

DNA Methyltransferase and Alcohol Dehydrogenase: Gene-Nutrient Interactions in Relation to Risk of Colorectal Polyps

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Abstract

Disturbances in DNA methylation are a characteristic of colorectal carcinogenesis. Folate-mediated one-carbon metabolism is essential for providing one-carbon groups for DNA methylation via DNA methyltransferases (DNMTs). Alcohol, a folate antagonist, could adversely affect one-carbon metabolism. In a case-control study of colorectal polyps, we evaluated three single nucleotide polymorphisms (–149C>T, –283T>C, –579G>T) in the promoter region of the *DNMT3b* gene, and a functional polymorphism in the coding region of the alcohol dehydrogenase *ADH1C* gene, *ADH1C* *2. Cases had a first diagnosis of colorectal adenomatous ($n = 530$) or hyperplastic ($n = 202$) polyps at the time of colonoscopy, whereas controls were polyp-free ($n = 649$). Multivariate logistic regression analysis was used to estimate odds ratios (OR) and corresponding 95%

confidence intervals (CI). There were no significant main associations between the *DNMT3b* or *ADH1C* polymorphisms and polyp risk. However, *DNMT3b* –149TT was associated with an increase in adenoma risk among individuals with low folate and methionine intake (OR, 2.00; 95% CI, 1.06–3.78, P interaction = 0.10). The *ADH1C* *2/*2 genotype was associated with a possibly elevated risk for adenomatous polyps among individuals who consumed >26 g of alcohol/d (OR, 1.95; 95% CI, 0.60–6.30), whereas individuals who were wild-type for *ADH1C* were not at increased risk of adenoma (P interaction = 0.01). These gene-diet interactions suggest that polymorphisms relevant to DNA methylation or alcohol metabolism may play a role in colorectal carcinogenesis in conjunction with a high-risk diet. (Cancer Epidemiol Biomarkers Prev 2008;17(2):330–8)

Introduction

Many studies have described associations between low folate intake and higher incidence of colorectal cancer or colorectal polyps (1, 2). Both epidemiologic and experimental studies have shown the importance of folate-mediated one-carbon metabolism in colorectal carcinogenesis (3). Folate functions as a donor of one-carbon units and has several essential functions, including DNA methylation, nucleotide synthesis, and DNA stability and repair (4–7). Other nutrients, such as methionine, are also important in DNA methylation (8). The folate and methionine cycles (see Fig. 1) are critical for providing methyl groups for DNA methylation (4). DNA methylation of CpG sites in the promoter regions (CpG islands) of genes can result in gene silencing. At the same time, “genomic DNA methylation,” which largely reflects methylation at repeat sequences and in satellite regions of the DNA, may be important in providing genomic stability (9).

DNA methyltransferases (DNMTs) catalyze the addition of a methyl group (CH₃) to the 5' position of a

cytosine base at CpG sites after DNA synthesis. Five active forms of DNMTs (DNMT1, DNMT2, DNMT3a, DNMT3b, and DNMT3l) have been characterized. DNMT1 is responsible for the maintenance of DNA methylation, whereas DNMT3a and DNMT3b are responsible for *de novo* DNA methylation (10–12). The biological role of DNMT2 in humans is currently unknown (13). DNMT3b is thought to be the most critical for carcinogenesis, and several polymorphisms in this gene have been described and investigated in relation to neoplastic outcomes (14–21).

The most extensively studied *DNMT3b* polymorphism is the –149C>T variant. This single nucleotide polymorphism has been associated with a decrease in breast cancer risk (21), an increased risk of prostate cancer (17) and lung cancer (15), and of developing hereditary nonpolyposis colorectal cancer earlier in life (19). The –149T allele has been associated with increased promoter activity (16) and two studies have shown decreased promoter methylation patterns among cancer patients with the T allele, although this may be considered contrary to the results of the promoter assay (18, 22).

