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OXFORD

Carcinogenesis, 2018, Vol. 39, No. 3, 375–388

doi:10.1093/carcin/bgy011
Advance Access publication January 30, 2018
Original Article

ORIGINAL ARTICLE

Alcohol intake, *ADH1B* and *ADH1C* genotypes, and the risk of colorectal cancer by sex and subsite in the Netherlands Cohort Study

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Abstract

The alcohol–colorectal cancer (CRC) association may differ by sex and *ADH1B* and *ADH1C* genotypes. ADH enzymes oxidize ethanol to acetaldehyde, both of which are human carcinogens. The Netherlands Cohort Study includes 120 852 participants, aged 55–69 years at baseline (1986), and has 20.3 years follow-up (case-cohort: $n_{subcohort}$ = 4774; n_{cases} = 4597). The baseline questionnaire included questions on alcohol intake at baseline and 5 years before. Using toenail DNA, available for ~75% of the cohort, we successfully genotyped six *ADH1B* and six *ADH1C* SNPs ($n_{subcohort}$ = 3897; n_{cases} = 3558). Sex- and subsite-specific Cox hazard ratios and 95% confidence intervals for CRC were estimated comparing alcohol categories, genotypes within drinkers and alcohol categories within genotype strata. We used a dominant genetic model and adjusted for multiple testing. Alcohol intake increased CRC risk in both sexes, though in women only in the (proximal) colon when in excess of 30 g/day. In male drinkers, *ADH1B* rs4147536 increased (distal) colon cancer risk. In female drinkers, *ADH1C* rs283415 increased proximal colon cancer risk. *ADH1B* rs3811802 and *ADH1C* rs4147542 decreased CRC risk in heavy (>30 g/day) and stable drinkers (compared to 5 years before baseline), respectively. Rs3811802 and rs4147542 significantly modified the alcohol-colon cancer association in women ($P_{for\ interaction}$ = 0.004 and 0.02, respectively). A difference in associations between genotype strata was generally clearer in men than women. In conclusion, men showed increased CRC risks across subsites and alcohol intake levels, while only colon cancer risk was increased in women at heavy intake levels. *ADH1B* rs3811802 and *ADH1C* rs4147542 significantly modified the alcohol–colon cancer association in women.

Introduction

Alcohol intake is a known risk factor for colorectal cancer (CRC), both the colon and rectum, and various other cancers including cancers of the oral cavity, pharynx, larynx, esophagus, liver and female breast (1,2). Cancer risk has been shown to increase as the volume of alcohol consumed increases (3). Sex differences may exist, with CRC risk being more strongly affected by alcohol intake in men than women (4) and a dose–response relation being less apparent in women than men (5). While this may be due to a restriction of range effect because women consume less alcohol than men, part of these differences may also simply be due to the scarcity of studies on the alcohol–CRC association in women (4).

The alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) gene families encode for enzymes responsible for the breakdown of alcohol in the body. Ethanol is oxidized by ADH to acetaldehyde, which is in turn oxidized by ALDH to acetate (6,7). The formation of acetaldehyde starts in the mouth and continues along the digestive tract, with the main production of

Received: October 12, 2017; **Revised:** December 19, 2017; **Accepted:** January 25, 2018

Abbreviations

ADH	alcohol dehydrogenase
ALDH	aldehyde dehydrogenase
CI	confidence interval
CRC	colorectal cancer
FFQ	food frequency questionnaire
FDR	false discovery rate
FFQ	food frequency questionnaire
NLCS	Netherlands Cohort Study
SNP	single nucleotide polymorphism

acetaldehyde occurring in the liver and the gut (1). Both ethanol and acetaldehyde have been classified as human carcinogens by the International Agency for Research on Cancer (IARC) (6). Genetic variants [i.e. single nucleotide polymorphisms (SNPs)] in *ADH* and *ALDH* genes have been shown to affect enzyme activities resulting in a slower or faster conversion of ethanol to acetaldehyde and acetaldehyde to acetate (7,8). The *ALDH2* rs671 gene variant strongly determines the slow rate at which most of the ethanol-derived acetaldehyde is oxidized, resulting in acetaldehyde accumulation when carrying the minor allele, especially when also carrying *ADH* genotypes associated with fast ethanol to acetaldehyde conversion, e.g. for *ADH1B* rs1229984. This results in the facial flushing syndrome known in Asian populations (7). In Caucasians, however, the rs671 allele causing slow oxidation of acetaldehyde is largely absent (9). *ADH1B* and *ADH1C*, on the other hand, have functional polymorphisms in Caucasian populations (7), but most previous epidemiological studies on SNPs in these genes and the risk of CRC had low sample sizes (10,11). In the absence of *ALDH* alleles causing slow oxidation of acetaldehyde, increased cancer risks may be explained by fast conversion of ethanol to acetaldehyde in the liver, resulting in higher systemic levels of acetaldehyde, potentially reaching the colorectum. Alternatively, slow conversion of ethanol to acetaldehyde in the liver results in ethanol circulating the blood for longer periods of time. Circulating ethanol may expose the colorectum to locally formed levels of acetaldehyde, depending on ADH activity in the colonic mucosa and conversion by intestinal bacteria (12–15). Therefore, in the absence of data on how much ethanol or acetaldehyde accumulates in the colorectum, an association between alcohol intake and CRC risk in both slow and fast ethanol metabolizers is conceivable.

The objective of this study was twofold in that we first aimed to investigate the alcohol–CRC association by sex and subsite, specifically addressing the limited evidence in women, and secondly we aimed to investigate whether this association was modified by genetic variants in *ADH1B* and *ADH1C*. *ADH1B* and *ADH1C* variants were also studied in relation to CRC risk by sex and subsite in drinkers, where an effect may be expected. The study setting was a prospective cohort including 120 852 participants, who were followed up for 20.3 years (16). A former study in this cohort after 13.3 years follow-up found no evidence of an influence of alcohol on CRC risk, overall and by subsite, though the number of cases among the heavy drinkers was rather limited (i.e. ≥30 g of alcohol per day), hampering sex-specific analyses (17). Another study in this cohort, investigating the alcohol–CRC association by *ADH1C* rs698 genotype strata after 7.3 years follow-up found no apparent evidence of effect modification, but the power was limited there as well (18). Therefore, we reinvestigated the alcohol–CRC association by sex and subsite and possible effect modification by rs698 and other *ADH1B* and *ADH1C* tagSNPs after 20.3 years of follow-up. Recently, Dutch recommendations with respect to alcohol intake for cancer prevention have been modified: from a maximum of two drinks daily for men and one drink daily for women to no alcohol intake for both sexes (2,19), and if consuming alcohol, no more than one drink daily (19). This revised recommendation may be particularly important for specific subgroups with an unfavorable genetic background.

Materials and methods

Design and study population

The Netherlands Cohort Study (NLCS) is a prospective cohort study which was initiated in 1986 and consists of 120 852 men and women who were aged 55–69 years old at baseline (16). Study participants completed a baseline self-administered questionnaire on dietary habits, lifestyle factors and other risk factors for cancer. For efficiency reasons, the NLCS uses a case-cohort approach in which cases are enumerated from the entire cohort and the person-time at risk is estimated from a subcohort (20). This subcohort, consisting of 5000 participants, was randomly selected immediately after baseline, independent of any exposure. After exclusion of participants who reported a history of cancer (other than skin cancer) at baseline, 4774 subcohort members were left. Follow-up of vital status and migration for these participants was done through the Central Bureau of Genealogy and the municipal population registries (>99.9% completeness). Follow-up for incident cancer cases was performed by record linkage to the Dutch Cancer Registry and PALGA (the Netherlands pathology database) (21,22) (>96% completeness) (23). After 20.3 years follow-up, 4597 CRC cases (ICD-O-3 codes C18-C20) were identified from the original cohort. The NLCS was approved by the institutional review boards of the Netherlands Organisation for Applied Scientific Research TNO (Zeist) and Maastricht University (Maastricht).

Exposure assessment

The baseline questionnaire included a 150-item semi-quantitative food frequency questionnaire (FFQ) containing questions on diet and alcohol intake. In addition to the questionnaire, participants in the NLCS were asked to return toenail clippings. Roughly 90 000 (~75%) of NLCS participants provided toenail clippings which were used as a source of DNA for genotyping (24,25). Using 20.3 years follow-up in the NLCS, DNA samples were available for 3558 CRC cases and 3897 subcohort members.

