimberly D. Siegmund, Carolyn M. Ervin, Anh Diep, Eric R. Lee, Harold D. Frankl, and Robert W. Haile**

Department of Preventive Medicine, University of Southern California, Los Angeles, California 90089-9181 [A. J. L., K. D. S., C. M. E., A. D., R. W. H.], and Divisions of Gastroenterology, Bellflower [E. R. L.], and Sunset [H. D. F.], Kaiser Permanente Medical Centers, Los Angeles, California 90027

## Abstract

**A common polymorphism in the *methylenetetrahydrofolate reductase* (*MTHFR*) gene, where a cytosine at nucleotide 677 is replaced by a thymine (677C→T), is associated with enzyme thermolability and a reduction in the conversion of 5,10-methyltetrahydrofolate (5,10-MTHF) into 5-methyltetrahydrofolate. We assessed the association between homozygosity for the *MTHFR* 677CT genotype (*TT*) and colorectal adenoma risk in a large sigmoidoscopy-based case-control study of members of a prepaid health plan in Los Angeles. *MTHFR* genotype was determined for 471 cases and 510 age-, sex-, clinic-, and sigmoidoscopy-date-matched controls. Information on RBC and plasma folate levels were analyzed for 331 cases and 350 controls. When compared with the presence of at least one wild-type allele (*CT/CC*), the odds ratio (OR) for the *TT* genotype was 1.19 [95% confidence interval (CI), 0.77–1.76] after adjusting for race and the matching factors. Compared with those in the lowest quartiles of RBC and plasma folate and a wild-type allele, adenoma risk was increased for *TT* homozygotes in the lowest folate quartiles (genotype: OR, 2.04 and 95% CI, 0.6–7.0; OR, 1.84 and 95% CI, 0.6–7.0 for RBCs and plasma folate, respectively) and decreased in *TT* homozygotes in the highest quartiles (genotype: OR, 0.82 and 95% CI, 0.32–2.10; OR, 0.65 and 95% CI, 0.22–1.95, respectively). There was also a significant interaction between *TT* genotype and the increased adenoma risk associated with alcohol. These data are consistent with an interaction between *MTHFR* genotype and folate availability.**

## Introduction

Folates are the primary methyl donors for all intracellular *trans*-methylation reactions, providing methyl and formyl groups to various substrates. The enzyme *MTHFR*[3] irreversibly converts intracellular 5,10-MTHF to 5-MTHF. This conversion is critical in controlling intracellular homocysteine and maintaining adequate SAM levels. SAM is the universal methyl donor, the ultimate source of methyl groups for all protein, lipid, and DNA methylation reactions. Cells synthesize *MTHFR* under conditions of relative 5-MTHF shortage (1, 2).

A common polymorphism in the *MTHFR* gene, in which a cytosine at position 677 is replaced by a thymine (677C→T), causes an alanine to valine substitution at that position. Those with the *TT* genotype have a thermolabile enzyme with about half the activity of the wild-type enzyme at 46°C (3). Homozygosity for the valine substitution was reported recently to be associated with increased plasma homocysteine but only in people with low plasma folate levels (4–9), demonstrating both the functional consequences of the *TT* genotype and the dependence of those consequences on available folates.

Three recent studies have reported on the relationship between the *MTHFR TT* genotype and CRC risk (10–12). Two groups reported a significantly decreased CRC risk in men with the *TT* genotype, relative to men with either the wild-type (*CC*) or heterozygous (*CT*) genotype (10, 11). Chen *et al.* (13) subsequently extended these studies to adenomatous polyps, reporting a nonsignificantly increased risk of adenomas in women with the *TT* genotype in the Nurses' Health Study. More recently, Ulrich *et al.* (14) reported an OR for *TT* compared with *CC* individuals of 0.8 (0.5–1.3) and a decreasing relative risk with increasing folate intake (OR, 0.7; 95% CI, 0.3–1.3) for *TT* homozygotes consuming >434 $\mu$g/day compared with *CC* homozygotes consuming >434 $\mu$g/day folate. The Ulrich *et al.* (14) data also suggested interactions between intakes of vitamins $B_{12}$, and $B_6$ with *MTHFR* genotype, with increased adenoma risk among those with lowest $B_{12}$, and $B_6$ intakes. Thus the relationship between *MTHFR* genotype and adenomatous polyps remains unclear.

