Alcohol, one-carbon nutrient intake, and risk of colorectal cancer according to tumor methylation level of *IGF2* differentially methylated region[1–6]

Reiko Nishihara, Molin Wang, Zhi Rong Qian, Yoshifumi Baba, Mai Yamauchi, Kosuke Mima, Yasutaka Sukawa, Sun A Kim, Kentaro Inamura, Xuehong Zhang, Kana Wu, Edward L Giovannucci, Andrew T Chan, Charles S Fuchs, Shuji Ogino, and Eva S Schernhammer

ABSTRACT

Background: Although a higher consumption of alcohol, which is a methyl-group antagonist, was previously associated with colorectal cancer risk, mechanisms remain poorly understood.

Objective: We hypothesized that excess alcohol consumption might increase risk of colorectal carcinoma with hypomethylation of insulin-like growth factor 2 (*IGF2*) differentially methylated region-0 (DMR0), which was previously associated with a worse prognosis.

Design: With the use of a molecular pathologic epidemiology database in 2 prospective cohort studies, the Nurses' Health Study and Health Professionals Follow-up Study, we examined the association between alcohol intake and incident colorectal cancer according to the tumor methylation level of *IGF2* DMR0. Duplication-method Cox proportional cause-specific hazards regression for competing risk data were used to compute HRs and 95% CIs. In addition, we investigated intakes of vitamin B-6, vitamin B-12, methionine, and folate as exposures.

Results: During 3,206,985 person-years of follow-up, we identified 993 rectal and colon cancer cases with an available tumor DNA methylation status. Compared with no alcohol consumption, the consumption of ≥15 g alcohol/d was associated with elevated risk of colorectal cancer with lower levels of *IGF2* DMR0 methylation [within the first and second quartiles: HRs of 1.55 (95% CI: 1.08, 2.24) and 2.11 (95% CI: 1.44, 3.07), respectively]. By contrast, alcohol consumption was not associated with cancer with higher levels of *IGF2* DMR0 methylation. The association between alcohol and cancer risk differed significantly by *IGF2* DMR0 methylation level (P-heterogeneity = 0.006). The association of vitamin B-6, vitamin B-12, and folate intakes with cancer risk did not significantly differ according to *IGF2* DMR0 methylation level (P-heterogeneity > 0.2).

Conclusions: Higher alcohol consumption was associated with risk of colorectal cancer with *IGF2* DMR0 hypomethylation but not risk of cancer with high-level *IGF2* DMR0 methylation. The association between alcohol intake and colorectal cancer risk may differ by tumor epigenetic features. *Am J Clin Nutr* 2014;100:1479–88.

Keywords molecular pathological epidemiology, biomarker, epigenetics, imprinting, one carbon metabolism

INTRODUCTION

DNA methylation plays a critical role as an epigenetic mechanism in the control of gene expression. Loss of imprinting (LOI)[7] of the insulin-like growth factor 2 (*IGF2*) gene is a common epigenetic aberration in various human cancers including colorectal, lung, bladder, esophageal, and prostate cancers (1–6). LOI of *IGF2* was previously associated with increased risks of colorectal cancer (7) and adenoma (8) as well as

[1] From the Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA (RN, ZRQ, MY, KM, YS, SAK, KI, CSF, and SO); the Departments of Nutrition (RN, KW, and ELG), Epidemiology (MW, ELG, SO, and ESS), and Biostatistics (MW), Harvard School of Public Health, Boston, MA; the Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA (MW, XZ, KW, ELG, ATC, CSF, and ESS); the Department of Gastroenterological Surgery, Graduate School of Medical Science, Kumamoto University, Kumamoto, Japan (YB); the Division of Gastroenterology, Massachusetts General Hospital, Boston, MA (ATC); and Applied Cancer Research–Institution for Translational Research Vienna, Vienna, Austria (ESS).

[2] Supported by NIH grants (P01 CA87969 to SEH; P01 CA55075 and UM1 CA167552 to WCW; K07CA190673 to RN; P50 CA127003 to CSF; R01 CA137178 to ATC; and R01 CA151993 to SO), a grant from the Bennett Family Fund and the Entertainment Industry Foundation through the National Colorectal Cancer Research Alliance, and postdoctoral fellowships from the Asan Medical Center (to SAK), the Japan Society for Promotion of Sciences (to KI), the Takashi Tsuruo Memorial Fund (to KI), and the Uehara Memorial Foundation (to KM). ATC is a Damon Runyon Clinical Investigator.

[3] The content of this article is solely the responsibility of the authors and does not necessarily represent the official view of the NIH. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

[4] Supplemental Tables 1–3 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

[5] RN, MW, and ZRQ contributed equally to this work. SO and ESS contributed equally to this work.

[6] Address correspondence to R Nishihara, Department of Medical Oncology, Dana-Farber Cancer Institute, Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Building 2, Room 304, Boston, MA 02115. E-mail: rnishiha@hsph.harvard.edu; or S Ogino, Dana-Farber Cancer Institute, Brigham and Women's Hospital, Harvard Medical School, 440 Brookline Avenue, M422, Boston, MA 02215. E-mail: shuji_ogino@dfci.harvard.edu.

[7] Abbreviations used: DMR0, differentially methylated region-0; HPFS, Health Professionals Follow-up Study; *IGF2*, insulin-like growth factor 2; LOI, loss of imprinting; NHS, Nurses' Health Study.

Received July 10, 2014. Accepted for publication September 19, 2014.
First published online October 8, 2014; doi: 10.3945/ajcn.114.095539.

a poor prognosis of colorectal cancer (9). *IGF2* is located in chromosome 11p15 and expressed predominately from the paternal allele. The *IGF2* gene encodes the IGF2 protein, which has antiapoptotic and mitogenic functions and plays a role in cell proliferation (10, 11). DNA methylation is an important epigenetic mechanism that plays a major role in gene regulation and imprinting (12, 13). Hypomethylation at the differentially methylated region-0 (DMR0) has been related with LOI of *IGF2* in colorectal cancer (6, 7), which leads to *IGF2* upregulation. The measurement of *IGF2* DMR0 methylation is a well-established surrogate for *IGF2* LOI (8, 14). Moreover, the methylation level of *IGF2* DMR0 is associated with the prognosis in colorectal cancer patients, indicating the clinical usefulness of this marker (9).

Alcohol antagonizes one-carbon metabolism, which is essential for DNA methylation and nucleotide biosynthesis. Excessive alcohol consumption has previously been related to higher colorectal cancer risk (15, 16), whereas adequate intakes of one-carbon nutrients, including vitamin B-6, vitamin B-12, methionine, and folate, are associated with lower colorectal cancer risk (17, 18). With consideration of the importance of the epigenetic regulation of *IGF2* DMR0 and the potential impact of alcohol on aberrant DNA methylation, we hypothesized that higher alcohol consumption might be associated with higher risk of colorectal cancer with *IGF2* DMR0 hypomethylation.

To test this hypothesis, we assessed whether the association of alcohol consumption with colorectal cancer risk differed according to *IGF2* DMR0 methylation level in 2 prospective cohort studies in which alcohol intake has been positively associated with risk of colorectal cancer (15, 19, 20). In secondary analyses, we examined intakes of one-carbon nutrients including vitamin B-6, vitamin B-12, methionine, and folate as exposures.

