

# Risk of metachronous squamous cell carcinoma in the upper aerodigestive tract of Japanese alcoholic men with esophageal squamous cell carcinoma: a long-term endoscopic follow-up study

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East Asian case-control studies have shown a strong relationship between alcohol consumption combined with inactive heterozygous aldehyde dehydrogenase-2 (*ALDH2\*1/\*2*) and the development of squamous cell carcinoma (SCC), especially multiple SCC, of the upper aerodigestive tract (UADT). This study aimed to identify determinants of the development of metachronous SCC in the UADT in alcoholics with esophageal SCC. Follow-up endoscopic examinations were carried out 4–160 months (median, 41 months) after initial diagnosis in 110 Japanese alcoholic men with esophageal SCC diagnosed by screening using endoscopy combined with oropharyngolaryngeal inspection and esophageal iodine staining. *ALDH2\*1/\*2* was significantly associated with the presence of multiple primary intraesophageal SCC at the time of initial diagnosis. Metachronous primary SCC of the esophagus was diagnosed in 29 of the 81 patients whose initial esophageal SCC was treated by endoscopic mucosal resection alone, and metachronous primary SCC of the oropharyngolarynx was diagnosed in 23 of the 99 patients without synchronous primary SCC of the oropharyngolarynx at the time of initial diagnosis. The risks of metachronous esophageal SCC and oropharyngolaryngeal SCC were significantly higher in *ALDH2\*1/\*2* heterozygotes than in *ALDH2\*1/\*1* homozygotes (age-adjusted and alcohol-adjusted hazard ratio = 3.38 [95% confidence interval: 1.45–7.85] and 4.27 [1.42–12.89], respectively), and in patients with multiple intraesophageal SCC at the time of initial diagnosis than in patients with a solitary intraesophageal SCC (3.09 [1.41–6.78] and 3.25 [1.41–7.47], respectively). *ALDH2\*1/\*2* and multiple synchronous intraesophageal SCC were found to be predictors of metachronous SCC in the UADT in this population. (*Cancer Sci* 2008; 99: 1164–1171)

Screening of Japanese alcoholic men by endoscopy combined with oropharyngolaryngeal inspection and esophageal iodine staining has revealed a very high prevalence of squamous cell carcinoma (SCC) in the upper aerodigestive tract (UADT).<sup>(1)</sup> We have reported findings that inactive heterozygous aldehyde dehydrogenase-2 (*ALDH2*) encoded by *ALDH2\*1/\*2*,<sup>(2–6)</sup> less-active alcohol dehydrogenase-1B (*ADH1B*, previously called *ADH2*) encoded by *ADH1B\*1/\*1*,<sup>(2,4,6)</sup> self-reported alcohol flushing,<sup>(6,7)</sup> macrocytosis with a mean corpuscular volume (MCV) of 106 fl or more,<sup>(6,7)</sup> heavy smoking,<sup>(1,6)</sup> and a low body mass index (BMI),<sup>(6,7)</sup> were risk factors for SCC in the UADT both in case-control studies<sup>(1–4,7)</sup> and prospective studies<sup>(5,6)</sup> in Japanese alcoholic men.

The increase in prevalence of multiple primary cancers in patients undergoing surgery at Japan's National Cancer Center Hospital for esophageal cancer, from 6% in 1969–1980, to 22% in 1981–1991, and then to 39% in 1992–1996,<sup>(8)</sup> has been alarming. The US National Cancer Institute's Surveillance,

Epidemiology, and End Results Program has reported a low frequency of synchronous (2%) and metachronous (3%) multiple cancers in patients with esophageal cancer during the period 1973–2003.<sup>(9)</sup> The increase in Japan might be partly explained by a dramatic increase in the proportion of heavy drinkers who are heterozygous for inactive *ALDH2*. In 1979, only 3% of Japanese alcoholics had inactive heterozygous *ALDH2*, compared to 8% in 1986, and 13% in 1992.<sup>(10)</sup> Inactive heterozygous *ALDH2* has been consistently associated with cancer multiplicity in Japanese patients with SCC in the UADT.<sup>(5,11–15)</sup> The International Agency for Research on Cancer has recently concluded that substantial mechanistic evidence in humans with *ALDH* deficiency indicates that acetaldehyde derived from the metabolism of ethanol in alcoholic beverages contributes to causing malignant esophageal tumors.<sup>(16)</sup> Acetaldehyde could act as a carcinogen on the entire epithelium of the UADT and induce multiple independent malignant foci in the UADT.

The majority of cancerous esophageal lesions detected in our screening program were treated by endoscopic mucosal resection (EMR) in accordance with established criteria,<sup>(1,17)</sup> and follow-up endoscopic examinations allowed us to observe the extremely high rate of development of metachronous primary SCC in the preserved esophagus and oropharyngolarynx. The aim of this study was to identify determinants of the development of metachronous SCC in the UADT in alcoholics with esophageal SCC by means of a long-term endoscopic follow-up study.

