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Dietary intake of folate and co-factors in folate metabolism, *MTHFR* polymorphisms, and reduced rectal cancer

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Abstract

Little is known about the contribution of polymorphisms in the methylenetetrahydrofolate reductase gene (*MTHFR*) and the folate metabolism pathway in rectal cancer alone. Data were from participants in a case-control study conducted in Northern California and Utah (751 cases and 979 controls). We examined independent associations and interactions of folate, B vitamins, methionine, alcohol, and *MTHFR* polymorphisms (*MTHFR C677T* and *A1298C)* with rectal cancer. Dietary folate intake was associated with a reduction in rectal cancer OR 0.66, 95% CI 0.48-0.92 (>475 mcg day compared to < = 322 mcg) as was a combination of nutrient intakes contributing to higher methyl donor status (OR 0.79, 95% CI 0.66-0.95). Risk was reduced among women with the *677 TT* genotype (OR 0.54, 95% CI 0.30-0.9), but not men (OR 1.11, 95% CI 0.70-1.76) and with the *1298 CC* genotype in combined gender analysis (OR 0.67, 95% CI 0.46-0.98). These data are consistent with a protective effect of increasing dietary folate against rectal cancer and suggest a protective role of the *MTHFR 677 TT* genotype in women and *1298* CC in men and women. Folate intake, low methyl donor status, and *MTHFR* polymorphisms may play independent roles in the etiology of rectal cancer.

Keywords

Diet; Pteroylpolyglutamic Acids; Rectal Neoplasms; Polymorphism; Genetic

Introduction

Methylation of DNA plays an important role in the regulation of gene activity [1,2] and DNA hypomethylation is associated with increased risk for colon adenomas [3]. In both benign and malignant colonic neoplasms, hypomethylation associated with hypermethylation of promoter regions of select tumor suppressor genes prevents transcription and promotes tumorigenesis [4-7]. However, recent review of the topic suggests that effects of folate on DNA methylation may be vary by the site, dependant on cell type, stage of transformation and the degree and duration of folate depletion [8]. Folate deficiency contributes to chromosomal instability and may increase susceptibility to radiation-induced DNA damage [9]. Thus, folate deficiency may contribute to carcinogenesis through several biological mechanisms and these mechanisms may be differentially important to colon and rectal cancer etiology.

Two functional polymorphisms of the *MTHFR* gene that are known to decrease MTHFR enzyme activity are *MTHFR C677T* [7,10] and *A1298C* [11,12]. These and other polymorphisms have been recently reviewed [13]. Reduced *MTHFR* activity has been reported

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in about 38% of the population; reduced mean enzyme activity has been shown (homozygotes being 30% of normal and heterozygotes being 65% of normal) although the association of folate deficiency and gene specific DNA methylation is not consistent [7,10-12]. We [14,15] and others [16-20] previously investigated the associations of these polymorphisms to risk for colon cancer. *MTHFR* polymorphisms are reported to be associated with greater risk for distal colon and rectal cancer than proximal colon tumors [20,21]. Greater chromosomal instability in the distal than the proximal colon may explain the difference in influence on colon and rectal cancer [22].

Low dietary intake of folate is associated with an increased risk of colorectal adenoma [23, 24] and colon cancer [14,25], perhaps through hypomethylation of DNA and resulting abnormal expression of oncogenes and tumor suppressor genes [26]. Alcohol, riboflavin, vitamins B_6 and B_{12}, and methionine are important co-factors in the folate metabolism pathway. The topic of alcohol, one-carbon transfer and colorectal cancer has been considered in detail recently [27]. We describe a CpG island colon tumor phenotype with a difference in location (distal and proximal) according to their micro-satellite stability status, other mutations, age and stage of cancer [28]. However, to our knowledge, interactions of *MTHFR* polymorphisms and dietary intakes of folate and other nutrients involved in folate metabolism specifically with respect to risk for rectal cancer have not been explored. Thus, the aim of this article was to examine whether *MTHFR C677T* or *A1298C* polymorphisms or their combined effects modify the association of dietary folate, riboflavin, vitamins B_6, and B_{12}, methionine, and alcohol with rectal cancer.

Materials and methods

Study population

Participants in the study were from the Kaiser Permanente Medical Care Program of Northern California (KPMCP), and the state of Utah. All eligible incident cases within these defined populations were identified and recruited for the study. Cases with a first primary tumor in the recto-sigmoid junction or rectum were identified between May 1997 and May 2001 using a rapid-reporting system. Case eligibility was determined by the Surveillance Epidemiology and End Results (SEER) Cancer Registries in Utah and Northern California. In Utah a rapid-reporting system was used to identify cases within 30 days of diagnosis. An on-line pathology reporting system was searched for rapid case-ascertainment of rectal cancer cases at KPMCP. Identified cases were confirmed through linkage to the Kaiser Permanente Northern California Cancer Registry. Cases with a previous colorectal tumor were not eligible for the study. Cases with familial adenomatous polyposis, ulcerative colitis, or Crohn's disease documented on the pathology report were not eligible. In addition to these criteria, participants were between 30 and 79 years of age at time of diagnosis, English speaking, and mentally competent to complete the interview. Institution review board approval was obtained from the University of Utah and KPMCP.

