Esophageal Cancer and Aldehyde Dehydrogenase-2 Genotypes in Japanese Males

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Abstract
Although drinking alcohol is an established esophageal cancer risk factor, the mechanisms by which alcohol induces this high-mortality rate cancer are not clear. To help elucidate this problem and develop an implementable preventive strategy, this genetic epidemiological study focused on aldehyde dehydrogenase 2 (ALDH2), the key enzyme for elimination of acetaldehyde generated by alcohol consumption. This enzyme is polymorphic; its mutant allele, ALDH2*2, which leads to the enzyme inactivity, is prevalent in Orientals. This Japanese case-control study of ALDH2-related risk for esophageal squamous cell carcinoma included alcoholics (40 cases and 55 controls) and nonalcoholic drinkers (29 cases and 28 controls). The analysis of the results of genotyping these subjects showed that the increased risk for esophageal cancer in those with one ALDH2*2 allele was substantially higher in both alcoholics (odds ratio = 7.6; 95% confidence interval = 2.8–20.7) and nonalcoholic drinkers (odds ratio = 12.1; 95% confidence interval = 3.4–42.8). The results strongly suggest that because persons who have this mutant ALDH2*2 allele have a high concentration of blood acetaldehyde after drinking alcohol, acetaldehyde (a recognized animal carcinogen) plays a pivotal role in the pathogenesis of alcohol-related esophageal cancer in humans. These results suggest that to help lower their risk for esophageal cancer, persons with the ALDH2*2 allele should be encouraged to reduce their consumption of alcoholic beverages.

Introduction
Epidemiological studies consistently show a strong relationship between esophageal cancer and the use of alcohol and tobacco (1–4). Although carcinogenic chemicals have been identified in tobacco (5), ethanol per se has not been proven carcinogenic, and the mechanistic pathway through which drinking causes cancer is not clear. One possible mechanism is that alcohol may generate metabolites that are carcinogenic in humans. Acetaldehyde, the major metabolite of ethanol and a recognized animal carcinogen (6, 7), is hypothesized to be the key substance in alcohol-related cancer in humans (2). However, due largely to the lack of practical experimental design, whether acetaldehyde plays a crucial role in the pathogenesis of esophageal cancer is still open to question.

Ethanol is eliminated from the body by oxidation to acetaldehyde and then to acetate, reactions catalyzed by alcohol dehydrogenase and ALDH2, respectively (8). Most of the acetaldehyde generated during alcohol metabolism is eliminated by ALDH2, the low Km form of ALDH (8). Although genetic polymorphism of alcohol dehydrogenase 2 and ALDH2 presents in Orientals, the genotype of ALDH2 is the major determinant of an individual’s blood acetaldehyde concentration after drinking (9).

Molecular epidemiological investigation of ALDH2 could provide some clue to the role of alcohol in esophageal cancer. The gene for the homotetrameric enzyme ALDH2 is located on chromosome 12, where the single bp difference in exon 12 produces a catalytically inactive isozyme (10, 11). The distribution of the ALDH2 allele has striking racial differences. The mutant allele ALDH2*2 encodes an inactive subunit with a single point mutation corresponding to an amino acid substitution (12). No ALDH2*2 allele has been found in Caucasians and African-Americans, but it is prevalent in Orientals. It is associated with the “flushing response” after consumption of a small amount of alcohol. This response, which includes facial flushing, tachycardia, headache, and other unpleasant symptoms, is caused by acetaldehydemia and prevents people from heavy drinking and developing of alcoholism (13). In Japan and China, approximately 10% of alcoholics have this mutant allele, compared with approximately 50% of control subjects (14–16). The mutant allele is dominant: persons with at least one ALDH2*2 allele lack the enzyme activity and show high blood acetaldehyde concentration after ethanol ingestion (9, 17). In ALDH2*2/ALDH2*2 homozygotes and ALDH2*1/ALDH2*2 heterozygotes, blood acetaldehyde concentrations are approximately 19 and 6 times that in ALDH2*1/ALDH2*1 homozygotes, respectively (9).

Because the ALDH2*2 allele serves as a determinant of an individual’s acetaldehyde exposure after drinking, we compared the distribution of the ALDH2*2 allele in esophageal cancer patients in the alcoholic and nonalcoholic population in this study. The primary goal was to improve our understanding of the etiological mechanisms and contribute to laboratory research on the pathogenesis of esophageal cancer.

Materials and Methods
Subjects. All of the subjects who participated in this study were Japanese. The alcoholic cases were 40 male patients (ages 44–75 years, mean 55 ± 7 years) who had esophageal cancer

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that was diagnosed in the National Institute on Alcoholism (Kurihama National Hospital, Kanagawa, Japan) between 1991 and 1995. The alcoholic controls were 55 randomly selected male patients who admitted to the Institute in 1991 (ages 50–66 years, mean 56 ± 4 years). The average ethanol consumption/day was 120 ± 53 g ethanol and 98 ± 82 g ethanol for the cancer alcoholics and the control alcoholics, respectively. All alcoholics met DSM-III R criteria for alcohol dependence (18).

The nonalcoholic cases were 29 male drinkers (ages 46–80 years, mean 61 ± 9 years) who had esophageal cancer that was diagnosed in the Tokyo Dental College, Ichikawa General Hospital between 1994 and 1995. All of these nonalcoholic patients drank every day (average ethanol consumption 59 ± 33 g/day). Twenty-eight nonalcoholic male controls (ages 41–77 years, mean 56 ± 11 years) who drank every day (average ethanol consumption 48 ± 13 g/day) were recruited from the staff and their acquaintances of the National Institute on Alcoholism. All nonalcoholic subjects did not meet the criteria for alcohol dependence of DSM-III R. All cancer subjects had histologically confirmed squamous cell carcinomas of esophagus.

