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Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics

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Aldehyde dehydrogenase-2 (ALDH2) eliminates most of the acetaldehyde produced during alcohol metabolism. In some drinkers, a mutant ALDH2 allele contributes to diminished activity of the enzyme, dramatically increasing the risk for esophageal cancer. This study was designed to evaluate the ALDH2 gene polymorphism as a predictor of the development of cancers prevalent in Japanese alcoholics. We performed ALDH2 genotyping on lymphocyte DNA samples from Japanese alcoholic men (487 cancer-free; 237 with cancer, including 34 oropharyngolaryngeal, 87 esophageal, 58 stomach, 46 colon, 18 liver, 7 lung, 9 other sites, and 19 multiple primary cancers in two or three organs). The frequencies of the mutant *ALDH2*2* allele were significantly higher in alcoholics with oropharyngolaryngeal (52.9%), esophageal (52.9%), stomach (22.4%), colon (21.7%) and esophageal cancer concomitant with oropharyngolaryngeal and/or stomach cancer (78.6%), than in cancer-free alcoholics (9.0%). After adjustment for age, daily alcohol consumption and amount of cigarette smoking, significantly increased risks (odds ratios) in the presence of the *ALDH2*2* allele were found for oropharyngolaryngeal (11.14), esophageal (12.50), stomach (3.49), colon (3.35), lung (8.20) and esophageal cancer concomitant with oropharyngolaryngeal and/or stomach cancer (54.20) but not for liver or other cancers. These results suggest a general role of acetaldehyde, a recognized animal carcinogen, in the development of human cancers.

Introduction

There is considerable epidemiologic evidence that drinking alcoholic beverages is associated with increased risk for certain kinds of cancer (1). For example, the risk for cancers of the oropharyngolaryngeal regions and the esophagus increases in proportion to the amount of alcohol consumed. For colon and stomach cancer, the relationship with alcohol drinking is controversial. In Japanese alcoholics, the oropharyngolaryngeal, esophageal and stomach cancers were detected at extremely high rates by endoscopic screening combined with inspection of oropharyngolaryngeal sites and esophageal iodine staining (2,3).

Despite definitive epidemiologic findings, questions pertaining to the general mechanisms through which alcohol causes cancer, much less site-specific effects, are largely unanswered. The first metabolite of orally ingested alcohol, acetaldehyde, has been suspected to play a pivotal role in the development of alcohol-related cancers because of its established carcinogenicity in laboratory animals (4).

Most of the acetaldehyde generated during alcohol metabolism is eliminated by aldehyde dehydrogenase-2 (ALDH2*) (5). A mutant allele in the ALDH2 gene, which is prevalent in Asians, dramatically diminishes this enzyme activity (5). Although alcohol dehydrogenase-2 (ADH2) in Asians is also polymorphic, and the different forms have different kinetic properties, the genotype of ADH2 has little effect on an individual's blood acetaldehyde concentration after drinking (6). The genotype of ALDH2 thus serves as an indicator of acetaldehyde exposure after alcohol consumption (6). If acetaldehyde is a key substance in certain kinds of cancer, one would expect the mutant *ALDH2*2* allele to be more prevalent in the affected population. The ideal subjects for a study of these associations are people repeatedly exposed to alcohol for a long period, i.e. alcoholic patients. Previous studies have shown a higher risk for esophageal cancer among people with the mutant allele, both everyday drinkers and alcoholics, provided that their alcohol consumption is similar to that of people without this allele (7). Furthermore, the *ALDH2*2* allele is strongly associated with multiple intraesophageal cancers (8) and upper aerodigestive tract cancer concurrent with esophageal cancer (3), both of which are frequent in alcoholics, suggesting that severe systemic acetaldehydemia caused multicentric field cancerization. However, the data demonstrating the association of the allele with oropharyngolaryngeal and stomach cancer were preliminary, and the association with other cancers prevalent in alcoholics remains unclear. To complement and extend these earlier findings, we undertook a comprehensive study of the ALDH2 genotype and cancers prevalent in Japanese alcoholics.

