



Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Jayasekara, H;MacInnis, RJ;Williamson, EJ;Hodge, AM;Clendenning, M;Rosty, C;Walters, R;Room, R;Southey, MC;Jenkins, MA;Milne, RL;Hopper, JL;Giles, GG;Buchanan, DD;English, DR

Title:

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

Date:

2017-04-01

Citation:

Jayasekara, H., MacInnis, R. J., Williamson, E. J., Hodge, A. M., Clendenning, M., Rosty, C., Walters, R., Room, R., Southey, M. C., Jenkins, M. A., Milne, R. L., Hopper, J. L., Giles, G. G., Buchanan, D. D. & English, D. R. (2017). Lifetime alcohol intake is associated with an increased risk of KRAS+ and BRAF-/KRAS- but not BRAF+ colorectal cancer. *International Journal of Cancer*, 140 (7), pp.1485-1493. <https://doi.org/10.1002/ijc.30568>.

Persistent Link:

<https://hdl.handle.net/11343/292259>

SCHOLARONE™
Manuscripts

Accepted Article

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version record](#). Please cite this article as [doi:10.1002/ijc.30568](https://doi.org/10.1002/ijc.30568).

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

Harindra Jayasekara^{1,2}, Robert J. MacInnis^{1,2}, Elizabeth J. Williamson^{3,4}, Allison M. Hodge¹, Mark Clendenning⁵, Christophe Rosty^{5,6,7}, Rhiannon Walters⁸, Robin Room^{9,10,11}, Melissa C. Southey¹², Mark A. Jenkins², Roger L. Milne^{1,2}, John L. Hopper^{1,2}, Graham G. Giles^{1,2}, Daniel D. Buchanan^{2,5} and Dallas R. English^{1,2}

¹Cancer Epidemiology Centre, Cancer Council Victoria, 615 St Kilda Road, Melbourne, Victoria 3004, Australia

²Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, 207 Bouverie Street, Melbourne, Victoria 3010, Australia

³Farr Institute of Health Informatics Research, London, NW1 2DA, United Kingdom

⁴Department of Medical Statistics, London School of Hygiene & Tropical Medicine, London, WC1E 7HT, United Kingdom

⁵Colorectal Oncogenomics Group, Genetic Epidemiology Laboratory, Department of Pathology, The University of Melbourne, Parkville, Victoria, Australia

⁶Envoi Specialist Pathologists, Brisbane, Queensland, Australia

⁷School of Medicine, The University of Queensland, Brisbane, Queensland, Australia

⁸Cancer and Population Studies Group, Queensland Institute of Medical Research, Herston, Queensland, Australia

⁹Centre for Alcohol Policy Research, La Trobe University, 215 Franklin Street, Melbourne, Victoria 3000, Australia

¹⁰Centre for Health Equity, Melbourne School of Population and Global Health, The University of Melbourne, 207 Bouverie Street, Carlton, Victoria 3010, Australia

¹¹Centre for Social Research on Alcohol and Drugs, Stockholm University, SE - 106 91, Stockholm, Sweden

¹²Genetic Epidemiology Laboratory, Department of Pathology, The University of Melbourne, Melbourne, Australia.

Correspondence to: Harindra Jayasekara, Cancer Epidemiology Centre, Cancer Council

Victoria, 615 St Kilda Road, Melbourne, Victoria 3004, Australia; Phone: +61 4 33469782;

Fax: +61 3 93495815; E-mail: harindra.jayasekara@cancervic.org.au

Keywords: Alcohol intake; *BRAF*; colorectal cancer; *KRAS*

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

Article category: Cancer Epidemiology

Abstract: 247 words **Text:** 3,316 words

Figures: 1 **Tables:** 3 **References:** 54

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*⁺ and *BRAF*⁻/*KRAS*⁻ tumors (originating via specific molecular pathways including the traditional adenoma-carcinoma pathway) but not with *BRAF*⁺ 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*⁺ (HR = 1.07, 95% CI: 1.00-1.15) and *BRAF*⁻/*KRAS*⁻ (HR = 1.05, 95% CI: 1.00-1.11) but not *BRAF*⁺ 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*⁺ and *BRAF*⁻/*KRAS*⁻ tumors originating via specific molecular pathways including the traditional adenoma-carcinoma pathway but not with *BRAF*⁺ 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 carcinogen¹ that increases the risk of colorectal cancer.²

