Jpn. J. Cancer Res. **90**, 711–719, July 1999

Genotype Difference of Aldehyde Dehydrogenase 2 Gene in Alcohol Drinkers Influences the Incidence of Japanese Colorectal Cancer Patients

Motoi Murata,[1] Masatoshi Tagawa,[2] Satoshi Watanabe,[3] Hideki Kimura,[4] Tatsuya Takeshita[5] and Kanehisa Morimoto[5]

[1]Division of Epidemiology, [2]Pathology, Chiba Cancer Center Research Institute, [3]Division of Gastrointestinal Surgery, [4]Thoracic Diseases, Chiba Cancer Center Hospital, 666-2 Nitona-cho, Chuoh-ku, Chiba, Chiba 260-0801 and [5]Department of Hygiene and Preventive Medicine, Osaka University School of Medicine, 2-2 Yamada-oka, Suita, Osaka 565-0871

A case-control study was conducted to explore the possible etiologic role of alcohol and aldehyde dehydrogenase 2 (*ALDH2*) gene among Japanese colorectal cancer patients. Information on their drinking, smoking and dietary habits was collected from 265 colon and 164 rectum cancer patients, and 794 non-cancer patients as a control group. Genotypes of the *ALDH2* gene at codon 487, glutamic acid (*ALDH2*1*) as a wild-type or lysine (*ALDH2*2*) as a mutated type with reduced enzyme activity, were analyzed by polymerase chain reaction in 160 colon and 110 rectum cancer patients and 121 control persons. Univariate analysis with the χ^2 statistical test showed that heavy alcohol drinking ($P<0.01$), frequent meat intake ($P<0.001$), and irregular ($P<0.01$), hasty ($P<0.01$) and excessive ($P<0.001$) eating habits were associated with the incidence of both colon and rectum cancers, whereas heavier smoking ($P<0.05$) and infrequent fish ($P<0.03$) and fruit ($P<0.01$) intake were solely associated with incidence of rectum cancer. Infrequent green vegetable intake was not correlated with the incidence of colorectal cancer. Multivariate unconditional logistic regression analysis confirmed the association of alcohol consumption ($P<0.01$) and meat intake ($P<0.05$). Homozygous and heterozygous carriers of *ALDH2*2* allele tended to be found in colon (trend $P=0.04$) but not in rectum cancer patients compared to controls. Risk elevation for colon cancer due to alcohol consumption was pronounced among the heterozygotes and it was statistically significant especially for distal colon cancer (trend $P=0.02$). We conclude that alcohol consumption is a risk factor for colorectal cancer and the risk can be enhanced in *ALDH2* heterozygotes.

Key words: Colorectal cancer — Alcohol drinking — *ALDH2* genotype

Alcohol intake has been shown to increase the risk of cancer of the upper aerodigestive organs, such as the oral cavity, pharynx, esophagus and larynx.[1)] Previous epidemiological investigations, however, have not demonstrated a definite etiologic role of alcohol in the incidence of colorectal cancer, and thus the issue remains controversial. An overview of the results of case-control and prospective studies on consumption of alcoholic beverages in relation to colorectal cancer indicates that beer intake, but not intake of other alcoholic beverages is likely to be positively associated with the incidence of rectum cancer.[2–13)] On the other hand, most studies have found no correlation between beer consumption and the incidence of colon cancer (see review by Kune and Vitetta[3)]). Nevertheless, several studies from Japan, including a study on Japanese living in Hawaii, have demonstrated a positive association between alcohol drinking and the incidence of colon cancer.[14–18)]

Mongoloid peoples are known to retain a genetic polymorphism of the aldehyde dehydrogenase 2 (*ALDH2*) gene, which encodes a mitochondrial enzyme responsible for the oxidation of acetaldehyde generated in alcohol metabolism, with two alleles, *ALDH2*1* and *ALDH2*2*, due to a single point mutation at codon 487 in exon 12.[19)] The former is the wild-type with full enzyme activity and the latter is the mutant type with reduced activity. While those with the wild-type homozygote (*ALDH2*1/*1*) have great tolerance to alcohol and can drink alcoholic beverages, those with the mutant homozygote (*ALDH2*2/*2*) are highly intolerant to alcohol and consequently do not drink. The tolerance to alcohol of heterozygotes (*ALDH2*1/*2*) is intermediate and they can drink moderately. Frequencies of the three genotypes, *ALDH2*1/*1*, *-*1/*2* and *-*2/*2* among Japanese are 56, 38 and 6%, respectively.[20)]

Takeshita *et al.*[21)] reported that the mean amount of alcohol consumption in the *ALDH2*1/*2* heterozygous group was about one half that in the *ALDH2*1/*1* homozygous group. Blood concentration of acetaldehyde after consuming alcohol was also different between the two groups. Since acetaldehyde, a substrate of *ALDH2*, is a carcinogenic agent, alcohol drinking must be more harm-

E-mail: mmurata@chiba-cc.pref.chiba.jp

ful for the heterozygotes than the homozygotes. Regarding upper aerodigestive tract cancers, Yokoyama *et al.*[22, 23] demonstrated that the *ALDH2* heterozygous individuals who regularly consume large amounts of alcohol belong to a high risk group. The statistical association of alcohol intake with the frequency of colon cancer, which has been demonstrated among Japanese,[14–18] might be influenced by this genotype difference. A case-control study was therefore conducted to explore a possible etiologic role of alcohol-drinking habit in the incidence of colorectal cancer with special reference to the *ALDH2* genotypes.

