REVIEW

Alcohol and Aldehyde Dehydrogenase Polymorphisms and a New Strategy for Prevention and Screening for Cancer in the Upper Aerodigestive Tract in East Asians

Akira Yokoyama,[1] Tai Omori[2] and Tetsuji Yokoyama[3]

[1]National Hospital Organization Kurihama Alcoholism Center, Kanagawa, Japan
[2]Department of Surgery, Kawasaki Municipal Hospital, Kanagawa, Japan
[3]Department of Human Resources Development, National Institute of Public Health, Saitama, Japan

(Received for publication on April 19, 2010)
(Revised for publication on May 31, 2010)
(Accepted for publication on June 17, 2010)

The ethanol in alcoholic beverages and the acetaldehyde associated with alcohol consumption are Group 1 human carcinogens (WHO, International Agency for Research on Cancer). The combination of alcohol consumption, tobacco smoking, the inactive heterozygous aldehyde dehydrogenase-2 genotype (*ALDH2*1/*2*) and the less-active homozygous alcohol dehydrogenase-1B genotype (*ADH1B*1/*1*) increases the risk of squamous cell carcinoma (SCC) in the upper aerodigestive tract (UADT) in a multiplicative fashion in East Asians. In addition to being exposed to locally high levels of ethanol, the UADT is exposed to a very high concentration of acetaldehyde from a variety of sources, including that as an ingredient of alcoholic beverages *per se* and that found in tobacco smoke; acetaldehyde is also produced by salivary microorganisms and mucosal enzymes and is present as blood acetaldehyde. The inefficient degradation of acetaldehyde by weakly expressed ALDH2 in the UADT may be critical to the local accumulation of acetaldehyde, especially in *ALDH2*1/*2* carriers. *ADH1B*1/*1* carriers tend to experience less intense alcohol flushing and are highly susceptible to heavy drinking and alcoholism. Heavy drinking by persons with the less-active *ADH1B*1/*1* leads to longer exposure of the UADT to salivary ethanol and acetaldehyde. The *ALDH2*1/*2* genotype is a very strong predictor of synchronous and metachronous multiple SCCs in the UADT. High red cell mean corpuscular volume (MCV), esophageal dysplasia, and melanosis in the UADT, all of which are frequently found in *ALDH2*1/*2* drinkers, are useful for identifying high-risk individuals. We invented a simple flushing questionnaire that enables prediction of the ALDH2 phenotype. New health appraisal models that include ALDH2 genotype, the simple flushing questionnaire, or MCV are powerful tools for devising a new strategy for prevention and screening for UADT cancer in East Asians. (Keio J Med 59 (4) : 115–130, December 2010)

***Keywords*: acetaldehyde, alcohol dehydrogenase, aldehyde dehydrogenase, esophageal cancer, head and neck cancer**

Introduction

Alcohol consumption and tobacco smoking are two major risk factors for squamous cell carcinoma (SCC) of the upper aerodigestive tract (UADT; the oral cavity, pharynx, larynx, and esophagus) in developed countries, and these factors synergistically increase the cancer risk. A large Japanese case-control study demonstrated that a combination of drinking (≥ 33 g ethanol/day) and heavy smoking (≥ 30 pack-years) posed the highest risk of esophageal and hypopharyngeal cancer [odds ratio (OR; 95% confidence interval) = 29.9 (10.9–81.9)] in compar-

Reprint requests to: Akira Yokoyama, MD, National Hospital Organization Kurihama Alcoholism Center, Kanagawa, 5-3-1 Nobi, Yokosuka, Kanagawa 239-0841, Japan, E-mail: a_yokoyama@kurihama1.hosp.go.jp

ison with the combination of never drinking and never smoking, whereas the OR was 8.2 (2.4–27.7) for the combination of drinking (≥ 33 g ethanol/day) and never smoking and 3.9 (1.3–11.8) for the combination of heavy smoking and never drinking.[1] Adequate intake of fruits and vegetables is associated with a reduced risk of SCC in the UADT. A recent Japanese prospective cohort study demonstrated that an increase in total fruit and vegetable intake by 100 g/day was associated with an 11% (1%–21%) decrease in the incidence of esophageal SCC,[2] and the protective effect of fruits and vegetables was greater in heavy drinkers and smokers. Low body mass index (BMI) is another risk factor for SCC in the UADT.[3] A case-control study[4] and a prospective study[5] in Japanese alcoholics showed that alcoholics with a BMI <19.0 (lowest quartile) had a substantially higher risk of SCC in the UADT than those with a BMI ≥ 23.2–23.7 [highest quartile; OR = 4.6 (1.8–11.7) for esophageal SCC and hazard ratio (HR; 95% confidence interval) = 3.6 (1.2–11.1) for SCC in the UADT]. Many Japanese alcoholic patients are also heavy smokers, and their poor nutritional status and low BMI are largely attributable to poor dietary habits, including a low intake of green and yellow vegetables, and smoking.[6]

We introduced an endoscopic screening program at Kurihama Alcoholism Center in 1993,[7] and by 2008, initial screening of 5210 Japanese alcoholic men by endoscopy combined with oropharyngolaryngeal inspection and esophageal iodine staining had detected esophageal SCC in 206 (4.0%) and oropharyngolaryngeal SCC in 55 (1.1%). Recent technical improvements in endoscopes and a growing understanding of the endoscopic findings of early SCC in the esophagus[7,8] and pharynx[9] have enabled very early detection of SCC in the UADT. Treatment of early esophageal SCC by endoscopic mucosectomy has become widespread in Japan and has succeeded in improving the outcome of this high-mortality cancer[10,11]; early pharyngeal SCC is also now being treated by endoscope-guided mucosectomy[12] with excellent outcomes.[13] It has thus become even more important to develop preventive strategies and screening programs based on risk factors.

Genetic Polymorphism of ALDH2 and Alcohol Metabolism

A mutant allele encoding an inactive subunit of aldehyde dehydrogenase-2 (ALDH2; rs671) was carried by the Han Chinese as they spread throughout East Asia.[14] Its highest frequency has been found in Southeast China, and inactive ALDH2 is observed in most areas of China, Japan, Korea, Mongolia, and Indochina, but is not found in most other populations. About 40% of Japanese have the inactive *ALDH2*2* allele (e.g., 7% are homozygous and 35% are heterozygous),[15] which acts in a dominant manner. After drinking 0.1 g ethanol/kg body weight, homozygotes for inactive ALDH2 and heterozygotes for inactive ALDH2 had 18 times higher and 5 times higher peak blood acetaldehyde concentrations, respectively, than active *ALDH2*1/*1* homozygotes who had consumed 0.8 g ethanol/kg body weight.[16] An alcohol challenge test showed a 7% slower ethanol elimination rate among Japanese with the *ALDH2*1/*2* genotype than with the *ALDH2*1/*1* genotype, suggesting product inhibition of ethanol oxidation by high blood acetaldehyde levels in *ALDH2*1/*2* heterozygotes.[17] After drinking a relatively small amount of alcohol, people with inactive ALDH2 tend to experience a flushing response, including facial flushing, palpitations, nausea, and drowsiness,[18] and they experience a hangover the next morning.[19] Having inactive ALDH2 generally prevents East Asians from drinking heavily. Homozygotes for inactive ALDH2 are usually non-drinkers or occasional drinkers because of their very intense flushing responses, but 26% of heavy drinkers[20] and 13% of alcoholics[15] in Japan have been found to be heterozygous for inactive ALDH2 in Japan.

ALDH2 and SCC in the UADT Esophageal SCC in Japan and Taiwan

In 1996, we first reported that the heterozygous inactive ALDH2 genotype is a strong risk factor for esophageal cancer in everyday drinkers and alcoholics.[21] Since then, Japanese and Taiwanese case-control studies have shown that approximately 65%–76% of esophageal cancer patients[20–28] and 53%–63% of alcoholics with esophageal cancer[21,29–31] had the *ALDH2*1/*2* genotype, and the studies have consistently demonstrated strong associations.[33] In a study in which alcoholics with no evidence of cancer based on endoscopic screening served as controls, the OR for detection of esophageal SCC by screening heterozygote alcoholics with inactive ALDH2 was 13.5-fold (8.1–22.6) the OR of alcoholics with active ALDH2.[31] We conducted a multicenter case-control study of 234 esophageal SCC cases and 634 controls[20] and found that the OR of moderate drinkers (198–365 g ethanol/week) with the *ALDH2*1/*2* genotype was 55.8 (15.4–202.5) when never/rare drinkers (< 22 g ethanol/week) were used as the reference group, and that far exceeded the OR of 10.4 (2.9–37.8) of heavy drinkers (≥ 396 g ethanol/week) with the *ALDH2*1/*1* genotype (**Fig. 1A**). Estimation of population attributable risk (PAR) revealed that alcohol-drinking *ALDH2*1/*2* heterozygotes accounted for 69% of the cancer cases. The PAR was 31% for preference for drinking strong alcoholic beverages straight, 54% for smoking, 26% for low intake of green-yellow vegetables, and 38% for low intake of fruits. If ALDH2 heterozygotes who consume 198 g ethanol/week or more consumed less than 198 g ethanol/week instead, 53% of esophageal cancers might be prevented in this population.[34] The ORs for the devel-

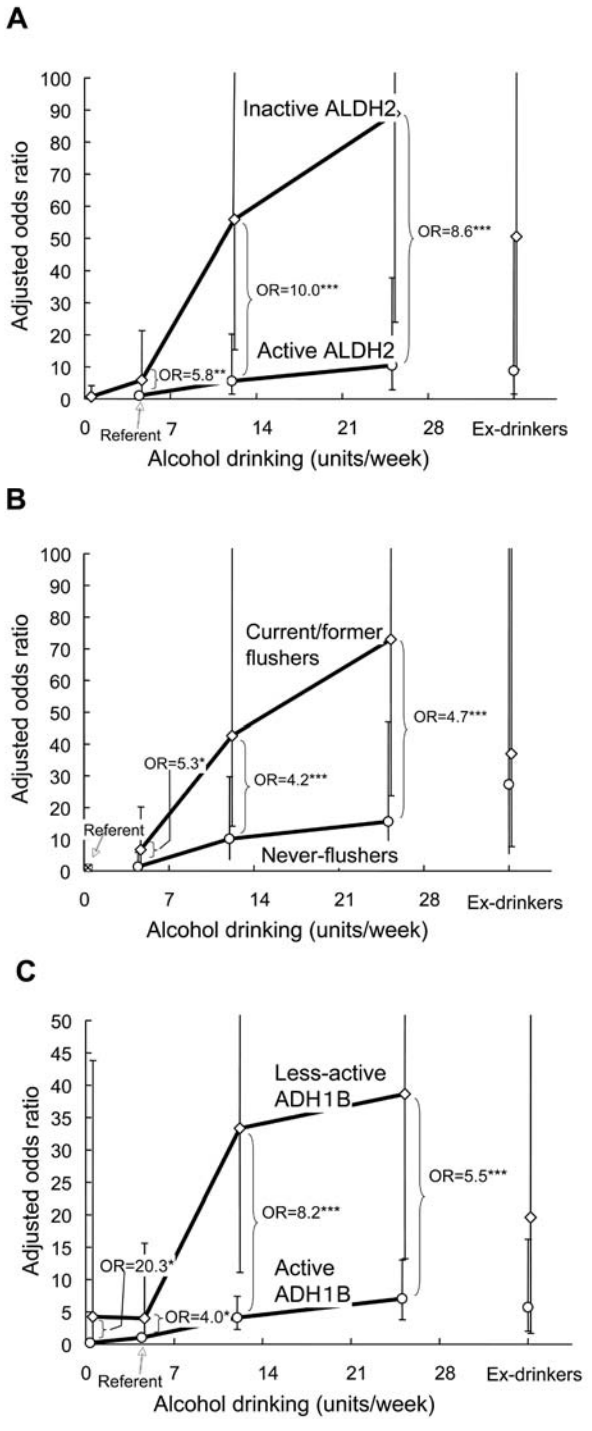

Fig. 1 Alcohol drinking by Japanese men and risk of esophageal squamous cell carcinoma according to (A) ALDH2 genotype,[20] (B) alcohol flushing,[106] and (C) ADH1B genotype.[20] The subjects were classified as never/rare drinkers, current drinkers who consumed 1 to 8.9 units/week (light drinkers; 1 unit = 22 g ethanol), 9 to 17.9 units/week (moderate drinkers), or 18+ units/week (heavy drinkers), or ex-drinkers. Odds ratios were adjusted for age, frequency of drinking strong alcoholic beverages straight, pack-years of smoking, frequency of intake of green and yellow vegetables, and frequency of fruit intake. The vertical lines indicate the 95% confidence interval. *p<0.05, **p<0.01, and ***p<0.001.

opment of esophageal cancer of Taiwanese drinkers with inactive heterozygous ALDH2 are also markedly high.[26,27] Persons who have the inactive *ALDH2*1/*2* genotype and are in the habit of drinking more than 30 g ethanol/day have a 103-fold (38–275) greater odds of developing esophageal cancer than non-drinkers with the active *ALDH2*1/*1* genotype, and a PAR of 58% for alcohol-drinking *ALDH2*1/*2* heterozygotes was found.[27] A meta-analysis showed that ALDH2-related susceptibility to esophageal cancer includes light-to-moderate drinkers as well as heavy drinkers.[35] The magnitude of the *ALDH2*1/*2*-associated risk was 7.07 (3.67–13.60) times in heavy drinkers and 2.49 (1.29–4.79) times in light-to-moderate drinkers.

