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Journal

Cancer, 121(20)

ISSN

0008-543X

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Publication Date

2015-10-15

DOI

10.1002/cncr.29465

Peer reviewed

HHS Public Access

Author manuscript

Cancer. Author manuscript; available in PMC 2016 October 15.

Published in final edited form as:

Cancer. 2015 October 15; 121(20): 3684–3691. doi:10.1002/cncr.29465.

Folate-mediated one-carbon metabolism genes and interactions with nutritional factors on colorectal cancer risk: Women's Health Initiative Observational Study

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Abstract

Background—Investigations of folate-mediated one-carbon metabolism (FOCM) genes and gene-nutrient interactions in relation to colorectal cancer (CRC) risk are limited to candidate polymorphisms and dietary folate. We comprehensively investigated associations between genetic variants in FOCM and CRC risk, and whether FOCM nutrient status modified these associations.

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Conflicts of interest: None.

Methods—We genotyped 288 candidate and tagging single nucleotide polymorphisms (SNPs) in 30 FOCM genes among 821 incident CRC case-control matched pairs in the Women's Health Initiative Observational Study cohort. FOCM biomarkers (red blood cell [RBC] folate, plasma folate, pyridoxal-5'-phosphate [PLP], vitamin B12, and homocysteine) and self-reported alcohol consumption were measured at baseline. Conditional logistic regression was implemented; effect modification was examined based on known enzyme-nutrient relationships.

Results—We observed statistically significant associations between CRC risk and functionally defined candidate SNPs of *MTHFD1* (K134R), *MTRR* (P450R), and *PRDM2* (S450N), and a literature candidate SNP of *TYMS* (g.676789A>T) (nominal P<0.05). In addition we noted suggestive associations for tagSNPs in *CBS*, *DHFR*, *DNMT3B*, *MAT1A*, *MTHFD1*, and *MTRR* (nominal P<0.05; non-significant adjusted P). Significant interactions between nutrient biomarkers and candidate polymorphisms were observed for (i) plasma/RBC folate and *FOLH1*, *PON1*, *TCN2*, *DNMT1*, and *DNMT3B*; (ii) plasma PLP and *TYMS* TS3; (iii) plasma B12 and *BHMT2*; (iv) homocysteine and *MTHFR* and *AARS*.

Conclusions—Genetic variants in FOCM genes are associated with CRC risk among postmenopausal women. FOCM nutrients continue to emerge as effect modifiers of genetic influences on CRC risk.

Keywords

Gene-nutrient interaction; one-carbon metabolism; colorectal cancer; postmenopausal women; biomarker

INTRODUCTION

Folate-mediated one-carbon metabolism (FOCM; Supplemental Figure 1) has important implications in colorectal carcinogenesis.[1,2] Folate functions as a donor of one-carbon units and is essential for methylation reactions and for nucleotide synthesis, and thus DNA stability and repair.[1] Low folate intakes, or biomarkers of low-folate status, have been implicated in increased risk of CRC.[3,4] Also, a number of functional polymorphisms in the genes encoding enzymes and co-enzymes[5–8] and their interactions with folate[7–9] are linked to CRC risk. However, genetic variants other than the known functional polymorphisms and influences of other important FOCM nutrients, namely vitamin B6 and B12,[6,7,10] on genetic polymorphisms in relation to CRC risk remain largely unexplored. Also, data are sparse on using biomarkers to indicate the status of FOCM nutrients in the investigations of gene-nutrient interaction on CRC risk.[8] Studying the impact of FOCM nutrients and genetics on CRC risk remains important in light of recent debate on whether the U.S. folic acid fortification program poses risks or benefits.[11]

The primary objective of this study was to comprehensively investigate whether genetic variants in FOCM-related genes were associated with CRC. In addition to known functional polymorphisms, we examined tagging-single-nucleotide-polymorphisms (tagSNPs) that capture genetic variation over the entire genetic region. We further evaluated interactions between genetic variants and biomarkers of folate, vitamin B6, vitamin B12, and homocysteine, an integrated marker of one-carbon status. We also investigated alcohol

consumption as an effect modifier because it can alter folate absorption and disturb DNA methylation.[12]

