

## Alcohol Flushing, Alcohol and Aldehyde Dehydrogenase Genotypes, and Risk for Esophageal Squamous Cell Carcinoma in Japanese Men

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### Abstract

**Alcohol flushing after light drinking is triggered mainly by severe acetaldehydemia in individuals possessing inactive aldehyde dehydrogenase (ALDH)-2. Inactive ALDH2 encoded by *ALDH2\*1/2\*2* and the low-activity form of alcohol dehydrogenase (ADH)-2 encoded by *ADH2\*1/2\*1* enhance the risk for esophageal cancer in Japanese light to heavy drinkers, a significant association that emphasizes the importance of screening tests for inactive ALDH2 based on alcohol flushing. The objectives of the present report were (a) to evaluate the reliability of a simple questionnaire that asks about both current and past flushing for detecting inactive ALDH2 and (b) to predict cancer risk based on flushing in a case-control manner. The study subjects consisted of 233 Japanese men with esophageal squamous cell carcinoma and 610 cancer-free Japanese men. When current or former flushing individuals were considered to have inactive ALDH2, the sensitivity and specificity of the test were 84.8% and 82.3%, respectively, for the cases and 90.1% and 88.0%, respectively, for the controls. To clarify the characteristics of men who had genetically inactive ALDH2 but did not report alcohol flushing, we analyzed individuals possessing the *ALDH2\*1/2\*2* genotype and found that those who also had *ADH2\*1/2\*1* (both cases and controls) tended not to report current flushing, and**

those who did not report current flushing (controls only) tended to be heavier drinkers. As compared with overall never or rare drinking, the cancer risks for light (1–8.9 units/week; 1 unit = 22 g of ethanol), moderate (9–17.9 units/week), and heavy (18+ units/week) drinkers with current or former flushing (odds ratio = 6.69, 42.66, and 72.86, respectively) significantly exceeded the risks for those who had never flushed (odds ratio = 1.27, 10.12, and 15.61, respectively), even after adjustment for age, smoking, and diet. The flushing questionnaire may be used in large-scale epidemiological studies as a surrogate marker of ALDH2 genotype to predict individual cancer risk.

### Introduction

Alcohol flushing responses, including facial flushing, palpitation, drowsiness, and other unpleasant symptoms, are frequently observed in East Asians (1–4). Intensive flushing after light drinking is mainly triggered by severe acetaldehydemia in those possessing the *ALDH2\*2* allele, which is prevalent in East Asians and encodes inactive forms of ALDH2<sup>11</sup> (5–8). Several studies have shown that the presence of the *ADH2\*2* allele, which is also prevalent in East Asians and encodes superactive forms of ADH2 (5), can also enhance alcohol flushing to some extent (9–12). The *ALDH2\*2* and *ADH2\*2* alleles thus act independently to prevent East Asians from developing alcoholism, probably by enhancing the unpleasant flushing responses (13–15).

Studies of various Japanese (12, 16–21) and Chinese (22) drinking populations have consistently shown that the inactive ALDH2 encoded by the *ALDH2\*1/2\*2* genotype and the less active *ADH2\*1/2\*1* form of ADH2 are strong risk factors for esophageal cancer. ALDH2/ADH2-related vulnerability for esophageal cancer includes not only heavy drinkers (12, 18–20) but also light to moderate drinkers (21). Given ample evidence of the carcinogenicity of acetaldehyde in experimental animals (23, 24), it is reasonable to speculate that acetaldehyde plays a crucial role in ALDH2-associated susceptibility to esophageal cancer. This risk association underscores the importance of attempting to develop screening tests for inactive ALDH2 based on alcohol flushing and to use those tests to predict cancer risk.