Controls were frequency matched to cases by sex and by five-year age groups with a ratio of cases to controls of 1:1. At KPMCP, controls were randomly selected from membership lists, and in Utah, controls younger than 65 were randomly selected from driver's license lists and controls 65 years and older were randomly selected from social security lists. The race/ethnicity of the study population was reported at the time of interview as 84% white, non-Hispanic, 4.1% African American, 7.6% Hispanic, 6.4% Asian, 3.9% American Indian, and 1.6% multiple races/ethnicity. A total of 742 rectal cancer cases and 970 matched controls are included in the analyses presented. Response rates were 65.2% for cases and 65.3% for controls; cooperation rates, or the number of people who participated of those we were able to contact was 73.2% for cases and 68.8% for controls.

Data collection

Data were collected for cases and controls by trained and certified interviewers for a calendar-year referent period that occurred one to two years prior to year of diagnosis or selection. Of the cases, 751 (79%) provided a blood sample and were genotyped; of the controls, 979 (81%) were genotyped. The detailed diet, lifestyle, medical and family history interview took approximately two hours. Rigorous quality control methods were used [29]. A detailed interviewer-administered dietary history questionnaire (DHQ) was used to assess diet. This DHQ was adapted and computerized [30] from the CARDIA DHQ [31,32]. Participants were asked to recall foods eaten, the frequency which they were eaten, serving size, and if fats were added in the preparation. Nutrient intake was calculated using the University of Minnesota Nutrition Coordinating Center's Nutrition Data System for Research (NDS-R), Database version 4.02_30, ©Regents of the University of Minnesota. Our database was updated to include folic acid fortified foods using data provided by NDS-R.

Dietary folate intake reflects actual dietary intake from folate, including synthetic folic acid from fortified foods. Data were collected both before and after folate fortification of enriched cereal-grain products became mandatory in 1998 [33,34]. Therefore, we initially examined dietary folate with and without synthetic folate. Using the different variables did not result in any substantive differences in associations. Therefore, only dietary folate (including synthetic folates) and total folates (dietary folate with supplements) are presented except where noted.

Current alcohol use was determined from reported alcohol consumption of beer, wine and liquor. Assessment of current alcohol was done for (1) ever and never and (2) median level of intake for men and women. Past alcohol use was derived from the average of use reported for 10 and 20 years ago; use from 10 years ago was used if data from 20 years ago was missing. Current use was not included in the past alcohol variable in order to avoid a variable that could be influenced by recent diagnosis. High past alcohol intake was considered >20 g/day alcohol for men and >10 g/day alcohol consumption for women.

The combination of folate, B_{12}, and alcohol were examined as reflecting high or low methyl donor status or influencing the folate metabolic pathway as follows. The lowest tertile of folate and B_{12} intake were categorized as low methyl donor status. The middle and high tertiles were coded as high methyl donor status. High alcohol use was categorized as low methyl donor status and no or low intake was categorized as high methyl donor status. If an individuals' folate and vitamin B_{12} intake were in the highest two tertiles and their alcohol use was high, however, they were coded as low methyl donor status.

Additional anthropometric and interview data used as covariates in analyses were collected. Height was measured at the time of interview and weight was reported for the two and five years prior to interview. The body mass index (BMI) of weight (kg)/height$(m)^2$ was calculated. Physical activity data were collected using a detailed physical activity questionnaire [35]. Recent hormone replacement therapy was determined by use within two years (recent HRT = Yes). Participants were asked to report number of years they smoked and usual number of cigarettes smoked per day when smoking. We calculated pack-years smoked for both current and former smokers. Non-steroidal anti-inflammatory medications (NSAIDS) and aspirin use was coded as (No) or ever (Yes) used regularly (for one month or more) within two years of the referent year. Estrogen-positive women were any women taking synthetic estrogen and women who had not gone through menopause (naturally or surgically). Estrogen negative women were women who experienced menopause and were not taking hormone replacement therapy.

MTHFR *677 C>T* and *1298 A>C* genotyping

Genotyping of the *MTHFR 677 C>T* polymorphism used a PCR-RFLP assay [19]. Briefly, 20 ng of genomic DNA was amplified using primers MTHFR-667-F (5′- TGA AGG AGA AGG TGT CTG CGG GA-3′) and MTHFR-667-R (5′- AGG ACG GTG CGG TGA GAG TG-3′) in the presence of 1.5 mM $MgCl^2$, dNTPs, and 0.25 Units of Amplitaq polymerase in a final volume of 12 ul. Following an initial denaturation of 2 min at 94°C, the reactions were cycled 30 times through 94°C for 10 s, 58°C for 10 s, and 72°C for 15 s. The PCR products were then subjected to *Hin*fI digestion according to the manufacturer (NEB) at 37°C for 16 h prior to electrophoretic separation on a 2% agarose gel containing ethidium-bromide and visualized with UV light. The *677* C allele produces a band of 197 bp (uncut) and the *677* T allele produced bands of 175 bp and 22 bp (cut).

