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Original Research Article

Alcohol consumption and molecular subtypes of colorectal cancer: pooled observational and Mendelian randomization analyses

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Abbreviations: BRAF, B-Raf proto-oncogene, serine/threonine kinase; CI, confidence interval; CCFR, Colon Cancer Family Registry; CIMP, CpG island methylator phenotype; CPS-II, Cancer Prevention Study II; CRC, colorectal cancer; DACHS, Darmkrebs: Chancen der Verhütung durch Screening Study; DALS, Diet, Activity and Lifestyle Study; EPIC, European Prospective Investigation into Cancer and Nutrition; GECCO, Genetics and Epidemiology of Colorectal Cancer Consortium; GWAS, genome-wide association study; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; IVW, inverse variance–weighted; KRAS, KRAS proto-oncogene, GTPase; MCCS, Melbourne Collaborative Cohort Study; MR, Mendelian randomization; MSI, microsatellite instability; NFCCR, Newfoundland Familial Colorectal Cancer Registry; NHS, Nurses' Health Study; NSHDS, Northern Sweden Health and Disease Study; OR, odds ratio; SNP, single-nucleotide polymorphism.

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A B S T R A C T

Background: Alcohol consumption is associated with colorectal cancer (CRC) risk, yet its association with distinct molecular subtypes remains unclear. Clarifying this could reveal insights into alcohol's carcinogenic mechanisms.

Objectives: We examined the association between alcohol consumption and the risk of CRC subtypes defined by individual tumor markers (and marker combinations), namely microsatellite instability (MSI) status, CpG island methylator phenotype (CIMP) status, *BRAF,* and *KRAS* mutations.

Methods: Pooled observational (n_{cases} = 11,826, $n_{controls}$ = 10,888; $n_{studies}$ = 10) and genome-wide association data (n_{cases} = 8178, $n_{controls}$ = 10,472; $n_{studies}$ = 10) were used. Multivariable logistic regression models and Mendelian randomization (MR) analyses were conducted to assess the association between alcohol consumption, modeled in MR as genetically predicted mean drinks per week per 1 SD increase (≈2.9 drinks/wk), and risk of CRC subtypes defined by individual tumor markers (and marker combinations). Case-only analyses tested for differences between molecular subtypes. Bonferroni correction was applied for multiple tests.

Results: Among drinkers, each additional 14 g/d of alcohol was associated with a 10% higher CRC risk [odds ratio (OR) = 1.10; 95% confidence interval (CI): 1.07, 1.13], but this association was primarily driven by heavy alcohol consumption (>28 g/d). Including nondrinkers revealed a J-shaped association (*P*-nonlinearity = 0.002). The associations with higher alcohol consumption were stronger in males compared with females. No significant heterogeneity was observed across MSI, CIMP, *BRAF*, or *KRAS*-defined subtypes. All associations were similar across smoking status, folate intake, tumor anatomical site, study design, early/late-onset CRC, and across individual studies (*P*-heterogeneity > 0.05). MR analyses supported that higher genetically predicted alcohol consumption was associated with CRC risk ($OR_{IVW\text{-}per\ 1SD}$ = 1.25; 95% CI: 1.01, 1.57), but similarly to the observational analysis, without evidence of heterogeneity across molecular subtypes.

Conclusions: Heavy alcohol consumption may initiate colorectal carcinogenesis through mechanisms that operate across all examined molecular pathways for CRC. Although the largest available data were used, power is lower for subtype heterogeneity analyses, and modest interaction effects cannot be excluded.

Keywords: alcohol, colorectal cancer, subtypes, GECCO, CCFR, molecular epidemiology

Introduction

Colorectal cancer (CRC) is among the most common cancers and contributes substantially to cancer-related mortality [1]. A key strategy for CRC prevention involves identifying modifiable lifestyle factors that influence disease risk. The International Agency for Research on Cancer classified alcoholic beverages as carcinogenic to humans (group 1) based on sufficient evidence of causality for CRC [2]. Epigenome-wide association studies have shown that alcohol consumption can alter DNA methylation in both blood and tissues [3,4]. Aberrant DNA methylation, an epigenetic mechanism that regulates gene expression, has also been implicated in the development of CRC [5]. Moreover, alcohol may interact with genetic polymorphisms in susceptibility genes, contributing to CRC tumorigenesis [6].

CRC is characterized by significant genetic and epigenetic diversity [7]. Detailed molecular characterization of CRC using clinically relevant genetic and epigenetic markers shows great potential for improving prognosis [8] and guiding treatment decisions [9]. KRAS proto-oncogene, GTPase (*KRAS)* mutations occur in ~30%–40% of sporadic CRC and are implicated in adenoma progression [10]. Microsatellite instability (MSI), found in ~15% of CRCs, is associated with a better prognosis [11]. Some MSI-high tumors also exhibit the CpG island methylator phenotype (CIMP) and B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) p.V600E mutations [7]. The latter phenotype is associated with a worse prognosis [12]. *BRAF* mutations predict a response to encorafenib plus cetuximab [13,14]; *KRAS* and *BRAF* mutations indicate a poor response to epidermal growth factor receptor inhibitors [15,16], and MSI-high status predicts a response to immunotherapy [17,18].

Although several studies have assessed whether established risk factors—including smoking, obesity, and diabetes—are differentially associated with CRC molecular subtypes [19–21], the role of alcohol consumption across these subtypes remains inadequately investigated. To date, over 10 studies have evaluated alcohol consumption in relation to CRC molecular characteristics; however, only 3 have been published in the past 12 y, and the results have been inconsistent [22–34]. In the largest and most recent study—a German case-control study, which is included in the present analysis, consisting of ~2500 CRC cases with molecular subtype information—no significant heterogeneity was observed for associations between alcohol consumption and CRC risk by *BRAF*, *KRAS*, CIMP, or MSI status (all *P*-heterogeneity > 0.05) [23]. However, the categorization of alcohol consumption was binary [≤24.6 g/d (reference group) compared with >24.6 g/d], precluding a nuanced understanding of dose–response associations. Moreover, the study did not distinguish nondrinkers, who may include individuals abstaining for health reasons, from light drinkers, limiting the ability to observe potential differential associations. Earlier studies employed more detailed categorizations of alcohol consumption but were constrained by small numbers of cases within molecular subtypes—often <600 cases per subtype—and particularly low numbers of participants with higher alcohol consumption [e.g., <20 cases consuming >1 drink per day (14 g/d)], limiting statistical power and the precision of the estimates.