MATERIALS AND METHODS

Study subjects The cases were 265 colon (157 males and 108 females) and 164 rectum (110 males and 54 females) cancer patients who underwent surgery in Chiba Cancer Center Hospital from 1989 through 1997. Patients who developed cancer of both the colon and rectum were classified as colon cancer patients. Since this study was started in April 1995, those patients who had died before that time were not included. For the study of *ALDH2* genotypes, 270 randomly selected patients (160 colon and 110 rectum cancers) were examined. As a control group, 794 outpatients (395 males and 399 females) with diseases other than cancer were used, and 121 of these (60 males and 61 females) were tested for the *ALDH2* genotypes. Gender and age distributions of the cases and controls are shown in Table I. The mean age of the male controls was slightly older than that of the cancer patients and that of the female controls was slightly younger than that of the rectum cancer patients.

Questionnaire study Information on the life styles of all subjects, including alcohol consumption, smoking histories and dietary habits was collected by a self-administered questionnaire. With respect to drinking and smoking, the subjects were categorized based on daily consumed amounts, irrespective of their current habitual status. Their favorite kind of alcoholic beverages (Japanese sake, shochu, beer, whisky or wine) and their drinking amounts per occasion were surveyed. Japanese sake was used as a standard for measuring alcohol consumed; a cup (180 ml) of sake corresponds to 27 ml of ethanol. Drinking amounts of other kinds of beverage were converted into that of Japanese sake according to their ethanol content. In the case of occasional drinkers, the amount of alcohol was calculated as one-fifth of the reported amount, and in nondrinkers the amount was categorized as zero. The following points were investigated in the questionnaire: eating attitude (regular or irregular), volume of meal (modest, ordinary, or excessive), eating speed (unhurried, ordinary, or hasty), taste preference for salty foods (prefer, ordinary, or dislike), taste preference for fatty foods (prefer, ordinary, or dislike), intake frequency of meat, fish, green vegetables, root vegetables, sea weed, fruit, milk and Japanese pickles (rare, 1–2/week, 3–4/week, or every day). One-unit moving scores were assigned for successive categories of each item.

***ALDH2* genotype test** DNA was obtained from peripheral blood lymphocytes after securing the informed consent of all donors and was amplified by the polymerase chain reaction (PCR) method using 5′ primer, 5′-CAAATTACAGGGTCAACTGCA-3′ and 3′ primer, 5′-CCACACTCACAGTTTTCTCTT-3′. Amplification consisted of 30 cycles under the following conditions: 1 min at 94°C for denaturing, 1 min at 60°C for primer annealing and 1 min at 72°C for primer extension with Ampli Taq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT). The

Table I. Numbers of Cancer Patients and Controls Classified by Gender and Age Classes

Patients/ controls	Sex[a)]	Age classes									Total
		<40	40–	45–	50–	55–	60–	65–	70–	75+	
Colon cancer	M	6	6	3	17	32	35	26	16	16	157
	F	4	7	8	17	11	17	14	22	8	108
ALDH2 tested	M	4	2	3	9	17	20	14	10	10	89
	F	3	4	4	14	6	11	10	14	5	71
Rectum cancer	M	3	2	8	11	26	27	16	8	9	110
	F	2	7	3	11	6	10	6	6	3	54
ALDH2 tested	M	1	1	7	8	18	17	10	6	6	74
	F	2	4	1	8	5	6	4	3	3	36
Controls	M	0	6	33	59	55	82	65	39	56	395
	F	0	9	54	74	78	62	51	38	33	399
ALDH2 tested	M	0	4	2	6	8	18	8	6	8	60
	F	0	6	3	9	10	12	8	8	5	61

a) Males (M) and females (F).

PCR-amplified DNA was digested with *Ear*I (New England Biolab, Beverly, MA) and the product was subjected to electrophoresis in 4% agarose gels to detect 112 bp and 135 bp bands, which corresponded to the *ALDH2*1* and *ALDH2*2* alleles, respectively.[20]

Statistical methods For the univariate analysis, we performed the Mantel-Haenszel χ^2 test on 2×2 contingency tables, or the Mantel-extension χ^2 test on $2\times k$ contingency tables, comparing numbers of cases and controls in different categories using the assigned scores.[24] In either case, the table was stratified by sex and age classes: <50, 50–59, 60–69 and 70+. Association of alcohol consumption with *ALDH2* genotype in colon cancer patients was further analyzed according to the sites of tumor: ascending, transverse, descending and sigmoid colon, recto-sigmoid junction, rectum, and multiple.

