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Gene-diet-interactions in folate-mediated one-carbon metabolism modify colon cancer risk

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Abstract

Scope—The importance of folate-mediated one-carbon metabolism (FOCM) in colorectal carcinogenesis is emphasized by observations that high dietary folate intake is associated with decreased risk of colon cancer (CC) and its precursors. Additionally, polymorphisms in FOCM-related genes have been repeatedly associated with risk, supporting a causal relationship between folate and colorectal carcinogenesis.

Methods and results—We investigated 10 candidate polymorphisms with defined or probable functional impact in eight FOCM-related genes (SHMT1, DHFR, DNMT1, MTHFD1, MTHFR, MTRR, TCN2 and TDG) in 1609 CC cases and 1974 controls for association with CC risk and for interaction with dietary factors. No polymorphism was statistically significantly associated with overall risk of CC. However, statistically significant interactions modifying CC risk were observed for DNMT1 I311V with dietary folate, methionine, vitamin B₂, and vitamin B₁₂ intake and for MTRR I22M with dietary folate, a pre-defined one-carbon dietary pattern, and vitamin B₆ intake. We observed statistically significant gene-diet interactions with five additional polymorphisms.

Conclusion—Our results provide evidence that FOCM-related dietary intakes modify the association between CC risk and FOCM allelic variants. These findings add to observations showing that folate-related gene-nutrient interactions play an important role in modifying the risk of CC.

Keywords

Folate-mediated one-carbon metabolism (FOCM); Polymorphisms; Diet; Colon cancer; Interaction

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers worldwide [1]. The majority of cases occur sporadically, indicating that health behaviors are strongly related to disease development [2, 3]. Risk factors include Western diet, sedentary lifestyle, obesity and chronic inflammation, while physical activity and the use of NSAIDs are protective of the disease [2–5]. In addition to these modifiable risk factors, genetic predisposition plays a crucial role in susceptibility to CRC [6]. Rare high penetrance mutations (e.g. in *APC*) confer strong risk of CRC in specific families, however, in the general population CRC is more likely to be caused by a combination of common alleles and environmental or lifestyle factors such as diet. The elucidation of gene-diet interactions is thus important to understand the full complexity of the association between nature and nurture in colorectal carcinogenesis.

Folate is a water-soluble B-vitamin that functions as a carrier of one-carbon groups. As an essential component of the folate-mediated one-carbon metabolism (FOCM), it is important for synthesizing nucleotides, such as thymidylate and purines, for DNA replication and DNA repair and for producing *S*-adenosylmethionine (SAM), the universal donor of methyl groups for DNA methylation [7, 8]. Folate has long been known to be important to human health and the role of B vitamins in CRC development has been studied for years.

Numerous studies have illustrated the significance of FOCM in colorectal carcinogenesis (Figure 1), showing that extrinsic (e.g. diet) as well as intrinsic (e.g. genetic variation) factors may contribute to the risk of CRC [7–12]. However, the exact mechanism underlying these relationships remains unclear and a combination of both seems likely. Most epidemiologic studies show a strong inverse association between folate status and colorectal neoplasia, consistently reporting that high dietary folate intake or biomarkers of folate intake are associated with a decreased risk of colon cancer or its precursors [2, 3, 7, 11–14]. In addition, investigations of polymorphisms in FOCM-related genes demonstrated the relevance of these genetic variants for colon cancer risk and gene-diet interaction studies support the connection between FOCM-related genes and nutrients in colorectal carcinogenesis [7, 15–20]. However, studies are often limited to small sample sizes and show some inconsistency. Yet the full elucidation of the complex interactions between genes and nutrients within the FOCM in colon and rectal carcinogenesis is important to build public guidelines for the general population.

We have previously reported statistically significant associations between colon cancer risk and functional polymorphisms in *MTHFR* and *TS* [21, 22]. In the present study, we have extended this work in our large case-control study to highlight further the importance of FOCM-related gene-diet interaction in susceptibility to colon cancer. We evaluated 10 common candidate polymorphisms (either non-synonymous or with defined functional impact) in *serine hydroxymethyltransferase 1* (*SHMT1*), *dihydrofolatereductase* (*DHFR*), *DNA (cytosine-5-) methyltransferase 1* (*DNMT1*), *methylenetetrahydrofolate dehydrogenase 1* (*MTHFD1*), *methylenetetrahydrofolatereductase* (*MTHFR*), *5-methyltetrahydrofolate-homocysteine methyltransferasereductase* (*MTRR*), *transcobalamin II* (*TCN2*), and *thymine-DNA glycosylase* (*TDG*), that show evidence for a functional impact on colon cancer risk. A major focus was the investigation of interactions of these polymorphisms with folate, methionine, vitamin B₂, vitamin B₆, and vitamin B₁₂ – nutrients that play crucial roles in FOCM – in order to identify gene-diet interactions that modify susceptibility to colon cancer.

MATERIALS AND METHODS

Study setting and design

This study is based on a large case-control study (n=1609 cases and n=1974 controls) of colon cancer. The participants were African-American, Caucasian, or Hispanic subjects from Kaiser Permanente Medical Care Program (KPMCP) of Northern California, an eight county area in Utah, and the metropolitan Twin Cities area of Minnesota.

Cases—Eligibility criteria for cases included the following: diagnosis with first-primary incident colon cancer (*International Classification of Diseases for Oncology*, 2nd edition codes 18.0, 18.2–18.9) between October 1, 1991 and September 30, 1994, between 30 and 79 years of age at time of diagnosis, and mentally competent to complete the interview. Proximal tumors were defined as cecum through transverse colon; and distal tumors were categorized as tumors in the splenic flexure and descending and sigmoid colon. Cases who had adenocarcinoma or carcinoma of the rectosigmoid junction or rectum (first 15 cm from the anal opening) or had known familial adenomatous polyposis, ulcerative colitis, or Crohn's disease were not eligible for the study. Response and participation proportions have been described previously [23]. Of the cases that were asked, 76% participated in the study.