To address these limitations, we conducted a pooled observational analysis and a 2-sample Mendelian randomization (MR) analysis leveraging data from 2 large, well-characterized consortia—the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) and the Colon Cancer Family Registry (CCFR)—to examine the association

between alcohol consumption and CRC risk across molecular subtypes defined by MSI, CIMP, and *BRAF* and *KRAS* mutation status.

Methods

Observational analysis

Study population

This study included CRC cases and controls nested within 6 cohorts [Cancer Prevention Study II (CPS-II) [35,36], Nurses' Health Study (NHS) [37], Health Professionals Follow-up Study (HPFS), European Prospective Investigation into Cancer (EPIC) Sweden [38], Melbourne Collaborative Cohort Study (MCCS) [39], Northern Sweden Health and Disease Study (NSHDS) [40]] and 4 case-control studies [Darmkrebs: Chancen der Verhütung durch Screening Study (DACHS) [41,42], Diet, Activity and Lifestyle Study (DALS) [43,44], CCFR Australia, Ontario, Seattle [45], and Newfoundland Familial Colorectal Cancer Registry (NFCCR) [46]]. These studies are part of the GECCO and the CCFR and contributed data on tumor molecular markers and alcohol consumption. CRC cases were defined as colorectal adenocarcinoma and confirmed via pathological records, medical records, and/or death certificate information. Additional information on the contributing studies is provided in the Supplements (Supplemental Table 1 and Appendix 1). All participants provided written informed consent, and each study was approved by the relevant institutional review or ethics committee.

Data harmonization and exposure definition

The cohort studies collected sociodemographic and lifestyle information at baseline through in-person interviews or structured self-administered questionnaires. In case-control studies, this information was gathered from cases and controls with reference to the period 1–2 y before enrolment. Dietary variables were ascertained using food frequency questionnaires. A multistep iterative data-harmonization procedure was applied, reconciling each study's unique protocols and data collection instruments [8,47,48]. Multiple quality-control checks were performed, and outlying values of variables were truncated to the minimum or maximum value of an established range for each variable. Variables were combined into a single dataset with standardized definitions, coding, and permissible values.

The exposure of interest was alcohol consumption, assessed using food frequency questionnaires and diet histories [49–51]. Participants were subsequently categorized into nondrinkers ($\leq$1 g/d accounting for trace alcohol from fermented foods), light (<7 g/d), moderate (7–28 g/d), and heavy (>28 g/d) drinkers, following the definitions used by the National Center for Health Statistics of the US Centers for Disease Control and Prevention. An intake of 14 g of alcohol per day is approximately equivalent to 1 standard alcoholic drink.

Tumor molecular subtypes of CRC

Testing for MSI, CIMP, and mutations in the *BRAF* and *KRAS* genes was conducted previously by each study and according to individual study protocols (Appendix 2). Briefly, MSI testing was primarily conducted using polymerase chain reaction following accepted guidelines (CCFR, CPS-II, MCCS, HPFS, and NHS) [52], with $\geq$4 interpretable markers typically required to classify tumors. DALS and DACHS used a mononucleotide panel of 3 and 2 markers, respectively. Tumors were classified as MSI-high if $\geq$30% of the markers showed instability. Other studies used immunohistochemistry for the correlated DNA mismatch repair proteins (NSHDS, EPIC Sweden, and subsets of CCFR and MCCS). Studies assessed *BRAF* and *KRAS* mutations using polymerase chain reaction, sequencing, and immunohistochemistry. Most studies evaluated *BRAF* c.1799T>A (p.V600E) mutations in exon 15 and *KRAS* mutations in codons 12 and 13, although any mutation identified by 1 of the studies in the *BRAF* and *KRAS* genes was included. CIMP status was determined using methylation analyses. The CCFR, CPS-II, HPFS, MCCS, NSHDS, EPIC Sweden, and NHS used MethyLight to determine CIMP status. CPS-II, HPFS, NSHDS, EPIC Sweden, and NHS used an 8-gene panel [53]; CCFR, DACHS, and MCCS used a 5-gene panel [54], and DALS determined CIMP status using a classic panel of CpG islands [55,56]. Additionally, we combined markers to create subtype classifications: subtypes 1–5 were created according to the Jass classification [57], and types 6–16 were numbered consecutively by the status of MSI, CIMP, *BRAF*, and *KRAS*.

Statistical analysis

Alcohol consumption was analyzed continuously (per 14 g/d) and categorically, as defined above. Logistic regression analysis was used to estimate the association between alcohol consumption and overall CRC. To assess potential nonlinear association, a logistic regression model with restricted cubic splines was also fitted. Knots were placed at the fifth, 50th, and 95th percentiles of alcohol consumption, with the median (50th percentile) serving as the reference point. Multinomial logistic regression models were used to estimate the relative risk ratio (RRR) for the association between alcohol consumption and CRC molecular subtypes (compared with controls) defined by tumor markers (MSI-high compared with microsatellite stable/MSI-low, CIMP-high compared with low/negative, and *BRAF* or *KRAS* mutated compared with wild type). To test for heterogeneity between subtypes, case-only multivariable logistic regression models were used. The multivariable models included study, age (continuous and years), sex (males and females), smoking status (never, former, and current smokers), BMI (continuous and kg/m^2), and education (less than high school graduate, high school graduate, some college, and college graduate). We also examined the above, considering Jass types instead of each molecular subtype for those subtypes with $\geq$50 cases.

To reduce potential bias from including nondrinkers (who may differ systematically from drinkers) [58], our primary analysis was restricted to current drinkers. The analysis included only participants with complete information. Separate analyses were conducted by study design (cohort, case-control studies), sex, smoking status, folate intake, CRC anatomical site (colon, rectum), and across the 10 included studies. Two sensitivity analyses were conducted to evaluate the robustness of our findings. First, we further adjusted our main model for diabetes, physical activity, total folate intake, and red and processed meat. Second, we reran all the above analyses, including nondrinkers. We applied a Bonferroni-corrected P value threshold of 0.004 [0.05/12 tests: 4 molecular markers $\times$ 3 groups (males, females, and combined)] and 0.002 [0.05/30 tests: 10 Jass types $\times$ 3 groups (males, females, and combined)] to assess statistical significance in primary subtype and Jass type analyses, respectively. For analyses, including overall CRC and subgroup evaluations, a 2-sided P value $<$ 0.05 was considered statistically significant. Analyses were performed using R v4.3.1 (R Foundation for Statistical Computing).