Two other promoter polymorphisms have also been studied in relation to cancer risk. One, –283T>C is in high linkage disequilibrium with –149C>T. This polymorphism has been associated with decreased risk of lung cancer (14); in the same study, the T allele was associated with decreased promoter activity compared with the C allele. The third polymorphism, –579G>T was

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Gene-nutrient interactions have been repeatedly described for folate-mediated one-carbon metabolism, in which the level of colorectal cancer risk associated with dietary factors depends, in part, on an interaction between exposure and relevant genetic variants (43-46). We investigated three polymorphisms in the *DNMT3b* gene, -149C>T, -283T>C, -579G>T, and a polymorphism in the *ADH1C* gene, *ADH1C* *2 in relation to risk of colorectal adenomas, established precursors of colorectal cancer. In these analyses, we included relevant interactions with one-carbon nutrients such as folate and methionine as well as alcohol intake.

Materials and Methods

Study Subjects. Cases with colorectal adenomatous and/or hyperplastic polyps and polyp-free control subjects were recruited through a large multiclinic private gastroenterology practice in metropolitan Minneapolis. Detailed participant recruitment for this case-control study have been described previously (47). Briefly, patients ages 30 to 74 years, who were scheduled for a colonoscopy between April 1991 and April 1994 were recruited prior to colonoscopy to blind patients and recruiters to the final diagnosis. The study was approved by the internal review boards of the University of Minnesota and each endoscopy site. Written informed consent was obtained. For the present study, the Institutional Review Board of the Fred Hutchinson Cancer Research Center approved all associated activities.

Cases had a first diagnosis of colon or rectal adenomatous ($n = 530$) or hyperplastic polyps ($n = 202$) at the time of the colonoscopy. Control subjects were free

of polyps at colonoscopy ($n = 649$). Patients whose colonoscopy did not reach the cecum were ineligible; removed polyps were examined histologically using standard diagnostic criteria (48). The participation rate for all patients who underwent colonoscopies was 68%. Information on the use of nonsteroidal anti-inflammatory drugs, lifestyle factors, anthropometry, demographics, and medical information, including family history of cancer and polyps, was obtained by questionnaire. Dietary intakes over the year prior to polyp diagnosis or reference date was obtained using an adaptation of the Willett semiquantitative food frequency questionnaire, which has been studied previously for validity and repeatability within the Nurses' Health Study cohort (49), the Iowa Women's Health Study cohort (50), and the Health Professionals Follow-up Study cohort (51). In the Iowa Women's Health study, correlation coefficients of this instrument on repeat administration were $r = 0.62$ for dietary folate, $r = 0.67$ for vitamin B₁₂ intake, and $r = 0.99$ for alcohol consumption (50). Giovannucci et al. (52) compared food frequency questionnaire values with folate levels measured in red blood cells (an indicator of long-term folate status) and reported correlations of $r = 0.55$ for women and $r = 0.56$ for men.

Genotyping. The *DNMT3b* (-149C>T, -283T>C, and -579G>T) and *ADH1C* (*2) polymorphisms were detected by allelic discrimination using a 5' nuclease assay on a 7900HT sequence detection system (Applied Biosystems). The 5' nuclease genotyping assays were validated by genotyping 92 individuals using both 5' nuclease assay and restriction fragment length polymorphism or sequencing. There were no discrepancies between the two assays. The 20 μ L genotyping reactions