For the multivariate analysis, unconditional logistic regression analysis was performed by adopting those study items which showed significant association with colon or rectum cancer in the univariate analysis.[25] The ages, amounts of alcohol and tobacco, and other items such as eating attitude, taste preference and intake frequency, were all treated as a continuous variable with the assigned scores. The population density of the residential areas of subjects was also included in the logistic regression analysis because it has been well documented that urbanization can be an indicator of Westernized lifestyles in Japan. Relative risk (RR) and its 95% confidence interval (CI) were calculated by using SPSS 7.5J for Medical Pack software (SPSS Inc., Chicago, Ill). The same logistic regression analysis was performed by adopting the *ALDH2* genotype and other items which showed significant association in the former multivariate analysis. This time, females were not included in the analysis because alcohol drinkers are infrequent among them.

RESULTS

Questionnaire study The numbers of colon or rectum cancer patients and controls were tabulated according to gender and the daily amount of alcohol consumed (Table II). They were also tabulated by the age classes, though this is not presented in the table. Among male drinkers, cases and controls, the favorite beverage was Japanese sake (39%), followed by beer (37%), shochu (15%) and others (8%) (data not shown). The proportion of Japanese sake was slightly larger in cancer cases (45%) than controls (36%). Among female drinkers, beer was the most popular beverage (68%). Beer was also more liked in younger than older age groups.

It is clearly seen in Table II that there was a larger proportion of heavy drinkers in the subject group than in the controls in males, while in females this was not seen, probably due to the small number of heavier drinkers. Among males, Mantel-Haenszel odds ratios controlled for age classes were calculated by comparing each class of drinkers with that of non-drinkers as a reference, and the results suggested that heavier drinkers (two or more cups a day) were predisposed to develop colon or rectum cancer (Table II). This tendency was statistically significant when

Table II. Numbers of Cancer Patients and Controls Classified by Amounts of Alcohol Drinking per Day and Statistical Tests

Patients/ controls	Sex	Amounts of alcohol (equivalent to cups of Japanese sake[a])					Total
		0	<1.0	1.0–1.9	2.0–2.9	3.0+	
Colon cancer	M	30	21	49	34	23	157
	F	77	27	3	0	1	108
Rectum cancer	M	20	12	38	26	14	110
	F	35	14	3	2	0	54
Controls	M	77	100	147	47	24	395
	F	238	141	15	2	3	399
		M-H OR (95% CI)[b]					M-ext χ (P)[c]
Colon cancer	M	1.0	0.53 (0.29–0.99)	0.81 (0.48–1.4)	1.66 (0.88–3.1)	2.19 (1.1–4.5)	3.4 (0.003)
Rectum cancer	M	1.0	0.48 (0.22–1.02)	0.84 (0.45–1.6)	2.04 (0.97–4.3)	2.10 (0.91–4.9)	3.0 (0.001)
Colon+rectum cancer	M	1.0	0.51 (0.30–0.87)	0.85 (0.54–1.3)	1.81 (1.03–3.2)	2.19 (1.2–4.2)	4.0 (0.000)

a) One cup (180 ml) of Japanese sake corresponds to 27 ml of ethanol.
b) Mantel-Haenszel odds ratio (95% confidence interval) adjusted for age classes.
c) Mantel-extension χ (P value).

Table III. Numbers of Cancer Patients and Controls Classified by Numbers of Cigarettes Smoked per Day and Estimated Odds Ratios

Patients/ controls	Sex	Numbers of cigarettes / day					Total
		0	<10	10–19	20–29	30+	
Colon cancer	M	42	2	27	5	31	157
	F	93	3	5	6	1	108
Rectum cancer	M	23	2	17	40	28	110
	F	45	3	3	3	0	54
Controls	M	108	23	55	132	77	395
	F	347	15	20	14	3	399
		M-H OR (95% CI)[a)]					M-ext χ (P)[b)]
Colon cancer	M	1.0		0.93 (0.54–1.6)	1.09 (0.68–1.8)	0.96 (0.55–1.7)	0.48 P=0.32
Rectum cancer	M	1.0		1.17 (0.60–2.3)	1.40 (0.77–2.5)	1.50 (0.82–2.7)	1.72 P=0.04
Colon+rectum cancer	M	1.0		1.03 (0.64–1.6)	1.20 (0.80–1.8)	1.16 (0.73–1.8)	1.30 P=0.10

a) Mantel-Haenszel odds ratio (95% confidence interval) adjusted for age classes. Numbers in the class of <10 were all pooled into the class of 10–19, because there were very few cases.
b) Mantel-extension χ (P value).