Mendelian randomization

Alcohol consumption and selection of genetic instruments

Genetic instruments for alcohol consumption were selected from the genome-wide association study (GWAS) by Liu et al. [59], which

included 941,280 individuals of European ancestry. Assessment of alcohol intake varied across studies included in the GWAS, typically asking about mean weekly intake over the past week or year. Alcohol consumption was therefore harmonized and defined as the mean number of drinks per week (combining all beverage types) and log-transformed before GWAS analysis to reduce the influence of outliers and minimize undue leverage on model estimates. One SD corresponds to ~2.9 drinks per week. We used genome-wide significant ($P < 5 \times 10^{-8}$), independent ($r^2 < 0.001$, clumping window: 10,000 kb) single-nucleotide polymorphisms (SNPs) associated with the phenotype. SNPs were harmonized with the outcome dataset, ensuring alignment of effect alleles and removal of palindromic SNPs with intermediate allele frequencies. The strength of the instruments was evaluated using F-statistics, with all SNPs exceeding the conventional threshold of $F > 10$, minimizing the risk of weak instrument bias [60].

CRC and molecular subtypes

Summary data for CRC molecular subtypes were drawn from a GWAS meta-analysis of 10 studies with participants of European descent [CCFR [45], CPS-II [35,36], NHS [37], HPFS, DACHS [41,42], DALS [43,44], Early Detection Research Network (EDRN) [61], EPIC Sweden [38], MCCS [39], NSHDS [40]] within the CCFR and the GECCO consortia (Supplemental Table 1). The current study included 10,472 controls and 8178 CRC cases with available information on the 4 molecular markers (Supplemental Tables 2 and 3). Polytomous regressions were performed for all Jass types and individual tumor markers, adjusting for age at diagnosis or selection, sex, GWAS set, and 3 principal components to account for underlying population structures.

Statistical analysis

The primary method was random-effects inverse variance–weighted (IVW) MR [62]. To account for potential horizontal pleiotropy, 2 MR sensitivity analyses (MR-Egger [63], weighted median [64]) were performed, each providing a valid MR estimate under different combinations of assumptions. We additionally implemented the MR pleiotropy residual sum and outlier test to detect and exclude potential outlying genetic variants [65]. Finally, the MR-accounting for Pleiotropy and Sample Structure (APSS) method was applied, which accounts for pleiotropy and sample structure using genome-wide summary statistics. It employs a foreground–background model to decompose observed SNP effect estimates, where the background component captures latent confounding, including polygenicity, correlated pleiotropy, and sample structure (e.g., population stratification, cryptic relatedness, and sample overlap), under linkage disequilibrium (LD) score regression assumptions. The foreground component is then used to estimate the causal effect while allowing for uncorrelated pleiotropy. MR analyses were performed using R v4.3.1 (R Foundation for Statistical Computing) and the "TwoSampleMR" and "MR-APSS" packages.

Results

Observational analysis

Study population characteristics

The study sample comprised 11,826 CRC cases and 10,888 controls from 10 observational studies (Table 1). Compared with controls, individuals with a CRC diagnosis were more likely to be former or current smokers (53.5% compared with 50.7%), obese (21.7% compared with 16.2%), and to have a first-degree relative with CRC (21% compared with 10%). Among cases, 14.7% were MSI-H (n =1739), 12.1% were CIMP-high (n = 1431), 10.9% were *BRAF* mutated (n = 1285), and 25.6% were *KRAS* mutated (n = 3026), with type 4 (n = 3381; 28.6%) being the most common Jass type. Compared with individuals without CRC, those with CRC were slightly more likely to report no alcohol consumption (40.2% compared with 37.2%) and heavy alcohol consumption (>28 g/d; 12.3% compared with 10.2%), and less likely to report moderate consumption of 7–28 g/d (21.3% compared with 24.9%). The characteristics by alcohol consumption category are also presented in Supplemental Table 4.

Alcohol consumption and molecular subtypes

Among drinkers, a 14 g/d increase (~1 drink/d) in alcohol consumption was linearly associated with a 10% higher risk of CRC [odds ratio (OR) = 1.10; 95% confidence interval (CI): 1.07, 1.13], with no evidence of nonlinearity (P-nonlinearity = 0.46) (Table 2 and Supplemental Figure 1A). When nondrinkers were included in the analysis, a J-shaped association was observed between alcohol consumption and overall CRC risk (P-nonlinearity = 0.002) (Supplemental Table 5 and Supplemental Figure 1B). Compared with light drinkers, both nondrinkers (OR = 1.11; 95% CI: 1.03, 1.20) and heavy drinkers (OR = 1.38; 95% CI: 1.24, 1.54) exhibited higher CRC risks, and light drinking was not associated with risk.

In both analyses, restricted to drinkers and including nondrinkers, alcohol consumption was positively associated with all examined molecular subtypes of CRC when compared with controls (Table 2 and Supplemental Table 5). Case-only heterogeneity tests showed no significant differences by any molecular subtype. The above associations did not vary by sex (Supplemental Table 5), smoking status (Supplemental Tables 6 and 7), folate intake (Supplemental Tables 8 and 9), tumor anatomical site (Supplemental Tables 10 and 11), study design (Supplemental Tables 12 and 13), across individual studies (Supplemental Table 14), or early/late-onset (Supplemental Table 15) (all P-interaction > 0.05). Further adjustment for additional potential confounders did not materially alter the results compared with the model with the initial adjustments (Supplemental Table 16).

Of the 16 possible combined CRC subtypes defined by MSI, CIMP, *BRAF,* and *KRAS* status, 10 subtypes had ≥50 cases and were included in the Jass classification analysis. When compared with controls, alcohol consumption (per 14 g/d increase) was associated with a higher risk of Jass types 3 (OR = 1.10; 95% CI: 1.05, 1.15), 4 (OR = 1.12; 95% CI: 1.09, 1.16), 9 (OR = 1.15; 95% CI: 1.03, 1.28), and 14 (OR = 1.31; 95% CI: 1.14, 1.50), with consistent finding across sexes. However, no heterogeneity was detected in case-only analyses across Jass subtypes, either in the sex-combined or sex-specific models, after correcting for multiple comparisons (P-heterogeneity >0.002) (Figure 1).