SUBJECTS AND METHODS

Study population

The Nurses' Health Study (NHS) is a prospective study established in 1976, including 121,701 female nurses aged 30–55 y. The Health Professionals Follow-up Study (HPFS) is a prospective study initiated in 1986, enrolling 51,529 male dentists, optometrists, osteopaths, pharmacists, podiatrists, and veterinarians aged 40–75 y. In this analysis, the baseline year was the first year for which detailed diet information was available. We included participants who provided baseline information on dietary intake in 1980 in the NHS and 1986 in the HPFS. We excluded participants with a history of cancer (except for nonmelanoma skin cancer), inflammatory bowel disease, or familial polyposis at baseline. This study was approved by Human Subjects Committees at Harvard School of Public Health and Brigham and Women's Hospital.

Assessment of dietary intake and other covariates

Alcohol consumption and dietary intakes of vitamin B-6, vitamin B-12, folate, and methionine were assessed with a self-administered questionnaire by using semiquantitative food-frequency questionnaires beginning from the baseline year of this analysis (21). As described in previous studies of these cohorts, we used baseline information of alcohol and one-carbon nutrient intakes for this analysis to take into account the long induction period of colorectal tumor in relation to alcohol and one-carbon nutrient intakes (21, 22). We assumed an ethanol content of 13.1 g for a 12-oz (38-dL) can or bottle of beer, 11.0 g for a 4-oz (12-dL) glass of wine, and 14.0 g for a standard portion of spirits (21). We computed nutrient intake by multiplying the consumption frequency of each unit of food by the nutrient content of the specified portions by using composition values from USDA sources (15, 23). In our analyses, we included any nutrient intake including from supplements to calculate daily intake of each nutrient. This method of dietary assessment was extensively validated by 1-wk diet records conducted in both cohorts (22, 24, 25). We categorized alcohol consumption into 3 fixed categories (none, 1–14 g/d, and $\geq$15 g/d) and folate intake into 4 fixed categories ($<$200, 200–299, 300–399, and $\geq$400 μg/d). All other one-carbon nutrients were categorized into quintiles. Information on lifestyle factors, including weight, smoking status, endoscopy status, regular aspirin use, and postmenopausal hormone use (only for women), were assessed every 2 y from questionnaires in both cohorts.

Assessment of colorectal cancer cases

Incident colorectal cancer cases were ascertained by using a biennial questionnaire, the National Death Index, and a medical record review. Study physicians, who were unaware of the exposure information, reviewed medical and pathologic records to retrieve information on tumor location and disease stage. The International Classification of Diseases (Ninth Edition) codes for colon and rectal cancers are 153 and154, respectively. A total of 3031 colorectal cancer cases were identified through 1 July 2008. We collected available tumor specimens from pathology laboratories across the United States, and data on *IGF2* DMR0 methylation analysis were obtained in 993 cases. As described previously, baseline characteristics of participants with colorectal cancer with available tissue molecular data were similar to those of participants without available molecular data (26). A single pathologist (SO) reviewed tumor tissue slides, and recorded pathologic features.

Pyrosequencing of *IGF2* DMR0 methylation

We measured methylation at *IGF2* DMR0 by using a previously described bisulfite-pyrosequencing assay (GenBank nucleotides 631–859, accession no. Y13633) (9). We categorized *IGF2* DMR0 methylation levels into quartiles.

Statistical analysis

We followed participants from the date of return of the baseline questionnaire through 1 July 2008. Participants whose *IGF2* DMR0 methylation level in tumor was unknown (n = 2038) and those who died of causes other than colorectal cancer (n = 21,970) were censored during 28 y of follow-up. To examine differential associations of baseline alcohol consumption with colorectal cancer risk by *IGF2* DMR0 methylation level, we used Cox proportional cause-specific hazards regression models with a duplication method for competing risk data (27, 28), which is also called a joint Cox proportional (29). This method accommodates different baseline hazard functions of each disease subtype and permits the estimation of separate associations

of a risk factor (e.g., alcohol consumption) with each tumor subtype and has been used to assess whether a risk factor has statistically different regression coefficients for different tumor subtypes (21, 30, 31). In the incidence analysis of one subtype, incidences of other tumor subtypes or tumor of unknown subtype were treated as censored data. A trend test across exposure categories was performed by assigning the median value to each category and treating these variables as continuous terms in the model. With the use of a random effects meta-regression analysis (32), we assessed whether the magnitude of the exposure-subtype association had an increasing or decreasing ordinal trend across quartiles of tumor *IGF2* DMR0 methylation level, and the statistical significance of this trend was presented as *P*-heterogeneity. Cox model analyses were based on the counting process data structure (33) and were stratified by age (in mo), sex (in the combined cohort analysis), and calendar year of the questionnaire cycle. In multivariable Cox model analyses, we further adjusted for BMI, a family history of colorectal cancer in any first-degree relative, pack-years smoked, lower endoscopy status, regular aspirin use, postmenopausal hormone use (for women only), leisure-time physical activity, number of servings of red meat consumed per day, total caloric intake, calcium intake, current multivitamin use, and each of the other nutrients under evaluation (i.e., intakes of alcohol, vitamin B-6, vitamin B-12, folate, and methionine). With the exception of alcohol, vitamin B-6, vitamin B-12, folate, and methionine, for which we used baseline information, we used the most-updated available information for covariates before each 2-y follow-up period. We did not observe evidence of a violation of the proportional hazard assumption on the basis of interaction terms between alcohol consumption and follow-up time ($P > 0.5$).

We used SAS software (version 9.3; SAS Institute) for all statistical analyses. All *P* values were 2 sided. Because multiple hypothesis testing is inherent to subgroup analyses in molecular pathologic epidemiology (34), we set a heterogeneity test between colorectal cancer subtypes according to *IGF2* DMR0 methylation level in relation to alcohol consumption as our primary hypothesis testing in which a *P* value for significance was set as 0.05. In the primary analysis, median intake within each of alcohol intake categories was used and tested for a statistical trend. All other analyses, including the evaluation of individual HR estimates for alcohol, and analyses of other exposures were secondary analyses, and any positive finding was to be interpreted cautiously, given multiple hypothesis testing. No analysis in this study was planned when cohort studies began, and all analyses were post hoc by definition.

RESULTS

Alcohol consumption and colorectal cancer risk by *IGF2* DMR0 methylation level

At baseline, there were 87,805 women in the NHS and 45,770 men in the HPFS. **Table 1** shows baseline characteristics of all participants according to the amount of alcohol consumption. During 3,206,985 person-years of follow-up, we identified 993 colorectal cancer cases with available data for *IGF2* DMR0 methylation level. Before pooling data from the NHS and HPFS, we conducted heterogeneity tests based on the *Q* statistic. We did not observe significant heterogeneity between cohorts for the association of alcohol consumption with risk of any specific cancer subtypes ($P > 0.2$ for Cochran's *Q* test) (**Supplemental Table S1**). Thus, the NHS and HPFS were combined to increase the statistical power.