Materials and methods

Subjects with cancer

The reference population comprised 4251 Japanese alcoholic men aged 40 years or older who were consecutively admitted to the National Institute on Alcoholism, Kurihama National Hospital, between January 1987 and October 1997. The diagnosis of alcohol dependence was based on the *Diagnostic and Statistical Manual of Mental Disorders* (3rd edn; 9). Included in this study were cancer patients who met the following criteria: no history of cancer before the onset of alcoholism and either (i) cancer that was histologically diagnosed in the Institute during admission for alcoholism treatment (170 patients), or (ii) history of cancer that was diagnosed at 40 years or older and preceded by the onset of alcoholism (73 patients).

Cancers diagnosed during the admission were detected mainly by screening procedures that included chest x-rays, upper gastrointestinal barium studies, upper gastrointestinal endoscopy combined with oropharyngolaryngeal inspection and esophageal iodine staining (2), fecal occult blood tests and subsequent double-contrast barium enema and/or colonoscopy, and abdominal ultrasound.

***Abbreviations:** ALDH2, aldehyde dehydrogenase-2; OR, odds ratio.

The association of the ALDH2 genotype with small subsets of the upper aerodigestive tract cancers detected by this screening have been reported previously (3,7,8).

The relationship between the onset of alcoholism and the diagnosis of cancer was judged on the basis of responses to the structured questionnaire systematically obtained by trained interviewers at admission from the patients and, when available, their significant others. Loss of control over drinking (i.e. either alcohol withdrawal syndromes or drinking bouts lasting >2 days) was considered a definite sign of alcoholism. Among the 73 patients with a history of cancer preceded by the onset of alcoholism, 64 had undergone surgical resection; two, endoscopic mucosal resection; three, chemotherapy and/or radiation therapy; and four, transcatheter arterial embolization or percutaneous ethanol injection therapy for liver cancer. The history of cancer in 48 patients was also ascertained by referral letters from other hospitals where the cancer treatment had been provided.

The exclusion of three patients in each group whose blood samples could not be obtained left 237 cancer patients for the analysis: 34 with cancers in oropharyngolaryngeal sites (16, oral cavity/oropharynx; 10, hypopharynx; 10, larynx); 87, esophagus; 58, stomach; 46, colon; 18, liver; seven, lung; six, kidney; one each, other sites (duodenum, pancreas, leukemia). Patients with multiple primary cancers in two or three organs numbered 19: five, hypopharyngeal and esophageal cancers; four, esophageal and stomach; and one each for all other combinations (gingival, esophageal and stomach; tongue, laryngeal and esophageal; oropharyngeal, laryngeal, esophageal and stomach; oropharyngeal and esophageal; laryngeal and esophageal; esophageal, colon and liver; stomach and colon; colon and liver; colon and lung; liver and duodenal).

Cancer-free control subjects

Among the above reference population, 558 alcoholic men aged 40 years or older consecutively admitted between January 1996 and April 1997 were systematically examined using chest x-rays, upper gastrointestinal endoscopy combined with oropharyngolaryngeal inspection and esophageal iodine staining (2), immunochemical fecal occult blood tests and subsequent double-contrast barium enema and/or colonoscopy, abdominal ultrasound and brain computed tomography scan. The exclusion of 71 patients who were diagnosed as having cancer or who had a history of cancer treatment left 487 cancer-free alcoholics who served as control subjects.

ALDH2 genotyping

Subjects' blood samples were obtained during admission for alcoholism treatment. ALDH2 genotyping was performed on lymphocyte DNA samples by polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) (10). Briefly, 100–200 ng genomic DNA was mixed with 5 pmol of each primer (5′-CAAATTACAGGGTCAACTGCT-3′sense; 5′-CCACAC-TCACAGTTTTCTCTT-3′ antisense) in a total volume of 50 μl containing 50 μM concentration of each dNTP, 1.5 mM $MgCl_2$, and 1 U of Taq DNA polymerase (Promega, Madison, WI). Thirty-five cycles of PCR (denaturation at 94°C for 15 s, annealing at 58°C for 1.5 min and polymerization at 72°C for 30 s) were performed in a Perkin Elmer Cetus GeneAmp PCR System 9600. After purification, each PCR product was digested with *Mbo*II, electrophoresed on 20% polyacrylamide gel, stained with ethidium bromide, and viewed. Genotypes were determined independently by two investigators who had no knowledge of the patients' status.