Controls—Controls were frequency-matched to cases by sex, race, and by 5-year age groups. At KPMCP, controls were randomly selected from membership lists. In Utah, controls 65 years and older were randomly selected from the Health Care Financing Administration lists and those younger than 65 years were randomly selected from driver's license lists. In Minnesota, controls were selected from driver's license and state-identification lists. Of the controls asked, 64% participated [24].

Data collection

In-person interviews using laptop computers were conducted by trained interviewers to collect diet and lifestyle data. Study quality-control methods have been previously described [25]. For this study, the reference period was the calendar year ~2 years before the date of diagnosis for cases or the date of selection for controls. Information on demographic factors such as age, sex, center, diet, physical activity, height and weight 2 years prior to diagnosis, regular use of aspirin and/or non-steroidal anti-inflammatory drugs (NSAIDs), cigarette-smoking history, and medical history were collected [23]. Study participants were asked to self-report their race/ethnicity. An adaptation of the validated Coronary Artery Risk Development in Young Adults (CARDIA) diet history questionnaire [26] was used to ascertain the participants' dietary intake data. The diet history interview was found to be a reasonably reliable and valid dietary survey method. The interviewers asked participants to determine which foods they had eaten and the frequency with which the foods had been eaten. The nutrients were calculated using the Minnesota Nutrition Coordinating Center's nutrient database version 19. Due to the imprecise measure of nutrient intakes from supplements, stratified analyses were restricted to individuals who indicated no regular intakes of vitamin supplements (1089 cases and 1315 controls) and, thus, reflect total dietary intakes. Participants were also asked to recall activity levels by level of intensity; consumption of alcoholic beverages for the referent year as well as 10 and 20 years ago; and usual number of cigarettes smoked in a day, along with when they started and stopped smoking. Blood was collected during the in-person interview or a clinic visit. Genomic DNA was extracted as described previously [27].

Genotyping

This study is a comprehensive evaluation of 10 candidate polymorphisms in FOCM, which were chosen on prior evidence regarding their probable impact on protein function. This was

based on laboratory or epidemiologic studies, and the *in silico* prediction tools SIFT [28–30] and PolyPhen [31–33]. The *DHFR* 19bp del, *MTHFD1* R134K, *MTHFD1* R653Q, and *TCN2* P259R polymorphisms were genotyped by allelic discrimination using the 5' nuclease assay on a 7900HT sequence detection system (Applied Biosystems, Foster City, CA) at the Fred Hutchinson Cancer Research Center (FHCRC, Seattle, WA). Genotyping results were validated by genotyping 100 individuals by both 5' nuclease assay and restriction fragment length polymorphism (RFLP) [34, 35]. Four negative controls and at least one positive control were included on each plate. For quality-control purposes, genotyping for 94 randomly selected samples was repeated.

The *SHMT1* L474F, *DNMT1* I311V, *MTHFR* R594Q, *MTRR* H622Y, *MTRR* I22M, and *TDG* G199S polymorphisms were genotyped using the Illumina™ GoldenGate bead-based genotyping platform at the Translational Genomics Institute (TGen, Phoenix, AZ). Blinded duplicates were included on all plates. Intraplate and interplate replicates were included at ~5%. Genotyping results were confirmed by comparing to genotype data from 30 CEPH trios (Coriell Cell Repository, Camden, NJ) who were genotyped by the HapMap project. Genotypes were excluded for any of the following criteria: GenTrain Score <0.4, 10%GC Score <0.25, AB T Dev >0.1239, Call Frequency <0.85, Replicate Errors >2, P-P-C Errors >2, <85% concordance with blinded or unblinded duplicates or with HapMap genotypes. The genotype frequencies among cases and controls for all polymorphisms were compatible with Hardy-Weinberg equilibrium (χ^2 test).

Data analysis

Heterozygous and homozygous variant genotypes were grouped together (i.e. dominant model) for the analysis of polymorphisms with minor allele frequencies <10%. For genes with multiple polymorphisms (*MTHFR* C677T (A222V), A1298C (E429A), G1793A (R594Q); *MTHFD1* G401A (R134K), A1958A (R653Q) and *MTRR* G66A (I22M), G401A (R134K)), we also analyzed combined genotypes to estimate the risk of colon cancer and interactions with one-carbon nutrients. Genotypes were grouped into categories according to putative phenotype and the combined major allele-genotype was considered a reference. Unconditional logistic regression models were used to estimate odds ratios (ORs) and corresponding 95% confidence intervals (95% CI) for the association between genotypes and risk of colon cancer. In these models, known risk factors were included as covariates to adjust for potential confounding: age at diagnosis or selection, sex (for models with men and women combined), race/ethnicity, study center, body mass index reported for the referent period, long-term vigorous leisure-time physical activity, total energy intake, dietary fiber, dietary calcium, usual number of cigarettes smoked per day on a regular basis, NSAID use, and hormone replacement therapy [21].

Categories for the different dietary exposures were calculated from the distribution of the control population and for men and women separately. Approximate tertile cut-points were used to designate categories of dietary intake. Smoking behavior was categorized as: never smoked regularly; 20 cigarettes/day; or >20 cigarettes/day [16]. High- or low-risk one-carbon dietary pattern was based on tertile intake of folate and methionine and long-term alcohol consumption as defined previously [16]. Because supplement use was only qualitatively assessed in the study, we restricted these analyses to individuals without relevant supplement use. Less than 1% reported taking individual supplements of folate, vitamin B₆ and vitamin B₁₂ and about 1% reported taking a B complex supplement.

Interactions between genotypes and diet on the risk of colon cancer were evaluated by testing for different slopes associated with nutrient intake across genotype. All statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Characteristics of the study population and polymorphism genotype frequencies are presented in Table 1 by case and control status. The study participants predominantly self-identified as Caucasian (92%), with the remainder self-identified as Hispanic (4%) or African-American (4%). Although allele frequencies for African-Americans were statistically significantly different from those of Caucasians or Hispanics (data not shown), stratification by race was not useful in determining associations for African-American participants due to the small sample size and imprecise estimates. The distribution of all genotypes among African-Americans or Hispanics did not differ between cases and controls. When we restricted our analysis to Caucasians, the results did not differ, thus we included all individuals in our analysis and adjusted for ethnicity.