As previously described (15, 19, 20), compared with no alcohol consumption, higher alcohol consumption at baseline was associated with higher risk of overall colorectal cancer [multivariable-adjusted HR: 1.28 (95% CI: 1.05, 1.55) for consumption of ≥15 g alcohol/d; *P*-trend = 0.043 across alcohol intake categories] (**Table 2**). Higher alcohol consumption was significantly associated with higher risk of colorectal cancer with first and second quartiles of *IGF2* DMR0 methylation [comparing consumption of ≥15 g alcohol/d to no consumption; multivariable-adjusted HRs: 1.55 (95% CI: 1.08, 2.24; *P*-trend = 0.009) and 2.11 (95% CI: 1.44, 3.07; *P*-trend = 0.0004), respectively]. In contrast, alcohol consumption was not associated with risk of colorectal cancer with third and fourth quartiles of *IGF2* DMR0 methylation (*P*-trend ≥ 0.15; *P*-heterogeneity = 0.006 across *IGF2* DMR0 methylation quartiles).

In sensitivity analyses, we used covariates measured at baseline and examined the association between baseline alcohol consumption and colorectal cancer incidence. Compared with no alcohol consumption, multivariable-adjusted HRs in the ≥15 g alcohol/d category were 1.44 (95% CI: 1.00, 2.07; *P*-trend = 0.027) and 1.89 (95% CI: 1.29, 2.77; *P*-trend = 0.002) for cancer with first and second quartiles of *IGF2* DMR0 methylation, respectively, whereas multivariate HRs were 1.13 (95% CI: 0.79, 1.63); *P*-trend = 0.27; and 0.82 (95% CI: 0.55, 1.23); *P*-trend = 0.21; for cancer with third and fourth quartiles of *IGF2* DMR0 methylation, respectively (*P*-heterogeneity = 0.012). In addition, we used the most-updated information for all the variables including alcohol, one-carbon nutrients, and other covariates measured before each 2-y follow-up and modeled these variables as time-varying variables. In the sensitivity analysis, results were also consistent with those in our main analysis; the consumption of ≥15 g alcohol/d was significantly associated with cancer with first and second quartiles of *IGF2* DMR0 methylation [multivariable-adjusted HRs: 1.86 (95% CI: 1.25, 2.77; *P*-trend < 0.0001) and 2.06 (95% CI: 1.36, 3.13; *P*-trend = 0.0001), respectively], whereas higher alcohol consumption was not significantly associated with cancer with third and fourth quartiles of *IGF2* DMR0 methylation [multivariable-adjusted HRs: 0.99 (95% CI: 0.66, 1.49; *P*-trend = 0.63) and 0.95 (95% CI: 0.62, 1.45; *P*-trend = 0.72), respectively; *P*-heterogeneity = 0.0008).

One-carbon nutrients and colorectal cancer risk by *IGF2* DMR0 methylation level

In secondary analyses, we examined the relation of one-carbon nutrient intakes with colorectal cancer risk according to *IGF2* DMR0 methylation level. In **Supplemental Tables S2** and **S3**, we show sex-specific results for the analysis of vitamin B-6, vitamin B-12, methionine, and folate. In both cohorts combined, we did not observe prominent differential associations between one-carbon nutrient intakes and colorectal cancer incidence by *IGF2* DMR0 methylation status (**Table 3**). Although the test for heterogeneity was significant in our methionine analyses (*P*-heterogeneity = 0.007), none of the tests for trend across

TABLE 1
Age-adjusted baseline characteristics of participants (1980 in NHS and 1986 in HPFS) according to the amount of alcohol intake[1]

	Alcohol intake, g/d								
	Women (NHS)			Men (HPFS)			Pooled		
	0 (n = 28,234)	1–14 (n = 49,038)	≥15 (n = 10,533)	0 (n = 10,851)	1–14 (n = 23,562)	≥15 (n = 11,357)	0 (n = 39,085)	1–14 (n = 72,600)	≥15 (n = 21,890)
Age,[2] y	46.9 ± 7.3[3]	46.3 ± 7.2	47.6 ± 6.8	54.6 ± 9.9	53.7 ± 9.7	54.7 ± 9.5	49.0 ± 8.8	48.7 ± 8.8	51.3 ± 9.0
Folate intake, μg/d	365.7 ± 293.4	367.6 ± 269.2	354.1 ± 255.1	485.1 ± 292.0	486.2 ± 276.2	462.1 ± 258.5	401.3 ± 298.2	407.4 ± 277.3	403.2 ± 262.4
Vitamin B-6, mg/d	3.1 ± 11.9	2.9 ± 7.2	2.9 ± 7.7	9.2 ± 26.9	8.5 ± 24.6	8.2 ± 23.0	4.9 ± 18.0	4.8 ± 15.6	5.4 ± 17.0
Vitamin B-12, μg/d	9.4 ± 25.5	8.9 ± 17.7	8.0 ± 15.7	12.7 ± 18.5	12.8 ± 19.5	12.1 ± 15.7	10.4 ± 23.9	10.2 ± 18.5	9.9 ± 16.2
Methionine, mg/d	1.9 ± 0.5	1.9 ± 0.5	1.7 ± 0.4	2.2 ± 0.5	2.2 ± 0.5	2.1 ± 0.4	2.0 ± 0.5	2.0 ± 0.5	1.9 ± 0.4
Current multivitamin use, %	33	34	36	41	42	44	35	37	40
Red meat,[4] servings/d	0.4 ± 0.3	0.4 ± 0.3	0.4 ± 0.3	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3
Calcium intake, mg/d	752 ± 344	740 ± 302	644 ± 270	969 ± 477	909 ± 420	806 ± 364	817 ± 401	796 ± 355	718 ± 325
Total calories, kcal/d	1,564 ± 517	1,543 ± 490	1,683 ± 498	1,922 ± 630	1,940 ± 608	2,145 ± 609	1,667 ± 575	1,676 ± 564	1,899 ± 599
Physical activity, MET-h/wk	12.2 ± 18.5	14.8 ± 21.3	15.1 ± 19.7	18.6 ± 27.0	21.4 ± 29.2	22.4 ± 31.2	14.4 ± 22.0	17.4 ± 24.9	19.2 ± 26.9
BMI, kg/m^2	25.0 ± 4.8	23.8 ± 3.9	23.0 ± 3.3	25.7 ± 3.6	25.5 ± 3.2	25.4 ± 3.0	25.2 ± 4.5	24.4 ± 3.8	24.1 ± 3.4
Family history of CRC, %	8	8	8	8	8	8	8	8	8
Pack-years smoked	8.9 ± 15.1	11.4 ± 15.4	18.0 ± 18.7	10.1 ± 17.9	12.1 ± 17.6	18.2 ± 20.5	9.4 ± 16.2	11.7 ± 16.2	17.6 ± 19.1
Lower endoscopy status, %									
No endoscopy	89	90	90	73	69	70	85	83	81
Endoscopy	11	10	10	26	30	29	15	17	19
Regular aspirin use, %	33	36	39	27	29	33	31	33	36
Postmenopausal hormone use, %	42	43	46	—	—	—	—	—	—

[1]Values were standardized to the age distribution of the study population. Alcohol and one-carbon nutrient intakes were assessed at baseline, and the most-updated information was used for other covariates in our main analysis. CRC, colorectal cancer; HPFS, Health Professionals Follow-up Study; MET-h, metabolic equivalent task-hours; NHS, Nurses' Health Study.

