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CLINICAL—ALIMENTARY TRACT

Alcohol Consumption and Multiple Dysplastic Lesions Increase Risk of Squamous Cell Carcinoma in the Esophagus, Head, and Neck

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This article has an accompanying continuing medical education activity, also eligible for MOC credit, on page e16. Learning Objective: Upon completion of this examination, successful learners will be able to recognize and identify the key and noticeable features associated with the prevention and pathophysiology of Esophageal Squamous Cell Carcinoma (ESCC).

See Covering the Cover synopsis on page 777.

BACKGROUND & AIMS: Some patients develop multiple squamous cell carcinomas (SCCs) in the upper aerodigestive tract, attributed to field cancerization; alcohol consumption has been associated with this process. We examined the association between multiple areas of dysplastic squamous epithelium with the development of SCC of the esophagus or head and neck cancer, as well as alcohol consumption and smoking. **METHODS:** We examined 331 patients with early stage esophageal SCC using Lugol chromoendoscopy to evaluate the dysplastic squamous epithelium in the esophagus. Patients then were assigned to 3 groups, based on the number of Lugol-voiding lesions: A, no lesion; B, 1–9 lesions; or C, 10 or more lesions. Participants completed lifestyle surveys on their history of drinking, smoking, and diet. All participants were evaluated by laryngopharyngoscopy before registration; only those without head and neck cancer were included, except for patients with superficial SCC limited to the subepithelial layer. Lesions detected in the esophagus and head and neck by surveillance were considered to be metachronous. The study end point was the cumulative incidence of metachronous SCCs in the esophagus and head and neck after endoscopic resection of esophageal SCC, according to the grade of Lugol-voiding lesions. At study entry, all patients were instructed to abstain from alcohol and smoking. **RESULTS:** Over the 2-year study period, metachronous SCCs of the esophagus were detected in 4% of patients in group A, in 9.4% of patients in group B, and in 24.7% of patients in group C ($P < .0001$ for patients in group A vs B or B vs C). Head and neck SCCs were detected in none of the patients in group A, in 1.7% of the patients in group B, and in 8.6% of the patients in group C ($P = .016$ for patients in group A vs C and $P = .008$ for patients in group B vs C). SCC of the esophagus or head and neck developed in 4.0% of patients in group A, in 10.0% of patients in group B, and in 31.4% of patients in group C ($P < .0001$ for group A vs B or A vs C). Alcohol abstinence decreased the risk of multiple SCCs of the esophagus (adjusted hazard ratio, 0.47, 95% confidence interval, 0.25–0.91; $P = .025$), whereas smoking abstinence did not. **CONCLUSIONS:** Multiple dysplastic lesions in the esophagus increase the risk of multiple SCCs. Alcohol abstinence reduces the risk of metachronous SCCs. Clinical Trials registry: UMIN000001676 and UMIN000005466.

Keywords: Drinking Alcohol; Carcinogenesis; Risk Factor; Genetics Methods.

Synchronous and metachronous development of squamous cell carcinoma (SCC) in the upper aerodigestive tract including the esophagus and the head and neck region has been referred to as the phenomenon of "field cancerization"[1] and adversely affects patient survival.[2] Recent endoscopic imaging technology such as narrow-band imaging can detect early SCC, and minimally invasive endoscopic treatment can achieve cure and organ preservation.[3–6] Conversely, cancer survivors after curative treatment are at risk for the development of metachronous multiple SCCs in the preserved organ. However, little is known about the interval from the first SCC to the metachronous SCC and the risk of metachronous SCC.

Squamous dysplasia has been believed to be a preneoplastic lesion of SCC, and it is identified easily as Lugol-voiding lesions (LVL) on Lugol chromoendoscopy.[7,8] Some patients with esophageal SCCs have multiple LVLs in the background esophageal mucosa.[9–13] However, prospective data on the association between the grade of LVL and the risk of metachronous SCC are lacking. In addition, mutation of the *TP53* gene in the background esophageal mucosa was shown as an early event of esophageal carcinogenesis. Moreover, *TP53* gene mutations were detected even in the microscopically normal epithelium.[14] However, data on the association between the grade of LVL and *TP53* status also are lacking. Follow-up data on LVL might provide important information about the risk of metachronous SCC.

Although field cancerization is associated closely with alcohol consumption and insufficient alcohol metabolism,[15,16] the benefit of alcohol abstinence on the field cancerization phenomenon remains unclear. In this study, we evaluated the cumulative incidence of metachronous multiple SCCs in the esophagus and the head and neck region according to the grade of LVL, as well as the risk factors of metachronous multiple SCCs and the effect of alcohol and smoking abstinence on field cancerization.

Methods

Study Design

From September 2005 through May 2010, we prospectively recruited patients from 16 hospitals throughout Japan. This cohort study was approved by the institutional review board at each hospital, and we obtained written informed consent from all patients (UMIN Clinical Trials Registry ID: UMIN000001676). The genetic analysis also was approved by the Institutional Review Board of Kyoto University Hospital, and all participants provided written informed consent (UMIN Clinical Trials Registry ID: UMIN000005466).

Study Population

Inclusion criteria of this cohort study were as follows: (1) newly diagnosed early SCC of the esophagus; (2) complete endoscopic resection by endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD); (3) tumor-negative vertical margins of resected specimens; (4) tumor invasion limited to the mucosa on histopathologic examination of resected specimens; (5) no additional treatment (surgical resection, radiotherapy, chemotherapy, and so forth) immediately after EMR or ESD; (6) no active head and neck cancer; (7) patients were evaluated by their attending physicians to be in good general condition, allowing at least 2 years of follow-up evaluation; and (8) written informed consent obtained from the patient. Exclusion criteria were as follows: (1) a history of chemotherapy for any other cancer or a history of surgical treatment or radiotherapy for head and neck cancer; (2) a history of iodine allergy; and (3) patients whom the investigator considered unsuitable as subjects.

We also prospectively examined *TP53* mutations in the background esophageal mucosa in other patients with early esophageal SCC who were treated at Kyoto University Hospital.

Grading of LVL

LVL was graded according to the number of LVLs per endoscopic view (A, no lesions; B, 1–9 lesions; C, $\geq$10 lesions) (Figure 1). Endoscopic images obtained from eligible patients at study entry were reviewed centrally in a blinded fashion by 3 endoscopists to determine the grade of LVL.