Alcohol intake and covariates

Alcohol intake during the year preceding the start of the study was measured by questions on six different types of alcohol: beer; red wine; white wine; sherry and other fortified wines; liquor types containing on average 16% alcohol; (Dutch) gin, brandy and whiskey. Participants were asked about the number of glasses they consumed during each drinking session and their usual frequency of alcohol drinking. Additionally, for the categories 'beer' and 'other alcoholic beverages', participants were asked to recall if they drank more, less, or the same amount of alcohol 5 years before baseline. The total amount of daily alcohol intake (g/day) was calculated using the information about how often the participants drank alcohol, the number of glasses they consumed during each drinking session and the types of alcohol they drank (i.e. their alcoholic content). We defined two alcohol categories: light-moderate alcohol intake as drinking >0 to <30 g of alcohol per day (>0 to <3 glasses of alcohol per day) and heavy alcohol intake as drinking 30 or more grams of alcohol per day (≥3 glasses of alcohol per day). Information on other covariates that were considered potential confounders on the basis of previous research was also available from the baseline questionnaire. The FFQ was validated against a 9-day diet record (26) and was tested for reproducibility (27). The adjusted Spearman correlation coefficient between mean daily alcohol intake assessed by the FFQ and that estimated from the 9-day diet record was 0.89 for all participants and 0.85 for users of alcoholic beverages. The absolute amount of alcohol reported in the FFQ by users of alcoholic beverages was, on average, 86% of that reported in the 9-day diet record (26).

Selection and genotyping of tagging SNPs

Tagging SNPs (tagSNPs) within *ADH1B* and *ADH1C* (including 5 kb up- and downstream) were selected as to potentially cover all of the genetic variation in these genes with a minor allele frequency of 5% or higher. In total,

13 tagSNPs were identified using the HapMap CEU (Utah Residents with Northern and Western European Ancestry) population, an r^2 threshold of 0.8 and aggressive tagging. Seven of these (i.e. rs1159918, rs2075633, rs1693439, rs9307239, rs4147536, rs3811802, rs17033) represented 84% of the genetic variation in *ADH1B* and six (i.e. rs698, rs1662033, rs3114046, rs4147542, rs283415, rs4699741) represented 96% of the genetic variation in *ADH1C* (28). SNPs were genotyped using 50 ng of toenail DNA per participant, which was carried out using the iPLEX™ assay for the MassARRAY® system (Agena Bioscience GmbH, Hamburg, Germany). Samples had a mean call rate of 97.1% (as based on the 13 SNPs in *ADH1B* and *ADH1C* studied here and 10 other SNPs that were included in the assay). SNP call rates were 94% or higher, except for rs4147542, which had a SNP call rate of 87%. Two SNPs, *ADH1C* rs4699741 and *ADH1C* rs9307239, violated Hardy–Weinberg equilibrium. When using a significance threshold of 0.05, one in twenty SNPs may be expected to show a violation on the basis of chance alone. Although two SNPs exceed this expectation by chance and we cannot check conditions needed for Hardy–Weinberg, e.g. random mating, all SNPs were genotyped using a single assay, which makes it unlikely that these violations represent genotyping errors. Therefore, we conservatively refrained from excluding these SNPs from the analysis. Genotyping for *ADH1B* rs17033 was unsuccessful (i.e. only the T allele was amplified) and therefore not included in our analyses as originally intended. Consequently, we used six *ADH1B* tagSNPs covering 76% of the genetic variation in *ADH1B* and six *ADH1C* tagSNPs covering 96% of the genetic variation in *ADH1C*.

Statistical analysis

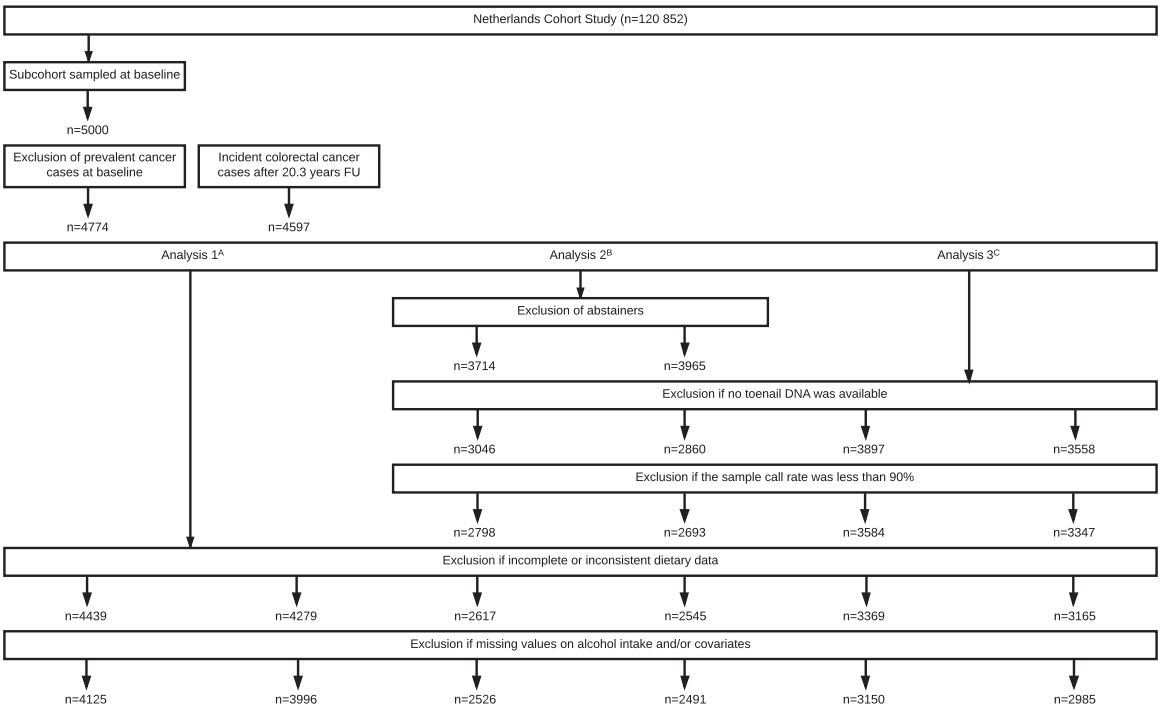
Statistical analyses were carried out using Cox regression to calculate hazard ratios (HR) and corresponding 95% confidence intervals (95% CI) for CRC by sex and subsite. Standard errors were estimated using the Huber–White sandwich estimator to account for additional variance introduced by sampling the subcohort from the entire cohort. *ADH1B* and *ADH1C* genotypes were defined according to a dominant model for reasons of power. Categories of total alcohol intake (0.1–29 and ≥30 g/day) were compared relative to abstaining (0 g/day). Trends were evaluated by including categorical variables as continuous variables in the Cox regression model. The proportional hazards assumption was tested using the scaled Schoenfeld residuals and by visually inspecting the –log–log transformed hazard curves. Multiplicative interactions were tested using the Wald test. All tests (two-tailed) were performed using Stata (version 14) and differences were regarded as statistically significant at $P < 0.05$.

Marginal effects of alcohol intake on CRC

Multivariable-adjusted models were used to study the alcohol–CRC associations. The covariates included were either *a priori*-selected risk factors based on the literature or variables that changed the HRs by at least 10% (using a backwards stepwise procedure). This resulted in the following confounder set: age (years), BMI (kg/m^2), smoking (never/ex/current), family history of CRC (yes/no), meat intake (g/day), processed meat intake (g/day), folate intake (µg/day) and physical activity based on baseline non-occupational physical activity (min/day) (29). Participants with incomplete or inconsistent questionnaires and missing information on alcohol intake and/or the predefined confounding factors were excluded from the analysis, leaving 4125 subcohort members and 3996 CRC cases (see Figure 1).

Associations between ADH1B and ADH1C tagSNPs and CRC risk in drinkers

We studied individual SNPs in relation to CRC risk in drinkers. Although small amounts of ethanol are produced endogenously, especially in the gastrointestinal tract (1), an effect of SNPs in alcohol-metabolizing genes may be expected in drinkers foremost as this is the group where the substrate (alcohol) is available. We conservatively refrained from adjusting for



Abbreviation: FU, follow-up.

[A] Analysis 1 is on the marginal effects of alcohol intake on CRC.

[B] Analysis 2 is on the associations between *ADH1B* and *ADH1C* tagSNPs and CRC risk in drinkers.

[C] Analysis 3 is on effect modification of the alcohol-CRC association by *ADH1B* and *ADH1C* tagSNPs.