Main associations

No statistically significant association between any of the 10 candidate polymorphisms and overall colon cancer risk was observed (Table 2). Stratifying by sex did not alter these associations. However, when stratified by age, statistically significant associations were found in women for the *MTHFD1* R134K variant ($p=0.03$) and in men for the *MTHFR* R594Q variant ($p=0.02$) (data not shown).

Dietary interactions

The major focus of this study was the investigation of potential interactions between 10 candidate polymorphisms in FOCM-related genes and dietary factors relevant to FOCM (folate, methionine, one-carbon dietary pattern, vitamin B₂, vitamin B₆, and vitamin B₁₂) (data not shown). Analyses were performed in men and women separately, as well as combined. Statistically significant interactions are shown in Table 3. A summary of all observed associations is provided in Table 4.

The *DNMT1* I311V variant was associated with a decreased risk of colon cancer among individuals with low dietary folate intake (OR=0.6; 95% CI=0.4–1.0) and a trend towards increased risk with moderate intake (p -interaction=0.04). Likewise, the variant was associated with a decreased risk of colon cancer in combination with low dietary methionine intake in women (OR=0.4; 95% CI=0.2–1.0), whereas high methionine reversed the association (p -interaction=0.04). In addition, *DNMT1* I311V showed statistically significant interactions with vitamin B intake. Low dietary vitamin B₂ or B₁₂ intake was inversely associated with colon cancer risk among variant carriers (B₂: OR=0.5; 95% CI=0.3–0.9; B₁₂: OR=0.6; 95% CI=0.4–0.9), whereas an increased risk was observed with higher vitamin B₂ or B₁₂ intake (B₂: p -interaction=0.02, B₁₂: p -interaction=0.05). These associations were largely explained by associations in women.

The second variant which showed statistically significant interactions with several dietary factors in the modification of colon cancer risk was *MTRR* I22M. Women homozygous for the variant were at an increased risk of developing colon cancer when their dietary folate intake was low (OR=2.2; 95% CI=1.1–4.2), whereas the risk decreased with high dietary folate intakes (p -interaction=0.02). A similar trend was observed for the one-carbon dietary pattern. Heterozygous women were at decreased risk when grouped in the lowest tertile of the one-carbon dietary pattern, whereas the risk increased for women with the same genotype grouped in the highest tertile (p -interaction=0.02). Moreover, the variant showed statistically significant interactions with vitamin B₆ intake. The variant was associated with increased risk of colon cancer for women with low vitamin B₆ intake (OR=3.0; 95% CI=1.5–6.0), whereas moderate intake decreased the risk of the disease (p -interaction=0.01).

The *SHMT* L474F polymorphism showed a statistically significant interaction (p-interaction <0.001) with methionine intake among women. The variant was associated with an increased risk of colon cancer in combination with moderate methionine intake, whereas the risk decreased with high methionine intake. A statistically significant interaction with this variant was also observed for dietary vitamin B₂ intake in men (p-interaction=0.03). Variant carriers who consumed the lowest amounts of vitamin B₂ were at higher risk compared to men with the same genotype but with moderate vitamin B₂ intake.

The analysis of the 19bp deletion polymorphism in the *DHFR* gene revealed a statistically significant interaction with methionine intake (p-interaction=0.01). An increased risk of colon cancer was observed for male variant carriers with moderate methionine intake (OR=1.7; 95% CI=1.0–3.0), whereas the risk of the disease showed a decreasing trend in variant carriers with a high methionine intake. A statistically significant interaction with this variant was also observed for the one-carbon dietary pattern in men (p-interaction=0.04). Men who were homozygous for the deletion were at statistically significantly decreased risk of colon cancer when grouped in the lowest tertile of the one-carbon dietary pattern (OR=0.3; 95% CI=0.1–0.8), whereas the risk increased when grouped in the highest tertile.

There were few interactions for the *MTHFD1* variants, except for *MTHFD1* R134K with dietary vitamin B₆ intake among women (p-interaction=0.01) and *MTHFD1* R653Q with dietary methionine intake (p-interaction=0.01) in both men and women. Finally, the *MTHFR* R594Q polymorphism showed a statistically significant interaction with dietary folate intake among women only (p-interaction=0.03).

We investigated combined genotypes for *MTHFR*, *MTRR*, and *MTHFD1*, to account for multiple polymorphisms in one gene. There were few statistically significant interactions (e.g., *MTRR* x high-risk dietary-pattern in men, p=0.03). However we consider these spurious, because sample sizes were low and patterns in the other sex or overall were not consistent.

DISCUSSION

For many years, diet has been recognized as an important modifiable factor that influences colorectal cancer risk and prevention. However, the exact underlying mechanisms are complex and are often modified by genetic predisposition. Understanding the interaction between nutrition and genetic variation is useful to distinguish between individuals who will and who will not benefit from diet intervention strategies. Folate and the FOCM are of special interest to colon and rectal cancer, as they are involved in DNA maintenance, repair and methylation. To further investigate the impact of FOCM on colon carcinogenesis, we investigated the associations between risk of colon cancer and polymorphisms in candidate genes involved in FOCM, namely *DHFR*, *DNMT1*, *MTHFD1*, *MTHFR*, *MTRR*, *SHMT*, *TCN2*, and *TDG*, with a special focus on dietary interactions in a large case-control study.

Overall, we observed no association between any of the investigated polymorphisms and colon cancer risk; however, several polymorphisms modified the risk of colon cancer in interacting with dietary intakes of folate, methionine, vitamin B₂, vitamin B₆, vitamin B₁₂, or the one-carbon dietary pattern as previously defined [16]. In particular, the variants *DNMT1* I311V and *MTRR* I22M showed multiple statistically significant diet interactions.