Survey of Lifestyle and Flushing Reaction After Drinking

At study entry, lifestyle surveys were conducted using a self-administered questionnaire (Supplementary Figure 1). The histories of drinking, smoking, and consumption of high-temperature foods, green-yellow vegetables, and fruit were documented carefully. The same questionnaire was used to obtain detailed information on each subject. Patient reports of facial flushing after drinking alcohol were presumed to be markers of insufficient alcohol metabolism owing to inactive aldehyde dehydrogenase-2 (ALDH2) genotype (present and past flushing were indicative of inactive ALDH2 and never flushing was indicative of active ALDH2).[17]

Definition of Synchronous and Metachronous Cancer

All candidates were surveyed by laryngoscopy by an otolaryngologist to confirm whether they had synchronous multiple cancers in the head and neck region before registration. The surveillance interval was within 6 months. Only patients without active head and neck cancer were eligible, except for patients with superficial SCCs limited to within the

Abbreviations used in this paper: **ALDH2, aldehyde dehydrogenase-2; CI, confidence interval; EMR, endoscopic mucosal resection; ESD, endoscopic submucosal dissection; LVL, Lugol voiding lesion; RR, relative risk; SCC, squamous cell carcinoma.**

Most current article

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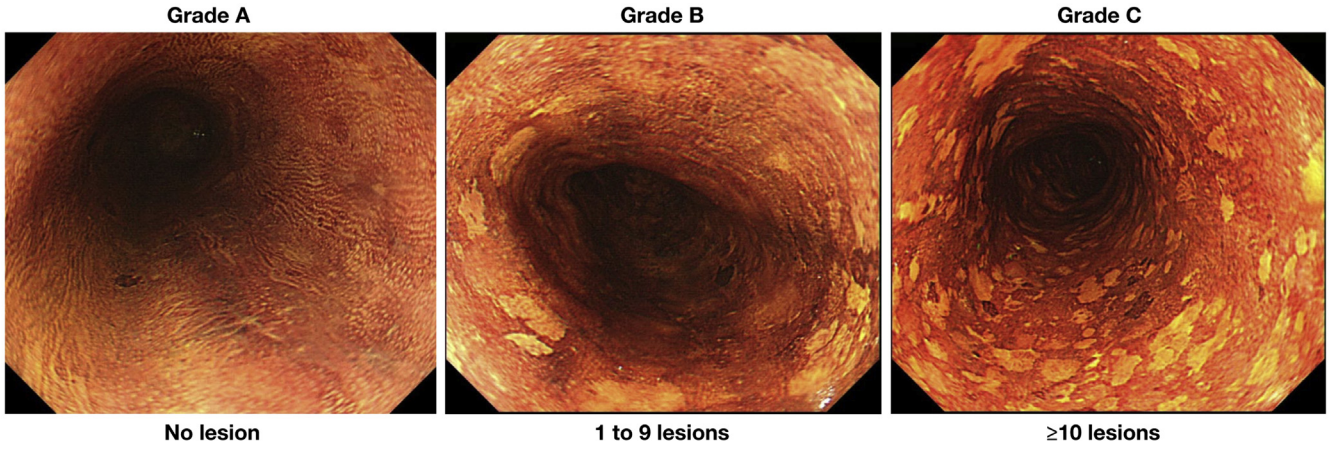


Figure 1. Grading of LVLs. The number of LVLs per endoscopic view was counted, and the grading was divided into the following 3 categories: (Group A) grade A, no lesions; (Group B) grade B, 1-9 lesions; and (Group C) grade C, 10 or more lesions.

subepithelial layer. Lesions detected by surveillance before registration and lesions including the esophagus were defined as *synchronous* lesions. After registration, all of the patients were followed up by a planned schedule according to the protocol. If a new lesion was detected at the first follow-up evaluation (3 months after registration) or thereafter, such a lesion was defined as a *metachronous* lesion.

Follow-Up Examinations

Endoscopic examinations of the head and neck, esophagus, and stomach, as well as Lugol chromoendoscopy, were performed at 3-month intervals for up to 6 months after EMR or ESD. Subsequently, these examinations were repeated every 6 months. The head and neck region was examined by laryngoscopy by an otolaryngologist at the time of EMR or ESD and at 1-year intervals thereafter.[18] In addition to otolaryngologists, gastrointestinal endoscopists also examined the oropharynx and hypopharynx during surveillance endoscopy.

Alcohol and Smoking Abstinence

At study entry, the physicians in charge handed a document describing the importance of alcohol and smoking abstinence to all patients and verbally instructed them to stop drinking and smoking. At each examination, we surveyed smoking status (nonsmoker or smoker; for smokers, the number of cigarettes smoked per day) and drinking status (nondrinker or drinker; for drinkers, the drinking frequency and amount of alcohol consumed) by self-reporting and instructed patients not to drink or smoke.

Histologic Analysis

Although the diagnosis of invasive SCC did not usually differ among the pathologists, the diagnosis of high-grade intraepithelial neoplasia sometimes was discordant. To control these differences, lesions with a diagnosis of high-grade intraepithelial neoplasia were reviewed centrally in a blinded fashion by 3 pathologists. Lesions present at enrollment as well as new lesions were examined. Lesions not diagnosed to be high-grade intraepithelial neoplasia or SCCs on central review were excluded from this analysis.

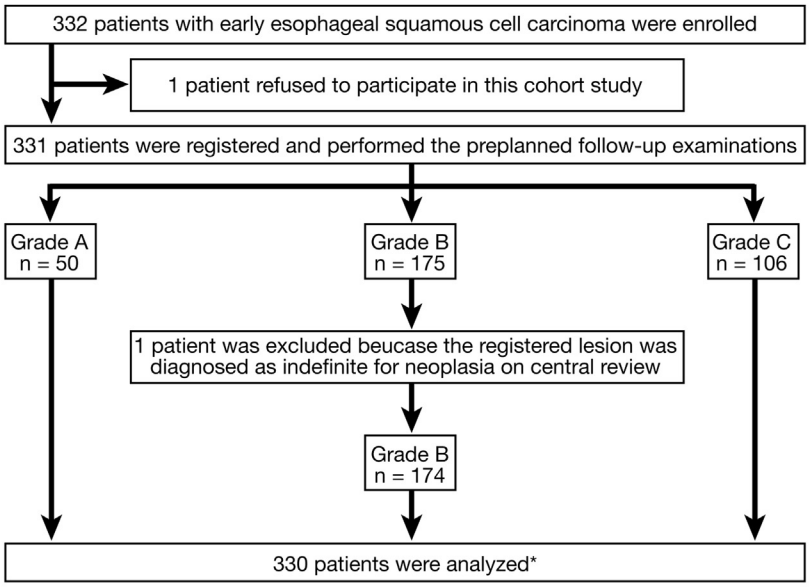
TP53 *Mutation in Background Esophageal Mucosa*

Biopsy specimens were taken from noncancerous background esophageal mucosa. Genomic DNA was extracted using the Gentra Puregene Tissue Kit protocol (Qiagen, Inc, Hilden, Germany) with several modifications, and a low frequency of *TP53* mutations was detected by deep sequencing of the amplicons by using the TruSeq Amplicon Cancer Panel (Illumina, Inc, San Diego, CA) to sequence mutational hotspots. This cancer panel includes hotspots of *TP53* mutation, but not all *TP53* exons. The amplicon libraries were subjected to massively parallel sequencing using MiSeq (Illumina, Inc) with 170-bp paired-end reads. The sequencing data were imported to the CLC Genomics Workbench software (version 6.0.1; CLC bio, Aarhus, Denmark), and low-quality data were trimmed by a quality limit of 0.001. The cleaned-up reads were mapped to the human genome (GRCh37_dbSNP135), and the somatic mutations were detected by the Quality-based Variant Detection command.

