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Alcohol Intake and Colorectal Cancer Risk by Molecularly-Defined Subtypes in a Prospective Study of Older Women

Anthony A. Razzak[1], **Amy S. Oxentenko**[1], **Robert A. Vierkant**[2], **Lori S. Tillmans**[3], **Alice H. Wang**[2], **Daniel J. Weisenberger**[4], **Peter W. Laird**[4], **Charles F. Lynch**[5], **Kristin E. Anderson**[6], **Amy J. French**[3], **Robert W. Haile**[7], **Lisa J. Harnack**[8], **Susan L. Slager**[2], **Thomas C. Smyrk**[3], **Stephen N. Thibodeau**[3], **James R. Cerhan**[9], and **Paul J. Limburg**[1]

[1]Department of Medicine, Mayo Clinic, Rochester, MN

[2]Division of Biomedical Statistics & Informatics, Mayo Clinic, Rochester, MN

[3]Department of Laboratory Medicine & Pathology, Mayo Clinic, Rochester, MN

[4]USC Epigenome Center, Los Angeles, CA

[5]Department of Epidemiology, University of Iowa, Iowa City, IA

[6]Department of Epidemiology, University of Minnesota, Minneapolis, MN

[7]Department of Preventive Medicine, USC Keck School of Medicine, Los Angeles, CA

[8]Masonic Cancer Center, University of Minnesota, Minneapolis, MN

[9]Division of Epidemiology, Mayo Clinic, Rochester, MN

Abstract

Increased alcohol consumption is a putative colorectal cancer (CRC) risk factor. However, existing data are less conclusive for women than men. Also, to date, relatively few studies have reported alcohol-related CRC risks based on molecularly-defined tumor subtypes. We evaluated associations between alcohol intake and incident CRC, overall and by microsatellite instability (MSI-H or MSI-L/MSS), CpG island methylator phenotype (CIMP-positive or CIMP-negative) and *BRAF* mutation (mutated or wild-type) status in the prospective, population-based Iowa Women's Health Study (IWHS; n = 41,836). Subjects were 55–69 years at baseline (1986) and exposure data were obtained by self-report. Incident CRCs were prospectively identified and archived, paraffin-embedded tissue specimens were collected from 732 representative cases, diagnosed through December 31, 2002. Multivariate Cox regression models were fit to estimate relative risks (RRs) and 95% confidence intervals (CIs). Among alcohol consumers, the median intake (range) was 3.4 (0.9–292.8) g/day. Compared to non-consumers, alcohol intake levels of ≤ 3.4 g/day (RR = 1.00; 95% CI = 0.86–1.15) and > 3.4 g/d (RR = 1.06; 95% CI = 0.91–1.24) were not significantly associated with overall CRC risk. Analyses based on alcohol intake levels of ≤ 30 g/d and > 30 g/d or quartile distributions yielded similar risk estimates. Null associations were also observed between each alcohol intake level and the MSI-, CIMP- or *BRAF*-defined CRC subtypes (p > 0.05 for each comparison). These data do not support an adverse effect from alcohol intake on CRC risk, overall or by specific molecularly-defined subtypes, among older women.

Corresponding Author: Paul J. Limburg, M.D., M.P.H.; Professor of Medicine, College of Medicine, Mayo Clinic; 200 First Street SW, Rochester, MN 55905. Tel: (507) 266-4338. Fax: (507) 266-0350. limburg.paul@mayo.edu..

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Keywords

Colorectal Cancer; Alcohol; Older Women; Cohort Study

Introduction

Worldwide, colorectal cancer (CRC) is the third most common malignancy, with over 1.2 million new cases reported each year (1). Environmental exposures are thought to play a functional role in colorectal carcinogenesis (2) and heightened awareness of potentially modifiable risk factors may serve to facilitate novel strategies to reduce the global health burden. Alcoholic beverages have been classified as "carcinogenic to humans" (group 1) by the International Agency for Cancer Research (3), with strong evidence for a potentially causal relationship between excess alcohol intake and seven specific cancer types, including CRC (4). According to a recent comprehensive report (5) from the World Health Organization and American Institute for Cancer Research (WHO/AICR), existing data suggest a dose-response influence on CRC risk, with alcohol intake > 30 g/d demonstrating a convincingly positive association for men, with greater uncertainty regarding alcohol intake-related CRC risks among women.

Several mechanisms have been proposed to account for the putatively pro-carcinogenic effects of excess alcohol consumption (2, 6, 7). Of particular interest is the biologically plausible relationship between increased alcohol intake and altered one-carbon metabolism, which could lead to growth promoting aberrancies in DNA methylation and/or other epigenetic modifications (8–13). Emerging data suggest that common environmental exposures, such as tobacco smoke, may be associated with molecularly distinct CRC subtypes defined by microsatellite instability (MSI), CpG island methylator phenotype (CIMP) and/or *BRAF* mutation status (14–18). However, inconsistent findings have been reported from previous case-control (19–23) and cohort studies (24–27) of associations between alcohol intake and MSI-, CIMP-, or *BRAF*-stratified CRC risks.

In the present study, we utilized data and tissue resources from the prospective, population-based Iowa Women's Health Study to evaluate alcohol intake as a potential risk factor for incident CRC, overall and with respect to subtypes characterized by microsatellite instability (MSI), CpG island methylator phenotype (CIMP), and *BRAF* mutation status, among older women. Since proximal and distal CRCs are known to exhibit different clinicopathologic features (28–30), we further analyzed associations between alcohol intake and CRC risk based on anatomic subsite (i.e., proximal colon and distal colorectum). These data update and extend a prior IWHS report of alcohol-associated CRC risks after 5 years of follow up (31) by including 12 years of additional follow-up time and the molecularly-defined, subtype-specific analyses.