## Materials and Methods

**Patients.** A total of 2953 Japanese alcoholic men (≥ 40 years old) with no history of gastrectomy or cancer in the UADT or stomach were screened using endoscopy combined with oropharyngolaryngeal inspection and esophageal iodine staining between January 1993 and December 2002 at the National Hospital Organization Kurihama Alcoholism Center. The screening detected esophageal SCC in 135 patients. The esophageal SCC of 125 of the 135 patients was treated at Kurihama Alcoholism Center, Ichikawa General Hospital, or Kawasaki Municipal Hospital by the authors, and the other 10 patients were referred to other hospitals. As of April 2007, two of the authors (A.Y. and T.O.) had carried out follow-up endoscopic examinations ranging from 4 to 160 months (median, 41 months) after the initial diagnosis of esophageal SCC in 110 of the 125 patients (56.7 ± 6.4 years old), but we

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**Table 1. Basic characteristics of esophageal squamous cell carcinoma (SCC) patients at baseline (n = 110)**

	n	%
Depth of esophageal SCC		
Epithelium	48	43.6
Mucosa	27	24.5
Muscularis mucosa	14	12.7
Submucosa	17	15.5
Proper muscle layer or deeper	4	3.6
Treatment		
EMR alone	81	73.6
EMR and chemo/radiation therapy	12	10.9
Radical surgery	12	10.9
Chemo/radiation therapy alone	5	4.5
Multiple intraesophageal SCC	34	30.9
Synchronous oropharyngolaryngeal SCC <sup>†</sup>	11	10.0
Oral cavity/oropharyngeal	5	4.5
Hypopharyngeal/epilaryngeal	5	4.5
Both oral cavity/oropharyngeal and hypopharyngeal/epilaryngeal	1	0.9
Age (years)		
Mean ± SD (range)	56.7 ± 6.4 (41–71)	NA
Alcohol consumption (units/day) <sup>‡</sup>		
Median (Q1, Q3)	5.0 (3.5, 6.0)	NA
Cigarette smoking (cigarettes/day)		
<20	41	37.3
20–29	31	28.2
30+	38	34.5
ALDH2 genotype		
*1/*1	50	45.5
*1/*2	60	54.5
*2/*2	0	0.0
ADH1B genotype		
*1/*1	47	42.7
*1/*2	25	22.7
*2/*2	38	34.5
Alcohol flushing (n = 106) <sup>§</sup>		
Current	7	6.6
Former	40	37.7
Never	59	55.7
MCV (≤7 days after the last drink, n = 80)		
Mean ± SD	103.4 ± 9.1	NA
≥106 fl	37	46.3

<sup>†</sup>Two patients had primary cancers of the esophagus, oropharyngolarynx, and stomach. <sup>‡</sup>1 unit = 22 g ethanol. <sup>§</sup>Alcohol flushing was classified on the basis of the results of an alcohol flushing questionnaire. ADH1B, alcohol dehydrogenase-1B; ALDH2, aldehyde dehydrogenase-2; EMR, endoscopic mucosal resection; fl, femtoliters; MCV, mean corpuscular volume; NA, not applicable; Q1, 25<sup>th</sup> percentile; Q3, 75<sup>th</sup> percentile; SD, standard deviation.

failed to follow-up the other 15 patients for more than 2 months. The 110 patients were evaluated as the subjects of this study. The esophageal SCC was confined to the epithelium in 48 of the patients and had invaded the mucosa in 27 patients, the muscularis mucosa in 14, the submucosa in 17, and the proper muscle layer or deeper in 4 (Table 1). The esophageal SCC was treated by EMR alone in 81 patients, by EMR and chemo/radiation therapy in 12, by radical surgery in 12, and by chemo/radiation therapy alone in 5. Synchronous primary carcinomas were defined as primary carcinomas diagnosed at the same time or within 2 months of finding the initial esophageal SCC, and metachronous carcinomas as carcinomas diagnosed more than 2 months after diagnosis of the initial lesion.<sup>(18)</sup> Thirty-four patients were diagnosed with synchronous multiple primary intraesophageal cancers, and 11 patients were diagnosed with synchronous primary oropharyngolaryngeal SCC (oral cavity/

oropharyngeal SCC in 5; hypopharyngeal/epilaryngeal SCC in 5; and both in 1). We evaluated the risk of metachronous primary SCC of the esophagus in the 81 patients whose initial esophageal SCC was treated by EMR alone, the risk of metachronous primary SCC of the oral cavity/oropharynx in the 104 patients without synchronous primary SCC of the oral cavity/oropharynx, the risk of metachronous primary SCC of the hypopharynx/epilarynx in the 104 patients without synchronous primary SCC of the hypopharynx/epilarynx, and the risk of metachronous primary SCC of the oropharyngolarynx as a whole in the 99 patients without synchronous primary SCC of the oropharyngolarynx (Fig. 1).

This study was reviewed and approved by the Ethics Committee of the Kurihama Alcoholism Center, and informed consent was obtained from all participating patients.