The ethanol patch test is a cutaneous model of the flushing response for predicting ALDH2 activity (4) and is used for health education of Japanese youth, especially those who have never consumed alcohol. Although the first large study of this test reported high sensitivity (93%) and specificity (94%) in

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<sup>11</sup> The abbreviations used are: ALDH, aldehyde dehydrogenase; ADH, alcohol dehydrogenase; OR, odds ratio.

identifying inactive ALDH2 (4), four other large studies failed to yield such high reliability (8, 10, 25, 26). The ethanol patch test is therefore considered unsatisfactory as an indicator of inactive ALDH2, especially for use with men age 50 years or older [sensitivity, 72%; specificity, 71% (25)], many of whom have a long history of drinking, because aging and acquired tolerance to acetaldehyde appear to influence the results.

An alternative approach to identify inactive ALDH2 by alcohol flushing is the assessment of facial flushing status after alcohol drinking. However, facial flushing after light drinking also diminishes in intensity among men with long or heavy drinking histories. Among male Japanese ALDH2 heterozygotes, 77.0% of those age 50 years or older (25), 70.1% of those with esophageal or oropharyngolaryngeal cancer (26), and 71.5% of those who were alcoholics and had esophageal cancer (12) recalled that they had at one time experienced facial flushing after drinking a glass of beer; however, only 64.6%, 25.4%, and 8.2% of these three groups, respectively, reported current facial flushing. These negative changes in the frequency of flushing responses suggest that long-time or heavy drinkers 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 (25).

Using this flushing questionnaire, we showed that among alcoholic men with esophageal cancer, only 47.8% of those with both the *ALDH2*\*1/2\*2 and *ADH2*\*1/2\*1 genotypes, compared with 92.3% of those with *ALDH2*\*1/2\*2 and the *ADH2*\*2 allele, reported current or former alcohol flushing (12). In individuals with the *ALDH2*\*1/2\*2 and *ADH2*\*1/2\*1 gene combination, which represents the highest risk for esophageal cancer (12, 17, 19, 21), the weak and/or diminished intensity of the alcohol flushing might have, in turn, enhanced the vulnerability to esophageal cancer. Examination of the relationship between alcohol flushing and esophageal cancer risk would offer a new strategic approach in the prevention of esophageal cancer.

To shed more light on these issues, we analyzed data from a multi-institutional case-control study in Japan. The present report had two main objectives: (a) to evaluate the reliability of a simple questionnaire about alcohol flushing for detecting inactive ALDH2; and (b) to predict cancer risk based on this questionnaire.

## Materials and Methods

**Subjects.** The study subjects consisted of esophageal cancer cases and cancer-free controls, and these data were used (a) to evaluate the reliability of the flushing questionnaire in each of the case and control groups separately and (b) to assess the risk of esophageal cancer in a case-control manner. The case participants were 234 male Japanese patients with primary esophageal squamous cell carcinomas undergoing treatment at the National Cancer Center Hospital, the National Cancer Center Hospital East, Kawasaki Municipal Hospital, or the National Osaka Hospital. The controls were 634 cancer-free Japanese men who visited two Tokyo clinics for annual health checkups. All were registered between September 2000 and December 2001 by a previously described method (21). The ethics committee of each collaborating institution reviewed and approved the proposed study, and each of the participants gave informed consent.

**Questionnaires.** Each participant was asked to fill out a simple questionnaire concerning alcohol flushing responses, and 233 cancer patients and 610 controls completed it. Although the

ADH2 genotype could also affect flushing responses, the effect did not seem to be as strong for the ALDH2 genotype (5–12), and therefore, this questionnaire was designed to identify inactive ALDH2, but not ADH2. The questions in this slightly modified version of our previous questionnaire (25) were: (a) Do you have tendency to flush in the face immediately after drinking a glass of beer (yes, no, or unknown)? (b) Did you have a tendency to flush in the face immediately after drinking a glass of beer during the first to second year after you started drinking (yes, no, or unknown)? Individuals who answered “yes” to question (a) were classified as “current flushing”; those who answered “yes” to question (b) but not to question (a) were classified as “former flushing.” The remaining subjects were classified as “never flushing.” Those who responded “unknown” to both questions (only 3.4% of cases and 1.6% of controls) were classified as “never flushing” because we speculated that those who had experienced unpleasant flushing should respond “yes” rather than “unknown.” Both current and former flushing individuals were considered to have inactive ALDH2.

