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Alcohol and the risk of colon and rectal cancer with mutations in the *K-ras* gene

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Abstract

The first metabolite of alcohol, acetaldehyde, may trigger replication errors and mutations in DNA, which may predispose to developing colorectal cancer (CRC). In a prospective study on colon and rectal cancer, we investigated the following hypotheses: alcohol consumption is associated with an increased risk of mutations in the *K-ras* oncogene, and beer consumption is associated with an increased risk of G → A mutations in this gene. Therefore, we studied the associations between consumption of alcohol and alcoholic beverages and the risk of CRC without and with specific *K-ras* gene mutations. In 1986, 120,852 men and women, aged 55–69 years, completed a questionnaire on risk factors for cancer. The case–cohort approach was used for data processing and analyses. After 7.3 years of follow-up, excluding the first 2.3 years, complete data from 4,076 subcohort members, 428 colon and 150 rectal cancer patients, were available for data analyses. Incidence rate ratios (RRs) and corresponding 95% confidence intervals (95% CIs) were estimated using Cox proportional hazards models. Compared to abstaining, a total alcohol consumption of 30.0 g/day and more was associated with the risk of colon and rectal cancer with and without a *K-ras* mutation in both men and women. Independent from alcohol intake, liquor consumption when compared to nonliquor consumption was associated with an increased risk of rectal cancer with a wild type *K-ras* in men (RR: 2.25, 95% CI: 1.0–5.0). Beer consumption was not clearly associated with the risk of colon and rectal tumors harboring G → A mutations in the *K-ras* gene in men. This association could not be assessed in women because of sparse beer consumption. In conclusion, alcohol does not seem to be involved in predisposing to CRC through mutations in the *K-ras* gene, and specifically beer consumption is not associated with colon and rectal tumors harboring a G → A mutation. © 2006 Elsevier Inc. All rights reserved.

Keywords: Alcohol; Colorectal cancer; *K-ras* mutations; Cohort study; The Netherlands

1. Introduction

Consumption of alcohol is well-known to be associated with several malignant and nonmalignant diseases. Although alcohol itself is not carcinogenic, there is increasing evidence that acetaldehyde, a cytotoxic, mutagenic, and carcinogenic metabolite of alcohol, rather than alcohol itself, is responsible for tumor enhancing effects (Salaspuro, 2003; Seitz et al., 2001). Acetaldehyde can bind to cellular proteins and DNA, and the additional formation of stable DNA adducts could trigger replication errors and mutations in tumor suppressor genes or oncogenes. Acetaldehyde may also interfere with DNA repair by inhibiting enzymes

important to the repair of adducts caused by alkylating agents (Ahmed, 1995; Salaspuro, 2003; Seitz et al., 2001). Regarding these mechanisms, it is plausible to consider alcohol consumption as a possible risk factor for developing colorectal cancer (CRC).

Development of CRC is a multistep process, characterized by an accumulation of several genetic alterations driving normal colorectal mucosa to transform into highly malignant derivatives (Fearon & Vogelstein, 1990; Vogelstein et al., 1988). An early event in colorectal tumorigenesis is often an activating mutation in the *K-ras* oncogene. *K-ras* mutations occur in 30–60% of colorectal carcinomas and are involved in the progression of small adenomas to the more clinically relevant larger adenomas. Therefore, *K-ras* mutations may be an important contributor to CRC development. Most *K-ras* mutations are G → A transitions and G → T transversions (Brink et al., 2003; Fearon &

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Vogelstein, 1990). Several studies have shown that patients with these types of *K-ras* point mutations have a poor prognosis and a high risk of recurrence of the disease (Cerottini et al., 1998; Span et al., 1996). Knowledge of the etiology of *K-ras* mutations arising in colorectal tumors may provide valuable clues for prevention strategies.

The association between alcohol consumption and CRC was long debated, and according to a recently published pooled analysis of eight prospective cohort studies, CRC risk is only increased when daily alcohol consumption levels exceed 30.0 g (Cho et al., 2004). This suggests that there may be a threshold in alcohol consumption above which the risk of CRC is increased. The role of different types of alcoholic beverages on the risk of CRC is less clear, although beer consumption has more consistently been implicated in the etiology of rectal cancer. In the past, beer in the Netherlands and in other European countries was contaminated with relatively large amounts of *N*-nitrosodimethylamine (NDMA) (mean 1.2 µg/kg) (Spiegelhalter et al., 1979; Stephany & Schuller, 1980). As such, beer drinking accounted for 90% of the NDMA intake in beer drinkers, whose total NDMA intake was as much as 10 times higher than that of nonbeer drinkers. Jacoby et al. (1992) have shown that nitrosamines have mutagenic properties and are able to induce mutations, especially G → A transitions in the *K-ras* gene in rodents. However, since 1979, changes in the beer-brewing process have greatly decreased the NDMA contaminations in beers (Ellen & Schuller, 1983). Yet it is likely that the adverse effects of NDMA still influences the risk of (rectal) cancer for up to 10 years later, and that this declines in the years thereafter.