The *MTHFR 1298 A>C* genotype was performed using a TaqMan assay as previously described by Curtin et al. [15]. Primers used were as follows: MTHFR 1298-F (5′ -GA GCA AGT CCC CCA AGG A-3′) and MTHFR 1298-R (5′- CTT TGT GAC CAT TCC GGT TTG-3′). TaqMan Probes were *MTHFR 1298 A>C* A-allele (5′-VIC-AGT GAA GAA AGT GTC TTT MGBNFQ-3′) and *MTHFR 1298 A>C* C-allele (5′-6-FAM-AGT GAA GCA AGT GTC TT MGBN FQ-3′). Each 17 ul PCR reaction contained 20 ng genomic DNA, 900 nM of each primer, 130 nM of each TaqMan probe, and 8.5 ul TaqMan Universal PCR Master Mix (contains AmpErase UNG and AmpliTaq Gold enzymes, dNTPs, and reaction buffer). PCR was carried out under the following conditions: 50°C for 2 min (UNG activation), 95°C for 10 min, followed by 40 cycles of 90°C for 15 s, and 60°C for 1 min using the BIO-RAD IQ detection system. The fluorescence of each sample was collected and analyzed version 3.0 of the iCycler IQ Real-Time detection software. For each marker genotyped, control samples representing all three possible genotypes were included in each 96-well tray. In addition, internal replicates representing >1% of the sample set were blinded and included.

Statistical methods

Unconditional logistic regression models were used to estimate risk of rectal cancer associated with folate, riboflavin, vitamins B_6 and B_{12}, methionine, and alcohol intake and *MTHFR* polymorphisms. Risk was determined across levels of dichotomous variables (yes, no), tertiles of intake, with tertiles determined by the sex-specific intake distribution in the control population. Our previous results suggest a stronger association of ibuprofen alone than all NSAIDS examined together with rectal cancer; therefore, we include recent ibuprofen use as a covariate in our models [36]. Assessment of the main effects of nutrients with respect to rectal cancer was conducted among all study participants with non-missing diet and covariate data (n = 941 cases and 1192 controls), and for only those for whom we had non-missing diet, covariate, and genotype information (n = 742 cases and 940 controls). Resulting odds ratios did not result in material differences in interpretation of the data. Therefore, the data in Table 2 reflects the former.

In multiple logistic regression models the following variables were included as covariates: sex, age at selection, physical activity, BMI, ibuprofen use in the last two years, pack-years of cigarettes smoked, energy, calcium, and fiber intake and *MTHFR* polymorphism. Adjustment for race did not significantly alter interpretation of data; therefore, we did not include race as an adjustment factor. We adjusted models examining main effects with the *MTHFR 677 C>T* polymorphism for the *1298 A>C* polymorphism and vice versa because both are known to reduce MTHFR enzyme activity. Linear trend was determined by evaluating significance of linear association across the categorized variable. Interaction or effect modification, between folate (and other nutrient) intake, *MTHFR* polymorphisms, and age, NSAIDS, and recent HRT use were evaluated by the cross-product of the exposures of interest. These cross products were created with four categories of genotype: (1) *677 CC* and 1*298AA*; 2 *C77 CT* and 1*298AA*,

677CT and *1298 AC*, *677 CC* and *1298 AC*; (3) *677 TT* and *1298AA*; (4) *677 CC* and *1298CC* and two of intake (collapsing the upper two tertiles of intake. We assessed interaction by using the common referent point (low methyl donor status and high risk genotype): *MTHFR 677 CC* genotype and *MTHFR 1298 AA* genotype, and low intakes of nutrients (the lowest tertile). We repeated analysis using cross products of one *MTHFR* genotype and diet. A significant multiplicative interaction with *MTHFR 677* polymorphism and gender was observed, therefore, further analyses were conducted and reported by gender.

Results

Fifty-eight percent of the population was male and 42% were female. The majority of participants were 50 years of age or older (Table 1, pp. 18-20) and the distribution was similar in cases and controls by virtue of the study design. The majority of participants were non-Hispanic white, a slightly greater proportion of cases were of Asian, Native American or mixed ethnicity than controls. The distribution of *MTHFR 677* genotypes was similar between male cases and controls, but female cases were slightly more likely to have the CC genotype. The distribution of *MTHFR 1298* genotypes was similar between female cases and controls, but fewer male cases had the *CC* genotype. In men and women combined, cases consumed more vitamin B_{12} (6.6 ± 0.38 vs. 6.0 ± 0.25) and methionine (2.19 ± 0.09 vs. 2.06 ± 0.06) than controls, but there were no significant differences in folate intake with or without synthetic sources of folate, or supplements, vitamins B_6, riboflavin, calcium, vitamin D, or alcohol.

