# 5,10-Methylenetetrahydrofolate Reductase Codon 677 and 1298 Polymorphisms and Colon Cancer in African Americans and Whites[1]

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## Abstract

**We evaluated polymorphisms in methylenetetrahydrofolate reductase (*MTHFR*), folate intake and alcohol consumption in relation to risk of colon cancer in a population-based case-control study in North Carolina. The study included 555 cases (244 African Americans and 311 whites) and 875 controls (331 African Americans and 544 whites). Total folate intake of <400 *versus* ≥400 μg/day showed a weak positive association with colon cancer among both African Americans [adjusted odds ratio (OR) = 1.4, 95% confidence interval (CI) = 1.0–2.0] and whites (OR = 1.6, 95% CI = 1.2–2.2). No association was observed with use of alcohol. Compared with wild-type genotypes, there was no association between the low activity *MTHFR* codon 677 TT genotype and colon cancer, but the low activity codon 1298 CC genotype was inversely associated with colon cancer in whites (OR = 0.5, 95% CI = 0.3–0.9). Unlike previous studies, we did not observe a strong protective effect of the codon 677 TT low-activity genotype when folate intake was high. Instead, we observed an increased risk of colon cancer when folate intake was low for participants with wild-type genotypes. Adjusted ORs for the combined effects of codon 677 CC and codon 1298 AA genotypes and folate intake <400 μg/day were 1.9 (95% CI = 1.1–3.4) in African Americans and 2.5 (95% CI = 1.2–5.2) in whites. Our results suggest that variation at *MTHFR* codon 1298 (within the COOH-terminal region) may be more important for colon cancer than variation at codon 677 ($NH_2$-terminal region), and in populations where folate intake is low, wild-type MTHFR activity may increase risk for colon cancer.**

## Introduction

Colon cancer is a complex disease involving multiple genetic and environmental factors (1). High intake of red meat, low intake of fruits, vegetables, and fiber, and high alcohol consumption combined with low intake of methionine and folate have been associated with increased risk of colon cancer (2, 3). The mechanisms by which folate intake and alcohol consumption affect risk of colon cancer are not fully understood. Folate is important in many biochemical pathways such as DNA methylation and DNA synthesis. Folate deficiency results in uracil misincorporation into DNA, DNA hypomethylation, and inhibition of excision repair of DNA in human colon epithelial cells (4–8). Defects in DNA methylation result in abnormal expression of oncogenes and tumor suppressor genes (9). Alcohol is metabolized to acetaldehydes by gastrointestinal flora, which may cause free radical damage to DNA of colon cells (10). High alcohol intake may deplete folate stores and may also affect bile composition and increase cell proliferation within the colon (11).

The influence of folate intake and alcohol consumption on colon cancer may be modified by inherited genetic factors (12). The enzyme *MTHFR*[3] functions at a critical juncture between DNA synthesis and methylation of DNA, proteins, and lipids (13). MTHFR activity catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The former is required as a substrate for DNA synthesis, whereas the latter plays a role in DNA methylation. The *MTHFR* gene is polymorphic, with single nucleotides variants within codon 677 in exon 4 (C→T, ala to val) and codon 1298 in exon 7 (A→C, glu to ala). The codon 677 polymorphism lies within the $NH_2$-terminal catalytic domain, whereas the codon 1298 polymorphism is in the COOH-terminal regulatory region (14). The codon 677 variant encodes a thermolabile enzyme with reduced activity (15) that leads to reduced plasma folate levels. Individuals who are homozygous for the codon 677 polymorphism also show hypomethylation of DNA in peripheral blood leukocytes, an effect that is especially pronounced when folate levels are low (16).

Several recent epidemiological studies demonstrated an inverse association between the *MTHFR* codon 677 TT genotype and colon cancer. In a cohort study of United States white male health professionals (Health Professionals Follow-up Study), Chen *et al.* (17) found that individuals homozygous for the *MTHFR* codon 677 T allele showed a weak inverse association with colon cancer risk. The inverse association was stronger among men with adequate folate intake. In the same study, codon 677 TT homozygotes showed an inverse associ-

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[3] The abbreviations used are: *MTHFR*, 5,10-methylenetetrahydrofolate reductase; NCCCS, North Carolina Colon Cancer Study; OR, odds ratio; CI, confidence interval; BMI, body mass index.

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ation with colon cancer when alcohol intake was low but a positive association when alcohol intake was high. In the Physician's Health Study, a randomized trial of mostly white United States male physicians, Ma *et al.* (18) observed a strong inverse association for codon 677 TT homozygotes and colon cancer when folate intake was high, and a weak positive association when folate intake was low. The authors also reported a strong inverse association between colon cancer and codon 677 TT genotype when alcohol intake was low, and a weak positive association when alcohol intake was high. In a case-control study conducted among mostly white male and female participants in the Kaiser Permanente Medical Care Program in Northern California, Utah, and Minnesota, Slattery *et al.* (19) observed a weak inverse association between colon cancer and *MTHFR* codon 677 TT genotype. The association was slightly stronger among people with high intake of dietary folate, but there was no difference according to dietary supplement use or alcohol intake. A weak inverse association for codon 677 TT genotype and colon cancer was also reported by Park *et al.* (20).

A potential biochemical explanation for the inverse association between colon cancer and the *MTHFR* TT genotype is that low MTHFR activity leads to buildup of 5,10-methylenetetrahydrofolate, which favors increased conversion of dUMP to dTMP. Less deoxyuridylate accumulates, so there is less DNA damage and more efficient DNA synthesis (4). However, low *MTHFR* activity also results in decreased levels of 5-methyltetrahydrofolate, a compound needed for synthesis of methionine. The current hypothesis to explain how low MTHFR activity could decrease risk of colon cancer is that the benefits of lessening deoxyuridylate-induced DNA damage outweigh the negative effects of reduced methionine synthesis, at least when folate intake is adequate. When folate intake is low, wild-type MTHFR activity could increase colon cancer risk by severely reducing the pool of 5,10-methylenetetrahydrofolate, resulting in buildup of dUMP. The theory would explain why Ma *et al.* (18) observed an inverse association for *MTHFR* 677 TT genotype in persons with high folate intake, and a positive association for *MTHFR* codon 677 C (wild-type)-containing genotypes when folate intake was low. The second *MTHFR* variant, codon 1298 A to C, is also associated with decreased enzymatic activity (14, 21) and would be expected to exert a similar protective effect on colon cancer risk when folate intake was high. Chen *et al.* (22) recently reported a weak but statistically nonsignificant inverse association between codon 1298CC genotype and colon cancer in the Physician's Health Study. There was no evidence of effect modification by folate intake.

Recent evidence suggests that the codon 677 T allele is present at lower frequency in Africans compared with Europeans (23) and in African Americans compared with United States whites (24). The codon 1298 C is also reported to occur a lower frequency in African Americans (13). We investigated the role *MTHFR* codon 677 and 1298 genotypes in a population-based case-control study of colon cancer among African Americans and whites in North Carolina. We estimated main effects for *MTHFR* genotypes, as well as joint effects with folate intake, use of dietary folate and vitamin supplements, and alcohol consumption.

