

Esophageal Cancer and Aldehyde Dehydrogenase-2 Genotypes in Japanese Males

Akira Yokoyama,¹ Taro Muramatsu, Tai Ohmori, Susumu Higuchi, Motoi Hayashida, and Hiromasa Ishii

National Institute on Alcoholism, Kurihama National Hospital, 5-3-1 Nobu Yokosuka, Kanagawa 239 [A. Y., T. M., S. H., M. H.], Department of Surgery, Tokyo Dental College, Ichikawa General Hospital, Ichikawa, Chiba 272 [T. O.], and Department of Internal Medicine, School of Medicine, Keio University, Shinjuku-ku, Tokyo 160 [H. I.], Japan

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 ALDH,² respectively (8). Most of the acetaldehyde generated during alcohol metabolism is eliminated by ALDH2, the low K_m 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

Received 7/7/95; revised 10/10/95; accepted 11/14/95.

¹ To whom requests for reprints should be addressed.

² The abbreviation used are: ALDH, aldehyde dehydrogenase; OR, odds ratio.

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), slightly modified. Briefly, 100–200 ng of genomic DNA were mixed with 5 pmol of each primer (5'-CAAATTACAGGGTCAAGGGCT-3' sense; 5'-CCA-CACTCACAGTTTTCTCTT-3' antisense) in a total volume of 50 μ l containing 50 μ M 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 *Mbo*II, 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 χ^2 test. To test for significant associations between the *ALDH2* genotype and cancer risk, ORs were calculated directly from the simplest 2 \times 2 cross-tabulation and their 95% confidence intervals by the logit method of Woolf (20) as estimates of the relative risks in alcoholics and nonalcoholics, respectively. The Mantel-Haenszel method was used to adjust for age and daily alcohol consumption, respectively (21). Crude ORs were virtually unchanged by adjustment for these factors; therefore, only crude ORs are presented.

Results

There were no significant differences in age or daily alcohol consumption between the cancer patients and controls in the alcoholic and nonalcoholic groups.

The restriction fragment patterns obtained are illustrated in Fig. 1. Of the three possible allele combinations, we found no *ALDH2*2/ALDH2*2* homozygotes among the subjects in this study.

Table 1 shows the genotype frequencies of the *ALDH2* loci in the four populations. The genotype frequency of the *ALDH2*1/ALDH2*2* heterozygote was significantly higher in

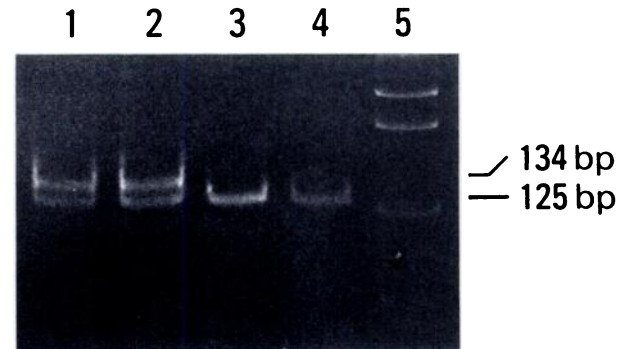


Fig. 1. DNA was amplified using PCR and subjected to *Mbo*II restriction enzyme digestion. Samples were analyzed by 20% PAGE: Lanes 1 and 2, *ALDH2*1/ALDH2*2*; Lanes 3 and 4, *ALDH2*1/ALDH2*1*; Lane 5, marker.

the esophageal cancer patients, in both alcoholic (52.5 versus 12.7%; $P < 0.001$) and nonalcoholic populations (72.4 versus 17.9%; $P < 0.001$).

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.

Table 1 ALDH2 genotype frequencies

	ALDH2*1/2*1	ALDH2*1/2*2
Alcoholics with esophageal cancer (n = 40)	19 (47.5%)	21 (52.5%) ^a
Control alcoholics (n = 55)	48 (87.3%)	7 (12.7%)
Drinkers with esophageal cancer (n = 29)	8 (27.6%)	21 (72.4%) ^b
Control drinkers (n = 28)	23 (82.1%)	5 (17.9%)

^a $P < 0.001$; $\chi^2 = 17.6$; degrees of freedom = 1.

^b $P < 0.001$; $\chi^2 = 17.1$; degrees of freedom = 1.

Table 2 ORs for the ALDH2*1/2*2 genotype

	OR	95% Confidence interval
Alcoholics	7.6	2.8–20.7
Nonalcoholics	12.1	3.4–42.8

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*1/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.

Acknowledgments

The authors extend their appreciation to H. Imazeki for her technical assistance and Drs. K. M. Parrish and T. Yokoyama for statistical analysis.

