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


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 high (MSI-H) or MSI low/microsatellite stable (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 to 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 (RR) and 95% confidence intervals (CI). Among alcohol consumers, the median intake (range) was 3.4 (0.9–292.8) g/d. Compared with nonconsumers, alcohol intake levels of 3.4 g/d or less (RR = 1.00; 95% CI, 0.86–1.15) and more than 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 or less and more than 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. Cancer Prev Res; 4(12); 2035–43.

Introduction

Worldwide, colorectal cancer (CRC) is the third most common malignancy, with more than 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 7 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 more than 30 g/d showing a convincingly positive association for men, with greater uncertainty about alcohol intake–related CRC risks among women.

Several mechanisms have been proposed to account for the putatively procarcinogenic 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 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 used data and tissue resources from the prospective, population-based Iowa Women's Health Study (IWHS) to evaluate alcohol intake as a potential risk factor for incident CRC, overall and with respect to subtypes characterized by MSI, CIMP, and BRAF mutation status, among older women. Because proximal and distal CRCs are known to exhibit different clinicopathologic features (28–30), we further analyzed associations between alcohol intake and CRC risk on the basis of 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 to 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 and colleagues previously reported that demographic characteristics and CRC rates were comparable between initial survey responders and nonresponders (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 nonresponders were crossmatched with the National Death Index to further identify decedents. Migration out of Iowa for IWHS subjects has been estimated at approximately 1% per year (34). For the present molecular epidemiology study, exclusion criteria consisted of (not mutually exclusive): follow-up less than 1 day (n = 10) and history of malignancy other than nonmelanoma 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 by 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 to 3 per month; 1 per week; 2 to 4 per week; 5 to 6 per week; 1 per day; 2 to 3 per day; 4 to 5 per day; and 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 to 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 CRCs 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 of 1,255 (58%) cases. CRC diagnoses were subsequently confirmed by an experienced gastrointestinal pathologist (T.C. Smyrk). Baseline demographics and general tumor characteristics (size and stage) for incident CRC cases with retrieved versus nonretrieved tissue specimens were not significantly different, as previously reported (18). Paraffin blocks were serially cut into 5- to 10-μm 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 by the QIAamp Tissue Kit (QIAGEN), 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.

MSI

MSI testing was conducted on paired tumor and normal DNA samples for each case subject, by 10 established markers as follows: 4 mononucleotide repeats (BAT25, BAT126, BAT140, and BAT34C4), 5 dinucleotide repeats (ACTC, D5S346, D18S55, D17S250, and D10197), and 1 complex marker (MYCL; ref. 38). MSI status was classified as MSI high (MSI-H) if 30% or more of the markers showed instability and MSI low or microsatellite stable
(MSI-L/MSS) if less than 30% of the markers showed instability (39, 40). MSI status was determined for 548 of 563 (97%) of the evaluable CRC cases.

CpG island methylation

Tumor DNA was treated with sodium bisulfite and subsequently analyzed by automated real-time PCR–based MethyLight to amplify methylated CpG sites in the promoter regions of an established 5-gene marker panel (CAGNA1G, IGF2, NEUROG1, RUNX3, and SOCS1; ref. 41). CIMP status was reported as CIMP positive if hypermethylation was observed in 3 markers or more or CIMP negative if hypermethylation was observed in 0 to 2 markers. CIMP status was determined for 535 of 563 (95%) of the evaluable CRC cases.

BRAF mutation

Tumor DNA was analyzed by fluorescent allele–specific PCR to detect the V600E point mutation in exon 15 of the BRAF gene. BRAF mutation and BRAF wild-type cases were defined by the presence or absence of the V600E point mutation, respectively. BRAF mutation status was determined for 545 of 563 (97%) of the evaluable CRC cases.

