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Original Article

Candidate pathway polymorphisms in one-carbon metabolism and risk of rectal tumor mutations

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Abstract: We examined candidate polymorphisms in genes involved in the folate-mediated, one-carbon metabolism pathway, *DNMT1* I311V, *MTHFD1* R134K and R653Q, *MTHFR* R594Q, *MTR* D919G, *MTRR* H595Y and I22M, *SHMT1* L474F, *SLC19A1* H27R, and *TDG* G199S, and associations with rectal tumor characteristics. We hypothesized that these candidate genes would influence CpG Island Methylator Phenotype and potentially *KRAS2* or *TP53* tumors. Data from a population-based study of 747 rectal cases (593 with tumor markers) and 956 controls were evaluated using generalized estimating equations. We observed an increased risk of *TP53* tumor mutations in homozygous carriers of the *MTHFD1* 134K allele (OR=2.0, 95%CI 1.2-3.1, *P*- trend=0.02). In the presence of low folate intake, the R134K variant was associated with increased risk of CIMP+ tumors (OR=2.8, 95%CI 1.04-7.7). The *MTRR* I22M variant genotype was associated with a modest increased risk of *TP53* mutations (OR=1.7, 95%CI 1.2-2.5, *P*-trend=0.001). Our findings offer limited support that polymorphisms in one-carbon metabolism genes influence rectal tumor phenotype, and that folate may interact with *MTHFD1* to alter CIMP+ risk.

Keywords: Folate-mediated one-carbon metabolism (FOCM), single nucleotide polymorphism, CpG island methylator phenotype (CIMP), *TP53*, *KRAS2*, rectal cancer

Introduction

Colorectal carcinogenesis appears to occur via distinct molecular pathways, including a pathway characterized by a large number of hypermethylated CpG islands with subsequent epigenetic transcriptional silencing[1-4]. This CpG island methylator phenotype (CIMP) includes the silencing of tumor suppressor genes such as the cell-cycle regulator *CDKN2A* [5-7]. *S*-adenosylmethionine (SAM), the universal donor of methyl groups in humans, and *S*-adenosylhomocysteine (SAH), the product of and an inhibitor of DNA methyltransferases, provide connections between folate-mediated, one-carbon metabolism (FOCM) and DNA methylation[8, 9]. Polymorphisms in folate-metabolizing genes have been reported to be associated with colon and rectal cancer in our investigations[10-12] and in other studies[8, 13], via a hypothesized effect on global DNA methylation and the availability of nucleotides for DNA synthesis and repair[14]. Thus, the provision of methyl groups and genetic variants in folate-mediated, one-carbon metabolism may play a role in defining rectal tumor subtypes [15]. Given the heterogeneity of acquired mutations in colorectal cancer (CRC), previous studies have examined associations of FOCM variants and colorectal tumors that exhibit promoter methylation[16-18] and other molecular characteristics, including *TP53* tumor mutations [19].

The purpose of this study was to evaluate associations between common genetic variants relevant to FOCM and rectal cancer risk, and to furthermore investigate the impact of dietary fac-

tors on these associations. We examined non-synonymous polymorphisms in: DNA (cytosine-5-)-methyltransferase 1 (*DNMT1*), I311V (rs2228612); methylenetetrahydrofolate dehydrogenase 1 (*MTHFD1*), R134K (rs1950902) and R653Q (rs2236225); 5,10-methylenetetrahydrofolate reductase (*MTHFR*), R594Q (rs2274976); 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR*), D919G (rs1805087); 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*), H595Y (rs10380) and I22M (rs1801394); serine hydroxymethyltransferase, cytosolic (*SHMT1*), L474F (rs1979277); solute carrier family 19 (folate transporter) member 1 (*SLC19A1*), H27R (rs1051266); and thymine-DNA glycosylase (*TDG*), G199S (rs4135113). These genes were selected based on their involvement in the production of the methyl-donor SAM, cellular folate availability, and other central roles in FOCM[20]. We evaluated genetic variants with folate and relevant B-vitamins, methionine, and alcohol to assess dietary interactions with polymorphisms, as little information is currently available in this regard. We previously reported findings that *MTHFR* 1298A>C (E429A, rs1801131) influenced folate in risk of CIMP+ male rectal cancer while *MTHFR* 677C>T (A222V, rs1801133) was not associated with rectal tumor subtypes[21]. To our knowledge, this is the first evaluation of *TP53* mutations, *KRAS2* mutations, and CIMP specifically in rectal tumors for relationships with FOCM coding polymorphisms in several genes.