Table 1. PCR conditions

Polymorphism	PCR	Primers/Probes	(Mg ²⁺)	[Primers, Probes]	Amplicon	Cycling
DNMT3b -149C>T	FP	5'TGTTCACTTCCAGTTGTCCTGAA3'	3 mmol/L	200 nmol/L	118 bp	50°C, 2 min, 95°C, 10 min, 40× 95°C, 30 s; 62°C, 60 s
	RP	5'GGACACTCACTGGGCCTTAG3'		200 nmol/L		
	C allele	5'VIC-CAGACCC C AGGCCT-3'NFQ		100 nmol/L		
	T allele	5'6FAM-AGACCC T AGGCC- TCCA-3'NFQ		100 nmol/L		
DNMT3b -283T>C	FP	5' GTTCGGGTTGAAAGGAGCC3'	6 mmol/L	200 nmol/L	86 bp	50°C, 2 min, 95°C, 10 min, 45× 95°C, 15 s; 65°C, 60 s
	RP	5' GAAGCCCTAAGCGGGAGG3'		200 nmol/L		
	T allele	5'VIC-CAAAACCAG A CTCCT-3'NFQ		100 nmol/L		
	C allele	5'6FAM-CAAAACCAG G CTCCT-3'NFQ		100 nmol/L		
DNMT3b -579G>T	FP	5'CAAAGGCAAGTGACTTGAAAA3'	4 mmol/L	200 nmol/L	82 bp	50°C, 2 min, 95°C, 10 min, 45× 95°C, 15 s; 60°C, 60 s
	RP	5'CCAGGATTAGATAGAG- AACGAGTAAAAA3'		200 nmol/L		
	G allele	5'VIC-AAATCCC C GCTGAAA-3'NFQ		100 nmol/L		
	T allele	5'6FAM-TAAAT C CC A GCT- GAAAC-3'NFQ		100 nmol/L		
ADH1C 1045A>G	FP	5' CAATGATATTTTCTTC- TTTTACGGCTTT3'	5 mmol/L	200 nmol/L	109 bp	50°C, 2 min, 95°C, 10 min, 50× 95°C, 15 s; 60°C, 1 min
	RP	5'GCGAAGCAGGTCAAATCCTT3'		200 nmol/L		
	A allele	5'VIC-CATTAATAACAAAT- ATTTTACC3'-NFQ		150 nmol/L		
	G allele	5'6FAM-CATTAATAACAAATG- TTTTACCT-3'NFQ		100 nmol/L		

contained 1× TaqMan Core Reagents (Applied Biosystems), 0.5 units of AmpliTaq DNA polymerase, 0.2 units of AmpErase UNG, primers, probes, and 4 ng of genomic DNA. Primers, probes, Mg²⁺ concentrations, and cycling conditions are listed in Table 1. Positive controls for all the genotypes as well as two negative controls were included on each plate. For quality control purposes, genotyping for 94 randomly selected samples was repeated. There were no discrepancies. All the genotypes were in Hardy-Weinberg equilibrium.

Statistical Data Analysis. Logistic regression analysis was used to estimate odds ratios (OR) and corresponding 95% confidence intervals (CI) comparing cases (with adenomatous or hyperplastic polyps) to polyp-free controls in association with *DNMT3b* or *ADH1C* genotypes, adjusting for age, sex, body mass index, smoking (pack-years), hormone use (females only), and intakes of fiber, alcohol, total energy, riboflavin, vitamin B₁₂, vitamin B₆, folate, and methionine.

Pairwise linkage disequilibrium between the three *DNMT3b* single nucleotide polymorphisms was calculated using SAS Genetics, and haplotypes were inferred. A generalized estimating equations weighted logistic regression model was used to analyze the haplotype effects, using the haplotype probabilities as weights and clustering the haplotypes for each individual (53). The analyses were adjusted for age, sex, body mass index, smoking (pack-years), hormone use (females only), and intakes of fiber, alcohol, total energy, riboflavin, vitamin B₁₂, vitamin B₆, folate, and methionine.

Results

The characteristics of the study population and risk factors for colorectal polyps in this population have been described previously (47, 54-56) and are shown in Table 2. Those with adenomatous polyps were older than individuals with hyperplastic polyps or polyp-free controls and were more likely to be male. Postmenopausal hormone and nonsteroidal anti-inflammatory drug use have previously been associated with decreased polyp risk in this study population (47, 56).

We investigated three polymorphisms within the promoter region of *DNMT3b*: -149C>T, -283T>C, and -579G>T, and a polymorphism within the coding region of *ADH1C*, *ADH1C* *2. Genotype frequencies among case and control groups are given in Table 2. The pairwise *r*² values between -149C>T and -283T>C, -149C>T and -579G>T, and -283T>C and -579G>T were 0.76, 0.80, and 0.90, respectively.

