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Germline polymorphisms in the one-carbon metabolism pathway and DNA methylation in colorectal cancer

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Abstract

Dietary intake of one-carbon nutrients (methyl donors) and germline variants in the one-carbon metabolism genes may influence global DNA methylation level and methylation in promoter CpG islands. In this study, we evaluated the relationship between single nucleotide polymorphisms (SNPs) in the one-carbon metabolism pathway and DNA methylation status in colorectal cancer. Utilizing 182 colorectal cancers cases in two prospective cohort studies, we determined the CpG island methylator phenotype (CIMP) status on eight CIMP-specific promoters and measured LINE-1 methylation level that correlates well with genome-wide DNA methylation level. We genotyped 23 nonsynonymous SNPs in the one-carbon metabolism genes using buffy coat DNA. Most of the 23 SNPs in the one-carbon metabolism pathway were not significantly associated with CIMP-high status (≥6/8 methylated promoters). However, the *MTHFR 429 Ala/Ala* variant (rs1801131) and the *TCN2* 259 *Arg/Arg* variant (rs1801198) were associated with CIMP-high status (*MTHFR 429* multivariate odds ratio (MV OR) = 7.56; 95% confidence interval (CI), 1.32–43.3; *p* trend = 0.10; *TCN2* 259 *Arg/Arg* variant MV OR = 3.82; 95% CI, 1.02–14.4; *p* trend =

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0.06). The one-carbon metabolism genotypes were not significantly associated with LINE-1 methylation, although there were modest differences in mean LINE-1 methylation levels between certain genotypes. Collectively, these exploratory data provide suggestive evidence for the association of *MTHFR 429 Ala/Ala* and *TCN2* 259 *Arg/Arg* and CIMP status in colorectal cancer.

Keywords

SNP; One-carbon metabolism; Colorectal cancer; CIMP; DNA methylation

Introduction

Genetic and epigenetic alterations are important in carcinogenesis [1, 2]. Genome-wide DNA hypomethylation involving repetitive DNA elements such as LINE-1 (long interspersed nucleotide element-1) is considered to play an important role in genomic instability by the reactivation of transposable DNA sequences [3, 4], leading to colorectal carcinogenesis [5–9]. In contrast, aberrant promoter CpG island methylation may also contribute to colorectal cancer development by silencing tumor suppressor genes [1]. The CpG island methylator phenotype (CIMP) is characterized by widespread promoter CpG island methylation [10, 11], and inversely associated with LINE-1 hypomethylation in colorectal cancer [12]. CIMP-high colorectal cancer shows a distinctive profile, including associations with female gender, proximal tumor location, poor differentiation, microsatellite instability-high (MSI-high), and *BRAF* mutation [13–17].

Genetic factors such as germline single nucleotide polymorphisms (SNPs), along with dietary intake of one-carbon nutrients such as folate and methionine [18, 19], likely influence cellular one-carbon metabolism and methyl-donor status [20, 21]. Recent studies have shown the potential relationship between germline variants in methyl-group metabolism genes and promoter CpG island methylation in colorectal tumors [22, 23].

We have previously shown that polymorphisms in the B_{12}-methionine-related pathway have been associated with colorectal cancer [24] and colorectal adenoma [25]. In this study, we examined the relationship between 23 germline nonsynonymous SNPs (nsSNPs) in 12 one-carbon metabolism genes and DNA methylation status (CIMP status and LINE-1 methylation) in colorectal cancer.

Materials and methods

Study population

The Nurses' Health Study (NHS) is an ongoing prospective study of 121,700 US female registered nurses. Details of the design and follow-up of this cohort have been previously described [26]. Briefly, at enrollment in 1976, the participants, who were aged 30–55, completed a questionnaire providing information on risk factors for cancer and cardiovascular disease. Exposure and disease information are updated biennially. From 1989 to 1990, blood samples were collected from 32,826 of the NHS participants. After blood collection through June 2000, 197 incident cases of colorectal cancer were confirmed through medical records or death reports, of which 190 cases were successfully genotyped.

The Health Professionals Follow-up Study (HPFS) began in 1986 when 51,529 US male health professionals (dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians), aged 40–75, responded to a mailed questionnaire. These men provided baseline information on age, marital status, height, weight, ancestry, medications, smoking history, medical history, physical activity, and diet. Information on exposure and medical

history is updated every 2 years. Blood samples were collected from 18,225 of the HPFS participants between 1993 and 1995; 168 incident cases of colorectal cancer were identified between the date of blood draw and January 2002.

On each biennial follow-up questionnaire, participants were asked whether they had had a diagnosis of colorectal cancer during the prior 2 years. When a participant reported a diagnosis of colorectal cancer, we asked for permission to obtain hospital records and pathology reports. For persistent nonresponders, we searched for National Death Index to identify potential colorectal cancer-related deaths. We identified more than 96% of incident colorectal cancers by these methods. Study physicians, blinded to exposure data, reviewed all medical records related to colorectal cancer, classifying disease stage according to the TNM (tumor-node-metastasis) classification.

In each cohort, individuals who were alive and free of diagnosed cancer at the time of case ascertainment were selected as controls and were matched to cases on year of birth and year and month of blood draw, as previously described [26, 27].

Previous studies that were based on the NHS and HPFS have described baseline characteristics of cohort participants and incident colorectal cancer cases and confirmed that our colorectal cancer cases were representative as a population-based sample [28]. We collected paraffin-embedded tissue blocks from hospitals where cohort participants with colorectal cancers had undergone resections of primary tumors. Specimens were selected based on availability of germline SNP analysis data and tumor analysis data at the time of this study. Table 1 indicates that there was no substantial bias in terms of clinical and pathologic features. Tumor content was more than 70% for all cases. All cases were confirmed by a single pathologist (S.O.) to be colorectal cancer, but not stromal tumor, carcinoid, lymphoma, or metastatic tumor from another organ site. Based on availability of tumor tissue specimens, a total of 182 colorectal cancer cases (100 from the HPFS and 82 from the NHS) were included. Overall, these cases were similar in epidemiologic features to the total number of samples in the NHS and HPFS with blood samples and similar in pathologic features to the total number of cases with CIMP and LINE-1 data. Cases were previously characterized for status of CIMP [29], LINE-1 methylation [12], and one-carbon metabolism germline SNPs [24, 25]. However, no study has been performed to correlate these 23 germline nonsynonymous SNPs in the one-carbon metabolism pathway with CIMP status and LINE-1 methylation in tumors. Blood collection, germline SNP analyses, and tumor tissue analyses were approved by the Institutional Review Boards of the Harvard School of Public Health, Dana-Farber Cancer Institute, and Brigham and Women's Hospital.

Genotyping methods

Genotyping was performed at the Dana-Farber/Harvard Cancer Center High-Throughput Polymorphism Core. DNA was extracted from 50-μl buffy coat fractions diluted with 150 μl of PBS by the Qiagen QIAamp Blood Kit (Qiagen, Chatsworth, CA) spin protocol. The genotypes of the one-carbon polymorphisms were determined by measuring end-point fluorescence using the 5′ nuclease assay (Taqman) on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) [30]. The 23 nonsynonymous SNPs were identified through the NCI SNP500Cancer database (http://snp500cancer.nci.nih.gov), the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP), and the International HapMap Project database (http://www.hapmap.org). The SNPs studied all resulted in amino acid changes and therefore are potentially functional. The *FOLH1 His475Tyr* SNP was not referenced in the above-mentioned databases but was investigated in relation to serum folate levels [31]. Our analysis included the 23 known nonsynonymous SNPs in 12 genes [24, 25] that could be evaluated by the Taqman assay (*SHMT* was

excluded from this analysis due to lower overlap of successfully genotyped blood samples with CIMP and LINE-1 data from somatic tissue).