Materials and methods

Participants in the study were from the Kaiser Permanente Medical Care Program of Northern California (KPMCP), and Utah. 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. Cases with a previous colorectal tumor, familial adenomatous polyposis, ulcerative colitis, or Crohn's disease were not eligible for the study. Participants were between 30 and 79 years of age at time of diagnosis, English speaking, and mentally competent to complete the interview. Controls were frequency matched to cases by sex and by five-year age cohort. At KPMCP, controls were randomly selected from membership lists. At Utah, controls younger than 65 were randomly selected from driver's license lists and controls 65 years and older were selected from social security lists. The study population was primarily white, non-Hispanic (83% of cases and 81% of controls), with the remainder of subjects reporting black, Hispanic, or Asian in roughly equal proportions. Response rates were 65% for both cases and controls; cooperation rates, the number of people who participated of those we were able to contact, were 73% for cases and 69% for controls [22]. Institution review board approval was obtained from the University of Utah and KPMCP. A total of 747 rectal cancer cases 593 with tumor markers) and 956 controls were genotyped [21, 23]. 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, as previously described in detail [21, 24, 25]. A dietary history questionnaire, adapted from the CARDIA dietary history, was used to assess diet and supplement intake. Participants were asked to recall foods eaten, the frequency which they were eaten, serving size, and supplemental vitamins used regularly. Nutrient intake was calculated using the University of Minnesota Nutrition Coordinating Center Nutrition Data System for Research (NDS-R), Database version 4.02_30,© Regents of the University of Minnesota, and include folic-acid fortified foods[26, 27]. Cases and controls had their blood collected during the in-person interview.

The genotype frequencies among cases and controls for all polymorphisms were compatible with Hardy-Weinberg equilibrium ($\chi 2$ test). Minor allele frequencies in study controls, by race and ethnicity, are shown in **Table 1**. Genomic DNA was extracted using methods described previously[10]. The *DNMT1* I311V, *MTHFR* R594Q, *MTRR* H622Y and I22M, *SHMT* L474F, and *TDG* G199S polymorphisms were detected using the Illumina™ GoldenGate bead-based genotyping platform at the Translational Genomics Institute (TGen, Phoenix, AZ). The reliability and reproducibility of the genotyping were confirmed by comparing to genotype data from 30 CEPH trios (Coriell Cell Repository, Camden, NJ) that were genotyped by the HapMap project. TGen included blinded duplicates on all plates and for every plate and batch, intraplate and interplate replicates were included at ~5%.

Table 1. Rectal study folate mediated, one-carbon metabolism SNPs and allele frequencies

							MAF in study controls			
Gene	Alias	SNP	dbSNP Id	Chr.	Exon	Base change	White	Hispanic	Black	Asian
DNMT1	*DNMT*	I311V	rs2228612[1]	19p13.2	12	931A>G	0.06	0.14	0.10	0.21
MTHFD1	*MTHFD*	R134K	rs1950902	14q24	6	401G>A	0.19	0.10	0.14	0.30
MTHFD1	*MTHFD*	R653Q	rs2236225	14q24	20	1958G>A	0.44	0.43	0.29	0.28
MTHFR		R594Q	rs2274976	1p36.3	12	1958G>A	0.05	0.09	0.04	0.20
MTR	*MS*	D919G	rs1805087	1q43	26	2756A>G	0.21	0.20	0.27	0.17
MTRR		H595Y[2]	rs10380	5p15.2-3	14	1783C>T	0.10	0.25	0.26	0.12
MTRR		I22M	rs1801394	5p15.2-3	2	66G>A	0.43	0.37	0.26	0.34
SHMT1	*cSHMT*	L474F	rs1979277	17p11.2	12	1420C>T	0.32	0.30	0.36	0.09
SLC19A1	*RFC1, FOLT*	H27R	rs1051266	21q22.3	2	80G>A	0.44	0.44	0.42	0.43
TDG		G199S	rs4135113	12q24.1	5	994G>A	0.02	0.03	0.14	0.10

Abbreviations: MAF, minor allele frequency; Chr, chromosome. [1]Previous dbSNP ID rs8111085. [2]Also known as H622Y.