Individuals with the highest dietary folate intake (including folic acid from fortified foods) had a significantly lower risk for rectal cancer (OR 0.66, 95% CI 0.48, 0.92) than those with the lowest intake (Table 2, pp. 21-22). Intakes of riboflavin, vitamins B_6 and B_{12}, methionine, and short- and past alcohol intake were not associated with risk for rectal cancer. There were no differences in association of folate, vitamins B_6 and B_{12}, methionine, or alcohol intake by sex. However, in men a high methyl donor status was associated with a decreased risk for rectal cancer (highest two tertiles diet and supplemental folate and B_{12} and low or no past alcohol intake, OR 0.69, 95% CI 0.54-0.87) whereas there was no reduction in risk among women (OR 1.05, 95% CI 0.79, 1.41).

Participants with the *MTHFR 677 TT* genotype were at a non-significantly lower risk of rectal cancer (OR = 0.83, 95% CI 0.58, 1.18) when compared to individuals who had the *CC* or *CT* genotype. Risk of rectal cancer differed by gender and genotype (multiplicative interaction $p = 0.03$) (Table 3, p. 23). In sex-stratified analysis, the *677 CT* and *TT* genotypes were associated with a lower risk of rectal cancer in women when compared to the *677 CC* genotype. Risk was particularly decreased among women 60 years or older (OR 0.32, 95% CI 0.12, 0.84) compared to younger women (OR 0.76, 95% CI 0.31-1.89). In men, the *MTHFR 677 TT* genotype was not associated with modified risk.

Individuals with the *MTHFR 1298 CC* genotype were at significantly lower risk of rectal cancer (OR = 0.67, 95% CI 0.46, 0.98) compared to individuals with the *AA* (referent) or *AC* genotypes (OR = 0.86, 95% CI 0.69, 1.08, Table 3). There was no significant interaction of the *677 C>T* and *1298 A>C* polymorphisms (Table 4, pp. 24-25).

We assessed whether the associations of folate, other nutrients or alcohol with rectal cancer were modified by the combined *MTHFR 677* and *1298* genotypes in comparison to wild type (*p*-value 0.11) (Table 5, pp. 26-29). No significant patterns were identified regardless of nutrient combination, level of cutoff for alcohol intake, consideration of diet and supplement use versus dietary intake only or grouping genotypes with similar associations with rectal cancer or sex stratified or sex combined analysis.

Discussion

We previously reported a modest association of folate, vitamin B_6, and vitamin B_{12} intake and *MTHFR* polymorphisms in relation to risk for colon cancer [14,15]. In this report, we provide support for an independent reduction in risk of rectal cancer with increasing intake of folate. Increased risk was observed among those consuming a low methyl donor status diet (low nutrient or high alcohol intake). Risk of rectal cancer was reduced among women with the *677C>T* polymorphism and men and women with the *1298 CC* genotype. There were no significant interactions between folate or other nutrient intakes or risk for methylation diet with *MTHFR* polymorphisms and rectal cancer. These reductions in risk may occur either through hypomethylation or through interruptions in normal DNA synthesis.

The association of increased folate intake with reduced risk of colorectal cancer is well documented [14,25,37-40]. Unfortunately, fewer studies have addressed the association in rectal cancer only, and the results are not consistent. Previously, a significant inverse trend of folate intake with rectal cancer was identified among men, but not women [38,41]. In the present study, the highest intake of total folates (synthetic and natural) from diet was associated with a 35% reduction in risk of rectal cancer when compared to the lowest intakes in men and women. Odds ratios were similar, but not significant, when stratified by gender. This similarity in association of folate with risk of colon and rectal cancer argues for similar a biological mechanism in these two tissues.

The associations of other B vitamins with risk of rectal cancer were not entirely as expected. Higher methyl donor status (medium or high dietary and supplemental folate and vitamin B_{12} and low alcohol intake) was associated with a decreased risk of rectal cancer in men, but other independent associations were absent. Intakes of dietary riboflavin, and vitamins B_6 and B_{12} were above the recommended levels for the vast majority of our participants. Therefore the failure to find associations might reflect adequate intake or inadequate variation in intake with which to drive an association. Greater intake of B6 is reported to be associated with lower risk of colorectal cancer [38,42,43], but was not associated with any change in risk of rectal cancer in this study. Although a role for B6 in reducing colorectal cancer is clear, evidence supporting importance, particularly in rectal cancer etiology is lacking. Whether this difference in findings is related to biological differences in mechanisms, or different study populations and methods is unknown.

The exact role of alcohol in folate metabolism is not clear, but alcohol may block folate release from hepatocytes, inhibit DNA methyltransferase, or induce malabsorption of folate [44]. In addition, low levels of riboflavin and flavin adenine dinucleotide (FAD) may reduce MTHFR activity. We previously reported independent associations of beer and wine with rectal cancer in men and women, respectively and past alcohol consumption with rectal cancer in women who did not use NSAIDS [45]. These associations were not modified by *MTHFR* polymorphisms, perhaps because the range of alcohol intake in this population is relatively low (both in Utah and California). Another possibility is the influence of folic acid fortification of foods which began in 1998. Increased folic acid intake might change the threshold of alcohols' effect. A more marked influence is noted among other populations where higher levels of alcohol consumption are apparent [46,47].