Statistical Analysis

We examined whether the grade of LVL was associated with the development of metachronous multiple SCCs in the esophagus and head and neck region. The number of patients required to detect a difference between grades A and B with a statistical power of 80% and a significance level of 5% was estimated assuming that the cumulative rate of metachronous SCCs in grades A, B, and C, would be 1%, 10%, and 30%, respectively, with a patient ratio of 4:4:2 and a drop-out rate of 10%. The total number of patients required was estimated to be 292. Under these conditions, the statistical power to detect a difference between grades A and C would be 99% or greater (significance level, 5%). The planned number of enrolled patients was set at 330, taking into account drop-outs and ineligible patients.

The Kaplan–Meier method and the log-rank test were performed for the analysis of development of a second primary SCC in the esophagus and the head and neck region. We



*The following 39 patients stopped the follow-up examinations

	Grade A (n = 3)	Grade B (n = 24)	Grade C (n = 12)	Total (n = 39)
Development of lesions in the esophagus that were considered to require surgical resection, radiotherapy, or chemotherapy	0	2	5	7
Development of second cancers in another organ that were considered to require chemotherapy	0	5	1	6
Follow-up observation were judged to be difficult by the physician	3	17	6	26

Figure 2. Enrollment and subjects for analysis.

defined the time to development from the day of endoscopic treatment to the day of endoscopic diagnosis of a second primary SCC. The person-year method was used to calculate the total number of multiple cancers arising in the esophagus and head and neck per 100 person-years (ie, a patient could have multiple events); the Poisson regression analysis was used to estimate the sex- and age-adjusted relative risk (RR) of all arising events. A Cox proportional-hazards model was used to estimate the sex- and age-adjusted hazard ratio (RR) and 95% confidence interval (CI) of the primary event. The analysis of end points was conducted after the completion of at least 2 years of follow-up observation for all patients from the time of enrollment. Data on patients in whom follow-up examinations were not completed were censored at the last observation. All data were analyzed with SAS (version 9; SAS Institute, Inc, Cary, NC). All authors had access to the study data and reviewed and approved the final manuscript.

Results

Study Population

A total of 332 patients with early esophageal SCC were enrolled. One patient refused to participate in this cohort study. The other 331 patients were registered and underwent the preplanned follow-up examinations. One patient was excluded because the lesion was not diagnosed definitively as high-grade intraepithelial neoplasia or invasive SCC on central review of the pathologic assessment (Figure 2). Table 1 summarizes the baseline characteristics of the 330 patients according to the grade of LVL. The median follow-up period was 49.4 months (range, 1.3–81.2 mo). The proportion of men was higher in the more severe LVL grades (62.0%, 85.1%, and 93.4%, respectively; $P < .0001$ for A or B vs C). The largest subgroup according to age was 60 to 69 years in grade C and 70 years or older in grades A and B. After adjusting for sex and age, the LVL grade was associated with progressively higher proportions of heavy drinkers (27.7%, 26.2%, and 52.8%, respectively; $P < .0001$ for trend, vs others), heavy smokers (41.1%, 65.9%, and 71.0%, respectively; $P < .0001$ for trend), not eating green-yellow vegetables almost every day (52.0%, 54.9%, and 71.3%, respectively; $P = .021$ for trend), and patients with a low body mass index (23.4%, 32.0%, and 37.4%, respectively; $P = .027$ for trend).

Primary and Secondary Outcomes

LVL grade was associated with progressive increases in the 2-year cumulative incidence of metachronous multiple SCCs (esophagus: 4.0%, 9.4%, and 24.7%, respectively; $P < .0001$ for A or B vs C; head and neck region: 0.0%, 1.7%, and 8.6%, respectively; $P = .016$ for A vs C and $P = .008$ for B vs C; both: 4.0%, 10.0%, and 31.4%, respectively; $P < .0001$ for A or B vs C) (Figure 3).

Table 1. Clinical Characteristics of Patients According to the LVL Grade

	LVL grade			
	A (n = 50)	B (n = 174)	C (n = 106)	*P*
Sex				
Male, %	62.0%	85.1%	93.4%	<.0001
Age, *y*				
40–59	22.0%	17.2%	19.8%	
60–69	36.0%	36.8%	55.7%	
≥70	42.0%	46.0%	24.5%	.005 [a]
Means ± SD	66.4 ± 9.9	67.7 ± 8.6	64.8 ± 6.5	.018 [a]
	Sex- and age-adjusted % or mean			
Drinking alcohol[b,c]				
Never/rare	25.9%	7.9%	4.7%	
Light	13.8%	21.4%	8.9%	
Moderate	27.8%	30.9%	25.5%	
Heavy[d]	27.7%	26.2%	52.8%	
Ex-drinker	4.7%	13.6%	8.2%	<.0001 [a]
Means ± SE[e]	10.4 ± 1.8	13.1 ± 0.9	19.9 ± 1.2	<.0001 [f]
Strong alcoholic beverages[b]				
Frequently	6.7%	11.8%	12.2%	
Sometimes	15.1%	20.1%	23.2%	
Never	78.2%	68.1%	64.6%	.17 [f]
Smoking, pack-years[b]				
Never, 0	22.7%	14.8%	12.4%	
Light, <30	36.2%	19.3%	16.6%	
Heavy, ≥30	41.1%	65.9%	71.0%	<.0001 [f]
High-temperature food[b]				
Likes very much	18.4%	20.8%	20.6%	
Likes somewhat	24.5%	25.1%	24.0%	
Neither likes nor dislikes	45.8%	41.8%	36.8%	
Dislikes somewhat	9.3%	9.9%	15.9%	
Dislikes very much	2.0%	2.3%	2.7%	.81 [f]
Green-yellow vegetables[b]				
Seldom	0.0%	3.8%	2.1%	
1–2 d/mo	3.8%	5.7%	5.5%	
1–2 d/wk	20.5%	16.5%	35.3%	
3–4 d/wk	27.7%	29.0%	28.4%	
Almost every day	48.0%	45.1%	28.7%	.021 [f]
Fruit[b]				
Seldom	11.1%	9.6%	12.8%	
1–2 d/mo	5.2%	15.2%	12.6%	
1–2 d/wk	33.4%	22.9%	33.1%	
3–4 d/wk	26.2%	20.2%	17.0%	
Almost every day	24.1%	32.0%	24.5%	.31 [f]
Body mass index, kg/m^2[g]				
<21.2	23.4%	32.0%	37.4%	
21.2–23.6	18.8%	37.1%	33.9%	
≥23.7	57.8%	30.9%	28.7%	.003 [f]
Means ± SE	23.5 ± 0.44	22.3 ± 0.24	22.1 ± 0.31	.027 [f]
Alcohol flushing[b]				
Never flushing	35.2%	31.5%	40.6%	
Flushing[h]	64.8%	68.5%	59.4%	.12 [a]

