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ORIGINAL ARTICLE

Alcohol dehydrogenase and aldehyde dehydrogenase gene polymorphisms, alcohol intake and the risk of colorectal cancer in the European Prospective Investigation into Cancer and Nutrition study

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BACKGROUND/OBJECTIVES: Heavy alcohol drinking is a risk factor of colorectal cancer (CRC), but little is known on the effect of polymorphisms in the alcohol-metabolizing enzymes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (*ALDH*) on the alcohol-related risk of CRC in Caucasian populations.

SUBJECTS/METHODS: A nested case–control study (1269 cases matched to 2107controls by sex, age, study centre and date of blood collection) was conducted within the European Prospective Investigation into Cancer and Nutrition (EPIC) to evaluate the impact of rs1229984 (*ADH1B*), rs1573496 (*ADH7*) and rs441 (*ALDH2*) polymorphisms on CRC risk. Using the wild-type variant of each polymorphism as reference category, CRC risk estimates were calculated using conditional logistic regression, with adjustment for matching factors.

RESULTS: Individuals carrying one copy of the rs1229984(A) (*ADH1B*) allele (fast metabolizers) showed an average daily alcohol intake of 4.3 g per day lower than subjects with two copies of the rs1229984(G) allele (slow metabolizers) ($P_{diff} < 0.01$). None of the polymorphisms was associated with risk of CRC or cancers of the colon or rectum. Heavy alcohol intake was more strongly associated with CRC risk among carriers of the rs1573496(C) allele, with odds ratio equal to 2.13 (95% confidence interval: 1.26–3.59) compared with wild-type subjects with low alcohol consumption ($P_{interaction} = 0.07$).

CONCLUSIONS: The rs1229984(A) (*ADH1B*) allele was associated with a reduction in alcohol consumption. The rs1229984 (*ADH1B*), rs1573496 (*ADH7*) and rs441 (*ALDH2*) polymorphisms were not associated with CRC risk overall in Western–European populations. However, the relationship between alcohol and CRC risk might be modulated by the rs1573496 (*ADH7*) polymorphism.

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INTRODUCTION

Alcohol intake has been identified as an important risk factor for the development of various types of cancer,[1] including colorectal cancer (CRC). Expert reviews from the International Agency for Research on Cancer[2] and the World Cancer Research Fund[3] confirm the involvement of alcohol in CRC aetiology. Among other hypotheses, alcohol may affect the intestinal absorption, hepatobiliary metabolism and renal excretion of folate[4] and may result in impaired retinol status, thus providing a more tumour-promoting environment,[5] or can promote oxidative stress.[6] In addition, although evidence from experimental animal models indicate that alcohol by itself is not carcinogenic,[2] alcohol intake might have a role in colorectal carcinogenicity through acetaldehyde, an oxidation product of alcohol.[7,8]

Acetaldehyde associated with alcoholic beverages has been labelled as carcinogenic to humans (IARC Group 1).[9] It is removed from the blood via a second reaction catalysed by aldehyde dehydrogenase (*ALDH*) enzymes, which oxidize it into acetate.[10] The efficiency of each of these reactions depends on multiple alcohol dehydrogenase (*ADH*) and *ALDH* enzymes, which are encoded by different genes, and which occur in several variants. Therefore, the risk of CRC incidence in relation to alcohol intake could be modulated by genetic factors, as the efficiency in converting ethanol to acetaldehyde, and its subsequent conversion into acetate is largely determined by the *ADH* and *ALDH* gene families.[11–13]

Although the few existing studies on CRC in Caucasian populations have been inconclusive,[14] studies in Japanese populations showed an increased CRC risk with the *ADH1B* polymorphism (slow metabolizer)[15] and a significant interaction between the *ALDH2* and *ADH2* polymorphisms, which was more apparent in low folate consumers than in high folate consumers.[16]

The *ADH* polymorphisms show sizeable heterogeneity among ethnic groups.[10] The allele rs1229984*2 (*His*) (rs1229984(A) in the present study), which codes for 'fast' metabolism of ethanol,[17] is a frequent allele in Asian populations, while the allele rs1229984*1 (*Arg*) (rs1229984(G) in the present study) is a major allele in Caucasians.[18]

The epidemiological investigations that have so far explored the association between *ADH* and *ALDH* polymorphisms and risk of CRC have been of relatively small size and very few data are available from European populations. In light of this, a nested case–control study was conducted within the EPIC, to investigate whether the rs1229984 (*ADH1B*), rs1573496 (*ADH7*) and rs441 (*ALDH2*) polymorphisms are associated with CRC risk in European populations, and whether these genes interact with alcohol intake in the aetiology of CRC. In our study, differences in the associations between single-nucleotide polymorphisms (SNPs) by anatomical sub-sites were also evaluated.