DNMT1 is involved in the establishment and regulation of tissue-specific methylation of cytosine residues. The investigated *DNMT1* I311V polymorphism, though predicted to be benign (PolyPhen-score: 0.826) and tolerated (SIFT-score: 0.46), leads to an amino acid change and may alter the properties of the protein. Concordant with our findings, a previous

study on *DNMT1* I311V and colon cancer risk did not observe any associations with overall colon cancer risk [36]. However, in this study, we observed that *DNMT1* I311V significantly modified the risk of colon cancer interacting with dietary folate, methionine, vitamin B₂ and vitamin B₁₂ intake. The interaction of the variant with vitamin B₁₂ intake may be explained by increased synthesis of methionine, resulting in higher B₁₂ levels, which in turn is converted to *S*-adenosylmethionine (SAM). Higher SAM levels, as a substrate for DNMT1, could result in increased DNMT1 activity and enhanced abnormal DNA methylation, thus, increasing the risk of colon cancer. However, a recent study that investigated the effect of *DNMT1* I311V on risk of rectal cancer, including gene-diet interactions, did not observe any associations with disease risk [37], consistent with the notion colon cancer and rectal cancer are distinct diseases.

The second polymorphism that modified the risk of colon cancer in association with several dietary factors (dietary folate, vitamin B₆, and the one-carbon dietary pattern) was *MTRR* I22M. *MTRR* triggers the reactivation of methionine synthase via reductive methylation and, thus, acts at the junction between the folate and methionine cycles. Kinetic investigations of the functional properties of the *MTRR* variant enzyme revealed a decreased affinity of the protein to methionine synthase [38]. The observed statistically significant interaction between the *MTRR* I22M variant and vitamin B₆ intake was consistent with a previous study on CRC, whereas two other studies, one of colorectal and one of rectal cancer did not observe any statistically significant interactions [37, 39, 40]. Based on its described functions, we expected to observe an interaction of the *MTRR* variant with both dietary folate and methionine intake; however, an interaction was observed only with folate. We also observed that the defined one-carbon dietary pattern modified the association between colon cancer risk and *MTRR* I22M in women, which is consistent with the decreased risk observed between variant genotypes and high folate intake in women.

SHMT triggers the synthesis of 5,10-methylene tetrahydrofolate, using vitamin B₆ as a cofactor. The ability of the variant enzyme to form a functional holo-enzyme is decreased [41]. We expected to find an interaction between *SHMT* L474F and both vitamin B₆ and folate intake, which, consistent with a previous study on colon cancer [42], was not observed. However, we observed a statistically significant interaction between *SHMT* L474F and dietary methionine intake in women and with vitamin B₂ intake in men, which has not been previously reported.

Our investigation of the *DHFR* 19bp deletion showed no interaction with folate intake. *DHFR* reduces folic acid (the synthetic form of folate used in supplements) to dihydrofolate (DHF), allowing DHF to enter the FOCM pathway; however, it is not relevant in the absorption of natural reduced folate from dietary sources. A study on breast cancer has reported decreased *DHFR* mRNA levels with the deletion and increased risk of breast cancer for women carrying the deletion and taking multivitamin supplements [43]. Our investigation of the *DHFR* 19bp deletion showed statistically significant interactions of the polymorphism with methionine intake and with the dietary-pattern-defined risk groups. As *DHFR* is linked to 5-methyl-THF, the primary methyl donor for methylating homocysteine to methionine, abnormalities in the methylation process due to variation in enzyme activity may alter carcinogenesis.

Two candidate polymorphisms in the *MTHFD1* gene were investigated within this study, both showing interactions with different dietary groups. The association between colon cancer risk and the *MTHFD1* R134K was modified by vitamin B₆ intake in women, which was also predicted due to its function as a cofactor for SHMT. In men, however, an opposite trend was observed, suggesting that these findings may be spurious. The *MTHFD1* R653Q variant allele interacted with methionine intake, but not with folate or vitamin B₆. The

variant allele is associated with decreased enzyme activity [44] and it has been shown that heterozygous individuals are at a decreased risk for hyperplastic polyps [45]. A study of rectal cancer did not find any association of the variant with overall risk or in combination with FOCM-related nutrients [37].

MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is a co-substrate for remethylation of homocysteine to methionine. We had previously reported that the combined genotypes of *MTHFR* 677CC/1298AA (wild-type/wild-type or heterozygous/wild-type) were associated with a trend towards reduced risk of colon cancer among women with a high folate intake [21]. Similarly, in this study a statistically significant interaction between *MTHFR* R594Q and folate intake was observed in women. The functional significance of the R594Q polymorphism is currently unknown. Although PolyPhen predicts it to be possibly damaging (score: 1.746), while SIFT does not (score: 1.00). A study by Xu *et al.* showed that, among Chinese women with high dietary folate intake, the R594Q variant allele was associated with a lower risk of endometrial cancer than those with a wild type allele and low folate intake [46]. The minor allele frequency of *MTHFR* R594Q is only 5%; thus, larger studies need to confirm the association of this polymorphism with colon cancer risk.

This study highlights the importance of investigating the joint effect of genetic susceptibility and nutrients. We did not observe any significant association between the individual polymorphisms with functional impact on FOCM and risk of colon cancer; however, including nutrient intake in our investigation, we observed several relevant gene-diet interactions. Our summary table suggests that for some functional genetic variants in FOCM (e.g. *DNMT1* I311V or *MTRR* I22M) nutrient intake “matters” and, thus, that these genetic polymorphisms deserve more attention in nutrigenetic strategies.

A strength of the present case-control study is the large sample size, which allowed us to investigate gene-diet interactions more reliably. The diet interviews were based on a comprehensive validated instrument which provides support for valid assessment. However, we recognize that, given the focused referent year, long-term dietary changes may not be captured. Another limiting factor was the small number of individuals in some strata, which could lead to imprecise estimates. We investigated specific hypotheses with respect to putative gene-diet interactions; thus, we did not adjust for multiple comparisons, yet we recognize that some associations are likely to be spurious.