**Follow-up endoscopy.** The follow-up plan was to examine the patients by endoscopy combined with oropharyngolaryngeal inspection and esophageal iodine staining every 6 months, and the examinations were carried out with Olympus endoscopes (models Q10, P20, XQ200, XQ230, Q240, and Q240Z, in chronological order of use; Olympus Optical, Tokyo, Japan).

**Multiple primary SCC in the UADT.** The diagnosis of multiple primary SCC of the UADT was based on the results of an endoscopic examination and histological evaluation. We used the following criteria proposed by Kuwano *et al.*:<sup>(19)</sup> (i) each cancerous lesion showed definite features of malignancy and was localized without continuity with the other; and (ii) each carcinoma was accompanied by areas of intraepithelial carcinoma.

**Drinking and smoking habits.** All of the participants in this study met the criteria for alcohol dependence as defined by the Diagnostic and Statistical Manual of Mental Disorders, 3rd edition revised.<sup>(20)</sup> Information on the patients' drinking profiles and smoking habits was obtained from the patients and, when available, from significant others. Daily alcohol consumption during the preceding year was expressed in grams of ethanol per day calculated by means of a standard conversion table for alcoholic beverages. Beer was assumed to be 5% ethanol (v/v); wine, 12%; sake, 16%; shochu, 25%; and whiskey, 40%.

**Flushing questionnaire.** We uniformly interviewed 106 of the 110 patients by asking two simple questions about flushing:<sup>(21,22)</sup> (a) Do you always flush in the face immediately after drinking a glass of beer (yes or no)? And (b) did you always flush in the face immediately after drinking a glass of beer during the first to second year after you started drinking (yes or no)? The designation 'current flushing' was applied to individuals who answered 'yes' to question (a) and 'former flushing' to those who answered 'no' to question (a) and 'yes' to question (b). The remaining subjects were classified as 'never flushing'. Current or former flushing individuals were assumed to have inactive ALDH2.

**BMI.** On their initial visit to the Center, each patient's body height was measured without shoes, and they were weighed wearing light clothing. The patients were classified into quartiles according to their BMI values.

**Blood testing.** During each patient's initial visit to the Center for the treatment of alcoholism we measured MCV by the electrical impedance method with an autoanalyzer (CELL-DYN 3500; Abbott, North Chicago, IL). MCV was measured in 80 patients within 7 days of their last drink. We then dichotomized them into an MCV <106 fl group and an MCV ≥106 fl group, because macrocytosis with an MCV value ≥106 fl within 7 days of taking their last alcoholic drink was found to be associated with an increased risk of esophageal SCC in our previous case-control study in Japanese alcoholic men.<sup>(6)</sup>

**ALDH2 and ADH1B genotyping.** Polymerase chain reaction-restriction fragment length polymorphism methods were used for ALDH2<sup>(23)</sup> and ADH1B<sup>(24)</sup> genotyping of lymphocyte DNA samples from all patients. To confirm the accuracy of genotype

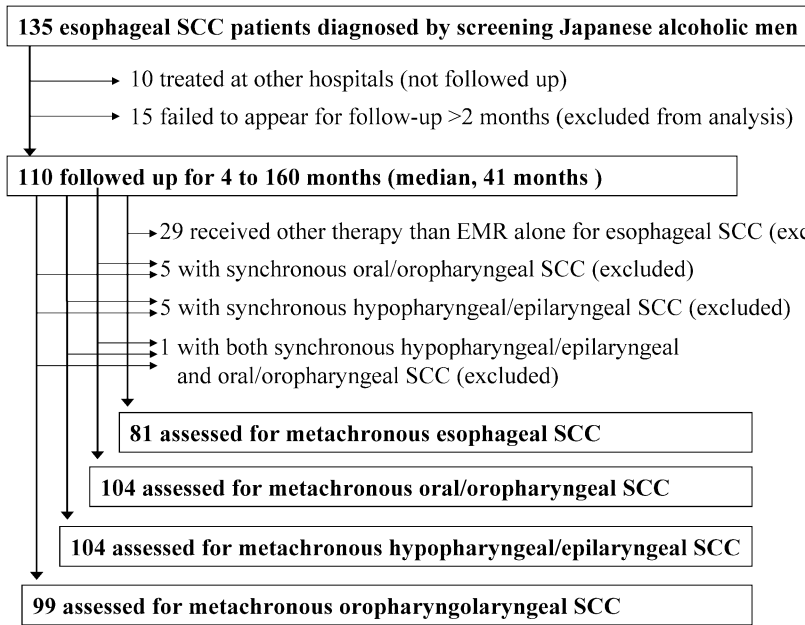


Fig. 1. Flow diagram tracking the numbers of participants in this study, detailing the subsets of subjects who were used for different analyses. EMR, endoscopic mucosal resection; SCC, squamous cell carcinoma.

