Genetic polymorphisms of alcohol and aldehyde dehydrogenases, and drinking, smoking and diet in Japanese men with oral and pharyngeal squamous cell carcinoma

Takahiro Asakage 1, Akira Yokoyama 1, Tatsumasa Haneda 2, Mitsuou Yamazaki 3, Manabu Muto 4, Tetsuji Yokoyama 5, Hoichi Kato 6, Hiroyasu Igaki 1, Toshimasa Tsujimaki 7, Yoshiya Kumaiga 1, Masako Yokoyama 1, Tai Omori 8 and Hiroshi Watanabe 11

Department of Otolarlaryngology, University of Tokyo, Bunkyo-ku, Tokyo 113-8655, Japan, 1National Hospital Organization Kurihama Alcoholism Center, Yokosuka, Kanagawa 239-0841, Japan, 2Kamio Memorial Hospital, Chiyoda-ku, Tokyo 104-0045, Japan, 3Division of Head and Neck Surgery and 4Division of Digestive Endoscopy and Gastrointestinal Oncology, National Cancer Center Hospital East, Kashiwa, Chiba 277-8577, Japan, 5Department of Technology Assessment and Biostatistics, National Institute of Public Health, Wako, Saitama 351-0104, Japan, 6Surgery Division, National Cancer Center Hospital, Chuoku, Tokyo 104-0045, 7Department of Surgery, Osaka National Hospital, Osaka, Osaka 540-0006, Japan, 8Kumagai Satellite Clinic, Shinjuku-ku, Tokyo 160-0074, Japan, 9Mitsubishi Health and Welfare Foundation, Shinjuku-ku, Tokyo 160-0023, Japan, 10Departments of Surgery and Gastroenterology, Kawasaki Municipal Hospital, Kawasaki, Kanagawa 210-0013, Japan and 11Department of Surgery, School of Medicine, Keio University, Shinjuku-ku, Tokyo 160-8582, Japan

*To whom correspondence should be addressed.
Email: tasakage-tky@umin.ac.jp

The genetic polymorphisms of aldehyde dehydrogenase-2 (ALDH2), alcohol dehydrogenase-1B (ADH1B, previously called ADH2), and ADH1C (previously called ADH3) affect the metabolism of alcohol. The inactive ALDH2 encoded by ALDH2*1/*2 and the less-active ADH1B encoded by ADH1B*I/*I increase the risk of esophageal squamous cell carcinoma in East Asian drinkers. This case–control study involved 96 Japanese men with oral and pharyngeal squamous cell carcinoma (hypopharyngeal cancer in 43 patients and oral/oropharyngeal cancer in 53) and 642 cancer-free Japanese men. The risk of the cancers overall and of hypopharyngeal cancer was increased 3.61- and 10.08-fold, respectively, by ALDH2*1/*2 among moderate-to-heavy drinkers (9+ units/week; one unit = 22 g of ethanol), but the risk of oral/oropharyngeal cancer was not significantly affected by the ALDH2 genotype. The results obtained with a simple alcohol flushing questionnaire were essentially comparable with those obtained by ALDH2 genotyping. Among moderate-to-heavy drinkers, men with the less-active ADH1B*I/*I had a significantly higher risk of the cancers overall, of hypopharyngeal cancer, and of oral/oropharyngeal cancer (OR = 5.56, 7.21 and 4.24, respectively). In view of the linkage disequilibrium between ADH1B and ADH1C, the ADH1C genotype does not significantly affect cancer risk. The significant independent risk factors for oral and pharyngeal cancer overall among moderate-to-heavy drinkers were inactive ALDH2*1/*2, less-active ADH1B*I/*I, frequent drinking of strong alcohol beverages straight, smoking, and lower intake of green–yellow vegetables. Educating these risks for cancer of the upper aerodigestive tract could be a useful new strategic approach to the prevention of these cancers in Japanese.

Introduction

Epidemiologic studies have demonstrated that drinking alcoholic beverages is causally related to the development of squamous cell carcinoma in the oral cavity, pharynx and esophagus (1). The mutant alleles encoding an inactive subunit of aldehyde dehydrogenase-2 (ALDH2*2) and a super-active subunit of alcohol dehydrogenase-1B (ADH1B*2; previously called ADH2*2) are highly prevalent among Japanese (42 and 93%, respectively) (2). Although the ALDH2 genotype, but not the ADH1B genotype, determines an individual’s peak blood acetaldehyde concentration (3), inactive ALDH2 and super-active ADH1B generally inhibit East Asians from drinking heavily (4) and developing alcoholism (5) by causing acetaldehydemia (3) and alcohol flushing responses, which include facial flushing, tachycardia and drowsiness (4,6,7), and 13 and 69% of Japanese alcoholics have the ALDH2*1/*2 allele and ADH1B*2 allele, respectively (2).

Case–control studies of various Japanese and Chinese drinking populations have consistently shown the inactive form of ALDH2 encoded by the gene ALDH2*1/*2 to be a risk factor for esophageal squamous cell carcinoma (8–12). ADH1B*I/*I has also been demonstrated to enhance the risk for esophageal cancer in Japanese (8–10), Chinese (8,11), Thai (13) and Central Europe populations (14), and the combination of ALDH2*1/*2 and ADH1B*I/*I increased the risk of esophageal cancer in a multiplicative fashion (9–11).