## Materials and Methods

**Study Participants.** The NCCCS is a population-based, case-control study of colon cancer in North Carolina. The study participants were from 33 counties in the central portion of North Carolina, an area that included rural, suburban, and urban counties. These counties were selected because of the good socioeconomic mix of African Americans and whites, and there were no referral or selection patterns that would impede case ascertainment or compliance with the study. Race was based on self-description by study participants. The racial composition of the study population was 39% African American and 61% white. People with a first diagnosis of histologically confirmed invasive adenocarcinoma of the colon between July 1, 1996, and June 30, 2000, were identified through the rapid ascertainment system of the North Carolina Central Cancer Registry. Other case eligibility criteria included age between 40 and 85 at the time of diagnosis, residing in the 33-county study area in North Carolina, ability to give informed consent and be mentally competent to complete the interview, have a North Carolina drivers license or identification card if <age 65 (because controls < age 65 were sampled from driver's license rosters), and permission from their primary physician to participate in the study. Controls were frequency matched to cases by race, sex, and by 5-year age group by sampling from two sources: (*a*) people younger than age 65 from Division of Motor Vehicle records of individuals with North Carolina driver's license or state identification cards; and (*b*) people age 65 and older from a list of Medicare eligible beneficiaries obtained from the Health Care Financing Administration. Each eligible subject was assigned a unique identifier.

The total number of participants enrolled was 1691, including 643 cases (294 African Americans and 349 whites) and 1049 controls (437 African Americans and 611 whites). For cases, the contact rate (contacted participants/eligible) was 78%, the cooperation rate (interviewed/eligible) was 84%, and overall response rate (product of the contact and cooperation rates) was 66%. For controls, the contact rate was 90%, the cooperation rate was 62%, and the overall response rate was 56%. The cooperation rates were 79% for African American cases, 89% for white cases, 59% for African American controls, and 65% for white controls. Nonparticipants (refusals and noncontactable people) were slightly younger than participants and more likely to be female (among cases) and male (among controls), but these differences were not statistically significant. We were unable to compare characteristics other than age, race, and gender on participants and nonparticipants.

**Data Collection.** Data were collected by in-person interviews in the participant's home or at another convenient location (such as the local hospital or health department) by trained nurse interviewers. Study participants were given a brief explanation of the interview process. Information was collected on physical activity, education, household income, occupational history, smoking habits, family history of cancer, and other risk factors. The time referent was 5 years before diagnosis in cases or selection in controls. Additional questions included lifetime medical history and current social ties and access to health care. Measurements of height and weight were taken at the time of interview.

Dietary information was collected using a modified version of the semiquantitative food frequency questionnaire developed at the National Cancer Institute, commonly referred to as the Block questionnaire (25, 26). The questionnaire has been validated in several different populations, including low-income African American populations (27, 28). Participants were asked to estimate their usual frequency of consumption and serving size of >100 foods during the previous year (or year before diagnosis for cases). A 1-year period was chosen to provide a full cycle of seasons so that responses would be independent of the time of the year (29). In addition, partici-

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pants were asked about vitamin and mineral supplementation, special diets, restaurant eating, sodium use, and fats used in cooking. The dietary questionnaire took ~35–45 min to complete. The questionnaire was modified by adding 10 food items consumed in North Carolina (several varieties of cooked greens and peas; Brunswick stew; pigs' ears, feet, tails, or chitterlings; fried shellfish; various types of snack foods; grits; various pies and cobblers).

Nutrient intake was calculated by an analysis program provided by the National Cancer Institute that incorporates the nutrient content of each food item, the consumption frequency, and a portion size based on age (25). The statistical program was modified to include the additional North Carolina food items. A quality control program was used to check for implausible values based upon age and sex of the participant. All outlying values were decided upon by committee of nutritionists who acted as consultants for the study. Dietary folate was estimated from the folate content, amount, and frequency of consumption for folate-containing foods. Participants were also asked if they took supplemental folate or multivitamins on a regular basis. Individual supplemental folate intake was imputed from the formulation of the most common over-the-counter daily multivitamin supplements used in the study population (400 $\mu$g folate). Total folate intake was defined as the sum of dietary and supplemental folate intake, as recommended by the Food and Drug Administration (30). We did not estimate intake of vitamin B-6, vitamin B-12, or methionine from food. Because of the low frequency of people who reported taking folate supplements alone, supplements containing folate as well as minerals, multivitamins, or vitamins B-6 and B-12 were analyzed as a single category.

Data on alcohol consumption (amount and frequency of consumption of beer, wine, and liquor) were obtained from the diet questionnaire. Alcohol was measured as a percentage of daily calories consumed from alcohol (25). Consumption was low in the study population, as observed in a previous case-control study conducted in this region of North Carolina (31). Thus, alcohol consumption was classified as ever *versus* never drinker. Never drinker was defined as having zero calories from alcohol/day and ever drinker was defined as consuming >0 calories from alcohol/day.

**Blood Collection and Processing.** At the time of the interview, 40 ml of blood was collected from consenting participants. The blood was kept in coolers with cold packs until delivered to the laboratory for processing. For cases who had a doctor's visit shortly after the interview and preferred to have the blood draw in their doctor's office, a FedEx specimen package was provided to have the blood drawn and shipped to the lab. Blood was usually in the lab within 24 h. Informed consent to obtain DNA was approved by the Institutional Review Board of the University of North Carolina School of Medicine.

The compliance rates for blood collection were 86% for cases and 83% for controls, resulting in a total of 1430 DNA samples for analysis of genotypes (555 cases and 875 controls). Participants who gave blood were more likely to be male, white, and never smokers compared with those who did not give blood, but these groups did not differ according to dietary intake or other risk factors, including stage at diagnosis in cases. ORs for colon cancer and dietary and other risk factors did not differ between those who gave blood and those who did not (data not shown).

Genomic DNA was extracted from blood using the PureGene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN). The purity and quantity of each DNA specimen was determined by UV absorption at 260 and 280 nm.

**MTHFR Codon 677 RFLP Assay.** The presence of the C→T polymorphism at codon 677 in the *MTHFR* gene was determined by PCR-RFLP analysis using methods developed by Chen *et al.* (17). Nontemplate controls and three positive amplification/genotype controls were included within each experimental run. The *MTHFR* 677-CC genotype (wild-type) was represented by a 198-bp band; *MTHFR* 677-CT genotype (heterozygous) was represented by 23, 175, and 198 bp bands; and *MTHFR* 677-TT genotype (homozygous mutant) was represented by 23 and 175 bp bands. The genotypes were determined independently by two reviewers who were blinded to the case/control status of the subjects.

***MTHFR* Codon 677 Taqman Assay.** The *MTHFR* codon 677 genotype was also determined using a 5′exonuclease (Taqman) assay, a high throughput genotyping method using allele-specific fluorescent probes (PE Biosystems). This fluorescence detection system relies on two allele-specific probes, which fluoresce only when bound to the template. For the codon 677 Taqman assay, PCR primers and probes were designed using Primer Express TM software (PE Applied Biosystems). Assay design and conditions were based on the allelic discrimination protocol from PE Applied Biosystems. The cDNA 667 C (ala) allele probe was labeled on the 5′ end with the VIC reporter dye and contained the following nucleotide sequence 5′-TGTCTGCGGGAGCCGATTTCA-3′, with a 57.1% G-C content and 21-bp length. The cDNA 667 T (val) allele probe was labeled on the 5′ end with the 6-FAM reporter dye and contained the following nucleotide sequence 5′-AGGTGTCTGCGGGAGTCGATTTCA-3′, with a 54.2% G-C content and a 24-bp length. Both probes had a melting temperature of 66.8°C and contained the quencher dye Tamra on the 3′ end. Forward and reverse primers were used to amplify the region surrounding the C667T polymorphism. The nucleotide sequence for the forward primer was 5′-AGGCTGACCTGAAGCACTTGAA-3′. The melting temperature was 60.0°C with a 50% G-C content and 22 bp in length. The nucleotide sequence for the reverse primer was listed 3′-CTCAAAGAAAAGCTGCGTGATGA-5′. The melting temperature was 60.5°C with a 43.5% G-C content and 23 bp in length. PCR reactions were performed in a 25-$\mu$l reaction volume using the Hot-start format. The reaction components were as follows: 1× Taqman Universal PCR Master Mix, 900 nM of each primer, 200 nM wild-type probe, 200 nM variant probe, and 25 ng of genomic DNA. The PCR was run on a Perkin-Elmer GenAmp 9700 thermocycler using the 9600 mode under the following conditions: 50°C for 2 min (AmpErase UNG Activation), 95°C for 10 min (AmpliTaq Gold Activation), followed by 40 cycles of 95°C for 15 s (denature) and 62°C for 1 min (anneal/extend). Negative (reagent) controls ($n = 5$) and positive controls consisting of synthetic oligonucleotides specific for each allele ($n = 8$) were included in each plate. Samples that could not be scored were repeated. Unreadable results on the second run were scored as missing.