Materials and Methods

Approvals for the present study were obtained from the Institutional Review Boards for Human Research at Mayo Clinic Rochester, the University of Minnesota and the University of Iowa.

Subjects

A detailed description of the methods used for IWHS subject recruitment and enrollment has been published elsewhere (32). In brief, a 16-page questionnaire was mailed out at baseline (January 1986) to 99,826 randomly selected women, ages 55–69 years, who resided in Iowa and held a valid driver's license. A total of 41,836 women (42%) returned the baseline questionnaire and these women comprise the full IWHS cohort. Bisgard, et al. previously reported that demographic characteristics and CRC rates were comparable between initial survey responders and non-responders (33). Vital status and state of residence were determined by mailed follow-up surveys in 1987, 1989, 1992, 1997 and 2004, as well as through linkage to Iowa death certificate records. Follow-up survey non-responders were cross-matched with the National Death Index to further identify decedents. Migration out of Iowa for IWHS subjects has been estimated at ~ 1% per year (34). For the present molecular epidemiology study, exclusion criteria consisted of (not mutually exclusive): follow up < 1 day (n=10) and history of malignancy other than non-melanoma skin cancer (n=3,830), leaving 38,001 subjects in the final analytic cohort.

Exposure Assessment

Self-reported exposure data were collected from IWHS subjects during the baseline evaluation. Dietary habits were assessed using a semiquantitative food frequency questionnaire adapted from the 126-item instrument developed by Willett and colleagues (35). Alcohol intake was ascertained by asking subjects to describe their average use during the past year for beer (1 glass, bottle or can), red wine (4 oz. glass), white wine (4 oz. glass) and liquor (1 drink or shot), with response levels of: never or less than once per month; 1–3 per month; 1 per week; 2–4 per week; 5–6 per week; 1 per day; 2–3 per day; 4–5 per day; 6+ per day. The reproducibility of alcohol intake estimated from the food frequency questionnaire and correlation with 24-hour dietary recall data in the IWHS cohort has been previously reported (36).

Case Ascertainment

Incident CRC cases were identified through annual linkage with the Iowa Cancer Registry, which participates in the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program (37). Each year, a computer-generated list of IWHS subjects was matched to the SEER registry data using combinations of first, last, and maiden names; zip code; birth date; and social security number. CRC cases were identified using International Classification for Diseases in Oncology (ICD-O) codes of 18.0, 18.2–18.9, 19.9, and 20.9. Proximal colon cancers were defined as tumors located in the cecum, ascending colon, hepatic flexure, transverse colon, and splenic flexure. Distal colorectal cancers were defined as tumors located in the descending colon, sigmoid colon, rectosigmoid junction, and rectum. Eleven CRCs did not have a subsite specified and were therefore excluded from the subsite analyses.

Tissue Collection and Processing

Archived, paraffin-embedded tissue specimens were recently requested from incident CRC cases diagnosed among IWHS subjects through December 31, 2002, with tissue specimens retrieved from 732/1,255 (58%) cases. CRC diagnoses were subsequently confirmed by an experienced gastrointestinal pathologist (TCS). Baseline demographics and general tumor characteristics (size and stage) for incident CRC cases with retrieved versus non-retrieved tissue specimens were not significantly different, as previously reported (18). Paraffin blocks were serially cut into 5- or 10-micron thick sections. One slide was stained with hematoxylin and eosin and areas of neoplastic (> 50%) and normal tissue were identified. Tumor and normal tissues were scraped from unstained slides and placed into separate tubes for DNA extraction using the QIAamp Tissue Kit (QIAgen, Valencia, California), according to the manufacturer's instructions. A total of 169 retrieved CRC cases were subsequently excluded from the present study due to inadequate/unusable tissue from the first primary CRC, or multiple primary CRCs at initial diagnosis leaving 563 incident CRC cases for the defined molecular analyses.

Microsatellite Instability

MSI testing was performed on paired tumor and normal DNA samples for each case subject, using 10 established markers: 4 mononucleotide repeats (BAT25, BAT26, BAT40, and BAT34C4), 5 dinucleotide repeats (ACTC, D5S346, D18S55, D17S250, and D10197), and 1 complex marker (MYCL) (38). MSI status was classified as microsatellite instability-high (MSI-H) if ≥ 30% of the markers demonstrated instability and microsatellite instability-low or microsatellite stable (MSI-L/MSS) if < 30% of the markers demonstrated instability (39, 40). MSI status was determined for 548/563 (97%) of the evaluable CRC cases.

CpG Island Methylation

Tumor DNA was treated with sodium bisulfite and subsequently analyzed using automated real-time PCR-based MethyLight to amplify methylated CpG sites in the promoter regions of an established 5-gene marker panel (*CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3*, and *SOCS1*) (41). CIMP status was reported as CIMP-positive if hypermethylation was observed in ≥ 3 markers or CIMP-negative if hypermethylation was observed in 0–2 markers. CIMP status was determined for 535/563 (95%) of the evaluable CRC cases.

BRAF Mutation

Tumor DNA was analyzed using fluorescent allele specific polymerase chain reaction to detect the V600E point mutation in exon 15 of the *BRAF* gene. BRAF-mutation and *BRAF*-wildtype cases were defined by the presence or absence of the V600E point mutation, respectively. *BRAF* mutation status was determined for 545/563 (97%) of the evaluable CRC cases.

Statistical Analyses

Data were descriptively summarized using frequencies and percents for categorical variables, and means and standard deviations for continuous variables. Among CRC cases, pair-wise associations between the various biomarker values were assessed using Pearson correlation coefficients, with negative and positive values for each marker coded as 0 and 1, respectively.

