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

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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 χ2 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.1-5 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.6-13 On the other hand, most studies have found no correlation between beer consumption and the incidence of colon cancer (see review by Kune and Vitetta13). 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.54-18

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-

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ful for the heterozygotes than the homozygotes. Regarding upper aerodigestive tract cancers, Yokoyama et al. 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, 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′-CAAAT-TACAGGGTCAACTGCA-3′ and 3′ primer, 5′-CCACA-CTCACAGTTTTTCTCTT-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).

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

| Patients/controls | Sex | Age classes | Total |
|------------------|-----|-------------|-------|
|                  | M   | 40– 45– 50– 55– 60– 65– 70+ |       |
| Colon cancer     | F   | 6  6  3  17  32  35  26  16  16 | 157   |
| ALDH2 tested     | M   | 4  7  8  11  11  17  14  22  8  | 108   |
|                  | F   | 4  2  3  9  17  20  14  10  10 | 89    |
| Rectum cancer    | M   | 3  2  8  11  26  27  16  8  9  | 110   |
| ALDH2 tested     | F   | 2  7  3  11  6  10  6  6  3  | 54    |
| Controls         | M   | 0  6  33  59  55  82  65  39  56 | 395   |
| ALDH2 tested     | F   | 0  9  54  74  78  62  51  38  33 | 399   |
|                  | 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 EarI (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.

Statistical methods
For the univariate analysis, we performed the Mantel-Haenszel $\chi^2$ test on $2 \times 2$ contingency tables, or the Mantel-extension $\chi^2$ test on $2 \times k$ contingency tables, comparing numbers of cases and controls in different categories using the assigned scores. 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 tumors: 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. 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 $\chi^2 (P_F)^c$

|                  |     | (0.53–0.99) | (0.81–1.66) | (2.19) | 3.4     | (0.003) |
| Colon cancer      | M   | (0.48–1.4)  | (0.88–3.1)  | (1.1–4.5) | 3.0     | (0.001) |
| Rectum cancer     | M   | (0.22–1.02) | (0.45–1.6)  | (0.97–4.3) | 2.10    | (0.001) |
| Colon+rectum cancer | M   | (0.30–0.87) | (0.54–1.3)  | (1.03–3.2) | 2.19    | (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 $\chi^2 (P$ value).
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 $\chi^2$, $P<0.001$) and rectum cancers ($\chi^2=3.0$, 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 $\chi^2$, $P=0.04$).

The contribution of other dietary habits, such as eating attitude, was analyzed by Mantel-Haenszel and Mantel-extension $\chi^2$ 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 ($P=0.03$) and preference for fatty foods ($P=0.06$) were also associated with rectum cancer. The frequencies of green and root vegetable intake were not associated with the incidence of colorectal cancer.

**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 $\chi^2 (P)$$^b$ |
|------------------|---------------------|-------------------------|
| Colon cancer     | 1.0                 | 0.93                    | 1.09 | 0.96 | 0.48 | 0.04 |
|                  | (0.54–1.6)          | (0.68–1.8)              | (0.55–1.7) |       |
| Rectum cancer    | 1.0                 | 1.17                    | 1.40 | 1.50 | 1.72 | 0.04 |
|                  | (0.60–2.3)          | (0.77–2.5)              | (0.82–2.7) |       |
| Colon+rectum cancer | 1.0              | 1.03                    | 1.20 | 1.16 | 1.30 | 0.10 |
|                  | (0.64–1.6)          | (0.80–1.8)              | (0.73–1.8) |       |

$^a$ 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 $\chi^2$ (P value).

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

| Patients/ controls | Genotypes | ALDH2$^1/1$ (%) | ALDH2$^1/2$ (%) | ALDH2$^2/2$ (%) | Total |
|--------------------|-----------|-----------------|-----------------|-----------------|-------|
| Colon cancer       | M         | 49 (55.0)       | 32 (36.0)       | 8 (9.0)         | 89    |
|                    | F         | 40 (56.4)       | 28 (39.4)       | 3 (4.2)         | 71    |
| Rectum cancer      | M         | 52 (70.3)       | 18 (24.3)       | 4 (5.4)         | 74    |
|                    | F         | 25 (69.4)       | 11 (30.6)       | 0 (0)           | 36    |
| 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 $\chi^2$ (P value) calculated by assigning scores of 0, 1 and 2 for ALDH2$^1/1$, -1/2, and -2/2 genotypes.
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those of controls, irrespective of gender (Table IV), though the differences were not statistically significant (Mantel-Haenszel $\chi^2=1.5$, d.f.=1, 0.30>$P>$0.20). In rectal cancer patients, this tendency was not observed. When doses of \textit{ALDH2}’2 allele were scored as 0, 1 and 2 for \textit{ALDH2}’1/’1, ’1/’2, and ’2/’2 genotypes, respectively, a significant association of the genotype with colon cancer (Mantel-extension $\chi=1.7$, $P=0.04$) but not with rectum cancer ($\chi=0.8$, $P=0.21$) was found. This result indicates that the presence of \textit{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 \textit{ALDH2}’1/’1 and ’1/’2 genotypes. The risk for colorectal cancer, and particularly for colon cancer, was higher in subjects with the \textit{ALDH2}’1/’2 than in those with the \textit{ALDH2}’1/’1 genotype, though this difference was not statistically significant. Among the \textit{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 \textit{ALDH2} also tended to develop colon cancer at the same sites (data not shown). Accordingly, the association of the genotype difference of \textit{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 V. Odds Ratios (95% Confidence Intervals)\textsuperscript{a} for Colon and Rectal Cancers in Males Based on the Amounts of Alcohol Drunk

| Cancer        | \textit{ALDH2}’1/’1 Alcohol intake | \textit{ALDH2}’2 Alcohol intake |
|---------------|-----------------------------------|---------------------------------|
|               | 0 <1.0 1.0+                        | 0 <1.0 1.0+                     |
| Colon cancer  | 1.0 1.9 1.0                         | 1.0 1.6 3.1                     |
|               | (0.2–8.6) (0.4–8.6)                | (0.3–7.8) (0.7–14.0)           |
| Rectum cancer | 1.0 1.4 1.0                         | 1.0 0.7 1.3                     |
|               | (0.1–5.8) (0.4–5.1)                | (0.1–3.7) (0.2–7.0)            |

\textsuperscript{a} Odds ratios were estimated in different \textit{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) |
significant in the heterozygous genotypes (ALDH2*1/2) (Mantel-extension $\chi^2=2.1, d.f.=1, P=0.02$), but not in the homozygotes (ALDH2*2/2) ($\chi^2=1.3, d.f.=1, P=0.10$) (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, 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. On the other hand, a protective effect of vegetables and fruit against colorectal cancer has been demonstrated by previous epidemiologic studies. 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.\(^{8,17,28}\) 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.\(^{21}\) 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\(^8,9,31\) 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\(^{25}\) 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 \(ALDH2\,^22\) 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 cannot 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.

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