Table IV. Distribution of *ALDH2* Genotypes in Cancer Patients and Controls with Statistical Tests

Patients/ controls	Sex	Genotypes			Total	M-ext χ (P)[a)]
		*ALDH2*1/*1* (%)	*ALDH2*1/*2* (%)	*ALDH2*2/*2* (%)		
Colon cancer	M	49 (55.0)	32 (36.0)	8 (9.0)	89	1.7
	F	40 (56.4)	28 (39.4)	3 (4.2)	71	(0.04)
Rectum cancer	M	52 (70.3)	18 (24.3)	4 (5.4)	74	0.8
	F	25 (69.4)	11 (30.6)	0 (0)	36	(0.21)
Controls	M	38 (63.3)	20 (33.3)	2 (3.4)	60	
	F	39 (63.9)	21 (34.4)	1 (1.7)	61	

a) Mantel-extension χ (P value) calculated by assigning scores of 0, 1 and 2 for *ALDH2*1/*1*, -**1/*2*, and -**2/*2* genotypes.

both incidence of colon and rectum cancers were included. In contrast, the relative risk for colorectal cancer among lighter drinkers (less than a cup) was reduced to 50%. The risk difference depending on the amount of alcohol drunk was statistically significant for both colon (Mantel-extension test, χ=3.4, d.f.=1, P<0.001) and rectum cancers (χ=3.0, d.f.=1, P<0.01). The association of alcohol consumption with the risk of colorectal cancer was not influenced by the kind of alcoholic beverages (data not shown). Similar tabulation with respect to the smoking history showed that neither the relative risk for colon cancer nor that for rectal cancer was different in any of the classes of smoking level (Table III). However a weak but significant dose-response relationship was observed for rectum cancer (Mantel-extension χ=1.7, P=0.04).

The contribution of other dietary habits, such as eating attitude, was analyzed by Mantel-Haenszel and Mantel-extension χ^2 tests. Significantly positive association with the incidence of colon cancer was observed in subjects who exhibited irregular (P<0.01), hasty (P<0.01) and excessive (P<0.001) eating habits, and in those who had a frequent intake of meat (P<0.001). Association with infrequent intake of milk (P=0.06) and preference for fatty foods (P=0.06) were both marginally significant. In addition to these factors, infrequent intake of fruit (P=0.01) and fish (P=0.03) were also associated with rectum cancer. The frequencies of green and root vegetable intake were not associated with the incidence of colorectal cancer.

***ALDH2* genotype test** Distributions of *ALDH2* genotypes in colon cancer patients showed that the wild-type homozygote (*ALDH2*1/*1*) was less frequent and the mutant homozygote (*ALDH2*2/*2*) and heterozygote (*ALDH2*1/*2*) were both more frequent as compared to

Table V. Odds Ratios (95% Confidence Intervals)[a] for Colon and Rectal Cancers in Males Based on the Amounts of Alcohol Drunk

Cancer	*ALDH2*1/*1* Alcohol intake			*ALDH2*1/*2* Alcohol intake		
	0	<1.0	1.0+	0	<1.0	1.0+
Colon cancer	1.0	1.3 (0.2–8.6)	1.9 (0.4–8.6)	1.0	1.6 (0.3–7.8)	3.1 (0.7–14.0)
Rectum cancer	1.0	0.9 (0.1–5.8)	1.4 (0.4–5.1)	1.0	0.7 (0.1–3.7)	1.3 (0.2–7.0)

a) Odds ratios were estimated in different *ALDH2* genotypes, separately, by the Mantel-Haenszel method after adjusting for age classes.

Table VI. Odds Ratios (95% Confidence Intervals) Obtained by Unconditional Logistic Regression Analysis for Colon and Rectum Cancers

Study item	Comparison	Colon cancer		Rectum cancer	
Sex	Males/females	1.04	(0.68–1.58)	1.18	(0.71–1.97)
Age	Quantitative	1.02	(1.01–1.04)	1.01	(0.62–1.14)
Population density	Quantitative	1.13	(1.05–1.23)	1.02	(0.92–1.13)
Meal time	Irregularity	1.53	(1.04–2.26)	1.46	(0.94–2.27)
Meal amount	Modest/excessive	0.58	(0.43–0.77)	0.74	(0.52–1.04)
Meal speed	Unhurried/hasty	0.89	(0.66–1.19)	0.86	(0.61–1.22)
Salty foods	Preference	0.96	(0.75–1.22)	0.75	(0.56–0.99)
Fatty foods	Preference	0.98	(0.76–1.25)	1.23	(0.91–1.65)
Meat	Eating frequency	1.41	(1.13–1.77)	1.33	(1.01–1.77)
Fish	Eating frequency	1.01	(0.80–1.26)	0.78	(0.59–1.03)
Green vegetables	Eating frequency	0.87	(0.67–1.12)	0.84	(0.62–1.14)
Fruit	Eating frequency	0.94	(0.78–1.13)	0.98	(0.79–1.22)
Milk	Eating frequency	0.98	(0.86–1.12)	0.83	(0.71–0.97)
Alcohol	Drinking amounts	1.35	(1.13–1.62)	1.37	(1.12–1.68)
Tobacco	Numbers/day	0.99	(0.98–1.01)	1.00	(0.99–1.02)

those of controls, irrespective of gender (Table IV), though the differences were not statistically significant (Mantel-Haenszel χ^2=1.5, d.f.=1, 0.30>*P*>0.20). In rectal cancer patients, this tendency was not observed. When doses of *ALDH2*2* allele were scored as 0, 1 and 2 for *ALDH2*1/*1*, -**1/*2*, and -**2/*2* genotypes, respectively, a significant association of the genotype with colon cancer (Mantel-extension χ=1.7, *P*=0.04) but not with rectum cancer (χ=0.8, *P*=0.21) was found. This result indicates that the presence of *ALDH2*2* allele confers susceptibility to colon cancer both in heterozygous drinkers and homozygous non-drinkers.