Follow-up for incident events was calculated as the time from completion of the baseline questionnaire until the age at first CRC diagnosis, date of move from Iowa, or date of death. If none of these events occurred, a woman was assumed to be alive, cancer-free, and living in Iowa through December 31, 2002. Cox proportional hazards regression analysis was used to estimate relative risks (RRs) and 95% confidence intervals (CIs) for associations between alcohol intake and incident CRC, overall and by anatomically- and molecularly-defined subsets. All eligible IWHS subjects were included in these Cox regression analyses, regardless of eventual cancer status. Incidence was modeled as a function of age (42). Baseline alcohol intake was analyzed with respect to quartile distribution and median split (3.4 g/d) among IWHS subjects who reported any consumption, and with respect to the proposed threshold value (5) for colorectal carcinogenicity (30 g/d). Alcohol non-consumers were defined as the reference group for all risk associations. Tests for trend were carried out by ordering the categorized alcohol intake levels from lowest to highest and including the resulting variable as a one degree-of-freedom linear term in a Cox proportional hazards model.

We first assessed associations of alcohol intake with any incident CRC. Subsequent analyses examined CRC risks defined by subtypes according to anatomic subsite (proximal or distal), microsatellite instability phenotype (MSI-H or MSS/MSI-L), CIMP status (CIMP-positive or CIMP-negative), and *BRAF* status (BRAF-mutation or *BRAF*-wildtype). For the subtype-specific analyses, the outcome variable was incident CRC with the molecular marker of interest; all other incident CRCs (including those with missing or unknown values for the molecular marker of interest) were considered censored observations at the date of diagnosis. We also examined associations of alcohol intake with CRC risk based on subsets defined by tissue availability (available versus not available), using the same multi-outcome analytic approach as described above to determine if incomplete tissue access introduced any bias. Two sets of Cox regression models were fit: one accounting for age and one additionally adjusting for other potential CRC risk factors, including body mass index (BMI; quartiles), waist to hip ratio (WHR; quartiles), smoking status (ever, never), exogenous estrogen use (ever, never), physical activity level (low, moderate, high), and daily intake (quartiles) of total energy (kcal/d), total fat (g/d), sucrose (g/d), red meat (g/d), calcium (mg/d), folate (μg/d), methionine (g/d) and vitamin E (mg/d). Family history of CRC and non-steroidal anti-inflammatory drug use were not systematically recorded at baseline and were not included in the current analyses. All statistical tests were two-sided, and all analyses were carried out using the SAS (SAS Institute, Inc., Cary, NC) and S-Plus (Insightful, Inc., Seattle, WA) software systems.

Results

In total, 21,464 (56%; 319,014 person-years) and 16,537 (44%; 245,675 person-years) IWHS subjects reported any alcohol consumption and no alcohol consumption at baseline, respectively. Among women with any alcohol intake, the median (range) was 3.4 g/d (0.9–292.8 g/d). With respect to the proposed WHO/AICR exposure threshold, 15,267 (40.2%) and 1,270 (3.3%) subjects reported alcohol intake levels of ≤ 30 g/d and > 30 g/d, respectively. Additional baseline demographics are provided in Table 1, by alcohol intake levels of none, any, ≤ 3.4 g/d and > 3.4 g/d. Among alcohol consumers, age, BMI and history of self-reported diabetes mellitus were lower, while smoking prevalence, physical activity level, exogenous estrogen use, and dietary intakes of total energy, total fat, calcium, folate, methionine and vitamin E were higher, compared to alcohol non-consumers. For cases with molecular marker data, the subtype-specific distributions were: 400 (73%) MSI-L/MSS and 148 (27%) MSI-H; 368 (69%) CIMP-negative and 167 (31%) CIMP-positive; and 391 (72%) *BRAF*-wildtype and 154 (28%) *BRAF*-mutated. Relatively strong Pearson correlations were observed between the MSI-H and CIMP-positive (0.70), MSI-H and *BRAF*-mutated (0.66), and CIMP-positive and *BRAF*-mutated (0.82) subtypes.

In the full analytic cohort, no statistically significant association was observed between incident CRC overall and alcohol intake levels of ≤ 3.4 g/d or > 3.4 g/d, based on comparisons to alcohol non-consumers in age-adjusted (RR = 0.96; 95% CI = 0.84–1.11 and RR = 1.03; 95% CI = 0.90–1.19; p trend = 0.79) and multivariable-adjusted (RR = 1.00; 95% CI = 0.86–1.15 and RR = 1.06; 95% CI = 0.91–1.24; p trend = 0.50) risk models, as shown in Table 2. Associations based on alcohol intake levels of ≤ 30 g/d or > 30 g/d, as well as by quartile distribution, were similarly unremarkable. With respect to anatomic subsite, none of the alcohol intake variables were significantly associated with either proximal colon or distal colorectal cancer (p > 0.05 for each comparison) (Table 2); further separation of distal colon and rectal cancers did not appreciably alter the observed, subsite-specific risk estimates (data not shown). When the incident CRC outcome was restricted to cases with evaluable tissue for molecular testing, the multivariate risk estimates for alcohol intake levels of ≤ 3.4 g/d and > 3.4 g/d (RR = 1.03; 95% CI = 0.83–1.28 and RR = 1.11; 95% CI = 0.88–1.39, respectively; compared to alcohol non-consumers; p trend = 0.40) were not appreciably different from estimates based on all incident CRC cases, providing reassurance that major selection bias was not introduced by tissue availability status.

Alcohol-related CRC risks based on the molecularly-defined subtypes did not reveal any evidence for differential associations by MSI, CIMP or *BRAF* mutation status (Table 3). Specifically, alcohol intake > 3.4 g/d was associated with comparable, non-statistically significant risk estimates for MSI-H and MSI-L/MSS tumors (RR=1.08; 95% CI = 0.70–1.65 and RR = 1.11; 95% CI = 0.85–1.46), CIMP-positive and CIMP-negative tumors (RR = 0.98; 95% CI = 0.65–1.49 and RR = 1.12; 95% CI = 0.84–1.50) and *BRAF*-mutated and *BRAF*-wildtype tumors (RR = 0.95; 95% CI = 0.61–1.46 and RR = 1.19; 95% CI = 0.91–1.57). Null associations with the molecularly-defined CRC subtypes were also observed for alcohol intake levels of ≤ 3.4 g/d, > 30 g/d, ≤ 30 g/d and quartile distributions (Table 3).