METHODS

Study population

The current study used a nested case-control design in the Women's Health Initiative Observational Study (WHI-OS) cohort.[13] We selected all confirmed invasive colorectal cancers as of April 24, 2008, and controls were matched by risk-set sampling on age (±3 years), race/ethnicity, enrollment date (±1 year), hysterectomy status, family history of CRC, and time of blood draw (±6 months). A total of 988 case-control pairs were selected for genotyping. The study was approved by the human subjects review boards; written informed consent was obtained from all participants.

Genotyping

A total of 30 FOCM genes were identified (Supplemental Table 1). Genotyped SNPs included both candidate polymorphisms with a high likelihood of functional impact, based on amino-acid change, genomic location, and laboratory and epidemiologic studies, and tagSNPs selected based on linkage-disequilibrium (LD). LD was determined from the CEPH population (Utah residents with ancestry from Northern and Western Europe) from HapMap 2 (data release #24, NCBI B36 assembly),[14] with a cutoff of minor allele frequency (MAF) at 5% and r^2=0.80.[15] TagSNPs covered from 10KB upstream to 5KB downstream of each gene, or through the end of LD blocks, whichever was greater. A total of 295 SNPs were selected (Supplemental Table 2). SNPs were genotyped using the Illumina 384-plex BeadXpress GoldenGate genotyping platform. Laboratory personnel were blinded to case-control status. For quality control, 30 CEPH trios (Coriell Cell Repository, Camden, NJ), genotyped by the HapMap project, and 5% blinded duplicates (42 case-control pairs) were included. Concordance among the blind duplicates was excellent: 38 pairs had 100% concordance and 3 pairs had a concordance of ≥95% (1 sample failed to genotype). SNPs were excluded for any of the following reasons: <95% call rate, <95% concordance with blind or non-blind duplicates, deviations from expected MAF, or Hardy-Weinberg equilibrium (HWE) p<0.0001; 254 SNPs successfully passed quality control (QC) procedures. Subsequently, 34 of the 41 SNPs which failed QC were re-genotyped using Sequenom iPLEX MassARRAY Typer v3.4 (Sequenom, San Diego, CA) or pre-designed TaqMan assays (Applied Biosystems, Carlsbad, CA). A total of 288 of the 295 SNPs (98%) were successfully genotyped. Samples were also genotyped for insertion-deletion and repeat polymorphisms in the thymidylate synthase gene (*TYMS*): *TS3* (3'UTR 1494delTTAAAG, rs151264360) and *TS5* (5'-UTR enhancer region with two or three repeats of a 28-bp sequence, a.k.a., *TSER*, rs34743033) using PCR and fluorescent size discrimination by capillary electrophoresis. Individuals carrying the 3 repeat *TS5* genotype were further interrogated for the G>C SNP (TSER*3 G>C) by restriction fragment length polymorphism method.[16] We excluded 74 samples due to average call rates <95% and 26 samples due to potential errors during blood or DNA processing. We further excluded participants with race/ethnicities other than white (167 case-control pairs) due to large MAF differences among racial groups. Subsequently, 821 case-control pairs entered statistical analysis.

FOCM biomarkers and covariates

A 12-hour fasting blood specimen was obtained at baseline clinic visit. The mean duration from blood draw to CRC diagnosis was 5.3 years (standard deviation=3.1 years). Total plasma homocysteine and pyridoxal-5'-phosphate (PLP), indicating vitamin B6 status, were determined by high-performance liquid chromatography (HPLC) with post-column fluorescence detection.[17,18] Red blood cell (RBC) and plasma folate and plasma vitamin B12 were determined by radioassays (SimulTRAC, MP Biomedicals, Orangeburg, NY). Inter-assay coefficients of variation (CV) of blinded duplicate control samples for each of the assays were: plasma homocysteine, 6.5%; RBC folate, 10.2%; plasma folate, 4.8%; plasma PLP, 4.8%; and plasma vitamin B12, 6.2%.

