Alcoholic beverages are causally related to cancer of the oral cavity, pharynx, larynx and esophagus. Ethanol is oxidized to acetaldehyde and then to acetate by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), both of which have genetic polymorphisms. A review of case-control studies of the effects of ALDH2, ADH2 and ADH3 genotypes shows consistently positive associations between inactive heterozygous ALDH2 and the less-active ADH2 genotypes and the risk for esophageal cancer in East Asian heavy drinkers and this enzyme-related vulnerability may extend to light-to-moderate drinkers. Some studies suggest similar associations with the risk for head and neck cancer in moderate-to-heavy-drinking Japanese. An established carcinogen in experimental animals, acetaldehyde can interact with human DNA. ALDH2-associated cancer susceptibility fits into a scenario in which acetaldehyde plays a critical role in the development of human cancer. Alcohol flushing and drinking behavior may partly explain this carcinogenic effect in carriers of less-active ADH2 genotypes. Whether the ADH3 genotype influences head and neck cancer risk in Western nations is controversial. Professional and public education about risky conditions connected to the ALDH2 and ADH2 genotypes and environmental factors is important in a new strategic approach to the prevention of alcohol-related cancers in East Asians. The use of simple tests to identify inactive ALDH2 on the basis of alcohol flushing responses could benefit many people, by helping them to identify their own cancer risks. Such testing could also help clinicians diagnose esophageal cancer earlier, through the use of endoscopic screening in the high-risk population.

Key words: alcohol dehydrogenase – aldehyde dehydrogenase – esophageal cancer – head and neck cancer

INTRODUCTION

Epidemiological surveys offer convincing evidence that alcoholic beverages are carcinogenic to humans and causally related to cancer of the oral cavity, pharynx, larynx and esophagus (1). Alcohol-metabolizing enzymes’ genetic polymorphisms modulate individual differences in alcohol-oxidizing capability and drinking behavior (2), but how enzyme polymorphisms influence individual cancer susceptibility is a new area of research. This review summarizing data on enzyme-related risks for esophageal and head and neck cancers represents an attempt to broaden the concept of what constitutes high-risk drinking.

ALCOHOL METABOLISM, DRINKING BEHAVIOR AND ALDH2 GENOTYPE

Ethanol is eliminated from the body by its oxidation, first to acetaldehyde and then to acetate. These reactions are mainly catalyzed by alcohol dehydrogenase and aldehyde dehydrogenase, respectively, in the liver. Most of the acetaldehyde generated during alcohol metabolism in vivo is promptly eliminated by aldehyde dehydrogenase-2 (ALDH2), the low-$K_m$ mitochondrial ALDH (2).

The gene for the homotetrameric enzyme ALDH2 has a polymorphism and its mutant ALDH2*2 allele (Glu487Lys)
encodes a catalytically inactive subunit (3). Individuals with ALDH2*1/2*2 genotype should have only 6.25% of normal ALDH2*1 protein; other molecules containing one or more ALDH2*2 subunits is considered to be inactive, explaining the dominant effect of ALDH2*2 (4). Approximately 40% of Japanese have inactive forms of ALDH2 with the mutant ALDH2*2 allele (5). Distribution of the ALDH2*2 allele varies by race (6): it is prevalent in East Asians but has not been found in Caucasians or Africans. When this enzyme is inactive, the body fails to metabolize acetaldehyde rapidly, leading to excessive accumulation of acetaldehyde. Alcohol challenge tests have shown that after drinking a small amount of ethanol (0.1 g/kg body weight), ALDH2*2/2*2 homozygous and ALDH2*1/2*2 heterozygous individuals’ average peak blood acetaldehyde concentrations are 18 times and five times, respectively, the average peak blood acetaldehyde in active ALDH2*1/2*1 homozygotes who have consumed a moderate (0.8 g/kg body weight) amount of ethanol (7). After drinking alcohol, persons with inactive ALDH2 exhibit the so-called ‘flushing response,’ which includes facial flushing, palpitations, drowsiness and other unpleasant symptoms (8). By causing acetaldehydeemia with the flushing response, the inactive forms of ALDH2 prevent many Japanese from drinking heavily and developing alcoholism (9).

The preventive effect of heterozygous ALDH2*1/2*2 is incomplete, however, and it is influenced by sociocultural factors. In addition to a rapid increase in Japanese per capita alcohol consumption, recent decades have seen a dramatic increase in the proportion of heavy drinkers who have inactive heterozygous ALDH2. For example, 2.5% of Japanese alcoholics had the inactive heterozygous ALDH2 in 1979, 8.0% in 1986 and 13.0% in 1992 (10).

CARCINOGENICITY OF ACETALDEHYDE

Several possible mechanistic pathways through which drinking alcohol may cause cancer have been discussed (11): (a) alcohol’s contact-related local effects on the upper gastrointestinal tract; (b) alcohol’s solvent effects on tobacco and other carcinogens; (c) the induction of microsomal enzymes involved in carcinogen metabolism; (d) the generation of oxygen radicals and lipid peroxidation products; (e) nutritional deficiency, especially vitamin and mineral deficiencies; and (f) suppressed immune function.