It has been also consistently reported that ALDH2*1/*2 is a strong risk factor for synchronous and metachronous multiple cancers in the upper aerodigestive tract in both Japanese alcoholic (8,9,15) and general (16,17) populations. Acetaldehyde plays a critical role in field cancerization throughout the entire mucosal surface of the upper aerodigestive tract. However, four Japanese case–control studies investigating alcohol intake, ALDH2 genotype, and cancer risk in the oropharyngolarynx have reported conflicting patterns of association (9,18–20). In a study in which alcohol consumption was found not to be associated with increased risk of oral cancer, the ALDH2 genotype was also found to have no effect (18). Another study, in which alcohol consumption was found to be a risk factor for oral cancer, reported a relatively weak but significantly increased risk associated with ALDH2*1/*2 (19). In alcoholic populations, although the case sample sizes were small, ALDH2*1/*2 strongly increased the cancer risk at all anatomical subsites in the upper aerodigestive tract (9,20), and the combination of ALDH2*1/*2 and ADH1B*1/*I increased the risk of...
oropharyngolaryngeal cancer in a multiplicative fashion (9). A prospective follow-up study of Japanese alcoholic men with esophageal SCC showed that hypopharyngeal SCC, but not oral cavity SCC, developed significantly more frequently in inactive ALDH2 carriers than in active ALDH2 carriers (15). ADH2/ADH1B gene polymorphisms may act differently according to the level of alcohol exposure or anatomical subsite in the oropharyngolarynx. A large case–control study in Central Europe showed that ADH1B*1/*1 was a strong risk factor for the upper aerodigestive tract cancer as a whole, although the results of subsite analyses for oral cancer and pharyngeal cancer did not reach significance (14).

The enzyme encoded by the ADH1C*2 allele (previously called ADH3*1) produces acetaldehyde twice as fast as enzyme encoded by the ADH1C*2 allele (21). It has been reported that in East Asians the ADH1C*2 allele increases the risk of esophageal cancer (10) as well as of alcoholism (5) through linkage with the ADH1B*1 allele, which is a true risk factor for both.

The aim of this study was to define the individual and combined roles of the ADH2, ADH1B, and ADH1C gene polymorphisms, drinking, smoking and diet in the risk of oral and pharyngeal cancer in Japanese men.

Materials and methods

The case participants in this study were 96 Japanese males with primary oral or pharyngeal squamous cell carcinoma (hypopharyngeal cancer in 43 patients and oral/oropharyngeal cancer in 53 patients) treated at the National Cancer Center Hospital East, the National Cancer Center Hospital, Osaka National Hospital, or Kawasaki Municipal Hospital. All were registered between September 2000 and December 2003 and met the following inclusion criteria: (i) age between 40 and 79 years and (ii) oral or pharyngeal (but not nasopharyngeal) squamous cell carcinoma newly diagnosed by histologically within 3 years before registration. Of the 96 cancer patients 18 were included in our earlier case–control study of esophageal squamous cell carcinoma, because they had squamous cell carcinomas simultaneously in both the oral cavity/pharynx and esophagus. The controls consisted of cancer-free men who received annual health checkups at two Tokyo clinics between September 2000 and December 2001. Most of the controls were ordinary residents or workers who lived in Tokyo or neighboring areas, who had registered for annual health checkups at the clinics. Potentially eligible subjects were sent letters of invitation and then contacted at the clinics. Of the eligible subjects contacted 86% were enrolled in the study, yielding a total of 642 controls.

The ethics committee of each collaborating institution reviewed and approved the proposed study, and each of the participants gave his informed consent.

Each participant independently completed a structured questionnaire concerning his drinking, smoking and dietary habits; and those with cancer were instructed to report their habits before they were diagnosed with cancer. Each was asked to classify himself as a never drinker, a current drinker or an ex-drinker, and to report alcohol intake as the frequency of consumption and usual amount(s) and type(s) of alcoholic beverage(s) consumed. Frequency of consumption was divided into five categories: never; 1–3 days/month; 1–2 days/week; 3–4 days/week; and ≥5 days/week. Weekly frequency of drinking was calculated according to a score assigned to each frequency category (0, 0.5, 1.5, 3.5 and 6, respectively). Weekly ethanol consumption was calculated by using a standard conversion table for alcoholic beverages, in which 180 ml sake was considered to be equivalent to 22 g of ethanol; 180 ml shochu to 36 g; 633 ml beer to 25 g; 30 ml whiskey to 10 g; and 120 ml wine to 11 g. Weekly alcohol intake was then converted into the number of units per week by dividing the total ethanol consumption in grams by 22 g per unit (the ethanol content of one serving of sake). The subjects were classified as non-drinkers, ex-drinkers, or current drinkers who consumed 1–8.9 units/week (light drinkers), 9–17.9 units/week (moderate drinkers), or 18+ units/week (heavy drinkers). The questionnaire also asked about the frequency of drinking strong alcoholic beverages straight. In addition, the subjects reported on smoking and the frequency of intake of green and yellow vegetables and fruit, as well as their preference for hot food and drinks. Each subject was asked to classify himself as a never smoker, a current smoker, or an ex-smoker and to report tobacco consumption as the duration of consumption and usual number of cigarettes consumed, and the number of pack-years (number of cigarettes/20 per day × number of years of smoking) was then calculated. The subjects were also asked to indicate their frequency of intake of green and yellow vegetables and fruit by choosing one of five replies: seldom; 1–2 days/month; 1–2 days/week; 3–4 days/week; almost every day.

Each participant was also asked to answer the following simple questionnaire concerning flushing response to alcohol (7): (i) do you have a tendency to flush in the face immediately after drinking a glass of beer (yes, no or unknown)? (ii) 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 (yes, no, or unknown)? The designation ‘current flushing’ was applied to individuals who answered ‘yes’ to question (i), ‘former flushing’ to those who answered ‘no’ or ‘unknown’ to question (i) and ‘yes’ to question (ii). The remaining subjects were classified as ‘never flushing’. Current or former flushing individuals were assumed to have inactive ALDH2 (7).

Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) methods or a PCR method were performed on lymphocyte DNA samples from all participants without any knowledge of their cancer status to determine their ALDH2 (9,22), ADH1B (23) and ADH1C (24) genotypes.

Fisher’s exact test and the Mantel–Haenszel chi-squared test were used to compare group data. Associations between genotypes, and other potential risk factors, and cancer of the oral cavity, oropharynx and hypo-pharynx are expressed as odds ratio (OR) and 95% confidence interval (CI) adjusted for the effect of several possible confounders by using a multiple logistic regression model and the STEPWISE method. Difference in the strength of associations between genotypes and risk of cancer was compared between the alcohol drinking groups (never/rare-to-light versus moderate-to-heavy), and statistically tested by using the interaction term ‘alcohol drinking × genotype.’ The exact logistic regression model was also used in a subgroup analysis when the sample size was very small. Since the amount of alcohol drinking could differ between genotypes even within the same drinking category (i.e. never/rare-to-light category and moderate-to-heavy category), the ORs were also adjusted for ‘subcategory of alcohol drinking’ that represented the difference between never/rare and light drinkers and moderate and heavy drinkers. ‘Subcategory of alcohol drinking’ was coded as 0 for never/rare and moderate drinkers, and 1 for light and heavy drinkers. These analyses were done with the SAS statistical package (version 8.2; SAS Institute, Cary, NC). The maximum likelihood estimates of the linkage disequilibrium statistic D between ADH1B and ADH1C gene polymorphisms were obtained by using the ASSOCIATE program version 2.36 provided by Dr Ott, J. (http://linkage.rockefeller.edu/ott/linkutil.htm).