Demographic characteristics, medical history, and lifestyle factors were recorded at study enrollment using standardized questionnaires. Height and weight were measured by trained staff. Number of servings per week of beer, wine, and/or liquor based on a medium serving size (12 oz. of beer, 6 oz. of wine, and 1.5 oz. of liquor) was assessed by a food frequency questionnaire.

Statistical analysis

Baseline characteristics of CRC cases and controls were compared by Wilcoxon tests (for continuous variables) and Chi-square tests (for categorical variables). Conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) adjusted for age (continuous). OR estimates were assessed based on co-dominant inheritance models, unless the number of participants was <0 in a cell, in which cases a dominant model was used. Global P-values were obtained by likelihood ratio tests. P-values for trend were obtained via log-additive models. To account for multiple testing of tagSNPs on the gene level, p-values adjusted for correlated tests (P_{ACT}) were calculated.[19] P-values for candidate SNPs were not adjusted for multiple comparisons, as we had specific prior hypotheses.

We investigated interactions between FOCM SNPs and RBC and plasma folate, plasma PLP, plasma B12, plasma homocysteine, and alcohol intake. All interactions were planned *a priori* with specific hypotheses based on the enzyme-nutrient functions (Supplemental Table 6). The biomarker concentrations and intake values were categorized into tertiles based on the distribution in the control participants. Participants with the lowest category of biomarker concentrations or alcohol intake levels and the homozygous wild-type genotype were assigned as the reference group. The interaction analyses were adjusted for *a priori* selected potential confounders – baseline age, body mass index (BMI), and smoking pack-years (all in continuous). P-values of interactions were determined by likelihood ratio tests. We calculated false discovery rate (FDR) q-values at the gene level to account for multiple testing for interactions.[20] We used a q-value threshold of 0.25, and thus we expected to have a 3 in 4 chance of actually being a true positive among all declared significant. Statistical significance for nominal P-values and P_{ACT} was defined as $P<0.05$; all statistical tests were 2-sided.

RESULTS

Compared to controls, cases had a higher BMI, more pack-years of smoking, a higher proportion of polyp removal and higher concentrations of plasma homocysteine, but had a lower proportion of receiving colonoscopy or sigmoidoscopy screening (Table 1; see Supplemental Table 3 for the distributions by tumor location and stage). Our main effect analysis showed that candidate polymorphisms in methylenetetrahydrofolate dehydrogenase 1 (*MTHFD1*, K134R), 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*, P450R, in high LD with R442C and S284T), *PRDM2* (S450N), which encodes a zinc finger protein, and *TYMS* (g.676789A>T), were significantly associated with CRC risk (Table 2). In addition, we observed nominally significant associations between CRC risk and tagSNPs in genes encoding these enzymes: cystathionine-beta-synthase (*CBS*), dihydrofolate reductase (*DHFR*), DNA (cytosine-5-)-methyltransferase 3 beta (*DNMT3B*), methionine adenosyltransferase I alpha (*MAT1A*), *MTHFD1*, and *MTRR* (Table 3; see Supplemental Table 4 for tumor location-specific risks and Supplemental Table 5 for full results). These associations of tagSNPs did not remain statistically significant after correcting for multiple testing (P_{ACT} >0.05).

Table 4 presents a summary of nominally significant gene-nutrient interactions, which were pre-hypothesized according to the functions of FOCM enzymes and nutrients, for candidate polymorphisms. Notably, the variant genotypes of a candidate polymorphism in folate hydrolase 1 (*FOLH1*, rs10839236) were significantly associated with higher risk of CRC in the presence of lower (tertile 1), but not higher (tertile 3) plasma folate concentrations, compared to the wild-type genotype in the presence of lower plasma folate concentrations. In addition, plasma or RBC folate modified CRC risk associated with candidate polymorphisms in paraoxonase 1 (*PON1*, L55M), transcobalamin II (*TCN2*, R232P), and *DNMT1* (I311L), and *DNMT3A* (rs11695471). Interactions were also observed between plasma homocysteine and methylenetetrahydrofolate reductase (*MTHFR*) R594Q and alanyl-tRNA synthetase (*AARS*) rs2070203; plasma PLP and *TYMS* TS3; plasma B12 and betaine-homocysteine S-methyltransferase 2 (*BHMT2*) rs626105; and alcohol intake and *DNMT3B* –149C>T and –579G>T (all P-interaction<0.05 and FDR q-value<0.25). A number of tagSNPs in the above genes as well as in other FOCM genes were also significantly modified by the study nutrients in relation to CRC risk (see Supplemental Table 7 for the full summary by the functions of FOCM enzymes and Supplemental Table 8 for OR and 95% CI).