In summary, this study shows that genetic polymorphisms in genes relevant to the FOCM pathway may interact with intakes of one-carbon metabolism nutrients. Our results provide support for the hypothesis that dietary intakes modify the association between colon cancer risk and the two candidate polymorphisms, *DNMT1* I311V and *MTRR* I22M. The interactions observed were consistent with our hypotheses. Furthermore, variants in *SHMT*, *DHFR*, *MTHFD1*, and *MTHFR* modified susceptibility to colon cancer based on dietary consumption of folate, methionine, and vitamins B₂ and B₆. The findings of this study add to the present understanding that folate-related gene-nutrient interactions play an important role in the risk of colon cancer and should be considered in diet intervention strategies. Further research needs to be conducted to test our results and to contribute further to establishing which diet/allele interactions are crucial to the role of folate-mediated one-carbon metabolism in colon carcinogenesis.

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List of Abbreviations

CRC	Colorectal cancer
CBS	Cystathione beta-synthase
DHF	Dihydrofolate
DHFR	Dihydrofolatereductase
DNMT1	DNA (cytosine-5-) methyltransferase 1
FOCM	Folate-mediated one-carbon metabolism
MTHFD1	Methylenetetrahydrofolate dehydrogenase 1
MTHFR	Methylenetetrahydrofolatereductase
MTRR	5-methyltetrahydrofolate-homocysteine methyltransferasereductase
SHMT1	Serine hydroxymethyltransferase 1
SAM	S-adenosylmethionine
TCN2	Transcobalamin II
TDG	Thymine-DNA glycosylase

THF Tetrahydrofolat

Figure 1.

Overview of folate-mediated one-carbon metabolism (simplified), links to methylation reactions and nucleotide synthesis AICAR = 5-aminoimidazole-4-carboxamide ribonucleotide; AICARFT = 5-amino-imidazole-4-carboxamide ribonucleotide transformylase; CBS = cystathione beta-synthase; DHF = dihydrofolate; DHFR = dihydrofolate reductase; DNMT = DNA methyltransferases; dTMP = deoxythymidine monophosphate; dUMP = deoxyuridine monophosphate; **GAR** = glycinamide ribonucleotide; GART = glycinamide ribonucleotide transformylase; hFR = human folate receptor; MS = methionine synthase; MTHFD1 = methylenetetrahydrofolate dehydrogenase; MTHFR = 5,10-methylenetetrahydrofolate reductase; RFC = reduced folate carrier; SAH (AdoHcy) = S-adenosylhomocysteine; SAM (AdoMet) = S-adenosylmethionine; SHMT = serine hydroxymethyltransferase; TCN2 = transcobalamin II; THF = tetrahydrofolate; TS = thymidylate synthase; X = a variety of substrates for methylation. Adapted from Ulrich et al. (2003) with permission.

Table 1

Characteristics and genotype of the study population

	Cases(n=1609)	Controls(n=1974)	p ^{a)}
Clinical			
	Median (SD)^{b)}		
Age at diagnosis or selection (range, 30–79), years	64.9 (± 9.8)	65.0 (± 10.2)	0.80
	n (%)	n (%)	
Tumor site			
Proximal	794 (49)		
Distal	776 (48)		
Unknown site	39 (2)		
Sex			
Men	901 (56)	1043 (53)	
Women	708 (44)	931 (47)	0.06
Race/ethnicity			
Non-Hispanic White	1470 (91)	1837 (93)	
African-American	72 (4)	54 (3)	
Hispanic	63 (4)	79 (4)	
Black-Hispanic	2 (<1)	2 (<1)	
Other	2 (<1)	1 (<1)	0.06
Recent hormone-replacement therapy use ^{c)}			
No	470 (77)	551(69)	
Yes	144 (23)	243 (31)	<0.01
Dietary			
	Median (SD)		
Kilocalories			
Men	2774 (± 1216)	2638 (± 1160)	0.01
Women	2048 (± 876)	1972 (± 831)	0.07
	Median (IQR)^{b)}		
Folate (µg/day)			
Men	387 (280–495)	393 (286–500)	0.77
Women	320 (232–409)	320 (233–407)	0.79
Vitamin B ₁₂ (µg/day)			
Men	6.5 (4.1–8.9)	6.3 (3.9–8.7)	0.20
Women	4.5 (2.8–6.2)	4.5 (2.8–6.2)	0.69
Genotype^{d)}			
	n (%)	n (%)	

	Cases(n=1609)	Controls(n=1974)	p ^{a)}
Clinical	Median (SD) ^{b)}		
DNMT1 I311V ^{e)} – A931G ^{f)} – rs2228612 ^{g)}			
AA	1255 (89)	1561 (88)	0.67
AG	157 (11)	200 (11)	
GG	5 (0)	10 (1)	
MAF ^{b)}	0.06	0.06	
MTRR I22M – G66A – rs1801394			
GG	439 (31)	550 (31)	0.54
GA	717 (50)	869 (49)	
AA	264 (19)	356 (20)	
MAF	0.44	0.45	
MTRR H622Y – G1763A – rs10380			
GG	1161 (82)	1427 (80)	0.51
GA	242 (17)	328 (19)	
AA	17 (1)	18 (1)	
MAF	0.10	0.10	
Genotype	n (%)	n (%)	
SHMT1 L474F – C1420T – rs1979277			
CC	656 (46)	824 (46)	0.58
CT	627 (44)	767 (43)	
TT	131 (9)	183 (10)	
MAF	0.31	0.32	
DHFR 19bp del – rs70991108			
ins/ins	470 (32)	587 (32)	0.92
ins/del	713 (49)	880 (48)	
del/del	287 (20)	368 (20)	
MAF	0.44	0.44	
MTHFD1 R134K – G401A – rs1950902			
GG	1023 (70)	1213 (66)	0.08
GA	396 (27)	560 (31)	
AA	51 (3)	63 (3)	
MAF	0.17	0.19	
MTHFD1 R653Q – G1958A – rs2236225			
GG	448 (30)	580 (32)	0.76
GA	716 (49)	886 (48)	
AA	306 (21)	370 (20)	
MAF	0.45	0.44	