In Table V, Mantel-Haenszel odds ratios adjusted for age classes were calculated for the amounts drunk by males of the *ALDH2*1/*1* and -**1/*2* genotypes. The risk for colorectal cancer, and particularly for colon cancer, was higher in subjects with the *ALDH2*1/*2* than in those with the *ALDH2*1/*1* genotype, though this difference was not statistically significant. Among the *ALDH2* genotypes, the biphasic association of the amount of alcohol with the frequency of colon cancer, i.e., lower frequency in light drinkers and higher frequency in heavy drinkers (Table II) became invisible (Table V).

We analyzed the relation between the amount of alcohol consumed and the tumor sites, and found that heavier drinkers were inclined to have a cancer at the sigmoid colon or the recto-sigmoid junction (data not shown). Furthermore, the heterozygotes of *ALDH2* also tended to develop colon cancer at the same sites (data not shown). Accordingly, the association of the genotype difference of *ALDH2* with the incidence of colon cancer observed in Table IV should be primarily due to colon cancer at the distal portion, though this association was not statistically significant. When the amount of alcohol was compared between this group of male cancer patients and the controls, the dose-dependent risk elevation was statistically

Table VII. Odds Ratios (95% Confidence Intervals) Obtained by Unconditional Logistic Regression Analysis for Colon and Rectum Cancers in Males

Study item	Comparison	Colon cancer		Rectum cancer	
Age	Quantitative	0.99	(0.96–1.03)	0.99	(0.95–1.03)
Population density	Quantitative	1.28	(1.02–1.62)	1.13	(0.87–1.18)
Meal amount	Modest/excessive	0.66	(0.31–1.42)	0.88	(0.43–1.78)
Fatty foods	Preference	1.33	(0.71–2.48)	0.94	(0.51–1.75)
Meat	Eating frequency	1.46	(0.83–2.55)	1.69	(0.91–3.13)
Fish	Eating frequency	1.06	(0.61–1.84)	0.65	(0.35–1.20)
Green vegetables	Eating frequency	1.19	(0.75–1.87)	1.51	(0.95–2.39)
Milk	Eating frequency	1.05	(0.76–1.45)	0.88	(0.64–1.22)
Alcohol	Drinking amounts	1.52	(0.92–2.23)	1.21	(0.75–1.94)
Tobacco	Numbers/day	0.98	(0.95–1.00)	0.97	(0.97–1.02)
ALDH2 genotypes	Doses of mutant allele	2.13	(0.97–4.66)	1.03	(0.48–2.20)

significant in the heterozygous genotypes (*ALDH2*1/*2*) (Mantel-extension χ=2.1, d.f.=1, P=0.02), but not in the homozygotes (*ALDH2*1/*1*) (χ=1.3, d.f.=1, P=0.10) (data not shown).

Multivariate analysis Multivariate logistic regression analysis on the lifestyles and the incidence of colorectal cancer showed that the amount of alcohol consumed was significantly associated with the incidence of colon (P=0.001) and rectum cancers (P=0.002) (Table VI). Statistical associations of the intake frequency of meat (P=0.003), irregular meal time (P=0.03) and excessive eating habits (P=0.0003) with the incidence of colon cancer were also observed, whereas hasty eating habits (P=0.43) and frequent intake of milk (P=0.80) were not related to the incidence. Population density also showed a positive association with the incidence of colon cancer (P=0.002), indicating that this type of cancer is prevalent among the residents in urban rather than rural areas. On the other hand, preference for salty foods (P=0.04), frequent consumption of meat (P=0.04) and infrequent consumption of milk (P=0.02) were positively associated with the incidence of rectum cancer, whereas the associations with the frequency of fish intake (P=0.08) and with excessive eating habits (P=0.08) were marginal. Tobacco smoking showed no association with colon or rectum cancers. The weak dose-response observed in Table III for rectum cancer should be owing to the effect of confounding with the alcohol drinking habit, because both habits were correlated (Pearson's correlation coefficient=0.38, P<0.0001 in males).

Multivariate analysis including the genotype difference of *ALDH2* demonstrated that both alcohol consumption (P=0.10) and *ALDH2* genotype (P=0.06) were relatively associated with the incidence of colon cancer (Table VII), although the numbers of cases and controls were limited. In contrast, neither alcohol consumption nor *ALDH2* genotype showed a significant association with rectum cancer.

DISCUSSION

We conducted a case-control study to investigate the possible etiologic role of alcohol consumption in colorectal cancer, considering other dietary habits and tobacco smoking history as possible confounding factors, as well as *ALDH2* genotypes, which are known to influence alcohol metabolism. The present results indicate that excessive alcohol consumption is a risk factor for colorectal cancer, although alcohol consumption may be somewhat protective in smaller amounts, and that heavier drinkers who are heterozygous for the *ALDH2* gene are at high risk especially for distal colon cancer.