DISCUSSION

In this national sample of postmenopausal women, candidate genetic variants in *MTHFD1*, *MTRR*, *PDRM2* and *TYMS* were associated with CRC risk and there were suggestive associations for several tagSNPs. Data from our interaction analyses support the importance of FOCM nutrients in modifying the CRC risk associated with genetic factors.

Our findings on several commonly studied polymorphisms are consistent with those reported in the literature. For example, the variant genotypes compared to the wild type of *SHMT* L474F (rs1979277) and *TYMS* TS5 were associated with lower CRC risk both in a

recent meta-analysis[5] and in our study population (Supplemental Table 5), although the associations in our data were not statistically significant in part due to a relatively small number of participants with the variant genotypes. In addition, our findings for *MTRR* P450R may be important, as the polymorphism is associated with colorectal adenoma among White individuals.[5] However, the *MTHFD1* K134R homozygous variant versus wild-type was associated with an increase in rectal cancer with TP53 mutation,[21] but not CRC risk overall.[5] *PRDM2* S450N and *TYMS* g.676789A>T have not been reported for their associations with CRC risk.

The tagSNP approach allowed us to explore genetic regions and loci beyond candidate SNPs. For example, data from the Encyclopedia of DNA elements (ENCODE)[22] show that the tagSNP *CBS* rs11701048 is in high LD with a group of intronic SNPs in *CBS* in DNAse hypersensitivity sites containing histone modifications characteristic of weak transcriptional enhancers or promoters (Supplemental Table 9). These SNPs are also linked to active transcriptional start sites specifically within colorectal mucosal tissue, suggesting their regulatory functions in developing CRC.

We identified several novel FOCM gene-nutrient interactions. While plasma and RBC folate concentrations did not predict CRC risk in our study population,[23] the significant interaction between *FOLH1* and plasma folate suggests that the absorption and uptake of folate prior to the metabolic reactions in the cell may have important implications in colorectal carcinogenesis. Also, our data suggest that the relationship between *TCN2* R232P, in which the variant allele may result in a lower affinity and less efficient transport of B12 compared to the wild-type allele[24] and an increased CRC risk,[25] may depend on long-term folate status, represented by RBC folate concentrations.

Paraoxonase metabolizes homocysteine thiolactone to homocysteine (Supplemental Figure 1), and higher versus lower plasma homocysteine is associated with an increased CRC risk in our study population.[26] For the polymorphism *PON1* L55M, both our study (Supplemental Table 5) and another investigation[27] observed a non-significant increase in CRC risk associated with the M allele, which is linked to lower activity and serum concentrations of paraoxonase compared to the L allele. However, multiple significant interactions observed for *PON1* L55M and several tagSNPs (Supplemental Table 7) suggest that the paraoxonase-CRC risk connection may be dependent on the statuses of folate and homocysteine. These tagSNPs are related to DNA methylation, GATA2 binding (rs854540, tagged by rs8491), and miR-218, a moderately conserved microRNA binding site (rs854552, tagged by both rs854548 and rs854551),[22] although these lines of evidence were not directly observed in colorectal tissues.