For *MTHFR* codon 677, genotype data were obtained on a total of 1420 persons: 552 cases (244 African American and 308 white) and 868 controls (329 African American and 539 white). *MTHFR* codon 677 genotypes were missing (unreadable or did not amplify) on 3 cases and 7 controls. A total of 1327 individuals were genotyped using PCR-RFLP, and 166 individuals were genotyped using Taqman. A random sample of 10% of the entire dataset was genotyped by both methods, and the results were identical.

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**MTHFR Codon 1298 RFLP Assay.** The presence of the A→C polymorphism at the 1298 position in the *MTHFR* gene was determined by PCR-RFLP analysis, using methods developed by van der Put *et al.* (21). Nontemplate controls and three positive amplification/genotype controls within each batch. The *MTHFR* 1298 AA genotype (wild-type) was represented by 5 bands: 56, 31, 30, 21, and 18 bp. The 1298 CC genotype (variant) was represented by 4 bands, 84, 31, 30, and 18 bp. Two reviewers, who were blinded to the case/control status determined, the genotypes independently.

***MTHFR* Codon 1298 AC Taqman Assay.** This assay is similar to the *MTHFR* 677 Taqman assay described above. The 1298 A (glu) allele probe was labeled on the 5′ end with the VIC reporter dye and contained the following nucleotide sequence 5′-AAGACACTTTCTTCACTG-3′, with a 38.9% G-C content, melting temperature of 65.4°C, and 18-bp length. The cDNA 1298 C (Ala) allele probe was labeled on the 5′ end with the FAM reporter dye and contained the following nucleotide sequence 5′-AGACACTTGCTTCACT-3′, with a 43.7% G-C content, melting temperature of 65.1°C, and a 16-bp length. Both probes contained the nonfluorescing minor grove binding (MGB) quencher dye on the 3′ end. Forward and reverse primers were used to amplify the region surrounding the A1298C polymorphism. The nucleotide sequence for the forward primer was 5′-AAGGAGGAGCTGCTGAAGATGT-3′. The melting temperature was 58.2°C with a 50% G-C content and 22 bp in length. The nucleotide sequence for the reverse primer was listed 3′-TGTGACCATTCCGGTTTGG-5′. The melting temperature was 58.8°C with a 52.6% G-C content and 19 bp in length. The cycling conditions were 50°C for 2 min (AmpErase UNG Activation), 95°C for 10 min (AmpliTaq Gold Activation), followed by 35 cycles of 92°C for 15 s (denature) and 60°C for 1 min (anneal/extend). Negative (reagent) controls ($n = 5$) and positive controls consisting of synthetic oligonucleotides specific for each allele ($n = 8$) were included in each plate. Samples that could not be scored were repeated. Unreadable results on the second run were scored as missing.

*MTHFR* codon 1298 genotype information was obtained on a total of 1422 persons: 552 cases (243 African American and 309 white) and 870 controls (329 African American and 541 white). Genotype information was unreadable or missing on 3 cases and 5 controls. Participants with missing information did not overlap for *MTHFR* codons 677 and 1298. A total of 462 individuals was genotyped using PCR-RFLP, and 960 persons were genotyped using Taqman. A random sample of 10% of the entire dataset was genotyped by both methods and results were identical.

**Statistical Analysis.** Allele frequencies and genotype frequencies for *MTHFR* 677 and 1298 were determined, and the observed genotype frequencies in African Americans and whites were compared with expected genotype frequencies calculated on the basis of observed allelic frequencies, assuming Hardy-Weinberg equilibrium. Departure from Hardy-Weinberg equilibrium was tested among African-American and white control groups using a goodness-of-fit $\chi^2$ test (32). *MTHFR* codon 677 and 1298 haplotype frequencies were estimated using the EH program (33). Estimated haplotypes frequencies were compared in cases, controls, and cases + controls, using a $\chi^2$ test based on differences in log likelihoods (34).

ORs were calculated as a measure of association between *MTHFR* genotypes or environmental exposures and colon cancer. OR and 95% CIs were estimated from unconditional logistic regression models (35) using the software package SAS (version 6.11; SAS Institute, Cary, NC). Offset terms were included in the models to account for the age-, race-, and sex-specific sampling probabilities used to identify recruitable participants (36). Race was classified by self-report.

ORs for *MTHFR* 677 and 1298 genotypes were calculated after subdividing cases based upon tumor location, distal or proximal. Cases with proximal tumors were compared with all controls, and cases with distal tumors were compared with all controls. ORs were also calculated separately for men and women.

Total folate intake and alcohol were used to assess joint effects with *MTHFR* genotypes. Cut points for total folate intake were based upon the recommended daily allowance for total folate intake, 400 $\mu$g/day (37). ORs did not differ when we used other cut points, including 250 $\mu$g/day (adequate per United States Department of Agriculture guidelines; Ref. 37) or median total folate intake in African-American controls (274 $\mu$g/day) and white controls (409 $\mu$g/day). Results are presented separately for users and nonusers of dietary supplements. Although several alternative methods for calculating ORs for joint effects of environmental factors and *MTHFR* genotype have been used (17–19), we used the reference groups used by Ma *et al.* (18) because this paper has been widely cited.

Crude ORs for environmental factors (ignoring *MTHFR* genotypes) were calculated on the subset of 1430 individuals for whom *MTHFR* genotype information was available. Similarly, main and joint effects for *MTHFR* genotypes and environmental factors were calculated using the subset of participants who had complete genotype information (N = 1420 for *MTHFR* codon 677, N = 1422 for *MTHFR* 1298). Joint effects for *MTHFR* codon 677 and 1298 genotypes and interactions of combined genotypes with environmental factors were calculated on the subset for whom genotype information at both loci were available (N = 1412).

