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# **Lifetime alcohol intake is associated with an increased risk of *KRAS*+ and *BRAF*-/ *KRAS*- but not *BRAF*+ colorectal cancer**

**Running title:** Alcohol intake and molecular subtypes of colorectal cancer

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**Abbreviations:** CI, confidence interval; CIMP, CpG island methylator phenotype; HR, hazard ratio; ICD-O-3, International Classification of Diseases for Oncology; MCCC, Melbourne Collaborative Cohort Study; MSI, microsatellite instability; MSS, microsatellite stable; VCR, Victorian Cancer Registry

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## Novelty and Impact

Ethanol in alcoholic beverages has a causal association with colorectal cancer. Differences in associations of alcohol intake with colorectal cancer subtypes defined by the presence of somatic mutations in oncogenes *BRAF* and *KRAS* are not yet established. In the present study, lifetime alcohol intake was associated with increased risks of *KRAS*<sup>+</sup> and *BRAF*⁻/*KRAS*⁻ tumors (originating via specific molecular pathways including the traditional adenoma-carcinoma pathway) but not with *BRAF*<sup>+</sup> tumors, a hallmark of tumor development via the ‘serrated’ pathway.

## Abstract

Ethanol in alcoholic beverages is a causative agent for colorectal cancer. Colorectal cancer is a biologically heterogeneous disease, and molecular subtypes defined by the presence of somatic mutations in *BRAF* and *KRAS* are known to exist. We examined associations between lifetime alcohol intake and molecular and anatomic subtypes of colorectal cancer. We calculated usual alcohol intake for 10-year periods from age 20 using recalled frequency and quantity of beverage-specific consumption for 38,149 participants aged 40-69 years from the Melbourne Collaborative Cohort Study. Cox regression was performed to derive hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between lifetime alcohol intake and colorectal cancer risk. Heterogeneity in the HRs across subtypes of colorectal cancer was assessed. A positive dose-dependent association between lifetime alcohol intake and overall colorectal cancer risk (mean follow-up=14.6 years; n=596 colon and n=326 rectal cancer) was observed (HR = 1.08, 95% CI: 1.04-1.12 per 10 g/day increment). The risk was greater for rectal than colon cancer ( $p_{\text{homogeneity}}=0.02$ ). Alcohol intake was associated with increased risks of *KRAS*<sup>+</sup> (HR = 1.07, 95% CI: 1.00-1.15) and *BRAF*<sup>-</sup>/*KRAS*<sup>-</sup> (HR = 1.05, 95% CI: 1.00-1.11) but not *BRAF*<sup>+</sup> tumors (HR = 0.89, 95% CI: 0.78-1.01;  $p_{\text{homogeneity}}=0.01$ ). Alcohol intake is associated with an increased risk of *KRAS*<sup>+</sup> and *BRAF*<sup>-</sup>/*KRAS*<sup>-</sup> tumors originating via specific molecular pathways including the traditional adenoma-carcinoma pathway but not with *BRAF*<sup>+</sup> tumors originating via the serrated pathway. Therefore, limiting alcohol intake from a young age might reduce colorectal cancer originating via the traditional adenoma-carcinoma pathway.

## Introduction

Ethanol in alcoholic beverages is a carcinogen<sup>1</sup> that increases the risk of colorectal cancer.<sup>2</sup>

Although colorectal cancer is generally referred to as a single, broad disease entity, it is a heterogeneous group of diseases in terms of molecular pathology and prognosis.<sup>3,4</sup> A number of molecularly defined subtypes of colorectal cancer have been described related to the presence of key somatic events including microsatellite instability (MSI), the CpG island methylator phenotype (CIMP), chromosomal instability, and somatic mutations in the oncogenes *BRAF*, *KRAS* and *PIK3CA*.<sup>5</sup> For instance, colorectal cancers with *BRAF* mutation are considered a distinct group<sup>3,6</sup> while a combination of features sets *KRAS*-mutated colorectal cancers apart from tumors harboring neither *BRAF* nor *KRAS* mutation.<sup>7</sup>

Smoking has been consistently shown to have differences in associations with the risk of specific molecular subtypes of colorectal cancer.<sup>8-10</sup> Findings for alcohol thus far have been inconsistent: increased risks of MSI-low<sup>8</sup> and MSI-high<sup>11,12</sup> colorectal cancer as well as an absence of a difference in association with MSI<sup>13,14</sup> or *BRAF* and CIMP<sup>15,16</sup> subtypes have been reported; associations for *KRAS* or combined *BRAF/KRAS* subtypes are not available. Similarly, uncertainty remains whether alcohol consumption poses a greater risk for rectal cancer over colon cancer: mechanistically, this is plausible considering that the rectal mucosa is exposed to a greater carcinogenic effect of acetaldehyde due to its higher concentration.<sup>17</sup> In the present study, we examined the associations between lifetime alcohol intake and colorectal cancer risk, overall and by subtypes defined by *BRAF* V600E and *KRAS* codons 12 and 13 somatic mutation status, and anatomic location (colon versus rectal), using data from the Melbourne Collaborative Cohort Study (MCCS).