Our data also suggest complex FOCM gene-nutrient interactions in the processes of DNA methylation and translation. First, DNMT3B is postulated to be a *de novo* methyltransferase.[28] In our data, alcohol intake modified the associations of *DNMT3B* –149C>T, –579G>T and tagSNP 15193C>T (rs2424908) with CRC risk, as alcohol intake can interrupt DNA methylation.[12] Second, DNMT1 is considered the “maintenance” DNMT that copies methylation patterns after DNA replication. Our data suggest that long-term folate status can also play a role in this mechanism. Third, PRDM2 is an important

modulating factor of DNA methylation and has been linked to colorectal carcinogenesis.[29] Lastly, we observed an important interaction between plasma homocysteine and a genetic variant in *AARS* potentially because alanyl-tRNA synthetase maintains the accuracy of amino acid selection, including preventing homocysteine from disrupting translation.[30] The gene-nutrient interactions among these epigenetic regulators are novel and need further confirmation.

The strengths of our study include its population-based design, standardized specimen and data collection, and physician-adjudication of CRC cases. The studied SNPs comprehensively covered relevant FOCM gene regions. By using measured biomarkers, we had a more objective and reliable assessment of internal doses of nutrients compared to self-reported dietary assessment and use of single or multivitamin supplements.

Several limitations should be noted. First, the recruitment of WHI-OS spanned the pre- (1994–1995), peri- (1996–1997), and post- (1998) periods of nationally mandatory folic acid fortification, which led to an upshift in folate status.[31] Thus, the expression of FOCM genes and their influences on CRC risks might have been modified in the era of folic acid fortification.[32] Second, a certain number of false-positive results might have occurred when we investigated the gene-nutrient interactions despite that we implemented several measures to minimize this possibility. Third, the generalizability of our study findings may be limited because we performed the analyses among white women only, and WHI participants were healthy volunteers with high education attainments, higher use of colonoscopy, and overall a higher nutritional status than postmenopausal women in general.

In conclusion, our observations highlight the importance of nutrient status when investigating FOCM-related genetic influences on CRC risk in postmenopausal women. Future studies should pursue potentially causal loci in the regions prioritized by our tagSNPs and the influence of these SNPs on FOCM biomarkers to further elucidate their roles in CRC prevention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Katherine Howes for measurements of homocysteine, PLP, and RBC folate; Pamela Yang for measurements of plasma folate and vitamin B12; Stephanie Rosse for functional annotation; the ENCODE Consortium and the ENCODE production laboratories for generating the particular datasets. We also thank WHI participants and the Program Office (National Heart, Lung, and Blood Institute, Bethesda, MD) Jacques Rossouw, Shari Ludlam, Joan McGowan, Leslie Ford, and Nancy Geller; (the Clinical Coordinating Center at Fred Hutchinson Cancer Research Center, Seattle, WA) Garnet Anderson, Ross Prentice, Andrea LaCroix, and Charles Kooperberg; the Investigators and Academic Centers (Brigham and Women's Hospital, Harvard Medical School, Boston, MA); JoAnn E Manson; (MedStar Health Research Institute/Howard University, Washington, DC) Barbara V Howard; (Stanford Prevention Research Center, Stanford, CA) Marcia L Stefanick; (The Ohio State University, Columbus, OH) Rebecca Jackson; (University of Arizona, Tucson/Phoenix, AZ) Cynthia A Thomson; (University at Buffalo, Buffalo, NY) Jean Wactawski-Wende; (University of Florida, Gainesville/Jacksonville, FL) Marian Limacher; (University of Iowa, Iowa City/Davenport, IA) Robert Wallace; (University of Pittsburgh, Pittsburgh, PA) Lewis Kuller; (Wake Forest University School of Medicine, Winston-Salem, NC) Sally Shumaker. The WHI program is funded by the National Heart, Lung, and Blood Institute, NIH, US Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HSN268201100003C, HHSN268201100004C, and HHSN271201100004C.