Each participant also completed a structured questionnaire concerning his drinking, smoking, and dietary habits; those with cancer were instructed to report on their habits before they got sick. The contents of the questionnaire and the method of calculating alcohol consumption (1 unit = 22 g, the ethanol content of one serving of sake) have been described previously (21). The subjects were classified as never/rare 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).

**DNA Analysis.** PCR-RFLP methods were used to analyze lymphocyte DNA samples from all participants, without knowledge of cancer status, to determine the genotypes for ALDH2 (19, 27) and ADH2 (28).

**Statistical Analysis.** The allele frequency was determined by direct counting. Deviation of the genotype distribution from Hardy-Weinberg equilibrium was analyzed by the exact test. Fisher's exact test and the Cochran-Mantel-Haenszel  $\chi^2$  test were used where appropriate in comparing group statistics. The Spearman rank-correlation analysis was used as a nonparametric test for trend. The associations of the alcohol flushing pattern and ALDH2 and ADH2 genotypes with esophageal cancer were expressed in terms of the OR, adjusted for the effects of several possible confounders by the use of a multiple logistic regression model. The reliability of the questionnaire for detecting inactive ALDH2 (2\*1/2\*2 or 2\*2/2\*2) was assessed in terms of sensitivity (the proportion of men truly possessing inactive ALDH2 who were so identified by the questionnaire) and specificity (the proportion of men truly possessing active ALDH2 who were so identified by the questionnaire). These analyses were done with the SAS statistical package (version 8.2; SAS Institute, Cary, NC).

## Results

**Basic Characteristics of the Study Subjects.** The esophageal cancer patients were significantly older than the cancer-free control subjects. After adjustment for age, we observed that the cancer patients reported heavier drinking, more drinking of strong beverages, more smoking, lower intake of green-yellow vegetables, and lower intake of fruit than the controls (Table 1). The cases and controls differed significantly in the distribution of alcohol flushing categories and ALDH2 and ADH2 genotypes. These genotypes significantly deviated from the Hardy-Weinberg equilibrium in the cases, but not in the controls.

Table 1 Basic characteristics in esophageal cancer cases and control subjects

	Cases (n = 233)	Controls (n = 610)	P
Age (yrs)			
40–49	6.0%	6.7%	
50–59	34.8%	46.7%	
60–69	42.1%	41.6%	
70–79	17.2%	4.9%	<0.0001
Mean ± SD	61.7 ± 7.9	58.9 ± 7.1	<0.0001
Alcohol drinking <sup>a,b</sup>			
Never/rare	2.2%	26.0%	
Light	10.3%	32.1%	
Moderate	36.9%	26.2%	
Heavy	45.1%	14.0%	
Ex-drinker	5.6%	1.7%	<0.0001
Strong alcoholic beverages <sup>a</sup>			
Frequently	17.8%	2.5%	
Sometimes	32.2%	15.3%	
Never	50.0%	82.2%	<0.0001
Smoking (pack-years) <sup>a</sup>			
0	9.0%	28.2%	
<30	16.7%	29.5%	
≥30	74.3%	42.4%	<0.0001
Green-yellow vegetables <sup>a</sup>			
Seldom	0.9%	0.6%	
1–2 days/month	8.6%	2.7%	
1–2 days/week	35.6%	22.1%	
3–4 days/week	33.9%	34.5%	
Almost every day	21.0%	40.1%	<0.0001
Fruit <sup>a</sup>			
Seldom	7.4%	3.6%	
1–2 days/month	18.2%	7.8%	
1–2 days/week	36.4%	25.8%	
3–4 days/week	20.8%	22.9%	
Almost every day	17.3%	39.9%	<0.0001
Alcohol flushing <sup>a,c</sup>			
Current flushing	44.6%	41.2%	
Former flushing	22.3%	10.2%	
Never flushing	33.1%	48.6%	<0.0001
ALDH2 genotype <sup>a</sup>			
2*1/2*1	26.6%	50.2%	
2*1/2*2	72.5%	43.0%	
2*2/2*2	0.9%	6.8%	<0.0001
P for HW <sup>d</sup>	<0.0001	0.84	
ADH2 genotype <sup>a</sup>			
2*1/2*1	21.9%	4.8%	
2*1/2*2	31.3%	35.0%	
2*2/2*2	46.8%	60.3%	<0.0001
P for HW <sup>d</sup>	<0.0001	1.00	