Besides alcohol drinking habit, frequent meat intake also exhibited a significantly positive association with the incidence of both colon and rectum cancers (Table VI). It has been generally accepted that excessive meat consumption is a risk factor for colorectal cancer,[26] but most Japanese epidemiological studies have failed to confirm this, probably because amounts of per capita meat consumption are still smaller than those in Western countries.[27] On the other hand, a protective effect of vegetables and fruit against colorectal cancer has been demonstrated by previous epidemiologic studies.[28] Our data did not disclose any significant association between the consumed amounts of fruit and vegetables and the incidence of colorectal cancer (Table VI). This should be due to the small number of subjects who seldom eat fruit and vegetables. Previous studies have demonstrated that a habit of tobacco smoking is a risk factor for many types of cancers and is also strongly correlated with a habit of alcohol drinking. However, the result shown in Table VI indicates that the smoking habit is not associated with the incidence of colorectal cancer. Overall, past reports indicate that tobacco smoking is positively associated with the risk of adenoma or carcinoma *in situ* in the large intestine, but not with that of colorectal cancer, which suggests a possible etiologic role in an

earlier stage of colorectal carcinogenesis.[8, 17, 29] It may be difficult to identify its causative effect in studies performed at later stages of colorectal cancer.

Regarding the association of alcohol with colorectal cancer, the previously reported studies, including both case-control and cohort studies, gave conflicting results.[1–18] Yet a meta-analysis by Longnecker *et al.*[2] covering 27 epidemiological studies detected a weak positive association. According to a review by Kune and Vitetta,[3] elevation of colorectal cancer risk with alcohol consumption was observed in beer drinkers with rectal cancer, but not in those with colon cancer. Subsequent case-control[4–7, 10, 12, 13] and cohort[8, 9, 11] studies showed similarly conflicting results. Thus, even the results of recent epidemiological studies are inconsistent regarding the association of alcohol consumption with colorectal cancers.

Hirayama[14] was the first to demonstrate a significant positive association of alcohol consumption and colon (especially, sigmoid colon) cancer in a Japanese cohort population. In Japan, several case-control studies have been conducted in recent years.[15–17, 30] All but one[30] of these studies found a positive correlation between alcohol consumption and colorectal cancer. Yoshida *et al.* showed that the risk of colon rather than rectal cancer was significantly elevated in alcohol drinkers, but sub-sites of the colon were not discriminated.[15] Murata *et al.* found a risk elevation for colon but not rectum cancers in alcohol drinkers in a nested case-control study.[16] They demonstrated that the risk elevation was mainly attributable to proximal colon cancer, which finding does not agree with the present result. Yamada *et al.* did not separate the colon and rectum among their study subjects.[17] Chyou *et al.*, in their cohort study on Japanese people living in Hawaii, reported that the risk of both colon and rectum cancers was increased with alcohol intake.[18] The present study further confirmed that heavier alcohol drinking increases the risk of colorectal cancer incidence. It would thus seem, on the whole, that Japanese individuals are more sensitive to alcohol-induced colon cancer development than Caucasians.

The biological role of alcohol in colon carcinogenesis remains uncharacterized. Acetaldehyde, a metabolite of ethanol metabolism, is a possible candidate for tumorigenesis. Acetaldehyde easily reacts with cellular components and induces cytotoxicity,[31] DNA damage,[32] and carcinogenesis in rodents.[33] Furthermore, it enhances folate deficiency and thus may induce DNA hypomethylation of the intestinal mucosal cells.[34, 35] Such hypomethylation would generate transcriptional activation of various genes, and might lead to accelerated cell cycles or unlimited cell growth. As stated earlier, *ALDH2* mediates the catabolism of acetaldehyde and plays a major role in the blood concentration of acetaldehyde.

The variant of the *ALDH2* enzyme encoded by the mutant *ALDH2*2* allele is deficient in enzymatic activity and is responsible for the "oriental flush" after drinking. Higher blood concentrations of acetaldehyde have been observed in response to alcohol consumption in heterozygotes (*ALDH2*1/*2*), who have decreased ability to metabolize acetaldehyde, than in homozygotes (*ALDH2*1/*1*) who are alcohol-tolerant.[36] Thus investigation of these heterozygotes is crucial to our understanding of the etiologic role of alcohol in colorectal cancer.

Recently, Yokoyama *et al.*[37] extended their earlier studies[22, 23] and performed another epidemiologic analysis on cancer incidence among alcoholic men. They found that, among those heavy drinkers, the frequency of *ALDH2* heterozygotes was 9% in cancer-free subjects and was 32% in patients with cancer of all sites, including that of the upper aerodigestive tracts, stomach, colon and lung. From the data, they estimated that the relative risk to develop any type of cancer was 5.4 in the heterozygotes when compared with the homozygotes. This indicates that persons who are *ALDH2* heterozygotes belong to a high risk group for cancer of any organ when they have continued to drink heavily. The influence of tobacco smoking can not be ignored, as noted by the authors, especially for tobacco-related cancers, because most of their study subjects were smokers and acetaldehyde is one of the major constituents of tobacco smoke.