## Materials and Methods

### Study population

The MCCS is a prospective cohort study of 41,514 people (99.2% aged 40-69 years; 58.9% women) recruited during 1990-94 from Melbourne.<sup>18</sup> Participants were recruited through the electoral rolls (registration to vote is compulsory for adults in Australia), advertisements and community announcements in local media (such as television, radio, newspapers).

Participants attended clinics where demographic, anthropometric, lifestyle and dietary information were collected and anthropometric measurements were performed. Participants aged <40 (n=194) and 70+ years (n=131) at baseline, with a confirmed cancer diagnosis before baseline (n=1,467), missing alcohol consumption data for any age period (n=22), reporting implausibly high alcohol intake (n=616) or extreme values of total energy intake (<1<sup>st</sup> percentile and >99<sup>th</sup> percentile) (n=779), and missing data on any of the covariates modelled (n=156) were excluded, leaving 38,149 (91.9% of all participants) eligible for this analysis (Fig. 1). The study protocol was approved by the Cancer Council Victoria's Human Research Ethics Committee. Participants gave written informed consent to participate and for investigators to obtain access to their medical records.

### Baseline data collection

A structured interview schedule was used at baseline to obtain information on potential risk factors including age, sex, country of birth, education, smoking habits, physical activity, and previous medical conditions. A 121-item food frequency questionnaire was used to collect dietary information.<sup>19</sup> Waist circumference was measured using a standard protocol. Baseline residential addresses were used to classify participants into quintiles of an area-based measure of socioeconomic status.<sup>20</sup>

### **Assessment of alcohol consumption**

Participants were asked at baseline if they had ever drunk at least 12 alcoholic drinks in a year. Those who had ('non-lifetime abstainers') were then asked about their usual frequency of consumption and usual quantity consumed per drinking occasion for beer, wine and spirits separately during 10-year age periods commencing at age 20, up to the decade of their age at baseline attendance. Usual intake within each age period in grams per day for each beverage type was calculated by multiplying intake frequency by quantity and standard amount of alcohol per container using Australian food composition tables.<sup>21</sup> The alcohol intake for each age period in grams per day was calculated as the sum of intake from the three beverage types. The baseline (current) alcohol intake in grams per day was obtained from intake for the age period encompassing baseline. Beverage-specific total intakes within age periods were summed to obtain total lifetime intakes in grams. The average lifetime alcohol intake in grams per day was derived by dividing the total lifetime intake by the total number of days within the age intervals up to baseline attendance.

### **Cohort follow-up and ascertainment of cases and deaths**

Cases and vital status were ascertained through the Victorian Cancer Registry (VCR), the Victorian Registry of Births, Deaths and Marriages, the National Death Index and the Australian Cancer Database. Incident cases were men and women with a first histopathological diagnosis of adenocarcinoma of the colon or rectum during follow-up to 31 December 2008. Cancer incidence data was coded following the 3<sup>rd</sup> Revision of the International Classification of Diseases for Oncology (ICD-O-3): colon (C18.0, C18.2-18.9) and rectum (C19.9, C20.9). Carcinomas of the appendix, and anus and anal canal including overlapping lesions of rectum, anus and anal canal, were not included but censored at diagnosis. In-situ lesions diagnosed during follow-up were ignored.

### Tumor molecular characterization and subtype classification

Archival tumor tissue was sought for all tumors diagnosed in Victoria. Diagnosis was verified and pathology was reviewed by a gastrointestinal histopathologist (CR). Tumor DNA was tested for the V600E *BRAF* mutation, which accounts for approximately 90% of *BRAF* mutations in colorectal cancer,<sup>22</sup> using a fluorescent allele-specific PCR discrimination method as previously described.<sup>23</sup> Exon 1 of *KRAS* was analyzed by direct Sanger sequencing.<sup>24</sup> Three tumor molecular subtypes were defined as follows: *BRAF*<sup>+</sup>, *KRAS*<sup>+</sup> and *BRAF*<sup>-</sup>/*KRAS*<sup>-</sup> (*BRAF*<sup>+</sup>/*KRAS*<sup>+</sup> does not occur frequently).