<sup>a</sup> Percentage and *P*s adjusted for age by direct method (using case group as the standard population) and by Cochran-Mantel-Haenszel test, respectively; *P*s except alcohol drinking are for trend.

<sup>b</sup> Never/rare, <1 unit/week; light, 1–8.9 units/week; moderate, 9–17.9 units/week; heavy, ≥18 units/week (1 unit = 22 g of ethanol).

<sup>c</sup> See text for definitions (“Questionnaires”).

<sup>d</sup> Exact *P* for deviation from Hardy-Weinberg equilibrium.

**The Reliability of a Flushing Questionnaire for Detecting Inactive ALDH2.** The ALDH2 genotype strongly affected the distribution of alcohol flushing categories in both the cancer patients and the controls, whereas the ADH2 genotype had a weak effect only in the controls (Table 2).

When current or former flushing individuals were considered to have inactive ALDH2, the sensitivity and specificity for identifying inactive ALDH2 were 84.8% and 82.3%, respectively, in the cancer patients, and 90.1% and 88.0%, respectively, in the controls (Table 3). However, when only current flushing was used to detect inactive ALDH2, diminished sen-

sitivity was more marked in the cases than in the controls (56.7% and 74.3%, respectively; shown in parentheses in Table 3). The sensitivity of current/former flushing for detecting inactive ALDH2 was significantly lower in the presence of *ADH2\*1/2\*1* than in its absence among both the cancer patients (60.0% versus 93.7%) and the controls (70.0% versus 91.7%). Moreover, the sensitivity of this method in detecting inactive ALDH2 diminished in controls who drank more.

To further clarify the characteristics of men who had genetically inactive ALDH2 but did not report alcohol flushing, we analyzed a subset of individuals possessing the *ALDH2\*1/2\*2* genotype (Table 4). Among both cases and controls, those individuals with the *ADH2\*1/2\*1* genotype more frequently reported former or never flushing than did those without this (*P* < 0.0001). When the ADH2 genotype was identical in the cancer patients and controls, the prevalence of never flushing was similar; after adjustment for ADH2 genotype, the distribution of flushing categories was similar in the cancer patients and controls (*P* = 0.30). The amount of alcohol drinking significantly increased with diminished flushing in the controls but not in the cases.

**The Risk of Esophageal Cancer According to Flushing Responses.** After adjustments for age, the frequency of drinking strong beverages, smoking, and the intake of green-yellow vegetables and fruit, we estimated the risk for esophageal cancer in five drinking categories (never/rare, light, moderate, heavy, and ex-drinkers) by reported flushing response (Table 5). We used overall never/rare drinkers as the reference category because it was difficult to analyze current/former and never flushing subjects in this drinking category separately, due to the small number of cases (*n* = 5) and because the prevalence of current/former flushing did not differ between cases (1 of 5 = 20.0%) and controls (25 of 137 = 18.2%). Light drinking increased the risk for esophageal cancer among individuals with current/former flushing (OR = 6.69), but not among those who had never flushed. The risk for esophageal cancer in moderate and heavy drinkers with current/former flushing (OR = 42.66 and 72.86, respectively) markedly exceeded that risk in those who had never flushed (OR = 10.12 and 15.61, respectively). Thus, in comparison with the never flushing group, a significantly increased risk for esophageal cancer was associated with current/former flushing in all drinking categories from light to heavy, even when the amount of alcohol consumed was identical (ORs = 4.22–5.27).