Figure 1. Flow diagram of available subcohort members and colorectal cancer cases, Netherlands Cohort Study, 1986–2006. FU, follow-up. [A]Analysis 1 is on the marginal effects of alcohol intake on CRC. [B]Analysis 2 is on the associations between **ADH1B** and **ADH1C** tagSNPs and CRC risk in drinkers. [C]Analysis 3 is on effect modification of the alcohol–CRC association by **ADH1B** and **ADH1C** tagSNPs.

factors other than age because it is unlikely that *ADH1B* and *ADH1C* genotypes are influenced by CRC risk factors in lifestyle and diet. Participants were excluded from the analysis if no toenail DNA sample was available, the sample call rate was less than 90%, the baseline questionnaire was incomplete or inconsistent, or information on alcohol intake was missing (see Figure 1).This resulted in 2526 subcohort members and 2491 CRC cases.

Effect modification of the alcohol–CRC association by ADH1B and ADH1C tagSNPs

Multivariable-adjusted models were used to study effect modification of the alcohol–CRC association by *ADH1B* and *ADH1C tagSNPs*, using the confounder set as described for the alcohol–CRC analyses. After excluding participants without available DNA samples, with a sample call rate of less than 90%, with incomplete or inconsistent questionnaires and without complete information on alcohol intake and/or the predefined confounding factors, 3150 subcohort members and 2985 CRC cases were left for analysis (see Figure 1).

Multiple testing

Because multiple tests were conducted within each gene, we applied the false discovery rate (FDR) control method of Benjamini and Hochberg (30,31) to address the issue of multiple testing. To accomplish this, *P*-values calculated from our analyses were ranked in ascending order. Gene- and endpoint-specific Benjamini adjusted *P*-values were calculated by dividing the *P*-value rank order by the total number of *P*-values and then multiplying this number by the FDR [i.e. the recommended 20% (32)]. If the original *P*-value was less than 0.05 and fell below the adjusted *P*-value, it was considered significant.

Sensitivity analyses

Drinking patterns over a longer duration of time may influence CRC risk differently or more profoundly than when evaluated on a single time point. For instance, stronger alcohol–CRC associations may be expected in those with a relatively constant long-term exposure to alcohol. As the NLCS has data available on alcohol intake 5 years before baseline, we conducted sensitivity analyses using these data. This included restricting the analyses on alcohol–CRC associations and effect modification to participants who reported to have had the same alcohol intake 5 years before baseline, which included abstainers on both occasions (i.e. the stable subgroup). For the SNP-CRC analyses in drinkers, this included restriction to those drinking equal amounts of alcohol 5 years before baseline (i.e. the stable drinkers). We also evaluated whether there may be a threshold level of alcohol intake at which individual SNPs start to influence CRC risk by stratifying SNP–CRC associations on alcohol intake level (light-moderate and heavy). Furthermore, because changes in reported alcohol intake may indicate underlying reasons such as health issues or exposure misclassification, possibly due to underreporting, we repeated the alcohol–CRC analyses restricting once to baseline drinkers who reported drinking less alcohol 5 years before baseline and once to baseline drinkers who reported drinking more alcohol 5 years before baseline. Finally, we checked for the risk of protopathic bias by excluding the first 2 years of follow-up (with no essential changes in results), as this may be especially likely when investigating alcohol intake in relation to cancer risk.

Results

Baseline characteristics

Table 1 shows the baseline characteristics of subcohort members and CRC cases. As regards alcohol intake, men were less often abstainers as compared to women and men were more likely to consume higher levels of alcohol than women, especially male CRC cases.

Marginal effects of alcohol intake on CRC

Alcohol intake was positively associated with CRC risk in both men and women (Table 2). In women, however, only colon cancer risks, in particular proximal colon cancer risk, were increased, but not until alcohol intake exceeded 30 g/day [$HR_{\text{heavy drinkers versus abstainers}}$ (95% CI) = 1.52 (1.03–2.24), 1.70 (1.09–2.66) and 1.25 (0.71–2.20) for colon, proximal colon and distal colon cancer, respectively]. In men, colon and rectal cancer risks were both increased and there were also non-significantly increased risks in men who consumed a light-moderate amount of alcohol versus abstainers

Table 1. Distribution of potential confounders and alcohol intake among subcohort members and CRC cases in the NLCS, 1986–2006

	Male subcohort		Male CRC cases		Female subcohort		Female CRC cases	
	N (%)	Mean (SD)	*N* (%)	Mean (SD)	*N* (%)	Mean (SD)	*N* (%)	Mean (SD)
Age (years)		61.4 (4.2)		61.8 (4.2)		61.5 (4.3)		62.1 (4.1)
Alcohol intake (g/day)								
0	329 (14.5)		303 (12.1)		731 (32.7)		599 (31.7)	
0.1–4	479 (21.1)		549 (22.0)		806 (36.0)		692 (36.6)	
5–14	621 (27.3)		630 (25.2)		417 (18.6)		348 (18.4)	
15–29	505 (22.2)		593 (23.7)		207 (9.3)		168 (8.9)	
≥30	339 (14.9)		426 (17.0)		77 (3.4)		84 (4.4)	
BMI (kg/m^2)		25.0 (2.6)		25.3 (2.7)		25.1 (3.6)		25.1 (3.5)
Smoking								
Never	300 (12.9)		313 (12.3)		1431 (58.9)		1190 (58.7)	
Ex	1175 (50.4)		1463 (57.3)		491 (20.2)		438 (21.6)	
Current	856 (36.7)		777 (30.4)		509 (20.9)		398 (19.6)	
Family history of CRC								
Yes	118 (5.1)		219 (8.6)		134 (5.5)		189 (9.3)	
No	2213 (94.9)		2336 (91.4)		2298 (94.5)		1840 (90.7)	
Meat intake (g/day)		104.9 (44.1)		105.3 (43.2)		92.2 (41.1)		90.6 (40.9)
Processed meat intake (g/day)		15.6 (16.9)		16.3 (16.8)		10.3 (11.9)		10.2 (11.3)
Folate intake (μg/day)		222 (77)		219 (72)		195 (71)		194 (71)
Non-occupational physical activity (min/day)								
≤30	447 (19.5)		431 (17.1)		639 (26.8)		596 (29.9)	
>30–60	710 (30.9)		776 (30.8)		737 (30.9)		598 (30.0)	
>60–90	419 (18.3)		497 (19.7)		518 (21.7)		451 (22.6)	
>90	719 (31.3)		815 (32.4)		490 (20.6)		351 (17.6)	

Table 2. Hazard ratios and 95% confidence intervals for the association between alcohol intake and colorectal cancer risk in men and women stratified by subsite in the NLCS, 1986–2006