Drinking and smoking habits

Information on the subjects' drinking profiles and smoking habits was obtained at admission from the patients and, when available, their partners. The information included the daily alcohol consumption during the year preceding admission, the duration of habitual drinking and the daily number of cigarettes currently smoked. Daily alcohol consumption was expressed in grams per day of ethanol using standard conversion for alcoholic beverages: beer was considered to be 5% ethanol (v/v); wine, 12%; sake, 16%; shochu, 25%; and whiskey, 40%. The commonest alcoholic beverage choice during the preceding year was sake for 40%, shochu for 33%, whiskey for 17% and beer for 10% of the patients.

Statistical analysis

Data were expressed as mean ± SD or as a percentage, and the unpaired Student's *t*-test and Fisher's exact test were used in comparing group statistics. The association between ALDH2 genotype and cancer risk was expressed in terms of the odds ratio (OR), adjusted for the effects of several possible confounders using a multiple logistic regression model. All analyses were done with the SAS statistical package (version 6.11; SAS Institute, Cary, NC).

Results

Table I describes the subjects by cancer status, drinking and smoking habits, and age. The patients with esophageal, stomach, colon, lung or kidney cancer were significantly older than the cancer-free patients. We observed more smoking among oropharyngolaryngeal, hypopharyngeal and multiple cancer patients, and less smoking among those with liver cancer than among controls. As for daily alcohol consumption, however, there were no differences between the cancer patients and controls, except less drinking among the liver cancer patients.

All subjects with the *ALDH2*2* allele were *ALDH2*1/2*2* heterozygotes, which is consistent with previous findings for alcoholic patients (11). The frequency of the *ALDH2*1/2*2* genotype was 11.6% (65/558) among the consecutive patients, but 9.0% (44/487) among those who were cancer-free.

After adjustment for age at admission, daily alcohol consumption during the preceding year and amount of cigarette smoking, we calculated ORs for *ALDH2*1/2*2* by cancer site separately for patients with cancer newly diagnosed at admission for alcoholism treatment and for those with a history of cancer preceded by the onset of alcoholism, because the latter analysis may be influenced by survivor bias. However, because the differences between the two analyses were slight, they were combined (Table II).

The frequency of the *ALDH2*1/2*2* genotype was significantly increased among patients with oropharyngolaryngeal (52.9%; 18/34), oral cavity/oropharyngeal (50.0%; 8/16), hypopharyngeal (70.0%; 7/10), laryngeal (50%; 5/10), esophageal (52.9%; 46/87), stomach (22.4%; 13/58), and colon (21.7%; 10/46) cancers, compared with *ALDH2*1/2*2* frequency in the cancer-free patients. The frequency was extremely high in patients with esophageal cancer concomitant with oropharyngolaryngeal and/or stomach cancer (78.6%; 11/14). These tendencies were not observed with liver or other cancers. Of 18 patients with liver cancer, three tested positive for hepatitis C virus antibodies by a second-generation assay, and another three reacted positively to both hepatitis B surface antigens and antibodies to hepatitis B e antigens; 15 had cirrhosis. The increases in risk with the presence of the *ALDH2*1/2*2* were significant for oropharyngolaryngeal (OR = 11.14), oral cavity/oropharyngeal (OR = 11.13), hypopharyngeal (OR = 23.74), laryngeal (OR = 12.95), esophageal (OR = 12.50), stomach (OR = 3.49), colon (OR = 3.35), lung (OR = 8.20) and esophageal cancer concomitant with oropharyngolaryngeal and/or stomach cancer (OR = 54.20).