The study was reviewed and approved by the Ethics Committee of the Institute and informed consent to participate in the study was obtained from the subjects. No subjects denied to participate in the study.

**ALDH2 Genotyping.** DNA was extracted from the blood by a standard method, and ALDH2 genotyping was performed by PCR-RFLP method (19). Briefly, 100–200 ng of genomic DNA were mixed with 5 pmol of each primer (5'-CAAATTACAGGTCAGGGGTCC-3' sense; 5'-CCACACCTCATGTTTTCTCTTT-3' antisense) in a total volume of 50 μl containing 50 μM concentration of each dNTP, 1.5 mM MgCl2, and 1 unit 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 MboII, electrophoresed on 20% polyacrylamide gel, stained with ethidium bromide, and viewed. Genotypes were determined without knowledge of the subjects' status.

**Statistical Analysis.** The Mann-Whitney U test was used to compare mean values of age and alcohol consumption. Differences in genotype frequencies were analyzed for significance by the χ² test. To test for significant associations between the ALDH2 genotype and cancer risk, ORs were calculated directly from the staff and their acquaintances of the National Institute on Alcoholism.

The risks for cancer of the esophagus with reference to ALDH2*2 in the two populations are presented in Table 2. The increases in risk with the presence of the ALDH2*2 allele were significant for both groups (alcoholics, OR = 7.6; nonalcoholics, OR = 12.1).

**Discussion**

The knowledge of the prevalence and effect of the ALDH2*2 allele prompted us to choose a molecular epidemiological approach for study of the long-debated relationship between the drinking of alcoholic beverages and esophageal cancer. Although epidemiological studies have consistently shown that chronic ethanol consumption is a strong determinant of carcinogenesis of the esophagus in humans (1–4), animal feeding studies have provided no evidence of the carcinogenicity of ethanol per se (1). Rather than a carcinogen, ethanol has been considered a syncarcinogen or cocarcinogen through a variety of mechanisms, such as its local solvent effect, which increases the penetration of other carcinogens into exposed tissues or the induction of microsomal enzymes that activate a variety of procarcinogens (22). The generation of metabolites after ethanol absorption complicates the situation. Among these metabolites, the first, acetaldehyde, has been particularly suspect, given its carcinogenicity in animal studies (6, 7).

In this study focused on the genetic differences of ALDH2, the established determinant of blood acetaldehyde after drinking, we first compared the frequency of genotypes of this enzyme in both esophageal cancer patients and controls in the alcoholic population. The rationale for this experimental design was straightforward: if acetaldehyde plays a critical role in the pathogenesis of esophageal cancer, the proportion of persons with the ALDH2*2 allele should be higher among the cancer patients, provided the two groups’ ethanol consumption are similar. In fact, we observed no difference between the mean daily ethanol consumption by cancer and control alcoholics. Despite the lack of difference in alcohol consumption, the difference in the ALDH2 genotype and allele frequency between the cancer and control groups was striking. Among the alcoholic esophageal cancer patients, the genotype frequency of ALDH2*1/ALDH2*2 heterozygotes was approximately 4.1 times that in the control alcoholics.

![Image](https://example.com/image.png)
We observed a similar tendency in nonalcoholic subjects. In a group of everyday drinkers selected as controls to match the alcohol consumption, the frequency of the ALDH2*1/ALDH2*2 genotype was 17.9%, which was lower than the frequencies in general population ranging from 35.0 to 36.6% reported by Japanese studies (9, 23, 24). This could be because the large portion of Japanese with inactive ALDH2 do not drink habitually (25). In any event, the genotype frequency of ALDH2*/ALDH2*2 in our nonalcoholic esophageal cancer subjects far exceeded that in Japanese general population and was 4.0 times the frequency in the control everyday drinkers.

Finding no ALDH2*2/ALDH2*2 homozygotes in this study population indicates that the observed association of the ALDH2*2 allele and esophageal cancer is not attributable to linkage disequilibrium with other unknown susceptibility loci but is mediated by alcohol drinking because ALDH2*2/ALDH2*2 homozygotes drink little, if any (16, 23, 24).

Together, our data strongly suggest that acetaldehyde, a recognized animal carcinogen, is crucial to the pathogenesis of alcohol-related esophageal cancer in humans. The main limitation of the present study is sample size, which does not permit simultaneous analysis of other possible confounding factors, such as smoking and diet that have been reported to contribute to the development of esophageal cancer. Therefore, this institute-based study should be considered as a step to a large-scale epidemiological study. There have been no such studies that shed light on the inactive ALDH2, and it is tempting to speculate that the marked differences in the incidence of esophageal cancer observed in diverse geographical areas are, at least in part, attributable to the racial differences of the frequency of this mutant ALDH2*2 allele (26, 27).

Despite the limitation, the message we have derived from the data is intriguing, from the point of view of both carcinogenesis research and preventive medicine. This new evidence of its involvement in the pathogenesis of alcohol-related esophageal cancer could renew interest in acetaldehyde as a subject of future laboratory research. In the meantime, the suggestion that reduced consumption of alcoholic beverages should help to lower the risk of this high mortality cancer in persons with the ALDH2*2 allele, i.e., those who exhibit a flushing response after drinking small amounts of alcohol. Among the symptoms of the response, facial flushing has been proven to be the most reliable indicator of the inactive ALDH2 (28). This information is available through a simple questionnaire and, thus, can be included in a large-scale epidemiological study, which is needed to complete risk assessment for the mutant allele.

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