	Cases(n=1609)	Controls(n=1974)	p ^{a)}
Clinical	Median (SD) ^{b)}		
MTHFR R594Q – G1793A – rs2274976			
GG	1290 (91)	1595 (90)	0.79
GA	127 (9)	171 (10)	
AA	4 (0)	5 (0)	
MAF	0.05	0.05	
TCN2 P259R – G776C – rs1801198			
GG	479 (33)	551 (30)	0.19
GC	724 (49)	916 (50)	
CC	265 (18)	365 (20)	
MAF	0.43	0.45	
TDG G199S – G994A – rs4135113			
GG	1365 (96)	1704 (96)	0.54
GA	58(4)	73 (4)	
AA	1 (0)	0 (0)	
MAF	0.02	0.02	

^{a)}Based on χ^2 or *t* test

^{b)}SD: Standard deviation; IQR: Interquartile range; MAF: minor allele frequency

^{c)}Hormone replacement therapy within 2 years of diagnosis/selection, among postmenopausal women

^{d)}Frequencies reported for non-Hispanic Whites

^{e)}Amino acid change

^{f)}Nucleotide change

^{g)}rs number

Table 2

Association between polymorphism genotypes and colon cancer risk, stratified by sex^{a)}

	Combined				Men				Women			
	Cases (n)	Controls (n)	OR ^{b)}	95% CI	Cases (n)	Controls (n)	OR	95% CI	Cases (n)	Controls (n)	OR	95% CI
SHMTL474F												
CC	667	838	1.0	–	412	471	1.0	–	255	367	1.0	–
CT	644	749	1.1	0.9–1.3	377	428	1.0	0.9–1.3	267	321	1.2	0.9–1.5
TT	128	178	0.9	0.7–1.2	66	108	0.7	0.5–1.0	62	70	1.3	0.9–1.9
P interaction – 0.09												
DHFR19bp del												
Ins/ins	452	573	1.0	–	275	323	1.0	–	177	250	1.0	–
Ins/del	739	882	1.1	0.9–1.2	454	502	1.0	0.8–1.3	285	380	1.1	0.8–1.4
Del/del	310	371	1.1	0.9–1.3	164	213	0.9	0.7–1.2	146	158	1.3	0.9–1.7
P interaction – 0.18												
DNMT1311V												
AA	1259	1531	1.0	–	751	871	1.0	–	508	660	1.0	–
AG or GG	183	233	1.0	0.8–1.2	105	135	0.9	0.7–1.2	78	98	1.1	0.8–1.5
P interaction – 0.45												
MTHFD1R134K												
GG	1053	1222	1.0	–	632	690	1.0	–	421	532	1.0	–
GA	400	543	0.9	0.8–1.0	232	313	0.8	0.7–1.0	168	230	1.0	0.8–1.2
AA	48	62	0.9	0.6–1.3	29	35	0.9	0.5–1.5	19	27	0.9	0.5–1.6
GA or AA	448	605	0.9	0.8–1.0	261	348	0.8	0.7–1.0	187	257	1.0	0.8–1.2
P interaction (co-dominant model) – 0.72												
P interaction (dominant model) – 0.47												
MTHFD1R653Q												
GG	477	579	1.0	–	271	345	1.0	–	206	234	1.0	–
GA	721	886	1.0	0.8–1.1	438	498	1.1	0.9–1.4	283	388	0.8	0.6–1.1
AA	303	362	1.0	0.8–1.3	184	195	1.2	0.9–1.6	119	167	0.8	0.6–1.1

	Combined				Men				Women			
	Cases (n)	Controls (n)	OR ^{a)}	95% CI	Cases (n)	Controls (n)	OR	95% CI	Cases (n)	Controls (n)	OR	95% CI
P interaction – 0.08												
MTHFR594Q												
GG	1314	1592	1.0	–	777	902	1.0	–	537	690	1.0	–
GA or AA	132	172	0.9	0.7 – 1.2	79	104	0.9	0.7 – 1.2	53	68	1.0	0.7 – 1.5
P interaction – 0.63												
MTRRH622Y												
GG	1136	1387	1.0	–	681	776	1.0	–	455	611	1.0	–
GA	280	352	1.0	0.8 – 1.1	159	212	0.8	0.7 – 1.1	121	140	1.2	0.9 – 1.5
AA	29	28	1.1	0.7 – 2.0	17	18	1.0	0.5 – 2.0	12	10	1.4	0.6 – 3.5
GA or AA	309	380	1.0	0.8 – 1.1	176	230	0.8	0.7 – 1.1	133	150	1.2	0.9 – 1.6
P interaction (co-dominant model) – 0.17												
P interaction (dominant model) – 0.06												
GG	422	522	1.0	–	247	303	1.0	–	175	219	1.0	–
GA	714	857	1.0	0.9 – 1.2	419	482	1.1	0.9 – 1.3	295	375	1.0	0.8 – 1.3
AA	308	389	1.0	0.8 – 1.2	190	224	1.0	0.8 – 1.4	118	165	0.9	0.6 – 1.2
P interaction – 0.77												
TCN2P259R												
GG	522	581	1.0	–	305	339	1.0	–	217	242	1.0	–
GC	715	898	0.9	0.8 – 1.0	421	519	0.9	0.7 – 1.1	294	379	0.9	0.7 – 1.1
CC	261	343	0.9	0.7 – 1.1	165	178	1.1	0.8 – 1.4	96	165	0.7	0.5 – 0.9
P interaction – 0.08												
TDGG199S												
GG	1366	1683	1.0	–	802	956	1.0	–	564	727	1.0	–
GA or AA	83	87	1.0	0.8 – 1.4	57	53	1.2	0.8 – 1.7	26	34	0.9	0.5 – 1.5
P interaction – 0.44												

a) Adjusted for age, race/ethnicity, center, body mass index, lifetime vigorous activity, energy intake, dietary fiber, dietary calcium, usual number of cigarettes smoked, NSAID use, and hormone replacement therapy. Participants with missing data for any of these variables were excluded. Combined analyses were also adjusted for sex.