[2]Value is not age adjusted.

[3]Mean ± SD (all such values).

[4]Beef, pork, or lamb.

TABLE 2
Baseline alcohol intake and risk of colorectal cancer according to *IGF2* DMR0 methylation level[1]

	Alcohol intake, g/d				
	0	1–14	≥15	*P*-trend[2]	*P*-heterogeneity[3]
Person-years	946,353	1,765,554	495,078	—	—
All colorectal cancers					—
Cases, *n*	258	523	212	—	
Age-adjusted HR (95% CI)	1 (referent)	1.10 (0.95, 1.28)	1.32 (1.10, 1.59)	0.006	
Multivariable-adjusted HR (95% CI)	1 (referent)	1.13 (0.97, 1.32)	1.28 (1.05, 1.55)	0.043	
IGF2 DMR0 methylation level					0.006
First quartile (≤25%)					
Cases, *n*	63	125	59	—	
Age-adjusted HR (95% CI)	1 (referent)	1.08 (0.80, 1.46)	1.60 (1.12, 2.29)	0.003	
Multivariable-adjusted HR (95% CI)	1 (referent)	1.11 (0.82, 1.50)	1.55 (1.08, 2.24)	0.009	
Second quartile (26–50%)					
Cases, *n*	51	138	63	—	
Age-adjusted HR (95% CI)	1 (referent)	1.49 (1.08, 2.06)	2.15 (1.48, 3.12)	0.0001	
Multivariable-adjusted HR (95% CI)	1 (referent)	1.55 (1.12, 2.14)	2.11 (1.44, 3.07)	0.0004	
Third quartile (51–75%)					
Cases, *n*	72	123	53	—	
Age-adjusted HR (95% CI)	1 (referent)	0.94 (0.70, 1.25)	1.27 (0.89, 1.82)	0.080	
Multivariable-adjusted HR (95% CI)	1 (referent)	0.96 (0.71, 1.28)	1.22 (0.85, 1.76)	0.15	
Fourth quartile (>75%)					
Cases, *n*	72	137	37	—	
Age-adjusted HR (95% CI)	1 (referent)	1.05 (0.79, 1.40)	0.86 (0.58, 1.29)	0.33	
Multivariable-adjusted HR (95% CI)	1 (referent)	1.08 (0.81, 1.44)	0.84 (0.56, 1.26)	0.24	

[1]Cox proportional cause-specific hazards regression for competing risk data were used to compute HRs and 95% CIs. All analyses were stratified by age (in mo), year of questionnaire return, and sex. Multivariable-adjusted HRs were further adjusted for BMI (in kg/m^2; <25 compared with 25–29.9 compared with ≥30), pack-years smoked (0 compared with 1–19 compared with 20–39 compared with ≥40 pack-years), family history of colorectal cancer in any first-degree relative, endoscopy status (no endoscopy compared with history of adenomatous polyps compared with negative endoscopy), physical activity level (quintiles of mean metabolic equivalent task-hours per week), red meat intake (quintiles of servings/d), total calorie intake (quintiles of kcal/d), calcium intake (quintiles of mg/d), current multivitamin use, regular aspirin use, and intakes of vitamin B-6, vitamin B-12, folate, and methionine. DMR0, differentially methylated region-0; *IGF2*, insulin-like growth factor 2.

[2]Linear trend test by using the median value of each category.

[3]Test for the heterogeneity of the association between alcohol intake and colorectal cancer risk according to *IGF2* DMR0 methylation level.

methionine quintiles were significant (*P*-trend > 0.09), and HRs did not consistently show a significant risk elevation with increasing levels of methionine intake.

In sensitivity analyses, in which we used covariates measured at baseline, tests for trend across quintiles of baseline intakes of vitamin B-6, vitamin B-12, and folate were not significant in any levels of *IGF2* DMR0 methylation (*P*-trend > 0.11). Tests for heterogeneity were also not significant in analyses of vitamin B-6, vitamin B-12, and folate (*P*-heterogeneity > 0.18). We observe lower risk of colorectal cancer with the fourth quintile of *IGF2* DMR0 methylation with increasing baseline intake of methionine (*P*-trend = 0.0008; *P*-heterogeneity = 0.010). When we used the most-updated information for all variables, all trend tests across quintiles of vitamin B-6, vitamin B-12, methionine, and folate were not significant (*P*-trend > 0.075).

DISCUSSION

In 2 large, prospective cohort studies, we showed that excess alcohol consumption was associated with higher risk of colorectal cancer with *IGF2* DMR0 hypomethylation and lower levels of *IGF2* DMR0 methylation but not risk of colorectal cancer with higher levels *IGF2* DMR0 methylation. The association of alcohol intake with colorectal cancer risk significantly differed according to tumor *IGF2* DMR0 methylation level. Within the *IGF2* DMR0 hypomethylated subtype, the elevation in risk appeared to follow a linear dose-response with increasing risks associated with increasing levels of alcohol intakes. Overall, our data support a possible mechanistic link between alcohol intake and colorectal cancer risk through *IGF2* DMR0 hypomethylation during colorectal carcinogenesis. In our secondary analysis, we did not show prominent differential associations of vitamin B-6, vitamin B-12, methionine, and folate intakes with risk of colorectal cancer according to *IGF2* DMR0 methylation level.

Tumor molecular analyses of colorectal cancer are increasingly important in clinical and epidemiologic research (35–38). Previous studies assessed the relation of alcohol and one-carbon nutrients with changes in various molecular features, including CpG island methylation and TP53 expression status, in colorectal cancer (39–42). A previous study also indicated that high alcohol consumption was associated with higher risk of colon cancer with hypomethylation in long interspersed nucleotide element-1, which is an indicator of global DNA methylation

TABLE 3
Baseline one-carbon nutrient intake and risk of colorectal cancer according to *IGF2* DMR0 methylation level[1]