The present study demonstrated that the heterozygous genotype of *ALDH2* constitutes a high risk factor for colon cancer among ordinary drinkers and this result is compatible with that of Yokoyama *et al.*[37] From these findings, it may be concluded that acetaldehyde is a key substance in colorectal carcinogenesis in relation to alcohol consumption.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan. We are very grateful to Y. Hayakawa and Y. Yokohata for their technical assistance.

(Received February 12, 1999/Revised April 15, 1999/Accepted April 22, 1999)

REFERENCES

1) Blot, W. J. Alcohol and cancer. *Cancer Res.*, **52**, 2119s–2123s (1992).
2) Longnecker, M. P., Orza, M. J., Adams, M. E., Vioque, J. and Chalmers, T. C. A meta-analysis of alcoholic beverage consumption in relation to risk of colorectal cancer. *Cancer Causes Control*, **1**, 59–68 (1990).

3) Kune, G. A. and Vitetta, L. Alcohol consumption and the etiology of colorectal cancer: a review of the scientific evidence from 1957 to 1991. *Nutr. Cancer*, **18**, 92–111 (1992).
4) Barra, S., Negri, E., Franceschi, S., Guarneri, S. and Vecchia, C. L. Alcohol and colorectal cancer: a case-control study from northern Italy. *Cancer Causes Control*, **3**, 153–159 (1992).
5) Meyer, F. and White, E. Alcohol and nutrients in relation to colon cancer in middle-aged adults. *Am. J. Epidemiol.*, **138**, 225–236 (1993).
6) Newcomb, P. A., Storer, B. E. and Marcus, P. M. Cancer of the large bowel in women in relation to alcohol consumption: a case-control study in Wisconsin (United States). *Cancer Causes Control*, **4**, 405–411 (1993).
7) Goldbohm, R. A., Van den Brandt, P. A., Van't Veer, P., Dorant, E., Sturmans, F. and Hermus, R. J. J. Prospective study on alcohol consumption and the risk of cancer of the colon and rectum in the Netherlands. *Cancer Causes Control*, **5**, 95–104 (1994).
8) Boutron, M. C., Faivre, J., Dop, M. C. and Senesse, P. Tobacco, alcohol, and colorectal tumors: a multistep process. *Am. J. Epidemiol.*, **141**, 1038–1046 (1995).
9) Glynn, S. A., Albanes, D., Pietinen, P., Brown, C. C., Rautalahti, M., Tangrea, J. A., Taylor, P. R. and Virtamo, J. Alcohol consumption and risk of colorectal cancer in a cohort of Finnish men. *Cancer Causes Control*, **7**, 214–223 (1996).
10) Le Marchand, L., Wilkens, L. R., Kolonel, L. N., Hankin, J. H. and Lyu, L. Association of sedentary lifestyle, obesity, smoking, alcohol use, and diabetes with the risk of colorectal cancer. *Cancer Res.*, **57**, 4787–4794 (1997).
11) Thun, M. J., Peto, R., Lopez, A. D., Monaco, J. H., Henley, J., Heath, C. W. and Doll, R. Alcohol consumption and mortality among middle-aged and elderly U.S. adults. *N. Engl. J. Med.*, **337**, 1705–1714 (1997).
12) Slattery, M. L., Schaffer, D., Edwards, S. L., Ma, K. N. and Potter, J. D. Are dietary factors involved in DNA methylation associated with colon cancer? *Nutr. Cancer*, **28**, 52–62 (1997).
13) Tavani, A., Ferraroni, M., Mezzetti, M., Franceschi, S., Lo Re, A. and La Vecchia, C. Alcohol intake and risk of cancers of the colon and rectum. *Nutr. Cancer*, **30**, 213–219 (1998).
14) Hirayama, T. Association between alcohol consumption and cancer of the sigmoid colon: observation from a Japanese cohort study. *Lancet*, **ii**, 725–727 (1989).
15) Yoshida, K., Oku, T., Ohba, S., Masuoka, H., Watabe, K., Mukaiya, M., Sasaki, K., Tsutsumi, T., Hirata, K., Mitsuhashi, Y., Takeida, K., Nishi, M., Miyake, H. and Mori, M. A case-control study of colorectal cancer: association between smoking, drinking, family history of cancer and colorectal cancer. *Jpn. J. Cancer Clin.*, **38**, 371–378 (1992) (in Japanese).
16) Murata, M., Takayama, K., Choi, B. C. K. and Pak, A. W. P. A nested case-control study on alcohol drinking, tobacco smoking, and cancer. *Cancer Detect. Prev.*, **20**, 557–568 (1996).
17) Yamada, K., Araki, S., Tamura, M., Sakai, I., Takahashi, Y., Kashihara, H. and Kono, S. Case-control study of colorectal carcinoma *in situ* and cancer in relation to cigarette smoking and alcohol use (Japan). *Cancer Causes Control*, **8**, 780–785 (1997).
18) Chyou, P. H., Nomura, A. M. and Stemmermann, G. N. A prospective study of colon and rectum cancer among Hawaii Japanese men. *Ann. Epidemiol.*, **6**, 276–282 (1996).
19) Goedde, H. W., Harada, S. and Agarwal, D. P. Racial differences in alcohol sensitivity: a new hypothesis. *Hum. Genet.*, **5**, 331–334 (1985).
20) Takeshita, T., Morimoto, K., Mao, K., Hashimoto, T. and Furuyama, J. Phenotypic differences in low Km aldehyde dehydrogenase in Japanese workers. *Lancet*, **341**, 837–838 (1993).
21) Takeshita, T., Morimoto, K., Mao, X., Hashimoto, T. and Furuyama, J. Characterization of the three genotypes of low Km aldehyde dehydrogenase in a Japanese population. *Hum. Genet.*, **94**, 217–223 (1994).
22) Yokoyama, A., Muramatsu, T., Ohmori, T., Higuchi, S., Hayashida, M. and Ishii, H. Esophageal cancer and aldehyde dehydrogenase-2 genotypes in Japanese males. *Cancer Epidemiol. Biomarkers Prev.*, **5**, 99–102 (1996).
23) Yokoyama, A., Ohmori, T., Muramatsu, T., Higuchi, S., Yokoyama, T., Matsushita, S., Matsumoto, M., Maruyama, K., Hayashida, M. and Ishii, H. Cancer screening of upper aerodigestive tract in Japanese alcoholics with reference to drinking and smoking habits and aldehyde dehydrogenase-2 genotypes. *Int. J. Cancer*, **68**, 313–316 (1996).
24) Rothman, K. L. and Boice, J. D., Jr. "Epidemiologic Analysis with a Programmable Calculator," pp. 5–10 (1982). Epidemiology Resources Inc., Boston.
25) Breslow, N. E. and Day, N. E. "Statistical Methods in Cancer Research. Vol. 1 — The Analysis of Case-Control Studies," pp. 192–246 (1980). IARC, Lyon.
26) Potter, J. D., Slattery, M. L., Bostick, R. M. and Gapstur, S. M. Colon cancer: a review of the epidemiology. *Epidemiol. Rev.*, **15**, 499–545 (1993).
27) Kono, S. Dietary factors for gastrointestinal cancers: a worldwide overview. *Gann Monogr. Cancer Res.*, **44**, 29–39 (1996).
28) Steinmetz, M. H. and Potter, J. D. Vegetables, fruits and cancer. I. Epidemiology. *Cancer Causes Control*, **2**, 325–357 (1991).
29) Giovannucci, E., Colditz, G. A., Stampfer, M. J., Hunter, D., Rosner, B. A., Willett, W. C. and Speizer, F. E. A prospective study of cigarette smoking and risk of colorectal adenoma and colorectal cancer in U.S. women. *J. Natl. Cancer Inst.*, **86**, 192–199 (1994).
30) Hoshiyama, Y., Sekine, T. and Sasaba, T. A case-control study of colorectal cancer and its relation to diet, cigarettes, and alcohol consumption in Saitama Prefecture, Japan. *Tohoku J. Exp. Med.*, **171**, 153–165 (1993).