There is ample evidence for the carcinogenicity of acetaldehyde, the first metabolite formed during the breakdown of the ethanol in experimental animals (12,13). The inhalation of acetaldehyde produced tumors of the respiratory tract, specifically adenocarcinomas and squamous cell carcinomas of the nasal mucosa in rats (14) and laryngeal carcinomas in hamsters (15), in which this metabolite also served as a promoter in carcinogenesis attributable to benzo[a]pyrene (15). Acetaldehyde induces chromosomal aberrations, micronuclei and sister chromatid exchanges in cultured mammalian cells (16). It can also interact covalently with DNA to form DNA adducts, which may be a critical initiating event in the multistage process of chemical carcinogenesis (17). The formation of N\textsuperscript{2}-ethyl-2\textsuperscript{-}deoxyguanosine, one major stable acetaldehyde–DNA adduct (17), was detected in DNA from the liver of ethanol-treated mice (18). N\textsuperscript{2}-Ethyl-2\textsuperscript{-}deoxyguanosine can be used efficiently by eukaryotic DNA polymerase (19). Alcoholic patients’ levels of acetaldehyde adduct in lymphocyte and granulocyte DNA were much higher than the corresponding levels in healthy control individuals (20). In vitro experiments have shown that lymphocytes from habitual drinkers with the inactive form of ALDH2, which cannot detoxify acetaldehyde efficiently, have higher frequencies of sister chromatid exchanges than lymphocytes from individuals with normal, active ALDH2 (21).

ESOPHAGEAL CANCER AND THE ALDH2 GENOTYPE

Since our first report in 1996 (22), case-control studies of various Japanese drinking populations (23–28) and Chinese alcoholics (29) have consistently demonstrated that the inactive ALDH2 encoded by the ALDH2*1/2*2 genotype is a strong risk factor for esophageal cancer (Table 1). Whether individuals drink every day (22) or are heavy drinkers (27) or are alcoholics (26), those with the ALDH2*1/2*2 genotype have similarly high odds of getting esophageal cancer [odds ratios (ORs) = 12.1, 16.4 and 13.5, respectively]. When patients’ cancer risk is compared with that of healthy subjects without adjusting for alcohol consumption, the result may be underestimated.

In the largest study yet conducted (30), light drinkers with ALDH2*1/2*2 (ethanol consumption 1–8.9 units/week; 1 unit = 22 g) had an esophageal cancer risk 5.8 times that of light drinkers with ALDH2*1/2*1 (reference category). Their risk was similar to that of moderate drinkers with ALDH2*1/2*1, who consumed 9–17.9 units/week (OR = 5.6). The risk for moderate drinkers with ALDH2*1/2*2 (OR = 55.84) exceeded that for heavy drinkers with ALDH2*1/2*1 (OR = 4.4) (23), because of the strong protective effect of ALDH2*1/2*2 against drinking. The link between the results of epidemiological, animal and basic science studies fits a scenario in which acetaldehyde plays a critical role in the development of human esophageal cancer.
Confirmation of these associations is needed. One study (27) reported that enhanced risk for esophageal cancer due to the inactive heterozygous ALDH2 did not reach significance in non-heavy drinkers. That study’s non-heavy-drinking cancer population consisted of 34 cases, of whom 16 were female, and the small sample size and inclusion of women might have hampered obtaining significant results. To date, much of the research in this field has focused on male Japanese drinkers, owing to the small proportion of women among esophageal cancer patients. If the accumulation of acetaldehyde is one of the important causes of esophageal cancer, it is reasonable to assume that drinking by women who have inactive ALDH2 may enhance their cancer risk. Curiously, Japanese esophageal cancer mortality statistics show that age-adjusted female mortality rates declined steadily, whereas male-to-female ratios within birth cohorts were widening (31). These observations suggest male–female differences in lifestyle-associated etiologies of esophageal cancer, including drinking, smoking and dietary habits. Analyses of women’s risks will be the foci of future research.

Per capita alcohol consumption is lower in Japan than in Western nations, but Japanese men’s age-adjusted esophageal cancer death rates ranks higher than those of men in many Western populations (32). This paradox might be explained, in part, by the strong carcinogenic role of ALDH2*1/2*2 in male Japanese drinkers.

**HEAD AND NECK CANCER AND THE ALDH2 GENOTYPE**

Among Japanese alcoholic men, we have found strong associations between inactive heterozygous ALDH2 and cancers of the oropharyngolarynx (OR = 18.5), oral cavity/oropharynx (OR = 20.8) and hypopharynx/epilarynx (OR = 28.9), as shown in Table 2 (26). The findings of three population-based Japanese studies were mixed (33–35). In a study in which about 40% of the subjects were women and alcohol consumption was not associated with the oral cancer risk, the ALDH2 genotype had no effect (33). Another study in which about 40% of the subjects were women showed that alcohol consumption was a risk factor for oral cancer and that the risk was higher in drinkers with ALDH2*1/2*2, although the magnitude of increased risk (OR = 2.9) was considerably lower (34) than in our study (26).

The most recent study of general head and neck cancer patients showed an ALDH2 genotype effect only among moderate-to-heavy male drinkers (35). This stronger link of inactive ALDH2 to the head and neck cancer risk only emerged when there was higher alcohol exposure, particularly in men. Different anatomical subsites in the head and neck may account for the apparent inconsistency of these results. The oral cavity might be more loosely associated with ALDH2-related carcinogenesis than the pharynx and epilarynx in non-alcoholic populations. In such populations, alcohol action and ALDH2 effects on oral carcinogenesis might be less important than other risks, such as smoking, poor dentition and inadequate oral hygiene. Without a lot more data, that question cannot be answered.

**FIELD CANCERIZATION AND THE ALDH2 GENOTYPE**

Multiple upper aerodigestive tract and stomach cancers associated with esophageal cancer are common, especially in heavy drinkers (36,37). This multiplicity is often explained by the concept of field cancerization, which suggests common etiologies (38). Both drinking and smoking have been reported to predict the likelihood of developing multiple cancers in these regions (37,39). Inactive heterozygous ALDH2 is a risk factor for synchronous or metachronous multiple cancers associated with esophageal and oropharyngolaryngeal cancer in both the alcoholic (36,40) and general (41) Japanese male populations.