DNMT3b. ORs associated with the *DNMT3b* variants are given in Table 3. There was no difference in adenoma or hyperplastic polyp risk associated with *DNMT3b* polymorphisms. Stratification by polyp size, age, or sex did not affect risk estimates. Haplotypes of the three *DNMT3b* polymorphisms (-149C>T, -283T>C, -579G>T) were inferred. The most common was the CTG haplotype (i.e., wild-type at all three loci; Table 2.) We observed no associations between polyp risk and *DNMT3b* haplotypes (Table 3).

We evaluated whether the *DNMT3b* associations with adenomas differed by intakes of one-carbon nutrients (Table 4). Folate and methionine are most relevant to the

Table 2. Main characteristics of the study population

Variables	Adenomatous polyps, n (%)	Hyperplastic polyps, n (%)	Controls, n (%)
Total N	530	202	649
Age (y)	58.0 ± 9.7	53.6 ± 9.9	52.9 ± 11.0
Sex			
Male	331 (62.2)	116 (57.4)	251 (38.6)
Female	201 (37.8)	86 (42.6)	399 (61.4)
Folate intake (μg)			
<268	170 (32.4)	62 (31.3)	213 (33.8)
268-440	190 (36.2)	72 (36.4)	203 (32.2)
>440	165 (31.4)	64 (32.3)	215 (34.1)
Methionine intake (g)			
<1.55	165 (31.4)	71 (35.9)	217 (34.4)
1.55-2.16	177 (33.7)	63 (31.8)	197 (31.2)
>2.17	183 (34.9)	64 (32.3)	217 (34.4)
Alcohol intake (g)*			
0	202 (38.6)	64 (32.3)	283 (44.9)
1-7	128 (24.5)	53 (26.8)	185 (29.4)
8-25	122 (23.3)	54 (27.3)	116 (18.4)
>26	71 (13.6)	27 (13.6)	46 (7.3)
<i>DNMT3b</i> -149C>T genotype			
C/C	167 (31.8)	53 (27.3)	216 (34.7)
C/T	250 (47.7)	106 (54.6)	300 (48.2)
T/T	107 (20.4)	35 (18.0)	106 (17.0)
<i>DNMT3b</i> -283T>C genotype			
T/T	186 (35.5)	61 (31.6)	235 (37.8)
T/C	243 (46.4)	105 (54.4)	291 (46.9)
C/C	95 (18.1)	27 (14.0)	95 (15.3)
<i>DNMT3b</i> -579G>T genotype			
G/G	190 (36.1)	60 (31.1)	239 (38.4)
G/T	237 (45.1)	102 (52.9)	288 (46.3)
T/T	98 (18.7)	31 (16.1)	95 (15.3)
<i>ADH1C</i> genotype			
*1/*1	177 (34.0)	74 (38.3)	220 (35.4)
*1/*2	251 (48.3)	86 (44.6)	299 (48.1)
*2/*2	92 (17.5)	33 (17.1)	103 (16.6)
<i>DNMT3b</i> haplotype [†] (-149C>T, -283T>C, -579G>T)			
CCG	1.2	0.7	1.2
CCT	0.2	0.3	0.6
CTG	53.8	52.7	56.6
CTT	0.5	0.8	0.4
TCG	0.1	0.1	0.1
TCT	39.8	40.3	36.9
TTG	3.8	4.1	3.7
TTT	0.6	1.0	0.6

NOTE: The pairwise *R*² for the three polymorphisms were 0.76 between -149C>T and -283T>C, 0.80 between -149C>T and -579G>T, and 0.90 between -283T>C and -579G>T.

*Alcohol intake was assessed in grams per day.