Discussion

In this prospective cohort study, baseline alcohol intake was not significantly associated with incident CRC overall, by anatomic subsite, or with respect to distinct, molecularly-defined subtypes. These results do not support pathway specificity as a major source of heterogeneity in alcohol-related CRC risks. Findings from the current study are consistent with the generally null associations between alcohol intake and incident CRC reported from an earlier IWHS study (31), while the molecularly-defined risk estimates add to the existing literature by providing robust data from a large, population-based cohort of older women.

Many epidemiological studies have previously examined alcohol consumption as a possible risk factor, as recently reviewed (2, 5). However, knowledge gaps remain for select demographic subgroups, particularly women with alcohol consumption at or below 30 g/d. In a meta-analysis of 34 case-control and 23 cohort (including the IWHS) studies published before May 2010 (43), Fedirko and colleagues described progressively increasing pooled RR estimates for incident CRC of 1.00 (95% CI = 0.95–1.05), 1.21 (95% CI = 1.13–1.28) and 1.52 (95% CI = 1.27–1.81) across alcohol intake levels of ≤ 12.5 g/d, 12.6–49.9 g/d, and ≥ 50 g/d respectively, as compared to alcohol non-consumers or occasional consumers. Of note, separate data for female alcohol consumers were only available from 26 (46%) of the analyzed studies. When stratified by gender, the alcohol-associated risk estimates were appreciably lower among women than men, for CRC overall (RR = 1.00; 95% CI = 0.94–1.07 and RR = 1.25; 95% CI = 1.13–1.39, respectively) and for each dose level except ≥ 50 g/d. Subsequent data from the UK Dietary Cohort Consortium (n=153,000) also failed to demonstrate a statistically significant association between alcohol intake levels of ≤ 30 g/d and CRC risk among women (or among men) (44). Thus, the preponderance of available data, including findings from the present IWHS report, suggests that women with low or moderate alcohol intake are not at increased CRC risk.

Current models of colorectal carcinogenesis incorporate at least three molecularly-distinct pathways (45–47), which can be at least partially represented by MSI, CIMP, and/or *BRAF* mutation status in various combinations. To date, mixed results have been observed regarding alcohol-related CRC risks by MSI phenotype, albeit with slightly different designs employed across studies. In a multi-center, case-control study involving subjects from Northern California, Utah and Minnesota (48), Slattery, et al. classified 1,510 colon cancer cases from both men and women as MSI-positive or MSI-negative based on a 12-marker panel. Higher long-term alcohol consumption was found to be associated with a non-statistically significant 40% increase in risk for MSI-positive, but not MSI-negative, tumors as compared to controls (OR = 1.4; 95% CI = 0.9–2.2 and OR = 0.9; 95% CI = 0.7–1.1). Diergaarde et al. (20) used a 5-marker panel to identify MSI-H versus MSI-L/MSS tumors among 184 colon cancer cases from men and women in a subsequent Dutch case-control study. Again, alcohol intake appeared to be associated with MSI-H rather than MSI-L/MSS tumors, although the reported risk estimates were not statistically significant (OR = 1.9; 95% CI = 0.8–4.7 and OR = 1.0; 95% CI = 0.6–1.8, respectively for comparison of extreme alcohol intake tertiles). Interestingly, when Poynter et al. analyzed associations between alcohol intake and MSI-H, MSI-L and MSS tumors separately (as determined by a 10-marker panel) in a case-unaffected sibling study conducted with data and tissue resources from the international Colon Cancer Family Registry,(21) consumption of ≥ 12 alcohol drinks per week was associated with a higher risk for MSI-L (OR = 1.85; 95% CI = 1.06–3.24; p = 0.03) than either MSS (OR = 1.20; 95% CI = 0.95–1.50) or MSI-H (OR = 0.63; 95% CI = 0.35–1.13) tumors among men and women combined. With respect to cohort studies, alcohol intake was not statistically significantly associated with MSI-defined CRC risk in the Netherlands Cohort Study (RR = 0.74; 95% CI = 0.19–2.89 for comparison of alcohol intake levels of ≥ 30 g/d vs. 0 g/d) (27). Conversely, a positive association was observed for alcohol intake ≥ 15 g/d with MSI-L/MSS tumors (RR = 1.46; 95% CI = 1.13–1.88) but not MSI-H tumors (RR = 0.89; 95% CI = 0.52–1.53) in a combined analysis of samples from women and men enrolled in the Nurses' Health Study and the Health Professionals Follow-up Study, respectively (25). However, when data for women were considered separately, the association between alcohol intake and MSI-L/MSS phenotype was attenuated (RR = 1.17; 95% CI = 0.79–1.73).