### Statistical analysis

Follow-up began at baseline attendance and continued until diagnosis of first colorectal cancer, censoring, death, date of leaving Victoria or 31 December 2008, whichever came first. Cox regression<sup>25</sup> with age as the time axis was performed to calculate HRs and 95% CIs for colorectal cancer overall, by molecular subtypes and by anatomic site (colon versus rectum), comparing lifetime alcohol intake with lifetime abstention. The following intake categories were used: abstainers (reference category), >0-19 g/day, 20-29 g/day, 30-39 g/day and  $\geq 40$  g/day. Wald tests from Cox regression models were used to assess linear trends for a 10 g/day increment in alcohol intake and for intake categories as a continuous measure. To test for heterogeneity in the HRs across molecular and anatomic subtypes of colorectal cancer, Cox regression models were fitted using a competing risks method.<sup>26</sup> Dose-response relationships between lifetime alcohol intake (as a continuous variable) and colorectal cancer incidence were examined by comparing models that included alcohol as a linear term only and as restricted cubic splines (four knots).<sup>27</sup> We fitted interaction terms to test for differences in associations by attained age (by splitting the data by median age at diagnosis). Sub-group analyses by gender were performed.



A causal diagram (directed acyclic graph) and existing evidence were used to determine confounding variables to be included in the multivariable-adjusted models. These were sex, education (primary school, some high/technical school, completed high/technical school, completed tertiary degree/diploma), socioeconomic status (quintiles ranging from most to least disadvantaged), smoking (never, former, current), physical activity (none, low, moderate, high), total red meat intake (quartiles), energy from food not including alcoholic beverages (continuous), dietary fiber intake (continuous) and dietary folate intake (continuous), and all models were stratified by country of birth (Australia/New Zealand, United Kingdom, Italy, Greece). Because waist circumference might be a consequence rather than a cause of alcohol consumption, we fitted models with (continuous) and without adjustment for this variable. We considered the model without adjustment for waist circumference to be the primary analysis.

In the subtype analysis, cases missing tumor molecular data were censored at diagnosis. In a sensitivity analysis, all participants diagnosed with any cancer other than colorectal cancer were censored at diagnosis. In addition, associations for baseline ('current') alcohol intake were also assessed. Each model was examined for outliers and influential points.<sup>28</sup> Nested models were compared using the likelihood ratio test.<sup>29</sup> Tests based on Schoenfeld residuals showed no evidence that proportional hazard assumptions were violated.<sup>30</sup> All statistical tests were two-sided, and *P*-values less than 0.05 were considered statistically significant. All statistical analyses were performed using Stata 14.1 (StataCorp, College Station, TX).

## Results

Characteristics of all 38,149 participants and cases by molecular and anatomic subtype are given in Table 1. The study had more females (59.5%) than males, and the majority were born in Australia, New Zealand or the UK (76.1%) (Table 1). More than half had never smoked and only 11% were current smokers. Almost a third of the participants did not consume alcohol and about half the participants consumed less than 20 g/day (Table 1). Of those who consumed alcohol, men reported median intakes of 17.6 g/day, 6.4 g/day and 4.5 g/day for total alcohol, beer and wine respectively (very few drank spirits), while women reported a median alcohol intake of 6.3 g/day.

By the end of follow-up (average 14.6 years/person), 922 incident cases of colorectal cancer were diagnosed (596, 64.6% colon; 326, 35.4% rectum), 1,428 participants had left Victoria and 4,153 had died. Molecular pathology data were obtained for 670 (73%) of the tumors; Figure 1 shows the reasons why data on *BRAF/KRAS* status were not obtained. The participants missing *BRAF/KRAS* status were not different in terms of their baseline characteristics from those with this information (Supplementary Table 1).

There were 111 colorectal cancers (16.6%) that had *BRAF* mutations, 183 (27.3%) that had *KRAS* mutations and 376 (56.1%) that were *BRAF*<sup>-</sup>/*KRAS*<sup>-</sup>. Of all tumors with molecular data, 423 (63.1%) were located in the colon, including 85.6% of the *BRAF*<sup>+</sup> tumors, 63.4% of the *KRAS*<sup>+</sup> tumors and 56.4% of the *BRAF*<sup>-</sup>/*KRAS*<sup>-</sup> tumors. Nearly two-thirds of the patients with *BRAF*<sup>+</sup> tumors were female while there were more males than females that had the other two subtypes (Table 1). *BRAF*<sup>+</sup> tumors were rare for participants born in Italy or Greece (Table 1). Compared with patients whose tumors were *KRAS*<sup>+</sup> or *BRAF*<sup>-</sup>/*KRAS*<sup>-</sup>, a higher proportion of patients with *BRAF*<sup>+</sup> tumors were lifetime abstainers from alcohol and fewer consumed  $\geq 30$  g/day (Table 1).