TGen excluded genotypes 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.

Polymorphisms that were not conducive to high-throughput methods were genotyped at the Fred Hutchinson Cancer Research Center (FHCRC, Seattle, WA). The *MTHFD1* R134K and R653Q polymorphisms were detected by allelic discrimination using the 5' nuclease assay on a 7900HT sequence detection system (Applied Biosystems, Foster City, CA) at. The 5' nuclease genotyping assays were validated by genotyping 100 individuals by both 5' nuclease assay and RFLP [28, 29]. No discrepancies were found. Four negative controls and at least one positive control for all of the genotypes were included in each plate. For quality control purposes, genotyping for 94 randomly selected samples was repeated and no discrepancies were found.

Tumor DNA was obtained from paraffin-embedded tissue as described[30]. Tumors were characterized by their genetic profile that included: sequence data for exons 5 through 8; sequence data for *KRAS2* codons 12 and 13; and methylation specific PCR of sodium bisulfite modified DNA for five CpG Island markers, *CDKN2A*, *MLH1* and methylated in tumors (MINT) 1, 2 and 31[5]. Tumors with two or more methylated CpG islands were scored as CIMP+.

All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). Unconditional logistic regression models were used to estimate odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs) for the association between genotypes and risk of rectal cancer. All interviewed and genotyped cases were compared to all genotyped population controls to examine associations of individual coding SNPs with rectal cancer overall. We primarily examined an unrestricted co-dominant model, unless there were insufficient numbers of homozygous carriers of the minor allele, in which a dominant model was used. Tumors were defined by specific alterations detected; any *TP53* mutation, any *KRAS2* mutation, or CIMP+. In order to compare specific types of mutations to controls while adjusting for the other tumor mutations simultaneously, a generalized estimating equation (GEE) with a multinomial outcome was used as case subjects could contribute from one to three outcome observations depending upon how many tumor mutations (CIMP+, *KRAS2*, *TP53*) an individual had [31]. The GEE accounts for correlation introduced by including subjects multiple times and was implemented using the GENMOD procedure as described by Kuss and McLerran[32] .

All models were adjusted for sex, age at diagnosis or selection, study center, race/ethnicity, recent estrogen (women), total energy (kcal),

and fiber (previously shown to have a protective effect in rectal cancer and may confound the effect of folate[33, 34]). Other factors were included that have been related to rectal cancer including screening (sigmoidoscopy), smoking status (within 5 years of the referent year), recent NSAID use, and long-term vigorous physical activity. Family history, calcium, long-term alcohol, and body mass index did not impact the estimates and were not included in the adjustments. *P* for trend was assessed using a likelihood ratio test. The likelihood of a model with a variable representing ordered genotype categories was compared to the likelihood of a model without the variable (χ^2, 1 d.f.). Nominal *P* values are presented in our hypothesis-driven investigation.

Results

Polymorphisms in one-carbon metabolism were generally not associated with risk of rectal cancer or rectal tumor markers (**Table 2**). However, we observed an increased risk of *TP53* tumor mutation in homozygous carriers of the *MTHFD1* 134K allele compared to wildtype (OR=2.0, 95%CI 1.2-3.1; *P*-trend=0.02) which appeared stronger in men in a gender-stratified analysis (OR=3.0, 95%CI 1.4-6.4; data not shown). In individuals with a variant *MTRR* I22M genotype, a modestly increased risk was observed in rectal cancer overall (OR 1.3, 95%CI 1.01-1.8; *P*-trend=0.04). This increased risk was confined primarily to those cases with a *TP53* mutation (OR=1.7, 95%CI 1.2-2.5, *P*-trend=0.001). The *SHMT1* L474F appeared to be associated with having a *KRAS2* mutation (OR=2.0, 95%CI=1.2-3.1, *P*-trend=0.02).