A Japanese case-control study of women showed a markedly higher risk [OR = 59.1 (4.65–750)] of esophageal SCC in heavy drinkers (≥ 396 g ethanol/week) with the *ALDH2*1/*2* genotype than in never/rare drinkers with the *ALDH2*1/*1* genotype; this study also showed a somewhat lower risk in women than in men overall, except among heavy drinkers with the *ALDH2*1/*2* genotype.[36] The large recent increase in alcohol consumption among women in Japan may have been too recent for the full effects to have materialized. The reported number of years of alcohol consumption among drinkers was substantially fewer for women than for men, and the women were more health-conscious than men in regard to smoking and dietary habits.

The two largest case-control studies, one in Taiwan[37] and the other in Japan,[28] showed that the *ALDH2*1/*2* genotype increased the risk of esophageal SCC more in the younger population, suggesting that high acetaldehyde exposure accelerates the development of esophageal SCC at a young age.

Esophageal SCC in Mainland China

In high-risk areas for esophageal cancer in mainland China, there is pronounced heritability of esophageal cancer risk, and alcohol drinking plays a less important role in esophageal carcinogenesis than in Japan and Taiwan. In such a high-risk area, case-control studies have shown modest to moderate positive associations [ORs = 1.67 (1.02–2.72) to 3.12 (1.86–6.58)][38–41] or no associations[42] between inactive ALDH2 and esophageal cancer risk. The *ALDH2*1/*2*-associated risk in Chinese studies was stronger where the impact of alcohol consumption on esophageal carcinogenesis was stronger, and when only male populations were evaluated. Over the past 40 years, per-capita alcohol consumption has increased by 9 times in China, whereas it has doubled in Japan. Consumption has remained stable in recent years in Japan, but will probably increase further in China.[43] Chinese consumption is still comparable to Japanese consumption in the 1970s, when the number of heavy drinkers with inactive heterozygous ALDH2 started to

increase dramatically.[44] Alcohol-related esophageal cancer in mainland China will be a greater problem in the future.

Head and Neck Cancer

A meta-analysis of six Japanese case-control studies showed increased *ALDH2*1/*2*-related susceptibility to head and neck cancer in moderate drinkers as well as in heavy drinkers compared to *ALDH2*1/*1* genotype,[45] and the magnitude of the *ALDH2*1/*2* effect on head and neck cancer was greater in heavy drinkers [OR = 3.57 (1.41–9.05)] than in moderate drinkers [OR = 1.68 (1.27–2.22)]. Japanese studies of the *ALDH2*1/*2*-associated risk for head and neck cancer have reported different patterns of association according to anatomical site and drinking habit. One case-control study of oral cancer in which alcohol consumption was not a risk factor showed that ALDH2 genotype had no effect,[46] but another case-control study of oral cancer in which alcohol consumption was a risk factor reported a relatively weak but significantly increased risk [OR= 2.9 (1.1–7.8)] associated with *ALDH2*1/*2*.[47] A case-control study of oral and pharyngeal SCC showed that *ALDH2*1/*2* was a strong risk factor for SCC in the hypopharynx [OR = 10.1 (3.8–26.8)] among men who were moderate-to-heavy drinkers, but the OR of 1.80 (0.83–3.89) for SCC of the oral cavity and oropharynx did not reach the level of significance.[48] We conducted a nested case-control study based on the results of the initial endoscopic screening of 3016 Japanese alcoholic men.[49] In that study 55 cases of SCC of the oropharyngolarynx were detected, and 275 cancer-free controls matched for age and initial examination time were randomly selected to provide a case:control ratio of 1:5. The OR of inactive ALDH2 heterozygotes versus active ALDH2 homozygotes for SCC in the oropharyngolarynx as a whole was 4.70 (2.60–8.51), and the OR of inactive ALDH2 heterozygotes versus active ALDH2 homozygotes for SCC in the oropharynx/hypopharynx/epilarynx was 6.66 (3.27–13.6). The OR of 1.55 (0.40–5.95) for SCC in the tongue/gingiva/vocal cord, however, did not reach the level of significance. A prospective study of cancer-free Japanese alcoholic men showed similarly high HRs of 13.0 (5.2–32.1) and 11.7 (4.7–29.5) for SCC of the esophagus and oropharyngolarynx in inactive ALDH2 heterozygotes, respectively, but half of the oropharyngolaryngeal SCCs were hypopharyngeal.[5] Inactive heterozygous ALDH2 and initial multiplicity of intra-esophageal SCCs increased the risk of metachronous hypopharyngeal/epilaryngeal SCC in Japanese alcoholic men who had been treated for esophageal SCC [HR = 12.08 (1.52–95.89) and 3.73 (1.22–11.41), respectively], but the positive associations with oral cavity/oropharyngeal SCC did not reach the level of significance [HR = 2.53 (0.82–7.86) and 2.41 (0.96–6.05), respectively].[50] The impact of the carcinogenesis-inducing effect of acetaldehyde may differ according to anatomical site.

Field Cancerization

The prevalence of multiple-organ cancer among Japanese esophageal cancer patients in the National Cancer Center has increased at an alarming rate, from 6% in 1969–80 to 22% in 1981–91 and to 39% in 1992–96.[51] The most frequent sites of multiple cancers were the head, neck and stomach. The SEER Program of the U.S. National Cancer Institute has reported synchronous and metachronous multiple cancers in only 2% and 3%, respectively, of esophageal cancer patients between 1973 and 2003.[52] One reason for the increase in Japan is the improvement in survival rates, which increases the chance of developing other primary cancers. Another reason is the widespread use of esophageal iodine staining to screen head and neck cancer patients for esophageal cancer; this has yielded a very high esophageal cancer detection rate (e.g., 11.8%).[53] Yet another reason for the increase in multiple esophageal cancers in Japan is the dramatic increase in the proportion of inactive ALDH2 heterozygotes among Japanese alcoholics in the same period, from 3% in 1979 to 8% in 1986 and 13% in 1992.[44] The proportion of inactive ALDH2 heterozygotes among Han Chinese alcoholics in Taiwan also increased during the 1990s, from 10% to 18%.[54] The inhibitory impact of inactive heterozygous ALDH2 on heavy drinking is influenced by sociocultural factors, such as greater peer pressure and greater social acceptance of drinking in Japanese male culture.

Japanese case-control studies and endoscopic follow-up studies have consistently demonstrated that *ALDH2*1/*2* is a strong risk factor for multiple cancerization in the UADT.[29,31,50,55–59] Alcoholics with the *ALDH2*1/*2* genotype and those with synchronous multiple esophageal SCCs at baseline had a markedly increased likelihood of developing metachronous SCC in the UADT after treatment for esophageal SCC.[50] Acetaldehyde plays a critical role in multicentric field cancerization throughout the UADT.

The frequency of synchronous and metachronous gastric cancer among esophageal SCC patients is also high in the Japanese population,[51,60] suggesting the presence of common background factors underlying the gastric and esophageal cancers. We conducted a case-control study based on the results of initial endoscopic screening of 3497 Japanese alcoholic men in which gastric adenocarcinoma was detected at a high rate of 1.4%.[61] In the gastric cancer cases compared with the controls, there was a significantly higher age-adjusted prevalence of patients positive for *Helicobacter pylori* (78% vs. 57%, $P = 0.022$), of severe chronic atrophic gastritis (CAG) assessed by the serum pepsinogen test (49% vs. 15%, $P <$

0.001), of *ALDH2*1/*2* (36% vs. 14%, $P < 0.001$), of mean corpuscular volume (MCV) ≥ 106 fl (38% vs. 20%, $P = 0.008$), and of synchronous SCC in the UADT (18% vs. 5%, $P = 0.004$). Even among the gastric-cancer-free controls, the prevalence of severe CAG was higher than generally reported in Japan, and combinations of CAG and *ALDH2*1/*2* entailed a higher risk of gastric cancer [OR = 4.0 (1.1–14), 17.6 (5.3–58), 9.7 (2.4–39), 17.1 (3.9–76), and 39.2 (6.4–239) for non-severe CAG alone, severe CAG alone, *ALDH2*1/*2* alone, non-severe CAG plus *ALDH2*1/*2*, and severe CAG plus *ALDH2*1/*2*, respectively].

The risk of metachronous gastric cancer in Japanese men after surgery for esophageal cancer has been reported to be twice that of the general Japanese male population.[60] The risk of metachronous gastric cancer is especially high in Japanese alcoholic men with esophageal SCC: the estimated cumulative rate for metachronous gastric cancer within 5 years was 15% in an alcoholic study,[62] as opposed to the 10% within 10 years reported in a Japanese non-alcoholic study.[60] CAG assessed by the serum pepsinogen test and *H. pylori* status were compared in 90 Japanese alcoholic men with esophageal SCC and 180 age-matched Japanese gastric- and esophageal-cancer-free alcoholic men.[62] The results showed markedly accelerated progression of *H. pylori*-related severe CAG in those with esophageal SCC, indicating the existence of common mechanisms by which both esophageal SCC and severe CAG develop. Endoscopic follow-up in Japanese alcoholic men with esophageal SCC revealed an age-adjusted HR of 7.87 (1.43–43.46) in patients with severe CAG in comparison with those without CAG. Accelerated development of severe CAG partially explained the very high frequency of development of metachronous gastric cancer in this population.

Esophageal Dysplasia

Esophageal dysplasia is rarely detected by endoscopy in the absence of esophageal iodine staining. We have collected mucosal biopsy specimens from distinctly iodine-unstained lesions (DIULs) with a greatest dimension of 5 mm or more, but because we often observed multiple DIULs in alcoholics, especially in those with *ALDH2*1/*2*, we were puzzled as to how to prioritize lesions for biopsy. We noticed that the DIULs changed color, and that most of the cancerous lesions became partially or completely tinged light pink about 2 minutes after staining. We refer to this endoscopic finding as the "pink color sign (PC sign)."[63] A comparison of 112 dysplasia lesions and 138 very early cancers revealed that the PC sign was positive in 90% of the cancers but in only 3% of the dysplasia lesions. Another study demonstrated 92% sensitivity and 94% specificity of the PC sign as a means of differentiating high-grade intra-epithelial neoplasia or SCC from other non-cancerous lesions when targeted biopsy of 121 DIULs ≥ 5 mm was performed.[64] The PC sign is a useful endoscopic finding for identifying high-risk cancerous lesions during screening of high-risk drinkers.