[†]Percentages rather than total N are reported for haplotypes because they are inferred rather than determined.

provision of methyl groups for *DNMT3b*. Individuals with variant *DNMT3b* genotypes and low folate and low methionine intake showed an increased risk of colorectal adenoma (low folate and methionine: folate <268 μg, methionine <1.66 g; medium folate and methionine: folate 268-400 μg, methionine 1.55-2.16 g; high folate and methionine: folate >440 μg, methionine >2.16 g). The -149C>T variant (homozygous variant) was associated with an increase in adenoma risk among individuals with low folate and methionine intake (OR, 2.00; 95% CI, 1.06-3.78); whereas there was no association with -149C>T genotype among those with medium or high intake (*P* interaction = 0.10).

ADH1C. ORs for the association between the *ADH1C* polymorphism and polyp risk are shown in Table 3. There was no statistically significant difference in

Table 3. Association between DNMT3b and ADH1C polymorphisms and risk of adenomatous and hyperplastic polyps

Polymorphism	Controls (n)	Adenomas		Hyperplastic polyps	
		Cases (n)	OR (95% CI)	Cases (n)	OR (95% CI)
<i>DNMT3b</i> genotypes					
-149C>T					
C/C (ref.)	216	167	1.00 (ref.)	53	1.00 (ref.)
C/T	300	250	0.93 (0.69-1.26)	106	1.24 (0.82-1.86)
T/T	106	107	1.28 (0.88-1.87)	35	1.33 (0.78-2.28)
-283T>C					
T/T (ref.)	235	186	1.00 (ref.)	61	1.00 (ref.)
T/C	291	243	0.93 (0.69-1.24)	105	1.34 (0.90-1.98)
C/C	95	95	1.21 (0.82-1.78)	27	1.12 (0.63-1.98)
-579G>T					
G/G (ref.)	239	190	1.00 (ref.)	60	1.00 (ref.)
G/T	288	237	0.92 (0.69-1.23)	102	1.33 (0.89-1.97)
T/T	95	98	1.27 (0.87-1.87)	31	1.38 (0.80-2.40)
<i>DNMT3b</i> haplotypes*					
CTG (ref.)	56.6	53.8	1.00 (ref.)	52.7	1.00 (ref.)
TCT	36.9	39.8	1.09 (0.93-1.27)	40.3	1.12 (0.92-1.37)
Rare	6.5	6.3	1.03 (0.84-1.24)	7.0	0.90 (0.68-1.17)
<i>ADH1C</i>					
1/1 (ref.)	220	177	1.00 (ref.)	74	1.00 (ref.)
1/2	299	251	1.15 (0.86-1.55)	86	0.88 (0.59-1.30)
2/2	103	92	1.23 (0.83-1.81)	33	1.04 (0.62-1.73)

NOTE: Multivariate adjustment for age, sex, smoking (pack-years), alcohol, hormone use (females only), body mass index, caloric intake, fiber intake, folate intake, methionine intake, vitamin B₁₂ intake, vitamin B₆ intake, and riboflavin intake.

*Percentages rather than total N are reported for haplotypes because they are inferred rather than determined.

adenoma or hyperplastic polyp risk associated with the *ADH1C* *2 genotype. However, after stratification by alcohol intake, we observed that individuals with the *ADH1C* *2/*2 genotype were at an increasing risk with increasing alcohol consumption (for >26 g alcohol per day, OR, 1.95; 95% CI, 0.60-6.30). This was a pattern for the *2 genotype, whereas those who were wild-type had no increase in risk as alcohol consumption increased. We tested for a difference of slopes (i.e., trend interaction) within each genotype group and found a significant

difference between both variant genotypes and the *1 genotype (Table 5, overall *P* interaction = 0.03; *P* interaction = 0.03 for *1/*2 versus *1/*1 and 0.02 for *2/*2 versus *1/*1).