MATERIALS AND METHODS

Subjects

EPIC is a multicentre prospective cohort study with more than 520 000 participants enrolled from 23 centres in Denmark, France, Greece, Germany, Italy, The Netherlands, Norway, Spain, Sweden and the United Kingdom. EPIC is designed to investigate the relation between dietary and lifestyle/environmental factors and cancer incidence. The rationale and methods of the EPIC study have been previously reported in detail.[19] Between 1992 and 1998, standardized lifestyle questionnaires, anthropometric data and blood samples were collected from most study participants at recruitment, before disease onset or diagnosis. Dietary habits, including alcohol consumption, over the previous 12 months were assessed at recruitment by validated country-specific dietary questionnaires designed to ensure high compliance while accounting for geographical specificity of diet.[19,20]

The present study included subjects who, during an average of 7.2 years follow-up, developed cancer of the colon ($n = 797$), specifically in the caecum, appendix, ascending colon and hepatic flexure, transverse colon, splenic flexure (proximal) (C18.0–18.5; International Statistical Classification of Diseases, Injury and Causes of Death, 10th Revision), descending and sigmoid colon (distal) (C18.6–C18.7), tumours that were overlapping or unspecified (C18.8 and C18.9), as well as cancer of the rectum ($n = 472$), with tumours occurring at the rectosigmoid junction (C19) and rectum (C20). CRC is defined as a combination of all the colon and rectal cancer cases. Anal canal tumours were excluded.

For each case ($n = 1269$), upto two controls ($n = 2107$) were randomly selected by incidence density sampling among cohort members alive and free of cancer at the time of case diagnosis, with blood samples available, and matched by sex, age (± 2.5 years), centre and date of blood collection (± 45 days). Women were further matched by menopausal status (pre-menopausal, post-menopausal, peri-menopausal/unknown), phase of menstrual cycle at blood collection and usage of hormone replacement therapy at the time of blood collection (yes/no). The additional matching criteria for women were of necessity to other EPIC nested case–control studies that were being conducted using the same matched case–control sets.

This study was approved by relevant Ethical Review Boards of IARC and all EPIC centres.

SNP selection/genotyping and folate assessment

The rs1229984 (*ADH1B*), rs1573496 (*ADH7*) and rs441 (*ALDH2*) polymorphisms were chosen because investigations on Central European populations indicate that they are associated with the risk of upper and aero-digestive tract cancer and that the association is potentially modifiable by the level of alcohol consumption.[21]

Genotyping for rs1229984 (*ADH1B*), rs1573496 (*ADH7*) and rs441 (*ALDH2*) polymorphisms was performed by Taqman methodology in 384-well plates read with the Sequence Detection Software on an ABI-Prism7900 instrument, according to the manufacturer's instructions (Applied Biosystems / Life Technologies, Saint-Aubin, France). Primers and probes were supplied by Applied Biosystems (Assays-by-Design). Each plate included a negative control (no DNA). Positive controls were duplicated on a separate plate. A random selection of the study subjects (both cases and controls) was re-genotyped for each polymorphism to examine the reliability of the Taqman genotyping assays. Internal duplicate concordance was $>99.9\%$ and the genotyping success rate was at least 94%. Some subjects were excluded due to incomplete genotyping data. Thus, the final number of complete case–control sets utilized was: rs1229984 (*ADH1B*), (number colon = 781; number rectal = 466), rs1573496 (*ADH7*) (number colon = 761; number rectal = 436) and rs441 (*ALDH2*) (number colon = 762; number rectal = 451).

Blood folate measurements were conducted at the LOCUS for homocysteine and related vitamins, University of Bergen (Norway) using a *Lactobacillus casei* microbiological assay,[22] adapted to a microtiter plate format and carried out by a robotic workstation (Micro-lab AT plus 2; Hamilton Bonaduz AG, Bonaluz, Switzerland).[23] Blood collection and storage procedures of the EPIC study have been previously described in detail.[24]

Statistical analyses

For each polymorphism, genotype distributions in control subjects were tested for Hardy–Weinberg equilibrium. Means and associated measures of variation (10th and 90th percentiles) of total alcohol intake and intakes of alcoholic beverages were calculated for cases and controls. Differences between cases and controls in (log-transformed) total alcohol intake and intakes of alcoholic beverages at baseline were assessed with linear regression models adjusted by sex and country. Similarly, linear regression models were used to compute adjusted means of total alcohol intake and intakes of alcoholic beverages at baseline (and associated s.e.) for each variants of the rs1229984 (*ADH1B*), rs1573496 (*ADH7*) and rs441 (*ALDH2*) polymorphisms, controlling for sex, country and case–control status. *P*-values associated with differences of heterozygous and homozygous subjects compared with wild-type individuals were determined with Wald tests, according to a χ^2-distribution with 1 d.f. In addition, the frequency of subjects by category of alcohol consumption (low, medium and high) by the three categories of each SNP were calculated.