In a study in which five deep serial sections were obtained from each biopsy specimen of DIULs ≥ 5 mm detected in alcoholics and stained with hematoxylin and eosin, an expert pathologist in the field of esophageal neoplasia diagnosed 96% of the non-cancerous DIULs as esophageal dysplasia.[65] The presence of multiple esophageal iodine-unstained lesions and the presence of large esophageal DIULs ≥ 5 mm were correlated with inactive heterozygous ALDH2[58,66–68] and with increased risk of multiple SCCs in the UADT.[5,58,66,67,69,70] The 6 year cumulative rates of detection of metachronous SCC in the esophagus and oropharyngolarynx were 56% and 35%, respectively, in Japanese alcoholics after endoscopic mucosectomy of esophageal SCC, as opposed to 31% and 20%, respectively, in those with esophageal dysplasia ≥ 5 mm, and 4% and 4%, respectively, in those without DIULs ≥ 5 mm.[71] Esophageal dysplasia ≥ 5 mm is associated with a very high risk of SCC in the UADT. The presence of multiple esophageal iodine-unstained lesions is a strong predictor of the development of metachronous SCC in the esophagus[69] and head and neck[70] in Japanese patients after endoscopic mucosectomy for esophageal SCC.

Melanosis in the UADT

Melanosis of the palate, pharynx, and esophagus is characterized by flat, greenish-black pigmented areas, and it is frequently (11%, 10%, and 7%, respectively) detected during endoscopic screening of Japanese alcoholic men.[65,68] We have shown that the presence of melanosis indicates a high risk of neoplasia in the UADT. Esophageal dysplasia and SCC in the UADT in alcoholic men were frequently (25% and 49%, respectively) accompanied by melanosis in the UADT. Inactive ALDH2, advanced age, and heavy smoking are strongly associated with the presence of melanosis. Some of the causes of melanosis and UADT neoplasia are the same, and they include heavy drinking, inactive ALDH2, acetaldehyde exposure, heavy smoking, and advanced age. A high detection rate (19%) of esophageal melanosis by endoscopy has been reported in Japanese esophageal SCC patients at a university hospital.[72] The above findings are consistent with the results of an earlier pathological study that found a high frequency and high density of melanocytes in surgical specimens obtained from Japanese esophageal cancer patients.[73] The detection of melanosis in the UADT may encourage endoscopists to use esophageal iodine staining and to examine the oropharyngolaryngeal sites carefully.

Carcinogenicity of Acetaldehyde

Acetaldehyde is a mutagen, genotoxin, and DNA-damaging agent,[74] and sufficient evidence of the carcinogenicity of acetaldehyde has been obtained in experimental animals.[75] Acetaldehyde inhalation has been shown to result in the development of tumors in the respiratory tract, i.e., adenocarcinomas and SCCs of the nasal mucosa in rats[76] and laryngeal carcinomas in hamsters[77]; in addition, acetaldehyde promotes carcinogenesis by benzo(a)pyrene in hamsters.[77] We have demonstrated high blood levels of mutagenic acetaldehyde-DNA adducts in alcoholics with heterozygous inactive ALDH2.[78] The formation of DNA adducts may explain many of the genotoxic effects of acetaldehyde. Other human evidence includes a high frequency of sister chromatid exchanges[79] and micronuclei[80] and slower electrophoretic DNA migration[81] by leukocytes from habitual drinkers with inactive heterozygous ALDH2. The International Agency for Research on Cancer (IARC) of the WHO has noted that the substantial mechanistic evidence obtained in ALDH2-deficient humans indicates a causal role of acetaldehyde in esophageal cancer.[82] It classified the ethanol in alcoholic beverages as a Group 1 carcinogen in 2007[82] and the acetaldehyde associated with alcohol consumption as a Group 1 carcinogen in 2009.[83] Classification as a Group 1 carcinogen means there is sufficient evidence of carcinogenicity in humans.

Salivary Acetaldehyde

Alcohol challenge tests that monitor blood and salivary acetaldehyde concentrations after a moderate dose of alcohol[84,85] have shown much higher salivary acetaldehyde concentrations than blood acetaldehyde concentrations in both inactive ALDH2 heterozygotes (e.g., 37–76 μM vs. 12–25 μM)[85] and active ALDH2 homozygotes (e.g., 24–53 μM vs. 2–5 μM). Most experimental studies on mammalian or human cell cultures have shown induction of mutations, sister chromatid exchanges, and chromosomal aberrations after exposure to acetaldehyde concentrations ranging from 40 to 1000 μM in a highly dose-dependent manner.[86] The acetaldehyde levels measured in the saliva of healthy persons may be sufficient to cause mutagenic damages in the UADT. Normal oral bacteria and yeasts produce acetaldehyde from ethanol, and they make a large contribution to the high acetaldehyde level in saliva. The *in vitro* acetaldehyde producing ability of saliva is abolished by sterile filtration or centrifugation,[86] and it has been found to be correlated with both salivary microbial counts and salivary acetaldehyde levels after drinking.[87,88] The salivary acetaldehyde levels after drinking were reduced after using an antiseptic mouthwash[86]; 4-methylpyrazole, a weak inhibitor of microbial alcohol dehydrogenase (ADH) but a strong inhibitor of mucosal and hepatic ADH, decreased the salivary acetaldehyde concentration of *ALDH2*1/*2* subjects but not of *ALDH2*1/*1* subjects.[89] Heavy drinking,[87,88] smoking,[87] and poor oral hygiene,[90] all of which are common in alcoholics, are associated with increased salivary acetaldehyde-producing capacity, probably as a result of modification of the oral microflora. Both the oral microbial counts and *in vitro* salivary acetaldehyde-producing capacity of Japanese alcoholics decreased after 3 weeks of abstinence, and the decreases were mutually correlated.[88] Many factors in addition to abstinence, including tooth-brushing behavior and diet, may have changed during the 3-week abstinence period. Overgrowth of oral microorganisms resulting in excessive production of salivary acetaldehyde is another possible explanation for the very high rate of UADT cancer in alcoholics.

Acetaldehyde in Alcoholic Beverages

Alcoholic beverages themselves contain high levels of acetaldehyde.[91] The French apple brandy Calvados, the Japanese distilled liquor *shochu*, and other spirits in undiluted form have an acetaldehyde concentration of 1000 μM.[85,91] In a randomized cross-over design study in which 19 healthy Japanese volunteers ingested 0.6 g ethanol/kg body weight in the form of 13%-ethanol Calvados, 13%-ethanol *shochu*, 13%-ethanol red wine, and 5%-ethanol beer under fasting conditions at 3-week intervals,[85] the acetaldehyde concentration of the beverages consumed was 600 μM, 600 μM, 250 μM, and 140 μM, respectively. The results showed that the salivary acetaldehyde concentrations immediately after drinking the high-acetaldehyde beverages were higher than immediately after drinking the low-acetaldehyde beverages. Overall trends suggest that consumption of all types of alcoholic beverages increases the risk for SCC in the UADT, but Japanese studies have shown stronger associations between SCC in the UADT and a preference for drinking concentrated alcoholic beverages, such as whiskey and *shochu*, straight.[20,48,92] The high level of direct exposure of the UADT to acetaldehyde as well as to ethanol, at least in part, explains the increased susceptibility to cancer of the UADT as a result of the preference for drinking high ethanol/acetaldehyde beverages straight.

Acetaldehyde in Tobacco Smoke

The results of a large genome-wide association study involving 1070 Japanese esophageal SCC patients and 2836 controls demonstrated that four risk factors [i.e., inactive heterozygous ALDH2, less-active ADH1B (see below), drinking, and smoking] synergistically increase the risk of esophageal cancer.[28] The effects of combination of the high-risk genotypes were more prominent in smokers and in heavier drinkers. Interestingly, the increase in risk associated with having the *ALDH2*1/*2*

genotype was greater in smokers than in non-smokers, but the increase in risk associated with less-active ADH1B was similar in smokers and non-smokers. Although alcohol consumption in each drinking category may have been greater in the smokers than in the non-smokers, the acetaldehyde in tobacco smoke is another possible explanation for these findings. As one of the major chemical constituents of tobacco smoke, acetaldehyde dissolves in saliva during active smoking and reaches concentrations as high as 400 μM.[93] Inefficient degradation of the acetaldehyde in inactive ALDH2 heterozygotes may lead to accumulation of very high levels of acetaldehyde in the mucosa of the UADT after high exposure to acetaldehyde during active smoking.

Acetaldehyde Metabolism in the Mucosa of the UADT

High-Km ADH7 (class IV ADH) is strongly expressed in the UADT and it actively catalyzes conversion of ethanol to acetaldehyde upon exposure to a locally high dose of ethanol.[94,95] ADH7 activity is higher in esophageal cancer cells than in normal esophageal tissue, suggesting that increased ADH7 activity may be a factor intensifying carcinogenesis through increased mucosal acetaldehyde production.[96] Chronic alcohol consumption leads to induction of cytochrome P4502E1, which metabolizes ethanol to acetaldehyde,[97] and induction of P4502E1 has been demonstrated in the oropharyngeal mucosa of alcoholics with oropharyngeal cancer.[98] The epithelium of the UADT of alcoholics is exposed to a very high concentration of acetaldehyde from a variety of sources. ALDH activity is disproportionately lower than ADH activity in the UADT.[94,95] The results of immunohistochemical staining of the esophageal mucosa with ALDH2-antibody in Japanese esophageal cancer patients have suggested that both inactive and active forms of ALDH2 are induced in normal esophageal epithelium by heavy drinking,[99] and this enzyme induction may widen the difference in mucosal acetaldehyde elimination between *ALDH2*1/*1* and *ALDH2*1/*2* alcoholics. During exposure to very high levels of acetaldehyde, the inefficient degradation of acetaldehyde by tissue ALDH2 in the UADT may be more critical to the difference in local accumulation of acetaldehyde between individuals with *ALDH2*1/*1* and *ALDH2*1/*2*.

The Simple Flushing Questionnaire

ALDH2-associated cancer susceptibility emphasizes the importance of developing screening tests for inactive ALDH2 based on alcohol flushing.[34] The results regarding associations between alcohol flushing and ALDH2 genotype have been found to differ markedly according to how the question about alcohol flushing is posed. Without defining the alcohol dose, answers to the question, "Do you currently experience facial flushing after drinking alcohol?" are unreliable as a means of predicting the presence of inactive ALDH2. Half of active ALDH2 carriers have been found to be always flushers or occasional flushers when asked this question, probably because they experienced alcohol flushing when they drank a substantial amount of alcohol.[100] The results of a Japanese cohort study in which this question was asked showed that 49% of flushers had active ALDH2 and that the flushing-associated risk of esophageal cancer was modest, but that the hazard risk was greatest for flushers who were also both heavy drinkers and heavy smokers.[101]

There have been several important advances in the methods used to predict the presence of inactive ALDH2 based on replies to questionnaires on alcohol flushing responses. Takeshita and Morimoto developed an alcohol sensitivity screening test based on flushing responses that had an 89% sensitivity and 90% specificity when applied to 424 Japanese male workers.[102] The ethanol patch test, a cutaneous test for the flushing response, is used in Japan in health education for youths, especially those who have never consumed alcohol. The first study reported a high sensitivity (93%) and specificity (94%) for identifying inactive ALDH2,[103] but subsequent studies failed to confirm such high reliability levels.[102,104,105] The sensitivity of 72% and specificity of 71% of the ethanol patch test among men age 50 or older, many of whom had a long history of drinking, were unsatisfactory,[105] suggesting that aging and acquired tolerance to acetaldehydemia influence the test results.