Statistical analyses

Data were descriptively summarized using frequencies and percentages for categorical variables and means and SDs for continuous variables. Among CRC cases, pairwise 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 (RR) and 95% confidence intervals (CI) 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 nonconsumers 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), MSI phenotype (MSI-H or MSI-L/MSS), CIMP status (CIMP positive or CIMP negative), and BRAF status (BRAF mutation or BRAF wild-type). 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 multioutcome analytic approach as described earlier to determine whether incomplete tissue access introduced any bias. Two sets of Cox regression models were fit: one accounting for age and one, in addition, adjusting for other potential CRC risk factors, including body mass index (BMI; quartiles), waist-to-hip ratio (WHR; quartiles), smoking status (ever and never), exogenous estrogen use (ever and never), physical activity level (low, moderate, and 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 nonsteroidal anti-inflammatory drug use were not systematically recorded at baseline and were not included in the current analyses. All statistical tests were 2-sided, and all analyses were carried out with the SAS (SAS Institute, Inc.) and S-Plus (Insightful, Inc.) 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 or less and more than 30 g/d, respectively. Additional baseline demographics are provided in Table 1, by alcohol intake levels of none, any, 3.4 g/d or less and more than 3.4 g/d. Among alcohol consumers, age, BMI, and history of self-reported diabetes mellitus were lower, whereas smoking prevalence, physical activity level, exogenous estrogen use, and dietary intakes of total energy, total fat, calcium, folate, methionine, and vitamin E were higher than alcohol nonconsumers. For cases with molecular marker data, the subtype-specific distributions were as follows: 400 (73%) MSI-L/MSS and 148 (27%) MSI-H; 368 (69%) CIMP negative and 167 (31%) CIMP positive; and 391 (72%) BRAF wild-type 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 less or more than 3.4 g/d, based on comparisons to alcohol nonconsumers in age-adjusted (RR = 0.96; 95% CI, 0.84–1.11 and RR = 1.03;
95% CI, 0.90–1.19; \( P_{\text{trend}} = 0.79 \) and multivariable-adjusted (RR = 1.00; 95% CI, 0.86–1.15 and RR = 1.06; 95% CI, 0.91–1.24; \( P_{\text{trend}} = 0.50 \)) risk models, as shown in Table 2. Associations based on alcohol intake levels of 30 g/d or less or more than 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 CRC (\( P > 0.05 \) 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 or less and more than 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 with alcohol nonconsumers; \( P_{\text{trend}} = 0.40 \)) 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.

Null associations with the molecularly defined CRC subtypes were also observed for alcohol intake levels of 3.4 g/d or less, more than 30 g/d, 30 g/d or less, 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), although the molecularly defined risk estimates add to the existing evidence for differential associations by MSI, CIMP, or \( \text{BRAF} \) mutation status (Table 3). Specifically, alcohol intake more than 3.4 g/d was associated with comparable, nonstatistically 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 \( \text{BRAF} \)-mutated and \( \text{BRAF} \)-wild type 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 or less, more than 30 g/d, 30 g/d or less, and quartile distributions (Table 3).

### Table 1. Baseline subject characteristics, by alcohol intake level

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nonconsumers</th>
<th>Any</th>
<th>( \leq 3.4 \text{ g/d} )</th>
<th>( &gt;3.4 \text{ g/d} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N = 21,464 )</td>
<td>( N = 16,537 )</td>
<td>( N = 8,313 )</td>
<td>( N = 8,224 )</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>62.5 (4.25)</td>
<td>61.7 (4.17)</td>
<td>61.9 (4.19)</td>
<td>61.5 (4.14)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.7 (5.4)</td>
<td>26.2 (5.41)</td>
<td>26.7 (7.71)</td>
<td>25.6 (4.23)</td>
</tr>
<tr>
<td>WHR</td>
<td>0.8 (0.09)</td>
<td>0.8 (0.08)</td>
<td>0.8 (0.09)</td>
<td>0.8 (0.08)</td>
</tr>
<tr>
<td>Smoking status, N (%)</td>
<td>15,689 (74.4%)</td>
<td>8,949 (54.8%)</td>
<td>5,375 (65.6%)</td>
<td>3,574 (44%)</td>
</tr>
<tr>
<td>Exogenous estrogen use, N (%)</td>
<td>13,537 (63.7%)</td>
<td>9,753 (59.6%)</td>
<td>5,006 (60.9%)</td>
<td>4,747 (58.3%)</td>
</tr>
<tr>
<td>Physical activity, N (%)</td>
<td>10,448 (49.9%)</td>
<td>7,181 (44%)</td>
<td>3,629 (44.3%)</td>
<td>3,391 (41.7%)</td>
</tr>
</tbody>
</table>

**Note:** Results presented as mean (SD) unless otherwise indicated.

*Including supplements.*

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suggests that women with low or moderate alcohol intake data, including findings from the present IWHS report, among men; ref. 44). Thus, the preponderance of available statistically significant association between alcohol intake Cohort Consortium (50 g/d or more. Subsequent data from the UK Dietary CI, 1.13–1.39, respectively) and for each dose level except overall (RR were appreciably lower among women than men, for CRC stratified by gender, the alcohol-associated risk estimates available from 26 (46%) of the analyzed studies. When alcohol nonconsumers or occasional consumers. Of note, across alcohol intake levels of 12.5 g/d or less, 12.6 to 49.9 g/d, and 50 g/d or more respectively, as compared 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 select demographic subgroups, particularly women with recent reviewed (2, 5). However, knowledge gaps remain regarding alcohol consumption as a possible CRC risk factor, as Current models of colorectal carcinogenesis incorporate at least 3 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 used across studies. In a multicenter, case–control study involving subjects from Northern California, UT, and MN (48), Slattery and colleagues 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 nonstatistically significant 40% increase in risk for MSI-positive, but not MSI-negative, tumors as compared with controls (OR = 1.4; 95% CI, 0.9–2.2 and OR = 0.9; 95% CI, 0.7–1.1). Diergaard and colleagues (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 seemed to be associated with MSI-H rather than MSI-L/MSS tumors, although the reported risk estimates were not statistically significant (OR = 1.4; 95% CI, 0.9–2.2 and OR = 1.0; 95% CI, 0.6–1.8, respectively for comparison of extreme alcohol intake tertiles). Interestingly, when Poynter and colleagues 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 sibship study conducted with data and tissue resources from the international Colon Cancer Family Registry (21),