The association between variants of SNPs and CRC risk was assessed by odds ratio and corresponding 95% confidence interval estimated in conditional logistic regression models, in which the case–control set was used as stratification variable. For each SNP separately, keeping the wild-type category consistently as the reference group, heterozygous and rare homozygous categories were modelled with indicator variables. *P*-values for statistical significance of odds ratios for heterozygous and rare

homozygous compared with wild-type individuals were obtained with Wald test with 2 d.f. Adjustments by blood folate and alcohol intake, both modelled as continuous variables, were performed, but results were not meaningfully altered. Effect modification by sex-specific categories of alcohol intake at baseline (<5, 5–25, >25 g per day in women; <10, 10–50, >50 g per day in men), blood folate (EPIC-wide tertiles), sex and country was assessed using indicator variables (as many as the number of categories minus one), combining the heterozygous and rare homozygous variants, and comparing log-likelihood of models with and without interaction terms to a χ^2-distribution with degrees of freedom equal to the number of interaction terms minus one. For the evaluation of potential confounding and assessment of interaction with variants of SNPs, the total number of CRC case–control sets used was 850, due to missing folate measurement data. Potential differences in the associations by colon and rectal cancer were explored in separate models.

All statistical analyses were performed using Stata Software.[25] All tests were two-tailed and statistical significance was assessed at the 5% level.

RESULTS

The rs1229984 (*ADH1B*), rs1573496 (*ADH7*) and rs441 (*ALDH2*) polymorphisms were in Hardy–Weinberg equilibrium in controls. Baseline characteristics and description of the study population are shown in Table 1. Total alcohol and beer intakes were significantly higher in cases than controls. For each polymorphism, the frequency of study subjects by combination of genetic categories and groups of alcohol intake are displayed in Supplementary Table 1A. While frequencies of genetic variants are rather constant across category of alcohol consumption for the rs1573496 (*ADH7*) and rs441 (*ALDH2*) polymorphisms, the percentage of subjects with low consumption (<5 and 10 g per day in women and men, respectively) showed to be lower among carriers of the 'slow' rs1229984(G) (*ADH1B*) allele (49%) than subjects carrying one copy of the 'fast' rs1229984(A) allele (59%). Consistently, opposite trends were observed among individuals with elevated alcohol intake. In Table 2, means of total alcohol intake and intakes of wine, beer and spirits are reported by categories of SNP variants. Heterozygous subjects in the rs1229984 (*ADH1B*) polymorphisms showed lower intakes of total alcohol and wine intakes. The large mean values of total alcohol and wine intakes displayed in rs1229984(A) (*ADH1B*) carriers are largely explained by the limited sample size in this group ($n = 12$), thus undermining the precision of these values. Other drinking

Table 1. Baseline characteristics of colorectal cancer cases and matched controls and country-specific cases and controls frequencies

	Cases		*Matched controls*		
N	1269		2107		
Age at recruitment[a]	58.5 ± 7.1		58.3 ± 7.4		
Men	621		1001		
Women	648		1106		
Smoking status					
Non-smokers	533 (42%)				
Smokers	421 (33%)		955 (45%)		
Ex-smokers	304 (24%)		687 (33%)		
Unknown	11 (1%)		445 (21%)		
			9 (1%)		
Physical activity					
Inactive	310 (24%)				
Moderately inactive	377 (30%)		501 (24%)		
Moderately active	260 (21%)		622 (29%)		
Active	232 (18%)		383 (18%)		
Unknown	90 (7%)		439 (21%)		
			162 (8%)		
	%	*Mean*[c]	*%*	*Mean*	P-*value*[d]
Alcohol intake (g per day)	12.5	20.2 (0.8, 52.4)	14.5		0.16
Wine	20.3	11.2 (0.2, 31.6)	22.9	17.4 (0.8, 45.5)	0.94
Beer	40.1	10.5 (0.2, 37.2)	44.7	10.9 (0.3, 31.6)	0.02
Spirits	43.6	3.3 (0.2, 7.5)	48.0	7.8 (0.2, 21.2)	0.20
				3.0 (0.2, 7.1)	
*Country (*N)					
France		33			
Italy		147		59	
Spain		121		291	
United Kingdom		216		236	
The Netherlands		150		398	
Greece		27		287	
Germany		157		54	
Sweden		76		303	
Denmark		337		131	
Norway		5		339	
				9	

[a]Means ± s.d. [b]Percentage of study subjects who reported no consumption in the 12 months preceding the dietary questionnaire administration. [c]Means (10th, 90th percentiles) of total alcohol and alcoholic beverages intakes (g per day) in total alcohol- and beverage-specific non-zero consumers. [d]*P*-values for (log-transformed) total alcohol intake and intakes of alcoholic beverages at baseline in relation to case–control status assessed with adjusted linear regression models.