Colorectal adenomas are known to be clinical precursors of CRC (15), and low folate diets have been associated with an increased risk of colorectal adenomas. There are a number of advantages to studying colorectal adenomas instead of CRCs as the end point of interest in epidemiological studies involving dietary exposures. It is possible to select subjects who are relatively asymptomatic so that symptoms do not cause a change in diet. Similarly, most adenomas are too small to have an effect on blood or plasma nutrient levels. Finally, the induction period for adenomas is substantially shorter than for colon cancer; therefore, nutrient measurements made at the time of an epidemiological study are closer in time to the presumed etiological events.

We report here on a large sigmoidoscopy-based case con-

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[2] To whom requests for reprints should be addressed, at Department of Preventive Medicine, University of Southern California, Los Angeles, CA 90089-9181.
[3] The abbreviations used are: MTHFR, methylenetetrahydrofolate reductase; 5-MTHF, 5-methyltetrahydrofolate; 5,10-MTHF, 5,10-methylenetetrahydrofolate; SAM, *S*-adenosyl methionine; SAH, *S*-adenosyl homocysteine; tHCY, plasma total homocysteine; OR, odds ratio; CI, confidence interval; BMI, body mass index; CRC, colorectal cancer.

trol study of colorectal adenomas in which we estimated the effect of the *MTHFR* genotype and assessed modification of this effect by levels of RBC, plasma, and estimated total folate intake from foods and dietary supplements. Our results confirm previous findings of an interaction between *MTHFR TT* genotype and folate availability risk of colorectal adenomas.

## Materials and Methods

**Study Population.** The study was approved by the Human Subjects Protection Committee of the University of California, Los Angeles, and by the Kaiser Permanente Institutional Review Board. All subjects signed a written informed consent form approved by the Institutional Review Board. Information on subject eligibility and recruitment are described more completely elsewhere (16). Briefly, subjects were eligible for the study if they underwent screening sigmoidoscopy at either of two Southern California Kaiser Permanente Medical Centers (Bellfower and Sunset) from January 1, 1991, through August 25, 1993, were between the ages of 50 and 74, had no evidence of prior bowel disease, and no previous bowel surgery. Cases were subjects diagnosed for the first time with one or more histologically confirmed adenomas. Controls had no adenomas of any type at sigmoidoscopy, had no history of adenomas, and were individually matched to cases by gender, age (within 5-year category), date of sigmoidoscopy (within 3-month category), and Kaiser Clinic. If the control initially matched to a case was not interviewed, a replacement control was identified.

**Clinical and Questionnaire Data.** Additional details of subject recruitment and data collection have been described elsewhere (16). Briefly, cases were individuals diagnosed with their first adenoma during screening sigmoidoscopy, and controls were drawn from the same population but had no adenomas at sigmoidoscopy and no history of adenomas. Adenoma data (*e.g.,* location, size, and number) were obtained from Kaiser pathology reports. Participants provided nondietary data during a 45-min in-person interview an average of 5 months after sigmoidoscopy. For dietary data, we used a 126-item, semiquantitative food frequency questionnaire (17) that inquired about diet in the year before sigmoidoscopy. The questionnaire was mailed, to be completed before the personal interview and then reviewed by the interviewer at the time of the interview.

Standard methods were used to calculate nutrient intake (18). We used the Nutrition Data System (base version 21) as a nutrient database for foods (19). For each subject, the reported frequency of consumption of each item on the questionnaire was multiplied by the nutrient content of the amount generally eaten, and total nutrient intake was obtained by summing, across foods, the amount each nutrient contributed. Data on the nutrient content of supplements were obtained from the Harvard School of Public Health.[4] Vitamin and dietary supplement data were self reported by either brand name (for multivitamins) or by the nutrient content/day (for single nutrient supplements) on the food frequency questionnaire.

**Red Cell and Plasma Folate.** Details of RBC folate determination are described more completely in Bird *et al.* (20). Briefly, a fasting blood sample was drawn in the morning from 500 cases and 533 controls. RBCs, plasma, and whole blood folate were determined for a subsample of cases and controls, consisting of the first 368 samples collected from male subjects and the first 313 samples collected from female subjects.