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.^{3,4} 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*.⁵ For instance, colorectal cancers with *BRAF* mutation are considered a distinct group^{3,6} while a combination of features sets *KRAS*-mutated colorectal cancers apart from tumors harboring neither *BRAF* nor *KRAS* mutation.⁷

Smoking has been consistently shown to have differences in associations with the risk of specific molecular subtypes of colorectal cancer.⁸⁻¹⁰ Findings for alcohol thus far have been inconsistent: increased risks of MSI-low⁸ and MSI-high^{11, 12} colorectal cancer as well as an absence of a difference in association with MSI^{13, 14} or *BRAF* and CIMP^{15, 16} 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.¹⁷ 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.¹⁸ 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 (<1st percentile and >99th 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.¹⁹ 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.²⁰

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.²¹ 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 3rd 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,²² using a fluorescent allele-specific PCR discrimination method as previously described.²³ Exon 1 of *KRAS* was analyzed by direct Sanger sequencing.²⁴ Three tumor molecular subtypes were defined as follows: *BRAF*⁺, *KRAS*⁺ and *BRAF*⁻/*KRAS*⁻ (*BRAF*⁺/*KRAS*⁺ 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 regression²⁵ 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 abstinence. The following intake categories were used: abstainers (reference category), >0-19 g/day, 20-29 g/day, 30-39 g/day and ≥ 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.²⁶ 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).²⁷ 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.²⁸ Nested models were compared using the likelihood ratio test.²⁹ Tests based on Schoenfeld residuals showed no evidence that proportional hazard assumptions were violated.³⁰ 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*⁻/*KRAS*⁻. Of all tumors with molecular data, 423 (63.1%) were located in the colon, including 85.6% of the *BRAF*⁺ tumors, 63.4% of the *KRAS*⁺ tumors and 56.4% of the *BRAF*⁻/*KRAS*⁻ tumors. Nearly two-thirds of the patients with *BRAF*⁺ tumors were female while there were more males than females that had the other two subtypes (Table 1). *BRAF*⁺ tumors were rare for participants born in Italy or Greece (Table 1). Compared with patients whose tumors were *KRAS*⁺ or *BRAF*⁻/*KRAS*⁻, a higher proportion of patients with *BRAF*⁺ tumors were lifetime abstainers from alcohol and fewer consumed ≥ 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 ≥ 40 g/day compared with lifetime abstinence; 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*⁺ and *BRAF*⁻/*KRAS*⁻ 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*⁺ 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*⁻ (HR = 1.06, 95% CI: 1.01-1.11 for a 10 g/day increment) and a lower incidence of *BRAF*⁺ 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 (≤ 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*⁺, *KRAS*⁺ and *BRAF*⁻/*KRAS*⁻ 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.³¹ 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*⁺ and *BRAF*⁻/*KRAS*⁻ 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.² 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,³² direct cellular injury and gene mutations in the large intestine caused by acetaldehyde,³³ decreased glutathione levels and the elimination of free radicals,³⁴ increased cell proliferation in the rectal mucosa¹⁷ and aldehyde dehydrogenase and alcohol dehydrogenase genetic status which is thought to modify the association between alcohol and colorectal cancer.³⁵ 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.^{36, 37}

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*⁺ is associated with high degree of CIMP³⁸⁻⁴⁰ and *KRAS*⁺ with CIMP-low.^{39, 41, 42} CIMP is characterized by a propensity for widespread CpG island hypermethylation⁴³ and is important for defining a specific etiologic pathway of tumorigenesis among colorectal cancers under certain conditions.⁴⁴ *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.^{45, 46} Colorectal cancers can be divided into two broad subgroups: CIMP-high/*BRAF*⁺/*KRAS*⁻ and CIMP-low or CIMP⁻/*BRAF*⁻/*KRAS*⁺ or – tumors.^{3, 4} Substantial evidence exists to suggest that CIMP-high (hence *BRAF*⁺) colorectal tumors arise through

the ‘serrated’ pathway rather than the ‘traditional’ adenoma-carcinoma pathway.^{44, 47-51} A previous analysis using MCCS data had confirmed an association between *BRAF*⁺ and CIMP⁺ tumors, and an underlying genetic basis for differential etiologies of colorectal cancer.³¹ The association of lifetime alcohol intake with an increased risk of *BRAF*⁻ 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⁻ 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.⁴ 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*⁻ and 1.05 (95% CI: 0.71-1.56) for *BRAF*⁺ colon cancer associated with an alcohol intake of ≥ 15 g/day for women.¹⁵ 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*⁻ and 0.95 (95% CI: 0.61-1.46) for *BRAF*⁺ colorectal cancer associated with an intake of >3.4 g/day.¹⁶ Neither study observed a dose-dependent association between alcohol intake and overall colon¹⁵ or colorectal cancer risk.¹⁶ 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 ≥ 14 drinks/week.⁵²

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,⁵³ 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.⁵⁴ 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.¹⁷ 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.⁴⁴ Therefore, limiting alcohol intake from a young age might help prevent occurrence of a sizeable proportion of colorectal cancer.