[a] *P* for trend across the 3 groups.
[b] Percentage values were adjusted for sex and age by direct method (using all patients as the standard population) and statistically tested by the Cochran–Mantel–Haenszel test. Mean values were adjusted for sex and age and were statistically tested by analysis of covariance.
[c] Never/rare, <1 U/wk; light, 1–8.9 U/wk; moderate, 9–17.9 U/wk; heavy, ≥18 U/wk (1 U = 22 g ethanol).
[d] $P < .0001$ for trend, vs others.
[e] Excluding ex-drinkers.
[f] *P* for homogeneity among the 3 groups.
[g] Categorized by the overall tertile. Missing data for 1 patient.
[h] Present or past flushing.

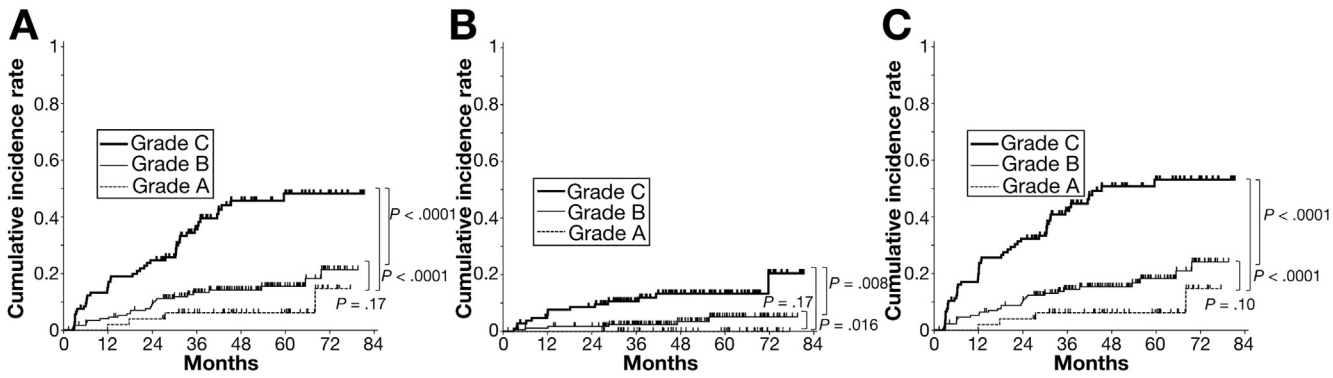


Figure 3. Cumulative incidence of metachronous multiple SCCs according to the LVL grade: (*A*) esophagus, (*B*) head and neck region, and (*C*) esophagus and head and neck region.

LVL grade was associated with progressive increases in the total number of metachronous multiple SCCs per 100 person-years (esophagus: RR, 1.91 and 7.41 for grades B and C, respectively, vs grade A; $P < .0001$ for trend; head and neck region: RR, 0.00 and 5.74 for grades A and C, respectively, vs grade B; $P < .0001$ for trend; both: RR, 2.19 and 9.05 for grades B and C, respectively, vs grade A; $P < .0001$ for trend) (Table 2). The frequency of events was notably high in grade C (18.9, 5.8, and 24.7 per 100 person-years in esophagus, head and neck region, and both, respectively).

Grade C LVL was the most significant predictor of metachronous multiple SCCs on analysis with a univariate or multivariate Cox proportional-hazards model (esophagus: multivariate RR, 8.76; 95% CI, 3.02–25.5; for C vs. A; head and neck region: multivariate RR, 3.51; 95% CI, 1.34–9.20; for C vs B; both: multivariate RR, 9.78; 95% CI, 3.41–28.0; for C vs A) (Table 3). On multivariate analysis, an alcohol flushing reaction after drinking was also a predictor of metachronous esophageal SCC (RR, 1.07; 95% CI, 1.02–2.82) and esophageal and head and neck SCC (RR, 1.60; 95% CI, 1.00–2.57). Small or large body mass index also was associated with metachronous esophageal SCC (RR, 2.09; 95% CI, 1.14–3.83; and RR, 1.86; 95% CI, 1.00–3.47, respectively) and esophageal and head and neck SCC (RR, 1.73; 95% CI, 0.99–3.02; and RR, 1.55; 95% CI, 0.88–2.73, respectively).

TP53 *Gene Alteration in Background Esophageal Mucosa*

To examine whether mutations of genes such as *TP53* occurred in the noncancerous esophageal mucosa with multiple LVLs and whether the mutation frequency was associated with the severity of LVLs, we performed deep sequencing of the *TP53* gene amplicon using biopsy specimens of noncancerous esophageal mucosa in patients with or without multiple LVLs. These samples were obtained from 38 early esophageal SCC cases independent from our present cohort study. The clinical characteristics are summarized in Supplementary Tables 1 and 2, and the detected low frequency of *TP53* mutations (≥2% of total reads) are listed in Supplementary Table 3. Our study showed that the number of cases that had a *TP53* mutation was 3 of 7 (42.9%) in grade A, 5 of 9 (55.6%) in grade B, and 17 of 22 (77.3%) in grade C (Supplementary Figure 2). Of note, several mutations (2–6 mutations) of the *TP53* gene were found simultaneously in biopsy specimens of noncancerous esophageal mucosa in patients with grade C LVL (Supplementary Figure 2). The median mutation frequency of *TP53* genes in biopsy specimens was 0%, 2.0%, and 10.1% in grades A, B, and C, respectively. The median in grade C was significantly higher than that in grade A ($P = .04$) (Supplementary Figure 3).