MR analysis

Alcohol consumption and molecular subtypes

Thirty-seven SNPs were used as genetic instruments for alcohol consumption, with the F-statistics ranging from 28.4 to 964.4, indicating sufficient instrument strength. Higher genetically predicted alcohol intake per week (per 1-SD increase in log-transformed drinks per week) was associated with a higher overall CRC risk (OR_{IVW}=1.25, 95% CI: 1.01, 1.57) (Figure 2A, Supplemental Table 17). When stratified by molecular subtypes, positive associations

TABLE 1
Baseline characteristics of cases and controls used in observational analysis

Characteristics	Cases (n = 11,826)	Controls (n = 10,888)
Study		
CCFR Australia	1349 (11.4)	179 (1.6)
CCFR Ontario	1708 (14.4)	1222 (11.2)
CCFR Seattle	1847 (15.6)	747 (6.9)
CPS-II	860 (7.3)	1003 (9.2)
DACHS	2009 (17)	2789 (25.6)
DALS	1095 (9.3)	1162 (10.7)
EPIC Sweden	146 (1.2)	381 (3.5)
HPFS	629 (5.3)	602 (5.5)
MCCS	490 (4.1)	674 (6.2)
NFCCR	573 (4.8)	472 (4.3)
NHS	793 (6.7)	1242 (11.4)
NSHDS	327 (2.8)	415 (3.8)
Age,[1] mean (SD), y	62 (13)	66 (11)
Unknown, *n* (%)	140 (1.2)	<5
Sex		
Males	6117 (51.7)	5523 (50.7)
Females	5709 (48.3)	5365 (49.3)
Alcohol consumption, mean (SD), g/d	11 (21)	10 (16)
Nondrinker	4750 (40.2)	4048 (37.2)
<7 g/d (<0.5 drink/d)	2.555 (21.6)	2592 (23.8)
7–28 g/d (0.5–2 drinks/d)	5230 (21.3)	2716 (24.9)
>28 g/d (>2 drinks/d)	1451 (12.3)	1113 (10.2)
Unknown, *n* (%)	556 (4.7)	419 (3.8)
Smoking status		
Never smoker	4843 (41)	4999 (45.9)
Former smoker	4612 (39)	4345 (39.9)
Current smoker	1718 (14.5)	1181 (10.8)
Unknown, *n* (%)	653 (5.5)	363 (3.3)
BMI (kg/m^2)		
Mean (SD)	27.1 (4.9)	26.3 (4.3)
Underweight (<18.5)	138 (1.2)	99 (0.9)
Normal (18.5 to <24.9)	3953 (33.4)	4348 (39.9)
Overweight (25–30)	4798 (40.6)	4511 (41.4)
Obese (>30)	2561 (21.7)	1762 (16.2)
Unknown, *n* (%)	376 (3.2)	168 (1.5)
Dietary intake, mean (SD)		
Red meat (servings/d)	0.8 (0.6)	0.7 (0.6)
Unknown, *n* (%)	751 (6.4)	629 (5.8)
Processed meat (servings/d)	0.4 (0.4)	0.4 (0.4)
Unknown, *n* (%)	4445 (38)	2000 (18)
Fruits (servings/d)	1.7 (1.5)	1.8 (1.6)
Unknown, *n* (%)	819 (6.9)	738 (6.8)
Vegetables (servings/d)	2.3 (1.9)	2.4 (2)
Unknown, *n* (%)	629 (5.9)	695 (6.4)
Fiber (g/d)	23 (11)	23 (10)
Unknown, *n* (%)	5849 (49)	4161 (38)
Total folate intake, (μg/d)	572 (599)	680 (605)
Unknown, *n* (%)	2009 (17)	2789 (26)
Education level		
Less than high school graduate	2287 (19.3)	1786 (16.4)
High school graduate or completed GED	2827 (23.9)	2610 (24)
Some college or technical school	2943 (24.9)	2304 (21.2)
College graduate	3380 (28.6)	3992 (36.7)
Unknown, *n* (%)	389 (3.3)	196 (1.8)
First-degree relative with CRC	2487 (21)	1084 (10)
Unknown, *n* (%)	417 (3.5)	543 (5)
Location of CRC		
Distal colon	3570 (30.2)	—
Proximal colon	4502 (38.1)	—
Rectum (including rectosigmoid junction)	3341 (28.3)	—
Unknown, *n* (%)	413 (3.5)	—
CRC stage		
Stage 1 or local	2732 (23.1	—
Stage 2/3 or regional	6829 (57.7)	—
Stage 4 or distant	1236 (10.5)	—
Unknown, *n* (%)	1029 (8.7)	—
BRAF		
Wild type	9469 (80.1)	—
Mutated	1285 (10.9)	—
Unknown, *n* (%)	1072 (9.1)	—
KRAS		
Wild type	6135 (51.9)	—
Mutated	3026 (25.6)	—
Unknown, *n* (%)	2665 (22.5)	—
Microsatellite instability (MSI)		
MSS/MSI-L	9097 (76.9)	—
MSI-H	1739 (14.7)	—
Unknown, *n* (%)	990 (8.4)	—
CIMP		
Low/negative	7338 (62)	—
High	1431 (12.1)	—
Unknown, *n* (%)	3057 (25.8)	—
Jass type (subtype combinations)		
[1] MSI-high, CIMP-high, *BRAF* mutated, *KRAS* wildtype	471 (4)	—
[2] MSS/MSI-low, CIMP-high, *BRAF* mutated, *KRAS* wildtype	212 (1.8)	—
[3] MSS/MSI-low, CIMP-low/negative, *BRAF* wildtype, *KRAS* mutated	1983 (16.8)	—
[4] MSS/MSI-low, CIMP-low/negative, *BRAF* wildtype, *KRAS* wildtype	3381 (28.6)	—
[5] MSI-high, CIMP-low/negative, *BRAF* wildtype, *KRAS* wildtype	231 (2)	—
[6] MSS/MSI-low, CIMP-low/negative, *BRAF* mutated, *KRAS* wildtype	187 (1.6)	—
[7] MSS/MSI-low, CIMP-low/negative, *BRAF* mutated, *KRAS* mut	25 (0.2)	—
[8] MSS/MSI-low, CIMP-high, *BRAF* wildtype, *KRAS* wildtype	147 (1.2)	—
[9] MSS/MSI-low, CIMP-high, *BRAF* wildtype, *KRAS* mutated	216 (1.8)	—
[10] MSS/MSI-low, CIMP-high, *BRAF* mutated, *KRAS* mut	3 (<0.001)	—
[11] MSI-high, CIMP-low/negative, *BRAF* wildtype, *KRAS* mutated	131 (1.1)	—
[12] MSI-high, CIMP-low/negative, *BRAF* mutated, *KRAS* wildtype	35 (0.3)	—
[13] MSI-high, CIMP-low/negative, *BRAF* mutated, *KRAS* mutated	3 (<0.001)	—
[14] MSI-high, CIMP-high, *BRAF* wildtype, *KRAS* wildtype	143 (1.2)	—
[15] MSI-high, CIMP-high, *BRAF* wildtype, *KRAS* mutated	26 (0.2))	—
[16] MSI-high, CIMP-high, *BRAF* mutated, *KRAS* mutated	10 (0.1)	—
Unknown, *n* (%)	4622 (39.1)	—