	PT at risk	Colorectum			Colon			Proximal Colon			Distal Colon			Rectum		
		N cases	HR[a]	95% CI	*N* cases	HR[a]	95% CI	*N* cases	HR[a]	95% CI	*N* cases	HR[a]	95% CI	*N* cases	HR[a]	95% CI
Men																
Alcohol intake																
Abstainers (0 g/day)	4548	267	1	(ref)	176	1	(ref)	81	1	(ref)	88	1	(ref)	69	1	(ref)
Light-moderate (0.1–29 g/day)	23 074	1625	1.22	(1.00, 1.48)	1050	1.21	(0.97, 1.50)	485	1.23	(0.93, 1.63)	538	1.23	(0.93, 1.63)	388	1.10	(0.82, 1.48)
Heavy (≥30 g/day)	4852	394	1.40	(1.10, 1.78)	246	1.35	(1.03, 1.77)	109	1.30	(0.92, 1.83)	126	1.42	(1.01, 1.98)	110	1.45	(1.02, 2.07)
P for trend			0.007			0.03			0.14			0.04			0.03	
Stable subgroup[b]																
Abstainers (0 g/day)	3514	211	1	(ref)	139	1	(ref)	65	1	(ref)	68	1	(ref)	52	1	(ref)
Light-moderate (0.1–29 g/day)	14 413	991	1.17	(0.93, 1.46)	628	1.13	(0.88, 1.46)	291	1.11	(0.81, 1.54)	324	1.21	(0.88, 1.68)	242	1.14	(0.80, 1.62)
Heavy (≥30 g/day)	2615	241	1.58	(1.17, 2.14)	155	1.58	(1.13, 2.21)	69	1.47	(0.97, 2.23)	78	1.70	(1.11, 2.59)	66	1.73	(1.11, 2.70)
P for trend			0.003			0.008			0.08			0.02			0.02	
Reported drinking more alcohol 5 years before baseline																
Light-moderate (0.1–29 g/day)	3754	317	1	(ref)	207	1	(ref)	92	1	(ref)	111	1	(ref)	80	1	(ref)
Heavy (≥30 g/day)	778	42	0.56	(0.34, 0.92)	23	0.45	(0.25, 0.82)	12	0.51	(0.25,1.08)	10	0.37	(0.17,0.84)	11	0.57	(0.26,1.23)
Reported drinking less alcohol 5 years before baseline																
Light-moderate (0.1–29 g/day)	2588	151	1	(ref)	104	1	(ref)	46	1	(ref)	51	1	(ref)	28	1	(ref)
Heavy (≥30 g/day)	989	67	1.12	(0.71, 1.77)	47	1.08	(0.66, 1.78)	21	1.04	(0.52,2.08)	25	1.22	(0.67,2.23)	14	1.45	(0.65,3.23)
Women																
Alcohol intake																
Abstainers (0 g/day)	11 373	531	1	(ref)	400	1	(ref)	232	1	(ref)	154	1	(ref)	93	1	(ref)
Light-moderate (0.1–29 g/day)	23 546	1101	1.01	(0.87, 1.17)	825	1.02	(0.87, 1.20)	481	1.02	(0.84,1.23)	324	1.06	(0.85,1.33)	199	0.99	(0.75,1.31)
Heavy (≥30 g/day)	1253	78	1.46	(1.02, 2.10)	58	1.52	(1.03, 2.24)	38	1.70	(1.09,2.66)	18	1.25	(0.71,2.20)	11	1.03	(0.51,2.07)
P for trend			0.29			0.23			0.21			0.46			1.00	
Stable subgroup[b]																
Abstainers (0 g/day)	9296	441	1	(ref)	336	1	(ref)	193	1	(ref)	132	1	(ref)	72	1	(ref)
Light-moderate (0.1–29 g/day)	13 059	632	1.01	(0.84, 1.20)	475	1.02	(0.84, 1.24)	286	1.06	(0.85,1.33)	180	1.00	(0.77,1.31)	112	1.06	(0.75,1.49)
Heavy (≥30 g/day)	701	46	1.52	(0.95, 2.42)	34	1.52	(0.91, 2.54)	21	1.59	(0.88,2.88)	11	1.33	(0.64,2.76)	6	1.12	(0.44,2.85)
P for trend			0.38			0.37			0.27			0.73			0.71	
Reported drinking more alcohol 5 years before baseline																
Light-moderate (0.1–29 g/day)	2818	113	1	(ref)	83	1	(ref)	47	1	(ref)	30	1	(ref)	24	1	(ref)
Heavy (≥30 g/day)	127	7	1.51	(0.48, 4.79)	5	1.53	(0.43, 5.48)	4			1			1		
Reported drinking less alcohol 5 years before baseline																
Light-moderate (0.1–29 g/day)	2427	101	1	(ref)	77	1	(ref)	44	1	(ref)	32	1	(ref)	19	1	(ref)
Heavy (≥30 g/day)	255	16	1.72	(0.75, 3.95)	11	1.57	(0.63, 3.93)	6	2.03	(0.63, 6.55)	5	1.31	(0.41, 4.15)	3		

Results were not shown when less than five cases were available. CI, confidence interval; HR, hazard ratio; NLCS, Netherlands Cohort Study; PT, person-time; ref, reference.

[a]Adjusted for age, BMI, smoking, family history of CRC, meat intake, processed meat intake, folate intake and non-occupational physical activity.

[b]Participants who reported drinking the same amount of alcohol intake at baseline and 5 years before baseline, including those who reported to be abstainers on both occasions.

[$HR_{light\text{-}moderate\ drinkers\ versus\ abstainers}$ (95% CI) = 1.21 (0.97–1.50), 1.23 (0.93–1.63), 1.23 (0.93–1.63) and 1.10 (0.82–1.48) for colon, proximal colon, distal colon and rectal cancer, respectively]. In addition, there was a statistically significant positive linear trend across alcohol intake categories in men, except for proximal colon cancer. When restricting to the stable subgroup, more pronounced associations were observed between alcohol intake and CRC risk in men, while statistically significant associations were no longer observed in women, but this may be explained by limited power. Remarkably, male heavy drinkers as compared to light-moderate drinkers reporting more alcohol intake 5 years before baseline had decreased CRC risks across subsites. Possibly, men who still reported to be heavy drinkers at baseline endure alcohol better than light-moderate drinkers who reported more alcohol intake 5 years before baseline. In the subanalysis in male drinkers reporting less alcohol intake 5 years before baseline, HRs were around the null for heavy drinkers as compared to light-moderate drinkers. In female drinkers who reported more and those who reported less alcohol intake 5 years before baseline, (non-significantly) increased risks of CRC were observed for heavy drinkers as compared to light-moderate drinkers, though the power was limited.

Associations between *ADH1B* and *ADH1C* tagSNPs and CRC risk in drinkers

Tables 3 and 4 show associations between *ADH1B* and *ADH1C* tagSNPs and CRC risk overall and by subsite in male and female drinkers, respectively, as analyzed according to a dominant model. Only FDR significant results will be mentioned below. *ADH1B* rs4147536 was associated with the risk of colon cancer and distal colon cancer in male drinkers [$HR_{CA/AA\ versus\ CC}$ (95% CI) = 1.25 (1.05–1.48) and 1.32 (1.07–1.62), respectively]. *ADH1C* rs283415 was associated with the risk of proximal colon cancer in female drinkers [$HR_{TC/CC\ versus\ TT}$ (95% CI) = 1.39 (1.08–1.80)]. Restricting these analyses to the stable drinkers revealed a statistically significant association between *ADH1C* rs4147542 and CRC risk in women [$HR_{TC/CC\ versus\ TT}$ (95% CI) = 0.73 (0.57–0.93)]. Stratifying these analyses by alcohol intake amount (i.e. light-moderate and heavy) to evaluate a potential threshold level of alcohol intake at which individual SNPs start to influence CRC risk revealed a statistically significant association between *ADH1B* rs3811802 and CRC risk in women who were heavy drinkers at baseline (>30 g/day) [$HR_{AG/GG\ versus\ AA}$ (95% CI) = 0.19 (0.07–0.50)], while no significant associations were observed in light-moderate drinkers. The results of both sensitivity analyses are presented in Supplementary Tables 1 and 2, available at *Carcinogenesis* Online.

Effect modification of the alcohol–CRC association by *ADH1B* and *ADH1C* tagSNPs

Table 5 shows the associations between alcohol intake and CRC risk in men and women, stratified by genotype, as analyzed according to a dominant model. The alcohol–CRC associations observed in genotype strata generally aligned with overall alcohol–CRC associations. HRs around the null were observed in one stratum and a pattern of increasing risks across alcohol categories was observed in the other stratum in men, while a difference in associations between genotype strata was less clear in women. Statistically significantly increased HRs for CRC were only present when comparing heavy drinkers with abstainers. Most interactions were not significant after FDR correction except for the interactions between alcohol intake and *ADH1B* rs3811802 and *ADH1C* rs4147542 in women. Risk was strongly increased in heavy (but not light-moderate) drinkers versus abstainers in female rs3811802 AA carriers, although the CI around this estimate is large [HR (95% CI) 5.72 (2.24–14.63)], while the risk across alcohol categories in female rs3811802 AG/GG carriers remained almost unchanged. In female rs4147542 TT carriers, there was a significant positive trend in CRC risk with increasing alcohol intake [$HR_{light\text{-}moderate\ drinkers\ versus\ abstainers}$ (95% CI) 1.25 (0.98–1.59) and $HR_{heavy\ drinkers\ versus\ abstainers}$ (95% CI) 1.78 (1.01–3.14)], while a decreased risk was observed for light-moderate drinkers and an increased risk for heavy drinkers relative to abstainers in female rs4147542 TC/CC carriers [HR (95% CI) 0.78 (0.60–1.02) and HR (95% CI) 1.74 (0.92–3.31), respectively]. After restricting the analysis to the stable subgroup (Supplementary Table 3, available at *Carcinogenesis* Online), only the interaction between alcohol intake and *ADH1C* rs4147542 in relation to CRC risk in women remained significant after FDR correction. In addition, in men, results were more pronounced, showing stronger increased HRs for CRC in heavy drinkers as compared with abstainers and more significant positive trends.