### Table 1. ALDH2*1/2*2 genotype-attributable risk for esophageal cancer in East Asians†

<table>
<thead>
<tr>
<th>Type of esophageal cancer</th>
<th>Subjects (No. of cases/controls)</th>
<th>Gender</th>
<th>Risk, OR† (CI)</th>
<th>Source (Ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Esophageal cancer</strong></td>
<td>Everyday drinkers (29/28)</td>
<td>M</td>
<td>12.1 (3.4–42.8)</td>
<td>Yokoyama et al., 1996 (22)</td>
</tr>
<tr>
<td></td>
<td>Alcoholics (40/50)</td>
<td>M</td>
<td>7.6 (2.8–20.7)</td>
<td>Yokoyama et al., 1996 (22)</td>
</tr>
<tr>
<td><strong>Esophageal SCC</strong></td>
<td>Overall population (94/70)</td>
<td>M/F</td>
<td>4.4 (2.5–7.7)</td>
<td>Hori et al., 1997 (23)</td>
</tr>
<tr>
<td><strong>Esophageal cancer</strong></td>
<td>Alcoholic (87/487)</td>
<td>M</td>
<td>12.5† (7.2–21.6)</td>
<td>Yokoyama et al., 1998 (24)</td>
</tr>
<tr>
<td><strong>Esophageal SCC</strong></td>
<td>Upper aerodigestive tract SCC patients (19/25)</td>
<td>M/F</td>
<td>Significantly increased</td>
<td>Tanabe et al., 1999 (25)</td>
</tr>
<tr>
<td><strong>Esophageal cancer</strong></td>
<td>Chinese alcoholics (59/222)</td>
<td>M/F</td>
<td>Significantly increased</td>
<td>Chao et al., 2000 (29)</td>
</tr>
<tr>
<td><strong>Esophageal SCC</strong></td>
<td>Alcoholics (112/526)</td>
<td>M</td>
<td>13.5† (8.1–22.6)</td>
<td>Yokoyama et al., 2001 (26)</td>
</tr>
<tr>
<td><strong>Esophageal cancer</strong></td>
<td>Heavy drinkers (68/26)</td>
<td>M/F</td>
<td>16.4† (4.4–61.2)</td>
<td>Matsuo et al., 2001 (27)</td>
</tr>
<tr>
<td></td>
<td>Other drinkers (34/215)</td>
<td>M/F</td>
<td>NS</td>
<td>Matsuo et al., 2001 (27)</td>
</tr>
<tr>
<td><strong>Esophageal cancer</strong></td>
<td>Habitual drinkers (65/151)</td>
<td>M</td>
<td>Significantly increased</td>
<td>Itoga et al., 2002 (28)</td>
</tr>
<tr>
<td><strong>Esophageal SCC</strong></td>
<td>General population (234/634)</td>
<td>M</td>
<td>7.5† (4.7–11.8)</td>
<td>Yokoyama et al., 2002 (30)</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; NS, not significant; SCC, squamous cell carcinoma. †Japanese unless otherwise noted. ‡OR adjusted for drinking and smoking habits.
There were 112 male alcoholics with esophageal cancer in one study, 40.1% had multiple intra-esophageal cancers and 19.6% had oropharyngolaryngeal and/or stomach cancer. In the presence of ALDH2*1/2*2, the risk for multiple intra-esophageal cancers (OR = 3.4) or esophageal cancer with oropharyngolaryngeal or stomach cancer (OR = 4.0) was higher than the risk for either solitary intra-esophageal cancer or esophageal cancer alone (26). In drinking men treated at Japan’s National Cancer Center, the risk for multiplicity as associated with esophageal or oropharyngolaryngeal cancer was also increased (ORs = 5.3 and 7.4, respectively) in the presence of the ALDH2*2 allele (41).

A study of esophageal iodine screening in Japanese oropharyngolaryngeal cancer patients also showed that the ALDH2*2 allele is an indicator of risk for earlier field cancerization, i.e. the presence of multiple esophageal dysplasias (42,43). The increasing prevalence of synchronous or metachronous multiple cancers in other organs (primarily in the head and neck and stomach) among all patients undergoing surgery at Japan’s National Cancer Center Hospital for esophageal cancer, from 6.3% in the 1969–80 period to 22.2% in 1981–91 and 39.0% in 1992–96 (44), is alarming. In addition to progress in the diagnosis and treatment of these cancers, widespread screening with esophageal iodine staining in head and neck cancer patients has detected extremely high rates (5.1–26.7%) of esophageal cancer (44–46).

The increased proportion of multiple cancers among Japanese esophageal cancer patients is linked to both the dramatic increase in the numbers of drinkers who have the ALDH2*2 allele and the rapid increase in per capita alcohol consumption in recent decades (10). These findings have very important implications for the development of strategies to prevent the secondary development of primary cancers, particularly in persons whose initial cancers were treated by endoscopic mucosal resection. Short-term follow-up after endoscopic mucosectomy of early esophageal cancer in alcoholics showed a 7.6-fold hazard ratio for the additional primary intra-esophageal cancers in those with ALDH2*1/2*2, compared with those with ALDH2*1/2*1 and a 5.5-fold hazard ratio in those with initial multiple primary intra-esophageal cancers, compared with those who had solitary intra-esophageal cancer (40). These results suggest that systemic acetaldehydemia plays a