Table 2. Metachronous primary squamous cell carcinoma (SCC) in the upper aerodigestive tract of Japanese alcoholic men with esophageal SCC

	Metachronous SCC			
	Esophagus	Oral cavity/oropharynx	Hypopharynx/epilarynx	Endolarynx
Subjects at baseline (n)	81	104	104	110
Events during follow-up (n)	29	19	13	2
Cancer depth				
Intraepithelium	20	8	5	1
Mucosa/muscularis mucosae (subepithelium <sup>†</sup> )	8	6	7	1
Submucosa	1	0	0	0
Proper muscle layer or deeper	0	5	1	0

<sup>†</sup>Oropharyngolarynx lacks the muscularis mucosae.

assignment, clear digestion patterns were used to determine the genotypes of all samples, and ALDH2 genotyping of each sample was carried out at least twice. Genotypes were determined without knowledge of the subjects' status.

**Statistical analysis.** Data are expressed as the mean ± SD, percentiles, or percentage. The cumulative percentages of patients with metachronous cancer in the UADT were calculated according to the Kaplan–Meier method, and groups were compared by the log–rank test. The Cox proportional hazards model was used to estimate the hazard ratio and 95% confidence interval (CI) after adjustment for confounding factors. Independent risk factors were selected by the stepwise procedure with  $P < 0.10$  for entry and removal. Age and alcohol drinking were forced into the model. Logistic regression analysis was used to compare categorical data between groups after adjustment for confounding factors, and the strength of associations was expressed as an odds ratio (OR) and 95% CI. All analyses were carried out using the SAS statistical package (version 9.1; SAS Institute, Cary, NC).

## Results

Table 2 shows the number and depth of invasion of metachronous primary SCC in the UADT. Follow-up endoscopy resulted in a diagnosis of metachronous primary esophageal SCC in 29 of the 81 patients whose initial esophageal SCC was treated by EMR alone. Metachronous SCC in the oral cavity/oropharynx was diagnosed in 19 of the 104 patients without SCC in the

sites at the time of the initial diagnosis, metachronous SCC in the hypopharynx/epilarynx in 13 of 104, and metachronous SCC in the endolarynx in 2 of 110. Ten of the patients had metachronous SCC in both the oral cavity/oropharynx and hypopharynx/epilarynx, and another patient with synchronous hypopharyngeal/epilaryngeal SCC had metachronous oral cavity/oropharyngeal SCC.

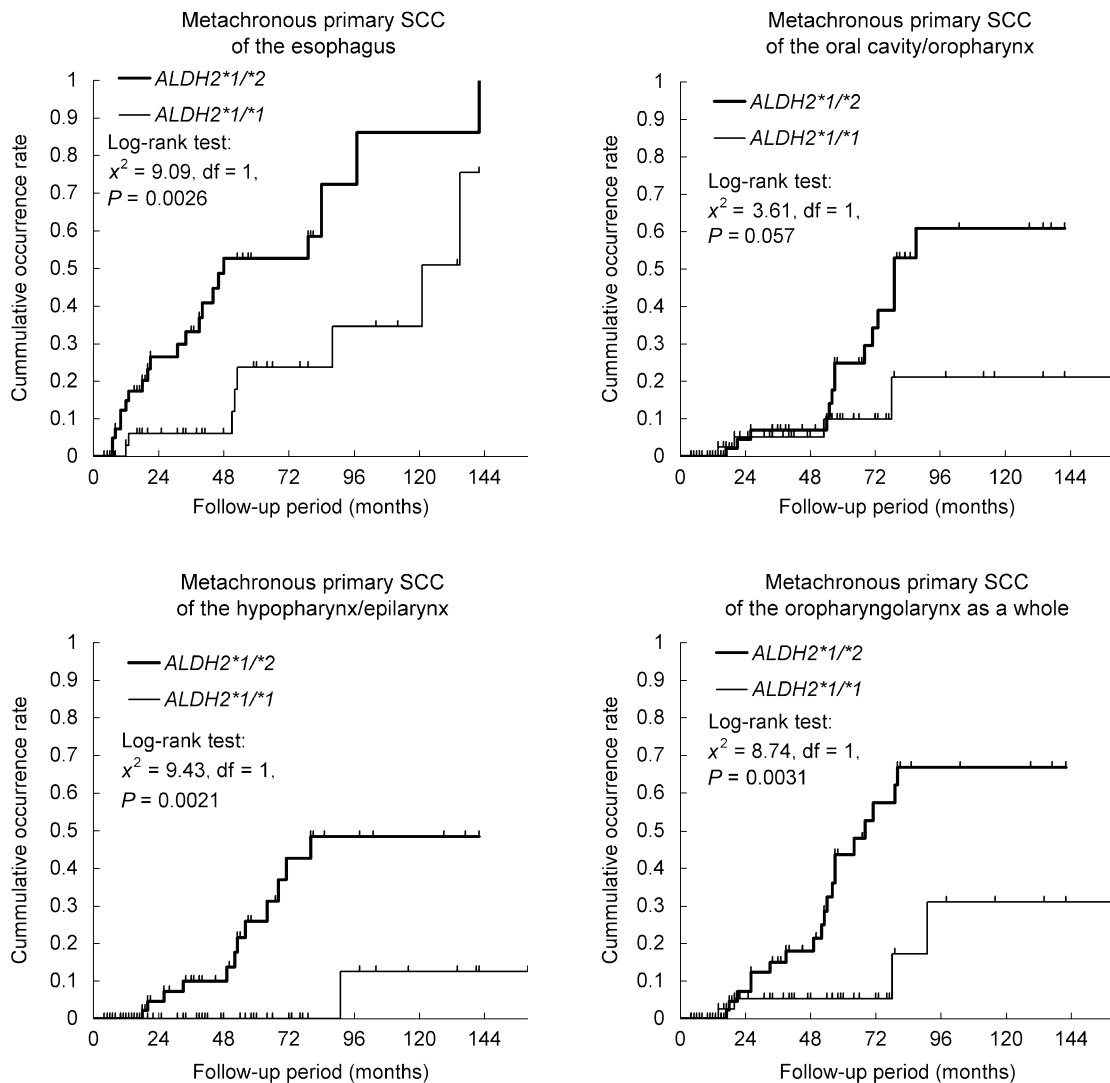
Thus, metachronous SCC in the oropharyngolarynx as a whole was diagnosed in 23 of the 99 patients without synchronous oropharyngolaryngeal SCC.