When the current flushing question, "Do you have a tendency to flush in the face immediately after drinking a glass of beer?" (a glass means about 180 mL, the most common Japanese beer glass size), was used to detect inactive ALDH2, its specificity for detecting active ALDH2 was very high, but its sensitivity for detecting inactive ALDH2 was only 74% in cancer-free men, 57% in men with esophageal cancer, and 8% in alcoholic men with esophageal cancer.[106,107] Alcohol flushing diminishes in intensity as a result of the development of tolerance to acetaldehydemia in higher-risk persons with a long or heavy drinking history.

We devised a flushing questionnaire to resolve the above problems (**Fig. 2**).[106] The simple flushing questionnaire consists of two questions: (A) Do you have a tendency to flush in the face immediately after drinking a glass (180 mL) of beer? (B) Did you have a tendency to flush in the face immediately after drinking a glass of beer during the first to second year after you started drinking? We classified the results as current flushing, former flushing, or never flushing. When current flushers and former flushers were assumed to have inactive ALDH2, the simple flushing questionnaire yielded 90% sensitivity and 88% specificity among 610 Japanese men aged 40 years or older[106] and 88% sensitivity and 92%

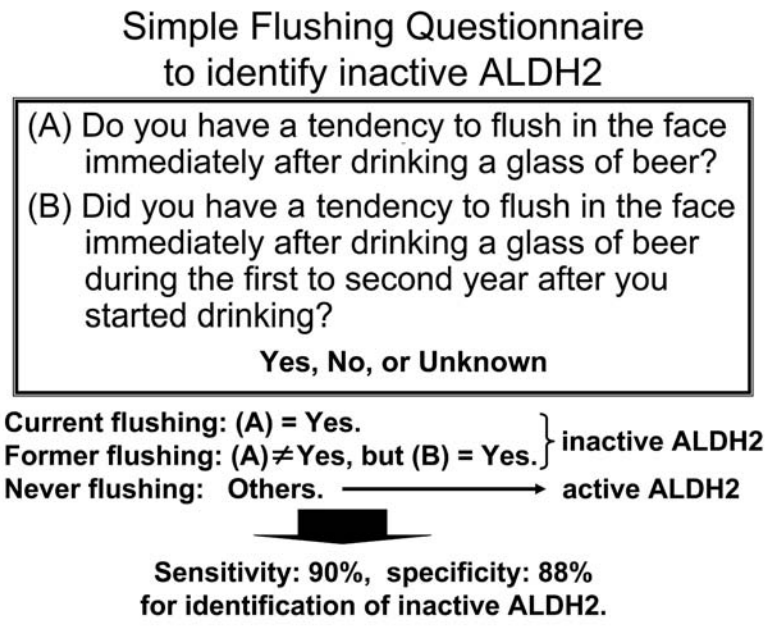

Fig. 2 The simple flushing questionnaire to identify inactive ALDH2. Sensitivity and specificity are both approximately 90% in Japanese subjects 40 years of age and over regardless of gender.[36,106]

specificity among 381 Japanese women aged 40 years or older.[36] The individuals' risks of UADT cancer estimated on the basis of the replies to the flushing questionnaire were slightly lower than, but basically comparable to, those estimated on the basis of ALDH2 genotyping (**Fig. 1B**).[36,48,106]

Screening for SCC in the UADT by Means of Health Risk Appraisal Models

Based on the results of a case-control study,[20] we devised health risk appraisal (HRA) models for esophageal cancer that include ALDH2 genotype or alcohol flushing.[108] The total risk score is calculated by adding the scores A to E. If the risk score is 11 or more according to the HRA-flushing model (**Fig. 3**), that person's risk of esophageal cancer is in the top 10% of the study population. A cross-validation study predicted that approximately 60% of the esophageal and hypopharyngeal SCCs in the entire population could be detected by examining only people whose risk scores were in the top 10% in the HRA models (i.e., sensitivity ≈60% and specificity ≈90%). The esophageal SCC detection rate among Japanese men aged 40 years or older whose risk score was in the top 10% (i.e., positive predictive value, PPV) was expected to be 1%–2%. Follow-up endoscopy with esophageal iodine staining (median follow-up period: 5.0 years) was performed on 404 cancer-free controls (aged 50–78 years), and resulted in the diagnosis of six esophageal SCCs and two pharyngeal SCCs. The risk scores of six of the eight cancer patients at baseline were in the top 10% according to the HRA-flushing model.[109] The cancer detection rate per 100 person-years in the top 10% risk group was 2.3 for esophageal cancer and 3.5 for esophageal or pharyngeal cancer. These figures have encouraged screening with our questionnaire in larger populations of Japanese men.

We are now applying this HRA-flushing questionnaire to mass-screening programs in large Japanese populations. This questionnaire enables many people to identify their risk of UADT cancer very easily, and public awareness campaigns using the questionnaire will encourage high-risk persons to undergo endoscopic screening or convince them to change their lifestyle to prevent UADT cancer. The HRA model using ALDH2 genotyping yielded a slightly better PPV[108] and a slightly higher cancer detection rate[109] than the HRA-flushing model. An initial cost would be necessary to genotype ALDH2 in all individuals in the target population. However, it should be emphasized that genotyping needs to be performed only once in a lifetime, that the data are always available, and that the unit cost would be greatly discounted if a huge number of samples were analyzed.

Screening for SCC in the UADT Based on Red Cell MCV

A high red cell mean corpuscular volume (MCV) is a traditional biological marker for alcohol abuse and alcoholism.[110] Recent epidemiological evidence indicates that the MCV of drinkers with *ALDH2*1/*2* increases in response to exposure to acetaldehyde.[4,111–115] Smoking, a low BMI, and folate deficiency also increase MCV.[113–115] MCV is used as a biomarker for risk of esophageal and

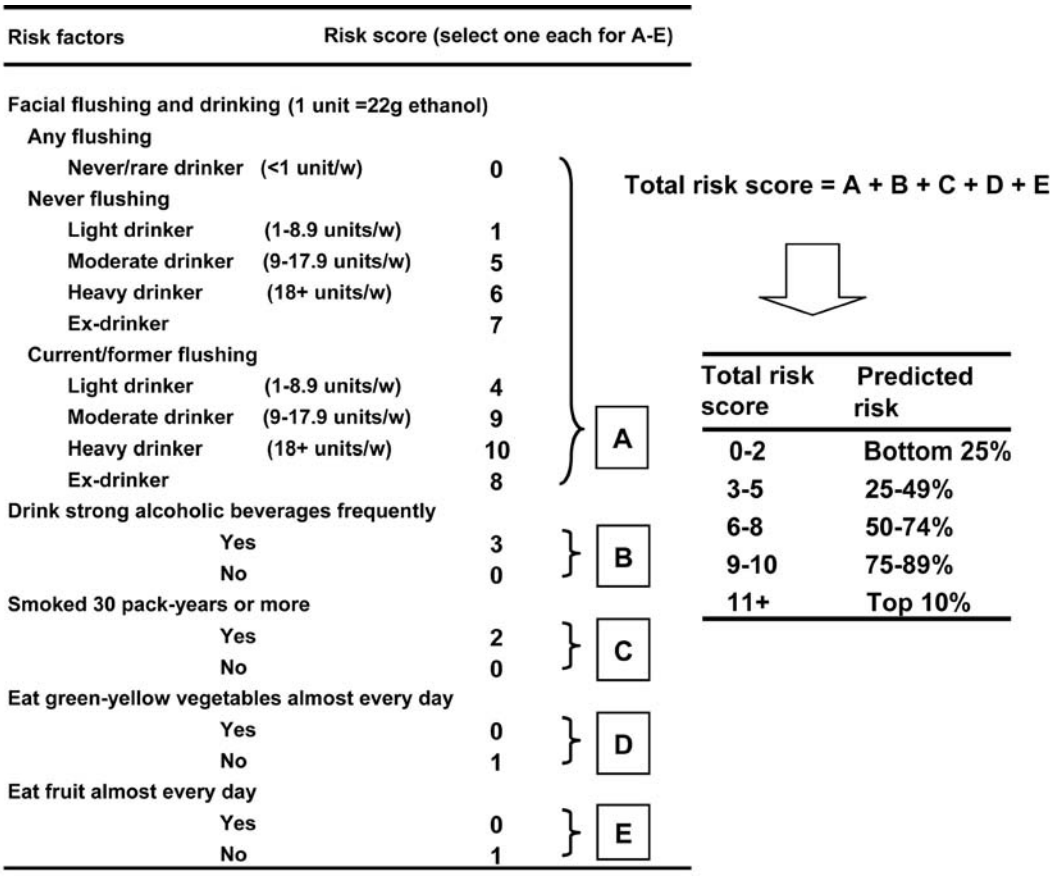

Fig. 3 Health risk appraisal model for esophageal cancer combined with the simple flushing questionnaire. The risk score is calculated as the sum of scores A to E. The expected esophageal cancer detection rate of Japanese men 40 years of age and over with a risk score of 11 or more who undergo endoscopic screening with esophageal iodine staining is 1%–2%.[108,109]

pharyngeal SCC in Japanese alcoholics.[4,5,49] A high MCV of 106 fl or more, the *ALDH2*1/*2* genotype, and the *ADH1B*1/*1* genotype synergistically increase the risk of esophageal SCC in Japanese alcoholics [OR = 320 (27 to >1000)].[4] The combination of high MCV and alcohol flushing is associated with a high risk of esophageal SCC, with an OR of 5.5 (2.2–13.8) in alcoholics. This way of estimating cancer risk can be easily applied in both public education and screening. An endoscopic follow-up study in cancer-free Japanese alcoholics revealed that cancer of the UADT developed much more frequently among alcoholics with a high MCV of 106 fl or more [HR = 2.52 (1.22–5.22)].[5]

We have demonstrated that MCV is useful as a marker for male Japanese drinkers who are at high risk of SCC in the UADT but who are not alcoholics.[108,113,114] Combining MCV with the traditional risk factors drinking and smoking had better predictive power for esophageal and pharyngeal SCC than the combination of drinking and smoking alone.[108,114] Approximately 30% of cancer-free Japanese men met the criterion of a combination of moderate-to-heavy drinking (≥ 198 g ethanol/week) and either 30+ pack-years or an MCV ≥ 99 fl, whereas approximately 70% of Japanese men with SCC of the esophagus or hypopharynx met the criterion.[108] MCV may be used to better select not only alcoholic male Japanese candidates to screen for this high mortality cancer but non-alcoholic male Japanese candidates as well. MCV is not a predictor of esophageal cancer in Caucasian heavy drinkers, probably because the main determinant of high MCV is severe acetaldehydemia after heavy drinking in inactive ALDH2 heterozygotes.[116]

Genetic Polymorphism of ADH1B, Alcohol Metabolism, and SCC in the UADT

Alcohol dehydrogenase-1B (ADH1B, previously called ADH2) also has a functional genetic polymorphism (rs1229984). A small fraction of East Asians have the less-active *ADH1B*1/*1* genotype (e.g., 7% of Japanese)[15] that is prevalent in Caucasians (present in approximately 90%); this less-active ADH1B is a strong risk factor for alcoholism in East Asians. Approximately 30% of Japanese and Taiwanese alcoholics have the less-active *ADH1B*1/*1* genotype,[15,54] and this genotype has been found to be more frequent in Japanese and Tai-

wanese alcoholics with cancer in the UADT[30,31,49] [e.g., OR = 3.46 (1.66–7.22) for oropharyngolaryngeal SCC[49] and 2.64 (1.62–4.31) for esophageal SCC[31]]. An endoscopic follow-up study in Japanese alcoholics demonstrated that SCC of the UADT was more common among alcoholics with the less-active *ADH1B*1/*1* [HR = 2.07 (1.01–4.26)].[5] With the exception of two negative Japanese studies,[25,36] the *ADH1B*1/*1* genotype has consistently been demonstrated to be associated with an increased risk of SCC in the UADT in non-alcoholic Japanese (**Fig. 1C**),[20,22,28,48,117] Chinese,[26,27,38–41] Thai[118] and Central European populations.[119] Stratification according to drinking categories has shown that the *ADH1B*1/*1* genotype increases the risk of UADT cancer in light drinkers[20,26–28,119] as well as in moderate-to-heavy drinkers.[20,26–28,38,41,48,117,119]