Results

The basic characteristics of the oral and pharyngeal cancer cases and cancer-free controls are shown in Table I. The cancer cases were significantly older than the controls. After adjustment for age, the cases reported more drinking, more drinking of strong beverages, more smoking, lower intake of green and yellow vegetables and lower intake of fruit than the controls. Although an association between intake of hot food and drinks and hypopharyngeal cancer was suggested, the suggestion of an association disappeared after adjusted for alcohol drinking, and therefore was omitted from subsequent analyses.

Table II shows the distribution of ALDH2, ADH1B and ADH1C genotypes and the results of the alcohol flushing questionnaire. All genotypes were in Hardy–Weinberg equilibrium (HWE) in the controls. The prevalence of the ALDH2 (2*1/*2) inactive heterozygotes was significantly higher in the cancer cases overall and in the hypopharyngeal cancer cases treated in the controls. The prevalence of the ADH1B (1B*1/*1) less-active homozygotes was significantly higher in the overall, hypopharyngeal, and oral/oropharyngeal cancer groups than in the controls. The prevalence of the ADH1C (IC*1 allele carrier) less-active...
forms was significantly higher in the overall and oral/oropharyngeal cancer cases than that in the controls. Significant linkage disequilibrium was detected between the ADH1B and ADH1C gene polymorphisms among both the controls \((D = 0.022, \chi^2 = 40.0, df = 1, P < 0.0001)\) and the cancer patients (overall: \(D = 0.042, \chi^2 = 13.9, df = 1, P = 0.0002\); hypo-pharynx: \(D = 0.037, \chi^2 = 9.03, df = 1, P = 0.0027\); oral cavity/oropharynx: \(D = 0.047, \chi^2 = 7.03, df = 1, P = 0.0081)\).

The frequency distributions and ORs for each of the combinations between alcohol drinking and ALDH2 genotype, ADH1B genotype, ADH1C genotype, and alcohol flushing are summarized in Tables III and IV, where the ORs have been adjusted for age, ‘subcategory of alcohol drinking’ (see statistical analysis section), drinking strong alcoholic beverages, smoking and green–yellow vegetable intake. The risk of the cancers overall and hypopharyngeal cancer was 8.26- and 9.39-fold, respectively, higher in moderate-to-heavy drinkers with inactive \(ALDH2^*1/*2\) than in the never/rare-to-light drinkers with the active \(ALDH2^*1/*1\) genotype. When the ORs were compared within each alcohol drinking category, the risk of the cancers overall and of hypopharyngeal cancer associated with \(ALDH2^*1/*2\) versus \(2^*1/*1\) was 3.61- and 10.08-fold greater among moderate-to-heavy drinkers, whereas no significant increase in risk was observed with \(ALDH2^*1/*2\) versus \(2^*1/*1\) among never/rare-to-light drinkers, clearly indicating a so-called gene–environment interaction \((P\) for interaction = 0.002 and 0.001, respectively; not shown in the table). The increased risk of moderate-to-heavy drinkers with \(ALDH2^*1/*2\) was seen in every smoking category, although the confidence interval was wide because of the small sample size (data not shown). For oral/oropharyngeal cancer, the difference in risk between \(ALDH2^*1/*2\) and \(2^*1/*1\) did not reach significance in the moderate-to-heavy drinkers \([OR (95\% CI) = 1.80 (0.83–3.89)]\).

The results for alcohol flushing were essentially comparable with those for \(ALDH2\) genotype. The risk of the cancers

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### Table I. Distribution of cases and controls by age, drinking, smoking and dietary habits