Supplementary Tables 4–7, available at *Carcinogenesis* Online, show the associations between alcohol intake and the risk of CRC by subsite, i.e. the colon, proximal colon, distal colon and rectum, in men and women, stratified by genotype. After FDR correction, the only statistically significant interactions observed were those between alcohol intake and *ADH1B* rs3811802 and *ADH1C* rs4147542 in relation to (proximal) colon cancer in women, consistent with the interactions observed for CRC in women. In men, a difference in associations was observed between genotype strata, when considering alcohol intake in relation to the risk of colon and proximal colon cancer, and, in particular, rectal cancer, but less so or not in relation to distal colon cancer. In women, the power was limited in analyses for distal colon and rectal cancer, hampering a proper comparison.

Discussion

This study addressed the lack of evidence regarding alcohol intake and CRC risk in women and found alcohol to be a CRC risk factor in men and women. Associations with CRC differed by sex. Alcohol intake increased CRC risk (non-)significantly at light-moderate and heavy intake levels across subsites in men. Only when alcohol intake exceeded 30 g/day, we observed increased colon cancer risks, particularly for the proximal colon, in women. We studied associations in *ADH1B* and *ADH1C* genetic subgroups, because these may be particularly susceptible to the deleterious effects of alcohol on CRC risk. *ADH1B* rs3811802 and *ADH1C* rs4147542 modified the association between alcohol intake and the risk of colorectal, colon and proximal colon cancer in women after FDR correction. The alcohol–CRC associations observed in genotype strata generally aligned with overall alcohol–CRC associations. A difference in associations between genotype strata was generally clearer in men than women but significant effect modification was only present in women. Restricting to participants with equal alcohol intake amounts 5 years before baseline resulted in (more) significant positive linear trends across alcohol intake categories within genotype strata in men but not women. Furthermore, *ADH1B* rs4147536 and *ADH1C* rs283415—which was in strong linkage disequilibrium (LD) with the commonly investigated *ADH1C* rs698 (r^2 = 0.9) in our data—were associated with an increased cancer risk at colon subsites in male and female drinkers, respectively, after FDR correction. *ADH1B* rs3811802 and *ADH1C* rs4147542 were associated with a decreased CRC risk in female heavy and stable drinkers, respectively, after FDR correction. These results substantiate the interplay between alcohol intake, *ADH1B* and *ADH1C* in relation to CRC risk.

Table 3. Hazard ratios and 95% confidence intervals for the association between single nucleotide polymorphisms in *ADH1B* and *ADH1C* and risk of overall colorectal cancer and subtypes in male drinkers in the NLCS, 1986–2006

	Allele	PT at risk	Colorectum		Colon		Proximal colon		Distal colon		Rectum	
			N cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI
ADH1B												
rs1159918	CC	8918	638	1 (ref)	407	1 (ref)	185	1 (ref)	209	1 (ref)	155	1 (ref)
	CA + AA	13 547	960	1.01 (0.87, 1.18)	632	1.05 (0.88, 1.24)	301	1.11 (0.89, 1.38)	318	1.02 (0.83, 1.26)	244	1.05 (0.83, 1.32)
	P-value (adjusted P-value)			0.89 (0.17)		0.59 (0.15)		0.36 (0.08)		0.86 (0.18)		0.69 (0.15)
rs2075633	TT	11 627	799	1 (ref)	519	1 (ref)	254	1 (ref)	251	1 (ref)	200	1 (ref)
	TC + CC	10 879	806	1.08 (0.93, 1.25)	524	1.08 (0.91, 1.27)	234	0.98 (0.79, 1.21)	278	1.19 (0.97, 1.46)	202	1.08 (0.86, 1.35)
	P-value (adjusted P-value)			0.32 (0.07)		0.38 (0.07)		0.84 (0.18)		0.10 (0.03)		0.49 (0.10)
rs1693439	GG	19 243	1367	1 (ref)	884	1 (ref)	404	1 (ref)	456	1 (ref)	342	1 (ref)
	GA + AA	3247	237	1.03 (0.84, 1.28)	159	1.07 (0.85, 1.36)	84	1.25 (0.94, 1.67)	73	0.95 (0.71, 1.28)	59	1.02 (0.75, 1.40)
	P-value (adjusted P-value)			0.76 (0.13)		0.55 (0.12)		0.13 (0.05)		0.75 (0.15)		0.88 (0.20)
rs9307239	CC	8625	557	1 (ref)	362	1 (ref)	174	1 (ref)	182	1 (ref)	145	1 (ref)
	CT + TT	13 841	1046	1.17 (1.00, 1.37)	680	1.17 (0.99, 1.40)	313	1.12 (0.90, 1.40)	347	1.19 (0.96, 1.48)	256	1.10 (0.87, 1.39)
	P-value (adjusted P-value)			0.04 (0.02)		0.07 (0.03)		0.30 (0.07)		0.11 (0.05)		0.41 (0.05)
rs4147536	CC	14 259	975	1 (ref)	617	1 (ref)	295	1 (ref)	305	1 (ref)	251	1 (ref)
	CA + AA	8221	630	1.16 (0.99, 1.35)	426	1.25 (1.05, 1.48)	193	1.20 (0.96, 1.49)	224	1.32 (1.07, 1.62)	151	1.06 (0.84, 1.34)
	P-value (adjusted P-value)			0.06 (0.03)		0.01[b] (0.02)		0.11 (0.03)		0.01[b] (0.02)		0.60 (0.13)
rs3811802	AA	6245	454	1 (ref)	295	1 (ref)	139	1 (ref)	150	1 (ref)	119	1 (ref)
	AG + GG	16 261	1150	0.96 (0.81, 1.14)	747	0.96 (0.80, 1.16)	349	0.94 (0.75, 1.20)	378	0.96 (0.76, 1.20)	283	0.91 (0.71, 1.16)
	P-value (adjusted P-value)			0.65 (0.12)		0.66 (0.17)		0.63 (0.13)		0.72 (0.13)		0.44 (0.07)
ADH1C												
rs698	TT	7777	549	1 (ref)	368	1 (ref)	180	1 (ref)	182	1 (ref)	134	1 (ref)
	TC + CC	14 729	1053	1.01 (0.86, 1.18)	674	0.96 (0.81, 1.14)	308	0.89 (0.71, 1.11)	346	1.00 (0.81, 1.24)	267	1.05 (0.83, 1.33)
	P-value (adjusted P-value)			0.93 (0.20)		0.65 (0.20)		0.31 (0.15)		1.00 (0.20)		0.69 (0.08)
rs1662033	TT	10 409	748	1 (ref)	496	1 (ref)	238	1 (ref)	248	1 (ref)	188	1 (ref)
	TG + GG	12 079	857	0.97 (0.83, 1.13)	547	0.93 (0.78, 1.10)	250	0.88 (0.71, 1.08)	281	0.96 (0.78, 1.18)	214	0.97 (0.77, 1.22)
	P-value (adjusted P-value)			0.68 (0.13)		0.38 (0.10)		0.22 (0.13)		0.69 (0.08)		0.79 (0.13)
rs3114046	CC	19 235	1367	1 (ref)	884	1 (ref)	404	1 (ref)	456	1 (ref)	342	1 (ref)
	CT + TT	3271	238	1.03 (0.84, 1.27)	159	1.07 (0.85, 1.35)	84	1.24 (0.93, 1.65)	73	0.95 (0.71, 1.27)	60	1.04 (0.76, 1.41)
	P-value (adjusted P-value)			0.77 (0.15)		0.58 (0.17)		0.14 (0.10)		0.72 (0.10)		0.83 (0.15)
rs4147542	TT	10 907	797	1 (ref)	514	1 (ref)	245	1 (ref)	258	1 (ref)	202	1 (ref)
	TC + CC	9628	754	1.07 (0.92, 1.25)	488	1.08 (0.91, 1.28)	219	1.01 (0.81, 1.26)	254	1.12 (0.91, 1.38)	188	1.06 (0.84, 1.33)
	P-value (adjusted P-value)			0.37 (0.12)		0.40 (0.12)		0.94 (0.20)		0.28 (0.03)		0.63 (0.05)

Table 3. *Continued*

	Allele	PT at risk	Colorectum		Colon		Proximal colon		Distal colon		Rectum	
			N cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI
rs283415	TT	6975	500	1 (ref)	334	1 (ref)	168	1 (ref)	160	1 (ref)	123	1 (ref)
	TC + CC	15 531	1104	0.99 (0.84, 1.16)	709	0.95 (0.79, 1.13)	320	0.85 (0.68, 1.06)	369	1.03 (0.83, 1.29)	279	1.02 (0.80, 1.30)
	P-value (adjusted P-value)			0.88 (0.17)		0.56 (0.15)		0.15 (0.12)		0.77 (0.13)		0.90 (0.18)
rs4699741	TT	19 601	1432	1 (ref)	931	1 (ref)	437	1 (ref)	470	1 (ref)	353	1 (ref)
	TC + CC	2905	173	0.80 (0.63, 1.01)	112	0.79 (0.61, 1.03)	51	0.77 (0.55, 1.08)	59	0.83 (0.60, 1.14)	49	0.92 (0.66, 1.30)
	P-value (adjusted P-value)			0.06 (0.03)		0.08 (0.03)		0.13 (0.08)		0.25 (0.02)		0.66 (0.07)

CI, confidence interval; HR, hazard ratio; NLCS, Netherlands Cohort Study; PT, person-time; ref, reference.
[a]Age-adjusted.
[b]Significant after adjusting for multiple testing.