Relatively fewer studies have evaluated alcohol intake as a potential risk factor for CRC subtypes defined by CIMP and/or *BRAF* mutation status. Colon cancer tissue specimens from the Slattery case-control study were further assessed using a 5-gene methylation marker panel to differentiate CIMP-high (≥ 2 positive markers) from CIMP-low (0 or 1 positive markers) cases, along with *BRAF* V600E mutation status (22). In the subset of MSI-positive tumors, long-term alcohol intake was positively associated with *BRAF*-wildtype tumors (OR = 2.2; 95% CI = 1.2–3.7; p trend = 0.01) and marginally associated with CIMP-low tumors (OR = 1.7; 95% CI = 0.7–4.3; p trend = 0.06), while no statistically significant associations between alcohol intake and CIMP or *BRAF* mutation status were observed in the subset of MSI-negative tumors. In a follow-up analysis of rectal cancer cases, total alcohol consumption did not appear to be associated with CIMP-positive status (23). Extended molecular analyses from the Netherlands Cohort Study demonstrated a non-statistically significant increased risk for *BRAF*-mutated tumors among women with alcohol intake ≥ 30 g/d (RR = 2.54; 95% CI = 0.70–9.19; compared to alcohol intake of 0 g/d), while the risk estimate for BRAF-wildtype tumors was not reported (27). Thus, coupled with results from the presently reported IWHS study, existing data remain inconclusive with respect to subtype-specific CRC risks associated with increased alcohol consumption.

Major strengths of our study include the large, prospective, population-based design; prolonged follow-up and ability to adjust for multiple potential confounding factors; comprehensive CRC case ascertainment; tissue availability from representative cases for detailed molecular analyses; extensive characterization of MSI, CIMP, and *BRAF* mutation status, and high success rates for the reported molecular assays. Importantly, our data were derived from a relatively homogeneous subject cohort (older, primarily Caucasian women) and may not be directly applicable to other, more diverse populations. The relatively low daily alcohol intake among IWHS participants may also have affected our ability to estimate molecularly-defined CRC risks associated with higher exposure levels. However, given current recommendations from groups such as the American Cancer Society (www.cancer.org/Healthy/index; accessed 7/27/2011) to limit alcohol intake to ≤ 2 drinks per day for men and ≤ 1 drink per day for women, associations between low-level alcohol consumption and CRC risk merit further consideration and may have even broader public health implications (with respect to the total at-risk population) than heavy alcohol intake. In addition, the consistency of our findings with previously reported IWHS data (31) and other cohort studies (25, 27) lends credence to the general and molecular associations observed in our study. We also employed a single, baseline exposure assessment to describe long-term alcohol consumption that introduced some degree of misclassification bias. Nonetheless, other investigators have shown that analyses of baseline, updated and cumulative average alcohol intake yield comparable CRC risk estimates (which may be attributable to low intraindividual variation during prolonged follow-up) (49), suggesting that this study design limitation likely had minimal influence on our reported associations. The CRC subtype distributions observed in our study were also slightly different than prevalence estimates for sporadic tumors arising in the general population (50), which is likely explained by the IWHS cohort demographics.

In summary, we found no evidence that low/moderate alcohol intake is a risk factor for incident CRC, overall or by MSI-, CIMP-, or *BRAF*-defined subtypes, among older women. Nonetheless, the full spectrum of benefits:consequences must be appreciated to reduce the societal burden imposed by alcohol consumption (51). According to recent data from the 2009 U.S. National Health Interview Survey, 43% of adult women are current, regular alcohol consumers (i.e., > 12 drinks in the past year) (52). Further research is needed to determine if other alcohol-related factors that were uncommon (i.e., heavy drinking) or not measured (i.e., latency period, binge drinking) in the IWHS cohort are associated with increased CRC risk among female alcohol consumers.

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References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer.
2. Chan AT, Giovannucci EL. Primary prevention of colorectal cancer. Gastroenterology. 2010; 138:2029–43. e10. [PubMed: 20420944]
3. IARC. IARC monograph on the evaluation of carcinogenic risks to humans: Alcohol consumption and ethyl carbamate. Lyon, France: 2010.
4. Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, et al. Carcinogenicity of alcoholic beverages. Lancet Oncol. 2007; 8:292–3. [PubMed: 17431955]
5. Food, nutrition, physical activity, and the prevention of cancer: A global perspective. Washington, DC: 2007. 2007
6. Boffetta P, Hashibe M. Alcohol and cancer. Lancet Oncol. 2006; 7:149–56. [PubMed: 16455479]
7. Guo R, Ren J. Alcohol and acetaldehyde in public health: from marvel to menace. Int J Environ Res Public Health. 2010; 7:1285–301. [PubMed: 20617031]
8. Slattery ML, Schaffer D, Edwards SL, Ma KN, Potter JD. Are dietary factors involved in DNA methylation associated with colon cancer? Nutr Cancer. 1997; 28:52–62. [PubMed: 9200151]
9. Potter JD. Methyl supply, methyl metabolizing enzymes and colorectal neoplasia. J Nutr. 2002; 132:2410S–2S. [PubMed: 12163702]
10. Giovannucci E. Alcohol, one-carbon metabolism, and colorectal cancer: recent insights from molecular studies. J Nutr. 2004; 134:2475S–81S. [PubMed: 15333745]
11. Mason JB, Choi SW. Effects of alcohol on folate metabolism: implications for carcinogenesis. Alcohol. 2005; 35:235–41. [PubMed: 16054985]
12. Arasaradnam RP, Commane DM, Bradburn D, Mathers JC. A review of dietary factors and its influence on DNA methylation in colorectal carcinogenesis. Epigenetics. 2008; 3:193–8. [PubMed: 18682688]
13. Mathers JC, Strathdee G, Relton CL. Induction of epigenetic alterations by dietary and other environmental factors. Adv Genet. 2010; 71:3–39. [PubMed: 20933124]
14. Slattery ML, Curtin K, Anderson K, Ma KN, Ballard L, Edwards S, et al. Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. J Natl Cancer Inst. 2000; 92:1831–6. [PubMed: 11078760]