References

- International Agency for Research on Cancer. Alcohol drinking. *In: IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans*, Vol. 44. Lyon, France: IARC, 1988.
- Blot, W. J. Alcohol and cancer. *Cancer Res.*, 52 (Suppl.): 2119s–2123s, 1992.
- Hirayama, T. Diet and cancer. *Nutr. Cancer*, 1: 67–81, 1979.
- Yokoyama, A., Ohmori, T., Makuuchi, H., Maruyama, K., Okuyama, K., Takahashi, H., Yokoyama, T., Yoshino, K., Hayashida, M., and Ishii, H. Successful screening for early esophageal cancer in alcoholics using endoscopy and mucosa iodine staining. *Cancer (Phila.)*, 76: 928–934, 1995.
- International Agency for Research on Cancer. Tobacco smoking. *In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 38. Lyon, France: IARC, 1986.
- International Agency for Research on Cancer. Allyl compounds, aldehydes, epoxides and peroxides. *In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, pp. 101–132. Vol. 36. Lyon, France: IARC, 1985.
- Woutersen, R. A., Appelman, L. M., Van Garderen-Hoetmer, A., and Feron, V. J. Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. *Toxicology*, 41: 213–231, 1986.
- Bosron, W. F., and Li, T-K. Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases, and their relationship to alcohol metabolism and alcoholism. *Hepatology*, 6: 502–510, 1986.
- Yamamoto, K., Ueno, Y., Mizoi, Y., and Tatsuno, Y. Genetic polymorphism of alcohol and aldehyde dehydrogenase and the effects on alcohol metabolism. *Jpn. J. Alcohol & Drug Dependence*, 28: 13–25, 1993.
- Hsu, L. C., Yoshida, A., and Mohandas, T. Chromosomal assignment of the genes for human aldehyde dehydrogenase-1 and aldehyde dehydrogenase-2. *Am. J. Hum. Genet.*, 38: 641–648, 1986.
- Yoshida, A., Hsu, L., and Yasunami, M. Genetics of human alcohol-metabolizing enzymes. *Prog. Nucleic Acid Res. Mol. Biol.*, 40: 255–287, 1991.
- Yoshida, A., Huang, I-Y., and Ikawa, M. Molecular abnormality of an inactive aldehyde dehydrogenase variant commonly found in Orientals. *Proc. Natl. Acad. Sci. USA*, 81: 258–261, 1984.
- Harada, S., Agarwal, D. P., Goedde, H. W., Takagi, S., and Ishikawa, B. Possible protective role against alcoholism for aldehyde dehydrogenase isozyme deficiency in Japan. *Lancet*, 2: 827, 1982.
- Thomasson, H. R., Edenberg, H. J., Crabb, D. W., Mai, X-L., Jerome, R. E., Li, T-K., Wang, S-P., Lin, Y-T., Lu, R-B., and Yin, S-J. Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *Am. J. Hum. Genet.*, 48: 677–681, 1991.
- Higuchi, S., Matsushita, S., Imazeki, H., Kinoshita, T., Takagi, S., and Kono, H. Aldehyde dehydrogenase genotypes in Japanese alcoholics. *Lancet*, 343: 741–742, 1994.
- Muramatsu, T., Wang, Z-C., Fang, Y-R., Hu, K-B., Yan, H., Yamada, K., Higuchi, S., Harada, S., and Kono, H. Alcohol and aldehyde dehydrogenase genotypes and drinking behavior of Chinese living in Shanghai. *Hum. Genet.*, 96: 151–154, 1995.
- Crabb, D. W., Edenberg, H. J., Bosron, W. F., and Li, T-K. Genotypes for aldehyde dehydrogenase deficiency and alcohol sensitivity: the inactive ALDH2*2 allele is dominant. *J. Clin. Invest.*, 83: 314–316, 1989.
- American Psychiatric Association. *The Diagnostic and Statistical Manual of Mental Disorders*, Ed. 3, Revised, DSM-III R, pp. 173–175. Washington, DC: American Psychiatric Association, 1987.
- Harada, S., and Zhang, S. New strategy for detection of ALDH2 mutant. *Alcohol Alcohol.*, 28: 11–13, 1993.
- Woolf, B. On estimating the relation between blood group and disease. *Ann. Hum. Genet.*, 19: 251–253, 1955.
- Mantel, N., and Haenszel, W. Statistical aspects of the analysis of data from retrospective studies of disease. *J. Natl. Cancer Inst.*, 22: 719–748, 1959.

22. Lieber, C. S., Garro, A., Leo, M. A., Mak, K. M., and Worner, T. Alcohol and cancer. *Hepatology*, 6: 1005–1019, 1986.
23. Higuchi, S., Matsushita, S., Muramatsu, T., Murayama, M., and Hayashida, M. Alcohol and aldehyde dehydrogenase genotypes and drinking behavior in Japanese. *Alcohol. Clin. Exp. Res.*, in press, 1996.
24. Takeshita, T., Morimoto, K., Mao, X-Q., 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.
25. Ohmori, T., Koyama, T., Chen, C. C., Yeh, E. K., Reyes, B. V., Jr., and Yamashita, I. The role of aldehyde dehydrogenase isozyme variance in alcohol sensitivity, drinking habits formation and the development of alcoholism in Japan, Taiwan and the Philippines. *Prog. Neuro-Psychopharmacol. & Biol. Psychiatry*, 10: 229–235, 1986.
26. Age-adjusted death rates for malignant neoplasm of esophagus in 1984–85 in 40 countries. *In: M. Kurihara, K. Aoki, and S. Hisamichi (eds.), Cancer Mortality Statistics in the World 1950–1985*, pp. 60. Nagoya, Japan: The University of Nagoya Press, 1989.
27. Goedde, H. W., Agarwal, D. P., Fritze, G., Meier-Tackmann, D., Singh, S., Beckmann, G., Bhatia, K., Chen, L. Z., Fang, B., Lisker, R., Paik, Y. K., Rothhammer, F., Saha N., Segal, B., Srivastava, L. M., and Czeizel, A. Distribution of ADH₂ and ALDH₂ genotypes in different populations. *Hum. Genet.*, 88: 344–346, 1992.
28. Higuchi, S., Muramatsu, T., Shigemori, K., Saito, M., Kono, H., Dufour, M. C., and Harford, T. C. The relationship between low Km aldehyde dehydrogenase phenotype and drinking behavior in Japanese. *J. Stud. Alcohol*, 53: 170–175, 1992.