^{b)} OR, odds ratio; CI, confidence interval

Table 3
Significant associations between polymorphism genotypes and colon cancer risk, stratified by dietary factors^(a)

	Tertile 1			Tertile 2			Tertile 3		
	Cases (n)	Controls (n)	OR ^(b)	95% CI	Cases (n)	Controls (n)	OR	95% CI	OR
<i>DNMT1/31IV</i>									
<i>Dietary folate intake^{(c)(d)}</i>									
Combined									
AA	302	332	1	–	271	347	0.8	0.6–1.0	0.8
AG or GG	35	60	0.6	0.4–1.0	48	45	1.1	0.7–1.8	0.8
P interaction – 0.04									
<i>Dietary methionine intake^{(c)(e)}</i>									
Women									
AA	111	131	1.0	–	119	152	0.9	0.6–1.3	0.7
AG or GG	8	20	0.4	0.2–0.9	22	25	0.9	0.5–1.8	1.4
P interaction – 0.04									
<i>Dietary vitamin B₂ intake^{(c)(f)}</i>									
Combined									
AA	281	333	1.0	–	270	326	1.0	0.8–1.3	1.0
AG or GG	30	61	0.5	0.3–0.9	47	45	1.3	0.8–2.1	1.2
P interaction – 0.02									
Women									
AA	141	145	1.0	–	81	124	0.6	0.4–0.9	0.6
AG or GG	13	24	0.5	0.2–0.9	19	17	1.1	0.5–2.3	0.8
P interaction – 0.04									
<i>Dietary vitamin B₁₂ intake^{(c)(g)}</i>									
Combined									
AA	256	341	1.0	–	301	335	1.2	0.9–1.5	1.0
AG or GG	27	60	0.6	0.4–0.9	54	50	1.4	0.9–2.2	1.2

	Tertile 1				Tertile 2				Tertile 3			
	Cases (n)	Controls (n)	OR ^{b)}	95% CI	Cases (n)	Controls (n)	OR	95% CI	Cases (n)	Controls (n)	OR	95% CI
P interaction – 0.05												
Women												
AA	132	143	1.0	–	93	138	0.7	0.5 – 1.0	93	123	0.7	0.5 – 1.2
AG or GG	11	25	0.4	0.2 – 0.8	16	20	0.9	0.4 – 1.8	22	15	1.5	0.7 – 3.4
P interaction – 0.01												
MTRR122M												
<i>Dietary folate intake^{c)}(d)</i>												
Women												
GG	38	66	1.0	–	30	40	1.2	0.6 – 2.4	34	39	1.5	0.7 – 3.1
GA	72	74	1.9	1.1 – 3.2	60	79	1.4	0.8 – 2.4	55	72	1.3	0.7 – 2.4
AA	31	25	2.2	1.1 – 4.2	26	34	1.3	0.7 – 2.6	22	35	0.9	0.4 – 2.0
P interaction – 0.02												
MTRR122M												
<i>One-carbon dietary pattern^{c)}(h)</i>												
Women												
GG	21	22	1.0	–	74	105	0.7	0.3 – 1.5	7	18	0.3	0.1 – 1.0
GA	27	35	0.8	0.4 – 1.8	150	177	0.9	0.4 – 1.8	10	12	0.7	0.2 – 2.2
AA	9	13	0.5	0.2 – 1.4	61	78	0.8	0.4 – 1.6	9	3	2.4	0.5 – 10.6
P interaction – 0.02												
<i>Dietary vitamin B₆ intake^{c)}(i)</i>												
Combined												
GG	89	135	1.0	–	92	92	1.6	1.1 – 2.4	102	133	1.2	0.8 – 1.8
GA	156	194	1.3	0.9 – 1.9	153	184	1.3	0.9 – 1.9	172	203	1.3	0.9 – 1.9
AA	72	67	1.7	0.1 – 2.6	67	92	1.2	0.8 – 1.8	80	82	1.4	0.9 – 2.3
P interaction – 0.02												
Women												
GG	40	62	1.0	–	25	40	1.1	0.5 – 2.1	37	43	1.4	0.7 – 2.8

	Tertile 1				Tertile 2				Tertile 3			
	Cases (n)	Controls (n)	OR ^{b)}	95% CI	Cases (n)	Controls (n)	OR	95% CI	Cases (n)	Controls (n)	OR	95% CI
GA	66	76	1.5	0.9 – 2.6	59	67	1.5	0.9 – 2.6	62	82	1.2	0.6 – 2.3
AA	34	19	3.0	1.5 – 6.0	19	42	0.7	0.3 – 1.4	26	33	1.1	0.5 – 2.3
P interaction – 0.01												
SHMT L474F												
<i>Dietary methionine intake^{c)(e)}</i>												
Women												
CC	52	68	1.0	–	48	91	0.7	0.4 – 1.1	60	61	1.0	0.5 – 1.9
CT	53	69	1.0	0.6 – 1.6	75	71	1.2	0.7 – 2.1	39	60	0.6	0.3 – 1.3
TT	12	14	1.1	0.5 – 2.6	20	14	1.8	0.8 – 4.0	8	16	0.6	0.2 – 1.5
P interaction – <0.001												
<i>Dietary vitamin B₂ intake^{c)(f)}</i>												
Men												
CC	77	112	1.0	–	124	99	1.7	1.1 – 2.6	97	120	1.1	0.6 – 1.9
CT	82	101	1.3	0.9 – 2.0	112	115	1.4	0.9 – 2.1	79	94	1.2	0.7 – 2.0
TT	15	18	1.2	0.6 – 2.6	14	34	0.7	0.3 – 1.3	17	23	1.0	0.5 – 2.2
P interaction – 0.03												
DHFR 19bp del												
<i>Dietary methionine intake^{c)(e)}</i>												
Men												
Ins/ins	58	78	1.0	–	72	104	0.9	0.6 – 1.5	66	60	1.4	0.7 – 2.5
Ins/del	83	117	0.9	0.6 – 1.5	140	124	1.5	0.9 – 2.3	112	116	1.2	0.7 – 2.1
Del/del	38	49	1.1	0.6 – 1.9	48	41	1.7	0.9 – 3.0	29	51	0.7	0.4 – 1.4
P interaction – 0.01												
<i>One-carbon dietary pattern^{c)(h)}</i>												
Men												
Ins/ins	24	23	1.0	–	147	183	0.8	0.4 – 1.6	25	36	0.7	0.3 – 1.5
Ins/del	41	53	0.8	0.4 – 1.7	238	253	0.9	0.5 – 1.8	56	50	1.1	0.5 – 2.2