31) Wickramasinghe, S. N., Gardner, B. and Barden, G. Cytotoxic protein molecules generated as a consequence of ethanol metabolism *in vitro* and *in vivo*. *Lancet*, **ii**, 823–826 (1986).
32) Fang, J. L. and Vaca, C. E. Development of a ^{32}P-postlabelling method for the analysis of adducts arising through the reaction of acetaldehyde with 2′-deoxyguanosine-3′-monophosphate and DNA. *Carcinogenesis*, **16**, 2177–2185 (1995).
33) Woutersen, R. A., Appelman, L. M., Van, G. H. A. and Feron, V. J. Inhalation toxicity of acetaldehyde in rat. III. Carcinogenicity study. *Toxicology*, **41**, 212–231 (1986).
34) Garro, A. J., Espina, N., McBeth, D. L., Wang, S. L. and Wu Wang, C. Y. Effects of alcohol consumption on DNA methylation reactions and gene expression: implications for increased cancer risk. *Eur. J. Cancer Prev.*, **1** (Suppl. 3), 19–23 (1992).
35) Cravo, M. L., Mason, J. B., Dayal, Y., Huchinson, M., Smith, D., Selhub, J. and Rosenberg, I. H. Folate deficiency enhances the development of colonic neoplasia in dimethylhydrazine-treated rats. *Cancer Res.*, **52**, 5002–5006 (1992).
36) Takeshita, T., Kawai, T. and Morimoto, K. Elevated levels of hemoglobin-associated acetaldehyde related to alcohol drinking in the atypical genotype of low K_m aldehyde dehydrogenase. *Cancer Res.*, **57**, 1241–1243 (1997).
37) Yokoyama, A., Muramatsu, T., Ohmori, T., Yokoyama, T., Okuyama, K., Takahashi, H., Hasegawa, Y., Higuchi, S., Maruyama, K., Shirakura, K. and Ishii, H. Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics. *Carcinogenesis*, **9**, 1383–1387 (1998).