Multivariate logistic regression models were used to identify and adjust for potential confounding factors (35), including current BMI (evaluated as $<27.5$, $\geq 27.5$, the median among controls, as well as continuous), dietary fiber ($<13.2$, $\geq 13.2$ g/day, the median among controls, and as continuous), energy intake ($<1708$, $\geq 1708$ kcal/day, the median among controls, as well as continuous), family history (yes/no for at least one first-degree relative with colon cancer), smoking (never, current, former), hormone replacement therapy (duration of use among postmenopausal women), and physical activity (frequency and level of activity). Current BMI, a measure of obesity, was calculated as weight divided by the square of height (kg/m$^2$), using measurements taken at the time of interview. A variable was included in final models if adjustment resulted in a 10% or greater change in the $\beta$ coefficient for the primary exposures (total folate intake, supplement use, alcohol use, *MTHFR* codon 677, and *MTHFR* 1298 genotype). Adjustment for energy intake as a continuous variable resulted in elevated ORs for joint effects of *MTHFR* and environmental factors. Thus, matching factors (age, race, and sex) were included in the models used to estimate main effects for genotypes, and the matching factors and energy intake were included in models to estimate ORs for folate intake, alcohol consumption, and joint effects for genotypes and environmental factors. Joint effects for alcohol intake and folate were estimated on an additive scale, using a common referent group (total folate $\geq$ 400 $\mu$g/day and never drinker). Joint effects for the combination of each *MTHFR* genotype (codon 677 or codon 1298) and folate intake, supplement use, and alcohol intake were also calculated on an additive scale using a common

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*Table 1* Characteristics of cases and controls in the NCCCS

| Characteristic | African Americans | | | | Whites | | | |
|---|---|---|---|---|---|---|---|---|
| | Cases ($n$ = 244) | | Controls ($n$ = 331) | | Cases ($n$ = 311) | | Controls ($n$ = 544) | |
| | $n$ | % | $n$ | % | $n$ | % | $n$ | % |
| Age (yr) | | | | | | | | |
| Mean | 61.7 | | 64.3 | | 64.3 | | 64.9 | |
| Median | 63.0 | | 66.0 | | 66.0 | | 67.0 | |
| Range | 40–79 | | 40–79 | | 40–79 | | 40–79 | |
| Gender | | | | | | | | |
| Male | 119 | 49.0 | 153 | 46.2 | 170 | 54.7 | 302 | 55.5 |
| Female | 124 | 51.0 | 178 | 53.8 | 141 | 45.3 | 242 | 44.5 |
| Total folate intake ($\mu$g/day) | | | | | | | | |
| Mean (SD) | 360.2 (217.2) | | 358.9 (219.3) | | 428.2 (231.4) | | 460.8 (241.3) | |
| Median | 292.5 | | 274.4 | | 353.0 | | 409.2 | |
| Range | 50.4–1375.4 | | 45.9–1125.0 | | 80.2–1225.3 | | 68.7–1293.3 | |
| Dietary supplement use | | | | | | | | |
| Yes | 58 | 24.0 | 90 | 27.3 | 109 | 35.3 | 229 | 42.2 |
| No | 184 | 76.0 | 240 | 72.7 | 200 | 64.7 | 314 | 57.8 |
| Alcohol (calories/day) | | | | | | | | |
| Mean (SD) | 56.0 (221.3) | | 20.2 (74.4) | | 70.4 (151.8) | | 45.8 (93.3) | |
| Median | 0 | | 0 | | 0 | | 0 | |
| Range | 0–2026.9 | | 0–739.5 | | 0–1023.1 | | 0–756.0 | |
| Family history | | | | | | | | |
| Yes | 45 | 18.4 | 29 | 8.8 | 68 | 22.0 | 50 | 9.3 |
| No | 199 | 81.6 | 301 | 91.2 | 241 | 78.0 | 488 | 90.7 |
| Energy intake (kcal/day) | | | | | | | | |
| Mean (SD) | 2035.8 (968.4) | | 1743.7 (770.2) | | 2021.7 (773.5) | | 1818.5 (628.3) | |
| Median | 1841.3 | | 1581.4 | | 1937.9 | | 1773.8 | |
| Range | 401.3–5795.7 | | 415.6–5474.2 | | 582.7–6368.9 | | 497.1–4454.0 | |
| Smoking status | | | | | | | | |
| Never | 50 | 20.8 | 76 | 23.0 | 42 | 13.6 | 82 | 15.1 |
| Current | 84 | 34.8 | 109 | 32.9 | 165 | 53.4 | 245 | 45.1 |
| Former | 107 | 44.4 | 146 | 44.1 | 102 | 33.0 | 216 | 39.8 |
| Current BMI (kg/m$^2$) | | | | | | | | |
| Mean (SD) | 28.5 (6.3) | | 29.7 (7.0) | | 27.4 (5.8) | | 27.7 (5.4) | |
| Median | 28.0 | | 29.1 | | 26.6 | | 26.7 | |
| Range | 14.8–51.2 | | 15.4–77.5 | | 16.7–63.5 | | 12.7–54.5 | |
| Annual household income (dollars) | | | | | | | | |
| <15,000 | 85 | 40.5 | 129 | 43.9 | 56 | 19.5 | 61 | 12.3 |
| 15,000–25,000 | 46 | 21.9 | 66 | 22.5 | 57 | 19.9 | 93 | 18.7 |
| >25,000–50,000 | 57 | 27.1 | 61 | 20.7 | 90 | 31.3 | 163 | 32.8 |
| >50,000 | 22 | 10.5 | 38 | 12.9 | 84 | 29.3 | 180 | 36.2 |

referent group. The combined effects of *MTHFR* 677 and 1298 genotypes were calculated using individuals who were homozygous wild-type activity at both loci as referent group. Joint effects of combined *MTHFR* codon 677 and 1298 genotypes with total folate intake, supplement use, and alcohol were also estimated.

For estimation of joint effects on an additive scale, indicator variables were created for each combination of categories of the potential modifying factors. Interactions on a multiplicative scale between *MTHFR* genotypes and total folate intake, supplement use, and alcohol were assessed by coding each variable using a separate internal referent group and entering these variables into logistic regression models. Models with main effects were compared with models containing main effects and an interaction term, using a likelihood ratio test (35).

## Results

Characteristics of study participants for whom blood samples were available (N = 1430; 555 cases and 875 controls) are presented in Table 1. Data were missing among cases for gender ($n$ = 1), total folate ($n$ = 4), supplement use ($n$ = 4), alcohol ($n$ = 4), family history ($n$ = 2), energy intake ($n$ = 4), smoking ($n$ = 5), BMI ($n$ = 1), and annual household income ($n$ = 7) and among controls for total folate ($n$ = 2), supplement use ($n$ = 2), family history ($n$ = 7), smoking ($n$ = 1), BMI ($n$ = 4), and annual household income ($n$ = 84). Mean total folate intake was approximately the same in African-American cases and controls but lower among white cases compared with controls. Dietary supplement use was lower in both African-American and white cases compared with controls, and alcohol intake was higher in cases compared with controls in both groups. African-American and white cases had a higher frequency of family history of colon cancer and higher energy intake than controls. Smoking and BMI were similar in cases and controls. Annual income was similar in African-American cases and controls, whereas white cases had lower annual income than controls. African Americans had considerably lower income than whites: 44% of African American controls but only 12% of white controls had annual household incomes of <$15,000/year.