The first 561 whole blood samples were assayed by using Quantaphase radioassay kits (Bio-Rad Laboratories, Hercules, CA) and a Beckman Gamma 4000 gamma counter (Beckman Instruments, Inc., Fullerton, CA). The last 120 samples were assayed with Quantaphase II kits. A direct comparison of individual folate values obtained by both kits, in our laboratory, indicated that transformation of Quantaphase II values into Quantaphase values could be obtained accurately by the linear regression formula derived by Bio-Rad Laboratories (21). We calculated red cell folate values from whole blood folate concentrations and corrected for hematocrit and plasma folate levels, according to an established formula (22).

***MTHFR* Genotype.** Genotype was determined by the PCR-RFLP method of Frosst *et al.* (3) using their published primer pairs. Amplification was performed in a total reaction mixture of 15 $\mu$l containing 1.5 $\mu$l each of 10$\times$ buffer, deoxynucleotide triphosphates, and each primer; 0.1 $\mu$l of DNA polymerase, 0.9 $\mu$l of $MgCl_2$, and 3 $\mu$l of template DNA, using an initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min. The reactions were stopped by chilling to 4°C. Restriction digestion was performed by adding 5 $\mu$l of digestion mix [5 units of *Hin*fI (Boehringer Mannheim, Mannheim, Germany) mixed with 0.5 $\mu$1 of digestion buffer H (Boehringer Mannheim)] and 4 $\mu$1 of double-distilled $H_2O$ directly to the PCR product and digesting at 37°C for 2 h. Digestion was stopped by chilling to 4°C. Digested PCR products were visualized by ethidium bromide on a 3.5% Native Agarose Gel (Life Technologies, Grand Island New York). All samples with inadequate DNA amplification or unclear results were genotyped at least three times by the same technician. A random 10% sample of each 96-well microtiter plate was genotyped twice by a technician who was blinded to case-control status and the original genotyping results. All retested samples gave the same genotype reading.

**Statistical Analysis.** Exposure was defined as homozygosity for the valine substitution (*TT*). Homozygous wild-type individuals (*CC*) were combined with heterozygotes (*CT*) as a single "unexposed" group, to increase statistical power in stratified analyses. Initial analyses in which heterozygotes were removed from the comparison group were essentially the same as those in which they were combined with homozygous wild-type individuals and are not presented.

RBC and plasma folate, alcohol consumption, and other stratification variables were categorized into quartiles. Category boundaries were determined from the exposure distribution of the entire sample. Nutritional and physiological exposures, such as calories and BMI were entered into the models as continuous variables.

We used *t* tests and ANOVA to compare mean plasma and RBC folate between levels of genotype and Pearson product correlation coefficients to determine correlations between the different measures of folate status. Statistical tests for correlations or differences in mean values were done using the natural log scale. Initial estimates of the primary genotype effect were obtained with both conditional logistic regression, controlling for race, and an unconditional logistic regression in which the matching factors, age, sex, clinic, and sigmoidoscopy examination date, as well as race, were included. Because the results were essentially identical, only the results of the unconditional analysis are reported here.

[4] L. Sampson, personal communication, 1992.