The mechanism of the increase in risk of alcoholism and UADT cancer associated with the *ADH1B*1/*1* genotype has remained a puzzle. Alcohol challenge tests have failed to demonstrate any associations between ADH1B genotype and the blood ethanol or acetaldehyde concentration after ingestion of light to moderate doses of ethanol by Japanese,[17] Chinese,[120] and Europeans.[121] However, the less-active form of ADH1B encoded by the *ADH1B*1/*1* genotype has an approximately 40 times lower Vmax *in vitro* than that encoded by *ADH1B*2/*2*,[122] and an experiment in which a clamping technique and intravenous alcohol infusion were used showed a modestly but significantly lower ethanol elimination rate (11%–18%) among Jews with the less-active *ADH1B*1/*1* genotype than with the *ADH1B*2* allele.[123] Heavy drinking may amplify the modest effect of *ADH1B*1/*1* on ethanol metabolism and lead to clear prolongation of the presence of ethanol in the body, including in the UADT. Although ethical considerations make it impossible to experimentally emulate the dangerously heavy drinking found in alcoholics, many alcoholic patients come to our Alcoholism Center after drinking the day before, and they have provided an opportunity to measure blood and salivary acetaldehyde and ethanol levels in that state. When we measured the blood and salivary ethanol and acetaldehyde levels of Japanese alcoholics in the morning when they first visited our Center after drinking the day before,[88,124] we found that ethanol and acetaldehyde remained in the blood and saliva for much longer periods and at much higher levels in those with the less-active *ADH1B*1/*1* genotype than in those with other genotypes, even after adjusting for age, body weight, the amount of alcohol consumed, and interval since the most recent drink. The blood and salivary ethanol levels were similar, but the acetaldehyde levels in saliva were strikingly higher than in the blood because of acetaldehyde production by oral microorganisms (e.g., median salivary acetaldehyde concentration of 47.4 μM in *ADH1B*1/*1* carriers vs. 1.6 μM in *ADH1B*2* allele carriers, $P = 0.009$).[88] However, none of the levels differed according to ALDH2 genotype.[124] The ADH1B and ALDH2 genotypes affect the blood and salivary ethanol and acetaldehyde levels of non-abstinent alcoholics in a different manner than in non-alcoholics, and clear effects of ADH1B genotype and less clear effects of ALDH2 have been observed in non-abstinent alcoholics. Alterations in alcohol metabolism as a result of alcoholism may modify the gene effects.[124]

East Asians[106,107,125–127] and Europeans[128] with the less-active *ADH1B*1/*1* genotype tend not to report alcohol flushing responses and tend to consume larger amounts of alcohol than those with the *ADH1B*2* allele. Alcohol-induced flushing is triggered by a steep initial rise in either blood or cutaneous acetaldehyde after drinking. This dramatic change may not occur in persons with the less-active ADH1B. The initial phase of acetaldehyde production may be difficult to monitor in conventional alcohol-challenge tests. Less-active *ADH1B*1/*1* markedly masked inactive *ALDH2*1/*2*-associated alcohol flushing. According to responses to the simple flushing questionnaire, 53% of alcoholics with cancer who had the combination of inactive *ALDH2*1/*2* and less-active *ADH1B*1/*1* were never flushers[107]; 28% of cancer-free Japanese men with this genotype combination reported never flushing, 38% reported former flushing, and only 37% reported current flushing.[106] The levels of alcohol consumption by inactive ALDH2 heterozygotes were clearly associated with their alcohol flushing categories.[106]

The above findings provide a clue as to why less-active ADH1B increases the risk of both alcoholism and UADT cancer. First, less-active ADH1B diminishes the intensity of alcohol flushing, thereby accounting for the greater susceptibility to alcoholism associated with it. Second, heavy drinking by alcoholics amplifies the modest effect of less-active ADH1B on ethanol elimination, which leads to much longer exposure to ethanol and, in turn, results in smelling of alcohol in the morning. When ethanol lingers in the body, the UADT is exposed to high levels of acetaldehyde as a result of acetaldehyde production in saliva, and that creates a condition that increases the risk of UADT cancer (**Fig. 4**).

The increase in risk of UADT cancer associated with the inactive *ALDH2*1/*2* genotype in Japanese alcoholics is higher than the increase associated with the less-active *ADH1B*1/*1* genotype.[5,31,49] Only the inactive *ALDH2*1/*2* genotype, and not the less-active *ADH1B*1/*1* genotype, increases the risk of synchronous and metachronous multiple UADT cancers in Japanese alcoholics.[50,59] The high tissue accumulation of acetaldehyde in alcoholics with the *ALDH2*1/*2* genotype may affect the risk of SCC in the UADT differently from the prolonged exposure of the UADT to ethanol and acetaldehyde in alcoholics with the *ADH1B*1/*1* genotype.

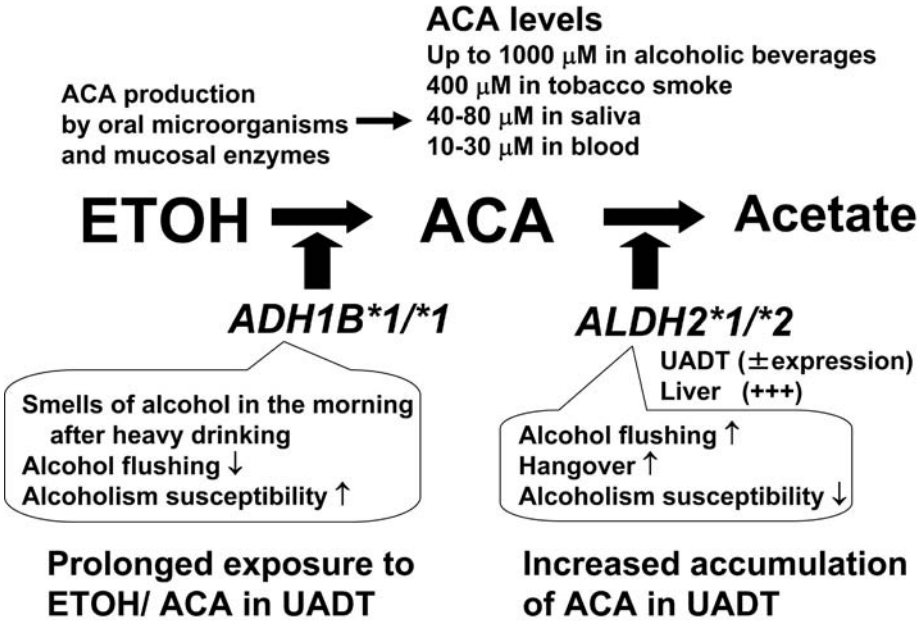

Fig. 4 Ethanol (ETOH) and acetaldehyde (ACA) exposure and accumulation in the upper aerodigestive tract (UADT) and the effects of *alcohol dehydrogenase-1B*1/*1* (*ADH1B*1/*1*) and *aldehyde dehydrogenase-2*1/*2* (*ALDH2*1/*2*) genotypes.

Combination of ADH1B and ALDH2 Genotypes and SCC in the UADT

The risk of SCC in the oropharyngolarynx and esophagus in Japanese alcoholics has been found to be greatly increased in a multiplicative fashion by the combination of less-active *ADH1B*1/*1* and inactive *ALDH2*1/*2* [OR = 122 (32–465) and 40 (18–91), respectively].[31] The PAR due to all combinations of ADH1B and ALDH2 genotypes in the alcoholic population has been found to be 82% for oropharyngolaryngeal SCC and 64% for esophageal SCC. Case-control studies in a general population confirmed a similar multiplicative effect of this genotype combination on the risk of SCC of the esophagus[20] and hypopharyx[48] in Japanese subjects and on the risk of SCC of the esophagus in Taiwanese subjects[26] [OR = 30.1 (13.2–68.9), 48.9, and 36.8 (9.4–144.7), respectively]. When the presence of these two genotypes was combined with consumption of 198–395 g ethanol/week or ≥ 396 g ethanol/week, the OR for esophageal SCC was estimated to be 248 and 417, respectively, in Japanese based on the multivariate OR for each risk factor.[20] A Taiwanese study also showed a multiplicative effect among the less-active ADH1B, inactive ALDH2, and drinking of more than 30 g ethanol/day [OR = 382 (47–3085)].[27] A Japanese genome-wide association study of esophageal SCC showed that individuals with both high-risk genotypes who smoked and drank >96.5 g ethanol/week had a 189 (95–377) times higher risk of esophageal SCC than those who had none of these risk factors.[28]

Genetic Polymorphism of ADH1C, Alcohol Metabolism, and SCC in the UADT

The enzyme encoded by the *ADH1C*1* allele (previously called *ADH3*1*) catalyzes acetaldehyde production twice as fast as the enzyme encoded by the *ADH1C*2* allele (rs1693482 and rs698).[122] The *ADH1C*2* allele has been reported to increase the risk of alcoholism in East Asians[15,54] and of both UADT cancer[20,48] and multiple iodine-unstained lesions[58] in Japanese drinkers. However, the associations with ADH1C were explained by linkage disequilibrium in which the *ADH1C*2* allele and the more influential *ADH1B*1* allele are linked; the associations disappeared after adjustment for the linkage.[15,20,48,54] A cross-sectional study in 2299 non-alcoholic Japanese subjects showed a significant positive association between the presence of an *ADH1C*2* allele and habitual drinking, regardless of the ADH1B genotype; however, the impact on habitual drinking was weak (3%–16% increase in OR).[129]

ADH1C polymorphism may be the rate-limiting factor in acetaldehyde metabolism in Western populations, in which the prevalence of the *ALDH2*2* allele and *ADH1B*2* allele is very low. Caucasians with the high-activity *ADH1C*1/*1* genotype have been found to have significantly higher salivary acetaldehyde concentrations after alcohol ingestion than *ADH1C*2* allele carriers.[130] Although published studies have not shown a consistent pattern of association,[131] several Western studies have shown that individuals in heavy drinking populations who had the *ADH1C*1* allele were at greater risk for UADT cancer.[130,132,133]

SCC in the UADT in Ex-drinkers

A recent pooled analysis including 13 studies and over 5000 cases demonstrated that the risk of esophageal cancer and risk of head and neck cancer only started to decline 5 years and 10 years, respectively, after alcohol intake ceased, probably because of the so-called "sick-quitter" effect.[134] Two Japanese case-control studies showed a very high risk of SCC in ex-drinkers with the *ALDH2*1/*2* or *ADH1B*1/*1* genotype.[20,48] Endoscopic follow-up studies in Japanese alcoholics after treatment of esophageal SCC failed to detect any effects of drinking cessation on the development of metachronous cancer,[56,62] suggesting that the molecular changes that predispose patients to metachronous cancer might be at the point of no return, and that intensive follow-up examinations are essential for cancer prevention in high-risk patients. Further study is needed to evaluate the longer-term effect of drinking cessation on the development of SCC in the UADT.

Conclusions

The ethanol in alcoholic beverages and the acetaldehyde associated with alcohol consumption are human carcinogens (Group 1, IARC). The inactive heterozygous *ALDH2*1/*2* genotype and the less-active *ADH1B*1/*1* genotype modify ethanol metabolism and greatly increase the susceptibility of the UADT to SCC in East Asian drinkers. The simple flushing questionnaire and MCV are useful tools that facilitate the assessment of ALDH2-associated cancer risk. Melanosis in the UADT and large or multiple esophageal dysplasia are endoscopic evidence of a high risk of developing SCC in the UADT. New health appraisal models that include the ALDH2 genotype, the simple flushing questionnaire, or MCV may provide powerful tools for cancer prevention and cancer screening in East Asians.