Discussion

An alcohol-challenge study has demonstrated that people who are *ALDH2*1/2*2* heterozygotes have blood acetaldehyde concentrations ~6 times higher than *ALDH2*1/2*1* homozygotes (6). In the present study, because the daily alcohol consumption did not differ between cancer and cancer-free alcoholics, the ALDH2 genotype was predictive of systematic acetaldehyde exposure. The most probable explanation for these results is that acetaldehyde, a recognized animal carcinogen (4), contributes significantly to alcohol-related carcinogenesis.

Perhaps the most intriguing aspect of this study was the connection with epidemiologic studies. The highest frequencies of the *ALDH2*1/2*2* genotype were observed in oropharyngolaryngeal and esophageal cancers, in which the associations with alcohol drinking have been definitively demonstrated (1). For colon and stomach cancers, the association has been controversial (1,12). Our results show that *ALDH2*1/2*2*

Table I. Age and drinking and smoking habits in Japanese alcoholics with and without cancer

Subjects, by cancer status	*n*	Age (years)	Ethanol (g/day)	Duration of drinking (years)	Cigarettes (no./day)
Cancer-free	487	53 ± 8	125 ± 65	28 ± 8	21 ± 13
Cancer, total	237	57 ± 8[e]	124 ± 61	32 ± 10[e]	20 ± 15
Oropharyngolaryngeal[a]	34	55 ± 8	143 ± 72	31 ± 7	30 ± 23[c]
Oral cavity/oropharyngeal[a]	16	55 ± 9	159 ± 83	31 ± 8	28 ± 14
Hypopharyngeal[a]	10	54 ± 6	119 ± 58	30 ± 8	41 ± 35[e]
Laryngeal[a]	10	58 ± 6	142 ± 58	32 ± 7	25 ± 12
Esophageal[a]	87	55 ± 7[d]	123 ± 53	30 ± 8[c]	23 ± 19
Stomach[a]	58	60 ± 9[e]	126 ± 62	34 ± 12[d]	18 ± 12
Colon[a]	46	58 ± 9[e]	119 ± 65	32 ± 12[c]	19 ± 11
Liver[a]	18	56 ± 7	105 ± 30[c]	30 ± 11	12 ± 9[d]
Lung[a]	7	62 ± 9[d]	115 ± 62	37 ± 10[d]	23 ± 14
Kidney	3	64 ± 4[e]	133 ± 87	36 ± 6[d]	18 ± 10
Other[a]	3	54 ± 4	104 ± 28	26 ± 5	8 ± 8
All multiple cancers	19	56 ± 5	121 ± 42	31 ± 9	36 ± 28[c]
Multiple esophageal cancers[b]	14	57 ± 6	125 ± 48	31 ± 10	40 ± 31[c]

The data presented are means ± SD.
[a]Includes patients with multiple cancers.
[b]Esophageal cancer concomitant with oropharyngolaryngeal and/or stomach cancers.
[c,d,e]Significantly different from the cancer-free alcoholics at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively (Student's *t*-test).

exists in relatively low frequencies among patients with colon and stomach cancers, compared with those in patients with oropharyngolaryngeal and esophageal cancers; however, the differences between *ALDH2*1/2*2* frequencies among colon and stomach cancer patients and cancer-free controls were marginally significant. This link between epidemiology and animal studies suggests the role of acetaldehyde, especially its role in organ-specific sensitivity in the development of alcohol-related cancer. Although the carcinogenic effects of acetaldehyde may also vary by anatomic subsite in the oropharyngolaryngeal area, the ORs for *ALDH2*1/2*2* were high for all of the oral cavity/oropharyngeal, hypopharyngeal and laryngeal cancers.

We observed no effects of the *ALDH2*2* allele on the development of liver cancer. The confounding effects of other risk factors, including the development of liver cirrhosis and concomitant hepatitis B or C virus infection, may more strongly predict carcinogenicity in the liver.