### Table 2. Associations between alcohol intake level and incident CRC, overall and by anatomic subsite

<table>
<thead>
<tr>
<th>Alcohol intake level</th>
<th>Person-years</th>
<th>Events, N</th>
<th>RR (95% CI)</th>
<th>Events, N</th>
<th>RR (95% CI)</th>
<th>Events, N</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonconsumers</td>
<td>319,014</td>
<td>721</td>
<td>1.00 (ref.)</td>
<td>360</td>
<td>1.00 (ref.)</td>
<td>344</td>
<td>1.00 (ref.)</td>
</tr>
<tr>
<td>Consumers</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Median split, g/d</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤3.4</td>
<td>125,073</td>
<td>266</td>
<td>1.00 (0.86–1.15)</td>
<td>137</td>
<td>1.05 (0.85–1.28)</td>
<td>120</td>
<td>0.92 (0.74–1.14)</td>
</tr>
<tr>
<td>&gt;3.4</td>
<td>120,602</td>
<td>268</td>
<td>1.06 (0.91–1.24)</td>
<td>136</td>
<td>1.08 (0.87–1.34)</td>
<td>130</td>
<td>1.07 (0.86–1.33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P_{trend} = 0.50</td>
<td></td>
<td>P_{trend} = 0.47</td>
<td></td>
<td>P_{trend} = 0.74</td>
</tr>
<tr>
<td>Threshold value, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤30</td>
<td>228,085</td>
<td>492</td>
<td>1.03 (0.91–1.16)</td>
<td>250</td>
<td>1.06 (0.89–1.25)</td>
<td>231</td>
<td>0.99 (0.83–1.18)</td>
</tr>
<tr>
<td>&gt;30</td>
<td>17,590</td>
<td>42</td>
<td>1.00 (0.71–1.40)</td>
<td>23</td>
<td>1.12 (0.71–1.77)</td>
<td>19</td>
<td>0.89 (0.54–1.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P_{trend} = 0.73</td>
<td></td>
<td>P_{trend} = 0.47</td>
<td></td>
<td>P_{trend} = 0.78</td>
</tr>
<tr>
<td>Quartiles, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 (x ≤ 1.8)</td>
<td>77,855</td>
<td>172</td>
<td>1.04 (0.88–1.24)</td>
<td>83</td>
<td>1.02 (0.80–1.31)</td>
<td>84</td>
<td>1.04 (0.81–1.33)</td>
</tr>
<tr>
<td>Q2 (1.8 &lt; x ≤ 3.4)</td>
<td>47,218</td>
<td>94</td>
<td>0.92 (0.74–1.15)</td>
<td>54</td>
<td>1.08 (0.81–1.46)</td>
<td>36</td>
<td>0.71 (0.50–1.02)</td>
</tr>
<tr>
<td>Q3 (3.4 &lt; x ≤ 11)</td>
<td>62,605</td>
<td>139</td>
<td>1.10 (0.91–1.33)</td>
<td>67</td>
<td>1.07 (0.82–1.41)</td>
<td>70</td>
<td>1.14 (0.87–1.49)</td>
</tr>
<tr>
<td>Q4 (11 &lt; x)</td>
<td>57,997</td>
<td>129</td>
<td>1.02 (0.83–1.25)</td>
<td>69</td>
<td>1.08 (0.82–1.44)</td>
<td>60</td>
<td>0.98 (0.73–1.33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P_{trend} = 0.66</td>
<td></td>
<td>P_{trend} = 0.46</td>
<td></td>
<td>P_{trend} = 1.00</td>
</tr>
</tbody>
</table>