Discussion

The results of this study indicate that polymorphisms in *DNMT3b* and *ADH1C* may not be associated with risk of colorectal adenoma, but that they may interact with

Table 4. DNMT3b genotype, combined folate, and methionine intake and risk of adenomatous polyps

<i>DNMT3b</i> Polymorphism	Combined folate and methionine intake*					
	Low			Medium or high		
	Controls (n)	Cases (n)	OR (95% CI)	Controls (n)	Cases (n)	OR (95% CI)
-149C>T						
C/C (ref.)	83	57	1.00 (ref.)	125	107	1.32 (0.78-2.21)
C/T	111	88	1.12 (0.68-1.83)	181	160	1.10 (0.67-1.81)
T/T	36	40	2.00 (1.06-3.78)	68	66	1.32 (0.75-2.34)
<i>P</i> interaction = 0.10						
-283T>C						
T/T (ref.)	90	67	1.00 (ref.)	138	116	1.14 (0.69-1.87)
T/C	108	86	1.02 (0.63-1.65)	174	155	0.99 (0.61-1.60)
C/C	32	32	1.47 (0.76-2.84)	61	62	1.25 (0.71-2.19)
<i>P</i> interaction = 0.47						
-579G>T						
G/G (ref.)	90	70	1.00 (ref.)	141	116	1.07 (0.65-1.74)
G/T	108	82	0.93 (0.58-1.50)	172	154	1.98 (0.61-1.58)
T/T	33	34	1.55 (0.82-2.96)	60	63	1.22 (0.70-2.14)
<i>P</i> interaction = 0.51						

NOTE: Multivariate adjustment for age, sex, body mass index, caloric intake, alcohol, dietary fiber intake, hormone use (females only), smoking (pack-years), vitamin B₁₂ intake, vitamin B₆ intake, and riboflavin intake.

*Tertiles were first created for folate and methionine separately using 1 for low (folate <268 μg, methionine <1.55 g), 2 for medium (folate 268-440 μg, methionine 1.55-2.16 g), and 3 for high (folate >440 μg, methionine >2.16 g). Folate and methionine tertile scores were added to create a combined folate and methionine variable with values ranging from 2 to 6. These combined scores were then divided as follows: combined folate and methionine intake was considered low if the score was 2 or 3, medium if the score was 4, and high if the score was 5 or 6.

Table 5. *ADH1C* genotype, alcohol intake, and risk of adenomatous polyps

<i>ADH1C</i> Polymorphism	Alcohol intake (g)											
	0			1-7			8-25			>26		
	Controls (n)	Cases (n)	OR (95% CI)	Controls (n)	Cases (n)	OR (95% CI)	Controls (n)	Cases (n)	OR (95% CI)	Controls (n)	Cases (n)	OR (95% CI)
1/1	92	84	1.00 (ref.)	68	36	0.62 (0.35-1.09)	41	30	0.76 (0.39-1.48)	16	24	1.09 (0.38-3.08)
1/2	130	86	0.75 (0.48-1.17)	83	65	1.00 (0.61-1.65)	51	56	1.12 (0.62-2.01)	22	43	2.25 (0.72-7.01)
2/2	48	25	0.61 (0.32-1.15)	29	25	1.15 (0.58-2.28)	14	21	1.71 (0.74-3.96)	10	19	1.95 (0.60-6.30)

P interaction = 0.03

NOTE: Multivariate adjustment for age, sex, body mass index, caloric intake, dietary fiber intake, hormone use (females only), smoking (pack-years), vitamin B₁₂ intake, vitamin B₆ intake, riboflavin intake, methionine intake, and folate intake.

nutrients in the one-carbon metabolism pathway to affect adenoma risk. Specifically, we found evidence that increasing alcohol intake may be more important among slow alcohol metabolizers. Additionally, we found that polymorphisms in *DNMT3b* may be associated with adenoma risk only in the context of low folate and methionine intake.

DNMT3b. Our results suggest that the -149C>T transition in *DNMT3b* was associated with an increased risk of adenomas among those with the -149TT genotype in combination with low folate and methionine intake. We found no association between the -283T>C polymorphism or the -579G>T polymorphism and polyp risk. The -149T allele has previously been associated with reduced levels of gene methylation in normal and tumor tissue compared with the C allele (18, 22); thus, the combination of low intake of one-carbon metabolism nutrients and the -149T allele may result in hypomethylation of genomic DNA, a state that has been observed for multiple cancer types (57-65). However, in another study, the -149T allele was associated with increased promoter activity (16), indicating that the function of the -149C>T polymorphism has not been fully elucidated. It may, like many other tumor processes, be dependent on stage of progression.