Effect of Alcohol and Smoking Abstinence on the Development of Metachronous SCC

As shown in Supplementary Figure 4, 69 (30.9%) of 223 patients who drank 1 U/day (22 g of ethanol) or more at baseline abstained from drinking alcohol, and 69 (53.5%) of 129 patients who had smoked at baseline abstained from smoking. The cumulative incidence of metachronous multiple SCCs in the esophagus was shown according to the status of drinking alcohol and smoking (Figure 4). Hazard ratios were adjusted for sex, age, LVL grade, alcohol intake at baseline, and alcohol flushing.

Alcohol abstinence decreased the risk of developing metachronous multiple SCCs of the esophagus (adjusted hazard ratio, 0.47; 95% CI, 0.25–0.91; $P = .025$) (Figure 4*A*). The risk reduction was especially large in grade C LVL (adjusted hazard ratio, 0.23; 95% CI, 0.09–0.60; $P = .003$) (Figure 4*B*). In contrast, smoking abstinence did not decrease the risk of developing metachronous multiple SCCs of the esophagus (all: adjusted hazard ratio, 0.59; 95% CI, 0.29–1.18; $P = .13$; grade C LVL: adjusted hazard ratio, 0.73; 95% CI, 0.32–1.67; $P = .45$) (Figure 4*C* and *D*, respectively).

Discussion

We found that patients with grade C LVL were at significant risk for the development of metachronous multiple SCCs in the esophagus and the head and neck region. Furthermore, *TP53* mutation in the background esophageal mucosa was associated with an increased grade of LVL. These results clearly supported the theory of field cancerization in which genetic alteration in the background

Table 2. Person-Years and Number of Primary and All Events According to the Grade of LVL

	LVL grade			
	A (n = 50)	B (n = 174)	C (n = 106)	Total (n = 330)
Esophagus				
Primary events				
Events, n	4	26	44	74
Person-years	208.1	652.0	327.1	1187.2
Per 100 person-years	1.9	4.0	13.5	6.2
RR[a]	1	2.32	7.68	$P < .0001$
95% CI	Referent	0.79–6.82	2.64–22.3	for trend
All events				
Events, n	6	33	85	124
Person-years	215.6	703.2	449.9	1368.6
Per 100 person-years	2.8	4.7	18.9	9.1
RR[b]	1	1.91	7.41	$P < .0001$
95% CI	Referent	0.78–4.69	3.12–17.6	for trend
Head and neck				
Primary events				
Events, n	0	6	14	20
Person-years	215.6	687.0	407.9	1310.4
Per 100 person-years	0.0	0.9	4.1	1.5
RR[a]	0.00	1	3.64	$P = .002$
95% CI	Incalculable	Referent	1.39–9.52	for trend
All events				
Events, n	0	7	26	33
Person-years	215.6	703.2	449.9	1368.6
Per 100 person-years	0.0	1.0	5.8	2.4
RR[b]	0.00	1	5.74	$P < .0001$
95% CI	Incalculable	Referent	0.08–0.40	for trend
Esophagus, head and neck				
Primary events[c]				
Events, n	4	29	50	83
Person-years	208.1	640.0	306.0	1154.1
Per 100 person-years	1.9	4.5	16.3	7.2
RR[a]	1	2.48	8.52	$P < .0001$
95% CI	Referent	0.85–7.19	2.97–24.4	for trend
All events[d]				
Events, n	6	40	111	157
Person-years	215.6	703.2	449.9	1368.6
Per 100 person-years	2.8	5.7	24.7	11.5
RR[b]	1	2.19	9.05	$P < .0001$
95% CI	Referent	0.91–5.26	3.87–21.2	for trend

[a]Sex- and age-adjusted hazard ratio by Cox proportional-hazards model.
[b]Sex- and age-adjusted relative risk by Poisson regression.
[c]Esophagus or head and neck cancer.
[d]Esophagus plus head and neck cancer(s).

squamous epithelium might accumulate and increase the potential to develop metachronous multiple SCCs. In addition, alcohol abstinence significantly decreased the development of metachronous SCCs, especially in the patients with grade C LVL. This result indicates that alcohol abstinence should be encouraged to reduce the risk of metachronous multiple SCCs after curative treatment.

Although the field cancerization phenomenon in the upper aerodigestive tract has been well known for more than half a century, it has been difficult to detect early cancer in this region. A randomized controlled trial found that narrow-band imaging effectively can detect early SCC in the esophagus and the head and neck region.[3] In patients with early SCC, organs can be preserved by endoscopic curative treatment, but the preserved organs are affected strongly by the field cancerization phenomenon. Therefore, an effective surveillance strategy after treatment and methods to prevent metachronous multiple SCCs are needed in survivors of esophageal SCC.

Data on the interval from the first SCC to the development of a second primary metachronous SCC have been limited, largely because of dismal survival, complete esophageal resection on diagnosis, poor follow-up evaluation, and lack of effective surveillance programs. In this study, we focused on patients with early esophageal SCC who could achieve cure and organ preservation. The

Table 3. Predictors of Metachronous Multiple Cancers by Cox Proportional-Hazards Model