A potential sex difference in intake levels at which alcohol intake increases CRC risk may in part be explained by differences in drinking pattern. Men perhaps are more likely to be more regular consumers than women. Regular alcohol exposure may be especially deleterious and may also increase CRC risk at light-moderate levels (33). In addition, sex differences in first-pass metabolism of alcohol (i.e. presystemic elimination of ethanol in, predominantly, the stomach and liver (34)) and ADH activity could lead to sex differences in the CRC risk associated with alcohol intake (35). Women have prolonged, higher blood ethanol concentrations than men upon similar intake levels due to differences in elimination of alcohol (i.e. the volume distribution is higher in men than women) (35). However, based on this, one would expect women to be affected by alcohol at lower intake levels than men, whereas we found a non-significant association with CRC risk at light-moderate alcohol intake levels in men but not women. A more plausible explanation, therefore, may be that there are interactions between alcohol intake and sex-specific factors. For example, women might be protected from the adverse effects of alcohol at light-moderate intake levels through a positive relationship between alcohol intake and estradiol levels (35). Increased estradiol levels were found to be protective against CRC in women (36,37). Alternatively, as suggested by Klatsky *et al.* (38), an increased risk of cancer among light-moderate drinkers may be due in part to the underreporting of heavy alcohol intake. This could explain the associations observed with light-moderate alcohol intake in men as the percentage of heavy drinkers is higher in men than women (even though underreporting may be expected more in women than men due to social desirability standards). Finally, the fact that a sex difference was very pronounced in relation to rectal cancer could indicate that there may have been some residual confounding by smoking, even after adjusting for smoking. Smoking has been more strongly associated with rectal cancer than colon cancer risk (39), and smoking correlated more strongly with alcohol intake in men than women [88% of male drinkers as compared to 48% of female drinkers in the subcohort were ever smokers].

We found that the association between alcohol intake and (proximal) colon cancer risk in women was significantly modified by *ADH1B* rs3811802 and *ADH1C* rs4147542 after FDR correction. The SNPs selected in this study were not selected on the basis of that these were strong causal variants *per se*, but on the basis of that these common SNPs (minor allele frequency >5%) tag the genetic variation in *ADH1B* and *ADH1C*. Considering that tagSNPs generally confer only minor risks, which may be explained by imperfect correlations with true causal variants and gene–gene interactions, it is difficult to show significance in gene-environment interaction studies, even with large sample sizes (40). Therefore, it may be considered remarkable that two SNPs modified the association between alcohol intake and (proximal) colon cancer risk in women after FDR correction. Especially *ADH1C* rs4147542 is noteworthy in this regard since it can be linked to functional evidence: it is an expression quantitative trait locus (eQTL) for *ADH1C* in several tissues including the transverse colon (41), and has been reported to be a methylation quantitative trait locus (mQTL) (42). DNA methylation might, in part, underlie our finding of a gene–environment interaction between rs4147542 and alcohol intake which was specific to proximal colon cancer risk and to women, in which the CpG island methylator phenotype (CIMP) is present more often (43,44). Curtin *et al.* (45) showed that *ADH1C* rs698 was associated with CRCs positive for CIMP in those with low folate intake. Alcohol may influence DNA methylation levels via influencing

Table 4. Hazard ratios and 95% confidence intervals for the association between single nucleotide polymorphisms in *ADH1B* and *ADH1C* and risk of overall colorectal cancer and subtypes in female drinkers in the NLCS, 1986–2006

	Allele	PT at risk	Colorectum		Colon		Proximal colon		Distal colon		Rectum	
			N cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI
ADH1B												
rs1159918	CC	8159	366	1 (ref)	270	1 (ref)	157	1 (ref)	109	1 (ref)	66	1 (ref)
	CA + AA	11 718	517	0.98 (0.82, 1.18)	384	0.99 (0.81, 1.20)	234	1.03 (0.81, 1.31)	141	0.90 (0.68, 1.19)	99	1.04 (0.75, 1.45)
	P-value (adjusted *P*-value)			0.82 (0.15)		0.88 (0.20)		0.80 (0.17)		0.45 (0.10)		0.82 (0.18)
rs2075633	TT	9702	457	1 (ref)	338	1 (ref)	216	1 (ref)	114	1 (ref)	89	1 (ref)
	TC + CC	10 233	429	0.89 (0.75, 1.07)	318	0.89 (0.74, 1.09)	177	0.78 (0.62, 0.99)	136	1.13 (0.86, 1.49)	76	0.81 (0.59, 1.13)
	P-value (adjusted *P*-value)			0.22 (0.05)		0.27 (0.05)		0.04 (0.02)		0.38 (0.08)		0.22 (0.02)
rs1693439	GG	16 820	746	1 (ref)	545	1 (ref)	328	1 (ref)	208	1 (ref)	141	1 (ref)
	GA + AA	3135	140	1.01 (0.79, 1.29)	111	1.09 (0.84, 1.42)	65	1.06 (0.77, 1.46)	42	1.08 (0.75, 1.57)	24	0.91 (0.58, 1.45)
	P-value (adjusted *P*-value)			0.95 (0.20)		0.51 (0.10)		0.70 (0.15)		0.67 (0.12)		0.71 (0.17)
rs9307239	CC	7622	348	1 (ref)	258	1 (ref)	158	1 (ref)	94	1 (ref)	67	1 (ref)
	CT + TT	12 313	536	0.95 (0.79,1.14)	396	0.95 (0.77, 1.16)	234	0.91 (0.72, 1.16)	155	1.02 (0.77, 1.35)	98	0.90 (0.65, 1.26)
	P-value (adjusted *P*-value)			0.58 (0.10)		0.59 (0.13)		0.45 (0.10)		0.91 (0.20)		0.55 (0.12)
rs4147536	CC	12 688	563	1 (ref)	426	1 (ref)	249	1 (ref)	171	1 (ref)	98	1 (ref)
	CA + AA	7247	321	0.99 (0.82, 1.19)	228	0.93 (0.75, 1.14)	144	1.00 (0.78, 1.27)	77	0.78 (0.58, 1.06)	67	1.19 (0.85, 1.66)
	P-value (adjusted *P*-value)			0.90 (0.18)		0.47 (0.08)		0.99 (0.20)		0.11 (0.07)		0.32 (0.03)
rs3811802	AA	6101	260	1 (ref)	195	1 (ref)	115	1 (ref)	78	1 (ref)	46	1 (ref)
	AG + GG	13 854	625	1.06 (0.87, 1.29)	460	1.05 (0.84, 1.29)	278	1.07 (0.83, 1.39)	171	0.97 (0.72, 1.31)	119	1.14 (0.79, 1.64)
	P-value (adjusted *P*-value)			0.53 (0.08)		0.68 (0.18)		0.59 (0.12)		0.84 (0.17)		0.47 (0.08)
ADH1C												
rs698	TT	7385	304	1 (ref)	225	1 (ref)	126	1 (ref)	95	1 (ref)	62	1 (ref)
	TC + CC	12 553	580	1.13 (0.94, 1.36)	430	1.13 (0.92, 1.39)	266	1.25 (0.98, 1.61)	155	0.97 (0.73, 1.28)	102	0.97 (0.69, 1.37)
	P-value (adjusted *P*-value)			0.20 (0.07)		0.23 (0.07)		0.08 (0.07)		0.81 (0.15)		0.88 (0.17)
rs1662033	TT	9720	410	1 (ref)	300	1 (ref)	169	1 (ref)	124	1 (ref)	80	1 (ref)
	TG + GG	10 215	476	1.11 (0.93, 1.33)	356	1.13 (0.93, 1.38)	224	1.27 (1.00, 1.60)	126	0.97 (0.74, 1.28)	85	1.01 (0.73, 1.41)
	P-value (adjusted *P*-value)			0.26 (0.10)		0.21 (0.05)		0.05 (0.03)		0.83 (0.17)		0.93 (0.20)
rs3114046	CC	16 820	745	1 (ref)	544	1 (ref)	328	1 (ref)	207	1 (ref)	141	1 (ref)
	CT + TT	3135	141	1.02 (0.79, 1.30)	112	1.10 (0.85, 1.44)	65	1.06 (0.77, 1.46)	43	1.11 (0.77, 1.61)	24	0.91 (0.58, 1.45)
	P-value (adjusted *P*-value)			0.90 (0.18)		0.46 (0.13)		0.70 (0.17)		0.56 (0.07)		0.71 (0.10)