Overexpression of *DNMT3b* has been found in tumors (66), although evidence has been equivocal (67). Many tumor suppressor genes and other regulatory genes are hypermethylated in colorectal cancer (11, 68). Moreover, Toyota et al. showed that CpG island methylation and subsequent silencing of the *CHFR* gene depended on the activity of DNMT3b (69). Conversely, in another study, DNA hypermethylation of the CpG islands of human colorectal tumor-suppressor genes was not associated with overexpression of *DNMT3b* in 25 cases of colon cancer (70). Concurrent with promoter hypermethylation, genomic hypomethylation is common in cancers and suggests a second possible mechanism for associations with cancer risk. Hypomethylation in these non-promoter regions of DNA may cause genomic instability (62), although some studies do not support this mechanism (71).

Nutrition throughout life influences DNA methylation because the one-carbon metabolism, which is highly dependent on dietary methyl donors and cofactors,

provides methyl groups for all biological methylation reactions (8). Dietary methionine and choline provide a major source of one-carbon units, and folic acid, vitamin B₁₂, and pyridoxal phosphate are cofactors in one-carbon metabolism, thus nutritional deficiencies or surpluses could induce DNA hypomethylation or DNA hypermethylation, respectively. Studies have shown that nutritional supplementation during early development could increase methylation at specific genes and cause permanent changes in gene expression (72). Mouse studies have shown that nutritional supplementation, particularly folate and methionine supplementation in adults, can increase methylation at specific genes. In a prospective, randomized trial, subjects who received folate supplementation had increased genomic DNA methylation, and decreased p53 strand breaks compared with those receiving placebo (73). Others found that folate supplementation was associated with decreased DNA hypomethylation in rectal mucosa in patients with colonic adenomas (74). Additionally, diets deficient in folate have been shown to induce genomic DNA hypomethylation in animal models (26, 75) and in humans (76, 77).

Two recent studies have reported associations between polymorphisms in the *DNMT3b* gene and colon cancer risk. Hong et al. investigated the -579G>T polymorphism and observed a statistically significantly decreased risk of adenomas for the combined GT and GG genotypes in younger Korean patients (20). In another study, Jones et al. reported that hereditary nonpolyposis colorectal cancer patients carrying one or two copies of the *DNMT3b* -149C>T variant T allele developed colorectal cancer significantly earlier than those patients homozygous for the wild-type gene in a population of mismatch repair gene mutation carriers (19).

Several other cancer types have been associated with the -149C>T polymorphism. The TT or combination CT and TT genotypes have been associated with increased risk of lung cancer (15, 16) and prostate cancer (17), but with decreased risk of or no association with breast cancer (21, 78). However, none of the studies referenced above took one-carbon metabolism nutrients into account; which is a critical component of these studies given the existing evidence for gene-nutrient interactions in folate-mediated one-carbon metabolism.

ADH1C. We observed no overall association between the *ADH1C* *2 polymorphism and polyp risk. However, we observed a statistically significant interaction between this polymorphism and alcohol intake. The *2/*2 genotype was associated with increased risk of cancer among those with the highest alcohol intake. The *2 allele is associated with ~2.5-fold lower enzyme activity (36). We had hypothesized that the highest risk would be found among those with the highest alcohol intakes and who were wild-type for *ADH1C* because these people would have the highest levels of acetaldehyde, a toxic alcohol metabolite, in their systems. Our findings contradict this hypothesis, possibly indicating that there are other mechanisms by which alcohol may influence cancer risk.

Alcohol intake has been inconsistently associated with increased risk of colorectal cancer (79). Alcohol has several procarcinogenic effects, including the production of acetaldehyde during metabolism, the production of reactive oxygen species, and antagonism of folate and other one-carbon metabolism nutrients (36). The lack of consistently elevated risks between alcohol intake and colorectal cancer risk may be due to the failure to take genetic variability in genes related to alcohol metabolism, such as the alcohol dehydrogenases and the aldehyde dehydrogenases, into account.