Quality control was ensured by including a random 10% of the samples in the 96-well plates as duplicates. These served as internal controls to validate the genotyping methods; there was 100% concordance. Laboratory personnel were blinded to the status (case, control, or quality control) of samples. The median genotyping success for the 23 SNPs included in this analysis was 95%.

Quantitative real-time PCR (MethyLight) for CIMP analysis

Sodium bisulfite treatment on tumor DNA and subsequent real-time PCR (MethyLight) assays were validated and performed as previously described [32]. We quantified promoter methylation in eight CIMP-specific genes (*CACNA1G*, *CDKN2A* (*p16*), *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3*, and *SOCS1*) [15, 33]. The PCR condition was initial denaturation at 95°C for 10 min followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. CIMP-high was defined as ≥6/8 methylated promoters using the 8-marker CIMP panel, CIMP-low as 1–5 methylated promoters, and CIMP-0 as 0/8 methylated promoters, according to the previously established criteria [33]. Accumulating evidence indicate that CIMP-high is a distinct entity with tight associations with MSI and BRAF mutation. Although CIMP-low is associated with KRAS mutation [32, 34], the relatedness between CIMP-low and CIMP-0 is closer to that between CIMP-low and CIMP-high [32]. Thus, because of limited power, we combined CIMP-low and CIMP-0 in this study.

Pyrosequencing to measure LINE-1 methylation

In order to accurately quantify relatively high LINE-1 methylation levels, we utilized Pyrosequencing technology as previously described [35, 36]. The amount of C relative to the sum of the amounts of C and T at each CpG site was calculated as percentage. The average of the relative amounts of C in the four CpG sites was used as overall LINE-1 methylation level in a given tumor. LINE-1 methylation level measured by Pyrosequencing has been shown to correlate well with overall 5-methylcytosine level (i.e., global DNA methylation level) in tumor cells [9, 35].

Statistical analysis

We evaluated the relationship between each of the 23 SNPs in the one-carbon metabolism pathway and DNA methylation status (CIMP status or LINE-1 methylation) in colorectal cancer cases (case–case analysis). We used the codominant genetic model (when appropriate) as well as the dominant genetic model, comparing variant carriers with the referent homozygous wild type. The genotype distributions for the SNPs were evaluated for agreement with Hardy–Weinberg equilibrium (HWE) by the trend test. We used unconditional logistic regression for the analyses to compute odds ratios (ORs) with 95% confidence intervals (CIs). The risk for CIMP-high was evaluated using logistic regression with an ordinal outcome variable, modeled as a three-level categorical model: CIMP-high, CIMP-low, and CIMP-0. Due to sample size constraints, the results from the collapsed two-level categorical variable: CIMP-high and CIMP-low/0 are presented. The association between LINE-1 levels (a continuous variable) and the SNPs was tested by the Wilcoxon rank sum method. Multivariate unconditional logistic regression (for CIMP data) and multivariate linear regression (for LINE-1 data) analyses were adjusted for age, sex, family history of colon cancer, pack years smoked, body mass index (BMI), postmenopausal hormone use, aspirin intake, physical activity, alcohol intake, total folate consumption, and red meat consumption. *p* values were obtained from the tests for linear trend of log-ORs were calculated using an ordered categorical variable by assigning scores to the genotypes: 0 (no variant allele), one (carrying one variant allele), and two (carrying two variant alleles).

Similar results were obtained with two-degree of freedom likelihood ratio test (2-df LRT) comparing models with and without the genotype variable. All statistical tests were two-sided. In our previous study of one-carbon metabolism SNPs and colorectal adenoma, we evaluated linkage disequilibrium of these 23 SNPs. The conservative Bonferroni-corrected *p* value for the independent tests is alpha/20 markers = 0.003. All statistical analyses were performed with SAS (version 9.1; SAS Institute, Cary, NC).

Results

The clinical characteristics of the 182 colorectal cancer cases are presented in Table 1. CIMP-high (defined as the presence of ≥6/8 methylated promoters) was detected in 31 (17%) of the 182 colorectal cancers. LINE-1 methylation levels in the 172 colorectal cancers were distributed approximately normally (mean 61.81, standard deviation 10.11, median 62.49, interquartile range 12.38).

We compared the distribution of the genotypes (Table 2) in the CIMP-high to a reference group of CIMP-low/0 (with ≤5/8 methylated promoters). Modest differences in genotype distributions were noted for *TCN2 (transcobalamin 2) Pro259Arg* (dbSNP ID: rs1801198; *p* trend = 0.06). In multivariate logistic regression analysis, the *MTHFR (methylenetetrahydrofolate reductase) 429 Ala/Ala* variant was associated with an increased risk of CIMP-high (dbSNP ID: rs1801131; OR = 7.56; 95% CI, 1.32–43.3; *p* trend = 0.11) (Table 3). In contrast, the *MTHFR Ala222Val* (dbSNP ID: rs1801133) genotypes were not significantly associated with CIMP-high. In the B_{12}-related cycle of the one-carbon metabolism pathway, the *TCN2 259 Arg/Arg* variant (OR = 3.82, 95% CI: 1.02–14.4; *p* value = 0.06) was associated with CIMP-high. All of the other SNPs in the one-carbon metabolism pathway were not significantly associated with CIMP-high (Table 3). Similar point estimates were obtained comparing CIMP-high to CIMP-low (excluding CIMP-0) in the age-adjusted model (data not shown). *MTHFR 429 Ala/Ala* was significantly associated with CIMP-high (OR = 7.39; 95% CI, 1.2–45.1, *p* trend = 0.03). The association for *TCN2 259 Arg/Arg* was slightly attenuated with OR = 2.91 (95% CI, 0.85–9.92, *p* trend = 0.06). Only a few cases with CIMP-high had distal or rectal tumors located in the rectum; therefore, we were unable to perform stratified analysis by location. However, these associations were not statistically significant after correcting for multiple comparisons.

We next measured LINE-1 methylation, which has been correlated with global DNA methylation level [35]. There was no significant difference in overall median LINE-1 methylation levels and the 23 nonsynonymous SNPs examined (Table 4) or by stratified analysis by tumor location (colon compared to rectal; data not shown). However, there were some differences in mean LINE-1 methylation levels between certain genotypes (i.e., *FTHFD 254*).

Discussion

We conducted this study to examine the relationship between germline polymorphisms in the one-carbon metabolism genes and DNA methylation status (CpG island methylator phenotype (CIMP) and LINE-1 methylation level) in colorectal cancer. We used quantitative PCR assays (MethyLight) [37] to determine the degree of DNA methylation, which is essential to reproducibly differentiate high-level from low-level methylation [32]. With the use of the CIMP-specific panel of eight promoters [15, 33], we were able to accurately identify CIMP-high in colorectal cancer. We have found that the *MTHFR 429 Ala/Ala* variant and the *TCN2 259 Arg/Arg* variant were associated with CIMP-high status in colorectal cancer. Our data support the possible link between these germline genetic variants and somatic promoter CpG island methylation in colorectal cancer.