Taking into account the mutagenic and carcinogenic potentials of acetaldehyde mentioned above, we hypothesized that alcohol consumption is associated with an increased risk of mutations in the *K-ras* oncogene involved in CRC. Hence, we examined the associations between consumption of alcohol and various alcoholic beverages and the risk of colon and rectal cancer with and without *K-ras* gene mutations. In a second hypothesis, we stated that beer consumption is associated with G → A mutations in the *K-ras* gene involved in CRC. To answer this hypothesis, we examined the associations between beer consumption and this specific type of *K-ras* mutation among the colon and rectal cancer cases in our study population.

2. Materials and methods

2.1. Study population

In September 1986, the Netherlands Cohort Study on diet and cancer was initiated. The study design has been described in detail elsewhere (Van den Brandt et al., 1990a). Briefly, the cohort included 58,279 men and 62,573 women, aged 55–69 years at baseline and originating from 204 municipal population registries throughout the country. All cohort members completed an extensive self-administered questionnaire on

daily dietary habits during the year preceding the start of the study, lifestyle factors, and other risk factors for cancer. Newly diagnosed cancer cases in the entire cohort were identified through annual record linkage to the Netherlands Cancer Registry (NCR, comprising nine cancer registries in the Netherlands) and to the Netherlands nationwide registry of pathology (PALGA), a nationwide database of histo- and cytopathology reports, used for identification and location of tumor tissue from eligible CRC cases stored in 54 Dutch Pathology laboratories (Van den Brandt et al., 1990b).

These analyses were carried out over the 7.3-year period of follow-up since September 1986. The first 2.3 years of follow-up were excluded, due to incomplete coverage of PALGA in some of the municipalities included and since subclinical symptoms may have influenced the exposure status in cases diagnosed during these 2.3 years. Using updated information from the NCR in 2004, 925 cases with histologically confirmed CRC were identified with an incidence date between January 1989 and January 1994, of whom 815 could be linked to a PALGA report of the lesion. CRC was classified according to site as follows, colon: cecum through sigmoid colon (ICD-O codes 153.0, 153.1, 153.2, 153.3, 153.4, 153.5, 153.6, 153.7, 153.8, 153.9); rectosigmoid colon (ICD-O code 154.0); and rectum (ICD-O code 154.1).

Processing and analysis of data were done according to the case-cohort approach: cases were identified for the entire cohort, whereas a random sample of the cohort (subcohort) was used to estimate person years at risk accumulating in the cohort (from the date of entry into the cohort until the date of a CRC diagnosis, death from any cause, leaving the study area, or the end of the 7.3-year follow-up period). This subcohort of 5,000 men and women was followed-up biennially to assess information on vital status and migration to calculate accumulated person time in the cohort. Prevalent cancer cases, other than nonmelanoma skin cancer were excluded from the subcohort, resulting in 4,748 subcohort members. During the first 2.3 years of follow-up, 101 (2%) subcohort members deceased or were diagnosed with cancer other than nonmelanoma skin cancer, leaving 4,647 subcohort members for analysis.

2.2. Assessment of exposure

Information on daily dietary habits was obtained by a 150-item semiquantitative food frequency questionnaire. Alcohol consumption was measured by six items: (1) beer, (2) red wine, (3) white wine, (4) sherry and other fortified wines, (5) liqueur (containing 16% alcohol), and (6) (Dutch) gin, brandy, and whiskey, which are presumed to cover all types of consumed alcoholic beverages. Participants were asked about their usual frequency of consumption (never/less than once per month, once per month, 2–3 times per month, once per week, 2–3 times per week, 4–5 times per week, and 6–7 times per week) and the number of glasses consumed at each drinking occasion. The total amount of alcohol per day (g/day) was calculated

by using the reported frequency of consumption, the quantity consumed, and the alcohol content of the consumed beverage.

The questionnaire has been validated against a 9-day diet record (Van den Brandt et al., 1990a). The Spearman correlation coefficient between mean daily alcohol intake assessed by the questionnaire and estimated from the 9-day diet record was 0.89 for all subjects and 0.85 for users of alcoholic beverages. The absolute amount of alcohol reported in the questionnaire by users of alcoholic beverages was, on average, 86% of that reported in the 9-day diet record.

Next to the dietary section, the questionnaire comprised a section on lifestyle and other risk factors for cancer. Information on, for instance, family history of CRC, sex, age at baseline, smoking, and body mass index (BMI) were retrieved from this section.