*Table 1* Characteristics of the study population

| Risk factor | Cases ($n$ = 518) $n$ (%) | Controls ($n$ = 554) $n$ (%) |
|---|---|---|
| Male[a] | 338 (65.3) | 370 (66.8) |
| Race/Ethnic group | | |
| White[b] | 284 (54.8) | 295 (53.3) |
| Black[c] | 87 (16.8) | 94 (17.0) |
| Hispanic | 90 (17.4) | 99 (17.9) |
| Asian | 57 (11.0) | 66 (11.9) |
| Smoker | | |
| Current | 103 (19.9) | 63 (11.4) |
| Former | 235 (45.4) | 253 (45.7) |
| Never | 180 (34.8) | 238 (43.0) |
| Physical activity >3 h/wk | 116 (22.4) | 169 (30.5) |
| Drink >0 g alcohol/day | 291 (56.2) | 290 (52.4) |
| NSAID use (some) | 149 (28.8) | 181 (32.7) |
| | Mean (SD) | Mean (SD) |
| Age[d] | 67.2 (6.9) | 67.1 (6.9) |
| BMI (kg/m$^2$)[e] | 27.5 (4.5) | 26.9 (4.8) |
| Mean daily dietary intakes | | |
| Total energy (kcal)[f] | 2044 (849) | 1924 (812) |
| Total fat (g/day) | 72.8 (35.3) | 65.9 (34.3) |
| Saturated fat (g/day) | 25.0 (13.0) | 22.2 (12.4) |
| Vegetable fat (g/day) | 35.8 (21.1) | 32.6 (18.5) |
| Folates | | |
| Total[g] ($\mu$g/day) | 447.8 (262.7) | 447.1 (260.7) |
| RBC (ng/ml)[h] | 261.4 (146.0) | 270.2 (153.2) |
| Plasma (ng/ml)[i] | 11.9 (8.8) | 13.5 (9.9) |
| | Median (1Q–3Q) | Median (1Q–3Q) |
| Exercise (met-hours/wk)[j] | 14.3 (9.0–29.0) | 18 (9.5–30.0) |
| Alcohol (g/day) for drinkers | 10.4 (2.3–26.1) | 6.4 (2.1–17.6) |
| Pack-years | | |
| Current smokers | 37.5 (15.5–52.5) | 37.0 (17.5–47.0) |
| Past smoker | 23.0 (8.0–42.0) | 20.0 (7.0–36.0) |

[a,d] Matching factors.
[b] Includes 18 individuals who listed their race/ethnic groups as "other."
[c] Includes three individuals who also considered themselves of Hispanic origin.
[e] $n$ = 516 cases and 553 controls.
[f] $n$ = 553 controls.
[g] Dietary + supplemental folate per questionnaire.
[h] $n$ = 331 cases and 350 controls.
[i] $n$ = 336 cases and 355 controls.
[j] Those who exercise >3 met-hours/week.

## Results

During the accrual period, we identified 628 cases and 689 controls who were potentially eligible. Of these, 70 cases and 94 controls refused interview, and we were unable to contact 29 cases and 32 controls. Thus, we obtained interview data for 529 cases and 563 controls. The response rate was 84% among cases and 82% among controls. Fifteen cases had carcinoma *in situ* in addition to adenomas and were excluded from further analysis. Analyses in which these 15 subjects were included in the case group did not change the results by >1%.

DNA for genotype determination had been used up in prior analyses for 20 of the remaining 1092 subjects (9 cases and 11 controls), leaving a total of 1072 cases for the current analysis. Genotype information was missing for 91 of these subjects (8.5%), 47 cases (9.1%) and 44 controls (7.9%). Missing genotype information generally occurred when subjects had refused to donate blood ($n$ = 59) or when no PCR product was obtained after three tries ($n$ = 30). Genotype could not be clearly assigned for two individuals with amplified DNA. An analysis in which we assumed that all subjects with missing genotypes were either *CC* or *CT* (unexposed) or that they were all *TT* (exposed) did not change the adjusted ORs by >2%. RBC and plasma folate values for stratified analyses were determined as described above.

*Table 2* *MTHFR* genotype prevalence and main effects

| Genotype | Cases | Controls | OR[a] | 95% CI |
|---|---|---|---|---|
| *TT* | 52 (11.0%) | 49 (9.6%) | 1.11 | 0.71–1.71 |
| *CT* | 163 (34.6%) | 198 (38.9%) | 0.85 | 0.65–1.13 |
| *CC* | 256 (54.3%) | 263 (51.5%) | 1.0 | Reference |
| *TT/CT + CC* | 52/419 | 49/461 | 1.19 | 0.78–1.81 |

[a] Adjusted for age, race, sex, clinic, and examination date.

Among interviewed subjects, the indications for sigmoidoscopy were routine for 45% of cases and 44% of controls, whereas 16% of cases and 13% of controls were referred for sigmoidoscopy for specific minor symptoms. The indication for sigmoidoscopy was not available for the remaining 39% of cases and 43% of controls. The average depth of penetration of the flexible sigmoidoscope was 55 cm for cases (SD, 11 cm) and 59 cm for controls (SD, 5 cm).

Table 1 shows the characteristics of the study population. This Kaiser Permanente population was predominantly male, white, and had a mean age >67 years.