## Discussion

**The Reliability of a Flushing Questionnaire for Detecting Inactive ALDH2.** Although it is well recognized that the current status of alcohol flushing is enhanced with inactive ALDH2, the relationship between past flushing and ALDH2 genotype has only been reported in single study by our group (25). The present study suggests that the flushing responses could disappear among a considerable number of individuals with inactive ALDH2, especially the cancer patients, during a long history of drinking. Thus, the approach of asking study participants about current and past alcohol flushing makes our flushing questionnaire uniquely useful for detecting inactive ALDH2 among both esophageal cancer patients and cancer-free individuals. When we considered only currently flushing individuals to have inactive ALDH2, we found that the decrease in the sensitivity for inactive ALDH2 was more marked in cancer patients than controls (Table 3; 56.7% and 74.3%, respectively; *p* = 0.0001). However, by considering both current and former flushing individuals to have inactive ALDH2,

Table 2 Frequency of alcohol flushing in 233 cases and 610 control subjects, by ALDH2 and ADH2 genotypes

Genotype	Cases				Controls			
	N	Current flushing <sup>a</sup>	Former flushing <sup>a</sup>	Never flushing <sup>a</sup>	N	Current flushing <sup>a</sup>	Former flushing <sup>a</sup>	Never flushing <sup>a</sup>
<i>ALDH2</i>								
2*1/2*1	62	11.3%	6.5%	82.3%	326	4.9%	7.1%	88.0%
2*1/2*2	169	56.2%	28.4%	15.4%	242	70.3%	18.6%	11.2%
2*2/2*2	2	100.0%	0.0%	0.0%	42	97.6%	0.0%	2.4%
<i>p</i> <sup>b</sup>			<0.0001				<0.0001	
<i>ADH2</i>								
2*1/2*1	51	33.3%	23.5%	43.1%	31	29.0%	22.6%	48.4%
2*1/2*2	73	43.8%	27.4%	28.8%	212	34.4%	7.1%	58.5%
2*2/2*2	109	50.5%	18.4%	31.2%	367	39.5%	12.5%	48.0%
<i>p</i> <sup>b</sup>			0.17				0.018	

<sup>a</sup> See text for definitions of flushing status ("Questionnaires").

<sup>b</sup> Fisher's exact test.

the sensitivity improved to 84.8% and 90.1% ( $P = 0.10$ ) among cancer patients and controls, respectively, at the cost of a relatively small decrease in the specificity. The observation that sensitivity and specificity were simultaneously high in both cases and controls is important, particularly for a case-control study (see discussions below for risk analysis). Thus, both the current and past status of alcohol flushing should be examined when the flushing response is used as a surrogate marker of ALDH2 genotype.

Some of the subjects with *ALDH2*\*1/2\*2 genotype reported never flushing. This negative response might be influenced by environmental and other genetic factors. For example, the *ADH2* genotype is another important determinant of intensity of alcohol flushing (9–12). We had reported earlier that among Japanese alcoholics with esophageal cancer, the much less active *ADH2*\*1/2\*1 form of *ADH2* tended to mask alcohol flushing, despite the presence of *ALDH2*\*1/2\*2 and severe acetaldehydemia (12). In the present study, we found that among individuals with *ALDH2*\*1/2\*2, both cases and controls possessing the *ADH2*\*1/2\*1 genotype tend not to experience current alcohol flushing, and among control subjects, only those who did not report current alcohol flushing tended to be heavier drinkers (Table 4). Thus, the *ADH2*\*1/2\*1 genotype's diminution of the intensity of alcohol flushing may enhance an

individual's vulnerability to drinking and subsequent acetaldehyde exposure. The dramatic initial response, which may be triggered by either an initially steep rise in blood or cutaneous acetaldehyde after drinking, may not occur in persons with the much less active form of *ADH2*, even in the presence of inactive heterozygous *ALDH2*.