Abbreviations: CCFR, Colon Cancer Family Registry; CIMP, CpG island methylator phenotype; CPS-II, Cancer Prevention Study II; CRC, Colorectal Cancer; DACHS, Darmkrebs: Chancen der Verhütung durch Screening Study; DALS, Diet, Activity and Lifestyle Study; EPIC, European Prospective Investigation into Cancer and Nutrition; GED, general educational development; HPFS, Health Professionals Follow-up Study; MCCS, Melbourne Collaborative Cohort Study; MSI, Microsatellite Instability; MSS, Microsatellite Stable; NFCCR, Newfoundland Familial Colorectal Cancer Registry; NHS, Nurses' Health Study; NSHDS, Northern Sweden Health and Disease Study.

[1] Age at diagnosis (cases) and selection (controls).

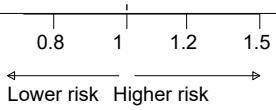
TABLE 2
Association between alcohol consumption, colorectal cancer, and its molecular subtypes among drinkers only according to the observational analysis[1]

Alcohol consumption	Overall CRC OR (95% CI)	*BRAF*		*KRAS*		CpG island methylator phenotype		Microsatellite instability	
		BRAF-wild type RRR (95% CI)	*BRAF* mutated RRR (95% CI)	*KRAS*-wild type RRR (95% CI)	*KRAS* mutated RRR (95% CI)	CIMP-low/negative RRR (95% CI)	CIMP-high RRR (95% CI)	MSS/MSI-L RRR (95% CI)	MSI-H RRR (95% CI)
Sex-combined									
n cases	6022	4881	587	3133	1592	3900	686	4747	835
<7 g/d (<0.5 drink/d)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
7–28 g/d (0.5–2 drinks/d)	1.00 (0.82, 1.09)	1.00 (0.91, 1.10)	0.97 (0.80, 1.19)	1.02 (0.92, 1.14)	0.90 (0.79, 1.03)	1.01 (0.92, 1.12)	0.94 (0.78, 1.13)	1.00 (0.91, 1.09)	1.01 (0.85, 1.20)
>28 g/d (>2 drinks/d)	1.34 (1.20, 1.50)	1.35 (1.20, 1.51)	1.22 (0.94, 1.58)	1.34 (1.18, 1.53)	1.28 (1.09, 1.51)	1.34 (1.19, 1.52)	1.26 (1.00, 1.60)	1.38 (1.23, 1.54)	1.19 (0.95, 1.49)
Per 14 g/d (1 drink/d)	1.10 (1.07, 1.13)	1.10 (1.07, 1.13)	1.05 (0.98, 1.12)	1.10 (1.07, 1.14)	1.10 (1.06, 1.14)	1.10 (1.07, 1.13)	1.09 (1.03, 1.16)	1.11 (1.07, 1.14)	1.07 (1.02, 1.12)
P-heterogeneity	—	0.1		0.9		0.78		0.16	
Males									
n cases	3803	3161	284	1983	993	2560	352	3071	461
<7 g/d (<0.5 drink/d)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
7–28 g/d (0.5–2 drinks/d)	1.08 (0.96, 1.21)	1.09 (0.96, 1.23)	1.08 (0.81, 1.45)	1.08 (0.94, 1.24)	1.01 (0.84, 1.20)	1.07 (0.94, 1.22)	0.98 (0.74, 1.28)	1.08 (0.96, 1.22)	1.03 (0.81, 1.31)
>28 g/d (>2 drinks/d)	1.43 (1.26, 1.64)	1.46 (1.27, 1.68)	1.24 (0.88, 1.74)	1.40 (1.19, 1.64)	1.41 (1.16, 1.72)	1.42 (1.23, 1.65)	1.28 (0.95, 1.74)	1.47 (1.28, 1.69)	1.10 (0.83, 1.46)
Per 14 g/d (1 drink/d)	1.11 (1.08, 1.14)	1.12 (1.08, 1.15)	1.06 (0.99, 1.14)	1.11 (1.08, 1.15)	1.11 (1.07, 1.16)	1.11 (1.07, 1.15)	1.11 (1.04, 1.19)	1.12 (1.08, 1.15)	1.06 (1.00, 1.13)
P-heterogeneity	—	0.14		1		0.95		0.06	
Females									
n cases	2219	1720	303	1150	599	1340	334	1676	374
<7 g/d (<0.5 drink/d)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
7–28 g/d (0.5–2 drinks/d)	0.91 (0.79, 1.04)	0.86 (0.75, 0.99)	0.84 (0.65, 1.09)	0.90 (0.77, 1.06)	0.78 (0.64, 0.95)	0.87 (0.75, 1.01)	0.90 (0.70, 1.15)	0.86 (0.74, 0.98)	0.88 (0.69, 1.11)
>28 g/d (>2 drinks/d)	1.21 (0.97, 1.52)	1.10 (0.86, 1.40)	1.14 (0.74, 1.75)	1.15 (0.88, 1.50)	1.06 (0.76, 1.49)	1.12 (0.87, 1.45)	1.17 (0.77, 1.76)	1.12 (0.88, 1.42)	1.39 (0.94, 2.06)
Per 14 g/d (1 drink/d)	1.05 (0.98, 1.12)	1.06 (0.99, 1.13)	1.01 (0.89, 1.16)	1.06 (0.98, 1.14)	1.04 (0.94, 1.14)	1.06 (0.98, 1.14)	1.03 (0.91, 1.17)	1.05 (0.98, 1.12)	1.10 (0.98, 1.23)
P-heterogeneity	—	0.55		0.78		0.59		0.72	

Abbreviations: CI, confidence interval; CIMP, CpG island methylator phenotype; CRC, colorectal cancer; MSI, microsatellite instability; MSS, microsatellite stable; OR, odds ratio; RRR, relative risk ratio.
[1] Controls were used as the reference for all effect estimates. Models were adjusted for the study population, age, sex (when not stratified), smoking status, alcohol consumption, education, BMI, and red meat intake. Case-only analysis used to calculate *P*-heterogeneity.