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls ((n = 642)) (%)</th>
<th>Oral and pharynx ((n = 96)) (%)</th>
<th>Hypo-pharynx ((n = 43)) (%)</th>
<th>Oral cavity/oropharynx ((n = 53)) (%)</th>
<th>(P) versus controls(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–49</td>
<td>6.4</td>
<td>8.3</td>
<td>4.7</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>50–59</td>
<td>47.8</td>
<td>36.5</td>
<td>32.6</td>
<td>39.6</td>
<td></td>
</tr>
<tr>
<td>60–69</td>
<td>41.1</td>
<td>39.6</td>
<td>48.8</td>
<td>32.1</td>
<td></td>
</tr>
<tr>
<td>70–79</td>
<td>4.7</td>
<td>15.6</td>
<td>14.0</td>
<td>17.0</td>
<td>0.0008</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>58.8 ± 7.1</td>
<td>60.9 ± 8.0</td>
<td>62.5 ± 7.3</td>
<td>59.6 ± 8.3</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Alcohol drinking(^b)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never/rare</td>
<td>22.6</td>
<td>5.2</td>
<td>4.7</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>31.6</td>
<td>13.5</td>
<td>14.0</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>27.0</td>
<td>29.2</td>
<td>32.6</td>
<td>26.4</td>
<td></td>
</tr>
<tr>
<td>Heavy</td>
<td>16.4</td>
<td>40.6</td>
<td>&lt;.0001</td>
<td>37.2</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Ex-drinker</td>
<td>2.5</td>
<td>11.5</td>
<td>(&lt;.0001)</td>
<td>11.6</td>
<td>(&lt;.0001)</td>
</tr>
<tr>
<td><strong>Strong alcoholic beverages</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequently</td>
<td>2.2</td>
<td>14.6</td>
<td>18.6</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>Sometimes</td>
<td>15.1</td>
<td>22.9</td>
<td>&lt;.0001</td>
<td>32.6</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Never</td>
<td>82.7</td>
<td>62.5</td>
<td>(&lt;.0001)</td>
<td>48.8</td>
<td>(&lt;.0001)</td>
</tr>
<tr>
<td><strong>Smoking (pack years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>27.9</td>
<td>5.2</td>
<td>4.7</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>27.4</td>
<td>16.7</td>
<td>&lt;.0001</td>
<td>11.6</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>30+</td>
<td>44.7</td>
<td>78.1</td>
<td>(&lt;.0001)</td>
<td>83.7</td>
<td>(&lt;.0001)</td>
</tr>
<tr>
<td><strong>Green-yellow vegetables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seldom</td>
<td>0.6</td>
<td>3.1</td>
<td>2.3</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>1–2 days/month</td>
<td>3.3</td>
<td>8.3</td>
<td>7.0</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>1–2 days/week</td>
<td>24.6</td>
<td>30.2</td>
<td>30.2</td>
<td>30.2</td>
<td></td>
</tr>
<tr>
<td>3–4 days/week</td>
<td>33.5</td>
<td>35.4</td>
<td>0.0002</td>
<td>39.5</td>
<td>0.014</td>
</tr>
<tr>
<td>Almost every day</td>
<td>38.0</td>
<td>22.9</td>
<td>(&lt;.0001)</td>
<td>20.9</td>
<td>(0.005)</td>
</tr>
<tr>
<td><strong>Fruit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seldom</td>
<td>4.5</td>
<td>13.5</td>
<td>14.0</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>1–2 days/month</td>
<td>8.3</td>
<td>18.8</td>
<td>16.3</td>
<td>20.8</td>
<td></td>
</tr>
<tr>
<td>1–2 days/week</td>
<td>27.6</td>
<td>26.0</td>
<td>25.6</td>
<td>26.4</td>
<td></td>
</tr>
<tr>
<td>3–4 days/week</td>
<td>23.2</td>
<td>20.8</td>
<td>&lt;.0001</td>
<td>23.3</td>
<td>0.002</td>
</tr>
<tr>
<td>Almost every day</td>
<td>36.5</td>
<td>20.8</td>
<td>(&lt;.0001)</td>
<td>20.9</td>
<td>(0.001)</td>
</tr>
<tr>
<td><strong>Hot food or drinks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Like very much /somewhat</td>
<td>25.4</td>
<td>27.1</td>
<td>0.71</td>
<td>37.2</td>
<td>0.11</td>
</tr>
<tr>
<td>Others</td>
<td>74.6</td>
<td>72.9</td>
<td>(0.53)</td>
<td>62.8</td>
<td>(0.066)</td>
</tr>
</tbody>
</table>

\(\*P\)-values are for homogeneity for age and alcohol drinking, and for trend for other variables. \(P\)-values in the parenthesis are adjusted for age by the Cochran–Mantel–Haenszel method.

\(\text{Never/rare, <1 unit/week; light, 1–8.9 units/week; moderate, 9–17.9 units/week; heavy, 18+ units/week; where 1 unit = 22 g of ethanol.}\)
calculated among moderate-to-heavy drinkers (Table V). The to the combinations of (OR oropharyngeal cancers among moderate-to-heavy drinkers significantly increased the risk of the overall and oral/oropharyngeal cancers: (OR ¼ 21.96-fold higher, respectively, than in never/rare-to-light drinkers who had never experienced flushing. When compared within each alcohol drinking category, the risk of the cancers overall and risk of hypopharyngeal cancer associated with current/former versus never flushing were 3.16- and 6.10-fold, respectively, higher among moderate-to-heavy drinkers, whereas no significant difference in risk was observed between current/former flushing versus never flushing among the never/rare-to-light drinkers. The results for the ADH1B genotype showed that only among moderate-to-heavy drinkers, the less-active ADH1B*1/*1 significantly increased the risk of the cancers overall, hypopharyngeal cancer and oral/oropharyngeal cancer (OR = 5.56, 7.21 and 4.24, respectively). Thus, the risk of these cancers in moderate-to-heavy drinkers with ADH1B*1/*1 was markedly higher, i.e. 26.40, 30.38 and 21.96-fold higher, respectively, than in never/rare-to-light drinkers with super-active ADH1B (1B*1/*2 + 1B*2/*2). The less-active form of ADH1C (1C*1/*1 + 1C*2/*2) significantly increased the risk of the overall and oral/oropharyngeal cancers among moderate-to-heavy drinkers (OR = 3.18 and 4.32, respectively). Because of the small sample size, the ORs of the ex-drinkers were very unstable and hence difficult to interpret. To examine the independent risks of cancers according to ADH1B and ADH1C gene polymorphisms, which were in highly significant linkage disequilibrium, the ORs according to the combinations of ADH1B and ADH1C genotypes were calculated among moderate-to-heavy drinkers (Table V). The risks of the cancers overall, hypopharyngeal cancer and oral/oropharyngeal cancer were significantly higher in men with ADH1B*1/*1 after adjustment for the ADH1C genotype. By contrast, the risks of these cancers were not unrelated to ADH1C genotype after adjustment for the ADH1B genotype. The multivariate ORs of cancer among moderate-to-heavy drinkers are summarized in Table VI, where adjustments were always made for age and alcohol drinking (= subcategory of alcohol drinking: heavy versus moderate) and other variables were selected by the STEPWISE method after exclusion of the ADH1C genotype because of its highly significant linkage disequilibrium with the ADH1B genotype. The significant independent risk factors for the cancers overall were inactive ALDH2*1/*2, less-active ADH1B*1/*1, frequent drinking of strong alcoholic beverages, smoking and lower intake of green–yellow vegetables. When analyzed separately for hypopharyngeal and oral/oropharyngeal cancers using relatively small sample size, smoking was not selected as a significant factor for hypopharyngeal cancer although the OR was fairly increased (OR ¼ 2.75, P = 0.081); green and yellow vegetables were not selected as significant factors for oral/oropharyngeal cancer although the OR was notably decreased (OR ¼ 0.28, P = 0.056); ALDH2 genotype was not related to the risk of oral/oropharyngeal cancer. Discussion The results of this study demonstrate that inactive ALDH2*1/*2 and less-active ADH1B*1/*1 are independent and significant risk factors for oral and pharyngeal cancer in