Despite the hereditarily small capacity for metabolism of blood acetaldehyde, 58% of the 233 cancer cases and 10% of the 610 controls in this study were moderate to heavy drinkers and had the *ALDH2*\*1/2\*2 genotype. Research still has not elucidated how drinkers with *ALDH2*\*1/2\*2 are able to overcome and adapt to acetaldehydemia and alcohol flushing. Our finding that a low sensitivity of current flushing in the detection of inactive *ALDH2* was associated with the drinking habits in the controls may have two possible explanations: (a) diminished flushing represents a primary phenomenon that makes such individuals prone to heavier drinking; and (b) diminished flushing may be due to tolerance to acetaldehydemia acquired during the individual's drinking history.

The higher frequency of never or former flushing among the cancer patients with *ALDH2*\*1/2\*2 than among cancer-free subjects with the same genotype can be explained in part by the higher frequency of *ADH2*\*1/2\*1 among the former as compared with the latter. This is consistent with our previous

Table 3 Sensitivity and specificity of the flushing questionnaire for detecting inactive ALDH2, by ADH2 genotype and alcohol drinking

	Inactive ALDH2 (2*1/2*2 or 2*2/2*2)					Active ALDH2 (2*1/2*1)				
	N		% of current/former flushing (sensitivity <sup>a</sup> )		<i>p</i> <sup>b</sup>	N		% of never flushing (specificity <sup>a</sup> )		<i>p</i> <sup>b</sup>
	Cases	Controls	Cases	Controls		Cases	Controls	Cases	Controls	
All subjects	171	284	84.8% (56.7%) <sup>c</sup>	90.1% (74.3%) <sup>c</sup>	0.10 (0.0001) <sup>c</sup>	62	326	82.3% (88.7%) <sup>c</sup>	88.0% (95.1%) <sup>c</sup>	0.21 (0.072) <sup>c</sup>
<i>ADH2</i> genotype										
2*1/2*1	45	20	60.0%	70.0%	0.58	6	11	66.7%	81.8%	0.58
2*1/2*2 or 2*2/2*2	126	264	93.7%	91.7%	0.55	56	315	83.9%	88.3%	0.38
<i>p</i> <sup>d</sup>			<0.0001	0.0078				0.29	0.63	
Alcohol drinking										
Never/rare to light	26	218	92.3%	95.4%	0.37	3	113	66.7%	83.3%	0.36
Moderate	63	35	82.5%	74.3%	0.43	23	131	91.3%	90.8%	1.00
Heavy	73	27	83.6%	70.4%	0.16	32	74	75.0%	87.8%	0.15
Ex-drinker	9	4	88.9%	75.0%	1.00	4	8	100.0%	62.5%	0.49
<i>p</i> <sup>d</sup>			0.75	<0.0001				0.26	0.12	

<sup>a</sup> Sensitivity and specificity of current/former flushing for detecting inactive ALDH2 (2\*1/2\*2 or 2\*2/2\*2).

<sup>b</sup> Comparison of the sensitivity and specificity between cases and controls by Fisher's exact test.

<sup>c</sup> Values in parentheses were percentage of current flushing among men with inactive ALDH2 (left columns); and percentage of former/never flushing among men with active ALDH2 (right columns).

<sup>d</sup> Comparison of the sensitivity and specificity between *ADH2* genotype groups and among alcohol drinking categories by Fisher's exact test.