### Table 2. ALDH2*1/2*2 genotype-attributable risk for head and neck cancer in Japanese

<table>
<thead>
<tr>
<th>Type of head and neck cancer</th>
<th>Subjects (No. of cases/controls)</th>
<th>Gender</th>
<th>Risk, OR† (CI)</th>
<th>Source (Ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oropharyngolaryngeal cancer</td>
<td>Alcoholics (34/487)</td>
<td>M</td>
<td>11.1† (5.2–24.4)</td>
<td>Yokoyama et al., 1998 (24)</td>
</tr>
<tr>
<td>Oral/oropharynx</td>
<td>Alcoholics (16/487)</td>
<td>M</td>
<td>11.1† (3.8–32.7)</td>
<td>Yokoyama et al., 1998 (24)</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>Alcoholics (10/487)</td>
<td>M</td>
<td>23.7† (5.2–107)</td>
<td>Yokoyama et al., 1998 (24)</td>
</tr>
<tr>
<td>Larynx</td>
<td>Alcoholics (10/487)</td>
<td>M</td>
<td>13.0† (3.3–51.3)</td>
<td>Yokoyama et al., 1998 (24)</td>
</tr>
<tr>
<td>Oral SCC</td>
<td>Overall population (147/92)</td>
<td>M/F</td>
<td>NS†</td>
<td>Katoh et al., 1999 (33)</td>
</tr>
<tr>
<td>Oral SCC</td>
<td>Drinkers (114/33)</td>
<td>M/F</td>
<td>2.9 (1.1–7.8)</td>
<td>Nomura et al., 2000 (34)</td>
</tr>
<tr>
<td>Oropharyngolaryngeal SCC</td>
<td>Alcoholics (33/526)</td>
<td>M</td>
<td>18.5* (7.7–44.4)</td>
<td>Yokoyama et al., 2001 (26)</td>
</tr>
<tr>
<td>Oral/oropharynx</td>
<td>Alcoholics (16/526)</td>
<td>M</td>
<td>20.8* (6.6–65.5)</td>
<td>Yokoyama et al., 2001 (26)</td>
</tr>
<tr>
<td>Hypopharynx/epilarynx</td>
<td>Alcoholics (18/526)</td>
<td>M</td>
<td>28.9* (8.7–96.6)</td>
<td>Yokoyama et al., 2001 (26)</td>
</tr>
<tr>
<td>Head and neck cancer</td>
<td>General population (67/634)</td>
<td>M</td>
<td>Increased in moderate-to-heavy drinkers</td>
<td>Asakage et al., 2002 (35)</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; NS, not significant; SCC squamous cell carcinoma. †OR adjusted for drinking and smoking habits.

### Table 3. Field cancerization risk attributable to the ALDH2*1/2*2 genotype in Japanese

<table>
<thead>
<tr>
<th>Type of field cancerization</th>
<th>Subjects (No. of cases/controls)</th>
<th>Gender</th>
<th>Risk, OR† (CI)</th>
<th>Source (Ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synchronous multiple esophageal SCCs</td>
<td>Alcoholics with esophageal SCC (18/15)</td>
<td>M</td>
<td>Significantly increased</td>
<td>Yokoyama et al., 1996 (36)</td>
</tr>
<tr>
<td>Metachronous multiple esophageal SCCs</td>
<td>Alcoholics with esophageal SCC (9/25)</td>
<td>M</td>
<td>Significantly increased</td>
<td>Yokoyama et al., 1998 (40)</td>
</tr>
<tr>
<td>Multiple esophageal dysplasias</td>
<td>Oropharyngolaryngeal SCC patients (14/17)</td>
<td>M/F</td>
<td>4.6 (1.0–21.1)</td>
<td>Muto et al., 2000 (42)</td>
</tr>
<tr>
<td>Multiple esophageal SCCs</td>
<td>Alcoholics with esophageal SCC (45/67)</td>
<td>M</td>
<td>3.4* (1.5–7.9)</td>
<td>Yokoyama et al., 2001 (26)</td>
</tr>
<tr>
<td>Oropharyngolaryngeal or stomach cancers</td>
<td>Alcoholics with esophageal SCC (22/90)</td>
<td>M</td>
<td>4.0* (1.2–13.0)</td>
<td>Yokoyama et al., 2001 (26)</td>
</tr>
<tr>
<td>Multiple cancers</td>
<td>Drinkers with esophageal SCC (26/48)</td>
<td>M</td>
<td>5.3* (1.1–51.1)</td>
<td>Yokoyama et al., 2002 (41)</td>
</tr>
<tr>
<td>Multiple cancers</td>
<td>Drinkers with oropharyngolaryngeal SCC (17/29)</td>
<td>M</td>
<td>7.4* (1.3–80.1)</td>
<td>Yokoyama et al., 2002 (41)</td>
</tr>
<tr>
<td>Multiple esophageal dysplasias</td>
<td>Oropharyngolaryngeal SCC patients (29/49)</td>
<td>M/F</td>
<td>17.6* (4.7–65.3)</td>
<td>Muto et al., 2002 (43)</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; SCC squamous cell carcinoma. †OR adjusted for drinking and smoking habits.
critical role in multicentric or field cancerization throughout the entire mucosal surface of the esophageal and oropharyngolaryngeal areas. They point to the need for a careful search for multiple-field cancerization both before and after beginning treatment of the index cancer in high-risk patients.