Intakes of methionine, alcohol, riboflavin, and vitamins B6 and B12 did not appear to modify risk of rectal tumors in relation to nonsynonymous SNPs included in this study (data not shown). However, those heterozygous or homozygous for the *MTHFD1* R134K variant who reported low folate intake from both food and supplements (<400 mcg/day, ~lower tertile in controls) were at increased risk of CIMP+ rectal cancer (OR 2.8, 95%CI 1.04-7.7) (**Table 3**). For individuals who carry one or two *MTRR* I22M variant alleles and who reported high folate intake (>750 mcg/day, ~higher tertile in controls) we observed a suggestive, nonstatstically significant increased risk of CIMP+ rectal cancer (OR 2.7, 95%CI 0.99-7.6) (data not shown).

Discussion

The strength of this investigation is our ability to study tumors and genetic and environmental risk factors in a large population-based collection of rectal tumors. Our study represents one of the very few case-control studies of rectal-site cancer specifically. As we have previously reported differences in risk between colon and rectal tumors as well as similarities [35-37], we believe it is important to elucidate what may be differences in their disease etiologies. Regarding nonsynonymous FOCM polymorphisms and risk of colon tumors, we generally did not find associations in the tumor markers. We did report a decreased risk of *MTHFR (*677C>T) and (1298A>C) variant genotypes in non-CIMP tumors only, and that the *TCN2* P259R variant allele conferred a modest reduce risk of a methylated colon tumor [16]. Thus, this investigation of rectal-site tumor markers was consistent in its lack of strong evidence for a role of FOCM coding SNPs in risk of CIMP tumors. We also observed that in individuals with the *MTRR* I22M variant genotype, a modestly increased risk was observed for rectal cancer overall, with a stronger effect in tumors harboring a *TP53* mutation (*P*-trend = 0.001). As a number of comparisons were made in our hypothesis-based investigation, it is possible this observation was a chance association and our findings must be interpreted cautiously.

In regard to FOCM variants and the effect of diet in colon cancer, Steck, et al. reported evidence of gene-diet interaction for *MTRR* I22M and folate intake in Caucasians [38]. In our investigation, we observed that those with a low intake of folate and the *MTHFD1* R134K variant were at increased risk of CIMP+ rectal cancer which may suggest that FOCM genetic risk factors could differ between cancers of the colon and rectum. Similar to our study of colon cancers [16], we found that few of the dietary factors hypothesized to affect DNA methylation were associated more strongly with CIMP+ than (non-CIMP+) *TP53*- or *KRAS2*-mutated rectal tumors.

The polymorphisms selected for this study were based on a candidate gene approach of investigating single nucleotide changes that alter an amino acid, and thus may be hypothesized to impact protein function. Similar to a recent report of associations in folate pathway genes and CRC[39], our findings offer only limited evidence for a broader role of genetic variants involved in

Table 2. Associations between nonsynonymous candidate polymorphisms in folate-mediated, one-carbon metabolism and rectal tumors[1]