References

1. Takezaki T, Shinoda M, Hatooka S, *et al*: Subsite-specific risk factors for hypopharyngeal and esophageal cancer (Japan). Cancer Causes Control 2000; **11**: 597–608.
2. Yamaji T, Inoue M, Sasazuki S, Iwasaki M, Kurahashi N, Shimazu T, Tsugane S, Japan Public Health Center-based Prospective Study Group: Fruit and vegetable consumption and squamous cell carcinoma of the esophagus in Japan: the JPHC study. Int J Cancer 2008; **123**: 1935–1940.
3. Kreimer AR, Randi G, Herrero R, Castellsagué X, La Vecchia C, Franceschi S, IARC Multicenter Oral Cancer Study Group: Diet and body mass, and oral and oropharyngeal squamous cell carcinomas: Analysis for the IARC multinational case-control study. Int J Cancer 2006; **118**: 2293–2297.
4. Yokoyama A, Yokoyama T, Muramatsu T, Omori T, Matsushita S, Higuchi S, Maruyama K: Macrocytosis, a new predictor for esophageal squamous cell carcinoma in Japanese men. Carcinogenesis 2003; **24**: 1773–1778.
5. Yokoyama A, Omori T, Yokoyama T, *et al*: Risk of squamous cell carcinoma of the upper aerodigestive tract in cancer-free alcoholic Japanese men: an endoscopic follow-up study. Cancer Epidemiol Biomarkers Prev 2006; **15**: 2209–2215.
6. Hosokawa Y, Yokoyama A, Yokoyama T, Wada N, Mori S, Matsushita T, Mizukami Y, Maesato H, Maruyama K: Relationship between drinking, smoking, and dietary habits and the body mass index of Japanese alcoholic men. Jpn J Alcohol & Drug Dependence 2010; **45**: 25–37.
7. Yokoyama A, Ohmori T, Makuuchi H, *et al*: Successful screening for early esophageal cancer in alcoholics using endoscopy and mucosa iodine staining. Cancer 1995; **76**: 928–934.
8. Arima M, Arima H, Tada M, Tanaka Y: Diagnostic accuracy of tumor staging and treatment outcomes in patients with superficial esophageal cancer. Esophagus 2007; **4**: 145–153.
9. Muto M, Nakane M, Katada C, Sano Y, Ohtsu A, Esumi H, Ebihara S, Yoshida S: Squamous cell carcinoma in situ at oropharyngeal and hypopharyngeal mucosal sites. Cancer 2004; **101**: 1375–1381.
10. Makuuchi H: Endoscopic mucosal resection for mucosal cancer in the esophagus. Gastrointest Endosc Clin N Am 2001; **11**: 445–458.
11. Momma K: Endoscopic treatment of esophageal mucosal carcinomas: indications and outcomes. Esophagus 2007; **4**: 93–98.
12. Sato Y, Omori T, Yokoyama A, *et al*: Treatment of superficial carcinoma in the pharynx and the larynx. Shokaki Naishikyo 2006; **18**: 1407–1416 (in Japanese with English abstract).
13. Kawakubo H, Omori T, Ando T, Sato Y, Sugiura H, Yokoyama A: Clinical outcome of treatment for superficial carcinoma of the oropharynx and hypopharynx. Stomach and Intestine (Tokyo) 2010; **45**: 265–281 (in Japanese with English abstract).
14. Li H, Borinskaya S, Yoshimura K, *et al*: Refined geographic distribution of the Oriental ALDH2*504Lys (nee 487Lys) variant. Ann Hum Genet 2009; **73**: 335–345.
15. Higuchi S, Matsushita S, Murayama M, Takagi S, Hayashida M: Alcohol and aldehyde dehydrogenase polymorphisms and the risk for alcoholism. Am J Psychiatry 1995; **152**: 1219–1221.
16. Enomoto N, Takase S, Yasuhara M, Takada A: Acetaldehyde metabolism in different aldehyde dehydrogenase-2 genotypes. Alcohol Clin Exp Res 1991; **15**: 141–144.
17. Mizoi Y, Yamamoto K, Ueno Y, Fukunaga T, Harada S: Involvement of genetic polymorphism of alcohol and aldehyde dehydrogenase in individual variation of alcohol metabolism. Alcohol Alcohol 1994; **29**: 707–710.
18. Harada S, Agarwal DP, Goedde HW: Aldehyde dehydrogenase deficiency as cause of facial flushing reaction to alcohol in Japanese. Lancet 1981; **2**: 982.
19. Yokoyama M, Yokoyama A, Yokoyama T, Funazu K, Hamana G, Kondo S, Yamashita T, Nakamura H: Hangover susceptibility in relation to aldehyde dehydrogenase-2 genotype, alcohol flushing, and mean corpuscular volume in Japanese workers. Alcohol Clin Exp Res 2005; **29**: 1165–1171.
20. Yokoyama A, Kato H, Yokoyama T, *et al*: Genetic polymorphisms of alcohol and aldehyde dehydrogenases and glutathione S-transferase M1 and drinking, smoking, and diet in Japanese men with esophageal squamous cell carcinoma. Carcinogenesis 2002; **23**: 1851–1859.
21. Yokoyama A, Muramatsu T, Ohmori T, Higuchi S, Hayashida M, Ishii H: Esophageal cancer and aldehyde dehydrogenase-2 genotypes in Japanese males. Cancer Epidemiol Biomarkers Prev 1996; **5**: 99–102.
22. Hori H, Kawano T, Endo M, Yuasa Y: Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and human esophageal squamous cell carcinoma susceptibility. J Clin Gastroenterol 1997; **25**: 568–575.
23. Matsuo K, Hamajima N, Shinoda M, Hatooka S, Inoue M, Takezaki T, Tajima K: Gene-environment interaction between an

aldehyde dehydrogenase-2 (ALDH2) polymorphism and alcohol consumption for the risk of esophageal cancer. Carcinogenesis 2001; **22**: 913–916.
24. Itoga S, Nomura F, Makino Y, *et al*: Tandem repeat polymorphism of the CYP2E1 gene: an association study with esophageal cancer and lung cancer. Alcohol Clin Exp Res 2002; **26**(Suppl.): 15S–19S.
25. Yang CX, Matsuo K, Ito H, *et al*: Esophageal cancer risk by ALDH2 and ADH2 polymorphisms and alcohol consumption: Exploration of gene-environment and gene-gene interactions. Asian Pacific J Cancer Prev 2005; **6**: 256–262.
26. Wu CF, Wu DC, Hsu HK, Kao EL, Lee JM, Lin CC, Wu MT: Relationship between genetic polymorphisms of alcohol and aldehyde dehydrogenases and esophageal squamous cell carcinoma risk in males. World J Gastroenterol 2005; **11**: 5103–5108.
27. Lee CH, Lee JM, Wu DC, *et al*: Carcinogenetic impact of ADH1B and ALDH2 genes on squamous cell carcinoma risk of the esophagus with regard to the consumption of alcohol, tobacco and betel quid. Int J Cancer 2008; **122**: 1347–1356.
28. Cui R, Kamatani Y, Takahashi A, *et al*: Functional variants in ADH1B and ALDH2 coupled with alcohol and smoking synergistically enhance esophageal cancer risk. Gastroenterology 2009; **137**: 1768–1775.
29. Yokoyama A, Muramatsu T, Ohmori T, *et al*: Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics. Carcinogenesis 1998; **19**: 1383–1387.
30. Chao YC, Wang LS, Hsieh TY, Chu CW, Chang FY, Chu HC: Chinese alcoholic patients with esophageal cancer are genetically different from alcoholics with acute pancreatitis and liver cirrhosis. Am J Gastroenterol 2000; **95**: 2958–2964.
31. Yokoyama A, Muramatsu T, Omori T, Yokoyama T, Matsushita S, Higuchi S, Maruyama K, Ishii H: Alcohol and aldehyde dehydrogenase gene polymorphisms and oropharyngolaryngeal, esophageal and stomach cancers in Japanese alcoholics. Carcinogenesis 2001; **22**: 433–439.
32. Yokoyama A, Mizukami T, Omori T, *et al*: Melanosis and squamous cell neoplasms of the upper aerodigestive tract in Japanese alcoholic men. Cancer Sci 2006; **97**: 905–911.
33. Yokoyama A, Omori T: Genetic polymorphisms of alcohol and aldehyde dehydrogenases and risk for esophageal and head and neck cancers. Jpn J Clin Oncol 2003; **33**: 111–121.
34. Brooks PJ, Enoch MA, Doldman D, Li TK, Yokoyama A: The alcohol flushing response: an unrecognized risk factor of esophageal cancer from alcohol consumption. PLoS Med 2009; **6**: e50.
35. Lewis SJ, Smith GD: Alcohol, ALDH2, and esophageal cancer: a meta-analysis which illustrates the potentials and limitations of a Mendelian randomization approach. Cancer Epidemiol Biomarkers Prev 2005; **14**: 1967–1971.
36. Yokoyama A, Kato H, Yokoyama T, *et al*: Esophageal squamous cell carcinoma and aldehyde dehydrogenase-2 genotypes in Japanese females. Alcohol Clin Exp Res 2006; **30**: 491–500.
37. Lee CH, Wu DC, Wu I: C, Goan YG., Lee JM, Chou SH, Chan TF, Huang HL, Hung YH, Huang MC, Lai TC, Wang TN, Lan CCE, Tsai S, Lin WY, Wu MT: Genetic modulation of *ADH1B* and *ALDH2* polymorphisms with regard to alcohol and tobacco consumption for younger aged esophageal squamous cell carcinoma diagnosis. Int J Cancer 2009; **125**: 1134–1142.
38. Guo YM, Wang Q, Liu YZ, Chen HM, Qi Z, Guo QH: Genetic polymorphisms in cytochrome P4502E1, alcohol and aldehyde dehydrogenases and the risk of esophageal squamous cell carcinoma in Gansu Chinese males. World J Gastroenterol 2008; **14**: 1444–1449.
39. Yang SJ, Wang HY, Li XQ, Du HZ, Zheng CJ, Chen HG, Mu XY, Yang CX: Genetic polymorphisms of ADH2 and ALDH2 on esophageal cancer risk in southwest China. World J Gastroenterol 2007; **13**: 5760–5764.
40. Ding JH: L. S., Li SP, Cao HX, Wu JZ, Su P, Gao CM, Ping S, Liu YT, Zhou JN, Chang J, Yao GH. Polymorphisms of alcohol dehydrogenase-2 and aldehyde dehydrogenase-2 and esophageal cancer risk in Southeast Chinese males. World J Gastroenterol 2009; **15**: 2395–2400.
41. Ding JH, Li SP, Cao HX, Wu JZ, Gao CM, Liu YT, Zhou JN, Chang J, Yao GH: Alcohol dehydrogenase-2 and aldehyde dehydrogenase-2 genotypes, alcohol drinking and the risk for esophageal cancer in a Chinese population. J Hum Genet 2010; **55**: 97–102.
42. Cai L, You NC, Lu H, *et al*: Dietary selenium intake, aldehyde dehydrogenase-2 and X-ray repair cross-complementing 1 genetic polymorphisms, and the risk of esophageal squamous cell carcinoma. Cancer 2006; **106**: 2345–2354.
43. WHO Global Status on Alcohol: 2004. Available: http://www.who.int/substance_abuse/publications/statusreportalcoholwpro/en/index.html. Accessed 20 March 2010.
44. Higuchi S, Matsushita S, Imazeki H, Kinoshita T, Takagi S, Kono H: Aldehyde dehydrogenase genotypes in Japanese alcoholics. Lancet 1994; **343**: 741–742.
45. Boccia S, Hashibe M, Galli P, *et al*: Aldehyde dehydrogenase 2 and head and neck cancer: a meta-analysis implementing a Mendelian randomization approach. Cancer Epidemiol Biomarkers Prev 2009; **18**: 248–254.
46. Katoh T, Kaneko S, Kohshi K, Munaka M, Kitagawa K, Kunugita N, Ikemura K, Kawamoto T: Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and oral cavity cancer. Int J Cancer 1999; **83**: 606–609.
47. Nomura T, Noma H, Shibahara T, Yokoyama A, Muramatsu T, Ohmori T: Aldehyde dehydrogenase 2 and glutathione S-transferase M1 polymorphisms in relation to the risk for oral cancer in Japanese drinkers. Oral Oncol 2000; **36**: 42–46.
48. Asakage T, Yokoyama A, Haneda T, *et al*: Genetic polymorphisms of alcohol and aldehyde dehydrogenases and drinking, smoking, and diet in Japanese men with oral and pharyngeal squamous cell carcinoma. Carcinogenesis 2007; **28**: 865–874.
49. Yokoyama A, Omori T, Yokoyama T, Sato Y, Kawakubo H, Mori S, Matsui T, Maruyama K: Risk factors of squamous cell carcinoma in the oropharynx, hypopharynx, and epilarynx of Japanese alcoholic men: A case-control study based on an endoscopic screening program. Stomach and Intestine (Tokyo) 2010; **45**: 180–189 (in Japanese with English abstract, tables, and figures).
50. Yokoyama A, Omori T, Yokoyama T, Sato Y, Kawakubo H, Maruyama K: Risk of metachronous squamous cell carcinoma in the upper aerodigestive tract of Japanese alcoholic men with esophageal squamous cell carcinoma: A long-term endoscopic follow-up study. Cancer Sci 2008; **99**: 1164–1171.
51. Watanabe H: Present status and management of multiple primary esophageal cancer associated with head and neck cancer. J Jpn Bronchoesophagol Soc 1998; **49**: 151–155 (in Japanese).
52. Hayat MJ, Howlader N, Reichman ME, Edwards BK: Cancer statistics, trends, and multiple primary cancer analyses from the Surveillance, Epidemiology, and End Results (SEER). Program. Oncologist 2007; **12**: 20–37.
53. Makuuchi H, Machimura T, Shimada H, *et al*: Endoscopic screening for esophageal cancer in 788 patients with head and neck cancers. Tokai J Exp Clin Med 1996; **21**: 139–145.
54. Chen CC, Lu RB, Chen YC, Wang MF, Chang YC, Li TK, Yin SJ: Interaction between the functional polymorphisms of the alcohol-metabolism genes in protection against alcoholism. Am J Hum Genet 1999; **65**: 795–807.
55. Yokoyama A, Muramatsu T, Ohmori T, *et al*: Multiple primary esophageal and concurrent upper aerodigestive tract cancer and the aldehyde dehydrogenase-2 genotype of Japanese alcoholics. Cancer 1996; **77**: 1986–1990.
56. Yokoyama A, Ohmori T, Muramatsu T, *et al*: Short-term follow-up after endoscopic mucosectomy of early esophageal cancer and aldehyde dehydrogenase-2 genotype in Japanese alcoholics. Can-