### Table II. Distribution of cases and controls by ALDH2, ADH1BP and ADH1C genotypes and alcohol flushing

<table>
<thead>
<tr>
<th>Genotype/flushing</th>
<th>Controls (n = 642) (%)</th>
<th>Cases of cancer</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oral and pharynx (n = 96) (%)</td>
<td>P, versus controls*</td>
<td>Hypo-pharynx (n = 43) (%)</td>
<td>P, versus controls*</td>
<td>Oral cavity/oropharynx (n = 53) (%)</td>
</tr>
<tr>
<td><strong>ALDH2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*1</td>
<td>53.7</td>
<td>46.9</td>
<td>30.2</td>
<td>60.4</td>
<td></td>
</tr>
<tr>
<td>*1/*2</td>
<td>39.4</td>
<td>53.1</td>
<td>0.038</td>
<td>69.8</td>
<td>0.0003</td>
</tr>
<tr>
<td>*2/*2</td>
<td>6.9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>(0.0007)</td>
</tr>
<tr>
<td>P for HWEb</td>
<td>0.84</td>
<td>0.0001</td>
<td>0.0004</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td><strong>ADH1B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*1</td>
<td>4.8</td>
<td>19.8</td>
<td>25.6</td>
<td>15.1</td>
<td></td>
</tr>
<tr>
<td>*1/*2</td>
<td>34.7</td>
<td>29.2</td>
<td>&lt;.0001</td>
<td>23.3</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>*2/*2</td>
<td>60.4</td>
<td>51.0</td>
<td>&lt;.0001</td>
<td>51.2</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>P for HWEb</td>
<td>1.00</td>
<td>0.0006</td>
<td>0.0011</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td><strong>ADH1C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*1</td>
<td>91.1</td>
<td>83.3</td>
<td>88.4</td>
<td>79.3</td>
<td></td>
</tr>
<tr>
<td>*1/*2</td>
<td>8.7</td>
<td>16.7</td>
<td>0.047</td>
<td>11.6</td>
<td>0.88</td>
</tr>
<tr>
<td>*2/*2</td>
<td>0.2</td>
<td>0.0</td>
<td>(0.022)</td>
<td>0.0</td>
<td>(0.66)</td>
</tr>
<tr>
<td>P for HWEb</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Alcohol flushing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current flushing</td>
<td>37.4</td>
<td>33.3</td>
<td>44.2</td>
<td>24.5</td>
<td></td>
</tr>
<tr>
<td>Former flushing</td>
<td>10.2</td>
<td>14.6</td>
<td>0.40</td>
<td>16.3</td>
<td>0.21</td>
</tr>
<tr>
<td>Never flushing</td>
<td>52.4</td>
<td>52.1</td>
<td>(0.23)</td>
<td>39.5</td>
<td>(0.23)</td>
</tr>
</tbody>
</table>

*P-values are for homogeneity between cases and controls. P-values in the parenthesis are adjusted for age by the Cochran–Mantel-Haenszel method.

**Significant linkage disequilibrium was detected between the ADH1B and ADH1C gene polymorphisms (controls: D = 0.022, χ² = 40.0, df = 1, P < 0.0001; oral and pharyngeal cancers: D = 0.042, χ² = 13.9, df = 1, P = 0.0002; hypo-pharynx cancers: D = 0.037, χ² = 9.03, df = 1, P = 0.0027; oral cavity/oropharyngial cancers: D = 0.047, χ² = 7.03, df = 1, P = 0.0081).**

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male Japanese moderate-to-heavy drinkers, and these findings are consistent with the results of previous studies of esophageal cancer in East Asian drinkers (9,10,11) and oropharyngolaryngeal cancer in Japanese alcoholics (9). This study is the first to link the genotype combination with the risk of oral and pharyngeal cancer in a general population. Individuals with inactive ALDH2 are incapable of rapidly eliminating acetaldehyde after drinking ethanol, and the carcinogenicity of acetaldehyde has been adequately demonstrated in experimental animals (24). Acetaldehyde has been found to interact covalently with DNA to form DNA adducts in humans, and DNA adduct levels have been reported to be much higher in alcoholics than in non-alcoholics (25). Higher levels of acetaldehyde-derived DNA adducts have recently been demonstrated in Japanese alcoholics with inactive ALDH2 than with active ALDH2 (26). Thus it is reasonable to speculate that the high risk for oral and pharyngeal cancer in persons with inactive ALDH2 is the consequence of repeated exposure to acetaldehyde.

Another intriguing finding is that ADH1B*1/*1 was associated with increased risk of both hypopharyngeal cancer and oral/oropharyngeal cancer, while ALDH2 was associated with high risk of hypopharyngeal cancer alone. Several explanations for the subsite differences are possible. First, the very small sample size of the cancer cases in this preliminary study may have hampered precise risk assessment among the subgroups. Second, the mechanisms of carcinogenesis differ among anatomically different sites, and the effect of ALDH2 on oral/oropharyngeal carcinogenesis is less important than other risk factors, such as direct exposure to ethanol, strong alcoholic beverages and tobacco smoke, poor dentition and inadequate oral hygiene. Third, ALDH2 activity in the tongue, gingival tissues (27) and esophagus (28) is extremely weak, if present at all. Subsite differences in the patterns of expression of ADH, ALDH and oral/pharyngeal cancer

### Table III. Odds ratios for the combinations of ALDH2, ADH1B and ADH1C genotypes and alcohol flushing with amount of alcohol drinking, adjusted for other main risk factors