ALCOHOL METABOLISM, DRINKING BEHAVIOR AND THE ADH2 AND ADH3 GENOTYPES

The class I group of ADHs consists of major isozymes that break down ethanol to acetaldehyde in vivo (2). The ADH holoenzyme can exist as either a homodimer or a heterodimer combination of α, β and γ subunits, each of which is encoded by a distinct locus, i.e. ADH1, ADH2 and ADH3, respectively. ADH2 is polymorphic and its mutant ADH2*2 allele (Arg47His) (3) is highly prevalent, existing in more than 90% of East Asians, but in fewer than 20% of either Caucasians or Africans. In vitro studies have shown that the ADH2*2 allele encodes a superactive subunit of ADH2 and that the superactive ADH2*2 homodimer has about a 40 times higher $V_{\text{max}}$ than the less-active ADH2*1/2*1 form of ADH2 (2). Striking differences have consistently been reported between the frequencies of the ADH2 genotypes in alcoholics and normal controls in both Japanese and Chinese populations (5,47). The less-active ADH2*1/2*1 genotype is more prevalent among alcoholics (31%) than among normal controls (7%) (5). A similar gene-related tendency, in which more cases of disease are associated with the less-active ADH2*1/2*1 form, has been reported for both Japanese and Chinese patients with alcoholic liver disease, compared with normal controls (48,49).

Oddly enough, investigators have found no correlation between the ADH2 genotype and peak blood acetaldehyde concentration after drinking alcohol (50), but alcohol-induced flushing has been reported to be less intensive in East Asians with ADH2*1/2*1 (51–53). Thus, many East Asians may be protected from alcoholism by the very low frequency of the ADH2*1/2*1 genotype, as well as by inactive ALDH2. The results are inconclusive for other races, but one study in a Jewish population (54) and two in other white populations (55,56) have reported that the ADH2*2 allele has a protective effect against heavy drinking and alcoholism.

The ADH3 homodimer encoded by the ADH3*1 allele produces acetaldehyde twice as fast as that encoded by the ADH3*2 allele (Arg271Glu, Ile349Val) (3) in vitro (2). In East Asians the ADH3*2 allele reportedly enhances the risk for alcoholism (47,57–59). Because of the proximity of the ADH2 and ADH3 loci on the human genome, ADH2 gene polymorphism is in linkage disequilibrium with ADH3 gene polymorphism (57,58). It is therefore reasonable to assume that the ADH3*2 allele (Arg271Glu, Ile349Val) (3) is highly prevalent, existing in more than 90% of East Asians, but in fewer than 20% of either Caucasians or Africans. In vitro studies have shown that the ADH2*2 allele encodes a superactive subunit of ADH2 and that the superactive ADH2*2 homodimer has about a 40 times higher $V_{\text{max}}$ than the less-active ADH2*1/2*1 form of ADH2 (2). Striking differences have consistently been reported between the frequencies of the ADH2 genotypes in alcoholics and normal controls in both Japanese and Chinese populations (5,47). The less-active ADH2*1/2*1 genotype is more prevalent among alcoholics (31%) than among normal controls (7%) (5). A similar gene-related tendency, in which more cases of disease are associated with the less-active ADH2*1/2*1 form, has been reported for both Japanese and Chinese patients with alcoholic liver disease, compared with normal controls (48,49).

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ESOPHAGEAL CANCER AND THE ADH2 GENOTYPE

All four Japanese case-control studies (23,26,30,53) and also the only Chinese case-control study (29) investigating the association between the ADH2 genotype and esophageal cancer have demonstrated that the less-active ADH2*1/2*1 form of ADH2 independently enhances cancer risk [e.g. ORs = 2.3 and 4.1 in Japanese male alcoholic (26) and general (30) populations, respectively], as shown in Table 4. Inasmuch as the frequency of ADH2*1/2*1 is higher among Japanese and Chinese patients with alcohol-related disorders than among the Japanese and Chinese general populations (47–49,57), the risk associated with ADH2*1/2*1 might be overestimated (OR = 6.2) (23), unless it is adjusted for alcohol consumption. The largest Japanese study (30) showed that, similarly to persons with the ALDH2*1/2*2 genotype, light drinkers with ADH2*1/2*1 have an esophageal cancer risk 4.0 times that for

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### Table 4. ADH2*1/2*1 genotype-attributable risk for esophageal and head and neck cancers in East Asians

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Subjects (No. of cases/controls)</th>
<th>Gender</th>
<th>Risk, OR (CI)</th>
<th>Source (Ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophageal SCC</td>
<td>Overall population (94/70)</td>
<td>M/F</td>
<td>6.2 (2.6–14.7)</td>
<td>Yokoyama et al., 1999 (53)</td>
</tr>
<tr>
<td>Esophageal SCC</td>
<td>Alcoholics (91/77)</td>
<td>M</td>
<td>2.0 (1.1–3.6)</td>
<td>Yokoyama et al., 1999 (53)</td>
</tr>
<tr>
<td>Esophageal SCC</td>
<td>General population (234/634)</td>
<td>M</td>
<td>4.1 (2.1–8.1)</td>
<td>Yokoyama et al., 2002 (30)</td>
</tr>
<tr>
<td>Oropharyngolaryngeal SCC</td>
<td>Alcoholics (33/526)</td>
<td>M</td>
<td>6.7 (2.8–15.9)</td>
<td>Yokoyama et al., 2001 (26)</td>
</tr>
<tr>
<td>Oral/oropharynx</td>
<td>Alcoholics (16/526)</td>
<td>M</td>
<td>5.5 (1.8–17.0)</td>
<td>Yokoyama et al., 2001 (26)</td>
</tr>
<tr>
<td>Hypopharynx/epilarynx</td>
<td>Alcoholics (18/526)</td>
<td>M</td>
<td>6.6 (1.6–21.3)</td>
<td>Yokoyama et al., 2001 (26)</td>
</tr>
<tr>
<td>Head and neck cancer</td>
<td>General population (67/634)</td>
<td>M</td>
<td>Increased in moderate-to-heavy drinkers</td>
<td>Asakage et al., 2002 (35)</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; NS, not significant; SCC, squamous cell carcinoma. †Japanese unless otherwise noted. §OR adjusted for drinking and smoking habits.
light drinkers without the ADH2*1/2*1 genotype (reference category) and this risk is similar to that for moderate drinkers without ADH2*1/2*1 (OR = 4.1). The esophageal cancer risk for moderate drinkers with ADH2*1/2*1 (OR = 33.3) exceeds that for heavy drinkers without this gene polymorphism (OR = 7.0). Moreover, in Japanese men representing both the alcoholic (26) and general (30) populations, the gene combination of ADH2*1/2*1 and ALDH2*1/2*1 enhances the cancer risk in multiplicative fashion (e.g., ORs = 40.4 and 30.1, respectively).