Although consuming a low risk for methylation diet was associated with a decreased risk of rectal cancer in men, this association was not modified by *MTHFR* genotype. This result also differs from the body of literature on colorectal cancer [14,46,47] that report an interaction of *MTHFR* genotype and methylation diet. A previous study that examined a high risk for hypomethylation reported a positive association between the high risk (low folate, low methionine and high alcohol consumption) diet and rectal cancer, but the study was based on

only 47 cases [41]. We hypothesized a greater effect of a low methyl donor diet in rectal cancer than colon cancer based on the suggestion that greater DNA instability exists more distally [22]. Further examination is necessary to determine whether the lower alcohol intake, folate fortification, or differences in study design or participants explain these disparate results and whether the biological mechanism is through hypomethylation or DNA stability.

The literature is mixed with regard to the independent association of the *677 C>T* polymorphism and risk for colorectal cancer. We found a reduction in risk of rectal cancer among women (38%), but not among men. Three recent studies reported no association between the *MTHFR 677 TT* polymorphism and rectal cancer [39,47,48] although they each had fewer cases of rectal cancer (n = 73, 220, and 290, respectively) than in the present study. A larger study, with 800 cases of rectal cancer, reported a 31% reduction in risk (not statistically significant) [20]. None of these studies reported gender specific effects. Our gender specific finding is in keeping with previously reported gender specific associations of rectal cancer and diet [45,49,50] or smoking and polymorphisms in genes for Phase I and Phase II metabolic enzymes [51]. They are also in keeping with our observation of greater reductions in rectal cancer noted with hormone replacement. It is yet unknown whether there is something particular about the participants from Utah and California in our study that leads to these gender differences or whether there is some other reason for the difference in results.

Few have reported on the association of *MTHFR 1298* polymorphisms with rectal cancer. Our finding of a 33% reduction in risk of rectal cancer among individuals with the *1298 CC* genotype (when men and women were analyzed together) is consistent with the idea that the *1298 CC* genotype is associated with a reduction in MTHFR enzyme activity and similar in magnitude to the reduction we found with the *MTHFR 677* TT genotype. Jiang et al. [48] also reported a significant (48%) reduction in rectal cancer among individuals with either the *1298 AC* or *1298 CC* genotypes in a Chinese study of 73 cases of rectal cancer, but a Japanese case-control study with 220 rectal cancer cases found no association of *MTHFR 1298* with rectal cancer [45]. Our finding is somewhat puzzling in light of the suggestion that the *1298 CC* genotype does not influence MTHFR enzyme activity as much as the *677 TT* genotype [7, 11]. Reasons for the disparities in findings are not immediately clear, although environmental exposures, racial differences in the expression of the polymorphisms and potential epigenetic interactions should be further explored.

Few studies have addressed whether there is an additive or synergistic effect of more than one polymorphism in the *MTHFR* gene. Risk was reduced among individuals (men and women) with *677CC* and *1298 CC* genotypes. We previously reported a reduction of a similar magnitude among women with *677CC* and *1298 CC* genotypes for colon cancer risk among women only [15]. The similarity in findings from women in our colon study and the present study points to similar mechanism for these polymorphisms with respect to colon and rectal cancer, particularly among women. However, a study investigating the same two *MTHFR* polymorphisms with colorectal cancer reported lower risk among individuals with the *677* TT and *1298 AA* genotype. And a non-significantly higher risk among individuals with the *677 CC* and *1298 CC* genotype [47]. Findings were similar in men and women with a caution that they had few people with the *1298 CC* genotype. The studies were similar in that neither our study nor theirs [47] had any individuals with the *677 TT* genotype and the *1298 AC* or *CC* genotypes and one individual had both the *677 CT* and *1298 CC* combined genotype in the previous study while we had none with this combined genotype. This previous study also reported an increased risk with 1298 CC genotype when alcohol intake was high, therefore, the alcohol intake and other differences between the Japanese study [47], our colon study [15] and our present rectal cancer study may contribute to differences in findings.

These dietary and other interview data come from a carefully monitored case-control study. Nonetheless, collection of dietary data referring to the year prior to diagnosis or selection for the study makes the data susceptible to recall bias. Recall bias could lead to over- or under-estimation of true associations, particularly if diet changed after the critical period with respect to etiology of rectal cancer. In addition, it is possible that the referent year does not correspond with the carcinogenic process. Such a mismatch in temporality could lead to either missed or spurious associations. Despite the large number of rectal cases, we were still limited in power when analyses were stratified by gender and interactions of two or more variables were examined, as in Table 4. Multiple comparisons do increase the risk of spurious findings, however, the consistency of our findings with those from colorectal cancer lend confidence to the suggestion that folate, the methylation diet, and *MTHFR* polymorphisms contribute to the etiology of rectal cancer. These findings point to the importance of folate nutrition and to the combined influence of nutrients and enzymes in folate metabolism on etiology of rectal cancer with differential importance to men and women.