Fifty-two cases and 49 controls were homozygous for the *TT* genotype (Table 2). Table 2 presents the association between *TT* genotype and colorectal adenomas in this population. There was no overall effect of genotype on colorectal adenoma risk (OR, 1.19; CI, 0.78–1.81). Additional adjustment for alcohol use, estimated methionine intake, dietary vitamin $B_{12}$, BMI, or any other adenoma risk factor identified previously in this study population did not result in a >10% change in this estimate. We had no data on vitamin $B_6$. The estimated OR for the *TT* genotype in those with adenomas ≥10 mm was 1.14 (CI, 0.72–1.81), whereas in those with adenomas <10 mm, the estimated OR was 1.19 (CI, 0.66–2.17).

There were notable differences between the four ethnic groups in the prevalence of the *TT* genotype in controls. Whites and Hispanics had the highest *TT* genotype prevalence (10.1 and 15.2% of controls, respectively), whereas among Asians, the prevalence was 8.8%. The prevalence of the *TT* genotype was quite low among Black controls, 2.4%.

Matching factor-adjusted ORs were computed for each of the four ethnic groups separately. The main effect of genotype was essentially identical for Whites and Hispanics (OR, 1.29 and 95% CI, 0.75–2.20; OR, 1.29 and 95% CI, 0.58–2.90, respectively). Adjusted ORs for Black and Asian subjects were both <1.0 (OR, 0.50 and 95% CI, 0.05–5.3; OR, 0.53 and 95% CI, 0.11–2.49, respectively), but heterogeneity tests were not statistically significant. Excluding Blacks and Asians from the analysis did not materially effect the results of the unstratified analysis, except to broaden the confidence intervals. The small number of exposed cases (3 Blacks and 8 Asians) were insufficient for further stratified analysis.

Table 3 shows the joint effect of *MTHFR* genotype and folate on adenoma risk. For those with RBC folate levels in the lowest quartile (<165 ng/ml), subjects with the *TT* genotype had approximately twice the adenoma risk of those with at least one wild-type allele. At the highest folate levels, adenoma risk was <1.0 for both *TT* homozygotes and those with a wild-type allele.

There was a statistically significant trend toward a protective effect of higher plasma folate ($P_{trend}$ = 0.04) among those with the *TT* genotype, compared with those in the lowest

*Table 3* *MTHFR* genotype interaction with RBC and plasma folate

| RBC folate[a] ng/ml ($n$ = 669) | 52.7–164.7 | 164.8–228.2 | 228.3–314.6 | 314.7–1454 | *P*-trend |
|---|---|---|---|---|---|
| *CC/CT* | | | | | |
| Case/Control | 81/95 | 74/78 | 63/85 | 65/76 | |
| OR[b] | 1.0 | 0.86 | 0.69 | 0.80 | 0.22 |
| 95% CI | (reference) | (0.55–1.35) | (0.43–1.08) | (0.51–1.28) | |
| *TT* | | | | | |
| Case/Control | 9/4 | 7/6 | 11/9 | 12/14 | |
| OR[b] | 2.04 | 1.02 | 1.12 | 0.80 | 0.21 |
| 95% CI | (0.60–6.96) | (0.32–3.22) | (0.44–2.87) | (0.33–1.78) | |
| *P* interaction | | | | | 0.45 |
| Plasma folate[c] ng/ml ($n$ = 691) | 2.0–5.78 | 5.8–9.51 | 9.52–17.4 | 17.5–56.6 | *P*-trend |
| *CC/CT* | | | | | |
| Case/Control | 78/74 | 75/72 | 74/85 | 69/87 | 0.14 |
| OR[b] | 1.0 | 0.97 | 0.82 | 0.73 | |
| 95% CI | (reference) | (0.62–1.54) | (0.52–1.29) | (0.46–1.15) | |
| *TT* | | | | | |
| Case/Control | 14/17 | 15/11 | 5/9 | 6/10 | 0.04 |
| OR[b] | 1.84 | 1.24 | 0.52 | 0.54 | |
| 95% CI | (0.60–6.96) | (0.52–2.93) | (0.16–1.64) | (0.19–1.59) | |
| *P* interaction | | | | | 0.14 |

[a] Four hundred three (196 cases and 207 controls) were missing values for RBC folate.
[b] Adjusted for age, gender, ethnicity, clinic, and exam date.
[c] Three hundred eighty-one (182 cases and 199 controls) were missing values for plasma folate.