2.3. Tissue samples and molecular analyses

Tumor material of CRC cases, identified in the entire cohort, was collected after approval of the Ethical Review Boards of University Maastricht, the NCR and PALGA. From 44 (5%) of the eligible 815 CRC cases, tissue samples could not be retrieved. Of the retrieved 771 tissue samples, 37 (5%) contained only healthy colorectal mucosa, did not yield sufficient DNA for molecular analyses, or were revised as a benign adenoma instead of a carcinoma by a pathologist. Hence, tumor tissue of 734 CRC cases was available for molecular analyses.

Mutation analysis of the exon 1 fragment of the *K-ras* oncogene, spanning codons 8–29, was performed on archived adenocarcinoma specimens of all 734 CRC patients (Brink et al., 2003).

2.4. Data analyses

Data analyses were based on subcohort members and cases for whom information on alcohol consumption and confounding variables were complete, i.e., 4,076 subcohort members, 428 colon cancer cases, and 150 rectal cancer cases. Since the rectosigmoid can be considered as a clinically applied term rather than an anatomically defined transitional zone between the colon and the rectum, cases with a rectosigmoid tumor ($n = 70$) were excluded from data analyses. Moreover, the group of cases with a rectosigmoid tumor was too small for adequate stratified analyses. Incidence rate ratios (RRs) and corresponding 95% confidence intervals (CIs) were estimated using Cox proportional hazards models. The proportional hazards assumption was tested using the scaled Schoenfeld residuals (Schoenfeld, 1982). Standard errors were estimated using the robust Huber–White sandwich estimator to account for additional variance introduced by sampling from the cohort.

Next to considering total amount of alcohol consumption, we examined three types of alcoholic beverages, i.e., beer, wine (red wine, white wine, sherry, and other fortified wines), and liquor (liqueur, gin, brandy, and whiskey). An extensive categorization of the alcohol variables was not

possible due to small numbers of cases per category. Only total alcohol consumption was divided into three categories: 0, <30.0, and ≥ 30.0 g/day. The threshold value of 30.0 g/day was chosen in line with the results of the pooled analysis by Cho et al. (2004).

Data analysis was conducted separately for male and female colon and rectal cancer with and without a *K-ras* mutation, described here as *K-ras+* (tumors harboring a *K-ras* mutation) and *K-ras-* (tumors harboring a wild type *K-ras* gene). However, since several cells comprised less than 10 cancer cases and some reference groups comprised relatively small numbers of cases, analyses were also conducted for all male plus female CRC cases with and without a *K-ras* mutation. In the overall analyses only, the rectosigmoid cancer cases were included.

In the light of our second hypothesis, cases with a *K-ras* mutation were further examined for associations between beer consumption and the risk of colon and rectal cancer harboring a G \rightarrow A mutation in the *K-ras* gene.

Age at baseline (years), BMI (kg/m^2), family history of CRC (yes vs. no), daily intake of total energy (kJ/day), daily folate intake ($\mu\text{g}/\text{day}$), daily intake of linoleic acid (g/day) and calcium (mg/day), physical activity (min/day), and smoking (never, ex, current) were considered as potential confounders. Those variables that changed the regression coefficient for alcohol by more than 10%, or those that were found to statistically significantly contribute to the multivariable model for colon and rectal cancer were included in the analyses. Analyses for beer, wine, and liquor were additionally adjusted for total alcohol consumption (as a categorical variable), to evaluate the effect of the beverage on the risk of colon and rectal cancer independent of the beverage's alcoholic content. Interactions between sex and alcohol consumption were investigated as well as interactions between smoking and alcohol, and folate intake and alcohol, but none of the tested interactions were found to be statistically significant. P -values $< .05$ were considered statistically significant. All analyses were performed with the STATA statistical software package.

3. Results

Alcohol intake and baseline characteristics of the study population are presented in Table 1. Overall, both male and female colon and rectal cancer patients tended to be older in age than subcohort members. For female colon cancer cases, cases with a *K-ras+* tumor were older in age than cases with a *K-ras-* tumor ($P < .05$). Furthermore, for both men and women, there were no significant differences in alcohol consumption or lifestyle characteristics when comparing the *K-ras-* colon tumors with the *K-ras+* colon tumors or when comparing the *K-ras-* rectal tumors with the *K-ras+* rectal tumors.