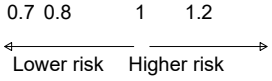
A) Both sexes

Jass−type	Cases	RRR* (95% CI)	Pvalue	Pheterogeneity
[4] MSS/MSI−low, CIMP-low/negative, *BRAF* wt, *KRAS* wt	1772	1.12 (1.09 to 1.16)	<0.001	Reference
[3] MSS/MSI−low, CIMP-low/negative, *BRAF* wt, *KRAS* mut	1051	1.10 (1.05 to 1.15)	<0.001	0.44
[1] MSI−high, CIMP-high, *BRAF* mut, *KRAS* wt	195	1.05 (0.94 to 1.18)	0.39	0.30
[5] MSI−high, CIMP-low/negative, *BRAF* wt, *KRAS* wt	117	1.03 (0.91 to 1.16)	0.66	0.33
[9] MSS/MSI−low, CIMP-high, *BRAF* wt, KRAS mut	117	1.15 (1.03 to 1.28)	0.01	0.62
[2] MSS/MSI−low, CIMP-high, *BRAF* mut, *KRAS* wt	108	1.01 (0.87 to 1.17)	0.94	0.38
[6] MSS/MSI−low, CIMP-low/negative, *BRAF* mut, *KRAS* wt	91	0.89 (0.73 to 1.08)	0.24	0.05
[8] MSS/MSI−low, CIMP-high, *BRAF* wt, *KRAS* wt	80	1.14 (1.00 to 1.30)	0.05	0.77
[11] MSI−high, CIMP-low/negative, *BRAF* wt, *KRAS* mut	71	0.93 (0.77 to 1.13)	0.47	0.12
[14] MSI−high, CIMP-high, *BRAF* wt, *KRAS* wt	60	1.31 (1.14 to 1.50)	<0.001	0.06



B) Males

Jass−type	Cases	RRR* (95% CI)	Pvalue	Pheterogeneity
[4] MSS/MSI−low, CIMP-low/negative, *BRAF* wt, *KRAS* wt	1206	1.14 (1.10 to 1.19)	<0.001	Reference
[3] MSS/MSI−low, CIMP-low/negative, *BRAF* wt, *KRAS* mut	643	1.12 (1.06 to 1.17)	<0.001	0.35
[5] MSI−high, CIMP-low/negative, *BRAF* wt, *KRAS* wt	74	1.01 (0.86 to 1.18)	0.92	0.19
[1] MSI−high, CIMP-high, *BRAF* mut, *KRAS* wt	73	1.09 (0.95 to 1.25)	0.22	0.45
[9] MSS/MSI−low, CIMP-high, *BRAF* wt, *KRAS* mut	69	1.18 (1.05 to 1.32)	0.01	0.58
[2] MSS/MSI−low, CIMP-high, *BRAF* mut, *KRAS* wt	55	1.06 (0.90 to 1.25)	0.47	0.40
[8] MSS/MSI−low, CIMP-high, *BRAF* wt, *KRAS* wt	53	1.19 (1.04 to 1.35)	0.01	0.59
[11] MSI−high, CIMP-low/negative, *BRAF* wt, *KRAS* mut	50	0.84 (0.66 to 1.08)	0.17	0.02



C) Females

Jass−type	Cases	RRR* (95% CI)	Pvalue	Pheterogeneity
[4] MSS/MSI−low, CIMP-low/negative, *BRAF* wt, *KRAS* wt	566	1.14 (1.10 to 1.19)	<0.001	Reference
[3] MSS/MSI−low, CIMP-low/negative, *BRAF* wt, *KRAS* mut	408	1.12 (1.06 to 1.17)	<0.001	0.61
[1] MSI−high, CIMP-high, *BRAF* mut, *KRAS* wt	122	1.09 (0.95 to 1.25)	0.22	0.54
[2] MSS/MSI−low, CIMP+, *BRAF* mut, *KRAS* wt	53	1.06 (0.90 to 1.25)	0.47	0.83

*Per 14g/day (1 drink/day) increase

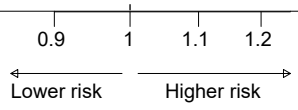


FIGURE 1. Association between alcohol consumption [per 14 g/d (1 drink/d) increase] and Jass classified types of colorectal cancer, overall and stratified by sex, according to the observational analysis. Controls were used as the reference for all relative risk ratios. The models were adjusted for age, sex, study population, BMI, smoking status, and education. Jass types with >50 cases were included. *P* value was calculated using multinomial logistic regression, comparing CRC cases to cancer-free controls separately for each defined Jass type. *P*-heterogeneity was calculated using multinomial logistic regression, comparing cases of each Jass type to all additional cases not belonging to that type. CI, confidence interval; CIMP, CpG island methylator phenotype; CRC, colorectal cancer; MSI, microsatellite instability; MSS, microsatellite stable; OR, odds ratio; RRR, relative risk ratio.

were observed across all subtypes, including MSI status, CIMP status, *BRAF*, and *KRAS* mutation status; however, these did not reach statistical significance, likely due to limited statistical power. No evidence of heterogeneity was detected across all subtypes (*P*-heterogeneity > 0.05). Identical findings were found when we employed the MR-APSS method (Supplemental Table 17). Similarly, in Jass type stratified analyses, all examined subtypes showed directionally positive associations, with no significant heterogeneity compared with the reference Jass type 4 (Figure 2B, Supplemental Table 18).