Table 2. Number of subjects (% cases), means (s.d.) of intake of baseline intake[a] (g per day) of total alcohol, wine, beer and spirits, adjusted by sex, country and case–control status, of total alcohol and wine intakes by variants of rs1229984 (*ADH1B*), rs1573496 (*ADH7*) and rs441 (*ALDH2*) polymorphisms

	N *(%)*	*Total alcohol*		*Wine*		*Beer*		*Spirits*	
		Mean (±s.e.)	P-*value*[b]	*Mean (±s.e.)*	P-*value*[b]	*Mean (±s.e.)*	P-*value*[b]	*Mean (±s.e.)*	P-*value*[b]
rs1229984 *(ADH1B)*									
G/G	2984 (38.3)	16.4 ± 0.4	—	9.0 ± 0.3	—	5.1 ± 0.2	—	1.7 ± 0.1	—
A/A	278 (36.0)	12.1 ± 1.2	<0.01	5.4 ± 0.9	<0.01	4.6 ± 0.7	0.72	1.7 ± 0.3	0.48
A/A	12 (41.7)	14.5 ± 5.8	0.40	9.8 ± 4.2	0.92	2.4 ± 3.4	0.20	1.7 ± 1.3	0.70
rs1573496 *(ADH7)*									
G/G	2614 (37.3)	16.1 ± 0.4	—	8.7 ± 0.3	—	5.1 ± 0.2	—	1.7 ± 0.1	—
C/G	545 (38.3)	15.7 ± 0.9	0.63	8.8 ± 0.6	0.71	4.5 ± 0.5	0.65	1.7 ± 0.2	0.79
C/C	33 (39.4)	15.5 ± 3.5	0.25	8.9 ± 2.5	0.97	4.6 ± 2.1	0.74	1.6 ± 0.8	0.70
rs441 *(ALDH2)*									
T/T	2153 (37.7)	15.9 ± 0.4	—	8.7 ± 0.3	—	5.0 ± 0.3	—	1.7 ± 0.5	—
T/C	997 (37.3)	16.1 ± 0.6	0.84	8.6 ± 0.5	0.96	5.0 ± 0.4	0.23	1.7 ± 0.5	0.25
C/C	103 (29.1)	16.1 ± 2.0	0.59	8.3 ± 1.4	0.58	5.9 ± 1.2	0.62	1.4 ± 0.5	0.22

[a]Study subjects' alcohol intake refers to the 12 months preceding the dietary questionnaire administration. [b]*P*-value associated with differences of log-transformed total alcohol intake and intakes of alcoholic beverages at baseline for heterozygous and rare homozygous variants in relation to wild-type individuals, assessed using adjusted linear regression models. For each genetic category, a Wald test was performed according to a χ^2-distribution with 1 d.f.

Table 3. Odds ratios (ORs) and 95% confidence interval (CI) for colorectal, rectum and colon cancer risk with rs1229984 (*ADH1B*), rs1573496 (*ADH7*) and rs441 (*ALDH2*) polymorphisms

Variants	*Colorectal*			*Rectum*			*Colon*		
	Case/control	*OR*	*95% CI*	*Case/control*	*OR*	*95% CI*	*Case/control*	*OR*	*95% CI*
rs1229984 *(ADH1B)*									
G/G	1129/1800	1	—	429/651	1	—	700/1149	1	—
G/A	97/176	0.95	0.72–1.24	30/66	0.77	0.48–1.21	67/110	1.07	0.77–1.49
A/A	5/6	1.36	0.41–4.53	2/—	—	—	3/6	0.86	0.21–3.50
P-value[a]			0.81			0.52			0.89
rs1573496 *(ADH7)*									
G/G	947/1549	1	—	337/543	1	—	610/1,006	1	—
C/G	205/311	1.09	0.90–1.32	79/110	1.17	0.85–1.61	126/201	1.04	0.81–1.32
C/C	13/19	1.30	0.64–2.64	6/5	2.73	0.78–9.57	7/14	0.87	0.35–2.18
P-value[a]			0.55			0.19			0.91
rs441 *(ALDH2)*									
T/T	794/1273	1	—	288/458	1	—	506/815	1	—
T/C	358/604	0.95	0.81–1.12	134/204	1.06	0.80–1.38	224/400	0.90	0.73–1.10
C/C	30/70	0.67	0.43–1.05	12/27	0.71	0.35–1.43	18/43	0.65	0.37–1.14
P-value[a]			0.20			0.56			0.22

[a]*P*-value for difference in risk between heterozygous and rare homozygous compared with wild-type individuals, determined with a Wald test according to a χ^2-distribution with 2 d.f.

levels according to variants of the rs1573496 (*ADH7*) and rs441 (*ALDH2*) SNPs were not clearly apparent.