15. Yang P, Cunningham JM, Halling KC, Lesnick TG, Burgart LJ, Wiegert EM, et al. Higher risk of mismatch repair-deficient colorectal cancer in alpha(1)-antitrypsin deficiency carriers and cigarette smokers. Mol Genet Metab. 2000; 71:639–45. [PubMed: 11136557]
16. Samowitz WS, Albertsen H, Sweeney C, Herrick J, Caan BJ, Anderson KE, et al. Association of smoking, CpG island methylator phenotype, and V600E BRAF mutations in colon cancer. J Natl Cancer Inst. 2006; 98:1731–8. [PubMed: 17148775]
17. Curtin K, Samowitz WS, Wolff RK, Herrick J, Caan BJ, Slattery ML. Somatic alterations, metabolizing genes and smoking in rectal cancer. Int J Cancer. 2009; 125:158–64. [PubMed: 19358278]
18. Limsui D, Vierkant RA, Tillmans LS, Wang AH, Weisenberger DJ, Laird PW, et al. Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. J Natl Cancer Inst. 2010; 102:1012–22. [PubMed: 20587792]
19. Slattery ML, Anderson K, Curtin K, Ma K, Schaffer D, Edwards S, et al. Lifestyle factors and Ki-ras mutations in colon cancer tumors. Mutat Res. 2001; 483:73–81. [PubMed: 11600135]
20. Diergaarde B, Braam H, van Muijen GN, Ligtenberg MJ, Kok FJ, Kampman E. Dietary factors and microsatellite instability in sporadic colon carcinomas. Cancer Epidemiol Biomarkers Prev. 2003; 12:1130–6. [PubMed: 14652271]
21. Poynter JN, Haile RW, Siegmund KD, Campbell PT, Figueiredo JC, Limburg P, et al. Associations between smoking, alcohol consumption, and colorectal cancer, overall and by tumor microsatellite instability status. Cancer Epidemiol Biomarkers Prev. 2009; 18:2745–50. [PubMed: 19755657]
22. 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; 120:656–63. [PubMed: 17096326]
23. Slattery ML, Wolff RK, Herrick JS, Curtin K, Caan BJ, Samowitz W. Alcohol consumption and rectal tumor mutations and epigenetic changes. Dis Colon Rectum. 2010; 53:1182–9. [PubMed: 20628283]
24. Bongaerts BW, de Goeij AF, de Vogel S, van den Brandt PA, Goldbohm RA, Weijenberg MP. Alcohol consumption and distinct molecular pathways to colorectal cancer. Br J Nutr. 2007; 97:430–4. [PubMed: 17313702]
25. Schernhammer ES, Giovannuccci E, Fuchs CS, Ogino S. A prospective study of dietary folate and vitamin B and colon cancer according to microsatellite instability and KRAS mutational status. Cancer Epidemiol Biomarkers Prev. 2008; 17:2895–8. [PubMed: 18843035]
26. van Engeland M, Weijenberg MP, Roemen GM, Brink M, de Bruine AP, Goldbohm RA, et al. Effects of dietary folate and alcohol intake on promoter methylation in sporadic colorectal cancer: the Netherlands cohort study on diet and cancer. Cancer Res. 2003; 63:3133–7. [PubMed: 12810640]
27. de Vogel S, Bongaerts BW, Wouters KA, Kester AD, Schouten LJ, de Goeij AF, et al. Associations of dietary methyl donor intake with MLH1 promoter hypermethylation and related molecular phenotypes in sporadic colorectal cancer. Carcinogenesis. 2008; 29:1765–73. [PubMed: 18339680]
28. Breivik J, Lothe RA, Meling GI, Rognum TO, Borresen-Dale AL, Gaudernack G. Different genetic pathways to proximal and distal colorectal cancer influenced by sex-related factors. Int J Cancer. 1997; 74:664–9. [PubMed: 9421366]
29. Lindblom A. Different mechanisms in the tumorigenesis of proximal and distal colon cancers. Curr Opin Oncol. 2001; 13:63–9. [PubMed: 11148689]
30. Ward R, Meagher A, Tomlinson I, O'Connor T, Norrie M, Wu R, et al. Microsatellite instability and the clinicopathological features of sporadic colorectal cancer. Gut. 2001; 48:821–9. [PubMed: 11358903]
31. Gapstur SM, Potter JD, Folsom AR. Alcohol consumption and colon and rectal cancer in postmenopausal women. Int J Epidemiol. 1994; 23:50–7. [PubMed: 8194924]
32. Folsom AR, Kaye SA, Prineas RJ, Potter JD, Gapstur SM, Wallace RB. Increased incidence of carcinoma of the breast associated with abdominal adiposity in postmenopausal women. Am J Epidemiol. 1990; 131:794–803. [PubMed: 2138863]