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Table 1

Population characteristics

	Men					Women				
	Cases		Controls		*p*-value[a]	Cases		Controls		*p*-value[a]
	n = 446	(%)	n = 554	(%)		n = 305	(%)	n = 425	(%)	
Age at diagnosis or selection										
30-39	12	(2.7)	10	(1.8)		8	(2.6)	12	(2.8)	
40-49	46	(10.3)	55	(9.9)		48	(15.7)	46	(10.8)	
50-59	120	(26.9)	136	(24.6)		75	(24.6)	112	(26.4)	
60-69	161	(36.1)	207	(37.4)		97	(31.8)	132	(31.1)	
70-79	107	(24.0)	146	(26.4)		77	(25.3)	123	(28.9)	
Race										
Non-Hispanic, white	362	(81.2)	460	(83.3)		255	(83.9)	373	(87.8)	
Hispanic	29	(6.5)	38	(6.8)		20	(6.6)	24	(5.7)	
Black	16	(3.6)	28	(5.0)		13	(4.3)	14	(3.3)	
Other	39	(8.7)	26	(4.7)	0.02	16	(5.3)	14	(3.3)	0.12
	Men Cases		Controls		*p*-value*	Women Cases		Controls		*p*-value*
	N	(%)	*n*	(%)		n	(%)	N	(%)	
MTHFR 677C>T										
CC	195	(43.7)	267	(48.2)		167	(54.8)	202	(47.5)	
CT	193	(43.3)	222	(40.1)		112	(36.7)	176	(41.4)	
TT	58	(13.0)	65	(11.7)	0.19	26	(8.5)	47	(11.1)	0.05
MTHFR 1298A>C										
AA	223	(50.0)	252	(45.5)		141	(46.2)	186	(43.8)	
AC	186	(41.7)	235	(42.4)		134	(43.9)	195	(45.9)	
CC	37	(8.3)	67	(12.1)	0.05	30	(9.8)	44	(10.4)	0.54
	Mean	(SD)	Mean	(SD)	*p*-value*	Mean	(SD)	Mean	(SD)	*p*-value*
Energy (kcal/day)†	3,001	(1, 567)	2, 835	(1, 3)	0.07	2, 400	(1, 230)	2, 283	(996)	0.16
Calcium (mg/day)†	1,241	(726)	1,234	(749)	0.88	1,015	(568)	1,076	(528)	0.14
Fiber (g/day)†	28.2	(14.9)	28.1	(13.8)	0.98	24.4	(13.0)	24.8	(11.1)	0.66
Folate (mcg/day)†	455	(248)	449	(210)	0.69	384	(210	387	(169)	0.87
	Mean†	(SD)	Mean	(SD)	*p*-value*	Mean	(SD)	Mean	SD	*p*-value*
Total folates (diet and supplements) (mcg/day)†	627	(386)	678	(594)	0.10	626	(397)	663	(414)	0.23
Riboflavin (mg/day)†	2.8	(1.5)	2.7	(1.3)	0.16	2.2	(1.1)	2	(0.9)	0.70
Vitamin B_6 (mg/day)†	2.6	(1.4)	2.5	(1.0)	0.09	2.1	(1.0)	2.1	(0.8)	0.71
Vitamin B_{12} (mcg/day)†	7.5	(5.7)	6.7	(4.6)	0.02	5.4	(4.6)	5.1	(3.2)	0.40
Methionine (g/day)†	2.4	(1.3)	2.2	(1.1)	0.04	1.9	(1.0)	1.8	(0.8)	0.40
Alcohol, past (g/day)†‡	20.7	(39.4)	16.3	(50.9)	0.13	6.2	(17.2)	5.2	(10.2)	0.32

*Based on Mantel-Haenzel χ^2 or *t* test

†Mean ± SD

‡High past alcohol intake >20 g/day alcohol for men and >10 g/day alcohol consumption for women, use from 10 years ago was used if data from 20 years ago was missing

Table 2

Associations of nutrients involved in the folate metabolism pathway and rectal cancer