	Tertile 1			Tertile 2			Tertile 3		
	Cases (n)	Controls (n)	OR ^{b)}	95% CI	Cases (n)	Controls (n)	OR	95% CI	OR
Del/del	7	22	0.3	0.1 – 0.8	88	93	1.1	0.6 – 2.2	26
P interaction – 0.04									
MTHFDI R134K									
<i>Dietary vitamin B₆ intake^{c)}(i)</i>									
Women									
GG	90	109	1.0	–	80	95	1.0	0.7 – 1.6	122
GA or AA	52	54	1.2	0.7 – 1.9	25	64	0.5	0.3 – 0.8	43
P interaction – 0.01									
MTHFDI R653Q									
<i>Dietary methionine intake^{c)}(e)</i>									
Combined									
GG	117	143	1.0	–	119	114	1.3	0.9 – 1.9	96
GA	128	198	0.8	0.6 – 1.1	185	210	1.0	0.7 – 1.5	176
AA	52	61	1.1	0.7 – 1.7	70	99	0.9	0.6 – 1.3	81
P interaction – 0.01									
MTHFR R594Q									
<i>Dietary folate intake^{c)}(d)</i>									
Women									
GG	127	155	1.0	–	111	136	1.0	0.7 – 1.4	99
GA or AA	15	10	2.0	0.9 – 4.7	5	16	0.3	0.1 – 0.9	13
P interaction – 0.03									

a) Non-supplement users

b) OR, odds ratio; CI, confidence interval

c) Adjusted for age, race/ethnicity, center, body mass index, lifetime vigorous activity, energy intake, dietary fiber, dietary calcium, usual number of cigarettes smoked, NSAID use, and hormone replacement therapy. Participants with missing data for any of these variables were excluded. Combined analyses were also adjusted for sex.

d) Tertile cut-points: combined: 295/425 µg/day; men: 330/465 µg/day; women: 275/380 µg/day

e) Tertile cut-points: combined: 1.5/2.2 g/day; men: 1.7/2.5 g/day; women 1.3/1.9 g/day.

f) Tertile cut-points: combined: 1.8/2.6 µg/day; men: 2.0/3.0 µg/day; women: 1.6/2.2 µg/day.

g) Tertile cut-points: combined: 4.2/6.9 µg/day; men: 4.9/7.9 µg/day; women: 3.6/5.6 µg/day.

h) Low risk defined as highest tertile folate and methionine and no or moderate long-term alcohol intake; high risk defined as lowest two tertiles of folate and methionine along with high long-term alcohol intake; intermediate risk defined as those not defined as high- or low- risk diet.

i) Tertile cut-points: combined: 1.8/2.5 µg/day; men: 2.0/2.8 µg/day; women: 1.6/2.2 µg/day.

Table 4

Summary of associations^(a-f)

Variant	Dietary Factors					
	Folate	Methionine	Dietary Pattern	Vitamin B ₂	Vitamin B ₆	Vitamin B ₁₂
<i>DNMT1 I311V</i>						
Combined	P=0.04 ↓T1;↑T2	P=0.06↓T1;↑T3	n.s.	P=0.02 ↓T1↑T2	n.s.	P=0.05 ↓T1↑T2↑T3
Men	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Women	n.s.	P=0.04 ↓T1;↑T3	n.s.	P=0.04 ↓T1↑T2	P=0.09↓T1↑T3	P=0.01 ↓T1↑T2↑T3
<i>MTRR I22M</i>						
Combined	n.s.	n.s.	n.s.	n.s.	P=0.02 ↑T1;↓T2	n.s.
Men	n.s.	n.s.	n.s.	n.s.	n.s.	P=0.07↑T3
Women	P=0.02 ↑T1;↓T3	n.s.	P=0.02 ↓T1↑T3	n.s.	P=0.01 ↑T1;↓T3	n.s.
<i>MTRR H622Y</i>						
Combined	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Men	n.s.	n.s.	n.s.	P=0.07↓T1&T2	n.s.	n.s.
Women	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>SHMT L474F</i>						
Combined	n.s.	P=0.09↓T2	n.s.	n.s.	n.s.	n.s.
Men	n.s.	n.s.	n.s.	P=0.03 ↑T1↓T2	n.s.	n.s.
Women	n.s.	P<0.001 ↑T2;↓T3	n.s.	n.s.	n.s.	n.s.
<i>DHFR 19bp del</i>						
Combined	n.s.	P=0.06↑T2;↓T3	P=0.09↓T1↑T3	n.s.	n.s.	n.s.
Men	n.s.	P=0.01 ↑T2;↓T3	P=0.04 ↓T1↑T2	n.s.	n.s.	n.s.
Women	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>MTHFD R134K</i>						
Combined	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Men	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Women	n.s.	n.s.	n.s.	n.s.	P=0.01 ↓T2↑T3	P=0.08↑T2;↓T3

Variant	Dietary Factors					
	Folate	Methionine	Dietary Pattern	Vitamin B ₂	Vitamin B ₆	Vitamin B ₁₂
<i>MTHFD R653Q</i>						
Combined	n.s.	P=0.01 ↓T2:↑T3	n.s.	n.s.	n.s.	n.s.
Men	n.s.	P=0.07↑T3	n.s.	P=0.07↑T3	n.s.	n.s.
Women	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>MTHFR R594Q</i>						
Combined	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Men	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Women	P=0.03 ↑T1:↓T2	n.s.	n.s.	n.s.	n.s.	n.s.
<i>TCN2 P259R</i>						
Combined	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Men	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Women	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>TDG G199S</i>						
Combined	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Men	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Women	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

a) ↑: increasing from wt →het → mutant
b) ↓: decreasing from wt →het → mutant
c) T1: tertile 1, T2:tertile 2, T3:tertile 3
d) codom=codominant model; dom = dominant model
e) n.s.: not significant
f) Restricted to non-supplement users