### Lifetime alcohol intake and colorectal cancer risk

These analyses included all 922 cases of colorectal cancer. Lifetime alcohol consumption was associated with an increased incidence of colorectal cancer (HR = 1.08, 95% CI: 1.04-1.12 for a 10 g/day increment,  $p$  for trend= $<0.001$ ; HR = 1.50, 95% CI: 1.15-1.95 for a lifetime intake of  $\geq 40$  g/day compared with lifetime abstention;  $p$  for trend=0.001) (Table 2). The model with the cubic splines fitted no better than a model with a single linear term for lifetime intake ( $p=0.5$ ). This association was significant for males (HR = 1.06, 95% CI: 1.02-1.11 for a 10 g/day increment,  $p$  for trend=0.003) and females (HR = 1.10, 95% CI: 1.00-1.21 for a 10 g/day increment,  $p$  for trend=0.05) (Table 2). For males, a HR of 1.08 (95% CI: 1.03-1.14,  $p$  for trend=0.004) for colorectal cancer was observed for a 10 g/day increment in beer intake while the evidence for an association for wine was weaker (HR = 1.07, 95% CI: 0.99-1.17 for a 10 g/day increment,  $p$  for trend=0.09); too few women drank beer to undertake a similar comparison. Associations did not change materially when waist circumference was included in the models (Supplementary Table 2).

### Associations with molecular subtypes of colorectal cancer

Lifetime alcohol intake was associated with increased incidence of *KRAS*<sup>+</sup> and *BRAF*<sup>-</sup>/*KRAS*<sup>-</sup> tumors (HR = 1.07, 95% CI: 1.00-1.15 and HR = 1.05, 95% CI: 1.00-1.11 respectively, for a 10 g/day increment) but not *BRAF*<sup>+</sup> tumors (HR = 0.89, 95% CI: 0.78-1.01 for a 10 g/day increment) ( $p_{\text{homogeneity}}=0.01$ ) (Table 3). Using *BRAF* status alone, a higher incidence of *BRAF*<sup>-</sup> (HR = 1.06, 95% CI: 1.01-1.11 for a 10 g/day increment) and a lower incidence of *BRAF*<sup>+</sup> tumors (HR = 0.89, 95% CI: 0.78-1.01 for a 10 g/day increment) associated with lifetime alcohol intake was observed ( $p_{\text{homogeneity}}=0.003$ ) (Table 3). The associations between lifetime alcohol intake and the two *KRAS* molecular subtypes did not differ ( $p_{\text{homogeneity}}=0.3$ ) (Table 3).

### Site-specific associations (colon versus rectum)

An increment in lifetime alcohol intake by 10 g/day was associated with a greater incidence of rectal cancer (HR = 1.08, 95% CI: 1.03-1.14) but not colon cancer (HR = 1.00, 95% CI: 0.96-1.05) ( $p_{\text{homogeneity}}=0.02$ ) (Table 3). For males, this pattern was observed for beer (HR = 1.11, 95% CI: 1.03-1.20 for rectal cancer and HR = 1.06, 95% CI: 0.98-1.13 for colon cancer, for a 10 g/day increment) and for wine (HR = 1.12, 95% CI: 0.99-1.27 for rectal cancer and HR = 1.05, 95% CI: 0.94-1.16 for colon cancer, for a 10 g/day increment) although the HR for rectal cancer for wine was not statistically significant (results not shown). However, there was no persuasive evidence for a difference in incidence between colon and rectal cancer for *BRAF*- tumors alone ( $p_{\text{homogeneity}}=0.4$ ) (Table 3). There was no evidence of interactions with attained age for colon ( $p = 0.6$ ) or rectal cancer ( $p = 0.09$ ) when the data were split according to median age at diagnosis ( $\leq 70$  and  $> 70$  years).

### Sensitivity analysis

HRs for overall colorectal cancer or molecular and anatomic subtypes did not change when individuals diagnosed with any cancer (apart from colorectal cancer) were censored at diagnosis (results not shown). In addition, current alcohol intake at baseline was also associated with an increased incidence of colorectal cancer (HR = 1.05, 95% CI: 1.01-1.09 for a 10 g/day increment,  $p$  for trend=0.02) but a difference in association between *BRAF*<sup>+</sup>, *KRAS*<sup>+</sup> and *BRAF*<sup>-</sup>/*KRAS*<sup>-</sup> subtypes was not observed ( $p_{\text{homogeneity}}=0.2$ ).

## Discussion

Our results confirm an association between lifetime alcohol intake and risk of colorectal cancer. A greater risk was observed for rectal than for colon cancer in the present analysis.

Alcohol intake was positively related to risk of *BRAF*- tumors irrespective of their *KRAS* status but not to risk of *BRAF*+ tumors. For *BRAF*- tumors, alcohol intake was positively associated with both colon and rectal tumors, but the association was weaker and not significant for colon cancer.