*Table 4* Correlations between folate measures

| | RBC folate ($n$ = 699) | Plasma folate ($n$ = 691) | Dietary folate ($n$ = 1059)[a] | Supplemental folate ($n$ = 1059) |
|---|---|---|---|---|
| RBC folate | | | | |
| Coefficient | 1.0 | 0.48 | 0.13 | 0.35 |
| *P*[b] | | 0.0001 | 0.0012 | 0.0001 |
| Plasma folate | | | | |
| Coefficient | | 1.0 | 0.17 | 0.40 |
| *P*[b] | | | 0.0001 | 0.0001 |
| Dietary folate | | | | |
| Coefficient[b] | | | 1.0 | 0.06 |
| *P* | | | | 0.11 |

[a] Twenty subjects (6 cases and 7 controls) were missing dietary questionnaire data.
[b] All *P*s estimated from natural log transformed folate data.

*Table 5* Relationships between *MTHFR* genotype and measures of folate status

| Genotype | RBC folate (ng/ml) | | Plasma folate (ng/ml) | | Total dietary folate ($\mu$g/day) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| *TT* | 305.8 | 151.0 | 11.3 | 9.3 | 486.4 | 286.0 |
| *CT* | 263.0 | 142.4 | 13.2 | 9.8 | 449.3 | 261.3 |
| *CC* | 260.2 | 154.7 | 12.7 | 9.2 | 439.8 | 253.3 |
| P[a] | 0.03 | | 0.23 | | 0.19 | |

[a] All *P*s estimated from natural log transformed folate data.

plasma folate quartile and at least one wild-type allele. The statistical interaction between plasma folate and *MTHFR* genotype was of borderline statistical significance, considering the small stratum-specific sample sizes ($P_{interaction}$ = 0.14).

There was a small correlation between dietary folate intake and either RBC or plasma folate, although at this sample size, all values were statistically significant (Table 4). RBC folate, but not plasma folate, varied significantly by genotype (Table 5).

The joint effect of *MTHFR* genotype and daily alcohol use, estimated vitamin $B_{12}$ intake, and dietary methionine intakes were also assessed and are presented in Table 6. The *MTHFR* TT genotype significantly modified the effect of alcohol on adenoma risk ($P_{interaction}$ = 0.012). Neither methionine nor vitamin $B_{12}$ intakes were associated with adenoma risk in this population, and *MTHFR* genotype did not modify these null results.

## Discussion

The data from this study confirm previous reports of an *MTHFR*/folate interaction on colorectal neoplasia risk (10–14). Compared with those with at least one wild-type allele, *TT* homozygotes in the lowest quartiles of either RBC or plasma folate had an approximate doubling of adenoma risk, whereas adenoma risk in *TT* homozygotes in the highest folate quartile appeared to decrease by 20% (RBC folate) to 50% (plasma folate) compared with the same baseline group. Similarly, in the case of alcohol use, which may be associated with folate depletion (23, 24), *TT* homozygotes with the highest quartile of alcohol consumption had an ~150% greater adenoma risk than those in the lowest quartile of alcohol consumption who had at least one wild-type allele. However, for those reporting no alcohol consumption, *TT* homozygotes appeared to have a 40% lower adenoma risk than those in the same alcohol group with at least one wild-type allele.

It is important to note that because this was a sigmoidoscopy-based study and the entire colon was not examined in controls, these results are limited to adenomas occurring on the left side of the colon, within reach of the sigmoidoscope. If the *MTHFR TT* genotype is differentially associated with right-sided adenomas, as reported recently for right-sided CRCs (12), then we may have underestimated the genotype/folate associations in this population by including a small number of individuals with right-sided adenomas in the control group. Approximately 15–17% of subjects with no family history and no adenomas at sigmoidoscopy may have one or more