<table>
<thead>
<tr>
<th>Alcohol drinking</th>
<th>Genotype/flushing</th>
<th>Controls (n = 642) (%)</th>
<th>Cases of cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n = 96)</td>
<td>Adjusted-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(%)</td>
<td>OR* (95% CI)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ALDH2</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Never/rare-to-light</td>
<td>*1/*1</td>
<td>18.4</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>*1/*2</td>
<td>29.0</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>*2/*2</td>
<td>6.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Moderate-to-heavy</td>
<td>*1/*1</td>
<td>33.6</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>*1/*2</td>
<td>9.7</td>
<td>36.5</td>
</tr>
<tr>
<td></td>
<td>Ex-drinker *1/*1</td>
<td>1.7</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>*1/*2</td>
<td>0.8</td>
<td>7.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ADH1B</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Never/rare-to-light</td>
<td>*1/*2 + *2/*2</td>
<td>51.6</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td>*1/*1</td>
<td>2.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Moderate-to-heavy</td>
<td>*1/*2 + *2/*2</td>
<td>48.3</td>
<td>53.1</td>
</tr>
<tr>
<td></td>
<td>*1/*1</td>
<td>4.0</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>Ex-drinker *1/*2</td>
<td>2.3</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>*1/*1</td>
<td>0.2</td>
<td>2.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ADH1C</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Never/rare-to-light</td>
<td>*1/*1</td>
<td>49.4</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>*1/*2 + *2/*2</td>
<td>4.8</td>
<td>3.1</td>
</tr>
<tr>
<td>Moderate-to-heavy</td>
<td>*1/*1</td>
<td>40.0</td>
<td>57.3</td>
</tr>
<tr>
<td></td>
<td>*1/*2 + *2/*2</td>
<td>3.3</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>Ex-drinker *1/*1</td>
<td>1.7</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>*1/*2 + *2/*2</td>
<td>0.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alcohol flushing</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Never/rare-to-light</td>
<td>Non-flushing</td>
<td>17.7</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>Flushing</td>
<td>36.8</td>
<td>9.4</td>
</tr>
<tr>
<td>Moderate-to-heavy</td>
<td>Non-flushing</td>
<td>32.9</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>Flushing</td>
<td>10.7</td>
<td>36.5</td>
</tr>
<tr>
<td></td>
<td>Ex-drinker Non-flushing</td>
<td>1.0</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>Flushing</td>
<td>1.0</td>
<td>5.2</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; NC, not calculable.

*ORs were calculated separately in each model of ALDH2, ADH1B and ADH1C genotype, or alcohol flushing, adjusted for strong alcoholic beverages, smoking, green–yellow vegetables (selected by STEPWISE method with P < 0.05 for entry and staying) and subcategory of alcohol drinking (see statistical analysis section) and age (forced to enter).%

*Adjusted ORs of ALDH2*1/*2 versus 2*1/*1, ADH1B*1/*1 versus 2*1/*2 + 2*2/*2 and flushing versus non-flushing in each drinking category.
Table IV. Site-specific odds ratios for the combinations of ALDH2 and ADH1B genotypes and alcohol flushing with amount of alcohol drinking, adjusted for other main risk factors

<table>
<thead>
<tr>
<th>Alcohol drinking</th>
<th>Genotype/flushing</th>
<th>Controls (n = 642) (%)</th>
<th>Cases of cancer</th>
<th>Oral cavity/oropharynx (n = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n = 43) (%)</td>
<td>Adjusted-OR(^a) (95% CI)</td>
<td>Adjusted-OR(^b) (95% CI)</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td></td>
<td>OR</td>
<td></td>
<td>OR</td>
</tr>
<tr>
<td>ALDH2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never/rare-to-light</td>
<td>*1/*1</td>
<td>18.4</td>
<td>9.3</td>
<td>1 Referent</td>
</tr>
<tr>
<td></td>
<td>*1/*2</td>
<td>29.0</td>
<td>9.3</td>
<td>0.51 (0.12–2.27)</td>
</tr>
<tr>
<td></td>
<td>*2/*2</td>
<td>6.9</td>
<td>0.0</td>
<td>0.00 (NC)</td>
</tr>
<tr>
<td>Moderate-heavy</td>
<td>*1/*1</td>
<td>33.6</td>
<td>20.9</td>
<td>0.93 (0.23–3.71)</td>
</tr>
<tr>
<td>Ex-drinker</td>
<td>*1/*2</td>
<td>9.7</td>
<td>48.8</td>
<td>9.39 (2.64–33.40)</td>
</tr>
<tr>
<td></td>
<td>*1/*1</td>
<td>1.7</td>
<td>0.0</td>
<td>0.00 (NC)</td>
</tr>
<tr>
<td></td>
<td>*1/*2</td>
<td>0.8</td>
<td>11.6</td>
<td>40.62 (5.81–284.0)</td>
</tr>
<tr>
<td><strong>ADH1B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never/rare-to-light</td>
<td>*1/*2 + *2/*2</td>
<td>51.6</td>
<td>16.3</td>
<td>1 Referent</td>
</tr>
<tr>
<td></td>
<td>*1/*1</td>
<td>2.7</td>
<td>2.3</td>
<td>2.68 (0.24–29.39)</td>
</tr>
<tr>
<td>Moderate-heavy</td>
<td>*1/*2 + *2/*2</td>
<td>41.3</td>
<td>48.8</td>
<td>4.22 (1.59–11.18)</td>
</tr>
<tr>
<td>Ex-drinker</td>
<td>*1/*2 + *2/*2</td>
<td>2.0</td>
<td>20.9</td>
<td>30.38 (8.08–114.2)</td>
</tr>
<tr>
<td></td>
<td>*1/*1</td>
<td>2.3</td>
<td>9.3</td>
<td>16.37 (3.26–82.12)</td>
</tr>
<tr>
<td></td>
<td>*1/*2 + *2/*2</td>
<td>0.2</td>
<td>2.3</td>
<td>120.56 (4.98–999)</td>
</tr>
<tr>
<td><strong>ADH1C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never/rare-to-light</td>
<td>*1/*1</td>
<td>49.4</td>
<td>16.3</td>
<td>1 Referent</td>
</tr>
<tr>
<td></td>
<td>*1/*2 + *2/*2</td>
<td>4.8</td>
<td>2.3</td>
<td>1.61 (0.17–15.04)</td>
</tr>
<tr>
<td>Moderate-heavy</td>
<td>*1/*1</td>
<td>40.0</td>
<td>60.5</td>
<td>4.97 (1.85–13.33)</td>
</tr>
<tr>
<td>Ex-drinker</td>
<td>*1/*2 + *2/*2</td>
<td>3.3</td>
<td>9.3</td>
<td>13.90 (2.98–64.93)</td>
</tr>
<tr>
<td></td>
<td>*1/*1</td>
<td>1.7</td>
<td>11.6</td>
<td>44.52 (9.07–218.5)</td>
</tr>
<tr>
<td></td>
<td>*1/*2 + *2/*2</td>
<td>0.8</td>
<td>0.0</td>
<td>0.00 (NC)</td>
</tr>
<tr>
<td>Alcohol flushing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never/rare-to-light</td>
<td>Non-flushing</td>
<td>17.7</td>
<td>9.3</td>
<td>1 Referent</td>
</tr>
<tr>
<td></td>
<td>Flushing</td>
<td>36.8</td>
<td>9.3</td>
<td>0.40 (0.09–1.79)</td>
</tr>
<tr>
<td>Moderate-heavy</td>
<td>Non-flushing</td>
<td>32.9</td>
<td>23.3</td>
<td>1.17 (0.30–4.52)</td>
</tr>
<tr>
<td>Ex-drinker</td>
<td>Flushing</td>
<td>10.7</td>
<td>46.5</td>
<td>7.11 (1.99–25.38)</td>
</tr>
<tr>
<td></td>
<td>Non-flushing</td>
<td>1.0</td>
<td>2.3</td>
<td>4.58 (0.35–59.52)</td>
</tr>
<tr>
<td></td>
<td>Flushing</td>
<td>1.0</td>
<td>9.3</td>
<td>23.90 (3.19–178.8)</td>
</tr>
</tbody>
</table>