cer Epidemiol Biomark Prev 1998; **7**: 473–476.
57. Yokoyama A, Watanabe H, Fukuda H, Haneda T, Kato H, Yokoyama T, Muramatsu T, Igaki H, Tachimori Y: Multiple cancers associated with esophageal and oropharyngolaryngeal squamous cell carcinoma and the aldehyde dehydrogenase-2 genotype in male Japanese drinkers. Cancer Epidemiol Biomarkers Prev 2002; **11**: 895–900.
58. Muto M, Takahashi M, Ohtsu A, Ebihara S, Yoshida S, Esumi H: Risk of multiple squamous cell carcinomas both in the esophagus and the head and neck region. Carcinogenesis 2005; **26**: 1008–1012.
59. Yokoyama A, Omori T, Tanaka Y, *et al*: p53 protein accumulation, cancer multiplicity, and aldehyde dehydrogenase-2 genotype in Japanese alcoholic men with early esophageal squamous cell carcinoma. Cancer Lett 2007; **247**: 243–252.
60. Matsubara T, Yamada K, Nakagawa A: Risk of second primary malignancy after esophagectomy for squamous cell carcinoma of the thoracic esophagus. J Clin Oncol 2003; **21**: 4336–4341.
61. Yokoyama A, Yokoyama T, Omori T, *et al*: *Helicobacter pylori*, chronic atrophic gastritis, inactive aldehyde dehydrogenase-2, macrocytosis, and multiple upper aerodigestive tract cancers and the risk for gastric cancer in alcoholic Japanese men. J Gastroenterol Hepatol 2007; **22**: 210–217.
62. Yokoyama A, Omori T, Yokoyama T, Kawakubo H, Mori S, Matsui T, Maruyama K: Chronic atrophic gastritis and metachronous gastric cancer in Japanese alcoholic men with esophageal squamous cell carcinoma. Alcohol Clin Exp Res 2009; **33**: 898–905.
63. Omori T, Yokoyama A: The pink color sign: A high-risk endoscopic finding of iodine-unstained lesions [Abstract]. Nihon Shokaki Naishikyo Gakkai Zasshi 2001; **43**(suppl 2): 1613 (in Japanese).
64. Shimizu Y, Omori T, Yokoyama A, Yoshida T, Hirota J, Ono Y, Yamamoto J, Kato M, Asaka M: Endoscopic diagnosis of early squamous neoplasia of the esophagus with iodine staining: high-grade intra-epithelial neoplasia turns pink within a few minutes. J Gastroenterol Hepatol 2008; **23**: 546–550.
65. Yokoyama A, Mizukami T, Omori T, *et al*: Melanosis and squamous cell neoplasms of the upper aerodigestive tract in Japanese alcoholic men. Cancer Sci 2006; **97**: 905–911.
66. Muto M, Hitomi Y, Ohtsu A, Ebihara S, Yoshida S, Esumi H: Association of aldehyde dehydrogenase 2 gene polymorphism with multiple oesophageal dysplasia in head and neck cancer patients. Gut 2000; **47**: 256–261.
67. Muto M, Nakane M, Hitomi Y, Yoshida S, Sasaki S, Ohtsu A, Yoshida S, Ebihara S, Esumi H: Association between aldehyde dehydrogenase gene polymorphisms and the phenomenon of field cancerization in patients with head and neck cancer. Carcinogenesis 2002; **23**: 1759–1765.
68. Yokoyama A, Omori T, Yokoyama T, *et al*: Esophageal melanosis, an endoscopic finding associated with squamous cell neoplasms of the upper aerodigestive tract, and inactive aldehyde dehydrogenase-2 in alcoholic Japanese men. J Gastroenterol 2005; **40**: 676–684.
69. Shimizu Y, Tsukagoshi H, Fujita M, Hosokawa M, Kato M, Asaka M: Metachronous squamous cell carcinoma of the esophagus arising after endoscopic mucosal resection. Gastrointest Endosc 2001; **54**: 190–194.
70. Shimizu Y, Tsukagoshi H, Fujita M, Hosokawa M, Kato M, Asaka M: Head and neck cancer arising after endoscopic mucosal resection for squamous cell carcinoma of the esophagus. Endoscopy 2003; **35**: 322–326.
71. Yokoyama A, Omori T, Yokoyama T, Sato Y, Kawakubo H, Mizukami T: Risk of cancer in the upper aerodigestive tract in Japanese alcoholic men with superficial esophageal cancer, esophageal dysplasia, or inactive aldehyde dehydrogenase-2: an endoscopic follow-up study. Stomach and Intestine (Tokyo) 2007; **42**: 1365–1374 (in Japanese with English abstract, tables, and figures).
72. Nishi T, Makuuchi H, Shimada H, *et al*: Esophageal melanosis and esophageal cancer [Abstract]. J Jpn Bronchoesophagol Soc 2005; **56**: 221 (in Japanese).
73. Ohashi K, Kato Y, Kanno J, Kasuga T: Melanocytes and melanosis of the esophagus in Japanese subjects − analysis of factors affecting their increase. Virchows Arch A Path Anat Histol 1990; **417**: 137–143.
74. Brooks PJ, Theruvathu JA: DNA adducts from acetaldehyde: implications for alcohol-related carcinogenesis. Alcohol 2005; **35**: 187–193.
75. International Agency for Research on Cancer: Allyl compounds, aldehydes, epoxides and peroxides. IN: IARC Monographs on the Evaluation on the Carcinogenic Risk of Chemicals to Humans. IARC scientific publication. Vol. 36. Lyon: IARC; 1985. p. 101–132.
76. Woutersen RA, Appelman LM, Van Garderen-Hoetmer A, Feron VJ: Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. Toxicology 1986; **41**: 213–231.
77. Feron VJ, Kruysse A, Woutersen RA: Respiratory tract tumours in hamsters exposed to acetaldehyde vapour alone or simultaneously to benzo[a]pyrene or diethylnitrosamine. Eur J Cancer Clin Oncol 1982; **18**: 13–31.
78. Matsuda T, Yabushita H, Kanaly RA, Shibutani S, Yokoyama A: Increased DNA damage in ALDH2-deficient alcoholics. Chem Res Toxicol 2006; **19**: 1374–1378.
79. Morimoto K, Takeshita T: Low Km aldehyde dehydrogenase (ALDH2) polymorphism, alcohol-drinking behavior, and chromosome alterations in peripheral lymphocytes. Environ Health Perspect 1996; **104**(Suppl. 3): 563–567.
80. Ishikawa H, Yamamoto H, Tian Y, Kawano M, Yamauchi T, Yokoyama K: Effects of ALDH2 gene polymorphisms and alcohol-drinking behavior on micronuclei frequency in non-smokers. Mutat Res 2003; **541**: 71–80.
81. Lu Y, Morimoto K: Is habitual alcohol drinking associated with reduced electrophoretic DNA migration in peripheral blood leukocytes from ALDH2-deficient male Japanese? Mutagenesis 2009; **24**: 303–308.
82. Baan R, Straif K, Grosse Y, Secretan B, Ghissassi FE, Bouvard V, Altieri A, Cogliano V, WHO International Agency for Research on Cancer Monograph Working Group: Carcinogenicity of alcoholic beverages. Lancet Oncol 2007; **8**: 292–293.
83. Secretan B, Straif K, Baan R, *et al*: A review of human carcinogens – Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. Lancet Oncol 2009; **10**: 1033–1034.
84. Väkeväinen S, Tillonen J, Agarwal DP, Srivastava N, Salaspuro M: High salivary acetaldehyde after a moderate dose of alcohol in ALDH2-deficient subjects: strong evidence for local carcinogenetic action of acetaldehyde. Alcohol Clin Exp Res 2000; **24**: 873–877.
85. Yokoyama A, Tsutsumi E, Imazeki H, Suwa Y, Nakamura C, Mizukami T, Yokoyama T: Salivary acetaldehyde concentration according to alcoholic beverage consumed and aldehyde dehydrogenase-2 genotype. Alcohol Clin Exp Res 2008; **32**: 1607–1614.
86. Homann N, Jousimies-Somer H, Jokelainen K, Heine R, Salaspuro M: High acetaldehyde levels in saliva after ethanol consumption: methodological aspects and pathogenetic implication. Carcinogenesis 1997; **18**: 1739–1743.
87. Homann N, Tillonen J, Meurman H, Rintamäki H, Lindqvist C, Rautio M, Jousimies-Somer H, Salaspuro M: Increased salivary acetaldehyde levels in heavy drinkers and smokers: a microbiological approach to oral cavity cancer. Carcinogenesis 2000; **21**: 663–668.
88. Yokoyama A, Tsutsumi E, Imazeki H, Suwa Y, Nakamura C, Yokoyama T: Contribution of the alcohol dehydrogenase-1B genotype and oral microorganisms to high salivary acetaldehyde concentrations in Japanese alcoholic men. Int J Cancer 2007; **121**: 1047–1054.