The combination of dietary factors and genetic and epigenetic variation contributes to colorectal cancer risk. Intake of folate, especially among alcohol consumers, is protective against the risk of colorectal cancer [20]. Diet, lifestyle, and environment [17, 38] contribute to carcinogenesis by inducing both genetic and epigenetic changes that in combination result in the disruption of key cellular process leading to neoplastic transformation. Folate from diet is directly linked to DNA methylation via the one-carbon metabolism pathway, where S-adenosylmethionine (SAM) [39] is the universal methyl donor for several biological methylation reactions and for de novo deoxynucleoside triphosphate synthesis. The reduced availability of methyltetrahydrofolate (methyl-THF), the main circulating form of folate, decreases the biosynthesis of SAM, thus limiting the availability of methyl groups for methylation reactions [40].

Thus, dietary folate consumption may modify the association between DNA methylation and colorectal cancer risk [23, 28, 41–44]. Examining epigenetic changes in colorectal cancer in relation to genetic and dietary factors is important because epigenetic heterogeneity that exists among colorectal cancers may be caused by genetic and environmental factors. However, few epidemiologic studies have investigated the role of epigenetic changes induced by dietary and environmental exposures [17]. In the one-carbon metabolism pathway, the *MTHFR Ala222Val (677C > T)* polymorphism (NM_005957.3) has been associated with decreased enzyme activity, folate status and global hypomethylation detected in lymphocytes [40]. The *MTHFR 429 (1298A > C)* polymorphisms is also associated with decreased enzymatic activity and hyperhomocysteinemia [45]. Intracellular folate levels (5,10-methylenetetrahydrofolate and tetrahydrofolate) have also been associated with promoter methylation of *MLH1, TIMP3, ARF (CDKN2A/p14)*, and the *MTHFR* SNPs [46]. However, a recent large population-based study did not find an association among folate, vitamin B_6, vitamin B_{12}, methionine, and CIMP-high [17]. Furthermore, studies have not found an association between the *MTHFR* haplotypes and promoter methylation status in proximal colon cancer [47] or colorectal adenoma [48]. Thus, other risk factors may be indirectly related to CIMP-high in colorectal cancer.

Analysis of genetic and epigenetic alterations, such as DNA hypermethylation and global hypomethylation is important in cancer research [1, 49–62]. We have previously described CIMP status and LINE-1 methylation in this large population-based sample of colorectal cancer [12, 29]. In this exploratory study, we evaluated the relationship of CIMP status and LINE-1 methylation with 23 nonsynonymous polymorphisms (SNPs) in 12 genes in the one-carbon pathway. Most of the SNPs in the one-carbon metabolism pathway were not associated with CIMP-high or LINE-1 methylation levels in colorectal cancer, although we may be underpowered to evaluate these associations given our sample size and due to multiple comparisons. We have shown an association between the *MTHFR 429 Ala/Ala* variant and CIMP-high in colorectal cancer, which was modestly stronger than the association observed in a larger population-based study of colon cancer evaluating SNPs in eight one-carbon metabolism genes [23]. In addition to sample size differences or chance, this difference in the magnitude of association may be in part due to differences in the CIMP markers and criteria in these two studies; Curtin et al. [23] used the classic CIMP marker panel (*MINT1, MINT2, MINT31, CDKN2A*, and *MLH1*), which is different from our 8-marker CIMP panel [15, 33]. Another recent large study suggests that the *MTHFR* 222 *Val/Val* variant may be associated with MSI cases of colorectal cancer [63]. Although CIMP status was not determined in this study, these results collectively support the relationship between the *MTHFR* SNPs and CIMP in colorectal cancer.

In the B_{12}-related cycle of the one-carbon metabolism pathway, the *TCN2 259 Arg/Arg* variant had an association with CIMP-high. However, Curtin et al. [23] reported a reduced

risk of colon cancer for the variant carriers of *TCN2*. In the Nurses' Health Study (NHS), the *TCN2 259 Pro/Arg + Arg/Arg* genotypes were associated with increased risk of colorectal adenoma [25] but not cancer [24]. Additional studies are necessary to examine the relation between the *TCN2 Pro259Arg* polymorphism and DNA methylation status in colorectal cancer. The relationship of germline genetic variation and somatic methylation is an emerging area of investigation [22, 23, 47, 48]. This exploratory population-based study provides evidence for the possible relationship between germline nonsynonymous SNPs in the one-carbon metabolism pathway and DNA methylation in tumors and suggests the need for further studies on the *MTHFR 429* polymorphism, the methionine-cycle and B_{12}-related genes, and the *FTHFD* gene. We were limited in this analysis by the overlap of genotype data available on incident colorectal cancer cases and methylation data from colorectal cancer tissue. In addition to dietary methyl status, other risk factors, such as smoking [17, 38], may be indirectly related to CIMP-high. The association of DNA methylation and lifestyle factors [57, 64] remains an important area of cancer research and the current findings should be evaluated in larger studies with prospective data on risk factors for cancer.

In summary, *MTHFR 429 Ala/Ala* and *TCN2* 259 *Arg/Arg* in the one-carbon metabolism pathway are associated with CIMP-high in colorectal cancer, although these findings may be due to chance. Further studies are necessary to elucidate the exact mechanisms of this association.

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Abbreviations

BHMT	Betaine–homocysteine methyltransferase
BMI	Body mass index
CI	Confidence interval
CIMP	CpG island methylator phenotype
HPFS	Health Professionals Follow-up Study
LINE-1	Long interspersed nucleotide element-1
MSI	Microsatellite instability
MTHFR	Methylenetetrahydrofolate reductase
MTRR	5-Methyltetrahydrofolate–homocysteine methyltransferase reductase (methionine synthase reductase)
NHS	Nurses' Health Study

nsSNP	Nonsynonymous single nucleotide polymorphism
OR	Odds ratio
SNP	Single nucleotide polymorphism
TCN2	Transcobalamin 2