Associations between drinking habits in men and the risk of *K-ras-* and *K-ras+* colon and rectal tumors are presented in Table 2. Consumption equal to or exceeding

Table 1
Baseline characteristics and drinking habits (mean [S.D.]) of the study population by sex, tumor site, and *K-ras* status, the Netherlands Cohort Study (1986–1993)

	Men					Women				
	Subcohort	Colon cancer		Rectal cancer		Subcohort	Colon cancer		Rectal cancer	
		Wild type <i>K-ras</i>	Mutated <i>K-ras</i>	Wild type <i>K-ras</i>	Mutated <i>K-ras</i>		Wild type <i>K-ras</i>	Mutated <i>K-ras</i>	Wild type <i>K-ras</i>	Mutated <i>K-ras</i>
<i>N</i>	2,029	148	82	63	36	2,047	140	58	24	27
Age (years)	61.3 (4.2)	62.5 (4.2)	63.4 (4.2)	62.4 (4.1)	62.3 (4.0)	61.4 (4.3)	62.7 (4.3) ^a	64.0 (3.5)	63.5 (3.7)	61.5 (3.7)
Alcohol consumption										
Total alcohol (g/day)	15.0 (16.8)	15.6 (16.5)	15.7 (16.3)	17.4 (19.7)	14.7 (12.7)	5.8 (9.4)	6.0 (12.5)	4.1 (7.1)	5.3 (6.5)	6.9 (3.7)
Beer (glasses/week)	3.2 (7.1)	3.1 (7.2)	2.3 (4.3)	3.3 (6.3)	3.1 (4.7)	0.2 (1.5)	0.3 (2.0)	0.04 (0.2)	0.1 (0.7)	0.1 (0.3)
Wine (glasses/week)	2.5 (5.2)	2.7 (4.9)	3.1 (6.1)	2.4 (5.7)	2.9 (4.6)	2.7 (4.8)	2.5 (5.2)	1.7 (3.5)	2.9 (4.1)	3.4 (6.4)
Liquor (glasses/week)	4.1 (6.4)	4.3 (6.5)	4.5 (6.4)	5.8 (8.7)	3.7 (4.9)	0.9 (2.8)	1.1 (4.4)	1.0 (2.5)	0.4 (1.1)	0.9 (2.1)
Total alcohol (%)										
Abstainers	14	15	16	11	11	33	37	46	38	26
0.1–4.9 g/day	21	24	23	25	22	36	35	28	21	41
5.0–14.9 g/day	27	22	20	21	17	19	13	15	33	18
15.0–29.9 g/day	23	22	23	21	36	9	9	9	8	11
≥30.0 g/day	15	17	18	22	14	3	6	2	0	4
Other characteristics:										
Family history of CRC (%)	5.5	12.8	12.2	7.9	16.7	6.1	12.9	6.9	16.7	7.4
Body mass index (kg/m ²)	25.0 (2.6)	25.3 (2.6)	25.9 (3.1)	12.9 (2.5)	25.1 (2.7)	25.0 (3.6)	25.6 (3.7)	25.5 (3.7)	25.0 (3.7)	25.9 (3.1)
Calcium intake (mg) ^b	947 (293)	976 (3,001)	930 (277)	933 (214)	843 (227)	903 (268)	899 (256)	913 (254)	1,005 (342)	860 (214)
Linoleic acid intake (g) ^c	16.2 (8.2)	16.7 (7.7)	18.3 (7.8)	17.1 (8.4)	17.0 (9.1)	12.7 (6.2)	11.9 (5.9)	13.6 (6.6)	11.4 (5.2)	11.4 (5.3)
Smoking (%)										
Never	13	11	11	19	14	58	62	74	67	56
Exsmoker	52	63	67	59	53	21	24	16	8	33
Current smoker	35	26	22	32	33	21	14	10	25	11

^aP < .05, for comparisons between cases without and with a *K-ras* mutation.

^bAdjusted for energy intake from dairy products.

^cAdjusted for total energy intake.

Table 2

RRs^a for male colon and rectal cancer cases with a wild type or a mutated *K-ras* gene according to drinking habits, adjusted for confounders, the Netherlands Cohort Study (1986–1993)

Drinking habits	Person years in subcohort	Colon cancer				Rectal cancer			
		Wild type <i>K-ras</i>		Mutated <i>K-ras</i>		Wild type <i>K-ras</i>		Mutated <i>K-ras</i>	
		Number of cases	RR (95% CI) ^a	Number of cases	RR (95% CI) ^a	Number of cases	RR (95% CI) ^a	Number of cases	RR (95% CI) ^a
Total alcohol (g/day)									
No alcohol	2,035	22	1.00	13	1.00	7	1.00	4	1.00
<30.0	10,190	100	0.95 (0.6–1.6)	54	0.84 (0.4–1.6)	42	1.23 (0.5–2.8)	27	1.30 (0.4–4.0)
≥30.0	2,121	26	1.24 (0.7–2.3)	15	1.15 (0.5–2.6)	14	2.00 (0.8–5.3)	5	0.98 (0.2–4.0)
Beer ^b									
No beer	6,082	69	1.00	40	1.00	30	1.00	14	1.00
Beer drinker	8,264	79	0.84 (0.6–1.2)	42	0.78 (0.5–1.3)	33	0.68 (0.4–1.2)	22	1.09 (0.5–2.4)
Wine ^b									
No wine	7,094	69	1.00	43	1.00	32	1.00	18	1.00
Wine drinker	7,252	79	1.11 (0.7–1.7)	39	0.85 (0.5–1.4)	31	0.79 (0.4–1.4)	18	0.92 (0.4–1.9)
Liquor ^b									
No liquor	5,234	51	1.00	29	1.00	14	1.00	11	1.00
Liquor drinker	9,112	97	1.08 (0.7–1.7)	53	0.97 (0.5–1.7)	49	2.25 (1.0–5.0)	25	1.15 (0.5–2.7)

RR = incidence risk ratio; CI = Confidence interval.