	Geno-	Ctrl.	Case	All cases[2]				*TP53* mutation				*KRAS2* mutation				CIMP+			
SNP	type	*n*	*n*	OR	(95% CI)		*P*[3]	*n*	OR	(95% CI)	*P*[3]	*n*	OR	(95% CI)	*P*[3]	*n*	OR	(95% CI)	*P*[3]
DNMT1 I311V	AA	809	622	-1-				228	-1-			136	-1-			53	-1-		
rs2228612	AG/GG	123	115	1.1	(0.8,	1.5)	*0.47*	43	1.0	(0.7, 1.5)	*0.65*	32	1.4	(0.9, 2.1)	*0.15*	6	0.7	(0.3, 1.6)	*0.52*
MTHFD1 R134K	GG	622	486	-1-				169	-1-			123	-1-			37	-1-		
rs1950902	GA	268	207	1.0	(0.8,	1.3)		77	1.2	(0.9, 1.5)		37	0.7	(0.5, 1.0)		17	1.1	(0.6, 2.0)	
	AA	37	38	1.2	(0.8,	2.0)	*0.57*	22	2.0	(1.2, 3.1)	*0.02*	5	0.5	(0.2, 1.2)	*0.12*	4	1.4	(0.6, 3.5)	*0.43*
MTHFD1 R653Q	GG	299	242	-1-				90	-1-			47	-1-			13	-1-		
rs2236225	GA	449	339	0.9	(0.7,	1.2)		127	0.9	(0.7, 1.2)		80	1.1	(0.8, 1.6)		32	1.6	(0.8, 3.1)	
	AA	180	155	1.1	(0.8,	1.5)	*0.68*	53	0.9	(0.6, 1.3)	*0.73*	41	1.4	(0.9, 2.1)	*0.18*	12	1.4	(0.7, 3.1)	*0.29*
MTHFR R594Q	GG	844	656	-1-				246	-1-			148	-1-			53	-1-		
rs2274976	GA/AA	89	77	1.2	(0.8,	1.6)	*0.40*	23	0.9	(0.5, 1.4)	*0.58*	17	1.2	(0.7, 2.1)	*0.63*	3	0.5	(0.2, 1.7)	*0.23*
MTR D919G	AA	599	488	-1-				176	-1-			114	-1-			36	-1-		
rs1805087	AG	302	228	0.9	(0.7,	1.1)		88	1.0	(0.7, 1.3)		50	0.8	(0.6, 1.2)		20	1.2	(0.7, 2.0)	
	GG	45	25	0.7	(0.4,	1.1)	*0.10*	9	0.8	(0.4, 1.7)	*0.34*	4	0.5	(0.2, 1.5)	*0.07*	1	0.4	(0.1, 2.6)	*0.58*
MTRR H595Y	CC	736	556	-1-				203	-1-			131	-1-			43	-1-		
rs10380	CT/TT	201	185	1.2	(0.9,	1.5)	*0.18*	70	1.2	(0.9, 1.6)	*0.32*	37	0.9	(0.6, 1.3)	*0.76*	15	1.4	(0.7, 2.5)	*0.33*
MTRR I22M	GG	278	187	-1-				62	-1-			43	-1-			15	-1-		
rs1801394	GA	464	363	1.2	(0.9,	1.5)		124	1.2	(0.9, 1.7)		83	1.2	(0.8, 1.7)		22	0.9	(0.5, 1.8)	
	AA	211	193	1.3	(1.01,	1.8)	*0.04*	86	1.7	(1.2, 2.5)	*0.001*	44	1.1	(0.7, 1.7)	*0.20*	21	1.9	(0.9, 3.7)	*0.03*
SHMT1 L474F	CC	455	362	-1-				142	-1-			69	-1-			27	-1-		
rs1979277	CT	363	287	1.0	(0.8,	1.3)		102	0.9	(0.7, 1.2)		70	1.4	(1.0, 1.9)		24	1.1	(0.7, 2.0)	
	TT	110	77	0.9	(0.7,	1.3)	*0.91*	24	0.7	(0.4, 1.1)	*0.27*	28	2.0	(1.2, 3.1)	*0.02*	4	0.6	(0.2, 1.6)	*0.60*
SLC19A1 H27R	GG	280	226	-1-				79	-1-			55	-1-			21	-1-		
rs1051266	GA	459	351	0.9	(0.7,	1.2)		130	1.1	(0.8, 1.4)		71	0.8	(0.6, 1.1)		23	0.7	(0.4, 1.2)	
	AA	183	147	1.0	(0.7,	1.3)	*0.83*	57	1.1	(0.8, 1.6)	*0.64*	36	1.0	(0.6, 1.5)	*0.69*	12	0.8	(0.4, 1.7)	*0.53*
TDG G199S	GG	888	697	-1-				252	-1-			162	-1-			55	-1-		
rs4135113	GA/AA	56	45	0.9	(0.6,	1.4)	*0.77*	20	1.2	(0.7, 2.0)	*0.76*	6	0.5	(0.2, 1.2)	*0.12*	3	0.8	(0.3, 2.5)	*0.64*

[1]Adjusted for sex, age, race, center, energy, fiber, screening, smoking status, recent NSAID use, physical activity, and recent estrogen (women); reference group are individuals homozygous for the common allele. [2]Includes rectal cases without tumor marker data.[3]Likelihood ratio test *P* for trend.