References

1. Esteller M. Epigenetics in cancer. N Engl J Med. 2008; 358(11):1148–1159. [PubMed: 18337604]
2. Feinberg AP. Epigenetics at the epicenter of modern medicine. Jama. 2008; 299(11):1345–1350. [PubMed: 18349095]
3. Ji W, Hernandez R, Zhang XY, Qu GZ, Frady A, Varela M, et al. DNA demethylation and pericentromeric rearrangements of chromosome 1. Mutat Res. 1997; 379(1):33–41. [PubMed: 9330620]
4. Gaudet F, Hodgson JG, Eden A, Jackson-Grusby L, Dausman J, Gray JW, et al. Induction of tumors in mice by genomic hypomethylation. Science. 2003; 300(5618):489–492. [PubMed: 12702876]
5. Yamada Y, Jackson-Grusby L, Linhart H, Meissner A, Eden A, Lin H, et al. Opposing effects of DNA hypomethylation on intestinal and liver carcinogenesis. Proc Natl Acad Sci USA. 2005; 102(38):13580–13585. [PubMed: 16174748]
6. Rodriguez J, Frigola J, Vendrell E, Risques RA, Fraga MF, Morales C, et al. Chromosomal instability correlates with genome-wide DNA demethylation in human primary colorectal cancers. Cancer Res. 2006; 66(17):8462–9468. [PubMed: 16951157]
7. Suzuki K, Suzuki I, Leodolter A, Alonso S, Horiuchi S, Yamashita K, et al. Global DNA demethylation in gastrointestinal cancer is age dependent and precedes genomic damage. Cancer Cell. 2006; 9(3):199–207. [PubMed: 16530704]
8. Karpf AR, Matsui S. Genetic disruption of cytosine DNA methyltransferase enzymes induces chromosomal instability in human cancer cells. Cancer Res. 2005; 65(19):8635–8639. [PubMed: 16204030]
9. Estecio MR, Gharibyan V, Shen L, Ibrahim AE, Doshi K, He R, et al. LINE-1 hypomethylation in cancer is highly variable and inversely correlated with microsatellite instability. PLoS ONE. 2007; 2(5):e399. [PubMed: 17476321]
10. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. Proc Natl Acad Sci USA. 1999; 96(15):8681–8686. [PubMed: 10411935]
11. Shen L, Toyota M, Kondo Y, Lin E, Zhang L, Guo Y, et al. Integrated genetic and epigenetic analysis identifies three different subclasses of colon cancer. Proc Natl Acad Sci USA. 2007; 104(47):18654–18659. [PubMed: 18003927]
12. Ogino S, Kawasaki T, Nosho K, Ohnishi M, Suemoto Y, Kirkner GJ, et al. LINE-1 hypomethylation is inversely associated with microsatellite instability and CpG methylator phenotype (CIMP) in colorectal cancer. Int J Cancer. 2008; 122:2767–2773. [PubMed: 18366060]
13. Kambara T, Simms LA, Whitehall VL, Spring KJ, Wynter CV, Walsh MD, et al. BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. Gut. 2004; 53(8):1137–1144. [PubMed: 15247181]
14. Samowitz W, Albertsen H, Herrick J, Levin TR, Sweeney C, Murtaugh MA, et al. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. Gastroenterology. 2005; 129(3):837–845. [PubMed: 16143123]
15. Weisenberger DJ, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet. 2006; 38(7):787–793. [PubMed: 16804544]
16. Kawasaki T, Nosho K, Ohnishi M, Suemoto Y, Kirkner GJ, Fuchs CS, et al. IGFBP3 promoter methylation in colorectal cancer: relationship with microsatellite instability, CpG island methylator phenotype, and p53. Neoplasia. 2007; 9(12):1091–1098. [PubMed: 18084616]

17. Slattery ML, Curtin K, Sweeney C, Levin TR, Potter J, Wolff RK, et al. Diet and lifestyle factor associations with CpG island methylator phenotype and BRAF mutations in colon cancer. Int J Cancer. 2007; 120(3):656–663. [PubMed: 17096326]

18. Flood A, Caprario L, Chaterjee N, Lacey JV Jr, Schairer C, Schatzkin A. Folate, methionine, alcohol, and colorectal cancer in a prospective study of women in the United States. Cancer Causes Control. 2002; 13(6):551–561. [PubMed: 12195645]

19. Boyapati SM, Bostick RM, McGlynn KA, Fina MF, Roufail WM, Geisinger KR, et al. Folate intake, MTHFR C677T polymorphism, alcohol consumption, and risk for sporadic colorectal adenoma (United States). Cancer Causes Control. 2004; 15(5):493–501. [PubMed: 15286469]

20. Giovannucci E. Alcohol, one-carbon metabolism, and colorectal cancer: recent insights from molecular studies. J Nutr. 2004; 134(9):2475S–2481S. [PubMed: 15333745]

21. Chen J, Kyte C, Valcin M, Chan W, Wetmur JG, Selhub J, et al. Polymorphisms in the one-carbon metabolic pathway, plasma folate levels and colorectal cancer in a prospective study. Int J Cancer. 2004; 110(4):617–620. [PubMed: 15122597]

22. Murrell A, Heeson S, Cooper WN, Douglas E, Apostolidou S, Moore GE, et al. An association between variants in the IGF2 gene and Beckwith–Wiedemann syndrome: interaction between genotype and epigenotype. Hum Mol Genet. 2004; 13(2):247–255. [PubMed: 14645199]

23. Curtin K, Slattery ML, Ulrich CM, Bigler J, Levin TR, Wolff RK, et al. Genetic polymorphisms in one-carbon metabolism: associations with CpG island methylator phenotype (CIMP) in colon cancer and the modifying effects of diet. Carcinogenesis. 2007; 28(8):1672–1679. [PubMed: 17449906]

24. Koushik A, Kraft P, Fuchs CS, Hankinson SE, Willett WC, Giovannucci EL, et al. Nonsynonymous polymorphisms in genes in the one-carbon metabolism pathway and associations with colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2006; 15(12):2408–2417. [PubMed: 17164363]

25. Hazra A, Wu K, Kraft P, Fuchs CS, Giovannucci EL, Hunter DJ. Twenty-four non-synonymous polymorphisms in the one-carbon metabolic pathway and risk of colorectal adenoma in the Nurses' Health Study. Carcinogenesis. 2007; 28(7):1510–1519. [PubMed: 17389618]

26. Colditz GA, Hankinson SE. The Nurses' Health Study: lifestyle and health among women. Nat Rev Cancer. 2005; 5(5):388–396. [PubMed: 15864280]

27. Tranah GJ, Bugni J, Giovannucci E, Ma J, Fuchs C, Hines L, et al. O6-methylguanine-DNA methyltransferase Leu84Phe and Ile143Val polymorphisms and risk of colorectal cancer in the Nurses' Health Study and Physicians' Health Study (United States). Cancer Causes Control. 2006; 17(5):721–731. [PubMed: 16633920]

28. Tranah GJ, Giovannucci E, Ma J, Fuchs C, Hunter DJ. APC Asp1822Val and Gly2502Ser polymorphisms and risk of colorectal cancer and adenoma. Cancer Epidemiol Biomarkers Prev. 2005; 14(4):863–870. [PubMed: 15824157]

29. Ogino S, Cantor M, Kawasaki T, Brahmandam M, Kirkner G, Weisenberger DJ, et al. CpG island methylator phenotype (CIMP) of colorectal cancer is best characterised by quantitative DNA methylation analysis and prospective cohort studies. Gut. 2006; 55:1000–1006. [PubMed: 16407376]

30. Livak KJ. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal. 1999; 14(5–6):143–149. [PubMed: 10084106]

31. Devlin AM, Ling EH, Peerson JM, Fernando S, Clarke R, Smith AD, et al. Glutamate carboxypeptidase II: a polymorphism associated with lower levels of serum folate and hyperhomocysteinemia. Hum Mol Genet. 2000; 9(19):2837–2844. [PubMed: 11092759]

32. Ogino S, Kawasaki T, Brahmandam M, Cantor M, Kirkner GJ, Spiegelman D, et al. Precision and performance characteristics of bisulfite conversion and real-time PCR (MethyLight) for quantitative DNA methylation analysis. J Mol Diagn. 2006; 8(2):209–217. [PubMed: 16645207]

33. Ogino S, Kawasaki T, Kirkner GJ, Kraft P, Loda M, Fuchs CS. Evaluation of markers for CpG island methylator phenotype (CIMP) in colorectal cancer by a large population-based sample. J Mol Diagn. 2007; 9(3):305–314. [PubMed: 17591929]