Total folate intake of <400 *versus* ≥400 $\mu$g/day showed a weak positive association with colon cancer. ORs adjusted for

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*Table 2* Allele and genotype frequencies for *MTHFR* codon *677* and *1298* polymorphisms among African-American and white participants in NCCCS

| | African Americans | | Whites | |
|---|---|---|---|---|
| | Cases | Controls | Cases | Controls |
| *MTHFR 677* | *n* = 244 | *n* = 329 | *n* = 308 | *n* = 539 |
| Allele frequencies[a] (95% CI) | | | | |
| C (ala) | 0.90 (0.87–0.92) | 0.89 (0.86–0.91) | 0.69 (0.66–0.73) | 0.70 (0.67–0.73) |
| T (val) | 0.10 (0.07–0.13) | 0.11 (0.08–0.13) | 0.31 (0.27–0.34) | 0.30 (0.27–0.33) |
| *P*[b] | 0.68 | | 0.87 | |
| Genotype frequencies[c] | *n* (%) | *n* (%) | *n* (%) | *n* (%) |
| CC | 198 (81.2%) | 264 (80.2%) | 144 (46.7) | 265 (49.2) |
| CT | 43 (17.6%) | 59 (17.9%) | 140 (45.5) | 223 (41.4) |
| TT | 3 (1.2%) | 6 (1.8%) | 24 (7.8) | 51 (9.4) |
| *P*[b] | 0.85 | | 0.45 | |
| *MTHFR 1298* | *n* = 243 | *n* = 329 | *n* = 309 | *n* = 541 |
| Allele frequencies[a] (95% CI) | | | | |
| A (glu) | 0.81 (0.77–0.84) | 0.81 (0.78–0.84) | 0.72 (0.68–0.75) | 0.66 (0.63–0.69) |
| C (ala) | 0.19 (0.16–0.23) | 0.19 (0.16–0.22) | 0.28 (0.25–0.32) | 0.34 (0.32–0.37) |
| Genotype frequencies[c] | *n* (%) | *n* (%) | *n* (%) | *n* (%) |
| AA | 157 (64.6%) | 217 (66.0%) | 156 (50.5%) | 237 (43.8%) |
| AC | 78 (32.1%) | 99 (30.0%) | 132 (42.7%) | 236 (43.6) |
| CC | 8 (3.3%) | 13 (4.0%) | 21 (6.8%) | 68 (12.6%) |
| *P*[b] | 0.82 | | 0.02 | |

[a] No. of alleles/number of chromosomes.
[b] $\chi^2$ test comparing cases and controls (Fisher's exact text when cell counts $< 5$).
[c] No. of participants with genotype/total no. of participants.

sampling fractions, age, gender, and total energy intake were 1.4 (95% CI = 1.0–2.0) among African Americans and 1.6 (95% CI = 1.2–2.2) in whites. Compared with never-users, users of dietary supplements showed a weak inverse association in whites (adjusted OR = 0.7, 95% CI = 0.5–1.0) and no association in African Americans (OR = 0.9, 95% CI = 0.6–1.3). There was no association with ever-use of alcohol in whites (adjusted OR = 0.9, 95% CI = 0.7–1.2) or African Americans (OR = 1.0, 95% CI = 0.6–1.5). There was no evidence for joint effects of low folate intake and alcohol consumption.

Allele and genotype frequencies for *MTHFR* codon 677 and 1298 polymorphisms are presented for African Americans and whites in Table 2. No significant departures from Hardy-Weinberg equilibrium were observed for *MTHFR* 677 or 1298 genotypes among neither African-American nor white cases or controls. Observed allele frequencies for codon 677 T among whites in the NCCCS were similar to published estimates for Europeans and United States whites (17–19, 23, 24). Among African Americans, codon 677 T allele frequencies were within the range of published estimates for Africans and African Americans [0–0.09 in sub-Saharan Africans (23), 0.05–0.24 in Brazilian blacks, and African Americans (13, 38–42)]. Allele frequencies for codon 1298 C among whites were similar to published estimates among European and American whites (0.10–0.33; Refs. 14, 21) and among African Americans were similar to previous studies of Africans (43).

*MTHFR* haplotype frequencies were estimated for combinations of codon 677 and 1298 alleles in African Americans and whites. There were no statistically significant case-control differences in estimated haplotypes frequencies for African Americans ($P = 0.9$) or whites ($P = 0.8$). Combining cases and controls, estimated haplotype frequencies among African Americans were: 70% codon 677 C + codon 1298 A; 19% codon 677 C + codon 1298 C; 11% codon 677 T + codon 1298 A; and 0% codon 677 T + codon 1298 C. Among whites, estimated haplotype frequencies were: 38% codon 677 C + codon 1298 A; 32% codon 677 C + codon 1298 C; 29% codon T + codon 1298 A; and 1% codon 677 T + codon 1298 C.

ORs for *MTHFR* genotypes and colon cancer are presented in Table 3 for African Americans and whites. For codon 677, a slight inverse association was observed for the TT genotype *versus* CC + CT genotypes in African Americans and whites. For codon 1298, a slight inverse association was observed for the CC genotype in African Americans, and a modest inverse association was found in whites. Slight differences in ORs were observed for *MTHFR* codon 677 genotypes after subdividing cases based upon tumor location. The adjusted OR for codon 677 TT *versus* CC + CT genotypes and proximal tumors was 0.6 (95% CI = 0.1–4.7) in African Americans and 0.6 (95% CI = 0.3–1.3) in whites and close to 1.0 for distal tumors. OR for codon 1298 CC genotype did not differ by tumor location. ORs for *MTHFR* genotypes were similar in men and women (data not shown).

Frequencies and ORs for combined *MTHFR* codon 677 and 1298 genotypes are presented in Table 4 for African Americans and whites. For whites, the OR for codon 1298 CC genotype on a wild-type codon 677 background (CC) was more strongly inverse than for codon 677 TT genotype on a wild-type codon 1298 background (AA). The frequency of compound heterozygotes was 5% (16 of 329) in African-American controls and 20% (109 of 539) in white controls. There was no association between compound heterozygosity and colon cancer.

ORs for the joint effects of *MTHFR* codon 677 genotypes, total folate intake, and supplement use are presented in Table 5. Among whites, no association with colon cancer was observed for the TT genotype, but a positive association was found for codon 677 C-containing genotypes (CC or CC + CT) when folate intake was $<400$ $\mu$g/day. A positive association was also observed for TT + CT genotypes at low total folate intake, but this association appeared to be driven by the more highly prevalent CT genotype because there was no association for TT genotype with low folate intake. Results were similar but ORs

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*Table 3* ORs and 95% CI for colon cancer in relation to *MTHFR* codon *677* and *1298* genotypes among all participants, African Americans and whites

| | Overall OR[a] (95% CI) | African Americans OR[b] (95% CI) | Whites OR[b] (95% CI) |
|---|---|---|---|
| *MTHFR 677* genotype | | | |
| CC | Reference | Reference | Reference |
| CT | 1.1 (0.9–1.4) | 1.0 (0.6–1.5) | 1.2 (0.9–1.6) |
| TT | 0.8 (0.5–1.4) | 0.8 (0.2–3.1) | 0.9 (0.5–1.5) |
| TT + CT *versus* CC as reference group | 1.1 (0.8–1.3) | 1.0 (0.6–1.5) | 1.1 (0.8–1.5) |
| TT *versus* CC + CT as reference group | 0.8 (0.5–1.3) | 0.8 (0.2–3.1) | 0.8 (0.5–1.4) |
| *MTHFR 1298* genotype | | | |
| AA | Reference | Reference | Reference |
| AC | 0.9 (0.7–1.2) | 1.1 (0.8–1.6) | 0.9 (0.6–1.1) |
| CC | 0.6 (0.4–0.9) | 0.8 (0.3–2.1) | 0.5 (0.3–0.8) |
| AC + CC *versus* AA as reference group | 0.9 (0.7–1.1) | 1.1 (0.7–1.5) | 0.8 (0.6–1.0) |
| CC *versus* AA + AC as reference group | 0.6 (0.4–0.9) | 0.8 (0.3–2.0) | 0.5 (0.3–0.9) |

[a] Adjusted for sampling fractions, age, gender, and race.
[b] Adjusted for sampling fractions, age, and gender.