33. Bisgard KM, Folsom AR, Hong CP, Sellers TA. Mortality and cancer rates in nonrespondents to a prospective study of older women: 5-year follow-up. Am J Epidemiol. 1994; 139:990–1000. [PubMed: 8178787]
34. Folsom AR, Kushi LH, Anderson KE, Mink PJ, Olson JE, Hong CP, et al. Associations of general and abdominal obesity with multiple health outcomes in older women: the Iowa Women's Health Study. Arch Intern Med. 2000; 160:2117–28. [PubMed: 10904454]
35. Willett WC, Sampson L, Browne ML, Stampfer MJ, Rosner B, Hennekens CH, et al. The use of a self-administered questionnaire to assess diet four years in the past. Am J Epidemiol. 1988; 127:188–99. [PubMed: 3337073]
36. Munger R, Folsom A, Kushi L, Kaye S, Sellers T. Dietary assessment of older Iowa women with a food frequency questionnaire: Nutrient intake, reproducibility, and comparison with 24-hour dietary recall interviews. Am J Epidemiol. 1992; 136:192–200. [PubMed: 1415141]
37. Hankey BF, Ries LA, Edwards BK. The surveillance, epidemiology, and end results program: a national resource. Cancer Epidemiol Biomarkers Prev. 1999; 8:1117–21. [PubMed: 10613347]
38. Cunningham JM, Kim CY, Christensen ER, Tester DJ, Parc Y, Burgart LJ, et al. The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. Am J Hum Genet. 2001; 69:780–90. [PubMed: 11524701]
39. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res. 1998; 58:5248–57. [PubMed: 9823339]
40. Thibodeau SN, French AJ, Cunningham JM, Tester D, Burgart LJ, Roche PC, et al. Microsatellite instability in colorectal cancer: different mutator phenotypes and the principal involvement of hMLH1. Cancer Res. 1998; 58:1713–8. [PubMed: 9563488]
41. 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; 38:787–93. [PubMed: 16804544]
42. Korn EL, Graubard BI, Midthune D. Time-to-event analysis of longitudinal follow-up of a survey: choice of the time-scale. Am J Epidemiol. 1997; 145:72–80. [PubMed: 8982025]
43. Fedirko V, Tramacere I, Bagnardi V, Rota M, Scotti L, Islami F, et al. Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. Ann Oncol. 2011
44. Park JY, Dahm CC, Keogh RH, Mitrou PN, Cairns BJ, Greenwood DC, et al. Alcohol intake and risk of colorectal cancer: results from the UK Dietary Cohort Consortium. Br J Cancer. 2010; 103:747–56. [PubMed: 20648013]
45. Pino MS, Chung DC. The chromosomal instability pathway in colon cancer. Gastroenterology. 2010; 138:2059–72. [PubMed: 20420946]
46. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010; 138:2073–87. e3. [PubMed: 20420947]
47. Leggett B, Whitehall V. Role of the serrated pathway in colorectal cancer pathogenesis. Gastroenterology. 2010; 138:2088–100. [PubMed: 20420948]
48. Slattery ML, Anderson K, Curtin K, Ma KN, Schaffer D, Samowitz W. Dietary intake and microsatellite instability in colon tumors. Int J Cancer. 2001; 93:601–7. [PubMed: 11477566]
49. Thygesen LC, Wu K, Gronbaek M, Fuchs CS, Willett WC, Giovannucci E. Alcohol intake and colorectal cancer: a comparison of approaches for including repeated measures of alcohol consumption. Epidemiology. 2008; 19:258–64. [PubMed: 18300715]
50. Pritchard CC, Grady WM. Colorectal cancer molecular biology moves into clinical practice. Gut. 2011; 60:116–29. [PubMed: 20921207]
51. Thavorncharoensap M, Teerawattananon Y, Yothasamut J, Lertpitakpong C, Chaikledkaew U. The economic impact of alcohol consumption: a systematic review. Subst Abuse Treat Prev Policy. 2009; 4:20. [PubMed: 19939238]
52. Summary health statistics for U.S. adults: National Health Interview Survey. Hyattsville; 2009. 2010

Table 1

Baseline Subject Characteristics, by Alcohol Intake Level

	Alcohol Intake Level			
	Non-Consumers	Consumers		
Characteristic	**N=21464**	**Any N=16537**	**≤ 3.4 g/d N=8313**	**> 3.4 g/d N=8224**
Age, years	62.5 (4.25)	61.7 (4.17)	61.9 (4.19)	61.5 (4.14)
Body Mass Index, kg/m^2	27.7 (5.4)	26.2 (4.51)	26.7 (4.71)	25.6 (4.23)
Waist to Hip Ratio	0.8 (0.09)	0.8 (0.08)	0.8 (0.09)	0.8 (0.08)
Smoking Status, N (%)				
never	15689 (74.4%)	8949 (54.8%)	5375 (65.6%)	3574 (44%)
ever	5387 (25.6%)	7374 (45.2%)	2817 (34.4%)	4557 (56%)
Exogenous Estrogen Use, N (%)				
never	13537 (63.7%)	9753 (59.6%)	5006 (60.9%)	4747 (58.3%)
ever	7699 (36.3%)	6600 (40.4%)	3209 (39.1%)	3391 (41.7%)
Physical Activity, N (%)				
low	10448 (49.9%)	7181 (44%)	3629 (44.3%)	3552 (43.8%)
moderate	5458 (26.1%)	4814 (29.5%)	2426 (29.6%)	2388 (29.4%)
high	5024 (24%)	4308 (26.4%)	2136 (26.1%)	2172 (26.8%)
Total Energy, kcal/d	1751.3 (734.6)	1843.6 (726.4)	1812.6 (692.4)	1875 (758.1)
Total Fat, g/d	67.0 (32.1)	69.5 (31.8)	69.3 (30.3)	69.8 (33.2)
Sucrose, gm/d	41.6 (24.9)	40.9 (24.1)	43.2 (24.2)	38.5 (23.8)
Red Meat, g/day	89.7 (76.4)	90.2 (72.4)	89.6 (70.1)	90.8 (74.7)
Calcium, mg/d[a]	1074.1 (577.0)	1112.5 (564.0)	1117.6 (564.3)	1107.5 (563.8)
Folate, µg/d[a]	420.9 (266.6)	438.5 (260.8)	438.9 (260.0)	438.2 (261.7)
Methionine, g/d	1.8 (0.9)	1.9 (0.9)	1.9 (0.8)	1.9 (0.9)
Vitamin E, mg/d[a]	66.5 (149.5)	68.0 (150.5)	68.9 (150.6)	67.1 (150.4)

Results presented as mean (standard deviation) unless otherwise indicated.

[a]including supplements.