^aAdjusted for age, family history of colorectal cancer (yes/no), body mass index (kg/m²), calcium intake (mg/day), linoleic intake (g/day), and smoking (never/ex/current).

^bAdditionally adjusted for total alcohol consumption (0, <30.0, ≥30.0 g/day).

30.0 g of alcohol per day, compared to abstaining was associated with an increased risk of colon cancer with and without a *K-ras* mutation, although none of these results reached statistical significance. For rectal cancer, both with and without *K-ras* mutations, the reference group and the group of cases who consumed 30.0 g of alcohol per day or more was very small, making it difficult to interpret the results correctly. Most of the associations with the different beverage types adjusted for total alcohol consumption were not consistent, suggesting that in general no clear associations with *K-ras*– and *K-ras*+ colon and rectal tumors existed. Compared to nonliquor consumers, liquor consumption was associated with an increased risk of a *K-ras*– rectal tumor, independent of the consumed alcohol (RR: 2.25, 95% CI: 1.0–5.0).

Table 3 shows the associations between drinking habits in women and the risk of *K-ras*– and *K-ras*+ colon and rectal tumors. When compared to abstaining, consumption of 30.0 g of alcohol per day and more was positively associated with *K-ras*– colon tumors. The *K-ras*+ colon cancer subgroup and both rectal cancer subgroups comprised too few cases to adequately interpret the results for total alcohol consumption. The results for the different beverage types independent of alcohol content, suggested that there were no clear associations with the risk of colon and rectal cancer with and without a *K-ras* mutation.

For the overall analyses (Table 4), combining all male plus female CRC cases (including rectosigmoid cancer cases), results pointed in the same direction as the results for the stratified analyses presented in Tables 2 and 3. Compared to abstainers, an increased risk was observed for *K-ras*– and *K-ras*+ colorectal tumors for cases whose daily

consumption exceeded 30.0 g of alcohol. Independent of consumed alcohol, only liquor consumption was positively associated with the risk of *K-ras*– colorectal tumors, although statistical significance was not reached (RR: 1.22, 95% CI: 0.9–1.6).

To examine the hypothesis that beer consumption is associated with G → A mutations in the *K-ras* gene, a further distinction of *K-ras*+ tumors was made into tumors with a G → A transition (results not shown). There were 41 male and 29 female colon cancer cases with a G → A transition and 20 male and 9 female rectal cancer cases harboring this transition. When compared to the nonbeer drinkers, consumption of beer was not associated with the risk of colon tumors harboring a G → A mutation in men, independent of the alcohol consumed. A positive association however was observed between beer drinking, independent of the alcohol consumed, and the risk of rectal tumors harboring a G → A mutation (RR: 3.48, 95% CI: 1.1–11.0). In women, these associations could not be assessed since there were only two female colon cancer cases and no female rectal cancer cases harboring a G → A mutation that consumed beer.

Next to beer intake, we also considered associations between total alcohol, wine, and liquor consumption and risk of colon and rectal tumors harboring G → A mutations, but no significant relationships were found.

4. Discussion

4.1. Alcohol, CRC, and *K-ras* gene mutations

In the current prospective study, we have investigated associations between alcohol consumption and the risk of

Table 3

RRs^a for female colon and rectal cancer cases with a wild type or a mutated *K-ras* gene according to drinking habits, adjusted for confounders, the Netherlands Cohort Study (1986–1993)

Drinking habits	Person years in subcohort	Colon cancer				Rectal cancer			
		Wild type <i>K-ras</i>		Mutated <i>K-ras</i>		Wild type <i>K-ras</i>		Mutated <i>K-ras</i>	
		Number of cases	RR (95% CI) ^a	Number of cases	RR (95% CI) ^a	Number of cases	RR (95% CI) ^a	Number of cases	RR (95% CI) ^a
Total alcohol (g/day)									
No alcohol	4,727	52	1.00	27	1.00	9	1.00	7	1.00
<30.0	9,467	80	0.78 (0.5–1.1)	30	0.62 (0.4–1.1)	15	0.98 (0.4–2.2)	19	1.28 (0.5–3.2)
≥30.0	503	8	1.83 (0.8–4.1)	1	0.65 (0.1–5.5)	0	—	1	1.25 (0.1–11.7)
Beer ^b									
No beer	13,239	129	1.00	55	1.00	22	1.00	24	1.00
Beer drinker	1,458	11	0.74 (0.4–1.5)	3	0.80 (0.2–2.8)	2	1.22 (0.3–6.0)	3	0.88 (0.2–3.4)
Wine ^b									
No wine	6,507	71	1.00	33	1.00	10	1.00	11	1.00
Wine drinker	8,190	69	0.79 (0.5–1.3)	25	1.05 (0.4–2.6)	14	3.80 (0.5–30.0)	16	0.88 (0.3–2.8)
Liquor ^b									
No liquor	9,649	90	1.00	40	1.00	18	1.00	14	1.00
Liquor drinker	5,048	50	1.16 (0.7–1.8)	18	1.20 (0.6–2.5)	6	0.56 (0.2–1.7)	13	1.72 (0.7–4.4)