	Esophagus		Head and neck		Esophagus or head and neck	
	Univariate RR (95% CI)[a]	Multivariate RR (95% CI)[b]	Univariate RR (95% CI)[a]	Multivariate RR (95% CI)[b]	Univariate RR (95% CI)[a]	Multivariate RR (95% CI)[b]
Grade of LVLs						
A	1 (ref)	1 (ref)	0.00 (incalculable)	0.00 (incalculable)	1 (ref)	1 (ref)
B	2.33 (0.79–6.85)	2.46 (0.84–7.24)	1 (ref)	1 (ref)	2.49 (0.86–7.23)	2.64 (0.91–7.68)
C	7.66 (2.64–22.3)	8.76 (3.02–25.5)	3.61 (1.38–9.46)	3.51 (1.34–9.20)	8.51 (2.97–24.4)	9.78 (3.41–28.0)
Drinking alcohol, per +1 U	1.12 (1.02–1.23)	–	1.18 (1.01–1.38)	–	1.11 (1.01–1.22)	–
Alcohol flushing						
Never flushing	1 (ref)	1 (ref)	1 (ref)	–	1 (ref)	1 (ref)
Flushing	1.39 (0.84–2.29)	1.70 (1.02–2.82)	1.29 (0.49–3.37)	–	1.30 (0.82–2.07)	1.60 (1.00–2.57)
Strong alcoholic beverages						
Sometimes/never	1 (ref)	–	1 (ref–)	–	1 (ref)	–
Frequently	0.95 (0.45–1.99)	–	1.17 (0.34–4.05)	–	1.07 (0.55–2.08)	–
Smoking, pack-years						
Never/light (<30)	1 (ref)	–	1 (ref)	–	1 (ref)	–
Heavy (≥30)	1.12 (0.66–1.92)	–	1.80 (0.57–5.66)	–	1.15 (0.69–1.91)	–
High-temperature food						
Likes very much	1.04 (0.57–1.90)	–	0.93 (0.27–3.20)	–	1.09 (0.62–1.92)	–
Others	1 (ref)	–	1 (ref)	–	1 (ref)	–
Green-yellow vegetables						
Almost every day	0.77 (0.46–1.26)	–	0.59 (0.21–1.68)	–	0.64 (0.39–1.03)	–
Others	1 (ref)	–	1 (ref)	–	1 (ref)	–
Fruit						
Almost every day	0.46 (0.24–0.87)	0.42 (0.22–0.82)	0.32 (0.07–1.45)	0.32 (0.07–1.43)	0.43 (0.23–0.81)	0.39 (0.21–0.74)
Others	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Body mass index, kg/m^2						
<21.2	2.06 (1.13–3.75)	2.09 (1.14–3.83)	1.29 (0.46–3.60)	–	1.65 (0.95–2.85)	1.73 (0.99–3.02)
21.2–23.6	1 (ref)	1 (ref)	1 (ref)	–	1 (ref)	1 (ref)
≥23.7	1.59 (0.86–2.94)	1.86 (1.00–3.47)	0.67 (0.21–2.13)	–	1.33 (0.76–2.33)	1.55 (0.88–2.73)

NOTE. One patient with missing data for body mass index was excluded (n = 329).
–, Not selected ($P \geq .15$). Sex and age also were adjusted but are not shown in the table.
[a]Sex- and age-adjusted RR for each variable.
[b]Variables were selected from all the variables listed above by the stepwise procedure with $P < .15$ for entry and removal, and the relative risks were shown for the selected variables.

frequency of events was notably low in grade A. The 2-year cumulative incidence of metachronous multiple SCCs and the total number of metachronous multiple SCCs per 100 person-years were 4.0%/2.8, 0.0%/0.0, and 4.0%/2.8 in the esophagus, head and neck region, and both, respectively. On the other hand, the frequency of events was notably high in grade C. The 2-year cumulative incidence of metachronous multiple SCCs and the total number of metachronous multiple SCCs per 100 person-years were 24.7%/18.9, 8.6%/5.8, and 31.4%/24.7 in the esophagus, head and neck region, and both, respectively. These results indicated that effective surveillance programs according to the grade of LVL or effective adjuvant strategies according to the grade of LVL are required to detect metachronous SCC earlier.

There has been no effective biomarker for the risk of the field cancerization phenomenon. In this study, we clearly showed that an increase in the grade of LVL is associated closely with the risk of metachronous SCC. In addition, grade C LVL and a flushing reaction after drinking alcohol are predictors of metachronous multiple SCCs. We previously reported that multiple LVLs in patients with SCC in the esophagus and the head and neck region was associated with ALDH2 inactive genotype and metachronous SCC in the esophagus and the head and neck region.[13,19,20] These data mean that cancer survivors with flushing reaction after drinking alcohol or multiple LVLs in the background mucosa, or both, should be considered as having a higher risk of field cancerization.

A recent meta-analysis showed that the alcohol-related risk of esophageal SCC is reversible after alcohol abstinence, and 16 years are required until all increased risk disappears.[21] In our study, with a median follow-up period of only 49.4 months (range, 1.3–81.2 mo), the risk of metachronous SCC significantly decreased after alcohol abstinence, especially among the patients with grade C LVL, in whom the hazard ratio decreased to 0.23. These data are very important for reducing the risk of metachronous SCC in cancer survivors who have received curative treatment.

The median follow-up time in our study was 49.4 months, which might be too short to draw strong recommendations about the value of surveillance intervals. However, even in this short period, the patients with grade C LVL had a risk of metachronous multiple SCCs in the esophagus and the head and neck region. This means that close