*Table 6* *MTHFR* genotype interaction with dietary intakes of alcohol, methionine, and Vitamin $B_{12}$

| Alcohol[a] g/day | 0 | 0.86–1.04 | 1.05–9.49 | >9.49 | *P*-trend |
|---|---|---|---|---|---|
| *CC/CT* | | | | | |
| Case/Control | 197/220 | 27/32 | 84/106 | 103/111 | |
| OR[b] | 1.0 | 0.96 | 0.91 | 1.26 | 0.36 |
| 95% CI | reference | (0.55–1.66) | (0.64–1.29) | (0.89–1.78) | |
| *TT* | | | | | |
| Case/Control | 25/15 | 2/5 | 14/11 | 21/8 | |
| OR | 0.69 | 0.48 | 1.46 | 3.13 | |
| 95% CI | (0.35–1.35) | (0.09–2.53) | (0.64–3.30) | (1.34–7.34) | 0.003 |
| *P* interaction | | | | 0.054 | 0.012 |
| Methionine[a] g/day | 0.15–1.3 | 1.3–1.7 | 1.7–2.2 | 2.2–8.8 | *P*-trend |
| *CC/CT* | | | | | |
| Case/Control | 109/124 | 98/108 | 103/122 | 109/107 | |
| OR | 1.0 | 1.03 | 0.98 | 1.17 | 0.502 |
| 95% CI | reference | (0.01–1.51) | (0.67–1.42) | (0.81–1.70) | |
| *TT* | | | | | |
| Case/Control | 13/15 | 13/12 | 14/9 | 12/13 | |
| OR | 1.03 | 1.26 | 1.77 | 1.08 | 0.781 |
| 95% CI | (0.46–2.27) | (0.55–2.90) | (0.73–4.28) | (0.47–2.48) | |
| *P* interaction | | | | | 0.966 |
| Vitamin $B_{12}$[a] μg/day | 0.1–3.5 | 3.6–5.4 | 5.4–8.8 | 8.8–66.3 | *P*-trend |
| *CC/CT* | | | | | |
| Case/Control | 104/114 | 101/116 | 106/124 | 108/107 | |
| OR | 1.0 | 0.97 | 0.94 | 1.12 | 0.634 |
| 95% CI | reference | (0.66–1.41) | (0.65–1.37) | (0.76–1.63) | |
| *TT* | | | | | |
| Case/Control | 14/12 | 15/16 | 6/11 | 17/10 | |
| OR | 1.29 | 1.05 | 0.61 | 1.92 | 0.599 |
| 95% CI | (0.57–2.94) | (0.49–2.25) | (0.22–1.71) | (0.84–4.42) | |
| *P* interaction | | | | | 0.76 |

[a] Twenty subjects (6 cases and 7 controls) were missing dietary questionnaire data.
[b] All ORs are adjusted for age, gender, ethnicity, clinic, and exam date.

adenomas beyond the reach of the sigmoidoscope (25, 26). Because adenoma prevalence is high in this age group (27), the use of endoscopically screened controls minimized the likelihood of disease misclassification for left-sided adenomas.

Additionally, only subjects with minor or no symptoms were eligible for this study, and the majority of subjects for whom a reason for sigmoidoscopy referral was recorded were either asymptomatic or had only minor symptoms, implying that symptoms from colorectal disease were not important causes of selection into this study or of changed dietary or other behavior that would bias responses to either the dietary questionnaire or the folate measures. Finally, our response rates were relatively high (84% for cases and 82% for controls). We believe these characteristics helped to minimize any potential selection and information biases in this study population.

A possible interaction between *MTHFR* genotype and plasma folate on colorectal neoplasia risk has been reported by some groups but not by others. Ulrich *et al.* (14) reported an OR of 1.5 for those with a *TT* genotype compared with an OR of 0.9 for those with the *CC* genotype, relative to those in the highest folate tertile and the *CC* genotype. Ma *et al.* (11) studied the association between *MTHFR* genotype and CRC, reporting a significant decrease in CRC risk in men with plasma folate above 3 ng/ml and a slight increase in risk (OR, 1.33; 95% CI, 0.34–5.17) relative to those with a wild-type allele and plasma folate >3 ng/ml. However, CRC risk was similarly increased, compared with the same baseline, in those with low folate intakes and at least one wild-type allele (OR, 1.49; 95% CI, 0.76–2.94). Neither Chen *et al.* (10) nor Slattery *et al.* (12) observed an increase in CRC risk in *TT* homozygotes in any folate group, whereas both noted a decreased risk among those with the highest folate intakes and two thermolabile alleles in some groups. In a prospective study of colorectal adenoma, Chen *et al.* (13) reported an increasing risk with increasing dietary folate intake among those with a *TT* genotype and a decreasing risk with increasing folate among those with a wild-type allele, when both groups were compared with those in the lowest folate tertile and at least one wild-type allele (13).