34. Barault L, Charon-Barra C, Jooste V, de la Vega MF, Martin L, Roignot P, et al. Hypermethylator phenotype in sporadic colon cancer: study on a population-based series of 582 cases. Cancer Res. 2008; 68(20):8541–8546. [PubMed: 18922929]
35. Yang AS, Estecio MR, Doshi K, Kondo Y, Tajara EH, Issa JP. A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. Nucleic Acids Res. 2004; 32(3):e38. [PubMed: 14973332]
36. Ogino S, Kawasaki T, Nosho K, Ohnishi M, Suemoto Y, Kirkner GJ, et al. LINE-1 hypomethylation is inversely associated with microsatellite instability and CpG island methylator phenotype in colorectal cancer. Int J Cancer. 2008; 122(12):2767–2773. [PubMed: 18366060]
37. Eads CA, Danenberg KD, Kawakami K, Saltz LB, Blake C, Shibata D, et al. MethyLight: a high-throughput assay to measure DNA methylation. Nucleic Acids Res. 2000; 28(8):E32. [PubMed: 10734209]
38. Liu Y, Lan Q, Siegfried JM, Luketich JD, Keohavong P. Aberrant promoter methylation of p16 and MGMT genes in lung tumors from smoking and never-smoking lung cancer patients. Neoplasia. 2006; 8(1):46–51. [PubMed: 16533425]
39. Alonso-Aperte E, Gonzalez MP, Poo-Prieto R, Varela-Moreiras G. Folate status and S-adenosylmethionine/S-adenosylho-mocysteine ratio in colorectal adenocarcinoma in humans. Eur J Clin Nutr. 2008; 62(2):295–298. [PubMed: 17375119]
40. Friso S, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, et al. A common mutation in the 5, 10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci USA. 2002; 99(8):5606–5611. [PubMed: 11929966]
41. Giovannucci E, Stampfer MJ, Colditz GA, Rimm EB, Trichopoulos D, Rosner BA, et al. Folate, methionine, and alcohol intake and risk of colorectal adenoma. J Natl Cancer Inst. 1993; 85(11): 875–884. [PubMed: 8492316]
42. Sharp L, Little J, Brockton NT, Cotton SC, Masson LF, Haites NE, et al. Polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene, intakes of folate and related B vitamins and colorectal cancer: a case–control study in a population with relatively low folate intake. Br J Nutr. 2008; 99(2):379–389. [PubMed: 18053312]
43. Brockton NT. Localized depletion: the key to colorectal cancer risk mediated by MTHFR genotype and folate? Cancer Causes Control. 2006; 17(8):1005–1016. [PubMed: 16933051]
44. Brockton NT. Systemic folate status and risk of colorectal cancer. Cancer Causes Control. 2008; 19(9):1005–1007. (author reply 3). [PubMed: 18438717]
45. Weisberg IS, Jacques PF, Selhub J, Bostom AG, Chen Z, Curtis Ellison R, et al. The 1298A → C polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine. Atherosclerosis. 2001; 156(2):409–415. [PubMed: 11395038]
46. Kawakami K, Ruszkiewicz A, Bennett G, Moore J, Watanabe G, Iacopetta B. The folate pool in colorectal cancers is associated with DNA hypermethylation and with a polymorphism in methylenetetrahydrofolate reductase. Clin Cancer Res. 2003; 9(16 Pt 1):5860–5865. [PubMed: 14676107]
47. Oyama K, Kawakami K, Maeda K, Ishiguro K, Watanabe G. The association between methylenetetrahydrofolate reductase polymorphism and promoter methylation in proximal colon cancer. Anticancer Res. 2004; 24(2B):649–654. [PubMed: 15161007]
48. van den Donk M, van Engeland M, Pellis L, Witteman BJ, Kok FJ, Keijer J, et al. Dietary folate intake in combination with MTHFR C677T genotype and promoter methylation of tumor suppressor and DNA repair genes in sporadic colorectal adenomas. Cancer Epidemiol Biomarkers Prev. 2007; 16(2):327–333. [PubMed: 17301267]
49. Yu J, Yu J, Almal AA, Dhanasekaran SM, Ghosh D, Worzel WP, et al. Feature selection and molecular classification of cancer using genetic programming. Neoplasia. 2007; 9(4):292–303. [PubMed: 17460773]
50. Tsareva SA, Moriggl R, Corvinus FM, Wiederanders B, Schutz A, Kovacic B, et al. Signal transducer and activator of transcription 3 activation promotes invasive growth of colon carcinomas through matrix metalloproteinase induction. Neoplasia. 2007; 9(4):279–291. [PubMed: 17460772]

51. Ateeq B, Unterberger A, Szyf M, Rabbani SA. Pharmacological inhibition of DNA methylation induces proinvasive and prometastatic genes in vitro and in vivo. Neoplasia. 2008; 10(3):266–278. [PubMed: 18320071]
52. Nagasaka T, Koi M, Kloor M, Gebert J, Vilkin A, Nishida N, et al. Mutations in both KRAS and BRAF may contribute to the methylator phenotype in colon cancer. Gastroenterology. 2008; 134(7):1950–1960. [PubMed: 18435933]
53. Samowitz WS. Genetic and epigenetic changes in colon cancer. Exp Mol Pathol. 2008; 85(1):64–67. [PubMed: 18482722]
54. Sawan C, Vaissiere T, Murr R, Herceg Z. Epigenetic drivers and genetic passengers on the road to cancer. Mutat Res. 2008; 642(1–2):1–13. [PubMed: 18471836]
55. Thorstensen L, Lind GE, Lovig T, Diep CB, Meling GI, Rognum TO, et al. Genetic and epigenetic changes of components affecting the WNT pathway in colorectal carcinomas stratified by microsatellite instability. Neoplasia. 2005; 7(2):99–108. [PubMed: 15802015]
56. Rocken C, Neumann K, Carl-McGrath S, Lage H, Ebert MP, Dierkes J, et al. The gene polymorphism of the angiotensin I-converting enzyme correlates with tumor size and patient survival in colorectal cancer patients. Neoplasia. 2007; 9(9):716–722. [PubMed: 17898867]
57. Campos AC, Molognoni F, Melo FH, Galdieri LC, Carneiro CR, D'Almeida V, et al. Oxidative stress modulates DNA methylation during melanocyte anchorage blockade associated with malignant transformation. Neoplasia. 2007; 9(12):1111–1121. [PubMed: 18084618]
58. Blanco D, Vicent S, Fraga MF, Fernandez-Garcia I, Freire J, Lujambio A, et al. Molecular analysis of a multistep lung cancer model induced by chronic inflammation reveals epigenetic regulation of p16 and activation of the DNA damage response pathway. Neoplasia. 2007; 9(10):840–852. [PubMed: 17971904]
59. Kuester D, Dar AA, Moskaluk CC, Krueger S, Meyer F, Hartig R, et al. Early involvement of death-associated protein kinase promoter hypermethylation in the carcinogenesis of Barrett's esophageal adenocarcinoma and its association with clinical progression. Neoplasia. 2007; 9(3): 236–245. [PubMed: 17401463]
60. Litkouhi B, Kwong J, Lo CM, Smedley JG III, McClane BA, Aponte M, et al. Claudin-4 overexpression in epithelial ovarian cancer is associated with hypomethylation and is a potential target for modulation of tight junction barrier function using a C-terminal fragment of *Clostridium perfringens* enterotoxin. Neoplasia. 2007; 9(4):304–314. [PubMed: 17460774]
61. Lim U, Flood A, Choi SW, Albanes D, Cross AJ, Schatzkin A, et al. Genomic methylation of leukocyte DNA in relation to colorectal adenoma among asymptomatic women. Gastroenterology. 2008; 134(1):47–55. [PubMed: 18166347]
62. Bjornsson HT, Sigurdsson MI, Fallin MD, Irizarry RA, Aspelund T, Cui H, et al. Intra-individual change over time in DNA methylation with familial clustering. Jama. 2008; 299(24):2877–2883. [PubMed: 18577732]
63. Hubner RA, Lubbe S, Chandler I, Houlston RS. MTHFR C677T has differential influence on risk of MSI and MSS colorectal cancer. Hum Mol Genet. 2007; 16(9):1072–1077. [PubMed: 17350979]
64. Anacleto C, Leopoldino AM, Rossi B, Soares FA, Lopes A, Rocha JC, et al. Colorectal cancer "methylator phenotype": fact or artifact? Neoplasia. 2005; 7(4):331–335. [PubMed: 15967110]