Alcohols’ estimated population-attributable risk for esophageal cancer due to their ADH2/ALDH2 gene polymorphisms is 63.9% (26). There are several possible explanations for this finding. First, ADH2 is the predominant enzyme among low-Km class I ADHs expressed in the esophagus, although the expression of high-Km ADH7 is stronger (60). In ADH2*1/2*1 homozygotes, low concentrations of ethanol may linger in the esophageal mucosa during the slow oxidation of ADH2 and thus may be associated with esophageal carcinogenesis. Second, individuals with both ALDH2*1/2*2 and ADH2*1/2*1 genotypes tend not to experience alcohol flushing (51,53). The diminished intensity of the aversive flushing response may enhance both individual vulnerability to drinking and exposure to acetaldehyde. Third, alcohols with the ADH2*1/2*1 genotype tend to have experienced ‘binge-drinking’ and withdrawal syndrome earlier in life than those with other genotypes (61). Thus, ADH2*1/2*1-mediated acceleration of the occurrence of alcohol-related events may contribute to the enhancement of alcohols’ risk for cancer.

The association of the ADH2 genotype and multiple or field cancerization in esophageal cancer patients has been assessed only in alcoholic male Japanese (26). The negative results of that study support the hypothesis that the role of ADH2*1/2*1 in carcinogenesis differs from the simple acetaldehyde scenario.

### HEAD AND NECK CANCER AND THE ADH2 GENOTYPE

Only two Japanese case-control studies showing enhanced cancer risk in the presence of ALDH2*1/2*2 (26,35) have also investigated the association between the ADH2 genotype and the risk for head and neck cancer (Table 4). Among alcoholic men, ADH2*1/2*1 increased the risk for cancer in the oropharyngolarynx (OR = 6.7), oral cavity/oropharynx (OR = 5.5) and hypopharynx/epilarynx (OR = 6.6) (26). As in esophageal cancer, the gene combination of ADH2*1/2*1 and ALDH2*1/2*2 enhanced the risk for oropharyngolaryngeal cancer in multiplicative fashion (OR = 122) among this population. We have estimated alcohols’ population-attributable risk due to ADH2/ALDH2 gene polymorphisms to be 82.0% for oropharyngolaryngeal cancer (26). Among a general male population, ADH2*1/2*1 increased the risk for head and neck cancer in moderate-to-heavy drinkers (35). Because it is associated with cancer risk through its interaction with drinking habits or the ALDH2 genotype, the ADH2 genotype may be presumed to be associated with the risk for head and neck cancer in populations in which alcohol drinking or inactive ALDH2 poses that risk.

### UPPER AERODIGESTIVE TRACT CANCER AND THE ADH3 GENOTYPE

ADH3 gene polymorphism is the rate-limiting factor in acetaldehyde metabolism among most Western populations, in which the prevalence of the ALDH2*2 and ADH2*2 alleles is very low (2). Individuals possessing ADH3*1 are hypothesized to be at greater risk for alcohol-related cancer in these populations, because the homodimer encoded by the ADH3*1 allele produces acetaldehyde faster than the one encoded by the ADH3*2 allele (2); however, as shown in Table 5, six published studies of Western populations have yielded no consistent pattern of association (62–67). Although higher ADH3*1/
3*1-associated risk was shown for laryngeal cancer in a small alcoholic population (62) and for oral cavity and pharyngeal cancer in a heavy-drinking population (63), subsequent larger studies have provided conflicting results. Three studies reported negative results (64–66) and one reported the association of the ADH3*1 allele with a lower risk for oral cancer (67).

A Japanese study reported that the ADH3*2 allele enhances the risk for esophageal cancer, but after adjustment by ALDH2 and ADH2 genotypes, it showed no relationship between the ADH3 genotype and cancer risk (30). Haplotype analysis has revealed that the apparent effect of the ADH3*2 allele reflects its linkage with the ADH2*1 allele, which has a true effect on the cancer risk.

ALCOHOL METABOLISM IN THE UPPER AERODIGESTIVE TRACT

Poor dentition, inadequate oral hygiene and the use of mouthwash are other suspected risk factors for oral cancer. The largest investigation into the role of mouthwash was carried out in the USA, where mouthwash has been used regularly by over 40% of adults (68). The study revealed that the risk for oral or pharyngeal cancer was elevated with the duration and frequency of mouthwash use, but that significant association was limited to mouthwashes with an alcohol content of 25% or higher. Because few subjects reported swallowed mouthwash, the results indicate that the exposure of topical mucosal tissue to alcohol may be involved in oral carcinogenesis.