Table 4. *Continued*

	Allele	PT at risk	Colorectum		Colon		Proximal colon		Distal colon		Rectum	
			N cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI	*N* cases	HR[a] 95% CI
rs4147542	TT	9297	443	1 (ref)	324	1 (ref)	205	1 (ref)	114	1 (ref)	89	1 (ref)
	TC + CC	9566	402	0.87 (0.73, 1.05)	300	0.89 (0.73, 1.09)	169	0.79 (0.62, 1.01)	123	1.04 (0.79, 1.38)	71	0.77 (0.55, 1.08)
	P-value (adjusted *P*-value)			0.15 (0.05)		0.26 (0.08)		0.06 (0.05)		0.77 (0.12)		0.13 (0.03)
rs283415	TT	6906	272	1 (ref)	200	1 (ref)	109	1 (ref)	87	1 (ref)	55	1 (ref)
	TC + CC	13 049	614	1.20 (0.99, 1.46)	456	1.22 (0.99, 1.50)	284	1.39 (1.08, 1.80)	163	1.00 (0.75, 1.33)	110	1.06 (0.75, 1.51)
	P-value (adjusted *P*-value)			0.06 (0.02)		0.07 (0.02)		0.01[b] (0.02)		0.99 (0.18)		0.72 (0.12)
rs4699741	TT	17 642	769	1 (ref)	577	1 (ref)	347	1 (ref)	217	1 (ref)	136	1 (ref)
	TC + CC	2314	117	1.19 (0.91, 1.57)	79	1.08 (0.80, 1.46)	46	1.06 (0.73, 1.52)	33	1.18 (0.78, 1.78)	29	1.66 (1.06, 2.58)
	P-value (adjusted *P*-value)			0.20 (0.08)		0.62 (0.18)		0.77 (0.18)		0.43 (0.05)		0.03 (0.02)

CI, confidence interval; HR, hazard ratio; NLCS, Netherlands Cohort Study; PT, person-time; ref, reference.

[a]Age-adjusted.

[b]Significant after adjusting for multiple testing.

Table 5. Hazard ratios and 95% confidence intervals for the association between alcohol intake and colorectal cancer risk by *ADH1B* and *ADH1C* genotypes in men and women in the NLCS, 1986–2006

Gene	SNP	Allele	Alcohol intake										
			Abstainers			Light-moderate (0.1–29 g/day)			Heavy (≥30 g/day)				
			N cases/PT at risk	HR[a]	95% CI	*N* cases/PT at risk	HR[a]	95% CI	*N* cases/PT at risk	HR[a]	95% CI	*P* for trend	*P* for interaction (adjusted *P*-value)
Men													
ADH1B	rs1159918	CC	88/1232	1	(ref)	499/7191	0.92	(0.65, 1.31)	116/1445	1.05	(0.68, 1.64)	0.75	
		CA/AA	117/2071	1	(ref)	743/10 709	1.22	(0.91, 1.64)	182/2269	1.41	(0.98, 2.02)	0.07	0.41 (0.10)
ADH1B	rs2075633	TT	95/1596	1	(ref)	625/9152	1.14	(0.81, 1.59)	154/1960	1.34	(0.89, 2.01)	0.14	
		TC/CC	110/1707	1	(ref)	624/8769	1.09	(0.80, 1.49)	144/1774	1.20	(0.80, 1.79)	0.38	0.99 (0.20)
ADH1B	rs1693439	GG	185/2880	1	(ref)	1073/15305	1.07	(0.84, 1.36)	246/3219	1.16	(0.86, 1.56)	0.33	
		GA/AA	20/424	1	(ref)	175/2599	1.12	(0.53, 2.35)	52/515	1.98	(0.82, 4.81)	0.08	0.28 (0.05)
ADH1B	rs9307239	CC	73/1083	1	(ref)	434/6872	0.88	(0.61, 1.28)	108/1504	1.01	(0.64, 1.58)	0.86	
		CT/TT	132/2208	1	(ref)	814/11 009	1.22	(0.91, 1.62)	189/2230	1.37	(0.96, 1.96)	0.09	0.40 (0.08)
ADH1B	rs4147536	CC	127/1985	1	(ref)	760/11 432	0.99	(0.75, 1.31)	177/2395	1.09	(0.76, 1.55)	0.59	
		CA/AA	78/1319	1	(ref)	489/6483	1.29	(0.89, 1.88)	121/1338	1.53	(0.96, 2.46)	0.08	0.42 (0.12)
ADH1B	rs3811802	AA	59/1007	1	(ref)	350/5062	1.23	(0.80, 1.88)	82/1013	1.29	(0.75, 2.22)	0.38	
		AG/GG	146/2297	1	(ref)	898/12 859	1.04	(0.79, 1.37)	216/2721	1.21	(0.87, 1.69)	0.22	0.80 (0.17)
ADH1C	rs698	TT	69/1270	1	(ref)	419/6312	1.33	(0.90, 1.97)	105/1197	1.75	(1.07, 2.86)	0.03	
		TC/CC	136/2034	1	(ref)	827/11 609	1.02	(0.77, 1.36)	193/2537	1.11	(0.78, 1.57)	0.53	0.35 (0.10)
ADH1C	rs1662033	TT	97/1773	1	(ref)	576/8380	1.33	(0.96, 1.86)	144/1678	1.69	(1.11, 2.57)	0.02	
		TG/GG	108/1530	1	(ref)	673/9541	0.95	(0.68, 1.31)	154/2056	1.02	(0.68, 1.51)	0.86	0.17 (0.05)
ADH1C	rs3114046	CC	185/2880	1	(ref)	1073/15297	1.07	(0.84, 1.36)	246/3219	1.16	(0.86, 1.56)	0.33	
		CT/TT	20/424	1	(ref)	176/2623	1.13	(0.54, 2.38)	52/515	1.88	(0.78, 4.51)	0.10	0.27 (0.08
ADH1C	rs4147542	TT	107/1260	1	(ref)	631/8763	0.85	(0.61, 1.18)	144/1723	0.98	(0.65, 1.48)	0.95	
		TC/CC	94/1706	1	(ref)	570/7672	1.28	(0.91, 1.79)	149/1575	1.61	(1.06, 2.46)	0.03	0.12 (0.03)
ADH1C	rs283415	TT	65/1243	1	(ref)	385/5654	1.40	(0.94, 2.08)	93/1104	1.66	(1.00, 2.75)	0.05	
		TC/CC	140/2060	1	(ref)	863/12 266	1.00	(0.75, 1.32)	205/2630	1.12	(0.79, 1.58)	0.46	0.37 (0.12)
ADH1C	rs4699741	TT	179/2738	1	(ref)	1121/15601	1.05	(0.83, 1.35)	258/3230	1.17	(0.86, 1.58)	0.31	
		TC/CC	26/565	1	(ref)	128/2319	1.19	(0.65, 2.19)	40/503	1.61	(0.75, 3.44)	0.22	0.50 (0.15)
Women													
ADH1B	rs1159918	CC	147/3395	1	(ref)	335/7422	1.08	(0.82, 1.43)	20/392	1.43	(0.73, 2.81)	0.38	
		CA/AA	234/5460	1	(ref)	456/10 666	0.99	(0.79, 1.24)	38/572	1.75	(1.03, 2.97)	0.33	0.68 (0.15)
ADH1B	rs2075633	TT	199/4735	1	(ref)	412/8725	1.13	(0.89, 1.43)	28/570	1.27	(0.72, 2.23)	0.26	
		TC/CC	181/4120	1	(ref)	382/9420	0.94	(0.73, 1.21)	30/394	2.10	(1.13, 3.91)	0.46	0.16 (0.03)
ADH1B	rs1693439	GG	324/7497	1	(ref)	668/15 372	1.04	(0.86, 1.25)	47/808	1.61	(1.02, 2.54)	0.21	
		GA/AA	57/1358	1	(ref)	126/2793	1.11	(0.69, 1.81)	11/157	1.57	(0.54, 4.60)	0.46	0.96 (0.18)
ADH1B	rs9307239	CC	148/3546	1	(ref)	309/6954	1.08	(0.81, 1.44)	28/377	1.80	(0.95, 3.38)	0.18	
		CT/TT	233/5292	1	(ref)	483/11 191	0.98	(0.78, 1.22)	30/587	1.39	(0.79, 2.43)	0.71	0.62 (0.13)
ADH1B	rs4147536	CC	236/5557	1	(ref)	513/11 574	1.08	(0.86, 1.34)	32/637	1.37	(0.80, 2.33)	0.30	
		CA/AA	145/3298	1	(ref)	279/6572	0.94	(0.70, 1.25)	26/328	2.03	(1.03, 3.99)	0.45	0.32 (0.07)
ADH1B	rs3811802	AA	107/2509	1	(ref)	224/5709	0.96	(0.68, 1.37)	22/137	5.72	(2.24, 14.63)	0.13	
		AG/GG	274/6313	1	(ref)	569/12 457	1.04	(0.85, 1.28)	36/827	1.09	(0.67, 1.77)	0.63	0.004[b] (0.02)
ADH1C	rs698	TT	139/3189	1	(ref)	273/6696	0.94	(0.70, 1.27)	17/349	1.50	(0.73, 3.06)	0.86	
		TC/CC	240/5666	1	(ref)	520/11 453	1.09	(0.88, 1.36)	40/615	1.70	(1.02, 2.84)	0.11	0.60 (0.17)