*ALDH2*\*1/\*2 was significantly associated with the presence of multiple intraesophageal SCC and the number of intraesophageal SCC at the time of the initial diagnosis (Table 3). Kaplan–Meier estimates of the proportions of patients with metachronous primary carcinoma of the UADT as a function of time are shown in Figs 2 and 3. Metachronous primary SCC of the esophagus, hypopharynx/epilarynx, and oropharyngolarynx as a whole was significantly more frequent among *ALDH2*\*1/\*2 heterozygotes than among *ALDH2*\*1/\*1 homozygotes, and among patients with multiple intraesophageal SCC at the baseline diagnosis than among those without it. A non-significant increase in the frequency of metachronous primary SCC of the oral cavity/oropharynx was observed among *ALDH2*\*1/\*2 heterozygotes ( $P = 0.057$ ) and patients with multiple intraesophageal SCC ( $P = 0.051$ ). These associations persisted after adjustment for age and alcohol drinking by the Cox proportional hazards model: the hazard ratios for SCC of the esophagus, oral cavity/oropharynx,

**Table 3. Relationship between aldehyde dehydrogenase-2 (ALDH2) genotype and number of multiple intraesophageal squamous cell carcinomas (SCC) at the baseline in 110 Japanese alcoholic men**

No. of multiple intraesophageal SCC at the baseline	n	ALDH2*1/*2 genotype		
		Prevalence, n (%)	Adjusted OR <sup>†</sup>	95% CI
1	76	34 (44.7)	1	Referent
2	20	15 (75.0)	3.68	(1.17–11.57)
3+	14	11 (78.6)	4.73	(1.15–19.47)
Total	110	60 (54.5)	P = 0.006 for trend	

<sup>†</sup>Adjusted for age, amount of alcohol consumed, and number of cigarettes smoked, according to the logistic regression model. CI, confidence interval; OR, odds ratio.



**Fig. 2.** Kaplan-Meier estimate of 110 Japanese alcoholic men with esophageal squamous cell carcinoma (SCC) who developed metachronous primary SCC in the upper aerodigestive tract, according to aldehyde dehydrogenase-2 (ALDH2) genotype.

hypopharynx/epilarynx, and oropharyngolarynx as a whole (Table 4) were 3.38 (95% CI: 1.45–7.85), 2.53 (0.82–7.86), 12.08 (1.52–95.89), and 4.27 (1.42–12.89), respectively, in *ALDH2*\*1/\*2 heterozygotes in comparison with *ALDH2*\*1/\*1 homozygotes; and 3.09 (1.41–6.78), 2.41 (0.96–6.05), 3.73 (1.22–11.41), and 3.25 (1.41–7.47) in patients with multiple intraesophageal SCC at the baseline compared to those without it. The adjusted hazard ratio for SCC of the esophagus was 2.90

(1.35–6.22) in the patients with current/former flushing compared with those who had never experienced flushing. None of the other factors evaluated in the present study affected the risk of metachronous SCC in the UADT when adjusted for age and alcohol drinking. A multivariate stepwise analysis showed that *ALDH2*\*1/\*2 genotype and multiple intraesophageal SCC at the baseline were independent risk factors for metachronous SCC in the esophagus, and that cigarette smoking and multiple