One of the main strengths of the present study is the availability of alcohol consumption data from age 20 especially considering that carcinogenesis is a chronic process. Also, abstainers for current intake might be contaminated by quitters. Other strengths include the relatively large number of colorectal cancers for which tumor *BRAF* and *KRAS* status were assessed according to standardized protocols, the prospective nature of the study, the near complete follow-up of cases through the population cancer registry, the low rates of attrition, and the availability of a range of demographic, clinical and lifestyle data. Nevertheless, several limitations exist: measurement error due to respondents having to summarize their frequency and quantity of alcoholic beverage intake for 10-year age intervals into single 'usual' values, potential for present intake to influence recall of past intake and under-reporting of past intake, residual confounding by unmeasured factors, and the fact that alcohol intake could have changed after the baseline assessment. We were unable to obtain archival tissue from the primary lesion to establish *BRAF/KRAS* status for about one quarter of the cases. However, this is unlikely to have biased the observed associations because the proportions of cases with and without *BRAF/KRAS* status varied little by ethnicity or sex, which were both strongly associated with molecular subtype.<sup>31</sup> Also, the possible lower

sensitivity of the technique employed to detect *KRAS* mutation may have contributed to an absence of a difference in association between *KRAS*<sup>+</sup> and *BRAF*<sup>-</sup>/*KRAS*<sup>-</sup> tumors.

In a recent meta-analysis, we found a relative risk of 1.49 for colorectal cancer associated with long term alcohol intake comparing the highest with the lowest intake category.<sup>2</sup> The excess risk associated with heavy drinking in the present study for all colorectal cancer is similar. Biological mechanisms proposed for alcohol-associated colorectal carcinogenesis include effects on carcinogen metabolism and hormone levels,<sup>32</sup> direct cellular injury and gene mutations in the large intestine caused by acetaldehyde,<sup>33</sup> decreased glutathione levels and the elimination of free radicals,<sup>34</sup> increased cell proliferation in the rectal mucosa<sup>17</sup> and aldehyde dehydrogenase and alcohol dehydrogenase genetic status which is thought to modify the association between alcohol and colorectal cancer.<sup>35</sup> The plausible relationship between alcohol intake and altered one-carbon metabolism that could result in aberrations in DNA methylation with or without epigenetic modifications has been the focus of recent investigations.<sup>36, 37</sup>

*BRAF* and *KRAS* are oncogenes that affect intracellular signaling pathways and are associated with global molecular characteristics which cause alterations of gene function on a genome-wide scale. For example, *BRAF*<sup>+</sup> is associated with high degree of CIMP<sup>38-40</sup> and *KRAS*<sup>+</sup> with CIMP-low.<sup>39, 41, 42</sup> CIMP is characterized by a propensity for widespread CpG island hypermethylation<sup>43</sup> and is important for defining a specific etiologic pathway of tumorigenesis among colorectal cancers under certain conditions.<sup>44</sup> *BRAF* and *KRAS*, on the other hand, are now part of routine clinical assessments for screening for Lynch syndrome and for assessing response to anti-EGFR therapy, respectively, rather than assessment of CIMP.<sup>45, 46</sup> Colorectal cancers can be divided into two broad subgroups: CIMP-high/*BRAF*<sup>+</sup>/*KRAS*<sup>-</sup> and CIMP-low or CIMP-/*BRAF*<sup>-</sup>/*KRAS*<sup>+</sup> or – tumors.<sup>3, 4</sup> Substantial evidence exists to suggest that CIMP-high (hence *BRAF*<sup>+</sup>) colorectal tumors arise through

the ‘serrated’ pathway rather than the ‘traditional’ adenoma-carcinoma pathway.<sup>44, 47-51</sup> A previous analysis using MCCS data had confirmed an association between *BRAF*<sup>+</sup> and CIMP<sup>+</sup> tumors, and an underlying genetic basis for differential etiologies of colorectal cancer.<sup>31</sup> The association of lifetime alcohol intake with an increased risk of *BRAF*<sup>-</sup> tumors in the present study suggests that the effects of alcohol on colorectal cancer development are restricted to tumors that arise through the traditional adenoma-carcinoma pathway of tumorigenesis. This pathway results in the development of tumors that are predominantly microsatellite stable (MSS), CIMP<sup>-</sup> and frequently harbor *KRAS* mutations, although the Lynch syndrome subtype of tumors demonstrating high levels of MSI are also thought to develop via adenoma-carcinoma pathway.<sup>4</sup> Our evidence does not suggest that the risk differs for the adenoma-carcinoma pathway according to the presence or otherwise of a *KRAS* mutation. In contrast, we observed no positive association between lifetime alcohol intake and colorectal cancers that harbored the *BRAF V600E* somatic mutation, a hallmark of tumor development through the ‘serrated’ pathway. Previously, the Nurses’ Health Study has reported HRs of 1.36 (95% CI: 0.67-2.74) for *BRAF*<sup>-</sup> and 1.05 (95% CI: 0.71-1.56) for *BRAF*<sup>+</sup> colon cancer associated with an alcohol intake of  $\geq 15$  g/day for women.<sup>15</sup> Similar findings were reported for participants in the Iowa Women’s Health Study: HRs of 1.19 (95% CI: 0.91-1.57) for *BRAF*<sup>-</sup> and 0.95 (95% CI: 0.61-1.46) for *BRAF*<sup>+</sup> colorectal cancer associated with an intake of  $>3.4$  g/day.<sup>16</sup> Neither study observed a dose-dependent association between alcohol intake and overall colon<sup>15</sup> or colorectal cancer risk.<sup>16</sup> Further, a recent case-control study reported odds ratios of 1.30 (95% CI: 0.91-1.85) for adenomas and 0.99 (95% CI: 0.68-1.47) for serrated polyps associated with an alcohol intake of  $\geq 14$  drinks/week.<sup>52</sup>