Acknowledgements

We thank the original investigators and the diligent team, who recruited the participants and who continue working on follow-up, for their contribution. We also express our gratitude to the many thousands of Melbourne residents who continue to participate in the study.

Financial support

MCCS cohort recruitment was funded by VicHealth and Cancer Council Victoria. The MCCS was further supported by Australian National Health and Medical Research Council grants 209057, 251553 and 504711 and by infrastructure provided by Cancer Council Victoria. MAJ is an NHMRC Senior Research Fellow. JLH is a NHMRC Senior Principal Research Fellow. DDB is a University of Melbourne Research at Melbourne Accelerator Program (R@MAP) Senior Research Fellow. RR's position was funded by the Victorian Department of Health and the Foundation for Alcohol Research and Education. The funding sources played no role in the study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the article for publication.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Altieri A, Coglianò V. Carcinogenicity of alcoholic beverages. *Lancet Oncol* 2007; 8: 292-3.
2. Jayasekara H, MacInnis RJ, Room R, English DR. Long-term alcohol consumption and breast, upper aero-digestive tract and colorectal cancer risk: a systematic review and meta-analysis. *Alcohol Alcohol* 2015; 51(3): 315-30.
3. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. *Histopathology* 2007; 50(1): 113-30.
4. Phipps AI, Limburg PJ, Baron JA, Burnett-Hartman AN, Weisenberger DJ, Laird PW, Sinicrope FA, Rosty C, Buchanan DD, Potter JD, Newcomb PA. Association between molecular subtypes of colorectal cancer and patient survival. *Gastroenterology* 2015; 148: 77-87. e2.
5. Ogino S, Chan AT, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. *Gut* 2011; 60(3): 397-411.
6. Pai RK, Jayachandran P, Koong AC, Chang DT, Kwok S, Ma L, Arber DA, Balise RR, Tubbs RR, Shadrach B, Pai RK. *BRAF*-mutated, microsatellite-stable adenocarcinoma of the proximal colon. *Am J Surg Pathol* 2012; 36: 744-52.
7. Rosty C, Young JP, Walsh MD, Clendenning M, Walters RJ, Pearson S, Pavluk E, Nagler B, Pakenas D, Jass JR, Jenkins MA, Win AK, et al. Colorectal carcinomas with *KRAS* mutation are associated with distinctive morphological and molecular features. *Mod Pathol* 2013; 26: 825-34.
8. Poynter JN, Haile RW, Siegmund KD, Campbell PT, Figueiredo JC, Limburg P, Young J, Le Marchand L, Potter JD, Cotterchio M, Casey G, Hopper JL, et al. Associations between smoking, alcohol consumption, and colorectal cancer, overall and by tumor microsatellite instability status. *Cancer Epidemiol Biomarkers Prev* 2009; 18(10): 2745-50.
9. Samowitz WS, Albertsen H, Sweeney C, Herrick J, Caan BJ, Anderson KE, Wolff RK, Slattery ML. Association of smoking, CpG island methylator phenotype, and V600E *BRAF* mutations in colon cancer. *J Natl Cancer Inst* 2006; 98(23): 1731-8.
10. Limsui D, Vierkant RA, Tillmans LS, Wang AH, Weisenberger DJ, Laird PW, Lynch CF, Anderson KE, French AJ, Haile RW, Harnack LJ, Potter JD, et al. Cigarette smoking and colorectal cancer risk by molecularly defined subtypes: *J Natl Cancer Inst* 2010; 102(14): 1012-22.
11. 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.
12. Diergaarde B, Braam H, Muijen GNPv, Ligtenberg MJL, Kok FJ, Kampman E. Dietary factors and microsatellite instability in sporadic colon carcinomas. *Cancer Epidemiol Biomarkers Prev* 2003; 12: 1130-6.
13. Eaton AM, Sandler R, Carethers JM, Millikan RC, Galanko J, Keku TO. 5,10-methylenetetrahydrofolate reductase 677 and 1298 polymorphisms, folate intake, and microsatellite instability in colon cancer. *Cancer Epidemiol Biomarkers Prev* 2005; 14(8): 2023-9.
14. Satia JA, Keku T, Galanko JA, Martin C, Doctolero RT, Tajima A, Sandler RS, Carethers JM. Diet, lifestyle, and genomic instability in the North Carolina Colon Cancer Study. *Cancer Epidemiol Biomarkers Prev* 2005; 14(2): 429-36.