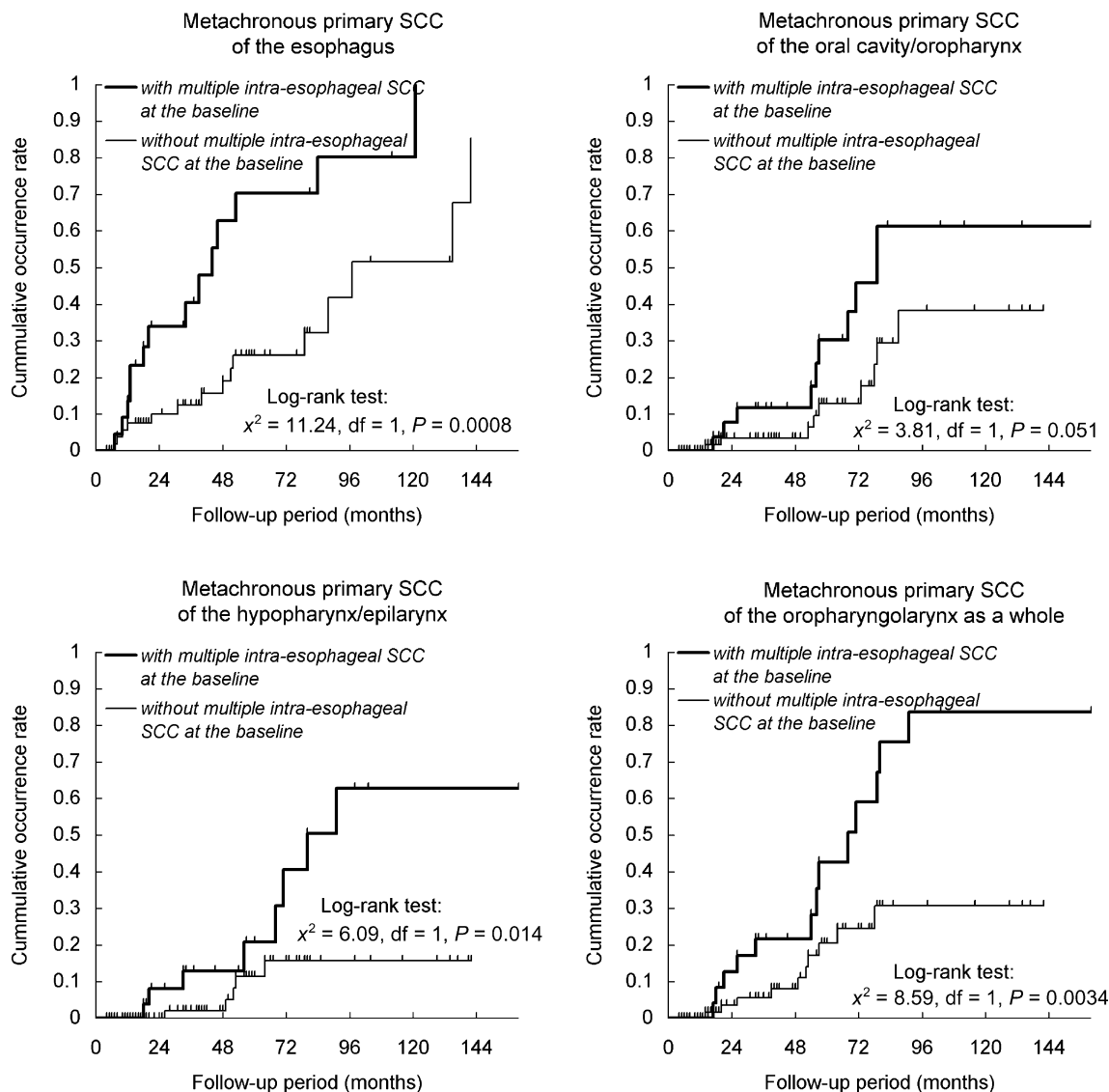


Fig. 3. Kaplan-Meier estimate of 110 Japanese alcoholic men with esophageal squamous cell carcinoma (SCC) who developed metachronous primary SCC in the upper aerodigestive tract, according to multiplicity of the initial esophageal cancer.

intraesophageal SCC at the time of the baseline were independent risk factors for metachronous SCC in the oropharyngolarynx as a whole (Table 4, bottom).

## Discussion

The results of this endoscopic follow-up study of Japanese alcoholic men with esophageal SCC showed very high prevalences of metachronous cancer in the UADT, that is, the cumulative rate for cancer in the oropharyngolarynx within 5 years were estimated as 39% in this alcoholic population, compared to 7% reported in a Japanese study of general patients after surgery for esophageal SCC.<sup>(25)</sup> The metachronous cancers tended to occur in several organs in the same alcoholic patients, suggesting a common strong genetic and environmental etiology of these cancers. Our previous short-term follow-up study after EMR of esophageal SCC showed a high prevalence of metachronous esophageal SCC among alcoholic men with *ALDH2\*1/\*2* and with synchronous multiple intraesophageal SCC,<sup>(5)</sup> and these findings were confirmed in this long-term follow-up study. The present study showed that the associated risks

of *ALDH2\*1/\*2* and esophageal cancer multiplicity extended to a risk of metachronous SCC in both the oropharyngolarynx as a whole and the hypopharynx/epilarynx in particular. *ALDH2\*1/\*2* has been consistently shown to be a strong risk factor for cancer multiplicity in Japanese patients with SCC in the UADT.<sup>(5,11-14)</sup> The results of the present study showed a positive association between *ALDH2\*1/\*2* and the number of intraesophageal SCC at the baseline. The presence of multiple areas of esophageal dysplasia, which is also associated with *ALDH2\*1/\*2*,<sup>(13,15)</sup> has been shown to increase the risk of metachronous SCC in the UADT in other follow-up studies of Japanese patients after EMR for esophageal SCC.<sup>(26,27)</sup> All of these findings suggest that *ALDH2\*1/\*2* plays a crucial role in multicentric or field cancerization throughout the entire mucosal surface of the UADT.