A growing body of evidence that acetaldehyde plays a pivotal role in the development of alcohol-related cancer highlights the possibility of topical ethanol oxidation by mucosal enzymes or bacteria in the lumen of the upper aerodigestive tract. Mucosal ADH and ALDH expression patterns and ethanol- and acetaldehyde-oxidizing abilities differ considerably along the alimentary tract (69). The esophagus and oral cavity are high in ADH activity and strongly express of ADH7 (60,70), which has a high Km and is very active in producing acetaldehyde upon exposure to a locally high dose of ethanol. The distribution of ADH7 is organ specific: it has been found in the mucosa of the oral cavity, esophagus and stomach, as well as in the cornea (69). The expression of ADH7 displays ethnic variability only in the stomach, being absent or markedly decreased in 70–80% of Japanese (71) and Chinese (69).

In individuals with the ALDH2*1/2*2 genotype, the salivary acetaldehyde levels after a moderate dose of alcohol are equal to nine times the blood levels and 2–3 times the salivary acetaldehyde levels in persons with ALDH2*1/2*1 genotype (72). Administration of 4-methylpyrazole, an ADH inhibitor, reduces the salivary acetaldehyde levels in those with ALDH2*1/2*2, but not in those with ALDH2*1/2*1 (73). Salivary acetaldehyde production decreases after treatment with the antiseptic chlorhexidine in vivo (74). Normal oral microflora also form acetaldehyde from ethanol and contribute to acetaldehyde levels in saliva (74,75). Smoking and heavy drinking (76) and poor oral hygiene (77) increase microbial salivary acetaldehyde production in vitro.

Alcoholic beverages themselves contain high levels of acetaldehyde. For example, after the dilution of beverages to 4% ethanol, acetaldehyde concentrations of 82 and 28 mM have been observed in whiskey and beer, respectively (78). Even in ALDH2*1/2*2 heterozygotes, the peak blood acetaldehyde was measured as only 23 μM after consumption of a moderate amount of alcohol (7).

Although overall trends suggest that all types of alcoholic beverages increase the risk for esophageal and oropharyngolaryngeal cancer, some epidemiological studies have shown stronger associations between these cancers and alcoholic beverages high in ethanol (1,30,79). These observations demonstrate not only the importance of the local effect of ethanol and its solvent action on tobacco and other carcinogens penetrating the mucosa but also the role of direct exposure to acetaldehyde as a carcinogenic ingredient in stronger alcoholic beverages. ALDH2 is expressed in most organs, including the stomach and the colon (69), but in neither the esophagus (60) nor the oral cavity (70), both of which appear to lack ALDH2 activity, expressing it extremely weakly, if at all. After exposure to acetaldehyde derived from systemic, mucosal, salivary or bacterial production and from alcoholic beverages per se, inefficient degradation of the acetaldehyde in the esophagus and oral cavity may enhance the chances for acetaldehyde-associated carcinogenesis. Comparison between the acetaldehyde concentrations in the esophagus and oral cavity and other organs would provide information crucial for the elucidation of this issue.

CANCER SCREENING IN HIGH-RISK JAPANESE POPULATIONS

The main purpose of genetic epidemiology in the field of cancer should include primary prevention, early detection and treatment of substance-induced cancers. The use of diagnostic and therapeutic technologies, such as endoscopy, vital staining and endoscopic mucosal resection for early digestive tract cancer, has become widespread in Japan. Therefore, the development of preventive efforts and screening programs based on these technologies is appropriate for use with high-risk Japanese populations.

At the National Institute on Alcoholism, Kurihama National Hospital, we routinely apply a regimented cancer-screening program, using endoscopy combined with oropharyngolaryngeal inspection and esophageal iodine staining in male alcoholic patients age 40 years or older (80). Between 1993 and 2000, systematic screening of 2672 male alcoholic patients showed cancer in 160 patients, an extremely high rate of 6.0%. Oropharyngolaryngeal squamous cell carcinoma was diagnosed in 33 of these patients (1.2%), esophageal squamous cell carcinoma in 112 (4.2%) and stomach adenocarcinoma in 38 (1.4%). Of the 112 with esophageal cancer, 22 were multiple-cancer patients who also had either oropharyngolaryngeal or stomach cancer or both. The rates of cancer detected via mass
screening by endoscopy in Japanese general populations had been reported as only 0.04% for esophageal cancer (81).

The esophageal iodine staining method influenced the high rate of esophageal cancer found in our screening. A large majority of the esophageal cancers detected were superficial: about 80% of the esophageal cancer patients were treated by endoscopic mucosal resection, alone or combined with irradiation. The detection rate and invasion depth of the esophageal cancers revealed by iodine staining are in sharp contrast to what has been shown by endoscopy only. In fact, when other investigators screened 1513 male Japanese alcoholics age 40 years or older by conventional endoscopy only, they detected only 10 patients with esophageal cancer (0.7%) and most of those cancers were at more advanced stages (82).

Mucosal biopsy specimens are taken from lesions that remain distinctly unstained by iodine when their greatest dimensions are ≥5 mm. We had often observed multiple iodine-unstained lesions in alcoholics, especially those with ALDH2*1/2*2, and were puzzled concerning which lesions to sample. In addressing this problem, we noticed the change of color in the unstained lesions. Most of the cancerous lesions were partially or totally tinged with light pink in the yellowish-white unstained areas about 2 min after iodine staining. We named this endoscopic finding ‘pink color (PC) sign.’ In a comparison of 112 dysplasia lesions and 138 mucosal cancer lesions, the PC sign showed high sensitivity (90%) and specificity (97%) in identifying cancer lesions. Thus, the PC sign is a useful endoscopic finding in the screening of high-risk drinkers (83).