Table 4 Prevalence of alcohol flushing and amount of alcohol drinking according to ADH2 genotypes in men with *ALDH2\*1/2\*2*

ADH2 genotype	Cases				P	Controls				P, cases vs. controls	
	Alcohol flushing			P		Alcohol flushing			P		
	Current	Former	Never			Current	Former	Never			
Prevalence of alcohol flushing <sup>a</sup>											
<i>ADH2*1/2*1</i>	(n = 44)	36.4%	25.0%	38.6%	<0.0001 <sup>b</sup>	(n = 16)	25.0%	37.5%	37.5%	<0.0001 <sup>b</sup>	0.68 <sup>b</sup>
<i>ADH2*1/2*2</i> or <i>2*2/2*2</i>	(n = 116)	61.2%	31.9%	6.9%		(n = 222)	73.4%	17.6%	9.0%		0.17 <sup>b</sup>
Total	(n = 160)	54.4%	30.0%	15.6%		(n = 238)	70.2%	18.9%	10.9%		0.0049 <sup>b</sup>
Adjusted for ADH2 genotype <sup>c</sup>		54.4%	30.0%	15.6%		60.1%	23.1%	16.8%		0.30	
Amount of alcohol drinking (units/week), median											
<i>ADH2*1/2*1</i>		18.82	18.00	15.27	0.81 <sup>d</sup>	3.51	7.25	9.90	0.0059 <sup>d</sup>		
<i>ADH2*1/2*2</i> or <i>2*2/2*2</i>		16.36	16.36	17.73	0.91 <sup>d</sup>	1.82	7.91	14.18	<0.0001 <sup>d</sup>		
Total		17.18	17.45	17.45	0.93 <sup>d</sup>	1.91	7.91	13.23	<0.0001 <sup>d</sup>		

<sup>a</sup> Shown as row percentages.

<sup>b</sup> P for trend by Cochran-Mantel-Haenszel test.

<sup>c</sup> Percentages values of control are adjusted for ADH2 genotype by a direct method using the cases as the standard population; P is for trend adjusted for ADH2 genotype by Cochran-Mantel-Haenszel test.

<sup>d</sup> P for trend by Spearman rank-correlation analysis.

finding that among both the alcoholic (12, 19) and present (21) populations, the less active form of ADH2 increased the carcinogenic effect of inactive ALDH2 on the esophagus in a multiplicative fashion. Because the ALDH2 genotype, but not the ADH2 genotype, is the basis on which the level of an individual's acetaldehyde exposure after drinking is determined (29), these results suggest that the ADH2 genotype affects cancer susceptibility, to some extent, through its influence on alcohol flushing.

**The Risk of Esophageal Cancer According to Flushing Responses.** In comparison with never flushing, the questionnaire's identification of current or former flushing status was associated with higher risk for esophageal cancer in light, moderate, and heavy drinkers (OR for flushing *versus* never flushing = 5.27, 4.22, and 4.67, respectively; Table 5). These high ORs were no longer significant after adjustment for ALDH2 genotype (OR = 1.12, 1.15, and 1.52, respectively; data not shown), whereas the risks were essentially unchanged when adjusted for ADH2 genotype (OR = 4.89, 4.45, and 5.19, respectively; data not shown), clearly indicating that our flushing questionnaire predicted cancer risk as a surrogate marker of

ALDH2 genotype but not of ADH2. Although ADH2 genotype was strongly linked to the risk of esophageal cancer (21) and weakly linked to alcohol flushing (Tables 2 and 3), the relationship between alcohol flushing and cancer risk mainly reflected the effect of ALDH2 genotype.

The ORs of alcohol flushing were not as strong as those of our earlier results concerning the presence of the inactive *ALDH2\*1/2\*2* genotype in the same population [OR for inactive *versus* active ALDH2 genotypes = 5.82, 10.01, and 8.56, respectively (21)], although these results were essentially comparable. Given that the flushing questionnaire is merely a surrogate marker of ALDH2 genotype, the weaker relationships in the present study would be explained mainly by the misclassification of ALDH2. That is, if we misclassify inactive ALDH2 as "active" (or *vice versa*) by the flushing questionnaire, and the misclassification occurs in equal proportion in the case and control groups [so-called "non-differential misclassification" (30)], the observed OR tends to be weaker (toward 1.0) than the true value under certain conditions (30). It should be noted that if the misclassification occurs differently in the case and control groups ("differential misclassification"), the OR can be either

Table 5 ORs for the combinations of alcohol flushing responses and amount of alcohol drinking, adjusted for other main esophageal cancer risk factors

Percentage values of controls are adjusted for age by a direct method using the cases as the standard population.