RR = incidence risk ratio; CI = confidence interval.

^aAdjusted for age, family history of colorectal cancer (yes/no), body mass index (kg/m²), calcium intake (mg/day), linoleic intake (g/day), and smoking (never/ex/current).

^bAdditionally adjusted for total alcohol consumption (0, <30.0, ≥30.0 g/day).

colon and rectal cancer with and without *K-ras* mutations, as our hypothesis implied a relationship between alcohol consumption and colorectal tumors harboring a *K-ras* mutation. When considering all male plus female colorectal tumors according to *K-ras* mutational status, our results showed that a daily alcohol consumption of 30.0 g/day and more was associated with an increased risk of CRC, which is consistent with current literature (Cho et al., 2004). However, in contradiction with our hypothesis this

increase in risk was independent of the presence or absence of *K-ras* mutations. When stratifying by sex and tumor site, this result was still observed for subgroups with sufficient numbers of cases. No clear associations were seen between alcohol intakes below 30.0 g/day and the risk of *K-ras*– and *K-ras*+ colon and rectal tumors in both men and women. There is evidence that acetaldehyde is directly involved in CRC (Taylor & Rehm, 2005), but also other mechanisms have been linked to colorectal tumorigenesis,

Table 4

RRs^a for male and female CRC cases (including the rectosigmoid cancer cases) with a wild type or a mutated *K-ras* gene according to drinking habits, adjusted for confounders, the Netherlands Cohort Study (1986–1993)

Drinking habits	Person years in subcohort	Wild type <i>K-ras</i>		Mutated <i>K-ras</i>	
		Number of cases	RR (95% CI) ^a	Number of cases	RR (95% CI) ^a
Total alcohol (g/day)					
No alcohol	6,761	96	1.00	56	1.00
<30.0	19,656	272	0.94 (0.7–1.2)	147	0.85 (0.6–1.2)
≥30.0	2,624	49	1.25 (0.8–1.8)	28	1.13 (0.7–1.9)
Beer ^b					
No beer	19,321	278	1.00	152	1.00
Beer drinker	9,722	139	0.84 (0.6–1.1)	79	0.86 (0.6–1.2)
Wine ^b					
No wine	13,601	202	1.00	117	1.00
Wine drinker	15,441	215	0.92 (0.7–1.2)	114	0.88 (0.6–1.2)
Liquor ^b					
No liquor	14,883	190	1.00	109	1.00
Liquor drinker	14,159	227	1.22 (0.9–1.6)	122	1.07 (0.8–1.5)

RR = incidence risk ratio; CI = confidence interval; CRC = colorectal cancer.

^aAdjusted for age, family history of CRC (yes/no), body mass index (kg/m²), calcium intake (mg/day), linoleic intake (g/day), and smoking (never/ex/current).

^bAdditionally adjusted for total alcohol consumption (0, <30.0, ≥30.0 g/day).

e.g., the induction of reactive oxygen species through the induction of cytochrome P-450 2E (Badger et al., 2003; Boffetta & Hashibe, 2006; Seitz et al., 2005). Many of these processes have been observed in heavy or chronic alcohol consumers. The amount of alcohol-related damage tends to follow a dose–response relationship and as such, higher levels of daily alcohol intakes may be needed for genetic mutations to occur as a result of alcohol consumption. Moderate consumption levels, as in our study population, might not be able to increase CRC risk through causing specific gene mutations. This may explain why we do not observe a differential association between alcohol consumption and *K-ras*– and *K-ras*+ tumors. Thus, moderate drinking apparently involves a certain risk of developing colorectal tumors, possibly arising through mechanisms other than those causative of genetic aberrations.

Liquor consumption as opposed to nonliquor consumption, increased the risk of a *K-ras*– rectal tumor in men independent of the alcohol consumed. Although this finding was statistically significant, we must warrant caution not to overinterpret this result. We performed many analyses and therefore it is possible that this is merely a chance finding. Moreover, the reference group was relatively small compared to the number of liquor drinking *K-ras*– rectal cancer cases. Also, liquor was the most frequently consumed beverage type among the male cancer cases and as such, a statistically significant result is more likely to be found for liquor than for beer or wine.