Table 1

Clinical and molecular characteristics of 182 colorectal cancer cases

Clinical feature	Total	LINE-1	CIMP-high	CIMP-low/0
	N (%)[a]	Mean (SD)	*N* (%)[a]	*N* (%)[a]
Age				
≤59	33 (18%)	62.11 (12.32)	1 (3.23)	29 (19.21)
60–69	66 (36%)	60.97 (9.00)	12 (38.71)	53 (35.10)
≥70	83 (46%)	61.91 (10.44)	18 (58.06)	69 (45.70)
Sex				
Male	100 (55%)	61.98 (11.60)	12 (38.71)	88 (58.28)
Female	82 (45%)	61.60 (8.08)	19 (61.29)	63 (41.72)
Tumor location				
Proximal[b]	80 (44%)	62.52 (9.60)	29 (96.67)	97 (65.54)
Distal[b]	98 (54%)	61.19 (10.67)	1 (3.33)	51 (34.46)
Unknown	4 (2.2%)	–	–	–
Tumor stage				
I	40 (22%)	61.17 (10.37)	5 (17.24)	35 (27.29)
II	55 (30%)	64.15 (9.98)	16 (55.17)	39 (30.47)
III	45 (25%)	58.37 (9.62)	4 (13.79)	41 (32.03)
IV	17 (9.3%)	60.04 (11.46)	4 (13.79)	13 (10.16)
Unknown	25 (14%)	–	–	–

[a]The percentages indicate the proportion of cases with a specific clinical feature

[b]Proximal colon includes cecum to transverse colon, and distal colorectum includes splenic flexure to rectum

Table 2

Polymorphisms in the one-carbon metabolism pathway and CIMP[b] status in colorectal cancer

	Gene/SNP of interest	CIMP-high, ≥6 genes *n* = 31 *N* (%)	CIMP-low/0, 0–5 genes *n* = 151 *N* (%)	Percentage in controls from Koushik et al. [24]
rs2372536	*5-Aminoimidazole-4-carboxamide ribonucleotide formyltransferase (ATIC)*			
	Codon 116			
	Cys/Cys	14 (46.7)	58 (39.7)	43.5
	Cys/Ser	14 (46.7)	72 (49.3)	44.9
	Ser/Ser	2 (6.7)	16 (11.0)	11.5
	p value		0.39	
rs3733890	*Betaine–homocysteine methyltransferase (BHMT)*			
	Codon 239			
	Arg/Arg	13 (44.8)	59 (41.6)	53.4
	Arg/Gln	16 (55.2)	67 (47.2)	39.5
	Gln/Gln	0	16 (11.3)	7.1
	p value		0.26	
rs8111085	*DNA methyltransferase 1 (DNMT1)*			
	Codon 311			
	Ile/Ile	24 (82.8)	122 (90.4)	86.8
	Ile/Val	4 (13.8)	13 (9.6)	12.3
	Val/Val	1 (3.5)	0	0.8
	p value		0.11	
rs202676	*Folate hydrolase/glutamate carboxypeptidase (FOLH1)*			
	Codon 75			
	Tyr/Tyr	19 (65.5)	90 (62.5)	63.7
	Tyr/His	9 (31.0)	50 (34.7)	31.4
	His/His	1 (3.5)	4 (2.8)	4.8
	p value		0.83	
Devlin et al. [31]	*Folate hydrolase/glutamate carboxypeptidase (FOLH1)*			
	Codon 475			
	His/His	27 (96.4)	132 (93.0)	91.3
	His/Tyr	1 (3.6)	10 (7.0)	8.4
	Tyr/Tyr	0	0	0.3
	p value		0.50	
rs3796191	*Formyltetrahydrofolate dehydrogenase (FTHFD)*			
	Codon 254			
	Leu/Leu	30 (96.8)	134 (97.1)	92.0
	Leu/Pro	1 (3.2)	4 (2.9)	7.7
	Pro/Pro	0	0	0.3
	p value		0.92	

	Gene/SNP of interest	CIMP-high, ≥6 genes *n* = 31 *N* (%)	CIMP-low/0, 0–5 genes *n* = 151 *N* (%)	Percentage in controls from Koushik et al. [24]
rs1127717	*Formyltetrahydrofolate dehydrogenase (FTHFD)*			
	Codon 793			
	Asp/Asp	21 (72.4)	87 (60.8)	62.9
	Asp/Gly	7 (24.1)	45 (31.5)	33.2
	Gly/Gly	1 (3.5)	11 (7.7)	4.9
	p value		0.21	
rs4646750	*Formyltetrahydrofolate dehydrogenase (FTHFD)*			
	Codon 812			
	Ile/Ile	29 (93.5)	126 (87.5)	87.4
	Ile/Val	2 (6.5)	18 (12.5)	12.2
	Val/Val	0	0	0.3
	p value		0.34	
rs9984077	*Glycinamide ribonucleotide transformylase (GART)*			
	Codon 421			
	Ile/Ile	20 (64.5)	90 (62.5)	58.2
	Val/Ile	8 (25.8)	46 (31.9)	36.7
	Val/Val	3 (9.7)	8 (5.6)	5.1
	p value		0.86	
rs8971	*Glycinamide ribonucleotide transformylase (GART)*			
	Codon 752			
	Gly/Gly	23 (74.2)	85 (59.0)	57.2
	Asp/Gly	7 (22.6)	53 (36.8)	35.4
	Asp/Asp	1 (3.2)	6 (4.2)	7.5
	p value		0.15	
rs1950902	*Methylenetetrahydrofolate dehydrogenase (MTHFD)*			
	Codon 134			
	Lys/Lys	24 (80.0)	92 (67.2)	68.8
	Arg/Lys	6 (20.0)	39 (28.5)	28.7
	Arg/Arg	0	6 (4.4)	2.5
	p value		0.12	
rs2236225	*Methylenetetrahydrofolate dehydrogenase (MTHFD)*			
	Codon 653			
	Gln/Gln	6 (20.0)	23 (16.2)	24.1
	Gln/Arg	14 (46.7)	65 (45.8)	47.5
	Arg/Arg	10 (33.3)	54 (38.0)	28.5
	p value		0.55	
rs1801133	*Methylenetetrahydrofolate reductase (MTHFR)*			
	Codon 222[C]			
	Ala/Ala	16 (53.3)	58 (42.7)	44.7
	Ala/Val	12 (40.0)	61 (44.9)	41.2