Sources of financial support: NIH R01 CA120523, N01WH22110

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Table 1

Baseline characteristics of the colorectal cancer cases and controls in the Women's Health Initiative Observational Study, 1983–2008

	Cases (n=821)	Controls (n=821)	P-value
Characteristic	**Mean (SD) or %**	**Mean (SD) or %**	
Age, years	67 (7)	67 (7)	0.49
Body-mass index, kg/m^2	27.8 (5.7)	26.8 (5.8)	<0.01
Pack-years of smoking	14 (22)	9 (18)	<0.01
History of colonoscopy or sigmoidoscopy (yes)	54%	62%	<0.01
History of colon polyp removal (yes)	26%	19%	0.01
Family history of colorectal cancer	21%	19%	0.31
Any one-carbon supplement use (yes)[1]	57%	56%	0.79
Alcohol intake, drinks/week	2.4 (5.1)	2.4 (4.6)	0.72
Red blood cell folate, ng/mL	610 (254)	627 (265)	0.25
Plasma folate, ng/mL	19.9 (15.1)	20.7 (14.4)	0.06
Plasma B6 (pyridoxal-5'-phosphate), nmol/L	96.1 (92.9)	105.2 (104.7)	0.05
Plasma B12, pg/mL	515 (280)	529 (264)	0.06
Plasma homocysteine, μmol/L	8.81 (2.99)	8.39 (2.58)	0.003
Tumor location		–	
Proximal	59%		
Distal	21%		
Rectal	18%		
Overlapping lesion/Unknown	2%		
Tumor grade		–	
Well differentiated	7%		
Moderately differentiated	63%		
Poorly differentiated	20%		
Anaplastic	1%		
Unknown/not done	8%		
Tumor stage (SEER staging)		–	
Localized	44%		
Regional	42%		
Distant	13%		
Unknown/not done	2%		

[1] Any current consumption of dietary supplements containing vitamins B2, B6, B12, or folic acid.

Table 2

Significant associations of candidate polymorphisms with CRC risk

Gene	SNP	Genotype	No. cases	No. controls	Odds ratio (95% CI)[1]	Global *P*-value	*P*-trend
MTHFD1	rs1950902 (K134R)	GG	538	523	1.00		
		GA	233	256	0.82 (0.66–1.03)		
		AA	23	27	0.62 (0.34–1.14)	0.09	0.03
MTRR	rs16879334 (P450R)[a]	CC	758	744	1.00		
		CG+GG	38	62	0.61 (0.40–0.94)	0.02	–
MTRR	rs2287780 (R442C)[a]	GG	757	743	1.00		
		GA+AA	38	63	0.61 (0.40–0.93)	0.02	–
MTRR	rs2303080 (S284T)[a]	AA	759	742	1.00		
		AT+TT	36	60	0.62 (0.40–0.95)	0.03	–
PRDM2	rs17350795 (S450N)	GG	780	772	1.00		
		GA+AA	15	31	0.52 (0.28–0.98)	0.04	–
TYMS	rs10502289 (g.676789A>T)	AA	539	503	1.00		
		AT	222	256	0.81 (0.65–1.00)		
		TT	32	47	0.61 (0.37–0.98)	0.03	0.01

[1] Adjusted for age and matching variables, including race/ethnicity, enrollment date, hysterectomy status, family history of CRC, and time of blood draw.

[a] The group of SNPs are in high linkage disequilibrium (LD, r^2>0.9).

Table 3

Nominally significant associations of tagSNPs with CRC risk

Gene	SNP	Genotype	No. cases	No. controls	Odds ratio (95% CI)[1]	Global *P*-value	*P*-trend	P_{ACT}
CBS	rs11701048	GG	632	674	1.00			
		GA+AA[4]	164	132	1.41 (1.07–1.85)	0.01	–	0.11
DHFR	rs10474632	GG	665	698	1.00			
		GA+AA[4]	130	108	1.38 (1.03–1.85)	0.03	–	0.22
DNMT3B	rs13045669	AA	725	757	1.00			
		AG+GG[4]	71	49	1.48 (1.00–2.18)	0.046	–	0.31
MAT1A	rs10887718	AA	187	232	1.00			
		AG	427	411	1.29 (1.00–1.66)	0.04	0.02	0.26
		GG	181	160	1.43 (1.06–1.93)			
MTHFD1	rs8003567	AA	682	668	1.00			
		AG+GG[4]	114	138	0.72 (0.54–0.96)	0.02	–	0.14
MTRR	rs9282787	AA	486	531	1.00			
		AG	274	254	1.23 (0.98–1.55)			
		GG	35	21	1.85 (1.04–3.28)	0.03	0.01	0.22

[1] Adjusted for age and matching variables, including race/ethnicity, enrollment date, hysterectomy status, family history of CRC, and time of blood draw.