	One-carbon nutrient intake						
	First quintile	Second quintile	Third quintile	Fourth quintile	Fifth quintile	*P*-trend[2]	*P*-heterogeneity[3]
Vitamin B-6, mg/d							
Person-years	651,952	645,266	641,446	637,991	630,330	—	—
All colorectal cancers							—
Cases, *n*	209	218	207	167	192	—	
Age-adjusted HR (95% CI)	1 (referent)	0.97 (0.80, 1.18)	0.84 (0.69, 1.02)	0.68 (0.55, 0.83)	0.79 (0.64, 0.96)	0.010	
Multivariable-adjusted HR (95% CI)	1 (referent)	1.04 (0.84, 1.27)	0.96 (0.76, 1.21)	0.83 (0.63, 1.10)	0.98 (0.74, 1.31)	0.75	
IGF2 DMR0 methylation level						—	0.23
First quartile (≤25%)							
Cases, *n*	50	52	53	46	46	—	
Age-adjusted HR (95% CI)	1 (referent)	0.95 (0.64, 1.41)	0.86 (0.58, 1.27)	0.75 (0.50, 1.12)	0.76 (0.51, 1.13)	0.70	
Multivariable-adjusted HR (95% CI)	1 (referent)	0.96 (0.64, 1.42)	0.89 (0.59, 1.34)	0.78 (0.50, 1.22)	0.82 (0.52, 1.30)	0.39	
Second quartile (26–50%)							
Cases, *n*	56	58	55	44	39	—	
Age-adjusted HR (95% CI)	1 (referent)	0.98 (0.68, 1.42)	0.84 (0.58, 1.23)	0.68 (0.46, 1.02)	0.59 (0.39, 0.89)	0.15	
Multivariable-adjusted HR (95% CI)	1 (referent)	0.99 (0.68, 1.44)	0.88 (0.59, 1.31)	0.72 (0.46, 1.12)	0.64 (0.40, 1.02)	0.87	
Third quartile (51–75%)							
Cases, *n*	53	61	47	46	41	—	
Age-adjusted HR (95% CI)	1 (referent)	1.09 (0.75, 1.57)	0.76 (0.51, 1.13)	0.76 (0.51, 1.13)	0.67 (0.44, 1.01)	0.074	
Multivariable-adjusted HR (95% CI)	1 (referent)	1.12 (0.77, 1.63)	0.80 (0.53, 1.21)	0.81 (0.52, 1.26)	0.74 (0.47, 1.18)	0.66	
Fourth quartile (>75%)							
Cases, *n*	50	47	52	31	66	—	
Age-adjusted HR (95% CI)	1 (referent)	0.87 (0.58, 1.30)	0.86 (0.58, 1.28)	0.53 (0.34, 0.83)	1.11 (0.77, 1.61)	0.12	
Multivariable-adjusted HR (95% CI)	1 (referent)	0.88 (0.58, 1.32)	0.90 (0.60, 1.36)	0.55 (0.34, 0.90)	1.21 (0.79, 1.86)	0.006	
Vitamin B-12 (μg/d)							
Person-years	780,801	547,678	574,728	661,972	641,806	—	—
All colorectal cancers							—
Cases, *n*	254	171	192	168	208	—	
Age-adjusted HR (95% CI)	1 (referent)	0.86 (0.71, 1.05)	0.91 (0.75, 1.10)	0.68 (0.56, 0.82)	0.86 (0.71, 1.03)	0.11	
Multivariable-adjusted HR (95% CI)	1 (referent)	0.89 (0.73, 1.09)	1.01 (0.82, 1.23)	0.78 (0.62, 0.97)	1.01 (0.79, 1.28)	0.81	
IGF2 DMR0 methylation level							0.63
First quartile (≤25%)							
Cases, *n*	67	44	47	35	54	—	
Age-adjusted HR (95% CI)	1 (referent)	0.85 (0.58, 1.24)	0.84 (0.58, 1.22)	0.53 (0.35, 0.80)	0.81 (0.57, 1.17)	0.62	
Multivariable-adjusted HR (95% CI)	1 (referent)	0.87 (0.59, 1.28)	0.93 (0.64, 1.36)	0.60 (0.39, 0.92)	0.94 (0.63, 1.39)	0.50	
Second quartile (26–50%)							
Cases, *n*	58	50	47	50	47	—	
Age-adjusted HR (95% CI)	1 (referent)	1.12 (0.76, 1.63)	0.98 (0.67, 1.45)	0.90 (0.62, 1.32)	0.86 (0.58, 1.27)	0.91	
Multivariable-adjusted HR (95% CI)	1 (referent)	1.16 (0.79, 1.70)	1.11 (0.75, 1.65)	1.02 (0.68, 1.52)	0.99 (0.65, 1.51)	0.30	
Third quartile (51–75%)							
Cases, *n*	64	43	47	43	51	—	
Age-adjusted HR (95% CI)	1 (referent)	0.90 (0.61, 1.32)	0.92 (0.63, 1.35)	0.71 (0.48, 1.05)	0.87 (0.60, 1.27)	0.82	
Multivariable-adjusted HR (95% CI)	1 (referent)	0.94 (0.64, 1.39)	1.03 (0.70, 1.51)	0.81 (0.54, 1.22)	1.02 (0.68, 1.53)	0.33	

(Continued)

TABLE 3 (*Continued*)

	One-carbon nutrient intake						
	First quintile	Second quintile	Third quintile	Fourth quintile	Fifth quintile	*P*-trend[2]	*P*-heterogeneity[3]
Fourth quartile (>75%)							
Cases, *n*	65	34	51	40	56	—	
Age-adjusted HR (95% CI)	1 (referent)	0.70 (0.46, 1.06)	0.97 (0.67, 1.41)	0.63 (0.42, 0.93)	0.90 (0.63, 1.29)	0.77	
Multivariable-adjusted HR (95% CI)	1 (referent)	0.71 (0.47, 1.09)	1.08 (0.75, 1.58)	0.71 (0.47, 1.07)	1.04 (0.70, 1.55)	0.16	
Methionine (g/d)							
Person-years	551,331	821,344	639,180	515,328	679,801	—	
All colorectal cancers							—
Cases, *n*	195	270	173	166	189	—	
Age-adjusted HR (95% CI)	1 (referent)	1.01 (0.83, 1.21)	0.77 (0.63, 0.95)	0.88 (0.72, 1.09)	0.75 (0.61, 0.92)	0.001	
Multivariable-adjusted HR (95% CI)	1 (referent)	1.03 (0.85, 1.24)	0.81 (0.65, 1.01)	0.95 (0.76, 1.19)	0.83 (0.66, 1.04)	0.064	
IGF2 DMR0 methylation level							0.006
First quartile (≤25%)							
Cases, *n*	47	55	42	43	60	—	
Age-adjusted HR (95% CI)	1 (referent)	0.80 (0.54, 1.19)	0.77 (0.51, 1.17)	0.93 (0.61, 1.41)	0.94 (0.64, 1.37)	0.20	
Multivariable-adjusted HR (95% CI)	1 (referent)	0.82 (0.55, 1.21)	0.80 (0.52, 1.22)	0.99 (0.65, 1.51)	1.05 (0.70, 1.55)	0.056	
Second quartile (26–50%)							
Cases, *n*	48	76	42	39	47	—	
Age-adjusted HR (95% CI)	1 (referent)	1.10 (0.76, 1.58)	0.77 (0.51, 1.16)	0.82 (0.54, 1.26)	0.73 (0.49, 1.09)	0.69	
Multivariable-adjusted HR (95% CI)	1 (referent)	1.12 (0.78, 1.62)	0.81 (0.53, 1.23)	0.88 (0.57, 1.36)	0.82 (0.54, 1.24)	0.74	
Third quartile (51–75%)							
Cases, *n*	45	79	39	39	46	—	
Age-adjusted HR (95% CI)	1 (referent)	1.27 (0.88, 1.84)	0.77 (0.50, 1.18)	0.91 (0.59, 1.40)	0.80 (0.53, 1.20)	0.20	
Multivariable-adjusted HR (95% CI)	1 (referent)	1.31 (0.90, 1.89)	0.81 (0.52, 1.25)	0.98 (0.63, 1.53)	0.89 (0.58, 1.36)	0.58	
Fourth quartile (>75%)							
Cases, *n*	55	60	50	45	36	—	
Age-adjusted HR (95% CI)	1 (referent)	0.74 (0.52, 1.08)	0.77 (0.52, 1.13)	0.84 (0.57, 1.25)	0.48 (0.32, 0.73)	0.009	
Multivariable-adjusted HR (95% CI)	1 (referent)	0.76 (0.53, 1.11)	0.81 (0.54, 1.19)	0.91 (0.61, 1.37)	0.54 (0.35, 0.84)	0.063	
Folate (μg/d)	<200	200–299	300–399	≥400	—	—	
Person-years	573,056	943,363	594,407	1,096,159	—	—	
All colorectal cancers							—
Cases, *n*	156	292	200	345	—	—	
Age-adjusted HR (95% CI)	1 (referent)	0.90 (0.74, 1.10)	0.80 (0.64, 1.00)	0.75 (0.61, 0.91)	—	0.004	
Multivariable-adjusted HR (95% CI)	1 (referent)	0.96 (0.78, 1.19)	0.93 (0.72, 1.20)	1.00 (0.76, 1.32)	—	0.69	
IGF2 DMR0 methylation level							0.68
First quartile (≤25%)							
Cases, *n*	31	76	46	94	—	—	
Age-adjusted HR (95% CI)	1 (referent)	1.21 (0.80, 1.85)	1.04 (0.65, 1.64)	1.14 (0.75, 1.72)	—	0.87	
Multivariable-adjusted HR (95% CI)	1 (referent)	1.32 (0.86, 2.02)	1.26 (0.78, 2.02)	1.69 (1.08, 2.64)	—	0.026	
Second quartile (26–50%)							
Cases, *n*	33	83	54	82	—	—	
Age-adjusted HR (95% CI)	1 (referent)	1.32 (0.88, 1.98)	1.21 (0.78, 1.87)	0.95 (0.63, 1.43)	—	0.17	
Multivariable-adjusted HR (95% CI)	1 (referent)	1.44 (0.95, 2.17)	1.47 (0.94, 2.32)	1.41 (0.90, 2.20)	—	0.40	