Discussion

In this large, pooled observational and MR study, we found consistent evidence that higher alcohol consumption is associated with

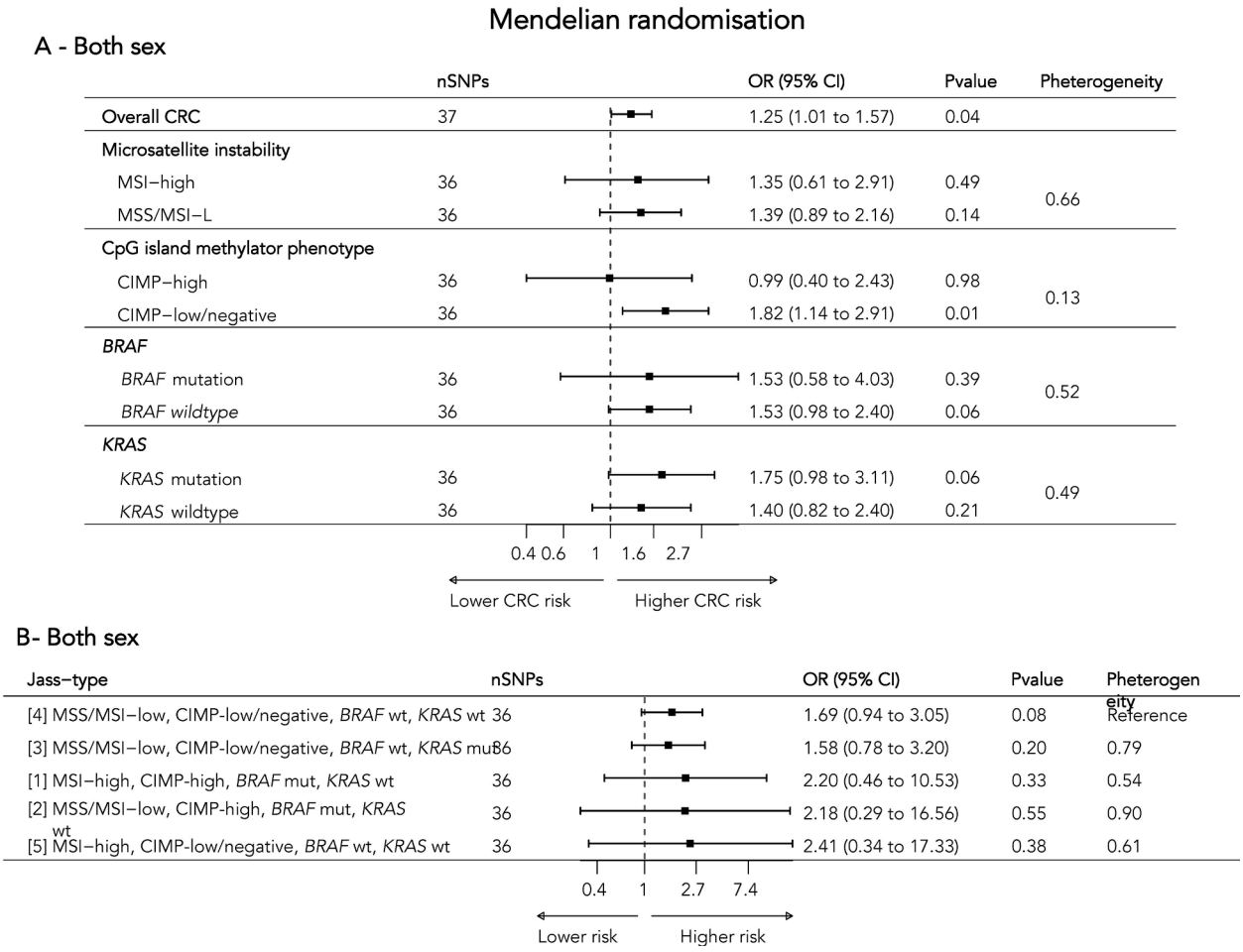


FIGURE 2. The association of genetically predicted alcohol intake (per 1-SD Increase In log-transformed drinks per week) with (A) CRC risk overall and by individual tumor molecular markers and (B) Jass classified types of CRC, according to the 2-sample Mendelian randomization inverse variance–weighted analysis. *P value was calculated using the inverse variance–weighted method, comparing CRC cases to cancer-free controls.* *P*-heterogeneity was calculated using the inverse variance–weighted method, comparing cases of each molecular subtype. CI, confidence interval; CIMP, CpG island methylator phenotype; CRC, colorectal cancer; MSI, microsatellite instability; MSS, microsatellite stable; OR, odds ratio; SNP, single-nucleotide polymorphism.

a higher risk of CRC. Among drinkers, each additional 14 g/d of alcohol (~1 drink/d) was associated with a 10% higher CRC risk, but this association was primarily driven by heavy alcohol consumption (>28 g/d). A J-shaped association was observed when nondrinkers were included. Alcohol consumption was positively associated with all major molecular subtypes across *BRAF*, *KRAS*, CIMP, or MSI status, with no significant heterogeneity by subtype. Results were consistent across strata defined by smoking, folate intake, tumor site, study design, and sex. Consistently, the MR analysis found no evidence of heterogeneity in the association between alcohol consumption and CRC risk across molecular subtypes.

Although previous research has shown subtype-specific associations for exposures such as smoking [19], the evidence for alcohol remains limited and inconclusive. In this study, we found that alcohol consumption was associated with a higher risk of CRC, consistently across individuals with tumors defined by CIMP, MSI, *BRAF*, and *KRAS* status, without evidence of heterogeneity. These findings are broadly consistent with a body of molecular pathological epidemiology literature, although previous studies vary in design, population, and methodological detail. Our findings are consistent with those of Razzak et al. [26], who reported no associations specific to molecular subtypes, including those defined by MSI and CIMP status, in the Iowa Women's Health Study. Our results also align with those of 3 prior studies based on cohorts included in our pooled analysis. Amitay et al. [23] found no heterogeneity by CIMP, *BRAF*, or *KRAS* status in the DACHS study, and Schernhammer et al. [25] reported no differences by CIMP status in the NHS. In contrast, Jayasekara et al. [24] found an association with *BRAF* wildtype [hazard ratio (HR) = 1.06, 95% CI: 1.01, 1.11] but not with *BRAF* mutated (HR = 0.89, 95% CI: 0.78, 1.01) tumors (*P*-heterogeneity = 0.003) in the MCCS, although their analysis was based on a smaller sample. Other investigations did not report associations between alcohol consumption and risk of specific CRC molecular subtypes compared with controls; however, they did not examine for heterogeneity by mutation status within cases [30, 32–34].