A screening in which alcoholic patients identified their alcoholic beverage preferences (79) showed that stronger beverages (whiskey and shochu), in contrast to lighter beverage choices (sake and beer), increase the risk for oropharyngolaryngeal and esophageal cancers and for multiple cancers (ORs = 4.8, 3.2 and 10.5, respectively). That study (79) also showed that heavy smoking (≥50 pack-years) also increased the risk for those cancers (ORs = 5.1, 2.8 and 11.8, respectively).

Epidemiological studies have provided ample evidence that the combination of alcohol and tobacco has a striking synergistic effect in the development of esophageal and oropharyngolaryngeal cancers (1,84,85). A very large Japanese case-referent study involving 346 male cases with hypopharyngeal/esophageal cancer and 11,936 male cancer-free referents (84) demonstrated that drinkers (≥1.5 units/day; 1 unit = 22 g ethanol) who were also smokers (≥30 pack-years) had the highest risk (OR = 29.9), whereas the risks of drinking (≥1.5 units/day)/never smoking and smoking (≥30 pack-years)/never drinking were lower (ORs = 8.2 and 3.9, respectively). Indeed, the Japanese screening in which esophageal iodine staining was used in individuals who met high-risk criteria (men ≥55 years old, heavy drinking or heavy smoking) showed high detection rates (0.4–0.7%) for esophageal cancer (81).

New evidence concerning ADH2/ALDH2-related cancer susceptibility could renew interest in acetaldehyde as an important subject for future cancer research and it has special public health implications for East Asian populations. This knowledge emphasizes the importance of our development of simple screening tests for inactive ALDH2 on the basis of alcohol flushing and of utilizing the tests to predict cancer risk. If the screening tests can be applied reliably in both high-risk and general populations, they could serve as a powerful tool in cancer prevention and cancer screening.

ETHANOL PATCH TEST

The ethanol patch test, a cutaneous test for the flushing response, is used for the health education of youth, especially those who have never consumed alcohol, in Japan. The first large study reported its high sensitivity (93%) and specificity (94%) in identifying inactive ALDH2 (8), but four other large studies failed to show such high reliability (51,86–88). The 72% sensitivity and 71% specificity of the ethanol patch test were unsatisfactory, especially for men age 50 years or older, many of whom had a long history of drinking (86), suggesting that aging and acquired tolerance to acetaldehyde influence the test results. In addition, the ethanol patch test in persons with both ALDH2*1/2*2 and ADH2*1/2*1 usually shows false-negative results (51). Hence the test is less reliable than the flushing questionnaire in alcoholics, probably because of both acquired tolerance to acetaldehyde after long-term heavy exposure and the relatively high prevalence of ADH2*1/2*1 genotype.

FLUSHING QUESTIONNAIRE

Alcohol flushing is frequently observed in East Asians and generally inhibits them from drinking heavily. Intensive flushing after light drinking is mainly triggered by severe acetaldehydemia in those possessing the ALDH2*2 allele. In young general populations, the current status of flushing responses predicts an individual’s ALDH2 phenotype fairly well (87); however, alcohol flushing after light drinking diminishes in intensity in men with long or heavy drinking histories. Among Japanese ALDH2 heterozygotes, 77.0% of all men age 50 years or older (86), 70.1% of men with esophageal or oropharyngolaryngeal cancer (41) and 71.5% of alcoholic men with esophageal cancer (53) recalled ‘past always facial flushing’ after drinking a glass of beer, yet only 64.6, 25.4 and 8.2% of these three groups reported ‘current always facial flushing.’

These negative changes in the frequency of flushing responses suggest that individuals with long or heavy drinking histories develop tolerance to severe acetaldehydemia. For that reason, we designed a questionnaire to detect changes in flushing responses over time, by asking about both current and past flushing (86): (a) Do you flush in the face immediately after drinking a glass of beer: always, sometimes or never? (b) Did you flush in the face immediately after drinking a glass of beer in the first to second year after you started drinking: always, sometimes or never? In tests of the reliability of this questionnaire in detecting inactive ALDH2, blinded genotyping of 266 elderly Japanese male respondents age 50 years or older showed inactive ALDH2 in 94% of those who reported...
currently or formerly always flushing and in 48% of those who reported sometimes flushing, whereas 96% of the subjects who reported never flushing had active ALDH2 (86). When all three categories of flushing (current always, past always and sometimes) were considered to have inactive ALDH2, 96% of those with inactive ALDH2 and 87% of the total subjects were discriminated correctly. These results suggest the utility of this simple flushing questionnaire in daily practice and also in large-scale cohort studies and in activities aimed at preventing alcohol-related cancer in East Asians.

Despite the apparent promise of the flushing questionnaire, only 47.8% of the alcoholic esophageal cancer patients with the ‘at risk’ ALDH2*1/2*2/ADH2*1/2*1 genotype combination, compared with 92.3% of those with the ALDH2*1/2*2 genotype and the ADH2*2 allele, reported current or former alcohol flushing (53). Alcohol flushing may be triggered by an initially steep rise in blood or cutaneous acetaldehyde after drinking, but this dramatic change may not occur in persons with the much less active form of ADH2, in spite of the presence of inactive heterozygous ALDH2. Hence developing screening tests for inactive ALDH2 in the high-risk alcoholic population is also important.

CONCLUSION

Professional and public education about risky conditions connected with genetic and environmental factors are important components of a new strategic approach to the prevention of alcohol-related cancers in the esophagus and head and neck. The use of our simple tests to identify the inactive ALDH2 based on alcohol flushing responses will benefit many people who can identify their own cancer risks. Use of the ethanol patch test and the flushing questionnaire, and in the future a screening test for inactive ALDH2, could also help clinicians diagnose cancer earlier, by indicating individuals in the high-risk population who might benefit from endoscopic screening.

References