15. Schernhammer ES, Giovannucci E, Baba Y, Fuchs CS, Ogino S. B vitamins, methionine and alcohol intake and risk of colon cancer in relation to BRAF mutation and CpG island methylator phenotype (CIMP). *Plos One* 2011; 6(6): e21102.
16. Razzak AA, Oxentenko AS, Vierkant RA, Tillmans LS, Wang AH, Weisenberger DJ, Laird PW, Lynch CF, Anderson KE, French AJ, Haile RW, Harnack LJ, et al. Alcohol intake and colorectal cancer risk by molecularly defined subtypes in a prospective study of older women. *Cancer Prev Res* 2011; 4(12): 2035-43.
17. Seitz HK, Simanowski UA. Alcohol and carcinogenesis. *Annu Rev Nutr* 1988; 8: 99-119.
18. Giles GG, English DR. The Melbourne Collaborative Cohort Study. In: IARC Sci Publ 156:69-70. Lyon, France: IARC, 2002.
19. Ireland P, Jolley D, Giles G, O'Dea K, Powles J, Rutishauser I, Wahlqvist ML, Williams J. Development of the Melbourne FFQ: a food frequency questionnaire for use in an Australian prospective study involving an ethnically diverse cohort. *Asia Pac J Clin Nutr* 1994; 3: 19-31.
20. McLennan B. *Socio-economic indexes for areas 96 [electronic resource]*. Canberra: Australian Bureau of Statistics, 1998.
21. Lewis J, Milligan G, Hunt A. *Nuttab95: nutrient data table for use in Australia*. Canberra: Australian Government Publishing Service, 1995.
22. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, et al. Mutations of the *BRAF* gene in human cancer. *Nature* 2002; 417(6892): 949-54.
23. Buchanan DD, Sweet K, Drini M, Jenkins MA, Win AK, English DR, Walsh MD, Clendenning M, McKeone DM, Walters RJ, Roberts A, Pearson S-A, et al. Risk factors for colorectal cancer in patients with multiple serrated polyps: a cross-sectional case series from genetics clinics. *Plos One* 2010; 5: e11636-e.
24. Rosty C, Buchanan DD, Walsh MD, Pearson S-A, Pavluk E, Walters RJ, Clendenning M, Spring KJ, Jenkins MA, Win AK, Hopper JL, Sweet K, et al. Phenotype and polyp landscape in serrated polyposis syndrome: a series of 100 patients from genetics clinics. *Am J Surg Pathol* 2012; 36: 876-82.
25. 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.
26. Lunn M, McNeil D. Applying cox regression to competing risks: *Biometrics* 1995; 51(2): 524-32.
27. Harrell FE, Jr., Lee KL, Pollock BG. Regression models in clinical studies: determining relationships between predictors and response. *J Natl Cancer Inst* 1988; 80: 1198-202.
28. Cleves MA, Gould WW, Gutierrez RG. *An introduction to survival analysis using Stata*, rev. ed. College Station, Texas: Stata Press, 2004.
29. Kirkwood BR, Sterne JAC. *Essential medical statistics*, 2nd ed. Blackwell Science, 2010.
30. Collett D. *Modelling survival data in medical research*, 2nd ed. Boca Raton, FL: Chapman & Hall/CRC, 2003.
31. English DR, Young JP, Simpson JA, Jenkins MA, Southey MC, Walsh MD, Buchanan DD, Barker MA, Haydon AM, Royce SG, Roberts A, Parry S, et al. Ethnicity and risk for colorectal cancers showing somatic *BRAF* V600E mutation or CpG island methylator phenotype. *Cancer Epidemiol Biomarkers Prev* 2008; 17: 1774-80.