Few studies have examined the potential associations between *ADH1C* genotype and risk of colorectal neoplasia in relation to alcohol and/or folate intake. To our knowledge, three studies, in addition to ours, have been done (31, 37, 39). Giovannucci et al. reported on 375 adenoma cases and 727 controls from the Health Professionals Follow-up Study that high intakes of alcohol (at least two drinks daily) are associated with an increased risk of colorectal adenomas, particularly among those with the *ADH1C* *2/*2 genotype. Additionally, they observed that the interaction between alcohol and *ADH1C* genotypes was stronger among those with low folate intake (37). However, in the same study population (211 cases of colorectal cancer and 1,104 controls), Chen et al. found that, although the association between colorectal cancer risk and alcohol consumption was stronger among individuals with the *ADH1C* *2/*2 genotype compared with other genotypes, the *ADH1C* polymorphism did not confer a significant risk of colorectal cancer independently (39). Conversely, in a study of 433 adenoma cases and 436 controls from the Netherlands, the risk of adenomas was highest among subjects who had the *ADH1C* *1/*1 genotype and were in the upper tertile of alcohol consumption. They described stronger associations between alcohol consumption and colorectal adenomas in carriers of the *ADH1C* *1/*1 genotype than those with other *ADH1C* genotypes (31).

Our results are consistent with the results of Giovannucci et al. Furthermore, their study and ours showed that alcohol was not appreciably related to the risk of colorectal adenoma among those with the *ADH1C* *1/*1 genotype, but the highest level of consumption was associated with a higher risk among individuals with the *ADH1C* *1/*2 genotype. However, in the study by Giovannucci et al., the majority of participants underwent sigmoidoscopy, rather than full colonoscopy (52), indicating that there could be undiagnosed colon polyps in the upper portions of the colon among controls. Similarly, the study by Tiemersma et al. also included

participants who had had either sigmoidoscopy or colon X-ray as a method for identifying polyps (18% of cases and 40% of controls; ref. 31). All participants in the present study underwent full colonoscopy; thus, reducing the chance that undiagnosed colon polyps were present among control participants and thus reducing misclassification and subsequent bias of ORs towards the null.

This study has several limitations. First, intakes of one-carbon metabolism nutrients and alcohol were collected via food frequency questionnaire and thus may not be accurate measures of true intakes. However, the food frequency questionnaire was administered prior to polyp diagnosis, and thus, any misclassification is likely to be nondifferential with respect to case status and the associations observed here would be underestimates of the true associations between one-carbon nutrients, alcohol, and polyp risk. Similarly, because alcohol consumption was generally low in this study population, we were limited in our ability to evaluate the combined effects of deficient one-carbon nutrient intake and high alcohol intake. The mechanism for increased polyp risk associated with *ADH1C* may be related to the effects of alcohol on folate absorption and metabolism; stronger interactions may be observed in populations with higher alcohol intake. Second, we limited our analyses to genetic variation in two genes related to methylation and alcohol metabolism, rather than considering the joint effects of several genes in these pathways. However, we chose genes and polymorphisms that are likely to be functionally relevant. Future studies of these pathways should consider multiple genes/variants together.

Additionally, we did not have access to polyp tissue in this study; thus, we were unable to directly measure aberrant methylation patterns, somatic mutations (such as *APC*), or microsatellite instability. Information on these molecular characteristics would help to more clearly delineate biological mechanisms of adenoma development.

In summary, we report here that intakes of one-carbon metabolism nutrients and alcohol may interact with genetic polymorphisms in genes related to DNA methylation and alcohol metabolism. These results add further to the data showing that folate-related gene-nutrient interactions play an important role in the risk of colorectal neoplasia.

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DNA Methyltransferase and Alcohol Dehydrogenase: Gene-Nutrient Interactions in Relation to Risk of Colorectal Polyps

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