While published studies which predominantly used current intake have not established a clear difference in risk for the associations of colon and rectal cancer with alcohol,<sup>53</sup> the

European Prospective Investigation into Cancer and Nutrition reported HRs of 1.12 (95% CI: 1.06-1.18) for rectal and 1.05 (95% CI: 1.00-1.11) for colon cancer for a 15 g/day increment in lifetime alcohol intake but did not report a formal test result comparing HRs.<sup>54</sup> We have shown a greater risk of rectal than colon cancer associated with alcohol in line with the explanation for greater exposure of distal colorectal mucosa to the carcinogenic effects of acetaldehyde than the proximal part.<sup>17</sup> We are unable to confirm whether there is a definitive site-specific difference in risks and found little evidence suggestive of a site-specific difference in risks for *BRAF*-tumors. Further epidemiologic evidence is needed to confirm a gradient of increasing associations from proximal to the distal colorectum for alcohol intake along with further mechanistic explanations for this putative relationship.

In summary, we have confirmed that the association between alcohol intake and the risk of colorectal cancer might be limited to specific molecular pathways including the ‘traditional’ adenoma-carcinoma pathway, the etiologic pathway for the majority of colorectal cancer.<sup>44</sup> Therefore, limiting alcohol intake from a young age might help prevent occurrence of a sizeable proportion of colorectal cancer.



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**Conflicts of interest**

The authors declare that they have no conflicts of interest.

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**Figure legend**

**Figure 1.** Flow diagram showing selection of participants

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**Table 1.** Baseline characteristics of participants and colorectal cancer cases in the Melbourne Collaborative Cohort Study

	All participants (n=38,149)	All cases (n=922)	Colorectal cancer cases				
			According to tumor molecular subtype <sup>1, 2</sup>			According to anatomic location <sup>1</sup>	
			<i>BRAF</i> + (n=111)	<i>KRAS</i> + (n=183)	<i>BRAF</i> -/ <i>KRAS</i> - (n=376)	Colon (n=596)	Rectum (n=326)
Age at baseline, mean (SD), years	55.2 (8.6)	60.1 (7.6)	61.9 (6.8)	60.5 (7.5)	59.5 (7.7)	60.1 (7.8)	59.9 (7.3)
Sex, n (%)							
Male	15,462 (40.5)	468 (50.8)	38 (11.2)	101 (29.6)	202 (59.2)	282 (60.3)	186 (39.7)
Females	22,687 (59.5)	454 (49.2)	73 (22.2)	82 (24.9)	174 (52.9)	314 (69.2)	140 (30.8)
Country of birth, n (%)							
Australia/New Zealand/UK	29,046 (76.1)	696 (75.5)	99 (19.9)	133 (26.8)	265 (53.3)	457 (65.7)	239 (34.3)
Italy/Greece	9,103 (23.9)	226 (24.5)	12 (6.9)	50 (28.9)	111 (64.2)	139 (61.5)	87 (38.5)
Education, n (%)							
Primary school	7,337 (19.2)	210 (22.8)	17 (10.5)	46 (28.6)	98 (60.9)	131 (62.4)	79 (37.6)
Some high/technical school	14,492 (38.0)	355 (38.5)	42 (16.5)	71 (28.0)	141 (55.5)	232 (65.3)	123 (34.7)
Completed high/technical school	7,891 (20.7)	200 (21.7)	30 (21.1)	35 (24.7)	77 (54.2)	137 (68.5)	63 (31.5)
Completed tertiary degree/diploma	8,429 (22.1)	157 (17.0)	22 (19.5)	31 (27.4)	60 (53.1)	96 (61.2)	61 (38.8)
Smoking, n (%)							
Never	22,171 (58.1)	470 (51.0)	62 (18.2)	93 (27.4)	185 (54.4)	317 (67.5)	153 (32.5)
Former	11,794 (30.9)	353 (38.3)	33 (12.9)	72 (28.1)	151 (59.0)	217 (61.5)	136 (38.5)
Current	4,184 (11.0)	99 (10.7)	16 (21.6)	18 (24.3)	40 (54.1)	62 (62.6)	37 (37.4)
Lifetime alcohol intake (g/day), n (%)							
Abstainer	11,067 (29.0)	251 (27.2)	38 (21.2)	40 (22.4)	101 (56.4)	175 (69.7)	76 (30.3)
>0-19	19,453 (51.0)	427 (46.3)	51 (16.7)	91 (29.8)	163 (53.5)	283 (66.3)	144 (33.7)
20-29	3,220 (8.4)	91 (9.9)	14 (20.0)	17 (24.3)	39 (55.7)	54 (59.3)	37 (40.7)
30-39	1,816 (4.8)	54 (5.9)	1 (2.4)	13 (30.9)	28 (66.7)	28 (51.9)	26 (48.1)
≥40	2,593 (6.8)	99 (10.7)	7 (9.5)	22 (29.7)	45 (60.8)	56 (56.6)	43 (43.4)