*Table 4* ORs[a] and 95% CI for combined *MTHFR* codon *677* and *1298* genotypes among African Americans and whites

| | *MTHFR* codon 1298 genotypes | | |
|---|---|---|---|
| | AA Cases/ Controls OR (95% CI) | AC Cases/ Controls OR (95% CI) | CC Cases/ Controls OR (95% CI) |
| African Americans | | | |
| *MTHFR* codon 677 genotypes | | | |
| CC | Reference | 1.1 (0.8–1.7) | 0.8 (0.3–2.1) |
| n[b] | 122/167 | 67/82 | 8/13 |
| CT | 1.0 (0.6–1.7) | 0.90 (0.4–2.0) | nd[c] |
| n[b] | 32/43 | 11/16 | 0/0 |
| TT | 0.8 (0.2–3.2) | nd | nd |
| n[b] | 3/6 | 0/0 | 0/0 |
| Whites | | | |
| CC | Reference | 0.9 (0.6–1.4) | 0.5 (0.2–0.8) |
| n[b] | 50/75 | 74/125 | 19/65 |
| CT | 1.1 (0.7–1.7) | 0.8 (0.5–1.3) | 2.97 (0.3–33.7)[d] |
| n[b] | 79/112 | 58/109 | 2/1 |
| TT | 0.8 (0.4–1.4) | nd | nd |
| n[b] | 24/47 | 0/0 | 0/2 |

[a] Adjusted for sampling fractions, age, and gender.
[b] Cases/controls.
[c] nd, not determined.
[d] Unstable estimate because of small numbers.

slightly weaker for the combined effects of codon 677 genotype and dietary supplement use. Among African Americans, ORs for TT genotype were unstable because of small numbers. A positive association was observed for codon 677 C-containing genotypes when total folate intake was low, but these associations were not as strong as among whites.

Results for the joint effects of *MTHFR* codon 1298 genotypes, total folate intake, and dietary supplement use are presented in Table 6. Among whites, an inverse association with colon cancer was observed for the codon CC genotype when folate intake was <400 μg/day, and among nonusers of supplements. A positive association was observed for AA or AA + AC genotypes when total folate intake was <400 μg/day and among nonusers of supplements. A positive association was observed among African-American participants with codon 1298 AA genotype and low folate intake, but there was no modification of ORs according to supplement use. ORs for codon 1298 CC genotype were unstable among African Americans because of small numbers.

Table 7 shows ORs for the joint effects of *MTHFR* genotypes and alcohol intake. ORs were close to 1.0 for combinations of codon 677 genotype and alcohol use. For codon 1298, an inverse association was observed for the CC genotype among never drinkers in African Americans and whites.

We estimated the effects of combined codon 677 and 1298 genotypes in relation to total folate intake, using high-activity compound homozygotes (codon 677 CC + codon 1298 AA) having adequate folate intake (≥400 μg/day) as the referent group. We found a positive association with colon cancer for high-activity homozygotes having low total folate intake (<400 μg/day): adjusted OR = 2.5 (95% CI = 1.2–5.2) among whites and 1.9 (95% CI = 1.2–3.4) among African Americans. ORs for the remaining combined genotype/total folate intake groups were close to 1.0. A weak positive association was observed for high-activity homozygote genotypes among white nonusers of dietary supplements (OR = 1.4, 95% = 0.7–3.0), but there was no association in African Americans. ORs for combined codon 677 and 1298 genotypes and alcohol consumption were close to 1.0 in African Americans and whites.

Likelihood ratio tests for multiplicative interaction were not statistically significant for most of the interactions assessed. *P*s of <0.10 were observed for the following interactions: among whites, codon 1298 genotype and total folate intake ($P = 0.03$), and codon 1298 genotype and use of dietary supplements ($P = 0.01$); among African Americans, combined codon 677 and 1298 genotypes and total folate intake ($P = 0.09$).

## Discussion

We investigated the association of *MTHFR* genotypes and colon cancer in a population-based case-control study of African Americans and whites in North Carolina. The effects of *MTHFR* codon 677 and 1298 genotypes, as well as the combined effects of genotypes at both loci, were evaluated in relation to total folate intake, dietary supplement use, and alcohol consumption. Unlike two previous studies (17, 18), we did not observe a strong inverse association between *MTHFR* codon 677 TT genotype and colon cancer. We observed ORs of 0.8 in African Americans (95% CI = 0.2–3.1) and whites (95% CI = 0.5–1.4) for codon 677 TT *versus* CC + CT genotypes and colon cancer, whereas Ma *et al.* (18) reported an OR of 0.5 (95% CI = 0.3–0.8) and Chen (17) reported an OR of 0.6 (95% CI = 0.3–1.1). Slattery *et al.* (19) reported a stronger inverse association of *MTHFR* 677 TT genotype with proximal com-

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*Table 5* ORs and 95% CI for colon cancer in relation to *MTHFR* codon 677 genotypes, total folate intake, and supplement use in African-American and white participants

| *MTHFR 677* genotype | Folate intake | African Americans | | Whites | |
|---|---|---|---|---|---|
| | | Cases/controls | OR[a] (95% CI) | Cases/controls | OR[a] (95% CI) |
| CC + CT | ≥400[b] | 76/113 | Reference | 115/248 | Reference |
| TT | ≥400[b] | 2/3 | 0.9 (0.1–6.0)[c] | 12/26 | 1.1 (0.5–2.2) |
| CC + CT | <400[b] | 163/209 | 1.4 (1.0–2.1) | 167/239 | 1.7 (1.3–2.3) |
| TT | <400[b] | 1/3 | 0.7 (0.1–6.9)[c] | 12/25 | 1.2 (0.6–2.5) |
| CC | ≥400 | 63/96 | Reference | 57/135 | Reference |
| TT + CT | ≥400 | 15/20 | 1.3 (0.6–2.8) | 70/139 | 1.3 (0.8–2.0) |
| CC | <400 | 133/168 | 1.5 (1.0–2.3) | 86/129 | 1.8 (1.2–2.8) |
| TT + CT | <400 | 31/44 | 1.3 (0.7–2.4) | 93/135 | 1.9 (1.3–2.9) |
| CC + CT | Yes[d] | 58/87 | Reference | 96/204 | Reference |
| TT | Yes[d] | 0/2 | nd[e] | 11/22 | 1.1 (0.5–2.4) |
| CC + CT | No[d] | 181/235 | 1.1 (0.7–1.6) | 186/283 | 1.4 (1.1–2.0) |
| TT | No[d] | 3/4 | 1.0 (0.2–4.9)[c] | 13/29 | 1.0 (0.5–2.1) |
| CC | Yes[d] | 48/71 | Reference | 48/106 | Reference |
| TT + CT | Yes[d] | 10/18 | 0.9 (0.4–2.1) | 59/120 | 1.1 (0.7–1.8) |
| CC | No[d] | 148/193 | 1.1 (0.7–1.7) | 95/158 | 1.4 (0.9–2.1) |
| TT + CT | No[d] | 36/46 | 1.1 (0.6–2.0) | 104/154 | 1.6 (1.0–2.4) |

[a] Adjusted for sampling fractions, age, gender, and energy intake.
[b] Total folate (μg/day).
[c] Unstable estimate because of small numbers.
[d] Dietary supplement use.
[e] nd, not determined.