(Continued)

TABLE 3 (*Continued*)

	One-carbon nutrient intake						
	First quintile	Second quintile	Third quintile	Fourth quintile	Fifth quintile	*P*-trend[2]	*P*-heterogeneity[3]
Third quartile (51–75%)							
Cases, *n*	48	72	48	80	—	—	
Age-adjusted HR (95% CI)	1 (referent)	0.78 (0.54, 1.12)	0.72 (0.48, 1.08)	0.65 (0.45, 0.94)	—	0.047	
Multivariable-adjusted HR (95% CI)	1 (referent)	0.85 (0.59, 1.24)	0.89 (0.58, 1.36)	0.97 (0.65, 1.46)	—	0.77	
Fourth quartile (>75%)							
Cases, *n*	44	61	52	89	—	—	
Age-adjusted HR (95% CI)	1 (referent)	0.72 (0.49, 1.07)	0.89 (0.59, 1.34)	0.82 (0.57, 1.19)	—	0.77	
Multivariable-adjusted HR (95% CI)	1 (referent)	0.79 (0.53, 1.18)	1.09 (0.71, 1.66)	1.22 (0.81, 1.84)	—	0.08	

[1]Cox proportional cause-specific hazards regression for competing risk data were used to compute HRs and 95% CIs. All analyses were stratified by age (in mo), year of questionnaire return, and sex. Multivariable-adjusted HRs were further adjusted for BMI (in kg/m^2; <25 compared with 25–29.9 compared with ≥30), pack-years smoked (0 compared with 1–19 compared with 20–39 compared with ≥40 pack-years), family history of colorectal cancer in any first-degree relative, endoscopy status (no endoscopy compared with history of adenomatous polyps compared with negative endoscopy), physical activity level (quintiles of mean metabolic equivalent task-hours per week), red meat intake (quintiles of servings/d), total calorie intake (quintiles of kcal/d), calcium intake (quintiles of mg/d), current multivitamin use, regular aspirin use, and intakes of vitamin B-6, vitamin B-12, folate, and methionine. DMR0, differentially methylated region-0; *IGF2*, insulin-like growth factor 2.

[2]Linear trend test by using the median value of each category.

[3]The test for the heterogeneity of the association between one-carbon nutrient intake and colorectal cancer risk according to *IGF2* DMR0 methylation level.

(21). However, to our knowledge, no previous epidemiology study assessed the influence of alcohol and one-carbon nutrients on colorectal cancer risk according to tumor *IGF2* DMR0 methylation level. Alcohol has been implicated in colorectal cancer initiation possibly through the inhibition of one-carbon metabolism as well as the action of acetaldehyde (43). Excess alcohol has been reported to antagonize methyl donors including vitamin B-6, vitamin B-12, methionine, and folate, leading to a lower concentration of *S*-adenosylmethionine in the liver (43–45). In both human and animal studies, a reduction of *S*-adenosylmethionine concurrently increased *S*-adenosylhomocysteine and homocysteine concentrations in the plasma (46, 47), resulting in a lower methylation capacity and hypomethylation in various tissues including the colonic mucosa (48–50). The *IGF2* gene is maternally imprinted and expressed only from the paternal allele. *IGF2* controls cell development, growth, and proliferation, and LOI of *IGF2* has been implicated in colorectal cancer (6, 7) and various other cancers (51). Previous studies reported that *IGF2* expression is controlled by DMRs, which are close to the *IGF2* promoter (6, 7, 52–54). Particularly, the hypomethylation of *IGF2* DMR0 can be a surrogate marker of LOI of *IGF2* in colorectal cancer (8, 14). *IGF2* upregulation by DMR0 hypomethylation may promote tumorigenesis in colorectal tissue. Taken together, besides the reported global DNA hypomethylation, our findings suggest that excess alcohol consumption might cause DNA hypomethylation at *IGF2* DMR0, leading to the epigenetic dysregulation of *IGF2* activity and colorectal carcinogenesis. To our knowledge, our study provides new information about the role of excess alcohol consumption in transcriptional control through aberrant local DNA methylation changes.

Our study had several important strengths. First, because of the availability of detailed, updated information on several dietary and lifestyle covariates relevant to colorectal cancer over 28 y of follow-up, we were able to examine long-term exposures to alcohol and one-carbon nutrients and take into consideration important confounding factors. Second, because of the prospective nature of our study, differential recall bias, particularly with regard to our dietary assessments, was not of concern. Third, our molecular characterization of colorectal cancer enabled us to conduct molecular pathologic epidemiology research (34, 55), which could link the risk factor (alcohol) to a molecular signature of disease (*IGF2* DMR0 hypomethylation) and, hence, give us unique insights on pathogenic mechanisms and causal inference.

Limitations of note related to the relatively low alcohol consumption in our cohorts of health professionals. We also acknowledge that we could not completely exclude a possibility of residual and unmeasured confounding. In addition, we were unable to obtain tumor tissue from all cases of confirmed colorectal cancer in the 2 cohorts. Nonetheless, risk factors in cases unavailable for tissue analysis did not significantly differ from those in cases with tumor tissue available (31). We believe that the generalizability of our findings needs to be assessed by independent studies.