OR: odds ratio; CI: confidence interval; NC: not calculable.
\(^a\)ORs were calculated separately in each model of ALDH2 genotype, ADH1B genotype, or alcohol flushing, adjusted for strong alcoholic beverages, smoking, green–yellow vegetables (selected by STEPWISE method with \(P < 0.05\) for entry and staying) and subcategory of alcohol drinking (see statistical analysis section) and age (forced to enter).
\(^b\)Adjusted ORs of ALDH2*1/*2 versus *2/*1, ADH1B*1/*1 versus *1/*2 + *2/*2, and flushing versus non-flushing in each drinking category.
Table V. Odds ratios for the combinations of ADH1B and ADH1C genotypes among moderate-to-heavy drinkers

<table>
<thead>
<tr>
<th>Combinations of (or adjustment for) ADH1B and ADH1C genotypes</th>
<th>Controls</th>
<th>Oral cavity/oropharynx</th>
<th>Oral and pharynx</th>
<th>Hypopharynx</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>OR (95%CI)</td>
<td>n</td>
<td>OR (95%CI)</td>
</tr>
<tr>
<td>1/C3 or 2/C3</td>
<td></td>
<td></td>
<td>1/C3 or 2/C3</td>
<td></td>
</tr>
<tr>
<td>ADH1B 1/1 or 1/2</td>
<td>25</td>
<td>1.00 (Referent)</td>
<td>46</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>ADH1B 1/2 or 1/2</td>
<td>5</td>
<td>1.00 (Referent)</td>
<td>9</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>ADH1B 1/1 or 2/2</td>
<td>25</td>
<td>1.00 (Referent)</td>
<td>46</td>
<td>1.00 (Referent)</td>
</tr>
</tbody>
</table>

Odds ratios and 95% confidence intervals were estimated by the exact logistic regression adjusted for age (+5 years). 

There are unique mechanisms of topical acetaldehyde production from local ethanol in the upper aerodigestive tract. An alcohol-challenge test showed that acetaldehyde levels in saliva are 10–20 times higher than those in blood, and normal oral microflora form acetaldehyde from ethanol and make a large contribution to acetaldehyde levels in saliva (31). The salivary acetaldehyde levels of individuals with inactive ALDH2 are higher than those of individuals with active ALDH2 after ethanol ingestion (32). The additional salivary acetaldehyde in those with inactive ALDH2 is at least in part derived from the salivary glands (32). Chronic alcohol consumption leads to induction of cytochrome P-4502E1, which metabolizes ethanol to acetaldehyde and increases activation of the nitrosamines present in tobacco smoke into their ultimate carcinogens (33). Ethanol-induced P-4502E1 has been demonstrated in the oropharyngeal mucosa of alcoholics with oropharyngeal cancer (34). High-Km ADH4 (previously called ADH7) is strongly expressed in the upper aerodigestive tract, and ADH4 is active in producing acetaldehyde upon exposure to a locally high dose of ethanol (27,28). Although immunohistochemistry with ALDH2-antibody has demonstrated an expression of ALDH2 in the esophagus (35), the ALDH2 activity in the upper aerodigestive tract is extremely weak, if present at all (27,28). Because of the high local production of acetaldehyde as a result of prolonged exposure of the upper aerodigestive tract to ethanol in moderate-to-heavy drinkers with less-active ADH1B, inefficient degradation of acetaldehyde by ALDH2 in the mucosa and/or salivary glands may increase local accumulation acetaldehyde.

There are other possible explanations for the effects of ADH1B on carcinogenesis in the oral cavity and pharynx. Individuals with both the ADH2*1/*1 genotype and ADH1B*1/*1 genotype tend not to experience alcohol flushing (4,6,7), and diminished intensity of the aversive flushing response has been found to be positively associated with daily alcohol consumption among ALDH2 heterozygotes (7). Alcoholics with the ADH1B*1/*1 genotype tend to have experienced binge drinking and the withdrawal syndrome earlier in life than those with other genotypes (36). ADH1B*1/*1 may influence a behavior of episodic heavy drinking in non-alcoholic drinkers, although we have not yet investigated possible effect of irregular heavy drinking patterns on cancer risk.