32. Marshall JR, Freudenheim JO. Alcohol. In: Schottenfeld D, Fraumeni, JF. *Cancer Epidemiology and Prevention*, 3rd ed. Oxford University Press, 2006.
33. Seitz HK, Pöschl G, Simanowski UA. Alcohol and cancer. *Recent Dev Alcohol* 1998; 14: 67-95.
34. Lieber CS. Mechanisms of ethanol-drug-nutrition interactions. *J Toxicol Clin Toxicol* 1994; 32(6): 631-81.
35. Ferrari P, McKay JD, Jenab M, Brennan P, Canzian F, Vogel U, Tjønneland A, Overvad K, Tolstrup JS, Boutron-Ruault MC, Clavel-Chapelon F, Morois S, et al. Alcohol dehydrogenase and aldehyde dehydrogenase gene polymorphisms, alcohol intake and the risk of colorectal cancer in the European Prospective Investigation into Cancer and Nutrition study. *Eu J Clin Nutr* 2012; 66: 1303-8.
36. 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.
37. Mathers JC, Strathdee G, Relton CL. Induction of epigenetic alterations by dietary and other environmental factors. *Adv Genet* 2010; 71: 3-39.
38. Samowitz WS, Albertsen H, Herrick J, Levin TR, Sweeney C, Murtaugh MA, Wolff RK, Slattery ML. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. *Gastroenterology* 2005; 129: 837-45.
39. Nosho K, Irahara N, Shima K, Kure S, Kirkner GJ, Schernhammer ES, Hazra A, Hunter DJ, Quackenbush J, Spiegelman D, Giovannucci EL, Fuchs CS, et al. Comprehensive biostatistical analysis of CpG island methylator phenotype in colorectal cancer using a large population-based sample. *Plos One* 2008; 3: 1-12.
40. Sanchez JA, Krumroy L, Plummer S, Aung P, Merkulova A, Skacel M, DeJulius KL, Manilich E, Church JM, Casey G, Kalady MF. Genetic and epigenetic classifications define clinical phenotypes and determine patient outcomes in colorectal cancer. *B J Surg* 2009; 96: 1196-204.
41. Ogino S, Goel A. Molecular classification and correlates in colorectal cancer. *J Mol Diagn* 2008; 10(1): 13-27.
42. Ogino S, Kawasaki T, Kirkner GJ, Suemoto Y, Meyerhardt JA, Fuchs CS. Molecular correlates with MGMT promoter methylation and silencing support CpG island methylator phenotype-low (CIMP-low) in colorectal cancer. *Gut* 2007; 56(11): 1564-71.
43. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa J-PJ. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci U S A* 1999; 96(15): 8681-6.
44. Jass JR. Serrated adenoma of the colorectum and the DNA-methylator phenotype. *Nat Clin Pract Oncol* 2005; 2(8): 398-405.
45. Toon CW, Walsh MD, Chou A, Capper D, Clarkson A, Sioson L, Clarke S, Mead S, Walters RJ, Clendenning M, Rosty C, Young JP, et al. *BRAF*V600E immunohistochemistry facilitates universal screening of colorectal cancers for Lynch syndrome. *Am J Surg Pathol* 2013; 37: 1592-602.
46. Lièvre A, Bachet J-B, Le Corre D, Boige V, Landi B, Emile J-F, Côté J-F, Tomasic G, Penna C, Ducreux M, Rougier P, Penault-Llorca F, et al. *KRAS* mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 2006; 66: 3992-5.
47. O'Brien MJ. Hyperplastic and serrated polyps of the colorectum. *Gastroenterol Clin North Am* 2007; 36(4): 947-68, viii.
48. O'Brien MJ, Yang S, Mack C, Xu H, Huang CS, Mulcahy E, Amoroso M, Farraye FA. Comparison of microsatellite instability, CpG island methylation phenotype,

BRAF and *KRAS* status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. *Am J Surg Pathol* 2006; 30: 1491-501.

49. East JE, Saunders BP, Jass JR. Sporadic and syndromic hyperplastic polyps and serrated adenomas of the colon: classification, molecular genetics, natural history, and clinical management. *Gastroenterol Clin North Am* 2008; 37: 25-46.

50. Goldstein NS. Serrated pathway and APC (conventional)-type colorectal polyps: molecular-morphologic correlations, genetic pathways, and implications for classification. *Am J Clin Pathol* 2006; 125: 146-53.