Table 5. *Continued*

Gene	SNP	Allele	Alcohol intake										
			Abstainers			Light-moderate (0.1–29 g/day)			Heavy (≥30 g/day)				
			N cases/PT at risk	HR[a]	95% CI	*N* cases/PT at risk	HR[a]	95% CI	*N* cases/PT at risk	HR[a]	95% CI	*P* for trend	*P* for interaction (adjusted *P*-value)
ADH1C	rs1662033	TT	193/4346	1	(ref)	367/8777	0.92	(0.72, 1.19)	25/440	1.59	(0.87, 2.92)	0.84	
		TG/GG	188/4508	1	(ref)	427/9369	1.12	(0.87, 1.44)	33/525	1.63	(0.92, 2.87)	0.13	0.44 (0.13)
ADH1C	rs3114046	CC	324/7497	1	(ref)	667/15 372	1.04	(0.86, 1.25)	47/808	1.61	(1.02, 2.54)	0.21	
		CT/TT	57/1358	1	(ref)	127/2793	1.12	(0.69, 1.82)	11/157	1.55	(0.53, 4.54)	0.45	0.95 (0.20)
ADH1C	rs4147542	TT	182/4890	1	(ref)	399/8448	1.25	(0.98, 1.59)	29/458	1.78	(1.01, 3.14)	0.02	
		TC/CC	180/3474	1	(ref)	356/8747	0.78	(0.60, 1.02)	28/405	1.74	(0.92, 3.31)	0.58	0.01[b] (0.02)
ADH1C	rs283415	TT	134/2923	1	(ref)	245/6263	0.87	(0.64, 1.19)	14/324	1.27	(0.59, 2.72)	0.67	
		TC/CC	247/5932	1	(ref)	549/11 903	1.12	(0.90, 1.39)	44/640	1.83	(1.11, 3.02)	0.05	0.19 (0.07)
ADH1C	rs4699741	TT	326/7548	1	(ref)	692/16 037	1.00	(0.83, 1.20)	50/850	1.52	(0.97, 2.37)	0.39	
		TC/CC	55/1307	1	(ref)	102/2129	1.43	(0.83, 2.46)	8/114	2.08	(0.61, 7.12)	0.12	0.82 (0.18)

CI, confidence interval; HR, hazard ratio; NLCS, Netherlands Cohort Study; PT, person-time; ref, reference.

[a]Adjusted for age, BMI, smoking, family history of CRC, meat intake, processed meat intake, folate intake and non-occupational physical activity.

[b]Significant after adjusting for multiple testing.

one-carbon metabolism (46), and folate is an important methyl donor. On the other hand, other studies have not specifically linked alcohol intake to CIMP in CRC (47–49).

Of the four other tagSNPs (*ADH1B* rs4147536 and rs3811802 and *ADH1C* rs283415 and rs4147542) that were associated with CRC risk in drinkers—also suggesting interplay between alcohol intake, *ADH1B* and *ADH1C*, and CRC risk—rs283415 is in strong LD with the commonly investigated rs698, for which functional evidence is available. *ADH1C* rs698 C-allele carriers who also carry the rs1693482 A-allele, encoding Ile350Val and Arg272Gln substitutions, respectively (50), have a ~2.5 times slower alcohol metabolizing rate (51,52) and were found to be at an increased risk of alcohol dependence in Asian populations (7). However, the evidence linking rs698 to cancer was judged inconclusive by IARC due to the small number of studies (1). A meta-analysis of 35 case–control studies comparing rs698 slow with faster alcohol metabolizers found an association with the risk of cancer overall in African and Asian but not European populations (53). This suggests ethnicity is an important factor to take into account. For example, in Caucasian populations there is uncertainty around whether slow or fast alcohol metabolizers are at an increased CRC risk. Although Caucasians carry *ADH* alleles affecting ethanol oxidation, they lack *ADH* alleles causing very fast oxidation of ethanol and also lack *ALDH* alleles causing slow oxidation of acetaldehyde. As such, conflicting results may have emerged from Caucasian studies on rs698 (54,55) in the absence of data on how much ethanol or acetaldehyde accumulates in the colorectum, as explained in the introduction.

Strengths of the present study include the population-based prospective design and long follow-up, yielding large case numbers and making selection and information bias unlikely. In addition, the NLCS contains information on alcohol intake at baseline as well as 5 years before baseline, allowing us to investigate whether drinking patterns or fluctuations in alcohol intake affected the studied associations. Information on potential confounders was based on a single baseline measurement, and although changes over time cannot be excluded, the NLCS population has been found stable in its dietary habits (16). Importantly, the elaborate available baseline information enabled us to adjust for a large set of relevant confounders. This is essential considering that individuals who consume higher levels of alcohol may in general have an unhealthier lifestyle than those who have lower intake levels. Furthermore, the high genotyping quality may also be considered as a strength.

In conclusion, as opposed to men who showed increased CRC risks across subsites and alcohol intake levels, alcohol intake only increased colon cancer risk in women and only at heavy intake levels. *ADH1B* rs3811802 and *ADH1C* rs4147542 modified the alcohol–CRC association in women. These data indicate that alcohol may be a particularly important CRC risk factor in specific genetic subgroups. Previous literature indicates a functional role of rs4147542, supporting our finding of an effect of this variant on alcohol-associated colorectal carcinogenesis and strengthening our confidence in covering relevant genetic variation in *ADH1B* and *ADH1C*.

Supplementary material

Supplementary data are available at *Carcinogenesis* online.

Funding

This work was supported by the European Foundation for Alcohol Research (EA 14 39 to C.C.J.M.S., P.v.d.B. and M.P.W.), the Biobanking

and Biomolecular Research Infrastructure Netherlands (to M.P.W.) and the Health Foundation Limburg (to M.P.W.).

Acknowledgements

We are indebted to the participants of this study and wish to thank the Netherlands Cancer Registry and the Netherlands nationwide registry of pathology (PALGA). We also thank Drs. A. Volovics, and A. Kester for statistical advice; S. van de Crommert, H. Brants, J. Nelissen, C. de Zwart, M. Moll, W. van Dijk and A. Pisters for data management; H. Hoofs, H. van Montfort, T. van Moergastel, L. van den Bosch, R. Schmeitz and J. Berben for programming assistance; L. Jonkers, J. Goessens, K. Lemmens and S. Lumeij for the laboratory work involved; and the Biobank Maastricht UMC+ for sample storage.
Conflict of Interest Statement: None declared.

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