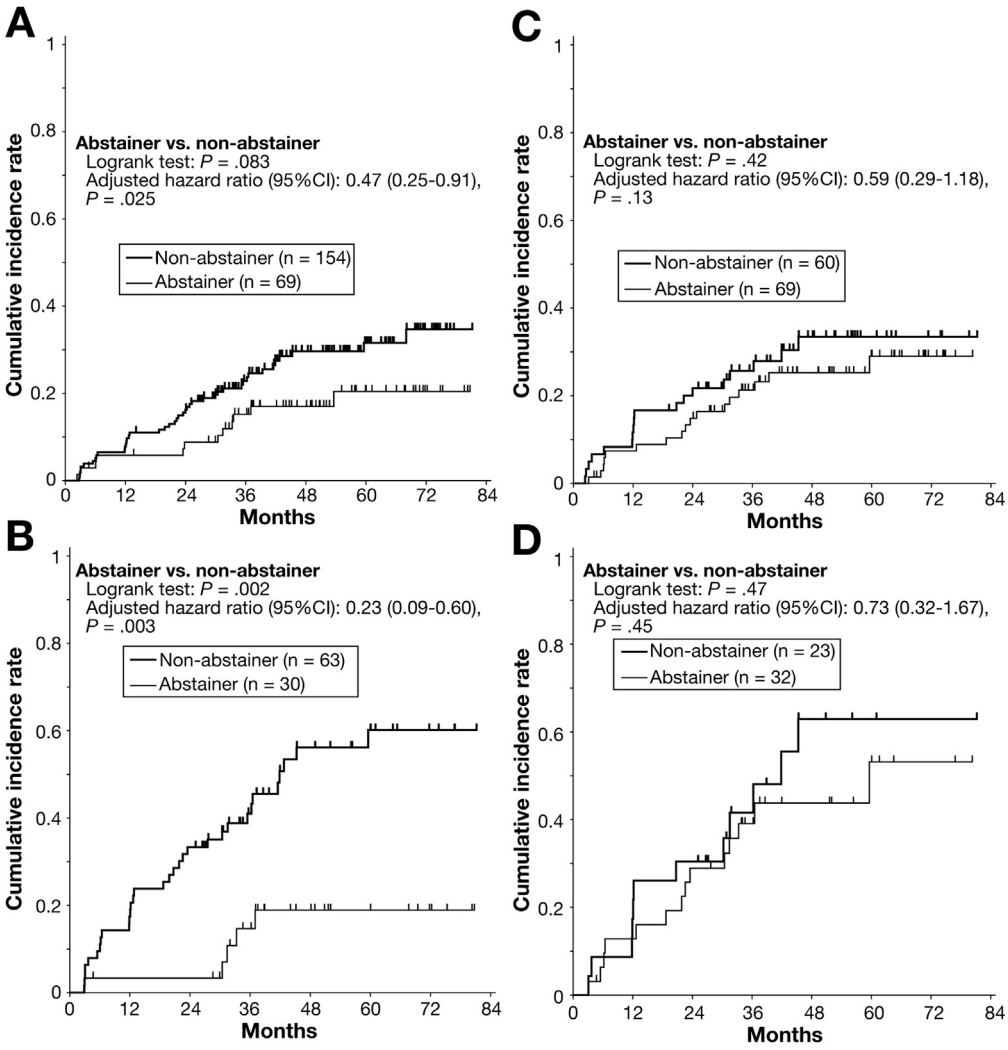


Figure 4. Cumulative incidence of metachronous multiple esophageal SCCs according to the status of drinking alcohol and smoking. (*A*) All patients according to the status of drinking alcohol. (*B*) Patients with grade C LVL according to the status of drinking alcohol. (*C*) All patients according to the status of smoking. (*D*) Patients with grade C LVL according to the status of smoking.

surveillance may be required for the patients with esophageal cancer who were treated successfully. Clinically, the grade of LVL on iodine staining of the background mucosa should be evaluated initially. If the grade suggests a risk of cancer, narrow-band imaging should be performed as surveillance for the early detection of cancer.

We showed that the *TP53* mutation was seen even in noncancerous lesions in patients with early esophageal SCC, and the frequency of the *TP53* mutation was associated with the severity of LVL grade. We believe these data help explain the mechanisms of metachronous esophageal carcinogenesis. In this study, we obtained biopsy samples from Lugol-stained lesions, in which glycogen granules are rich and structural atypia is not seen. Such mucosa was assumed to be "non-cancerous" esophageal epithelium.[22] However, we could not exclude the possibility that the greater the number of LVL lesions present in the patient, the more likely the possibility that biopsy specimens from normal mucosa still contain cryptic dysplasia that would not have been detected by Lugol staining alone. In addition, we could not address the difference regarding *TP53* mutations between the primary lesion and the noncancerous background esophageal mucosa because we did not obtain DNA samples from primary lesions. Moreover, we could not assess whether the different *TP53* mutations identified in the same case were derived from monoclonal or polyclonal origin. These limitations should be clarified further in future studies.

In conclusion, the grade of LVL is a useful predictor of the risk of metachronous multiple SCCs arising in the esophagus and the head and neck region in survivors of esophageal cancer. Alcohol abstinence is required to reduce the risk of metachronous multiple SCCs in the esophagus.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2016.07.040.

References

1. Slaughter DP, Southwick HW, Smejkal W. "Field cancerization" in oral stratified epithelium. Cancer 1953; 6:963–968.

2. Matsubara T, Yamada K, Nakagawa A. Risk of second primary malignancy after esophagectomy for squamous cell carcinoma of the thoracic esophagus. J Clin Oncol 2003;21:4336–4341.
3. Muto M, Minashi K, Yano T, et al. Early detection of superficial squamous cell carcinoma in the head and neck region and esophagus by narrow band imaging: a multicenter randomized controlled trial. J Clin Oncol 2010;28:1566–1572.
4. Katada C, Tanabe S, Koizumi W, et al. Narrow band imaging for detecting superficial squamous cell carcinoma of the head and neck in patients with esophageal squamous cell carcinoma. Endoscopy 2010;42:185–190.
5. Higuchi K, Tanabe S, Azuma M, et al. A phase II study of endoscopic submucosal dissection for superficial esophageal neoplasms (KDOG 0901). Gastrointest Endosc 2013;78:704–710.
6. Muto M, Satake H, Yano T, et al. Long-term outcome of transoral organ-preserving pharyngeal endoscopic resection for superficial pharyngeal cancer. Gastrointest Endosc 2011;74:477–484.
7. Muto M, Hitomi Y, Ohtsu A, et al. Association of aldehyde dehydrogenase 2 gene polymorphism with multiple oesophageal dysplasia in head and neck cancer patients. Gut 2000;47:256–261.
8. Yokoyama A, Hirota T, Omori T, et al. Development of squamous neoplasia in esophageal iodine-unstained lesions and the alcohol and aldehyde dehydrogenase genotypes of Japanese alcoholic men. Int J Cancer 2012;130:2949–2960.
9. Muto M, Hironaka S, Nakane M, et al. Association of multiple Lugol-voiding lesions with synchronous and metachronous esophageal squamous cell carcinoma in patients with head and neck cancer. Gastrointest Endosc 2002;56:517–521.
10. Shimizu Y, Tsukagoshi H, Fujita M, et al. Head and neck cancer arising after endoscopic mucosal resection for squamous cell carcinoma of the esophagus. Endoscopy 2003;35:322–326.
11. Urabe Y, Hiyama T, Tanaka S, et al. Metachronous multiple esophageal squamous cell carcinomas and Lugol-voiding lesions after endoscopic mucosal resection. Endoscopy 2009;41:304–309.
12. Hori K, Okada H, Kawahara Y, et al. Lugol-voiding lesions are an important risk factor for a second primary squamous cell carcinoma in patients with esophageal cancer or head and neck cancer. Am J Gastroenterol 2011;106:858–866.
13. Katada C, Muto M, Nakayama M, et al. Risk of superficial squamous cell carcinoma developing in the head and neck region in patients with esophageal squamous cell carcinoma. Laryngoscope 2012;122:1291–1296.
14. Mandard AM, Hainaut P, Hollstein M. Genetic steps in the development of squamous cell carcinoma of the esophagus. Mutat Res 2000;462:335–342.
15. Secretan B, Straif K, Baan R, et al. A review of human carcinogens-part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. Lancet Oncol 2009;10:1033–1034.
16. Lee YC, Wang HP, Wang CP, et al. Revisit of field cancerization in squamous cell carcinoma of upper aerodigestive tract: better risk assessment with epigenetic markers. Cancer Prev Res 2011;4:1982–1992.
17. Yokoyama T, Yokoyama A, Kato H, et al. Alcohol flushing, alcohol and aldehyde dehydrogenase genotypes, and risk for esophageal squamous cell carcinoma in Japanese men. Cancer Epidemiol Biomarkers Prev 2003;12:1227–1233.
18. Katada C, Muto M, Tanabe S, et al. Surveillance after endoscopic mucosal resection or endoscopic submucosal dissection for esophageal squamous cell carcinoma. Dig Endosc 2013;25(Suppl 1):S39–S43.
19. Muto M, Takahashi M, Ohtsu A, et al. Risk of multiple squamous cell carcinomas both in the esophagus and the head and neck region. Carcinogenesis 2005;26:1008–1012.
20. Katada C, Muto M, Tanabe S, et al. Risk for the development of multiple Lugol-voiding lesions of the esophageal mucosa in patients with esophageal squamous cell carcinoma. Dis Esophagus 2014;27:457–462.
21. Jarl J, Gerdtham UG. Time pattern of reduction in risk of oesophageal cancer following alcohol cessation – a meta-analysis. Addiction 2012;107:1234–1243.
22. Mori M, Adachi Y, Matsushima T, et al. Lugol staining pattern and histology of esophageal lesions. Am J Gastroenterol 1993;88:701–705.