Individuals with inactive ALDH2 are incapable of rapidly eliminating acetaldehyde after consuming ethanol. Acetaldehyde has been established as a carcinogen in experimental animals,<sup>(28)</sup> and is suspected of playing a critical role in carcinogenesis in humans.<sup>(16)</sup> Higher levels of mutagenic acetaldehyde-derived DNA adducts have recently been shown in Japanese alcoholics

**Table 4. Risk factors for metachronous primary squamous cell carcinoma (SCC) in the upper aerodigestive tract of Japanese alcoholic men with esophageal SCC**

Risk factors	Metachronous primary SCC							
	Esophagus (29 cancers in 81 patients) <sup>†*</sup>		Oral cavity/ oropharynx (19 cancers in 104 patients) <sup>†§</sup>		Hypopharynx/ epilarynx (13 cancers in 104 patients) <sup>†§</sup>		Oropharyngolarynx as a whole (23 cancers in 99 patients) <sup>†§</sup>	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
<b>Adjusted for age and alcohol consumption</b>								
<i>ALDH2</i> *1/*2 versus *1/*1	3.38	(1.45–7.85)	2.53	(0.82–7.86)	12.08	(1.52–95.89)	4.27	(1.42–12.89)
<i>ADH1B</i> *1/*1 versus *1/*2 + *2/*2	0.85	(0.38–1.88)	0.52	(0.20–1.38)	2.39	(0.75–7.56)	0.82	(0.34–1.95)
Alcohol flushing versus non-flushing <sup>¶</sup>	2.90	(1.35–6.22)	1.02	(0.41–2.51)	0.90	(0.30–2.71)	0.97	(0.42–2.21)
MCV ≥106 versus <106 (fl) (≤7 days after the last drink)	1.15	(0.46–2.86)	2.43	(0.78–7.58)	1.41	(0.39–5.11)	1.56	(0.59–4.16)
Multiple versus solitary intraesophageal SCC at baseline	3.09	(1.41–6.78)	2.41	(0.96–6.05)	3.73	(1.22–11.41)	3.25	(1.41–7.47)
BMI ≥22.2 versus <22.2 (kg/m <sup>2</sup> ) <sup>††</sup>	0.90	(0.40–2.07)	0.41	(0.12–1.44)	1.62	(0.50–5.25)	0.79	(0.30–2.09)
Smoking, per 10+ cigs/day	1.08	(0.85–1.38)	1.09	(0.87–1.35)	0.86	(0.59–1.24)	1.29	(0.98–1.70)
<b>Adjusted for age, alcohol consumption, and all selected variables<sup>†††</sup></b>								
<i>ALDH2</i> *1/*2 versus *1/*1	3.22	(1.36–7.64)	—	—	12.08	(1.52–95.89)	—	—
Multiple versus solitary intraesophageal SCC at baseline	2.84	(1.30–6.24)	2.41	(0.96–6.05)	—	—	4.27	(1.79–10.16)
Smoking, per 10+ cigs/day	—	—	—	—	—	—	1.46	(1.08–1.97)

<sup>†</sup>No. of subjects analyzed for alcohol flushing and mean corpuscular volume (MCV) (e.g. 72 and 55, respectively, for esophagus) was smaller because of missing values. <sup>‡</sup>Patients treated by methods other than endoscopic mucosal resection alone at baseline were excluded. <sup>§</sup>Patients who had oropharyngolaryngeal cancer at baseline were excluded. <sup>¶</sup>Alcohol flushing was classified on the basis of the results of an alcohol flushing questionnaire; <sup>††</sup>Selected by the stepwise procedure with  $P < 0.10$  from all risk factors listed above except alcohol flushing and MCV because of missing values. Age and alcohol consumption were forced into the model. Body mass index (BMI) and alcohol dehydrogenase-1B (*ADH1B*) genotype were not selected for any cancer site. <sup>†††</sup>75th percentile among all patients. —, not selected; CI, confidence interval; HR, hazard ratio.

with inactive *ALDH2* compared with active *ALDH2*.<sup>(29)</sup> Very high salivary acetaldehyde levels have also been shown in alcoholics, and they were found to be partly attributable to increased salivary acetaldehyde production as a result of oral microorganism overgrowth in addition to heavy alcohol consumption.<sup>(30)</sup> After a moderate oral dose of alcohol the salivary acetaldehyde levels of persons with inactive *ALDH2* are two to three times higher than those of persons with active *ALDH2*.<sup>(31)</sup> Inefficient degradation of the acetaldehyde in inactive *ALDH2* heterozygotes might result in a very high level of accumulation in the mucosa of the UADT after a high level of exposure to acetaldehyde, because *ALDH2* expression in the UADT is extremely weak, if present at all.<sup>(32)</sup> Thus it is reasonable to speculate that the high risk of metachronous SCC in the UADT in persons with inactive *ALDH2* is the consequence of repeated high exposure of the UADT to acetaldehyde.