ADH1C gene polymorphism may be the rate-limiting factor in acetaldehyde metabolism among non-Asian populations, in which the prevalence of the ALDH2*2 allele and ADH1B*2 allele is very low (21). Although the results of a large pooled analysis of ADH1C and head and neck cancer in Europeans were negative (37), several studies have shown that individuals with the more-active ADH1C*1 allele are at greater risk of upper aerodigestive tract cancer only in heavy drinking populations (38,39). The finding concerning ADH1C.
in our study is in contrast with the non-Asian studies. The less-active ADH1C*2 allele increased the risk of oral and pharyngeal cancer among the moderate-to-heavy drinkers in the present study. However, when we considered the linkage disequilibrium between the ADH1C*2 allele and ADH1B*1 allele (5,10), we found no significant relationship between ADH1C and oral and pharyngeal cancer, indicating that ADH1B*1 alone is the true risk allele in this population. This finding is also in good agreement with the results of previous East Asian studies of esophageal cancer (10) as well as of alcoholism (5).

Analyses of the effect of the type of alcoholic drink on carcinogenesis have not yielded consistent results, in partly because the actual ethanol concentration of alcoholic drinks varies depending on whether and how the drink is diluted with non-alcoholic mixers. Our study addressed risk by asking about the frequency of drinking strong alcoholic beverages straight, and demonstrated that frequent drinking of strong beverages straight is a strong independent risk factor for oral and pharyngeal cancer. The results of this study are consistent with the results of our previous studies of esophageal cancer in Japanese men (10) and women (12), and of upper aerodigestive tract cancer in Japanese alcoholic men (40). The highly concentrated alcohol may act as an efficient solvent for tobacco and other carcinogens to penetrate the mucosa. Alcoholic beverages themselves contain high levels of acetaldehyde (41), and the high levels of acetaldehyde in strong alcoholic beverages may also play a role in the carcinogenesis as another carcinogenic ingredient in addition to alcohol.

There is substantial evidence that smoking and drinking synergistically increase the risk of oral and pharyngeal cancer, and our risk estimates were consistent with previous studies (1). Acetaldehyde is one of the major chemical constituents of tobacco smoke. It dissolves in saliva during active smoking, and drinking and smoking have a synergistic effect on salivary acetaldehyde concentration (42). Smoking also alters the oral bacterial flora, which leads to higher acetaldehyde levels in saliva after drinking (42,43). High exposure to salivary acetaldehyde in subjects who smoke and drink may partly explain the synergistic effect of alcohol drinking and tobacco smoking in increasing the risk of oral and pharyngeal carcinogenesis. The possible contribution of the ALDH2- and ADH1B-polymorphisms to salivary acetaldehyde levels during smoking and drinking should be clarified in future studies, which may be extremely valuable in interpreting the results of this study.

The significant protective effect of green–yellow vegetable intake on the risk of oral and pharyngeal cancer is also consistent with its effect on the risk of esophageal cancer in our previous studies (10,12) and the results of a meta-analysis of oral cancer (44). Chronic alcohol consumption and metabolism result in the generation of several classes of DNA-damaging molecules, including reactive oxygen species and lipid peroxidation products (45). The high intake of antioxidants, including vitamin C and β-carotene, is a possible explanation for the protective role of high intake of green–yellow vegetables. Folate deficiency leads to alterations in DNA methylation and disruption of DNA integrity and repair, and low folate intake increases the risk of oral and pharyngeal cancer (46). Serum folate levels are more likely to be lowered by alcohol drinking in men with inactive ALDH2 than in men with active ALDH2 (47). This phenomenon is biologically plausible, because acetaldehyde increases folate catabolism in vitro (48). Thus, drinkers with inactive ALDH2 may require a high folate intake for cancer protection.

Alcohol flushing among ALDH2*1/*2 heterozygotes diminishes in intensity in individuals with a long or heavy drinking history, suggesting the development of tolerance to acetaldehydemia (6,7), and this tendency is more prominent among those with ADH1B*1/*1 (6,7). For that reason, we designed a questionnaire to detect changes in flushing response over time by asking about current and past flushing, and we demonstrated that the results of the flushing questionnaire predicted the risk of esophageal cancer as a marker of ALDH2 genotype in men (7) and women (12). When we assumed that current or former flushing men had inactive ALDH2, the sensitivity and specificity for inactive ALDH2 in the controls was 90 and 88%, respectively. Current or former flushing status identified by our flushing questionnaire was associated with a higher risk of oral and pharyngeal cancer than never flushing. The results obtained by the flushing questionnaire were essentially comparable to

### Table VI. Multivariate odds ratios of selected factors for oral and pharyngeal cancer among moderate-to-heavy drinkers

<table>
<thead>
<tr>
<th>Sites of cancer</th>
<th>Oral and pharynx (n = 67)</th>
<th>Hypo-pharynx (n = 30)</th>
<th>Oral cavity/oropharynx (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALDH2 genotype</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>*2/*2 versus *1/*1</td>
<td>3.12 (1.63–5.97)</td>
<td>11.58 (4.00–33.54)</td>
<td>—</td>
</tr>
<tr>
<td>ADH1B genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*1 versus *2/*2 + *1/*2</td>
<td>4.04 (1.55–10.56)</td>
<td>4.22 (1.23–14.50)</td>
<td>4.59 (1.54–13.71)</td>
</tr>
<tr>
<td>Strong alcoholic beverages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequently versus never/sometimes</td>
<td>7.46 (2.34–23.82)</td>
<td>23.41 (4.84–113.1)</td>
<td>5.21 (1.38–19.62)</td>
</tr>
<tr>
<td>Smoking (pack-years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30+ versus &lt;30</td>
<td>3.14 (1.49–6.64)</td>
<td>—</td>
<td>3.61 (1.48–8.81)</td>
</tr>
<tr>
<td>Green–yellow vegetables intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Every day versus others</td>
<td>0.34 (0.16–0.75)</td>
<td>0.25 (0.08–0.83)</td>
<td>—</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval.

Age and alcohol drinking were forcedly adjusted in all models. The other independent variables were selected by STEPWISE method with P < 0.05 for entry and staying.
those obtained by ALDH2 genotyping. The flushing questionnaire may become a useful tool for prevention of oral and pharyngeal cancer as well as esophageal cancer (7). Professional and public education about risky conditions in the upper aerodigestive tract could be useful in identifying high risk individuals for cancer in these regions and help persuade them to undergo cancer screening and change their lifestyle choices to prevent cancer from developing.

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References

p450 2E1 in the oropharyngeal mucosa in alcoholics with cancer [Abstract].

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