	Gene/SNP of interest	CIMP-high, ≥6 genes *n* = 31	CIMP-low/0, 0–5 genes *n* = 151	Percentage in controls from Koushik et al. [24]
		N (%)	*N* (%)	
	Val/Val	2 (6.7)	17 (12.5)	14.1
	p value		0.22	
rs1801131	*Methylenetetrahydrofolate reductase (MTHFR)*			
	Codon 429[C]			
	Glu/Glu	11 (37.9)	65 (46.8)	44.4
	Glu/Ala	13 (44.8)	66 (47.5)	37.9
	Ala/Ala	5 (17.2)	8 (5.8)	17.7
	p value		0.11	
rs8923	*Methylenetetrahydrofolate synthase (MTHFS)*			
	Codon 202			
	Ala/Ala	25 (83.3)	119 (83.2)	84.2
	Ala/Thr	5 (16.7)	24 (16.8)	15.3
	Thr/Thr	0	0	0.4
	p value		0.97	
rs1805087	*Methionine synthase (MTR)*			
	Codon 919			
	Asp/Asp	23 (74.2)	82(57.3)	65.8
	Asp/Gly	7 (22.6)	52 (36.4)	29.7
	Gly/Gly	1 (3.2)	9 (6.3)	4.5
	p value		0.10	
rs1801394	*Methionine synthase reductase (MTRR)*			
	Codon 22			
	Ile/Ile	9 (31.0)	33 (23.4)	20.2
	Ile/Met	10 (34.5)	68 (48.2)	49.4
	Met/Met	10 (34.5)	40 (28.4)	30.4
	p value		0.92	
rs1532268	*Methionine synthase reductase (MTRR)*			
	Codon 175			
	Ser/Ser	11 (39.2)	61 (43.3)	40.6
	Ser/Leu	15 (53.6)	69 (48.9)	43.2
	Leu/Leu	2 (7.1)	11 (7.8)	16.1
	p value		0.80	
rs2303080	*Methionine synthase reductase (MTRR)*			
	Codon 284			
	Ser/Ser	29 (100)	132 (92.3)	95.4
	Ser/Thr	0	11 (7.7)	4.6
	Thr/Thr	0	0	0
	p value		0.12	
rs162036	*Methionine synthase reductase (MTRR)*			
	Codon 350			

	Gene/SNP of interest	CIMP-high, ≥6 genes *n* = 31	CIMP-low/0, 0–5 genes *n* = 151	Percentage in controls from Koushik et al. [24]
		***N* (%)**	***N* (%)**	
	Lys/Lys	22 (71.0)	103 (73.1)	79.6
	Lys/Arg	9 (29.0)	35 (24.8)	18.6
	Arg/Arg	0	3 (2.1)	1.7
	p value		0.99	
rs2287780	*Methionine synthase reductase (MTRR)*			
	Codon 415			
	Arg/Arg	100 (100.0)	134 (91.8)	95.6
	Arg/Cys	0	12 (8.2)	4.4
	Cys/Cys	0	0	0
	p value		0.10	
rs10380	*Methionine synthase reductase (MTRR)*			
	Codon 595			
	His/His	23 (79.3)	111 (77.6)	82.5
	His/Tyr	6 (20.7)	29 (20.3)	16.5
	Tyr/Tyr	0	3 (2.1)	1.0
	p value		0.70	
rs1801198	*Transcobalamin 2 (TCN2)*			
	Codon 259			
	Pro/Pro	9 (31.0)	54 (38.6)	31.3
	Pro/Arg	10 (34.5)	66 (47.1)	49.9
	Arg/Arg	10 (34.5)	20 (14.3)	19.5
	p value		0.06	

[a] All *p* values are from chi-square comparing CIMP-high to CIMP-low

[b] CIMP, CpG island methylator phenotype

[c] *MTHFR 222* corresponds to nucleotide position and change: *677 C →*

T MTHFR 429 corresponds to nucleotide position and change: *1298 A → C*

Table 3

Association of nonsynonymous SNPs in the one-carbon metabolic pathway with CIMP-high[c] in colorectal cancer

	Gene/SNP of interest	Logistic regression[a] OR (95% CI)	Multivariate logistic[b] OR (95% CI)
rs2372536	*ATIC Codon 116*		
	Cys/Cys	1.0	1.0
	Cys/Ser + Ser/Ser	0.76 (0.34–1.72)	0.86 (0.33–2.25)
rs3733890	*BHMT Codon 239*		
	Arg/Arg	1.0	1.0
	Arg/Gln + Gln/Gln	0.77 (0.33–1.75)	0.61 (0.22–1.72)
rs8111085	*DNMT Codon 311*		
	Ile/Ile	1.0	1.0
	Ile/Val + Val/Val	1.80 (0.57–5.67)	0.93 (0.20–4.35)
rs202676	*FOLH Codon 75*		
	Tyr/Tyr	1.0	1.0
	Tyr/His + His/His	0.71 (0.30–1.69)	0.49 (0.17–1.39)
Devlin et al. [31]	*FOLH Codon 475*		
	His/His	1.0	1.0
	His/Tyr + Tyr/Tyr	0.49 (0.06–4.11)	1.38 (0.13–15.4)
rs3796191	*FTHFD Codon 254*		
	Leu/Leu	1.0	1.0
	Leu/Pro + Pro/Pro	1.51 (0.15–15.6)	1.33 (0.09–20.4)
rs1127717	*FTHFD Codon 793*		
	Asp/Asp	1.0	1.0
	Asp/Gly + Gly/Gly	0.59 (0.24–1.46)	0.35 (0.11–1.11)
rs4646750	*FTHFD Codon 812*		
	Ile/Ile	1.0	1.0
	Ile/Val + Val/Val	0.49 (0.11–2.32)	0.55 (0.09–3.52)
rs9984077	*GART Codon 421*		
	Ile/Ile	1.0	1.0
	Ile/Val + Val/Val	0.53 (0.12–2.28)	0.69 (0.13–3.67)
rs8971	*GART Codon 752*		
	Gly/Gly	1.0	1.0
	Gly/Asp + Asp/Asp	1.92 (0.21–17.3)	1.11 (0.08–14.9)
rs1950902	*MTHFD Codon 134*		
	Lys/Lys	1.0	1.0
	Lys/Arg + Arg/Arg	0.60 (0.22–1.63)	0.58 (0.17–1.96)
rs2236225	*MTHFD Codon 653*		
	Gln/Gln	1.0	1.0
	Gln/Arg	1.08 (0.43–2.70)	1.05 (0.32–3.42)
	Arg/Arg	1.37 (0.43–4.36)	1.91 (0.44–8.37)

	Gene/SNP of interest	Logistic regression[a] OR (95% CI)	Multivariate logistic[b] OR (95% CI)
	Gln/Arg + Arg/Arg	1.15 (0.49–2.72)	1.24 (0.42–3.72)
rs1801133	*MTHFR Codon 222*[d]		
	Ala/Ala	1.0	1.0
	Ala/Val + Val/Val	0.64 (0.28–1.44)	0.56 (0.21–1.51)
rs1801131	*MTHFR Codon 429*[d]		
	Glu/Glu	1.0	1.0
	Glu/Ala	1.29 (0.52–3.20)	1.29 (0.44–3.81)
	Ala/Ala	3.60 (0.95–13.7)	7.56 (1.32–43.3)
	Glu/Ala + Ala/Ala	1.59 (0.68–3.72)	1.78 (0.65–4.84)
rs8923	*MTHFS Codon 202*		
	Ala/Ala	1.0	1.0
	Ala/Thr + Thr/Thr	0.90 (0.31–2.67)	0.65 (0.18–2.44)
rs1805087	*MTR Codon 919*		
	Asp/Asp	1.0	1.0
	Asp/Gly	0.53 (0.21–1.34)	0.47 (0.15–1.48)
	Gly/Gly	0.35 (0.04–3.05)	0.22 (0.01–3.71)
	Asp/Gly + Gly/Gly	0.50 (0.20–1.21)	0.43 (0.14–1.30)
rs1801394	*MTRR Codon 22*		
	Ile/Ile	1.0	1.0
	Ile/Met	0.53 (0.19–1.47)	0.33 (0.09–1.15)
	Met/Met	1.03 (0.36–2.92)	0.71 (0.20–2.54)
	Ile/Met + Met/Met	0.71 (0.29–1.73)	0.46 (0.15–1.39)
rs1532268	*MTRR Codon 175*		
	Ser/Ser	1.0	1.0
	Ser/Leu + Leu/Leu	1.28 (0.54–3.00)	0.90 (0.32–2.52)
rs2303080	*MTRR Codon 284*		
	Ser/Ser	1.0	1.0
	Ser/Thr + Thr/Thr	–	–
rs162036	*MTRR Codon 350*		
	Lys/Lys	1.0	1.0
	Lys/Arg + Arg/Arg	1.1 (0.46–2.66)	1.84 (0.59–5.72)
rs2287780	*MTRR Codon 415*		
	Arg/Arg	1.0	1.0
	Arg/Cys + Cys/Cys	–	–
rs10380	*MTRR Codon 595*		
	His/His	1.0	1.0
	His/Tyr + Tyr/Tyr	0.98 (0.36–2.69)	1.65 (0.47–5.77)
rs1801198	*TCN 2 Codon 259*		
	Pro/Pro	1.0	1.0
	Pro/Arg	0.91 (0.34–2.44)	0.82 (0.25–2.71)
	Arg/Arg	2.67 (0.93–7.70)	3.82 (1.02–14.4)