Physical activity, n (%)							
None	8,431 (22.1)	218 (23.6)	27 (16.2)	39 (23.3)	101 (60.5)	129 (59.2)	89 (40.8)
Low	7,721 (20.2)	175 (19.0)	20 (16.9)	37 (31.4)	61 (51.7)	111 (63.4)	64 (36.6)
Moderate	13,464 (35.3)	347 (37.7)	40 (15.9)	76 (30.3)	135 (53.8)	241 (69.5)	106 (30.5)
High	8,533 (22.4)	182 (19.7)	24 (17.9)	31 (23.1)	79 (59.0)	115 (63.2)	67 (36.8)
Energy intake from food, mean (SD), kJ/day	8,777 (3,041)	9,003 (3,125)	8,588 (2,869)	9,450 (3,293)	8,935 (3,046)	9,116 (3,184)	8,797 (3,008)
Waist circumference, mean (SD), cm	85.4 (12.9)	89.4 (13.1)	86.8 (12.8)	90.3 (11.8)	90.0 (13.1)	88.9 (13.6)	90.3 (12.0)
Tumor molecular subtype							
<i>BRAF</i> +	-	111 (12.0)	-	-	-	95 (85.6)	16 (14.4)
<i>KRAS</i> +	-	183 (19.9)	-	-	-	116 (63.4)	67 (36.6)
<i>BRAF</i> -/ <i>KRAS</i> -	-	376 (40.8)	-	-	-	212 (56.4)	164 (43.6)
Missing	-	252 (27.3)	-	-	-	173 (68.6)	79 (31.4)

<sup>1</sup>Row percentages given.

<sup>2</sup>For individuals with data on tumor molecular subtype.

SD, standard deviation.



**Table 2.** Hazard ratios (HR) and 95% confidence intervals (CI) for colorectal cancer according to lifetime alcohol intake for participants in the Melbourne Collaborative Cohort Study

	Cases (%)	Person years	Multivariable-adjusted <sup>1</sup> HR (95% CI)	<i>p</i> for trend <sup>2</sup>
<i>All</i>				
For a 10 g/day increment in alcohol intake	922 (100)	558,871	1.08 (1.04-1.12)	<0.001
Alcohol intake categories (g/day)				0.001
Lifetime abstainer	251 (27.2)	166,390	1	
>0-19	427 (46.3)	283,526	1.03 (0.87-1.22)	
20-29	91 (9.9)	46,384	1.24 (0.95-1.60)	
30-39	54 (5.9)	26,167	1.24 (0.90-1.70)	
≥40	99 (10.7)	36,404	1.50 (1.15-1.95)	
<i>Men</i>				
For a 10 g/day increment in alcohol intake	468 (100)	221,107	1.06 (1.02-1.11)	0.003
Alcohol intake categories (g/day)				0.02
Lifetime abstainer	67 (14.3)	32,048	1	
>0-19	196 (41.9)	104,316	1.01 (0.76-1.34)	
20-29	70 (15.0)	31,598	1.20 (0.85-1.69)	
30-39	45 (9.6)	20,776	1.15 (0.78-1.69)	
≥40	90 (19.2)	32,369	1.38 (0.99-1.92)	
For a 10 g/day increment in beer intake	468 (100)	221,107	1.08 (1.03-1.14)	0.004
For a 10 g/day increment in wine intake	468 (100)	221,107	1.07 (0.99-1.17)	0.09
<i>Women</i>				
For a 10 g/day increment in alcohol intake	454 (100)	337,764	1.10 (1.00-1.21)	0.05
Alcohol intake categories (g/day)				0.1

Lifetime abstainer	184 (40.5)	134,342	1	
>0-19	231 (50.9)	179,211	1.00 (0.81-1.23)	
20-29	21 (4.6)	14,786	1.14 (0.72-1.83)	
30-39	9 (2.0)	5,390	1.46 (0.74-2.90)	
≥40	9 (2.0)	4,035	2.00 (1.01-3.96)	
For a 10 g/day increment in wine intake	454 (100)	337,750	1.12 (0.99-1.26)	0.07

<sup>1</sup>Adjusted for sex (for men and women combined), education, socioeconomic status, smoking, physical activity, energy intake from food, dietary fiber, dietary folate and total red meat, and stratified by country of birth.