*Table 6* ORs and 95% CI for colon cancer in relation to *MTHFR* codon 1298 genotype, total folate intake, and supplement use in African-American and white participants

| *MTHFR 1298* genotype | Folate intake | African Americans | | Whites | |
|---|---|---|---|---|---|
| | | Cases/controls | OR[a] (95% CI) | Cases/controls | OR[a] (95% CI) |
| AA + AC | ≥400[b] | 76/114 | Reference | 120/253 | Reference |
| CC | ≥400[b] | 1/2 | 1.0 (0.1–12.0)[c] | 10/23 | 0.9 (0.4–2.0) |
| AA + AC | <400[b] | 157/201 | 1.4 (1.0–2.1) | 167/220 | 1.8 (1.3–2.4) |
| CC | <400[b] | 7/11 | 1.1 (0.4–3.1) | 10/44 | 0.5 (0.3–1.1) |
| AA | ≥400[b] | 41/75 | Reference | 67/129 | Reference |
| CC + AC | ≥400[b] | 36/41 | 1.6 (0.9–3.0) | 63/147 | 0.8 (0.5–1.3) |
| AA | <400[b] | 114/142 | 1.8 (1.1–2.8) | 88/108 | 1.8 (1.2–2.7) |
| CC + AC | <400[b] | 50/70 | 1.6 (0.9–2.8) | 89/156 | 1.2 (0.8–1.8) |
| AA + AC | Yes[d] | 57/88 | Reference | 100/213 | Reference |
| CC | Yes[d] | 1/1 | 2.1 (0.1–35.2)[c] | 9/16 | 1.2 (0.5–2.8) |
| AA + AC | No[d] | 176/227 | 1.1 (0.8–1.7) | 187/260 | 1.6 (1.2–2.1) |
| CC | No[d] | 7/12 | 0.9 (0.3–2.4) | 11/51 | 0.5 (0.2–0.9) |
| AA | Yes[d] | 36/57 | Reference | 57/108 | Reference |
| CC + AC | Yes[d] | 22/32 | 1.1 (0.6–2.3) | 52/121 | 0.8 (0.5–1.3) |
| CC + AC | Yes[d] | 22/32 | 1.1 (0.6–2.3) | 52/121 | 0.8 (0.5–1.3) |
| AA | No[d] | 119/160 | 1.1 (0.7–1.9) | 98/129 | 1.5 (1.0–2.2) |
| CC + AC | No[d] | 64/79 | 1.2 (0.7–2.1) | 100/182 | 1.1 (0.7–1.6) |

[a] Adjusted for sampling fractions, age, gender, and energy intake.
[b] Total folate (μg/day).
[c] Unstable estimate because of small numbers.
[d] Dietary supplement use.

pared with distal tumors. We also observed a slightly stronger inverse association for *MTHFR* 677 TT genotype and proximal tumors. These differences could be attributable to chance or reflect physiological differences in the effects of folate metabolism in the colon. For codon 1298, we observed an inverse association codon 1298 CC *versus* AA + AC genotypes and colon cancer in whites (OR = 0.5, 95% CI = 0.3–0.9), whereas Chen *et al.* (22) reported an OR of 0.7 (95% CI = 0.4–1.4). In contrast to Chen *et al.* (22), our results suggest that *MTHFR* codon 1298 genotype may be more important than codon 677 genotype for colon cancer risk. Among whites, we observed an OR of 0.5 (95% CI = 0.2–0.8) for codon 1298 CC on wild-type codon 677 background and 0.8 (95% CI = 0.4–1.4) for codon 677 TT on wild-type codon 1298 background, whereas Chen *et al.* (22) reported ORs of 0.8 (95% CI = 0.4–1.7) and 0.5 (0.3–1.0), respectively. Additional large epidemiological studies are needed to more fully understand the relative contributions of *MTHFR* codon 677 and 1298 genotypes to colon cancer risk.

Previous studies of the joint effects of *MTHFR* codon 677

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*Table 7* ORs for colon cancer in relation to *MTHFR* codon 677 and 1298 genotypes and alcohol intake in African-American and white participants

| | | African Americans | | Whites | |
|---|---|---|---|---|---|
| | | Cases/controls | OR[a] (95% CI) | Cases/controls | OR[a] (95% CI) |
| *MTHFR 677* genotype | | | | | |
| CC + CT | Never[b] | 8/262 | Reference | 172/298 | Reference |
| TT | Never[b] | 3/4 | 1.0 (0.3–4.7) | 13/24 | 1.0 (0.5–2.1) |
| CC + CT | Ever[b] | 51/61 | 1.0 (0.7–1.6) | 110/190 | 0.9 (0.7–1.3) |
| TT | Ever[b] | 0/2 | nd[c] | 11/27 | 0.7 (0.3–1.4) |
| CC | Never[b] | 155/215 | Reference | 86/159 | Reference |
| TT + CT | Never[b] | 36/51 | 1.0 (0.6–1.6) | 99/163 | 1.2 (0.5–1.9) |
| CC | Ever[b] | 41/49 | 1.0 (0.6–1.6) | 57/106 | 0.9 (0.6–1.4) |
| TT + CT | Ever[b] | 10/14 | 1.0 (0.4–2.3) | 64/111 | 1.0 (0.7–1.5) |
| *MTHFR 1298* genotype | | | | | |
| AA + AC | Never[b] | 187/256 | Reference | 176/279 | Reference |
| CC | Never[b] | 4/10 | 0.6 (0.2–1.9) | 9/42 | 0.3 (0.2–0.7) |
| AA + AC | Ever[b] | 46/60 | 0.9 (0.6–1.5) | 111/194 | 0.8 (0.6–1.2) |
| CC | Ever[b] | 4/3 | 1.7 (0.4–8.1)[d] | 11/26 | 0.6 (0.3–1.3) |
| AA | Never[b] | 128/176 | Reference | 96/142 | Reference |
| CC + AC | Never[b] | 63/88 | 1.0 (0.7–1.5) | 89/179 | 0.7 (0.5–1.0) |
| AA | Ever[b] | 27/39 | 0.9 (0.5–1.5) | 59/95 | 0.9 (0.6–1.3) |
| CC + AC | Ever[b] | 19/21 | 1.1 (0.6–2.2) | 52/99 | 0.7 (0.5–1.1) |

[a] Adjusted for sampling fractions, age, gender, and energy intake.
[b] Alcohol intake.
[c] nd, not determined.
[d] Unstable estimate because of small numbers.

genotype and folate intake suggested that the variant TT genotype plus high folate intake was associated with a reduced risk of colon cancer among whites (17–19). We did not observe such an inverse association in African Americans nor in whites. Instead, we observed an increased colon cancer risk for persons with wild-type codon 677 alleles (CC or CC + CT) and low folate intake. For codon 677, our results at low folate intake are similar to previous studies. Among whites, our observed OR of 1.7 (95% CI = 1.2–2.3) for the combination of codon 677 CC + CT genotypes and low folate intake is similar in magnitude to the OR of 1.5 (95% CI = 0.8–2.9), as reported by Ma *et al.* (18) for MTHFR codon 677 C-containing genotypes and low folate. For codon 1298, our results at low levels of folate are the opposite of what one would expect based upon previous studies because the low activity codon 1298 CC genotype was associated with decreased colon cancer risk when folate levels were low but not when they were high.