Variants of rs1229984 (*ADH1B*), rs1573496 (*ADH7*) and rs441 (*ALDH2*) polymorphisms were overall not associated with colorectal, colon and rectal cancer risk (Table 3). Similarly to previous findings in the EPIC cohort,[26] in this study total alcohol intake was associated with a significant increase in the risk of colorectal, colon and rectal cancer (results not shown). As reported in Table 4, alcohol intake showed a more pronounced association with CRC risk among subject carriers of the rare rs1573496(C) (*ADH7*) allele ($P_{interaction} = 0.07$), with odds ratio = 1.80 (95% confidence interval: 1.02–3.17; $P = 0.04$) comparing high vs low alcohol intake among heterozygous and wild-type homozygous subjects (estimates obtained using a linear combination of model parameters).

No evidence of heterogeneity by blood folate ($P_{interaction} = 0.76$, 0.69, 0.73 for rs1229984 (*ADH1B*), rs1573496 (*ADH7*), rs441 (*ALDH2*), respectively), sex ($P_{interaction} = 0.90$, 0.37, 0.90 for rs1229984 (*ADH1B*), rs1573496 (*ADH7*), rs441 (*ALDH2*), respectively) or country ($P_{interaction} = 0.79$, 0.89, 0.26 for rs1229984 (*ADH1B*), rs1573496 (*ADH7*), rs441 (*ALDH2*), respectively) were apparent in the associations between each polymorphism and CRC risk (data not shown).

DISCUSSION

The results from this study nested within a European cohort suggest that heterozygous subjects with the 'fast' rs1229984(A) (*ADH1B*) allele have an average of 4.3 g per day lower alcohol intake compared with homozygous participants, carrying two

Table 4. Odds ratios and 95% confidence interval for colorectal cancer risk with rs1229984 (*ADH1B*), rs1573496 (*ADH7*) and rs441 (*ALDH2*) polymorphisms by sex-specific categories of alcohol intake (g per day) at baseline

SNP	*Variants*	*Total alcohol intake*[a]				
		Low	*Medium*	*High*	P-*value*[b]	$P_{interaction}$[c]
rs1229984 (*ADH1B*)	G/G	1 (ref)	1.16 (0.97–1.37)	1.38 (1.07–1.79)	0.73	
	G/A, A/A	1.09 (0.77–1.54)	0.94 (0.61–1.49)	1.35 (0.59–3.09)	0.61	0.61
rs1573496 (*ADH7*)	G/G	1 (ref)	1.20 (1.00–1.44)	1.21 (0.91–1.61)	0.12	
	G/C, C/C	1.18 (0.91–1.55)	1.02 (0.75–1.41)	2.13 (1.26–3.59)	0.04	0.07
rs441 (*ALDH2*)	T/T	1 (ref)	1.21 (0.99–1.48)	1.43 (1.06–1.94)	0.03	
	T/C, C/C	1.02 (0.81–1.27)	0.94 (0.73–1.21)	1.48 (1.00–2.18)	0.10	0.25

[a]Sex-specific categories of alcohol intake (g per day) were <5, 5–25, >25 in women, and <10, 10–50, and >50 in men, referring to study subjects' intake during the 12 months preceding dietary questionnaire administration. [b]*P*-value for difference in risk between high and medium compared with low alcohol intake by categories of genetic variants, computed using Wald test for contrasts according to a χ^2-distribution with 2 d.f. [c]*P*-value for interaction between categories of baseline alcohol intake (three categories) and variants of single-nucleotide polymorphisms (SNPs, two categories), comparing the log-likelihood of models with and without interaction terms, according to a χ^2-distribution with 2 d.f.

copies of the 'slow' rs1229984(G) (*ADH1B*). The reduction is mainly attributable to lower wine intake, whereas the consumption of beer and spirits are virtually unchanged in the two groups. No clear differences in drinking levels were observed across variants of the rs1573496 (*ADH7*) and rs441 (*ALDH2*) polymorphisms. Subjects carrying copies of the fast alcohol metabolizer show a tendency towards lower alcohol consumption, in line with previous observations among Caucasian populations.[27–30]

The present study also shows that polymorphisms in the rs1229984 (*ADH1B*), rs1573496 (*ADH7*) and rs441 (*ALDH2*) genes are not associated with risk of CRC or of colon or of rectal cancers in Caucasian populations. However, further analyses in the rs1573496 (*ADH7*) polymorphism showed that alcohol intake was associated with CRC risk among heterozygous and homozygous carriers of the rare rs1573496(C) (*ADH7*) allele, while among homozygous wild-type participants the alcohol CRC association was weak. With respect to SNPs in the *ALDH* family, similar genes alcohol interactions have been previously observed for upper aero-digestive tract cancer,[21] for head and neck cancer,[31] and for CRC.[15]