Overall	Referent	Intermediate			Upper tertile			
	Number of Cases/ Controls	Number of Cases/ Controls	OR*	95% CI	Number of Cases/ Controls	OR*	95% CI	*P* value
Dietary folates	351/398	310/398	0.84	0.67, 1.05	280/396	0.66	0.48, 0.92	0.01
Diet and supplement folates	373/395	287/395	0.78	0.63, 0.97	281/402	0.82	0.64, 1.05	0.09
Dietary riboflavin	306/398	292/395	1.04	0.82, 1.32	343/399	1.27	0.91, 1.77	0.19
Diet and supplement riboflavin	338/395	303/398	0.91	0.73, 1.13	300/399	0.94,	0.73., 1.22	0.65
Dietary vitamin B_6	319/397	317/394	0.99	0.78, 1.24	304/400	0.92	0.72, 1.17	0.59
	Referent	Intermediate					Upper Tertile	
Diet and supplement vitamin B_6	349/395	295/396	0.87	0.70, 1.08	297/401	0.92	0.72, 1.17	0.46
Vitamin B_{12}	283/394	321/401	1.08	0.87, 1.36	337/397	1.13	0.86, 1.51	0.37
Diet and supplement vitamin B_{12}	338/398	307/397	0.91	0.73, 1.13	296/397	0.91	0.71, 1.15	0.37
Methionine	302/396	299/398	0.96	0.76, 1.22	340/398	1.02	0.74, 1.40	0.95
	≤0.09 g/d	0.10-4.75 g				>4.75 g		
Current alcohol use	285/398	323/393	1.11	0.89, 1.38	333/401	1.05	0.84, 1.31	0.70
	None	Men >0 to <20 g/d; Women >0 to <10 g/d;					Men≥20 g/d; Women≥10 g/d	
Past alcohol use	404/546	296/409	0.93	0.76, 1.15	241/237	1.16	0.90, 1.48	0.36

Cutpoints: Dietary folates (mcg) ≤323; 324 to ≤475; >475; Diet and Supplement folates ≤441; 442 to ≤743; >743; Dietary riboflavin (mg) ≤1.84; 1.85 to ≤2.68; >2.68; Diet and Supplement riboflavin ≤2.49; 2.50 to <4.00; ≥4.00; Dietary vitamin B_6 (mg) ≤1.79; 1.80 to ≤2.6; >2.6. Diet and Supplement vitamin B_6 ≤2.44; 2.45 to ≤4.08; >4.08; Dietary vitamin B_{12} (mcg) ≤3.92; 3.93 to ≤6.57; >6.57; Diet and Supplement vitamin B_{12} <6.09; 6.10 to ≤11.17; >11.17; Methionine (mg) ≤1.55; 1.56 to ≤2.26; > 2.26; Current alcohol Use (g), ≤0.09; 0.10 to ≤4.75; > 4.75; Past alcohol use (g), none, >0 to <20; ≥20 men; none; >0 to <10 g; ≥10 women

*Odds ratios are adjusted for age, sex BMI, activity, energy, fiber, calcium, ibuprofen use, and smoking (pack-years)

Table 3

Association of *MTHFR 677* and *1298* with rectal cancer

Genotype	All			Men			Women		
	Number of Cases/ Controls	OR	95% CI	Number of Cases/ Controls	OR	95% CI	Number of Cases/ Controls	OR	95% CI
677 CC[*]	357/466	1.00		194/267	1.00		163/199	1.00	
CT	301/392	0.92	0.73, 1.14	191/219	1.14	0.85, 1.54	110/173	0.69	0.49, 0.99
TT	84/112	0.83	0.58, 1.18	58/65	1.11	0.70, 1.76	26/47	0.54	0.30, 0.98
1298 AA[*]	360/436	1.00		221/252	1.00		139/184	1.00	.
AC	317/424	0.86	0.69, 1.08	186/232	0.93	0.69, 1.25	131/192	0.78	0.55, 1.10
CC	65/110	0.67	0.46, 0.98	36/67	0.67	0.41, 1.11	29/43	0.68	0.38, 1.23

[*] Odds ratios are adjusted for age, BMI, activity, energy, fiber, calcium, ibuprofen use, smoking (pack-years), and the other *MTHFR* genotype; *p* for multiplicative interaction by genotype and sex = 0.03

Table 4

Association of combined genotypes of *MTHFR* 677 and 1298 with rectal cancer

A1298C									
	AA			***AC***			***CC***		
C677T*	**Number of Cases/ Controls**	**OR**	**95% CI**	**Number of Cases/ Controls**	**OR**	**95% CI**	**Number of Cases/ Controls**	**OR**	**95% CI**
All									
CC	119/122	1.00	.	173/234	0.78	0.56, 1.08	65/110	0.63	0.42, 0.94
CT	157/202	0.82	0.59, 1.15	144/190	0.78	0.55, 1.09	0/0		
TT	84/112	0.78	0.53, 1.14	0/0			0/0		
Men									
CC	64/76	1.00		94/124	0.93	0.60, 1.44	36/67	0.67	0.39, 1.15
CT	99/111	1.15	0.74, 1.78	92/108	1.06	0.68, 1.66	0/0		
TT	58/65	1.11	0.68, 1.83	0/0			0/0		
Women									
CC	55/46	1.00	.	79/110	0.61	0.37, 1.00	29/43	0.58	0.31, 1.10
CT	58/91	0.53	0.31, 0.90	52/82	0.52	0.31, 0.90	0/0		
TT	26/47	0.46	0.25, 0.87	0/0			0/0		