Differences between our results and those of previous studies could have resulted from the methods used to measure folate intake. In the Physicians Health Study (17, 22), folate intake was estimated using plasma because dietary information was not available. In the Health Professionals Follow-up Study (17), folate intake was estimated using a food frequency questionnaire administered at entry into the cohort. In the study by Slattery *et al.* (19), a food frequency questionnaire was used to estimate folate intake, but dietary supplements were not included. We were unable to use RBC or plasma folate levels to estimate folate intake in our study because blood samples were taken after disease diagnosis in cases. In our study, folate intake was estimated from specific food items listed in the National Cancer Institute (Block) food frequency questionnaire, an instrument that differed from the questionnaires used in other studies, as well as dietary supplements. Folate intake estimated from the food frequency questionnaire used in the Health Professionals Follow-up Study (Willet) showed a strong correlation with red cell folate levels (3). The Block questionnaire has not been validated against plasma folate but showed strong correlations for related metabolites (44), and the Willet and Block questionnaires show a strong correlation in estimated nutrient intake after energy adjustment (45). We adapted the Block questionnaire by adding additional regional food items. Although we did not validate these additions to the questionnaire, the modifications were slight and probably had minimal effect on estimates of dietary folate intake. Thus, we conclude that the modified Block questionnaire used in our study provided estimates of folate intake that are comparable with previous studies. Food frequency questionnaires tend to underestimate folate intake, particularly during an era of dietary fortification (30), so it is possible that all of the epidemiological studies of *MTHFR* genotype and folate intake underestimated folate intake. Additional misclassification in our study may have arisen because we assumed all folate supplement users took a daily tablet containing 400 $\mu$g, which could have overestimated folate intake in some people. However, results did not differ when we assumed lower values for folate supplements (data not shown).

There are several limitations to our study. First, we were unable to distinguish between intake of vitamin B-6, B-12, and folate in the form of supplements, thus our findings for supplement use could be attributable to folate, vitamin B6, vitamin B12, or other compounds found in dietary supplements. We did not estimate intake of B vitamins or methionine from food, thus we could not analyze the joint effects of these compounds and *MTHFR* genotype. Because dietary intake of folate and B vitamins is often highly correlated (44), some or all of the effect of total folate intake in our study could be attributable to vitamin B-6 or B-12. However, Slattery *et al.* (19) did not show strong differences in the effects of folate, vitamin B-6, or vitamin B-12 according to *MTHFR* genotype.

Differences in results for the joint effects of *MTHFR* genotypes and folate intake could also have resulted from differences in folate intake across study populations. Our study was based upon a largely rural population in North Carolina with significant numbers of low-income people, whereas pre-

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vious studies enrolled health professionals (17, 18) and participants in a health maintenance organization health plan (19). Previous studies appear to have enrolled persons with higher folate intake than our population. In the Physician's Health Study (18), 9% of controls were classified as having folate deficiency based upon plasma folate levels, and in the Health Professionals Follow-up Study (3), 20% of participants had estimated folate intake of $<269$ $\mu$g/day. In our study, 43% of African-American controls and 24% of white controls had inadequate total folate intake ($<250$ $\mu$g/day). The majority of study participants did not take dietary supplements. Median total folate intake in African-American and white controls in our study was considerably lower than the estimated median total folate intake among people of similar age in the Continuing Survey of Food Intakes by Individuals for 1994–1996 (30), a representative sample of noninstitutionalized people living in United States households. Wild-type MTHFR activity shunts folate toward DNA methylation and increases buildup of deoxyuridylate, an effect that would be especially pronounced when folate intake was low. Thus, in populations where folate intake is low, wild-type MTHFR activity may increase risk for colon cancer because not enough folate is available for DNA synthesis. One would also expect to see minimal protective effect of low MTHFR activity. The fact that a strong positive association with colon cancer was observed for people in our study with wild-type genotypes at codons 677 and 1298 and low folate intake is additional support for this theory. Comparisons across future studies would be facilitated by the use of absolute (rather than relative) cut points for folate intake as used in our study.

With respect to alcohol consumption and *MTHFR* genotypes, two previous studies reported strong inverse associations among whites for the interaction of low alcohol consumption and the codon 677 TT genotype (17, 18), whereas one study found no association (19). Studies of colorectal adenomas have also observed the combination of the *MTHFR* 677 TT genotype and moderate alcohol consumption resulted in lower risk (46, 47). In our study, ORs for *MTHFR* codon 677 genotype were close to 1.0 among both drinkers and nondrinkers, similar to the study of Slattery *et al.* (19). For codon 1298, we observed an inverse association with colon cancer for nondrinkers with the CC genotype, suggesting that codon 1298 genotype may be more relevant than codon 677 genotype. However, we had limited power to evaluate interactions with alcohol in our study because alcohol consumption was low.

Differences in results across studies of *MTHFR* genotype and colon cancer could be attributable to the methods used for *MTHFR* genotyping. Our observed allele frequencies were similar to previous studies, and genotypes in our study were confirmed using two different laboratory methods. In our study, 2 white cases and 1 white control were heterozygous, and 2 white controls were homozygous for the codon 677 T + codon 1298 C haplotype. Previous reports suggested that the codon 677 T allele occurred only on a codon 1298 A (wild-type) chromosome, and 1298 C variant occurred on a 677 C (wild-type) chromosome (21, 48). Individuals homozygous for low-activity *MTHFR* alleles at both loci (codon 677 TT and codon 1298 CC) were not observed in studies conducted in the Netherlands (21), Germany (48), and Israel (43). However, Chen *et al.* (22) observed 3 white individuals (3% of controls) who were heterozygous for a codon 677 T + codon 1298 C haplotype. In our study, codon 677 and 1298 genotypes were confirmed by two separate laboratory methods, but determination of haplotypes was based upon maximum likelihood statistical methods and not direct observations. With the exception of the 5 people with apparent codon 677 T + codon 1298 C haplotypes, our observed haplotypes are similar to those reported recent by Rosenberg *et al.* (43) for white Israelis and Ghanaian Africans.

In our study, response rates were modest but were comparable with many recent population-based studies (49). Cooperation rates for blood draws were high compared with other studies (49). The fact that *MTHFR* genotype and allele frequencies were similar to previous studies suggests that enrollment in our study was not differential with respect to genotype. ORs for environmental factors were similar to previous studies and did not differ among those who gave and blood and those who did not. Thus, selection bias is an unlikely explanation for our observed results. Recall bias must be considered because we used self-reported dietary information gathered after the diagnosis of disease in cases (50). Total folate intake less than the current recommended daily allowance (400 $\mu$g/day; Ref. 37) was associated with a slight increase in colon cancer risk in our study. Use of dietary supplements showed a weak inverse association. These results are consistent with previous prospective epidemiological studies of colon cancer (3, 50), as well as studies of colon polyps (51, 52), where recall bias is expected to be minimal. Recent laboratory evidence lends biological plausibility to these findings. Kim *et al.* (53) recently showed that in rat models, folate deficiency induces DNA strand breaks in the P53 tumor suppressor gene, and folate supplementation prevents them. Previous studies reported a positive association between alcohol consumption and risk of colon cancer (3) and colon polyps (52), but we did not observe such an association in our study. However, alcohol consumption was low in our study population, and very few participants consumed alcohol at the levels associated with increased risk in previous studies of colon cancer.

Our study was the first to examine *MTHFR* 677 and 1298 polymorphisms in a population-based study that included large numbers of African Americans and large numbers of people with less than adequate folate intake. Previous studies examined the role of *MTHFR* polymorphisms in mostly white populations where folate intake was fairly high. In contrast, we examined the effects of *MTHFR* genotype in a population where folate intake was low. For this reason, we may have detected more of the detrimental effects of wild-type *MTHFR* activity that are seen at low levels of folate intake. In our study, *MTHFR* codon 1298 genotype had a stronger effect on colon cancer risk than *MTHFR* codon 677, and future studies are needed to confirm or refute this finding. To date, epidemiological studies have only examined one of many genes involved in metabolism of folate (12). Use of polymorphisms for additional loci will provide additional important information regarding the role of folate intake in colon cancer risk and help to determine the amounts of folate supplementation required to reduce risk of colon cancer.

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