The alleles encoding the different *ADH* variants are heterogeneously distributed among ethnic groups.[10] The allele rs1229984(A) (*ADH1B*) codes for fast metabolism of ethanol,[17] and is a major allele in Asian populations. In contrast, the allele rs1229984(G) (*ADH1B*), which codes for slow metabolizer, is a major allele in Caucasians.[18] The frequency of subjects with two copies of the rs1229984(G) (*ADH1B*) allele ('slow' metabolizer), of the heterozygous subjects, and of the homozygous carrying two rs1229984(A) (*ADH1B*) ('fast' metabolizer) was 90%, 9% and <1% in our study, respectively, while it was around 5%, 35% and 60% in studies conducted in Japan.[16,19] This major difference in frequency, that is, degree of genetic exposure, may explain the inconsistency in risk estimates with respect to genetic factors as the statistical power to detect an association will be greater in the Japanese studies than ours.

The slow alcohol metabolizer of the rs1229984(G) (*ADH1B*) polymorphism has been shown to be associated with various alcohol-related conditions, such as an increased risk of alcoholic liver disease,[32] or cerebral infarction.[33] For alcohol-related cancers, an increased risk of upper aero-digestive tract cancers has been observed consistently in studies conducted in Europe[21,34] and in Japan.[35]

For CRC, no clear explanation for the increased risk associated with the slow alcohol metabolizer rs1229984(G) (*ADH1B*) allele observed in Asian populations[15,16] has been provided. High intracolonic acetaldehyde concentrations may result from oxidation of ethanol by some strains of colonic bacteria, which possesses *ADH* activity.[8] This has been observed *in vitro*[36] and *in vivo* where acetaldehyde production was inhibited by antibiotic treatment.[37,38] Low activity of *ALDH* in colonic aerobic bacteria and in mucosa may lead to acetaldehyde accumulation in the colon.[8,39] This so called bacterio-colonic pathway of ethanol oxidation could be a mechanism responsible for increased CRC risk associated with elevated alcohol intake, in conjunction with a lack of link with variants of the *ADH* genes, at least in Caucasian populations, where limited genetic variability in the *ADH* family is observed. However, testing this hypothesis is beyond the scope of the present work.

The association between alcohol and CRC risk has been consistently observed in several epidemiological investigations,[40–42] and in a pooled analysis of eight cohort studies.[43] Similarly, in a full cohort analysis of the EPIC study lifetime alcohol intake and alcohol intake at recruitment were positively associated with the risk of CRC, the association being stronger for rectum than for colon cancer.[26] Similarly, in the present study alcohol appeared to be positively associated with the risk of CRC (data not shown).

Given drinking avoidance behaviour of 4.3 g per day associated with rs1229984 (*ADH1B*) heterozygous subjects compared with rs1229984(A) homozygous individuals in the present study, relatively weak associations between alcohol-related SNPs and risk of CRC are likely to be captured in this context. High alcohol consumption may result in inadequate folate status by decreasing intestinal absorption and increasing renal excretion.[4] It is hypothesized that low folate status increases the risk of CRC by altering DNA methylation and DNA synthesis.[44,45] It is known that acetaldehyde, rather than alcohol itself, can cleave folate chemically. A high intracolonic concentration of acetaldehyde from bacterial production and metabolism of alcohol could thus lead to decreased folate levels in the colonic mucosa. However, the present study lacks the statistical power required to properly evaluate how intake of dietary folate or blood folate levels may modulate the CRC risk association between alcohol consumption and *ADH*/*ALDH* genes.