89. Väkeväinen S, Tillonen J, Salaspuro M: 4-Methylpyrazole decreases salivary acetaldehyde levels in ALDH2-deficient subjects but not in subjects with normal ALDH2. Alcohol Clin Exp Res 2001; **25**: 829–834.
90. Homann N, Tillonen J, Rintamäki H, Salaspuro M, Lindqvist C, Meurman JH: Poor dental status increases acetaldehyde production from ethanol in saliva: a possible link to increased oral cancer risk among heavy drinkers. Oral Oncol 2001; **37**: 153–158.
91. Linderborg K, Joly JP, Visapää JP, Salaspuro M: Potential mechanism for Calvados-related oesophageal cancer. Food Chem Toxicol 2008; **46**: 476–479.
92. Yokoyama A, Ohmori T, Muramatsu T, *et al*: Cancer screening of upper aerodigestive tract in Japanese alcoholics with reference to drinking and smoking habits and aldehyde dehydrogenase-2 genotype. Int J Cancer 1996; **68**: 313–316.
93. Salaspuro V, Salaspuro M: Synergistic effect of alcohol drinking and smoking on *in vivo* acetaldehyde concentration in saliva. Int J Cancer 2004; **111**: 480–483.
94. Dong YJ, Peng TK, Yin SJ: Expression and activities of class IV alcohol dehydrogenase and class III aldehyde dehydrogenase in human mouth. Alcohol 1996; **13**: 257–262.
95. Yin SJ, Chou FJ, Chao SF, Tsai S, Liao CS, Wang SL, Wu CW, Lee SC: Alcohol and aldehyde dehydrogenases in human esophagus: comparison with the stomach enzyme activities. Alcohol Clin Exp Res 1993; **17**: 376–381.
96. Jelski W, Kozlowski M, Laudanski J, Niklinski J, Szmitkowski M: The Activity of Class I, II, III, and IV alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) in esophageal cancer. Dig Dis Sci 2009; **54**: 725–730.
97. Lieber CS: Microsomal ethanol-oxidizing system (MEOS): the first 30 years (1968–1998) – a review. Alcohol Clin Exp Res 1999; **23**: 991–1007.
98. Baumgarten G, Waldherr R, Stickel F, Simanowski UA, Ingelmann-Sundverg M, Seitz HK: Enhanced expression of cytochrome p450 2E1 in the oropharyngeal mucosa in alcoholics with cancer [Abstract]. Alcohol Clin Exp Res 1996; **20**(Suppl 2): 80A.
99. Morita M, Oyama T, Kagawa N, *et al*: Expression of aldehyde dehydrogenase 2 in the normal esophageal epithelium and alcohol consumption in patients with esophageal cancer. Front Biosci 2005; **10**: 2319–2324.
100. Higuchi S, Muramatsu T, Shigemori K, Saito M, Kono H, Dufour MC, Harford TC: The relationship between low Km aldehyde dehydrogenase phenotype and drinking behavior in Japanese. J Stud Alcohol 1992; **53**: 170–175.
101. Ishiguro S, Sasazuki S, Inoue M, Kurahashi N, Iwasaki M, Tsugane S, JPHC Study Group: Effect of alcohol consumption, cigarette smoking and flushing response on esophageal cancer risk: a population-based cohort study (JPHC study). Cancer lett 2009; **275**: 240–246.
102. Takeshita T, Morimoto K: Development of a questionnaire method to discriminate between typical and atypical genotypes of low-Km aldehyde dehydrogenase in a Japanese population. Alcohol Clin Exp Res 1998; **22**: 1409–1413.
103. Muramatsu T, Higuchi S, Shigemori K, *et al*: Ethanol patch test: a simple and sensitive method for identifying ALDH phenotype. Alcohol Clin Exp Res 1989; **13**: 229–231.
104. Takeshita T, Yang X, Morimoto K: Association of the ADH2 genotypes with skin responses after ethanol exposure in Japanese male university students. Alcohol Clin Exp Res 2001; **25**: 1264–1269.
105. Yokoyama A, Muramatsu T, Ohmori T, Kumagai Y, Higuchi S, Ishii H: Reliability of a flushing questionnaire and the ethanol patch test in screening for inactive aldehyde dehydrogenase-2 and alcohol-related cancer risk. Cancer Epidemiol Biomarkers Prev 1997; **6**: 1105–1107.
106. Yokoyama T, Yokoyama A, Kato H, *et al*: Alcohol flushing, alcohol and aldehyde dehydrogenase genotypes, and risk for esophageal squamous cell carcinoma in Japanese men. Cancer Epidemiol Biomarkers Prev 2003; **12**: 1227–1233.
107. Yokoyama A, Muramatsu T, Ohmori T, *et al*: Alcohol and aldehyde dehydrogenase gene polymorphisms influence susceptibility to esophageal cancer in Japanese alcoholics. Alcohol Clin Exp Res 1999; **23**: 1705–1710.
108. Yokoyama T, Yokoyama A, Kumagai Y, *et al*: Health risk appraisal models for mass screening of esophageal cancer in Japanese men. Cancer Epidemiol Biomarkers Prev 2008; **17**: 2846–2854.
109. Yokoyama A, Kumagai Y, Yokoyama T, *et al*: Health risk appraisal models for mass screening for esophageal and pharyngeal cancer: an endoscopic follow-up study of cancer-free Japanese men. Cancer Epidemiol Biomarkers Prev 2009; **18**: 651–655.
110. Wu A, Chanarin I, Levi AJ: Macrocytosis of chronic alcoholism. Lancet 1974; **1**: 829–830.
111. Nomura F, Itoga S, Tamura M, Harada S, Iizuka Y, Nakai T: Biological markers of alcoholism with respect to genotypes of low-Km aldehyde dehydrogenase (ALDH2) in Japanese subjects. Alcohol Clin Exp Res 2000; **24**(4 Suppl): 30S–33S.
112. Hashimoto Y, Nakayama T, Futamura A, Omura M, Nakahara K: Erythrocyte mean cell volume and genetic polymorphism of aldehyde dehydrogenase 2 in alcohol drinkers. Blood 2002; **99**: 3487–3488.
113. Yokoyama M, Yokoyama A, Yokoyama T, Hamana G, Funazu K, Kondo S, Yamashita T, Yoshimizu H, Nakamura H: Mean corpuscular volume and the aldehyde dehydrogenase-2 genotype in male Japanese workers. Alcohol Clin Exp Res 2003; **27**: 1395–1401.
114. Yokoyama A, Yokoyama T, Kumagai Y, *et al*: Mean corpuscular volume, alcohol flushing and the predicted risk of squamous cell carcinoma of the esophagus in cancer-free Japanese men. Alcohol Clin Exp Res 2005; **29**: 1877–1883.
115. Yokoyama T, Saito K, Lwin H, Yoshiike N, Yamamoto A, Matsushita Y, Date C, Tanaka H: Epidemiological evidence that acetaldehyde plays a significant role in the development of decreased serum folate concentration and elevated mean corpuscular volume in alcohol drinkers. Alcohol Clin Exp Res 2005; **29**: 622–630.
116. Sun L, König IR, Jacobs A, Seitz HK, Junghanns K, Wagner T, Ludwig D, Jacrobs A, Homann N: Mean corpuscular volume and ADH1C genotype in white patients with alcohol-associated diseases. Alcohol Clin Exp Res 2005; **29**: 788–793.
117. Hiraki A, Matsuo K, Wakai K, Suzuki T, Hasegawa Y, Tajima K: Gene-gene and gene-environment interactions between alcohol drinking habit and polymorphisms in alcohol-metabolizing enzyme genes and the risk of head and neck cancer in Japan. Cancer Sci 2007; **98**: 1087–1091.
118. Boonyaphiphat P, Thongsuksai P, Sriplung H, Puttawibul P: Lifestyle habits and genetic susceptibility and the risk of esophageal cancer in the Thai population. Cancer Lett 2002; **186**: 193–199.
119. Hashibe M, Boffetta P, Zaridze D, *et al*: Evidence for an important role of alcohol- and aldehyde metabolizing genes in cancers of the upper aerodigestive tract. Cancer Epidemiol Biomarkers Prev 2006; **15**: 696–703.
120. Peng GS, Chen YC, Tsao TP, Wang MF, Yin SJ: Pharmacokinetic and pharmacodynamic basis for partial protection against alcoholism in Asians heterozygous for the variant ALDH2*2 gene allele. Pharmacogenet Genomics 2007; **17**: 845–855.
121. Martínez C, Galván S, Garcia-Martin E, Ramos M, Gutiérrez-Martín Y, Agúndez JA: Variability in ethanol biodisposition in whites is modulated by polymorphisms in the ADH1B and ADH1C genes. Hepatology 2010; **51**: 491–500.
122. Yin SJ, Bosron WF, Magnes LJ, Li TK: Human liver alcohol dehydrogenase: Purification and kinetic characterization of the β_2 β_2, $\beta_2\beta_1$, $\alpha\beta_2$ and $\beta_2\gamma_1$ 'Oriental' isozymes. Biochemistry 1984; **23**: 5847–5853.
123. Neumark YD, Friedlander Y, Durst R, Leitersdorf E, Jaffe D, Ramchandani VA, O'Connor S, Carr LG, Li TK: Alcohol dehy-

drogenase polymorphisms influence alcohol-elimination rates in a male Jewish population. Alcohol Clin Exp Res 2004; **28**: 10–14.
124. Yokoyama A, Tsutsumi E, Imazeki H, Suwa Y, Nakamura C, Yokoyama T: Polymorphisms of alcohol dehydrogenase-1B and aldehyde dehydrogenase-2 and the blood and salivary ethanol and acetaldehyde concentrations of Japanese alcoholic men. Alcohol Clin Exp Res 2010; **34**: 1246–1256.
125. Takeshita T, Mao XQ, Morimoto K: The contribution of polymorphism in the alcohol dehydrogenase β subunit to alcohol sensitivity in a Japanese population. Hum Genet 1996; **97**: 409–413.
126. Chen WJ, Chen CC, Yu JM, Cheng AT: Self-reported flushing and genotypes of ALDH2, ADH2, and ADH3 among Taiwanese Han. Alcohol Clin Exp Res 1998; **22**: 1048–1052.
127. Matsuo K, Wakai K, Hirose K, Saito T, Tajima K: Alcohol dehydrogenase 2 His47Arg polymorphism influences drinking habit independently of aldehyde dehydrogenase 2 Glu487Lys polymorphism: analysis of 2,299 Japanese subjects. Cancer Epidemiol Biomarkers Prev 2006; **15**: 1009–1013.
128. Macgregor S, Lind PA, Bucholz KK, *et al*: Associations of ADH and ALDH2 gene variation with self report alcohol reactions, consumption and dependence: an integrated analysis. Hum Mol Genet 2009; **18**: 580–593.
129. Matsuo K, Hiraki A, Hirose K, Ito H, Suzuki T, Wakai K, Tajima K: Impact of the alcohol-dehydrogenase (ADH) 1C and ADH1B polymorphisms on drinking behavior in nonalcoholic Japanese. Hum Mutat 2007; **28**: 506–510.
130. Visapää JP, Götte K, Benesova M, *et al*: Increased cancer risk in heavy drinkers with the alcohol dehydrogenase 1C*1 allele, possibly due to salivary acetaldehyde. Gut 2004; **53**: 871–876.
131. Brennan P, Lewis S, Hashibe M, *et al*: Pooled analysis of alcohol dehydrogenase genotypes and head and neck cancer: A HuGE review. Am J Epidemiol 2004; **159**: 1–16.
132. Harty LC, Caparoso NE, Hayes RB, *et al*: Alcohol dehydrogenase 3 genotype and risk of oral cavity and pharyngeal cancers. J Natl Cancer Inst 1997; **89**: 1698–1705.
133. Homann N, Stickel F, König IR, *et al*: Alcohol dehydrogenase 1C*1 allele is a genetic marker for alcohol-associated cancer in heavy drinkers. Int J Cancer 2006; **118**: 1998–2002.
134. Rehm J, Patra J, Popova S: Alcohol drinking cessation and its effect on esophageal and head and neck cancers: A pooled analysis. Int J Cancer 2007; **121**: 1132–1137.