Table 4

Summary of interactions between candidate polymorphisms, nutritional biomarkers, and alcohol intake[1,2]

Gene	SNP	Function, location	Direction of association in tertiles of the biomarker/nutrient[3,5]	P-interaction	q value[4]
FOLH1	rs10839236	Candidate, intron	Plasma folate: T1 ↑, T3 ↓	0.018	*0.11*
MTRR	rs1532268 (S202L)	Nonsynonymous	Plasma folate: T1 ↓, T3 ↑	0.045	0.46
	rs10380 (H622Y)	Nonsynonymous	B12: T1 ↓	0.020	0.42
BHMT2	rs626105	Candidate, intron	B12: T3 ↓	0.021	*0.11*
CBS	rs1801181	Candidate, synonymous codon	RBC folate: T3 ↓	0.035	0.26
PON1	rs854560 (L55M)	Nonsynonymous	Plasma folate: T3 ↑	0.021	*0.12*
MTHFR	rs2274976 (R594Q)	Nonsynonymous	Hcy: T1↑, T3↑	0.008	*0.09*
MTHFD2	rs702465	Candidate, intron	PLP: T1 ↓	0.049	0.34
TYMS	TS3	Candidate	PLP: T3 ↓	0.009	*0.10*
TCN2	rs1801198 (R232P)	Nonsynonymous	RBC folate: T1 ↑, T3 ↓	0.040	*0.23*
DNMT1	rs2228612 (I311L)	Nonsynonymous	RBC folate: T1 ↓, T3 ↓	0.016	*0.02*
DNMT3A	rs11695471	Candidate, intron	Plasma folate: T1 ↑, T3 ↓	0.002	*0.02*
DNMT3B	rs6087990 (–283T>C)[a]	Candidate, upstream variant 2KB	RBC folate: T1 ↑	0.043	0.29
	rs2424913 (–149C>T)	Candidate, intron	Alcohol: T1 ↑	0.023	*0.16*
	rs2424909 (–579G>T)[a]	Candidate, intron	Alcohol: T1 ↑	0.048	*0.17*
AARS	rs2070203	Synonymous codon	Hcy: T3 ↑	<0.001	*0.001*

Abbreviations: RBC, red blood cell; Hcy, homocysteine; PLP, pyridoxal-5'-phosphate; T, tertile

[1] Listed if an interaction had nominal P<0.05 and showed a trend of change in risk along with the genotype mutation.

[2] Adjusted for age, BMI, smoking pack-years, and matching variables, including race/ethnicity, enrollment date, hysterectomy status, family history of CRC, and time of blood draw.

[3] T1↑: CRC risk was higher for variants (heterozygous and/or homozygous genotypes) in the presence of lower (tertile 1) nutrients; T1↓: CRC risk was lower for variants in the presence of lower (tertile 1) nutrients; T3 ↑: CRC risk was higher for variants in presence of higher (tertile 3) nutrients; T3 ↓: CRC risk was lower for variants in presence of higher (tertile 3) nutrients; all comparisons were made to wild-type genotype in the presence of lower (tertile 1) nutrients.

[4] False discovery rate q-values; q-value <0.25 was italicized.

[5] Tertile cutoffs were RBC folate: 484 and 689 ng/mL; plasma folate: 12.4 and 23.5 ng/mL; plasma PLP: 52.0 and 93.1 nmol/L; plasma B12: 392 and 592 pg/mL; plasma homocysteine: 7.08 and 8.79 μmol/L; alcohol intake: 0 and 1.7 drinks/week.

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[a] SNPs are in high linkage disequilibrium (r^2>0.9).