Also intriguing was the association between multiple cancers and the *ALDH2*2* allele. Esophageal cancer concomitant with oropharyngolaryngeal and/or stomach cancer was strongly associated with the presence of the *ALDH2*2* allele in Japanese alcoholics, suggesting that these cancers share a common genetic and environmental risk in the combination of the *ALDH2*2* allele and heavy drinking. Three of 12 liver cancer patients without hepatitis B or C virus infection had primary digestive tract cancer and were *ALDH2*1/2*1* homozygotes. The detoxification of carcinogenic compounds may be inhibited by liver cirrhosis, or other unidentified mechanisms common to liver and digestive tract cancers may be present.

These new insights suggest the need for review of the epidemiology of alcohol-related cancers. The enormous variation in the incidence of esophageal cancer throughout the world may be partly attributable to the ethnic differences in *ALDH2*2* allele frequency (13). The relationship between colon cancer and alcohol is less clear, but most Asian studies have demonstrated dose-dependent positive results (14–16), which might be explained by the high frequency of the allele in this ethnic population. The prevalence of stomach cancer in Japanese is near the highest in the world, and it is particularly high in Japanese alcoholics (3). Therefore, we suggest that future epidemiologic studies concerning alcohol-related cancers in Asians should take the ALDH2 genotype into account. Several cohort studies have shown high relative risks of lung cancer for alcoholics compared with the general population, but the apparent increases in risk are likely to be attributable to residual confounding by tobacco (1). Although we found significantly increased risk for lung cancer in the presence of the *ALDH2*2* allele in Japanese alcoholics, the small number of lung cancer cases in this study prevents reaching any conclusion. Acetaldehyde is one of the major chemical constituents of tobacco smoke (0.5–1.2 mg/cigarette) (17). Inhalation of acetaldehyde enhanced the incidence of respiratory tract cancers induced by intratracheal instillation of benzo[*a*]pyrene in hamsters (4). Further investigation in this area is needed.

Because the patients were all heavy-drinking alcoholics, this study could not confirm the dose–response relationship between alcohol and cancers. The dose–response pattern combined with ALDH2 genotype needs further investigation. Furthermore, 89% of controls and 85% of cancer patients in the study were also current smokers. It would be worthwhile to evaluate associations within groups of smokers and non-smokers in a sufficiently large sample.

The histopathological characteristics of the cancers in patients with the *ALDH2*2* allele is another interesting area that may provide insight into alcohol-related carcinogenesis. Such studies are underway in our laboratory. By routine application of esophageal iodine staining, we have detected very early esophageal cancer, including carcinoma *in situ* (2,8). The relationship between carcinoma *in situ* and invasive cancer in the esophagus is not clear; however, of 87 esophageal cancer patients in this study, 60.0% (18/30) of those with carcinoma *in situ*, 51.2% (21/41) of those with newly diagnosed invasive cancer and 43.8% (7/16) of those with a history of cancer preceded by the onset of alcoholism had the *ALDH2*2* allele, suggesting that these cancers share a common background.

Whereas much work remains in elucidation of the underlying mechanisms that link the *ALDH2*2* allele and cancers, these