To date, some studies have been performed on the effect of different lifestyle factors on genetic alterations of the *K-ras* gene (Bautista et al., 1997; Martinez et al., 1999; Slattery et al., 2000, 2001), of which only two considered alcohol consumption (Martinez et al., 1999; Slattery et al., 2000). Martinez et al. (1999), who conducted cross-sectional analyses among adenoma patients in a phase III trial, found no associations between total alcohol consumption and *K-ras* gene mutations in adenomas. Slattery et al. (2000), used data collected as part of a large population-based case–control study on sporadic colon cancer. Without distinguishing between men and women, the authors found that levels of alcohol intake above 10 g/day reduced the risk of *K-ras* mutations in colon tumors (odds ratio [OR]: 0.7, 95% CI: 0.5–1.0). None of the above mentioned authors reported on the different types of alcoholic beverages.

4.2. Alcohol, CRC, and type of *K-ras* mutations

Our data showed no association between beer consumption, independent from alcohol intake, and colon tumors with a G → A mutation in men, whereas a statistically significant increased risk was observed for rectal tumors with a G → A mutation. Again, caution is warranted not to overinterpret this result. The increased risk was based on merely 13 male rectal cancer cases with a G → A mutation and a reference group of only 7 cases. As mentioned in Section 1, the adverse effects of elevated NDMA levels present in

beer before 1979 might influence the risk of cancer for up to 10 years later. We checked the exact year of cancer diagnosis of the 13 male rectal cancer cases that harbored a G → A mutation; however, only one of these cases was diagnosed between January 1979 and January 1990, namely in 1989. Also, the results in Table 2, based on 22 male *K-ras*+ rectal cancer cases and a reference group of 14 cases, indicated that beer, independent of consumed alcohol, was not associated with the risk of rectal tumors harboring a mutated *K-ras* gene. Unfortunately, we have not been able to gather information on the actual nitrosamine levels that were present in beer over the past few decades, or on whether these concentrations might vary across different types of beer and brands. We therefore will not emphasize the association we observed between beer consumption in men and rectal cancer harboring a G → A mutation too much.

To our knowledge, only one other study reported an association between total alcohol consumption, but not specific beverage types, and different types of *K-ras* mutations (Slattery et al., 2000). In this study, the authors found that levels of alcohol above 10 g/day were associated with a reduced risk of G → A mutations in their population-based case–control study on sporadic colon cancer (OR: 0.7, 95% CI: 0.4–1.1).

4.3. Strengths and limitations of the study

This prospective study was based on a large population. Due to record linkage to the NCR and to PALGA, losses to follow-up were minimized. The high completeness of follow-up of cancer incidence and of subcohort members also minimized selection and information bias. Exclusion of cases diagnosed during the first 2.3 years of follow-up, diminished the chance of information bias due to preclinical symptoms of CRC.

Our study is currently the largest prospective study that has investigated associations between alcohol consumption and colon and rectal cancer with and without *K-ras* mutations. Nevertheless, it should be noted that the number of colon and rectal cancer cases in certain subgroups are small.

4.4. Conclusion

This study does not show a clear relationship between alcohol consumption and risk of colon and rectal tumors with a *K-ras* mutation, nor was a positive association apparent between beer consumption and the risk of colon and rectal tumors harboring G → A transitions in the *K-ras* gene. Liquor consumption in men, seemed to be a potential risk factor for developing a *K-ras*– rectal tumor, independent of the alcohol consumed. We conclude that although daily alcohol consumption from a certain level of consumption may be a risk factor for developing CRC, alcohol consumption seems not involved in predisposing to CRC through (specific) mutations in the *K-ras* gene.