In conclusion, we showed that the association of higher alcohol consumption with colorectal cancer risk varies by tumor *IGF2* DMR0 methylation level and is stronger for tumor with *IGF2* DMR0 hypomethylation. Taken together with previous data, these results suggest that alcohol consumption may increase risk of a potentially more aggressive type of colorectal tumor

because of the poorer prognosis in colorectal cancer patients with *IGF2* DMR0 hypomethylation (9). Hypomethylation of *IGF2* DMR0 may be one mechanism by which alcohol consumption affects colorectal cancer risk. Additional studies are needed to further elucidate genetic and epigenetic alterations attributable to excess alcohol consumption.

We thank the NHS and HPFS for their valuable contributions as well as the following state cancer registries for their help: Alabama, Arizona, Arkansas, California, Colorado, Connecticut, Delaware, Florida, Georgia, Idaho, Illinois, Indiana, Iowa, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Nebraska, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, South Carolina, Tennessee, Texas, Virginia, Washington, and Wyoming. In addition, this study was approved by the Connecticut Department of Public Health Human Investigations Committee. Certain data used in this publication were obtained from the Department of Public Health.

The authors' responsibilities were as follows—CSF and SO: designed the research; RN, MW, SO, and ESS: analyzed data; RN, SO, and ESS: wrote the manuscript; RN and SO: had primary responsibility for the final content of the manuscript; and all authors: assumed full responsibility for analyses and interpretation of data, conducted the research, and read and approved the final manuscript. None of the authors had a conflict of interest.

REFERENCES

1. Murata A, Baba Y, Watanabe M, Shigaki H, Miyake K, Ishimoto T, Iwatsuki M, Iwagami S, Yoshida N, Oki E, et al. IGF2 DMR0 methylation, loss of imprinting, and patient prognosis in esophageal squamous cell carcinoma. Ann Surg Oncol 2014;21:1166–74.
2. Silva TD, Vidigal VM, Felipe AV, DE Lima JM, Neto RA, Saad SS, Forones NM. DNA methylation as an epigenetic biomarker in colorectal cancer. Oncol Lett 2013;6:1687–92.
3. Ribarska T, Goering W, Droop J, Bastian KM, Ingenwerth M, Schulz WA. Deregulation of an imprinted gene network in prostate cancer. Epigenetics 2014;9:704–17.
4. Kohda M, Hoshiya H, Katoh M, Tanaka I, Masuda R, Takemura T, Fujiwara M, Oshimura M. Frequent loss of imprinting of IGF2 and MEST in lung adenocarcinoma. Mol Carcinog 2001;31:184–91.
5. Byun HM, Wong HL, Birnstein EA, Wolff EM, Liang G, Yang AS. Examination of IGF2 and H19 loss of imprinting in bladder cancer. Cancer Res 2007;67:10753–8.
6. Cui H, Onyango P, Brandenburg S, Wu Y, Hsieh CL, Feinberg AP. Loss of imprinting in colorectal cancer linked to hypomethylation of H19 and IGF2. Cancer Res 2002;62:6442–6.
7. Cheng YW, Idrees K, Shattock R, Khan SA, Zeng Z, Brennan CW, Paty P, Barany F. Loss of imprinting and marked gene elevation are 2 forms of aberrant IGF2 expression in colorectal cancer. Int J Cancer 2010;127:568–77.
8. Cruz-Correa M, Cui H, Giardiello FM, Powe NR, Hylind L, Robinson A, Hutcheon DF, Kafonek DR, Brandenburg S, Wu Y, et al. Loss of imprinting of insulin growth factor II gene: a potential heritable biomarker for colon neoplasia predisposition. Gastroenterology 2004;126:964–70.
9. Baba Y, Nosho K, Shima K, Huttenhower C, Tanaka N, Hazra A, Giovannucci EL, Fuchs CS, Ogino S. Hypomethylation of the IGF2 DMR in colorectal tumors, detected by bisulfite pyrosequencing, is associated with poor prognosis. Gastroenterology 2010;139:1855–64.
10. Pan Y, He B, Lirong Z, Nie Z, Chen L, Gu L, Hoffman AR, Wang S, Hu J. Gene therapy for cancer through adenovirus vectormediated expression of the Ad5 early region gene 1A based on loss of IGF2 imprinting. Oncol Rep 2013;30:1814–22.
11. Nie ZL, Pan YQ, He BS, Gu L, Chen LP, Li R, Xu YQ, Gao TY, Song GQ, Hoffman AR, et al. Gene therapy for colorectal cancer by an oncolytic adenovirus that targets loss of the insulin-like growth factor 2 imprinting system. Mol Cancer 2012;11:86.
12. Smith ZD, Meissner A. DNA methylation: roles in mammalian development. Nat Rev Genet 2013;14:204–20.
13. Bardhan K, Liu K. Epigenetics and colorectal cancer pathogenesis. Cancers (Basel) 2013;5:676–713.
14. Ito Y, Koessler T, Ibrahim AE, Rai S, Vowler SL, Abu-Amero S, Silva AL, Maia AT, Huddleston JE, Uribe-Lewis S, et al. Somatically acquired hypomethylation of IGF2 in breast and colorectal cancer. Hum Mol Genet 2008;17:2633–43.
15. Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Willett WC. Alcohol, low-methionine–low-folate diets, and risk of colon cancer in men. J Natl Cancer Inst 1995;87:265–73.
16. Fedirko V, Tramacere I, Bagnardi V, Rota M, Scotti L, Islami F, Negri E, Straif K, Romieu I, La Vecchia C, et al. Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. Ann Oncol 2011;22:1958–72.
17. Kim YI. Folate, colorectal carcinogenesis, and DNA methylation: lessons from animal studies. Environ Mol Mutagen 2004;44:10–25.
18. Harnack L, Jacobs DR Jr, Nicodemus K, Lazovich D, Anderson K, Folsom AR. Relationship of folate, vitamin B-6, vitamin B-12, and methionine intake to incidence of colorectal cancers. Nutr Cancer 2002;43:152–8.
19. Giovannucci E, Stampfer MJ, Colditz GA, Hunter DJ, Fuchs C, Rosner BA, Speizer FE, Willett WC. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. Ann Intern Med 1998;129:517–24.
20. Nan H, Lee JE, Rimm EB, Fuchs CS, Giovannucci EL, Cho E. Prospective study of alcohol consumption and the risk of colorectal cancer before and after folic acid fortification in the United States. Ann Epidemiol 2013;23:558–63.
21. Schernhammer ES, Giovannucci E, Kawasaki T, Rosner B, Fuchs CS, Ogino S. Dietary folate, alcohol and B vitamins in relation to LINE-1 hypomethylation in colon cancer. Gut 2010;59:794–9.
22. Schernhammer ES, Giovannucci E, Baba Y, Fuchs CS, Ogino S. B vitamins, methionine and alcohol intake and risk of colon cancer in relation to BRAF mutation and CpG island methylator phenotype (CIMP). PLoS ONE 2011;6:e21102.
23. Giovannucci E, Stampfer MJ, Colditz GA, Rimm EB, Trichopoulos D, Rosner BA, Speizer FE, Willett WC. Folate, methionine, and alcohol intake and risk of colorectal adenoma. J Natl Cancer Inst 1993;85:875–84.
24. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
25. Willett WC, Sampson L, Browne ML, Stampfer MJ, Rosner B, Hennekens CH, Speizer FE. The use of a self-administered questionnaire to assess diet four years in the past. Am J Epidemiol 1988;127:188–99.
26. Nishihara R, Wu K, Lochhead P, Morikawa T, Liao X, Qian ZR, Inamura K, Kim SA, Kuchiba A, Yamauchi M, et al. Long-term colorectal-cancer incidence and mortality after lower endoscopy. N Engl J Med 2013;369:1095–105.
27. Lunn M, McNeil D. Applying Cox regression to competing risks. Biometrics 1995;51:524–32.
28. Lau B, Cole SR, Gange SJ. Competing risk regression models for epidemiologic data. Am J Epidemiol 2009;170:244–56.
29. Xue X, Kim MY, Gaudet MM, Park Y, Heo M, Hollenbeck AR, Strickler HD, Gunter MJ. A comparison of the polytomous logistic regression and joint cox proportional hazards models for evaluating multiple disease subtypes in prospective cohort studies. Cancer Epidemiol Biomarkers Prev 2013;22:275–85.
30. Ogino S, Nishihara R, Lochhead P, Imamura Y, Kuchiba A, Morikawa T, Yamauchi M, Liao X, Qian ZR, Sun R, et al. Prospective study of family history and colorectal cancer risk by tumor LINE-1 methylation level. J Natl Cancer Inst 2013;105:130–40.
31. Nishihara R, Lochhead P, Kuchiba A, Jung S, Yamauchi M, Liao X, Imamura Y, Qian ZR, Morikawa T, Wang M, et al. Aspirin use and risk of colorectal cancer according to BRAF mutation status. JAMA 2013;309:2563–71.
32. Stram DO. Meta-analysis of published data using a linear mixed-effects model. Biometrics 1996;52:536–44.
33. Therneau TM, Grambsch PM. Modeling survival data: extending the Cox model. New York: Springer; 2001.
34. Ogino S, Chan AT, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. Gut 2011;60:397–411.
35. Colussi D, Brandi G, Bazzoli F, Ricciardiello L. Molecular pathways involved in colorectal cancer: implications for disease behavior and prevention. Int J Mol Sci 2013;14:16365–85.
36. Bishehsari F, Mahdavinia M, Vacca M, Malekzadeh R, Mariani-Costantini R. Epidemiological transition of colorectal cancer in developing countries: environmental factors, molecular pathways, and opportunities for prevention. World J Gastroenterol 2014;20:6055–72.