To the best of our knowledge, this study is the largest study to date conducted regarding the role of rs1229984 (*ADH1B*), rs1573496 (*ADH7*) and rs441 (*ALDH2*) genes on CRC risk in Caucasian populations. Our study confirmed that the fast alcohol metabolizer allele in the *ADH1B* polymorphism is associated with a reduction of alcohol intake, mainly ascribable to wine consumption, and that the rs1229984 (*ADH1B*) and rs441 (*ALDH2*) polymorphisms showed no association with CRC risk. The relationship between alcohol intake and risk of CRC seemed to be modulated by the rs1573496(C) (*ADH7*) polymorphism.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Boffetta P, Hashibe M. Alcohol and cancer. *Lancet Oncol* 2006; **7**: 149–156.
2 Baan R, Straif K, Grosse Y, Secretan B, El GF, Bouvard V *et al.* Carcinogenicity of alcoholic beverages. *Lancet Oncol* 2007; **8**: 292–293.
3 WCRF/AICR. *Food, nutrition, physical activity and the prevention of cancer: a global perspective*. AICR: Washington DC, 2007.
4 Laird PW, Jaenisch R. DNA methylation and cancer. *Hum Mol Genet* 1994; **3**: 1487–1495.
5 Wang XD. Alcohol, vitamin A and cancer. *Alcohol* 2005; **35**: 251–258.
6 Hoek JB, Pastorino JG. Ethanol, oxidative stress, and cytokine-induced liver cell injury. *Alcohol* 2002; **27**: 63–68.
7 Seitz HK, Simanowski UA. Alcohol and carcinogenesis. *Annu Rev Nutr* 1988; **8**: 99–119.
8 Salaspuro M. Bacteriocolonic pathway for ethanol oxidation: characteristics and implications. *Ann Med* 1996; **28**: 195–200.
9 Secretan B, Straif K, Baan R, Grosse Y, El GF, Bouvard V *et al.* A review of human carcinogens--Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. *Lancet Oncol* 2009; **10**: 1033–1034.
10 Edenberg HJ. The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. *Alcohol Res Health* 2007; **30**: 5–13.
11 Seitz HK, Becker P. Alcohol metabolism and cancer risk. *Alcohol Res Health* 2007; **30**: 38–47.
12 Seitz HK, Stickel F. Acetaldehyde as an underestimated risk factor for cancer development: role of genetics in ethanol metabolism. *Genes Nutr* 2010; **5**: 121–128.
13 Druesne-Pecollo N, Tehard B, Mallet Y, Gerber M, Norat T, Hercberg S *et al.* Alcohol and genetic polymorphisms: effect on risk of alcohol-related cancer. *Lancet Oncol* 2009; **10**: 173–180.
14 Landi S, Gemignani F, Moreno V, Gioia-Patricola L, Chabrier A, Guino E *et al.* A comprehensive analysis of phase I and phase II metabolism gene polymorphisms and risk of colorectal cancer. *Pharmacogenet Genomics* 2005; **15**: 535–546.
15 Yin G, Kono S, Toyomura K, Moore MA, Nagano J, Mizoue T *et al.* Alcohol dehydrogenase and aldehyde dehydrogenase polymorphisms and colorectal cancer: the Fukuoka Colorectal Cancer Study. *Cancer Sci* 2007; **98**: 1248–1253.
16 Matsuo K, Wakai K, Hirose K, Ito H, Saito T, Suzuki T *et al.* A gene-gene interaction between ALDH2 Glu487Lys and ADH2 His47Arg polymorphisms regarding the risk of colorectal cancer in Japan. *Carcinogenesis* 2006; **27**: 1018–1023.
17 Bosron WF, Crabb DW, Li TK. Relationship between kinetics of liver alcohol dehydrogenase and alcohol metabolism. *Pharmacol Biochem Behav* 1983; **18**: 223–227.
18 Brennan P, Lewis S, Hashibe M, Bell DA, Boffetta P, Bouchardy C *et al.* Pooled analysis of alcohol dehydrogenase genotypes and head and neck cancer: a HuGE review. *Am J Epidemiol* 2004; **159**: 1–16.
19 Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M *et al.* European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* 2002; **5**: 1113–1124.
20 Kaaks R, Riboli E. Validation and calibration of dietary intake measurements in the EPIC project: methodological considerations. European Prospective Investigation into Cancer and Nutrition. *Int J Epidemiol* 1997; **26**: S15–S25.
21 Hashibe M, Boffetta P, Zaridze D, Shangina O, Szeszenia-Dabrowska N, Mates D *et al.* Evidence for an important role of alcohol- and aldehyde-metabolizing genes in cancers of the upper aerodigestive tract. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 696–703.
22 Eussen SJ, Vollset SE, Igland J, Meyer K, Fredriksen A, Ueland PM *et al.* Plasma folate, related genetic variants, and colorectal cancer risk in EPIC. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 1328–1340.
23 Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. *Methods Enzymol* 1997; **281**: 43–53.