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References

- Ahmed, F. E. (1995). Toxicological effects of ethanol on human health. *Crit Rev Toxicol* 25, 347–367.
- Badger, T. M., Ronis, M. J., Seitz, H. K., Albano, E., Ingelman-Sundberg, M., & Lieber, C. S. (2003). Alcohol metabolism: role in toxicity and carcinogenesis. *Alcohol Clin Exp Res* 27, 336–347.
- Bautista, D., Obrador, A., Moreno, V., Cabeza, E., Canet, R., Benito, E., Bosch, X., & Costa, J. (1997). Ki-ras mutation modifies the protective effect of dietary monounsaturated fat and calcium on sporadic colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 6, 57–61.
- Boffetta, P., & Hashibe, M. (2006). Alcohol and cancer. *Lancet Oncol* 7, 149–156.
- Brink, M., de Goeij, A. F., Weijenberg, M. P., Roemen, G. M., Lentjes, M. H., Pachen, M. M., Smits, K. M., de Bruïne, A. P., Goldbohm, R. A., & van den Brandt, P. A. (2003). K-ras oncogene mutations in sporadic colorectal cancer in The Netherlands Cohort Study. *Carcinogenesis* 24, 703–710.
- Cerottini, J. P., Caplin, S., Saraga, E., Givel, J. C., & Benhattar, J. (1998). The type of K-ras mutation determines prognosis in colorectal cancer. *Am J Surg* 175, 198–202.
- Cho, E., Smith-Warner, S. A., Ritz, J., van den Brandt, P. A., Colditz, G. A., Folsom, A. R., Freudenheim, J. L., Giovannucci, E., Goldbohm, R. A., Graham, S., Holmberg, L., Kim, D. H., Malila, N., Miller, A. B., Pietinen, P., Rohan, T. E., Sellers, T. A., Speizer, F. E., Willett, W. C., Wolk, A., & Hunter, D. J. (2004). Alcohol intake and colorectal cancer: a pooled analysis of 8 cohort studies. *Ann Intern Med* 140, 603–613.
- Ellen, G., & Schuller, P. L. (1983). N-nitrosamine investigations in the Netherlands: highlights from the last ten years. In: R. Preussmann (Ed.), *Das Nitrosamin-Problem* (pp. 81–92). Weinheim, Germany: Weinheim Verlag.
- Fearon, E. R., & Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. *Cell* 61, 759–767.
- Jacoby, R. F., Alexander, R. J., Raicht, R. F., & Brasitus, T. A. (1992). K-ras oncogene mutations in rat colon tumors induced by N-methyl-N-nitrosourea. *Carcinogenesis* 13, 45–49.
- Martinez, M. E., Maltzman, T., Marshall, J. R., Einspahr, J., Reid, M. E., Sampliner, R., Ahnen, D. J., Hamilton, S. R., & Alberts, D. S. (1999). Risk factors for Ki-ras mutation in sporadic colorectal adenomas. *Cancer Res* 59, 5181–5185.
- Salaspuro, M. P. (2003). Alcohol consumption and cancer of the gastrointestinal tract. *Best Pract Res Clin Gastroenterol* 17, 679–694.
- Schoenfeld, D. (1982). Partial residuals for the proportional hazards regression model. *Biometrika* 69, 239–241.
- Seitz, H. K., Matsuzaki, S., Yokoyama, A., Homann, N., Vakevainen, S., & Wang, X. D. (2001). Alcohol and cancer. *Alcohol Clin Exp Res* 25, 137S–143S.
- Seitz, H. K., Maurer, B., & Stickel, F. (2005). Alcohol consumption and cancer of the gastrointestinal tract. *Dig Dis* 23, 297–303.
- Slattery, M. L., Anderson, K., Curtin, K., Ma, K., Schaffer, D., Edwards, S., & Samowitz, W. (2001). Lifestyle factors and Ki-ras mutations in colon cancer tumors. *Mutat Res* 483, 73–81.
- Slattery, M. L., Curtin, K., Anderson, K., Ma, K. N., Edwards, S., Leppert, M., Potter, J., Schaffer, D., & Samowitz, W. S. (2000). Associations between dietary intake and Ki-ras mutations in colon tumors: a population-based study. *Cancer Res* 60, 6935–6941.
- Span, M., Moerkerk, P. T., De Goeij, A. F., & Arends, J. W. (1996). A detailed analysis of K-ras point mutations in relation to tumor progression and survival in colorectal cancer patients. *Int J Cancer* 69, 241–245.
- Spiegelhalter, B., Eisenbrand, G., & Preussmann, R. (1979). Contamination of beer with trace quantities of N-nitrosodimethylamine. *Food Cosmet Toxicol* 17, 29–31.
- Stephany, R. W., & Schuller, P. L. (1980). Daily dietary intakes of nitrate, nitrite and volatile N-nitrosamines in the Netherlands using the duplicate portion sampling technique. *Oncology* 37, 203–210.
- Taylor, B., & Rehm, J. (2005). Moderate alcohol consumption and diseases of the gastrointestinal system: a review of pathophysiological processes. *Dig Dis* 23, 177–180.
- Van den Brandt, P. A., Goldbohm, R. A., van 't Veer, P., Volovics, A., Hermus, R. J., & Sturmans, F. (1990a). A large-scale prospective cohort study on diet and cancer in The Netherlands. *J Clin Epidemiol* 43, 285–295.
- Van den Brandt, P. A., Schouten, L. J., Goldbohm, R. A., Dorant, E., & Hunen, P. M. (1990b). Development of a record linkage protocol for use in the Dutch Cancer Registry for Epidemiological Research. *Int J Epidemiol* 19, 553–558.
- Vogelstein, B., Fearon, E. R., Hamilton, S. R., Kern, S. E., Preisinger, A. C., Leppert, M., Nakamura, Y., White, R., Smits, A. M., & Bos, J. L. (1988). Genetic alterations during colorectal-tumor development. *N Engl J Med* 319, 525–532.