Although high acetaldehyde exposure due to *ALDH2*\*1/\*2 contributes to the development of synchronous multiple intraesophageal SCC, a multivariate analysis showed that the presence of the multiple intraesophageal SCC and *ALDH2*\*1/\*2 independently increased the risk of metachronous intraesophageal SCC after EMR for the initial esophageal SCC, and an analysis by the stepwise procedure showed a predominant effect of multiple intraesophageal SCC over *ALDH2*\*1/\*2 in increasing the risk of metachronous SCC in the oropharyngolarynx as a whole. Other factors associated with the presence of multiple intraesophageal SCC in alcoholics' lifestyle or in their hereditary traits might also be associated with a higher risk of metachronous SCC in the UADT.

*ALDH2*\*1/\*2 and the initial multiplicity of intraesophageal SCC increased the risk of metachronous hypopharyngeal/epilaryngeal SCC (hazard ratios = 12.08 and 3.73, respectively), but the positive associations for oral cavity/oropharyngeal SCC did not reach the level of significance (hazard ratios = 2.53 and 2.41, respectively). However, 10 of the 13 hypopharyngeal/epilaryngeal SCC patients also had metachronous SCC in the

oral cavity/oropharynx, and all 10 patients had *ALDH2*\*1/\*2. Previous case-control studies of Japanese alcoholics have shown similarly strong associations between *ALDH2*\*1/\*2 and both oral cavity/oropharyngeal SCC and hypopharyngeal/epilaryngeal SCC.<sup>(4,33)</sup> However, the site-specific difference observed is consistent with the results of previous Japanese case-control studies of oral and pharyngeal SCC conducted in a general population.<sup>(34)</sup> The mechanisms of metachronous carcinogenesis might differ among anatomically different sites.

Less-active *ADH1B*\*1/\*1, MCV ≥ 106 fl, and low BMI are independent risk factors for SCC in the UADT in Japanese alcoholic men,<sup>(4,6,7)</sup> but none of them were associated with an increased risk of metachronous cancer development among the alcoholics with esophageal SCC. Ethanol and acetaldehyde persist in the saliva for longer periods in alcoholics with less-active *ADH1B*\*1/\*1 than in alcoholics with other genotypes.<sup>(30)</sup> Macrocytosis in alcoholics has many causes, including heavy drinking, acetaldehyde exposure, aging, smoking, and poor nutritional status.<sup>(7)</sup> Alcoholics' low BMI is probably attributable to poor dietary habits that lead to vitamin deficiencies and suppressed immune function. A very high level of exposure of the UADT to acetaldehyde in persons with *ALDH2*\*1/\*2 could affect the risk of metachronous SCC in the UADT in a different manner from factors associated with less-active *ADH1B*\*1/\*1, MCV ≥ 106 fl, and low BMI. Our previous studies of Japanese alcoholics have also shown that the magnitude of the increase in risk of UADT cancer as a result of having inactive *ALDH2*\*1/\*2 was much greater than the increase in risk due to the less-active *ADH1B*\*1/\*1, MCV ≥ 106 fl, and low BMI<sup>(4,6,7)</sup> and that, of the three factors of inactive *ALDH2*\*1/\*2, less-active *ADH1B*\*1/\*1, and MCV ≥ 106 fl, only inactive *ALDH2*\*1/\*2 was linked to synchronous multiplicity of UADT cancer in Japanese alcoholics with esophageal SCC.<sup>(14)</sup>

Current/former flushing was associated with metachronous SCC in the esophagus, but not with metachronous SCC in the oropharyngolarynx. This finding is mainly explained by the

lower sensitivity of alcohol flushing as a marker of inactive ALDH2 in alcoholics with less-active *ADH1B\*1/\*1*.<sup>(21,22)</sup> When current/former flushing individuals were assumed to have inactive ALDH2, the sensitivity of current/former flushing for identifying inactive ALDH2 was 52% in the esophageal SCC alcoholics with less-active *ADH1B\*1/\*1*. Among the 23 patients with metachronous oropharyngolaryngeal SCC, 9 of the 19 *ALDH2\*1/\*2* heterozygotes had *ADH1B\*1/\*1*, and 6 of these 9 reported never having experienced alcohol flushing.

As alcoholic patients often fail to appear for regular follow-up examinations, especially when they relapse into alcohol use, we are unable to report the true incidence of metachronous carcinoma in the entire cohort, however, the reasons for the follow-up examinations included readmission because of a relapse of alcoholism. The background characteristics of the follow-up patients might have affected their risk of metachronous SCC compared to the patients who stopped appearing for follow-up examinations. Our endoscopic follow-up examinations detected metachronous carcinoma in the very early stages, and only 3 of the metachronous SCC patients had symptoms attributable to the SCC lesions prior to the follow-up screening. Thus, it is unlikely that the symptomatic cases biased the results greatly.

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