24 Bingham S, Riboli E. Diet and cancer--the European Prospective Investigation into Cancer and Nutrition. *Nat Rev Cancer* 2004; **4**: 206–215.
25 Stata Statistical Software: *Release 12 [computer program]*. *Version StataCorp*. StataCorp LP: College Station, TX, 2011.
26 Ferrari P, Jenab M, Norat T, Moskal A, Slimani N, Olsen A *et al.* Lifetime and baseline alcohol intake and risk of colon and rectal cancers in the European prospective investigation into cancer and nutrition (EPIC). *Int J Cancer* 2007; **121**: 2065–2072.
27 Whitfield JB, Nightingale BN, Bucholz KK, Madden PA, Heath AC, Martin NG. ADH genotypes and alcohol use and dependence in Europeans. *Alcohol Clin Exp Res* 1998; **22**: 1463–1469.
28 Lilla C, Koehler T, Kropp S, Wang-Gohrke S, Chang-Claude J. Alcohol dehydrogenase 1B (ADH1B) genotype, alcohol consumption and breast cancer risk by age 50 years in a German case-control study. *Br J Cancer* 2005; **92**: 2039–2041.
29 Macgregor S, Lind PA, Bucholz KK, Hansell NK, Madden PA, Richter MM *et al.* Associations of ADH and ALDH2 gene variation with self report alcohol reactions, consumption and dependence: an integrated analysis. *Hum Mol Genet* 2009; **18**: 580–593.
30 Zuccolo L, Fitz-Simon N, Gray R, Ring SM, Sayal K, Smith GD *et al.* A non-synonymous variant in ADH1B is strongly associated with prenatal alcohol use in a European sample of pregnant women. *Hum Mol Genet* 2009; **18**: 4457–4466.
31 Hiraki A, Matsuo K, Wakai K, Suzuki T, Hasegawa Y, Tajima K. Gene-gene and gene-environment interactions between alcohol drinking habit and polymorphisms in alcohol-metabolizing enzyme genes and the risk of head and neck cancer in Japan. *Cancer Sci* 2007; **98**: 1087–1091.
32 Tanaka F, Shiratori Y, Yokosuka O, Imazeki F, Tsukada Y, Omata M. High incidence of ADH2*1/ALDH2*1 genes among Japanese alcohol dependents and patients with alcoholic liver disease. *Hepatology* 1996; **23**: 234–239.
33 Suzuki Y, Fujisawa M, Ando F, Niino N, Ohsawa I, Shimokata H *et al.* Alcohol dehydrogenase 2 variant is associated with cerebral infarction and lacunae. *Neurology* 2004; **63**: 1711–1713.
34 Hashibe M, McKay JD, Curado MP, Oliveira JC, Koifman S, Koifman R *et al.* Multiple ADH genes are associated with upper aerodigestive cancers. *Nat Genet* 2008; **40**: 707–709.
35 Yokoyama A, Omori T, Yokoyama T, Sato Y, Mizukami T, Matsushita S *et al.* Risk of squamous cell carcinoma of the upper aerodigestive tract in cancer-free alcoholic Japanese men: an endoscopic follow-up study. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 2209–2215.
36 Jokelainen K, Siitonen A, Jousimies-Somer H, Nosova T, Heine R, Salaspuro M. In vitro alcohol dehydrogenase-mediated acetaldehyde production by aerobic bacteria representing the normal colonic flora in man. *Alcohol Clin Exp Res* 1996; **20**: 967–972.
37 Visapaa JP, Jokelainen K, Nosova T, Salaspuro M. Inhibition of intracolonic acetaldehyde production and alcoholic fermentation in rats by ciprofloxacin. *Alcohol Clin Exp Res* 1998; **22**: 1161–1164.
38 Tillonen J, Homann N, Rautio M, Jousimies-Somer H, Salaspuro M. Ciprofloxacin decreases the rate of ethanol elimination in humans. *Gut* 1999; **44**: 347–352.
39 Nosova T, Jokelainen K, Kaihovaara P, Heine R, Jousimies-Somer H, Salaspuro M. Characteristics of aldehyde dehydrogenases of certain aerobic bacteria representing human colonic flora. *Alcohol Alcohol* 1998; **33**: 273–280.
40 Corrao G, Bagnardi V, Zambon A, La VC. A meta-analysis of alcohol consumption and the risk of 15 diseases. *Prev Med* 2004; **38**: 613–619.
41 Moskal A, Norat T, Ferrari P, Riboli E. Alcohol intake and colorectal cancer risk: a dose-response meta-analysis of published cohort studies. *Int J Cancer* 2007; **120**: 664–671.
42 Fedirko V, Tramacere I, Bagnardi V, Rota M, Scotti L, Islami F *et al.* Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. *Ann Oncol* 2011; **22**: 1958–1972.
43 Cho E, Smith-Warner SA, Ritz J, van den Brandt PA, Colditz GA, Folsom AR *et al.* Alcohol intake and colorectal cancer: a pooled analysis of 8 cohort studies. *Ann Intern Med* 2004; **140**: 603–613.
44 Giovannucci E. Alcohol, one-carbon metabolism, and colorectal cancer: recent insights from molecular studies. *J Nutr* 2004; **134**: 2475S–2481S.
45 Kono S, Chen K. Genetic polymorphisms of methylenetetrahydrofolate reductase and colorectal cancer and adenoma. *Cancer Sci* 2005; **96**: 535–542.

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