	Gene/SNP of interest	Logistic regression[a] OR (95% CI)	Multivariate logistic[b] OR (95% CI)
	Pro/Arg + Arg/Arg	1.35 (0.56–3.25)	1.40 (0.49–4.00)

[a] Logistic regression, comparing CIMP-high to the referent of CIMP-low/0, adjusted for age

[b] Odds ratios for multivariate logistic regression, comparing CIMP-high to the referent of CIMP-low/0, adjusted for: age, sex, family history of colon cancer, pack years smoked, body mass index (BMI), postmenopausal hormone (PMH) use, aspirin intake, physical activity, alcohol intake, total folate consumption, and red meat consumption

[c] CIMP, CpG island methylator phenotype

[d] *MTHFR 222* corresponds to nucleotide position and change: *677 C → T*

MTHFR 429 corresponds to nucleotide position and change: *1298 A → C*

Table 4

SNPs in the one-carbon metabolism pathway and LINE-1 levels in 172 colorectal cancer cases

	Genes/SNPs	Wild type *n* Mean ± std Median (95% CI)	Het ± Var *n* Mean ± std Median (95% CI)	*p* value[a] (Wilcoxon)
rs2372536	*ATIC Codon 116*	67 61.25 ± 11.46 61.85 (58.45–64.04)	99 61.78 ± 9.15 62.42(59.95–63.6)	0.88
rs3733890	*BHMT Codon 239*	69 60.88 ± 11.17 62.85 (58.19–63.56)	92 62.43 ± 9.51 62.43 (60.46–64.40)	0.73
rs8111085	*DNMT Codon 311*	138 61.74 ± 10.26 62.59 (60.02–63.47)	18 61.59 ± 11.79 60.62 (55.73–67.46)	0.94
rs202676	*FOLH Codon 75*	101 62.66 ± 9.76 62.85 (60.73–64.59)	62 60.67 ± 10.74 61.81 (57.95–63.40)	0.33
Devlin et al. [31]	*FOLH Codon 475*	151 61.62 ± 10.18 62.55 (59.99–63.26)	10 58.56 ± 9.93 59.31 (51.46–65.67)	0.22
rs3796191	*FTHFD Codon 254*	156 61.79 ± 10.25 62.41 (60.16–63.41)	5 54.90 ± 9.70 58.20 (42.86–66.94)	0.13
rs1127717	*FTHFD Codon 793*	101 62.47 ± 10.01 63.10 (60.49–64.44)	61 60.50 ± 9.57 61.05 (58.05–62.95)	0.10
rs4646750	*FTHFD Codon 812*	147 61.39 ± 10.13 62.30 (59.73–63.04)	19 64.70 ± 8.34 65.87 (60.67–68.71)	0.16
rs9984077	*GART Codon 421*	11 63.27 ± 10.43 56.26–70.28	154 61.56 ± 9.93 62.38 (59.98–63.15)	0.60
rs8971	*GART Codon 752*	7 66.11 ± 6.41 63.82 (60.18–72.04)	158 61.60 ± 10.36 62.41 (59.97–63.22)	0.27
rs1950902	*MTHFD Codon 134*	109 61.64 ± 10.12 62.40 (59.72–63.56)	48 62.73 ± 9.17 62.59 (60.06–65.39)	0.75
rs2236225	*MTHFD Codon 653*	59 61.23 ± 8.69 60.46 (58.96–63.49)	103 61.83 ± 10.53 63.07 (59.77–63.88)	0.34

	Genes/SNPs	Wild type *n* Mean ± std Median (95% CI)	Het ± Var *n* Mean ± std Median (95% CI)	*p* value[a] (Wilcoxon)
rs1801133	*MTHFR*[b] *Codon 222*	67 62.64 ± 9.94 63.35 (60.22–65.07)	89 61.43 ± 10.39 62.35 (59.24–63.62)	0.47
rs1801131	*MTHFR*[b] *Codon 429*	73 61.27 ± 10.05 62.35 (58.92–63.61)	85 62.75 ± 9.57 63.17 (60.69–64.81)	0.39
rs8923	*MTHFS Codon 202*	134 62.03 ± 10.40 62.72 (60.25–63.81)	29 60.62 ± 9.21 61.47 (57.12–64.13)	0.31
rs1805087	*MTR Codon 919*	99 61.85 ± 8.32 62.30 (60.19–63.51)	65 61.37 ± 11.99 62.55 (58.40–64.34)	0.83
rs1801394	*MTRR Codon 22*	39 61.89 ± 12.91 63.27 (57.71–66.08)	121 61.68 ± 9.53 62.40 (57.97–63.40)	0.55
rs1532268	*MTRR Codon 175*	68 61.73 ± 10.28 61.81 (59.24–64.22)	91 61.96 ± 9.94 (59.89–64.03)	0.58
rs2303080	*MTRR Codon 284*	151 61.62 ± 10.27 62.42 (59.97–63.27)	11 62.32 ± 9.34 62.22 (56.04–68.59)	0.90
rs162036	*MTRR Codon 350*	120 62.46 ± 9.86 62.70 (60.67–64.24)	43 60.86 ± 9.60 62.22 (57.91–63.82)	0.43
rs2287780	*MTRR Codon 415*	154 61.62 ± 10.34 62.29 (59.98–63.26)	12 62.31 ± 8.90 62.26 (56.66–67.97)	0.90
rs10380	*MTRR Codon 595*	127 62.36 ± 10.02 62.82 (60.50–64.02)	35 60.09 ± 9.64 61.85 (56.77–63.40)	0.26
rs1801198	*TCN2 Codon 259*	59 61.20 ± 10.47 60.77 (58.47–63.93)	100 61.67 ± 9.76 62.49 (59.73–63.60)	0.72

[a] No significant differences for any SNP comparing wild type and variant carriers (wild type determined according to Koushik et al. [24]) by Wilcoxon test and/or linear regression using the codominant genetic model (data not shown)

[b] *MTHFR 222* corresponds to nucleotide position and change: *677 C → T*

MTHFR 429 corresponds to nucleotide position and change: *1298 A → C*