Table 3. Associations between nonsynonymous SNPs in folate-mediated, one-carbon metabolism and rectal tumors (folate intake<400 mcg/day)[1]

		Ctrl.	Case	All Cases[2]			*TP53* Mutation			*KRAS2* Mutation			CIMP+		
		n	*n*	OR	(95% CI)		*n*	OR	(95% CI)	*n*	OR	(95% CI)	*n*	OR	(95% CI)
DNMT1 I311V	AA	219	213	-1-			73	-1-		45	-1-		20	-1-	
rs2228612	AG/GG	38	36	1.0	(0.6,	1.7)	18	1.4	(0.8, 2.5)	6	0.7	(0.3, 1.8)	2	0.9	(0.2, 3.8)
MTHFD1 R134K	GG	166	160	-1-			54	-1-		41	-1-		9	-1-	
rs1950902	GA/AA	90	87	0.6	(0.3,	1.2)	37	1.3	(0.7, 2.1)	9	0.4	(0.2, 0.8)	13	2.8	(1.04, 7.7)
MTHFD1 R653Q	GG	80	80	-1-			30	-1-		12	-1-		8	-1-	
rs2236225	GA/AA	175	169	0.9	(0.6,	1.4)	61	0.9	(0.5, 1.4)	39	1.6	(0.8, 3.0)	13	0.7	(0.3, 1.5)
MTHFR R594Q	GG	228	222	-1-			84	-1-		44	-1-		17	-1-	
rs2274976	GA/AA	26	24	1.1	(0.4,	3.5)	6	0.6	(0.2, 1.7)	7	1.8	(0.7, 4.8)	3	1.9	(0.4, 8.2)
MTR D919G	AA	171	160	-1-			60	-1-		35	-1-		16	-1-	
rs1805087	AG/GG	90	92	1.1	(0.8,	1.6)	33	1.1	(0.7, 1.7)	17	1.0	(0.6, 1.8)	6	0.8	(0.3, 2.1)
MTRR I22M	GG	70	69	-1-			26	-1-		15	-1-		8	-1-	
rs1801394	GA/AA	196	182	1.5	(0.8,	3.0)	65	0.9	(0.5, 1.7)	38	0.9	(0.4, 1.8)	14	0.7	(0.3, 2.0)
MTRR H595Y	CC	197	181	1			66	-1-		41	-1-		16	-1-	
rs10380	CT/TT	62	69	1.3	(0.9,	2.0)	26	1.4	(0.8, 2.3)	10	0.7	(0.3, 1.4)	6	1.6	(0.6, 4.5)
SHMT1 L474F	CC	118	125	-1-			48	-1-		21	-1-		10	-1-	
rs1979277	CT/TT	136	120	0.7	(0.4,	1.4)	41	0.7	(0.4, 1.1)	29	1.1	(0.6, 2.2)	10	1.0	(0.4, 2.9)
SLC19A1 H27R	GG	77	74	-1-			30	-1-		12	-1-		8	-1-	
rs1051266	GA/AA	178	167	1.6	(0.8,	3.4)	57	0.9	(0.5, 1.6)	36	1.4	(0.6, 2.9)	12	0.6	(0.2, 1.7)
TDG G199S	GG	238	236	-1-			85	-1-		50	-1-		20	-1-	
rs4135113	GA/AA	20	15	0.6	(0.2,	2.6)	6	0.7	(0.3, 2.0)	1	0.3	(0.0, 2.1)	2	1.3	(0.2, 7.4)

[1]Adjusted for sex, age, race, center, energy, fiber, screening, smoking status, recent NSAID use, physical activity, and recent estrogen (women); reference group are individuals homozygous for the common allele. [2]Includes rectal cases without tumor marker data.

FOCM in rectal cancer. Based on a modeling approach, Ulrich, et al. suggest many longer-range regulatory mechanisms in folate metabolism have evolved to protect the rate of methylation at the cellular level against fluctuations in folate and methionine input[15]. Although the literature supports the relevance of FOCM polymorphisms and dietary factors for global DNA methylation, our data provide only limited support for a role of these factors in the promoter-specific methylation that characterizes the CIMP subset of rectal cancer, and further research is warranted.

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