51. Kambara T, Simms LA, Whitehall V, Spring KJ, Wynter C, Walsh MD, Barker MA, Arnold S, McGivern A, Matsubara N, Tanaka N, Higuchi T, et al. *BRAF* mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. *Gut* 2004; 53(8): 1137-44.

52. Burnett-Hartman AN, Passarelli MN, Adams SV, Upton MP, Zhu L-C, Potter JD, Newcomb PA. Differences in epidemiologic risk factors for colorectal adenomas and serrated polyps by lesion severity and anatomical site. *Am J Epidemiol* 2013;177: 625-37.

53. Moskal A, Norat T, Ferrari P, Riboli E. Alcohol intake and colorectal cancer risk: A dose-response meta-analysis of published cohort studies. *Int J Cancer* 2007; 120: 664-71.

54. Ferrari P, Jenab M, Norat T, Moskal A, Slimani N, Olsen A, Tjønneland A, Overvad K, Jensen MK, Boutron-Ruault MC, Clavel-Chapelon F, Morois S, et al. Lifetime and baseline alcohol intake and risk of colon and rectal cancers in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Int J Cancer* 2007; 121: 2065-72.

Accepted Article

Figure legend

Figure 1. Flow diagram showing selection of participants

Accepted Article

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 ^{1, 2}			According to anatomic location ¹	
			<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)	

¹Row percentages given.

²For 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 ¹ HR (95% CI)	<i>p</i> for trend ²
<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

¹Adjusted 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.

²Wald 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.

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) ¹	<i>p</i> value ²
<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-/KRAS-</i>	376 (56.1)	1.05 (1.00-1.11)	
<i>BRAF subtype</i>			
<i>BRAF+</i>	113 (16.7)	0.89 (0.78-1.01)	0.003
<i>BRAF-</i>	563 (83.3)	1.06 (1.01-1.11)	
<i>KRAS subtype</i>			
<i>KRAS+</i>	189 (27.7)	1.07 (1.00-1.15)	0.3
<i>KRAS-</i>	494 (72.3)	1.03 (0.98-1.08)	
<i>Anatomic location</i>			
For all colorectal cancer	922 (100.0)		0.02
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)	

¹Adjusted 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.

²Test of homogeneity.

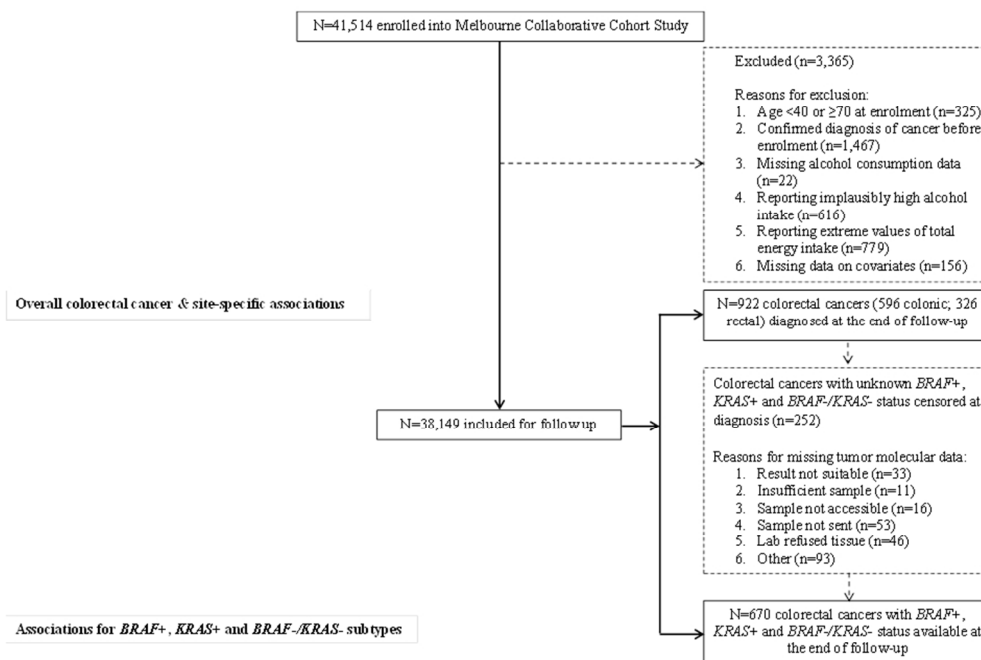


Figure 1. Flow diagram showing selection of participants

254x190mm (96 x 96 DPI)

Accep