The consistency of our findings across all examined molecular subtypes suggests that alcohol may increase CRC risk through common alcohol-related mechanisms rather than by acting specifically through epigenetic or mismatch repair-related pathways. Several factors may contribute to the absence of meaningful heterogeneity by subtype. First, alcohol-related mechanisms such as acetaldehyde exposure, oxidative stress, chronic inflammation, and impaired DNA

repair are thought to operate broadly and may promote colorectal carcinogenesis irrespective of downstream molecular characteristics. Second, although we examined established molecular subtypes for CRC, these classifications may not fully capture etiologically relevant heterogeneity in alcohol-related carcinogenesis.

Our study included a large number of cases with complete molecular data, allowing us to examine alcohol-related CRC risk across CIMP, MSI, *BRAF*, and *KRAS* subtypes with higher power to test for potential heterogeneity. Unlike earlier studies that often focused on a single cohort or used only binary (ever/never) definitions of alcohol consumption, we used harmonized alcohol exposure categories and molecular subtype definitions across multiple studies, enabling more detailed, stratified, and pooled analyses. Taken together, our results inform mechanistic pathways and provide robust evidence that alcohol contributes to CRC development, irrespective of molecular subtype. These findings support the need for broad-based alcohol reduction strategies as part of public health efforts to lower the overall burden of CRC. Although we evaluated well-established molecular subtypes (defined by CIMP, MSI, *BRAF*, and *KRAS*), a more comprehensive tumor characterization—including genome-wide DNA methylation profiling, histone modification analysis, gene expression studies, immune profiling, and spatial transcriptomics—could provide deeper insights into subtype-specific associations.

Although the precise biological mechanisms linking alcohol consumption to CRC development remain incompletely understood, several plausible pathways have been proposed. A central hypothesis involves the metabolic conversion of ethanol to acetaldehyde and the concurrent generation of reactive oxygen species, both of which can damage critical cellular components, including DNA, proteins, and lipids, leading to cellular dysfunction and increased oncogenic potential [66,67]. One proposed mechanism suggests that acetaldehyde disrupts mucosal adhesion in the colon by promoting tyrosine phosphorylation, particularly in the presence of specific gut microbiota that facilitate its accumulation within the colonic lumen [68]. This disruption compromises the integrity of adhesive proteins within the epithelium [69], thereby weakening intercellular junctions and enhancing cellular proliferation and migration processes central to tumor initiation and metastasis. Additionally, chronic alcohol consumption has been associated with altered folate metabolism [69–71], a micronutrient vital for DNA synthesis, repair, and methylation. Impaired folate status may therefore contribute to genomic instability and colorectal carcinogenesis. Moreover, alcohol-induced alterations in the gut microbiome—referred to as dysbiosis—may further promote inflammatory and carcinogenic processes within the colon [72].

To our knowledge, this is the largest study examining the associations between alcohol consumption and CRC molecular subtypes. A major strength of our study is the ability to pool individual-level data from 10 observational studies with available information on alcohol consumption measurements and tumor-marker status, providing insights into how it is associated with different pathways of colorectal tumorigenesis. Another key strength is the triangulation of evidence through a pooled observational and MR analysis. Triangulation integrates evidence from distinct methodologies, each with different sources of bias, thereby strengthening the robustness of the results [73]. Limitations should also be considered when interpreting the findings. Alcohol consumption was self-reported in observational studies, which may lead to underreporting, particularly among heavy drinkers, and could attenuate risk estimates. Moreover, the nondrinker category included former drinkers, some of whom may have stopped drinking due to ill health, potentially biasing comparisons; to minimize misclassification, our primary analyses focused on current drinkers. Reverse causation remains a potential concern, particularly for case-control studies, although stratified analyses by study design showed consistent results. Despite adjusting for multiple potential confounders, residual confounding cannot be entirely excluded, although sensitivity analyses were reassuring. Finally, as our study population was predominantly of European descent from the United States, Europe, Canada, and Australia, generalizability to other racial and ethnic groups may be limited.

In conclusion, this study provides evidence that heavy alcohol consumption is associated with a higher risk of CRC across all examined major molecular subtypes (*BRAF*, *KRAS*, CIMP, or MSI). These findings seem to suggest that alcohol may act through mechanisms that transcend specific tumor molecular profiles. Our results support existing public health guidelines advocating for reduced alcohol consumption and underscore the relevance of lifestyle-based prevention strategies across the 4 major molecular pathways defined by *BRAF*, *KRAS*, CIMP, and MSI status in colorectal carcinogenesis, although further stratification by alcohol-related molecular features may reveal subtype-specific differences. This analysis leverages the largest available consortium resource for molecularly characterized CRC; statistical power is inherently lower for subtype heterogeneity analyses, and modest interaction effects cannot be excluded.

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Author contributions

The authors' responsibilities were as follows – CVC, WCC, UP, AIP, KKT: designed research and designed methodology; CVC, WCC: data curation, analyzed data or performed statistical analysis, and wrote paper; UP, AIP, KKT: provided essential reagents or provided essential materials; CVC, UP, AIP, KKT: primary responsibility for final content; and all authors: conducted research and read and approved the final manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that no generative AI or AI-assisted technologies were used in the writing of the manuscr

Conflict of interest

UP was a consultant with AbbVie, and her husband holds individual stocks in the following companies: BioNTech SE—ADR, Amazon, CureVac BV, NanoString Technologies, Google/Alphabet Inc Class C, NVIDIA Corp, and Microsoft Corp. MG reports research funding from Janssen and Sunbird Bio; consulting fees from Nerviano Medical Sciences; and honoraria from PER and OncLive—all unrelated to the present work. CET is an epidemiology contractor for Pfizer, unrelated to the present study. BVG reports a lecturer honorarium from AstraZeneca AB, for educational activities unrelated to this study.

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Data availability

Tumor markers and epidemiologic data are available on request and permission. Please contact gecco@fredhutch.org to request the standardized proposal form. The principal investigators of each contributing study will evaluate and approve the proposal, and data access will be managed centrally.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2026.101308.

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