|aAs reported during the IWHS baseline evaluation (1986).
|bAdjusted 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).
|cMedian split among IWHS subjects who reported any alcohol consumption.
|dAccording to WHO/AICR report (5).
<table>
<thead>
<tr>
<th>Alcohol intake level</th>
<th>Person-years</th>
<th>MSI-L/MSS</th>
<th>MSI-H</th>
<th>CIMP negative</th>
<th>CIMP positive</th>
<th>BRAF wild-type</th>
<th>BRAF mutated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonconsumers</td>
<td>319,014</td>
<td>228</td>
<td>86</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
</tr>
<tr>
<td>Median split</td>
<td>125,073</td>
<td>89</td>
<td>26</td>
<td>0.81</td>
<td>(0.61–1.06)</td>
<td>0.74</td>
<td>(0.55–0.98)</td>
</tr>
<tr>
<td>&gt;3.4</td>
<td>120,602</td>
<td>83</td>
<td>36</td>
<td>1.08</td>
<td>(0.79–1.44)</td>
<td>0.98</td>
<td>(0.72–1.32)</td>
</tr>
<tr>
<td>Threshold Value</td>
<td>228,085</td>
<td>159</td>
<td>58</td>
<td>0.94</td>
<td>(0.68–1.29)</td>
<td>0.87</td>
<td>(0.63–1.20)</td>
</tr>
<tr>
<td>Q1 (&lt; 1.8)</td>
<td>77,855</td>
<td>64</td>
<td>16</td>
<td>0.79</td>
<td>(0.52–1.19)</td>
<td>0.74</td>
<td>(0.53–0.99)</td>
</tr>
<tr>
<td>Q2 (1.8 &lt; x ≤ 3.4)</td>
<td>47,218</td>
<td>25</td>
<td>10</td>
<td>0.83</td>
<td>(0.58–1.20)</td>
<td>0.74</td>
<td>(0.50–1.05)</td>
</tr>
<tr>
<td>Q3 (3.4 &lt; x ≤ 11)</td>
<td>62,605</td>
<td>44</td>
<td>18</td>
<td>1.06</td>
<td>(0.76–1.50)</td>
<td>0.83</td>
<td>(0.59–1.29)</td>
</tr>
<tr>
<td>Q4 (11 &lt; x)</td>
<td>57,997</td>
<td>39</td>
<td>18</td>
<td>1.09</td>
<td>(0.72–1.50)</td>
<td>0.86</td>
<td>(0.58–1.30)</td>
</tr>
<tr>
<td>P trend</td>
<td>0.51</td>
<td>0.21</td>
<td>0.25</td>
<td>0.26</td>
<td>0.20</td>
<td>0.25</td>
<td>0.20</td>
</tr>
</tbody>
</table>

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).
consumption of 12 or more 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 \( \geq 30 \) g/d vs. 0 g/d; ref. 27). Conversely, a positive association was observed for alcohol intake of 15 g/d or more 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 \( \text{BRAF} \) 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 (\( \geq 2 \) positive markers) from CIMP-low (0 or 1 positive markers) cases, along with \( \text{BRAF} \) V600E mutation status (22). In the subset of MSI-positive tumors, long-term alcohol intake was positively associated with \( \text{BRAF} \) wild-type tumors (\( RR = 2.2 \); 95% CI, 1.2–3.7; \( P_{\text{trend}} = 0.01 \)) and marginally associated with CIMP-low tumors (\( OR = 1.7 \); 95% CI, 0.7–4.3; \( P_{\text{trend}} = 0.06 \)), whereas no statistically significant associations between alcohol intake and CIMP or \( \text{BRAF} \) 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 seem to be associated with CIMP-positive status (23). Extended molecular analyses from the Netherlands Cohort Study showed a nonstatistically significant increased risk for \( \text{BRAF} \)-mutated tumors among women with alcohol intake of 30 g/d or more (\( RR = 2.54 \); 95% CI, 0.70–9.19; compared with alcohol intake of 0 g/d), whereas the risk estimate for \( \text{BRAF} \) wild-type 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 \( \text{BRAF} \) mutation status; and high success rates for 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 (49), to limit alcohol intake to 2 or less drinks per day for men and 1 or less 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 used 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 within-individual variation during prolonged follow-up; ref. 50), 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 (51), 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 \( \text{BRAF} \)-defined subtypes, among older women. Nonetheless, the full spectrum of benefits/consquences must be appreciated to reduce the societal burden imposed by alcohol consumption (52). 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; ref. 53). Further research is needed to determine whether other alcohol-related factors that were uncommon (i.e., heavy drinking) or not measured (i.e., latency period and binge drinking) in the IWHS cohort are associated with increased CRC risk among female alcohol consumers.

**Disclosure of Potential Conflicts of Interest**

Dr. Limburg served as a consultant for Genomic Health, Inc. from August 8, 2008, to April 19, 2010. Mayo Clinic has licensed the intellectual property of Dr. Limburg to Exact Sciences and he and Mayo Clinic have contractual rights to receive royalties through this agreement. No potential conflicts of interest were disclosed by the other authors.

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