37. Barrow TM, Michels KB. Epigenetic epidemiology of cancer. Biochem Biophys Res Commun 2014 Aug 11 (Epub ahead of print; DOI: 10.1016/j.bbrc.2014.08.002).
38. Suzuki H, Yamamoto E, Maruyama R, Niinuma T, Kai M. Biological significance of the CpG island methylator phenotype. Biochem Biophys Res Commun 2014 Jul 10 (Epub ahead of print; DOI: 10.1016/j.bbrc.2014.07.007).
39. Curtin K, Samowitz WS, Ulrich CM, Wolff RK, Herrick JS, Caan BJ, Slattery ML. Nutrients in folate-mediated, one-carbon metabolism and the risk of rectal tumors in men and women. Nutr Cancer 2011; 63:357–66.
40. Tillmans LS, Vierkant RA, Wang AH, Jewel Samadder N, Lynch CF, Anderson KE, French AJ, Haile RW, Harnack LJ, Potter JD, et al. Associations between cigarette smoking, hormone therapy, and folate intake with incident colorectal cancer by TP53 protein expression level in a population-based cohort of older women. Cancer Epidemiol Biomarkers Prev 2014;23:350–5.
41. Van Guelpen B, Dahlin AM, Hultdin J, Eklof V, Johansson I, Henriksson ML, Cullman I, Hallmans G, Palmqvist R. One-carbon metabolism and CpG island methylator phenotype status in incident colorectal cancer: a nested case-referent study. Cancer Causes Control 2010; 21:557–66.
42. Schernhammer ES, Ogino S, Fuchs CS. Folate and vitamin B6 intake and risk of colon cancer in relation to p53 expression. Gastroenterology 2008;135:770–80.
43. Varela-Rey M, Woodhoo A, Martinez-Chantar ML, Mato JM, Lu SC. Alcohol, DNA methylation, and cancer. Alcohol Res 2013;35:25–35.
44. Zakhari S. Alcohol metabolism and epigenetics changes. Alcohol Res 2013;35:6–16.
45. Halsted CH, Villanueva JA, Devlin AM, Niemela O, Parkkila S, Garrow TA, Wallock LM, Shigenaga MK, Melnyk S, James SJ. Folate deficiency disturbs hepatic methionine metabolism and promotes liver injury in the ethanol-fed micropig. Proc Natl Acad Sci USA 2002;99: 10072–7.
46. Cravo ML, Gloria LM, Selhub J, Nadeau MR, Camilo ME, Resende MP, Cardoso JN, Leitao CN, Mira FC. Hyperhomocysteinemia in chronic alcoholism: correlation with folate, vitamin B-12, and vitamin B-6 status. Am J Clin Nutr 1996;63:220–4.
47. Stickel F, Choi SW, Kim YI, Bagley PJ, Seitz HK, Russell RM, Selhub J, Mason JB. Effect of chronic alcohol consumption on total plasma homocysteine level in rats. Alcohol Clin Exp Res 2000;24:259–64.
48. Yi P, Melnyk S, Pogribna M, Pogribny IP, Hine RJ, James SJ. Increase in plasma homocysteine associated with parallel increases in plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation. J Biol Chem 2000;275:29318–23.
49. Duthie SJ. Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis. J Inherit Metab Dis 2011;34:101–9.
50. Choi SW, Stickel F, Baik HW, Kim YI, Seitz HK, Mason JB. Chronic alcohol consumption induces genomic but not p53-specific DNA hypomethylation in rat colon. J Nutr 1999;129:1945–50.
51. Ogawa O, Eccles MR, Szeto J, McNoe LA, Yun K, Maw MA, Smith PJ, Reeve AE. Relaxation of insulin-like growth factor II gene imprinting implicated in Wilms' tumour. Nature 1993;362:749–51.
52. Bell AC, Felsenfeld G. Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. Nature 2000;405: 482–5.
53. Hark AT, Schoenherr CJ, Katz DJ, Ingram RS, Levorse JM, Tilghman SM. CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. Nature 2000;405:486–9.
54. Cui H, Cruz-Correa M, Giardiello FM, Hutcheon DF, Kafonek DR, Brandenburg S, Wu Y, He X, Powe NR, Feinberg AP. Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. Science 2003; 299:1753–5.
55. Ogino S, Lochhead P, Chan AT, Nishihara R, Cho E, Wolpin BM, Meyerhardt JA, Meissner A, Schernhammer ES, Fuchs CS, et al. Molecular pathological epidemiology of epigenetics: emerging integrative science to analyze environment, host, and disease. Mod Pathol 2013;26:465–84.