Alcohol drinking <sup>a</sup>	Alcohol flushing <sup>b</sup>	Cases	Controls	OR <sup>c</sup>	95% CI <sup>d</sup>	OR <sup>e</sup>	95% CI
		N (%)	N (%)				
Never/rare	Any	5 (2.2)	137 (26.0)	1	Referent	5.27	1.43–19.48
	Light	3 (1.3)	85 (14.0)	1.27	0.27–5.88		
Moderate	Never-flushing	21 (9.0)	109 (18.1)	6.69	2.21–20.20	4.22	2.25–7.89
	Current/former flushing	32 (13.7)	128 (19.9)	10.12	3.45–29.69		
Heavy	Never-flushing	54 (23.2)	38 (6.3)	42.66	14.17–128.42	4.67	2.42–9.00
	Current/former flushing	36 (15.5)	73 (10.1)	15.61	5.19–46.91		
Ex-drinker	Never-flushing	69 (29.6)	28 (3.8)	72.86	23.75–223.57	1.36	0.23–8.06
	Current/former flushing	5 (2.2)	6 (0.8)	27.31	5.24–142.46		
Total	Current/former flushing	8 (3.4)	6 (0.9)	37.00	7.66–178.76		
		233 (100)	610 (100)				

<sup>a</sup> 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.

<sup>b</sup> See text for definitions ("Questionnaires").

<sup>c</sup> Adjusted for age, frequency of drinking strong alcohol beverages, pack-years and intake frequency of green-yellow vegetables and fruits by a multiple logistic regression model.

<sup>d</sup> CI, confidence interval.

<sup>e</sup> Adjusted OR of current/former flushing *vs.* never flushing in each drinking category.

exaggerated or underestimated, and the biased results are difficult to interpret (30). Therefore, if we use only current flushing to identify inactive ALDH2, the markedly differential sensitivity and specificity between cases and controls may cause a troublesome bias due to differential misclassification. To address this issue, we simulated how strongly these non-differential and differential misclassifications could bias the results (data not shown). This simulation study showed that when we considered only current flushing, the simulated ORs were markedly exaggerated or underestimated due to the differential misclassification, whereas when we considered both current and past flushing, the simulated ORs were approximately equal to (among never/rare and moderate drinkers) or greater than (among heavy drinkers) the ORs calculated by assuming the non-differential misclassification. This means that by considering both current and past flushing, our questionnaire predicts cancer risk at least as an approximate surrogate marker of ALDH2 genotype in never/rare to moderate drinkers and may be more predictive for cancer risk than a mere surrogate marker of ALDH2 in heavy drinkers.

After all, our simple questionnaire not only yielded high sensitivity and specificity for detecting inactive ALDH2 among both cancer patients and cancer-free controls but was also useful in predicting individuals' ALDH2-associated cancer risks.

**Conclusions.** The high reliability of our simple flushing questionnaire in detecting inactive ALDH2 and predicting individual risk for esophageal cancer suggests the utility of its application in large-scale epidemiological studies of potential ALDH2-related cancers of the head and neck (18, 19, 31, 32), esophagus (12, 18–21), stomach (18, 19), colorectum (18, 33, 34), and lung (18). Professional and public education about this association is a direct and cost-effective approach to the prevention of esophageal cancer. This flushing questionnaire will not only benefit many drinkers by helping them to identify their own risks for esophageal cancer but will also help clinicians to detect esophageal cancer even earlier by identifying high-risk individuals.

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## Alcohol Flushing, Alcohol and Aldehyde Dehydrogenase Genotypes, and Risk for Esophageal Squamous Cell Carcinoma in Japanese Men

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