Table 2

Associations Between Alcohol Intake Level and Incident Colorectal Cancer, Overall and by Anatomic Subsite

		ANY CRC (N = 1255)		PROXIMAL CRC (N = 633)		DISTAL CRC (N = 594)	
Alcohol Intake Level[a]	**Person-Years**	**Events, N**	**RR (95% CI)[b]**	**Events, N**	**RR (95% CI)[b]**	**Events, N**	**RR (95% CI)[b]**
Non-Consumers	319,014	721	1.00 (ref.)	360	1.00 (ref.)	344	1.00 (ref.)
Consumers							
Median Split[c], g/d							
≤3.4	125073	266	1.00 (0.86–1.15)	137	1.05 (0.85–1.28)	120	0.92 (0.74–1.14)
>3.4	120602	268	1.06 (0.91–1.24)	136	1.08 (0.87–1.34)	130	1.07 (0.86–1.33)
p trend			*0.50*		*0.47*		*0.74*
Threshold Value[d], g/d							
≤30	228085	492	1.03 (0.91–1.16)	250	1.06 (0.89–1.25)	231	0.99 (0.83–1.18)
>30	17590	42	1.00 (0.71–1.40)	23	1.12 (0.71–1.77)	19	0.89 (0.54–1.5)
p trend			*0.73*		*0.47*		*0.78*
Quartiles, g/d							
Q1 (x≤1.8)	77855	172	1.04 (0.88–1.24)	83	1.02 (0.80–1.31)	84	1.04 (0.81–1.33)
Q2 (1.8<x≤3.4)	47218	94	0.92 (0.74–1.15)	54	1.08 (0.81–1.46)	36	0.71 (0.50–1.02)
Q3 (3.4<x≤11)	62605	139	1.10 (0.91–1.33)	67	1.07 (0.82–1.41)	70	1.14 (0.87–1.49)
Q4 (11<x)	57997	129	1.02 (0.83–1.25)	69	1.08 (0.82–1.44)	60	0.98 (0.73–1.33)
p trend			*0.66*		*0.46*		*1.00*

[a]as reported during the IWHS baseline evaluation (1986);

[b]adjusted for age, BMI, WHR, smoking status, exogenous estrogen use, physical activity level, and daily intakes of total energy, total fat, sucrose, red meat, calcium, folate, methionine and vitamin E (mg/d);

[c]median split among IWHS subjects who reported any alcohol consumption;

[d]according to WHO/AICR report.(5)

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Table 3

Associations Between Alcohol Intake Level and Incident Colorectal Cancer, by Molecularly-Defined Subtypes

		Microsatellite Instability (MSI)				CpG Island Methylator Phenotype (CIMP)				*BRAF* mutation			
		MSI-L/MSS		MSI-H		CIMP-negative		CIMP-positive		*BRAF*-wildtype		*BRAF*-mutated	
Alcohol Intake Level[a]	**Person-Years**	**Events**	**RR (95% CI)**[b]	**Events**	**RR (95% CI)**[b]	**Events**	**RR (95% CI)**[b]	**Events**	**RR (95% CI)**[b]	**Events**	**RR (95% CI)**[b]	**Events**	**RR (95% CI)**[b]
Non-Consumers	319014	228	1.00 (ref.)	86	1.00 (ref.)	201	1.00 (ref.)	104	1.00 (ref.)	213	1.00 (ref.)	97	1.00 (ref.)
Consumers													
Median Split[c], g/d													
≤3.4	125073	89	1.09 (0.84–1.40)	26	0.81 (0.52–1.26)	90	1.21 (0.94–1.57)	27	0.74 (0.48–1.14)	91	1.17 (0.90–1.50)	24	0.69 (0.44–1.09)
>3.4	120602	83	1.11 (0.85–1.46)	36	1.08 (0.70–1.65)	77	1.12 (0.84–1.50)	36	0.98 (0.65–1.49)	87	1.19 (0.91–1.57)	33	0.95 (0.61–1.46)
p trend			*0.39*		*0.93*		*0.28*		*0.67*		*0.15*		*0.52*
Threshold Value[d], g/d													
≤30	228085	159	1.11 (0.90–1.37)	58	0.94 (0.66–1.34)	154	1.18 (0.94–1.47)	60	0.87 (0.62–1.22)	166	1.20 (0.97–1.48)	53	0.81 (0.57–1.16)
>30	17590	13	0.93 (0.49–1.76)	4	0.75 (0.26–2.16)	13	1.06 (0.56–2.01)	3	0.53 (0.16–1.74)	12	0.87 (0.45–1.70)	4	0.73 (0.25–2.08)
p trend			*0.51*		*0.61*		*0.21*		*0.25*		*0.26*		*0.23*
Quartiles, g/d													
Q1 (x≤1.8)	77855	64	1.26 (0.95–1.67)	16	0.79 (0.46–1.36)	66	1.44 (1.08–1.91)	17	0.74 (0.44–1.24)	66	1.37 (1.03–1.82)	16	0.74 (0.43–1.25)
Q2 (1.8<x≤3.4)	47218	25	0.79 (0.51–1.22)	10	0.83 (0.43–1.61)	24	0.83 (0.53–1.29)	10	0.74 (0.38–1.42)	25	0.82 (0.53–1.27)	8	0.62 (0.30–1.28)
Q3 (3.4<x≤11)	62605	44	1.21 (0.87–1.68)	18	1.06 (0.62–1.82)	38	1.12 (0.78–1.61)	21	1.12 (0.68–1.83)	45	1.25 (0.90–1.75)	18	1.01 (0.60–1.72)
Q4 (11<x)	57997	39	1.00 (0.69–1.46)	18	1.09 (0.63–1.90)	39	1.12 (0.76–1.63)	15	0.83 (0.47–1.49)	42	1.12 (0.78–1.62)	15	0.87 (0.48–1.56)
p trend			*0.75*		*0.81*		*0.60*		*0.68*		*0.39*		*0.56*

[a] as reported during the IWHS baseline evaluation (1986);

[b] adjusted for age, BMI, WHR, smoking status, exogenous estrogen use, physical activity level, and daily intakes of total energy, total fat, sucrose, red meat, calcium, folate, methionine and vitamin E (mg/d);

[c] median split among IWHS subjects who reported any alcohol consumption;

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