Table II. ALDH2 genotype and risk for cancer in Japanese alcoholics

Subjects, by cancer status	Newly diagnosed cancer				History of cancer					Total			
		ALDH2 genotype (%)		OR for *ALDH2**1/2*2[c]			ALDH2 genotype (%)		OR for *ALDH2**1/2*2[c]		ALDH2 genotype (%)		OR for *ALDH2**1/2*2[c]
	n	2*1/2*1	2*1/2*2	(95% CI)	*n*	Years since diagnosis	2*1/2*1	2*1/2*2	(95% CI)	*n*	2*1/2*1	2*1/2*2	(95% CI)
Cancer-free	487	91.0	9.0	–	487		91.0	9.0		487	91.0	9.0	
Cancer, total	167	67.1	32.9[f]	5.61 (3.50–8.99)	70	3 ± 3	70.0	30.0[f]	5.11 (2.70–9.69)	237	67.9	32.1[f]	5.43 (3.51–8.40)
Oropharyngolaryngeal[a]	19	47.4	52.6[f]	11.66 (4.28–31.73)	15	3 ± 3	46.7	53.3[f]	12.00 (3.80–37.93)	34	47.1	52.9[f]	11.14 (5.09–24.36)
Oral cavity/oropharyngeal[a]	8	50.0	50.0[e]	10.33 (2.41–44.27)	8	4 ± 3	50.0	50.0[e]	13.16 (2.74–63.20)	16	50.0	50.0[f]	11.13 (3.79–32.66)
Hypopharyngeal[a]	6	33.3	66.7[e]	22.16 (3.48–141.18)	4	4 ± 3	25.0	75.0[e]	29.25 (2.38–359.25)	10	30.0	70.0[f]	23.74 (5.24–107.55)
Laryngeal[a]	6	50.0	50.0[d]	17.44 (2.78–109.49)	4	2 ± 1	50.0	50.0[d]	10.42 (1.35–80.37)	10	50.0	50.0[e]	12.95 (3.27–51.26)
Esophageal[a]	71	45.1	54.9[f]	13.33 (7.45–23.84)	16	2 ± 2	56.3	43.8[f]	9.79 (3.23–29.68)	87	47.1	52.9[f]	12.50 (7.23–21.61)
Stomach[a]	35	80.0	20.0	3.35 (1.28–8.76)	23	4 ± 3	73.9	26.0[d]	4.05 (1.40–11.72)	58	77.6	22.4[e]	3.49 (1.64–7.44)
Colon[a]	35	80.0	20.0	3.01 (1.20–7.53)	11	3 ± 4	72.7	27.3	4.86 (1.18–19.94)	46	78.3	21.7[d]	3.35 (1.51–7.45)
Liver[a]	13	100	0		5	1 ± 2	80.0	20.0	2.93 (0.29–29.47)	18	94.4	5.6	0.71 (0.09–5.60)
Lung[a]	6	66.7	33.3	9.87 (1.41–69.12)	1	3	100	0		7	71.4	28.6	8.20 (1.27–53.15)
Kidney	3	100	0		3	4 ± 2	100	0		6	100	0	
Other[a]	1	100	0		2	8 ± 10	100	0		3	100	0	
All multiple cancers	13	38.5	61.5[f]	22.98 (6.03–87.61)	6	3 ± 3	33.3	66.7[e]	20.40 (3.10–134.05)	19	36.8	63.2[f]	21.02 (6.85–64.47)
Multiple esophageal cancers[b]	8	12.5	87.5[f]	240.28 (14.24–4054.30)	6	3 ± 3	33.3	66.7[e]	20.40 (3.10–134.05)	14	21.3	78.6[f]	54.20 (11.51–255.23)

ALDH2, aldehyde dehydrogenase-2; *ALDH2*1*, normal allele; *ALDH2*2*, mutant allele.

[a] Includes patients with multiple cancer.

[b]Esophageal cancer concomitant with oropharyngolaryngeal and/or stomach cancer.

[c]Logistic model adjusted for age at admission, daily alcohol consumption and amount of cigarette smoking.

[d,e,f] Significantly different from the cancer-free alcoholics at $P<0.05$, $P<0.01$ and $P<0.001$, respectively (Fisher's exact test).

new findings have an important clinical implication, especially for the population in which the mutant allele is prevalent. The *ALDH2*2* allele actually affects drinking behavior in the general population: Alcohol consumption is reduced among individuals who carry one or two *ALDH2*2* alleles. Because *ALDH2*2* generally serves as a strong protective factor against alcoholism by making drinking unpleasant (11), it is very likely that *ALDH2*2* protects against alcohol-induced cancers in the normal population. The *ALDH2*2* allele thus has opposing effects: It negatively influences drinking behavior, but it potentiates the carcinogenic effects of alcohol exposure in drinkers.

A simple questionnaire about facial flushing after drinking can indicate an individual's ALDH2 phenotype fairly well (18). Thus, information on ALDH2-associated genetic susceptibilities can be used routinely in daily practice. Moreover, communicating these implications to the public could contribute in a specific way to the prevention of certain kinds of alcohol-related cancer in Asians.

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