*OR are adjusted for age, BMI, activity, energy, fiber, calcium, ibuprofen use, smoking (packyears), and sex where appropriate

Table 5

Association of folates, vitamin B_{12}, alcohol and risk for methylation diet with *MTHFR* polymorphisms and rectal cancer in men and women

	Lowest Tertile					Higher 2 tertiles				
	Cases	Controls	OR	95% CI		Cases	Controls	OR	95% CI	
				Lower	Upper				Lower	Upper
Folates										
Men										
677 CC and 1298AA	25	26	1.00			39	50	0.75	0.36	1.59
Heterozygotes†	108	123	1.04	0.56	1.93	177	220	0.79	0.42	1.49
677 TT and 1298AA	18	22	0.92	0.40	2.15	40	43	0.94	0.45	1.98
677 CC and 1298CC	15	23	0.77	0.32	1.85	21	44	0.47	0.21	1.05
≠RERI = 0.91; Multiplicative = 0.85										
Women										
677 CC and 1298AA	17	15	1.00			38	31	1.07	0.44	2.60
Heterozygotes†	77	84	0.83	0.38	1.81	112	199	0.49	0.22	1.06
677 TT and 1298AA	10	20	0.46	0.16	1.31	16	28	0.52	0.19	1.37
677 CC and 1298CC	12	15	0.74	0.26	2.11	17	28	0.56	0.21	1.46
≠RERI = 0.66; Multiplicative = 0.46										
Vitamin B_{12}										
Men										
677 CC and 1298AA	17	21	1.00			47	55	0.94	0.43	2.06
Heterozygotes†	87	116	0.95	0.47	1.93	198	227	1.02	0.51	2.05
677 TT and 1298AA	16	17	1.11	0.43	2.87	42	48	1.05	0.48	2.32
677 CC and 1298CC	12	24	0.67	0.26	1.75	24	43	0.63	0.27	1.47
≠RERI = 0.98; and Multiplicative = 0.98										
Women										
677 CC and 1298AA	12	17	1.00			43	29	2.09	0.84	5.21
Heterozygotes†	71	86	1.19	0.53	2.70	118	197	0.82	0.36	1.86
677 TT and 1298AA	9	20	0.62	0.21	1.84	17	27	0.91	0.33	2.49
677 CC and 1298CC	13	16	1.18	0.41	3.40	16	27	0.88	0.32	2.38
≠RERI = 0.51; and Multiplicative = 0.11										
Current alcohol										
Men										
677 CC and 1298AA	23	27	1.00			41	49	0.98	0.48	2.00
Heterozygotes†	89	113	1.03	0.54	1.93	196	230	1.02	0.56	1.87
677 TT and 1298AA	11	24	0.57	0.23	1.43	47	41	1.40	0.69	2.86
677 CC and 1298CC	10	27	0.48	0.19	1.22	26	40	0.78	0.36	1.67
≠RERI = 0.18; and Multiplicative = 0.21										
677 CC and 1298AA	14	21	1.00			41	25	1.98	0.83	4.74
Heterozygotes†	63	85	1.11	0.51	2.41	126	198	0.79	0.37	1.66
677 TT and 1298AA	8	15	0.78	0.25	2.40	18	32	0.71	0.28	1.79
677 CC and 1298CC	6	14	0.59	0.18	1.97	23	29	1.08	0.44	2.66
≠RERI = 0.47; Multiplicative 0.12										
Methyl donor status	*Low*					*High*				
	Cases	Controls	OR	95% CI		Cases	Controls	OR	95% CI	
677 CC and 1298AA	53	60	1.00			11	16	0.86	0.36	2.08
Heterozygotes†	232	262	1.07	0.70	1.62	53	81	0.83	0.49	1.41
677 TT and 1298AA	44	45	1.15	0.65	2.03	14	20	0.92	0.41	2.06
677 CC and 1298CC	28	51	0.67	0.36	1.22	8	16	0.62	0.24	1.61
≠RERI = 0.96; Multiplicative = 0.99										
677 CC and 1298AA	39	29	1.00			16	17	0.90	0.38	2.16
Heterozygotes†	139	199	0.53	0.31	0.91	49	84	0.55	0.29	1.04

	Lowest Tertile					Higher 2 tertiles				
	Cases	Controls	OR	95% CI		Cases	Controls	OR	95% CI	
				Lower	Upper				Lower	Upper
677 TT and 1298AA	21	34	0.47	0.23	0.99	5	13	0.37	0.11	1.19
677 CC and 1298CC	21	38	0.44	0.21	0.92	8	5	1.69	0.48	5.99
‡RERI = 0.61; Multiplicative = 0.23										

Adjusted for age, physical activity, BMI, ibuprofen use, smoking (pack-years) and energy, calcium and fiber intake

†Heterozygotes includes *C77 CT* and 1*298AA, 677CT* and *1298AC , 677CC* and *1298AC*

‡*p* values for relative excess risk due to interaction (RERI); and multiplicative interaction