A possible interaction between folate status and *MTHFR* genotype has been reported consistently in studies of plasma tHCY (4, 5, 8, 28). Although adenoma risk may be etiologically more complex than moderately elevated plasma tHCY, it is possible that these two different outcomes generally reflect a similar set of biological changes in folate metabolism. *MTHFR* activity is the rate-limiting step in determining the distribution of methyl groups for *trans*-methylation activities and nucleotide synthesis. The MTHFR product, 5-MTHF, is well known to be a major component in the pathogenesis of moderate homocysteinemia (2, 29). Kim *et al.* (29) recently reported that plasma tHCY was a more efficient measure of functional 5-MTHF deficiency, when compared with either RBC or plasma folate. Recently, Deloughery *et al.* (4) reported that plasma tHCY reached undetectable levels at significantly lower plasma folate levels in *TT* homozygotes than in those with at least one wild-type allele. Similar findings were reported by Malinow *et al.* (8) and Nelen *et al.* (9) in separate studies of

*MTHFR* genotype and folate supplementation in moderate homocysteinemia. If generalizable, this finding suggests that intracellular homocysteine levels may be controlled with less available folate in those with a thermolabile *MTHFR* enzyme. If this is also the case in colonic epithelium, then a similar mechanism could also affect the shift from an increased to a decreased risk of colon neoplasia as plasma folate levels increase to moderate levels.

We can only speculate about the biological mechanisms underlying an apparently exaggerated response to folate availability in *TT* homozygotes. A simple explanation would be that chronic *MTHFR* inefficiency, over a lifetime, is associated with compensatory mechanisms. An increase in folate absorption, recycling efficiency, *MTHFR* synthesis, or all three processes could support a higher ratio of available to unavailable intracellular tetrahydrofolate, providing a relative advantage to *TT* homozygotes when 5-MTHF levels are sufficient or only moderately low. However, as folate levels fall, such compensations would eventually fail. The resulting shortage of 5,10-MTHF for maintaining nucleotide pool balances (30, 31) and eventually 5-MTHF for controlling the SAM:SAH ratio (2) might become severe, especially in rapidly dividing tissues such as the colon. At this point, we would expect an increase in the adverse outcomes associated with excess uracil incorporation into DNA (32) and, eventually, methylation abnormalities (24).

The *MTHFR TT* genotype was associated with significantly higher RBC and nonsignificantly lower plasma folate in this study population, and neither was substantively correlated with dietary folate. This is also similar to associations reported by others (4, 7, 11, 33, 34) and emphasizes the usefulness of RBC and plasma folate, rather than dietary folate, as indices of folate status in future studies. It is unclear why *MTHFR* genotype was significantly related only to plasma folate in this population.

Excess alcohol intake is associated with multiple potentially procarcinogenic changes, including the induction of several phase I enzymes (*e.g., CYP-2E1*) as well as folate inhibition (35). A significant interaction between *MTHFR* genotype and increasing alcohol intake, as observed here, implicates folate metabolism as an important intermediate in the higher adenoma risk of individuals with high alcohol intake, because any effect of *MTHFR* genotype must involve this critical folate metabolic pathway at some level. On the other hand, we did not see either the main effects of, or genotype interactions between, dietary methionine and $B_{12}$ intakes, (14) or age (12, 14) on adenoma risk in this study population, most likely because this group of largely older males did not vary enough with respect to these variables.

The thermolabile *MTHFR* variant may be one of the most common genetic determinants of tHCY levels discovered to date (3, 4, 7, 9, 28). In this study, we have observed an increased adenoma risk in those at the lowest plasma and RBC folate quartiles and a decreased adenoma risk in those with higher plasma or RBC Folates. These data are reminiscent of reports that plasma tHCY may reach undetectable levels at significantly lower plasma folate levels in *TT* homozygotes than in those with at least one wild-type allele (4, 8, 9), providing independent evidence that the functional consequences of the *MTHFR* genotype are dependent on folic acid levels. Larger studies of populations with a wide range of plasma folate values will be needed to specify the nature of this interaction.

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