<sup>2</sup>Wald test from Cox regression models assessing linear trends for a 10 g/day increment in alcohol intake and for intake categories as a continuous measure.

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**Table 3.** Hazard ratios (HR) and 95% confidence intervals (CI) for colorectal cancer for a 10 g/day increment in lifetime alcohol intake by tumor molecular subtype and anatomic location for participants in the Melbourne Collaborative Cohort Study

	Cases (%)	For a 10 g/day increment in alcohol intake	
		HR (95% CI) <sup>1</sup>	p value <sup>2</sup>
<i>Tumor molecular subtype</i>			
<i>BRAF/KRAS subtype</i>	670 (100.0)		0.01
<i>BRAF</i> +	111 (16.6)	0.89 (0.78-1.01)	
<i>KRAS</i> +	183 (27.3)	1.07 (1.00-1.15)	
<i>BRAF</i> -/ <i>KRAS</i> -	376 (56.1)	1.05 (1.00-1.11)	
<i>BRAF</i> subtype	676 (100.0)		0.003
<i>BRAF</i> +	113 (16.7)	0.89 (0.78-1.01)	
<i>BRAF</i> -	563 (83.3)	1.06 (1.01-1.11)	
<i>KRAS</i> subtype	683 (100.0)		0.3
<i>KRAS</i> +	189 (27.7)	1.07 (1.00-1.15)	
<i>KRAS</i> -	494 (72.3)	1.03 (0.98-1.08)	
<i>Anatomic location</i>	922 (100.0)		0.02
For all colorectal cancer			
Colon	596 (64.6)	1.00 (0.96-1.05)	
Rectum	326 (35.4)	1.08 (1.03-1.14)	
For <i>BRAF</i> - colorectal cancer			
Colon	330 (58.6)	1.03 (0.98-1.10)	0.4
Rectum	233 (41.4)	1.07 (1.00-1.14)	

<sup>1</sup>Adjusted for sex, education, socioeconomic status, smoking, physical activity, energy intake from food, dietary fiber, dietary folate and total red meat, and stratified by country of birth.

<sup>2</sup>Test of homogeneity.

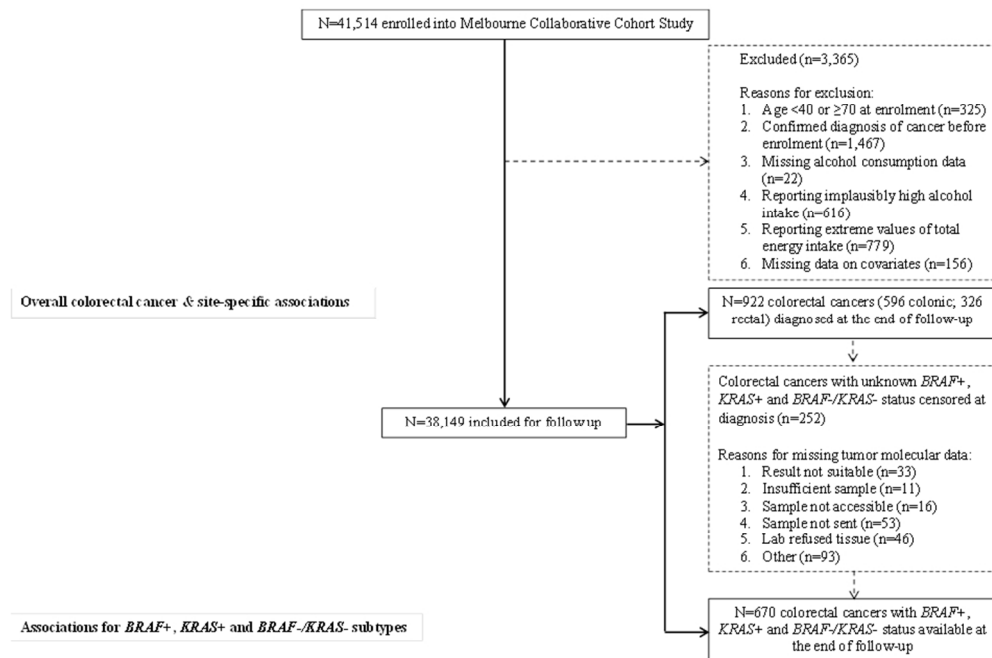


Figure 1. Flow diagram showing selection of participants

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