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Conflicts of interest
The authors disclose no conflicts.

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【Regarding alcohol consumption before disease onset】

Q1. Did you like to drink alcohol before disease onset?
1. Yes 2. No

Q2. Did you regularly drink alcohol before disease onset?
1. Yes, I did →Proceed to Q3
2. I quit drinking before disease onset. →Proceed to Q3
3. No, I did not →Proceed to Q8

Q3. At what age did you start drinking?
_____years old

Q4. For persons who quit drinking before disease onset, how old were you when you quit drinking?
_____years old

Q5. On average, how often did you drink before disease onset?
1. 1 to 3 days a month 2. 1 to 2 days a week
3. 3 to 4 days a week 4. 5 or more days a week

Q6. If you regularly drank alcohol before disease onset, how much did you drink per day?
Beer or low-malt beer-like beverages (large bottles):_____bottles
Beer or low-malt beer-like beverages (medium-sized bottles or long cans):_____bottles
Draft beer or low-malt beer-like beverages (350 mL):___bottles
Japanese sake:_____ x 180 mL Shochu:_____ x 180 mL Shochu highball:_____glasses
Wine:_____glasses Cocktails:_____glasses Whiskey or brandy (with water):_____glasses
Whiskey or brandy (straight or with ice):_____glasses Others types of alcohol beverages:_____. How much:_____.

Q7. Before disease onset, did you often drink strong liquors, such as whiskey, brandy, and shochu, straight?
1. Yes, I often drank. 2. Yes, I occasionally drank. 3. No, I did not drink.

All respondents, please fill in all of the following.

Q8. Do you have a tendency to develop facial flushing immediately after drinking a glass of beer?
1. Yes 2. No 3. Unknown

Q9. Did you have a tendency to develop facial flushing immediately after drinking a glass of beer in the first one or two years after you started drinking?
1. Yes 2. No 3. Unknown

【Regarding smoking status before disease onset】

Q10. Did you smoke before disease onset?
1. Yes 2. I quit smoking before disease onset. 3. No

Q11. At what age did you start smoking?
_____years old

Q12. If you quit smoking before disease onset, please write down your age when you quit smoking.
_____years old

Q13. Before disease onset, how many cigarettes did you smoke per day or before you stopped smoking?
_____cigarettes

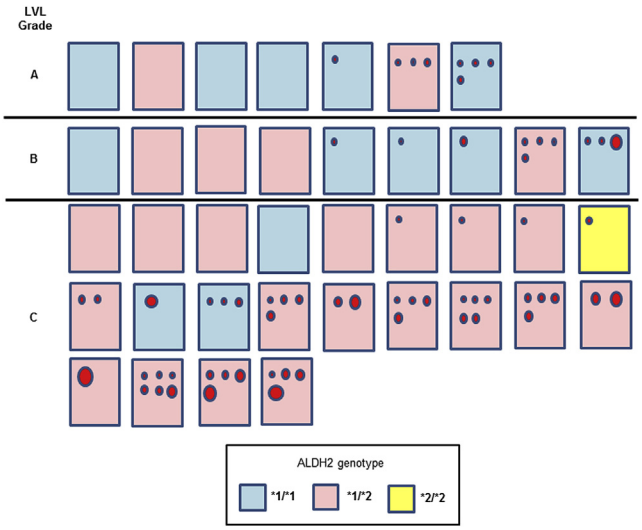
【Regarding eating habits before disease onset】

Q14. Please circle the number that best describes your eating habits before disease onset.
Hot dishes 1. Like very much 2. Like 3. Do not like or dislike 4. Do not like very much 5. Hate
Brightly colored vegetables (green leafy vegetables such as spinach and shungiku, carrots, pumpkin, tomatoes, etc.)
1. Rarely eat 2. Eat 1 to 2 times a month 3. Eat 1 to 2 times a week 4. Eat 3 to 4 times a week 5. Eat nearly every day
Fruit 1. Rarely eat 2. Eat 1 to 2 times a month 3. Eat 1 to 2 times a week 4. Eat 3 to 4 times a week 5. Eat nearly every day

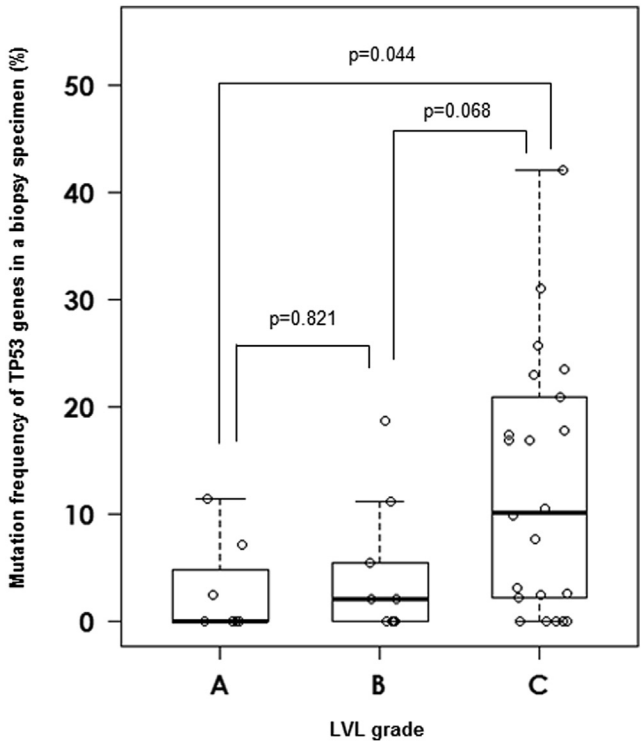
【Regarding your family history of cancer】

Q15. Did any of your blood relatives, such as your father, mother, brothers or sisters, and children, ever have cancer of the mouth, throat, or esophagus?
1. Yes 2. No

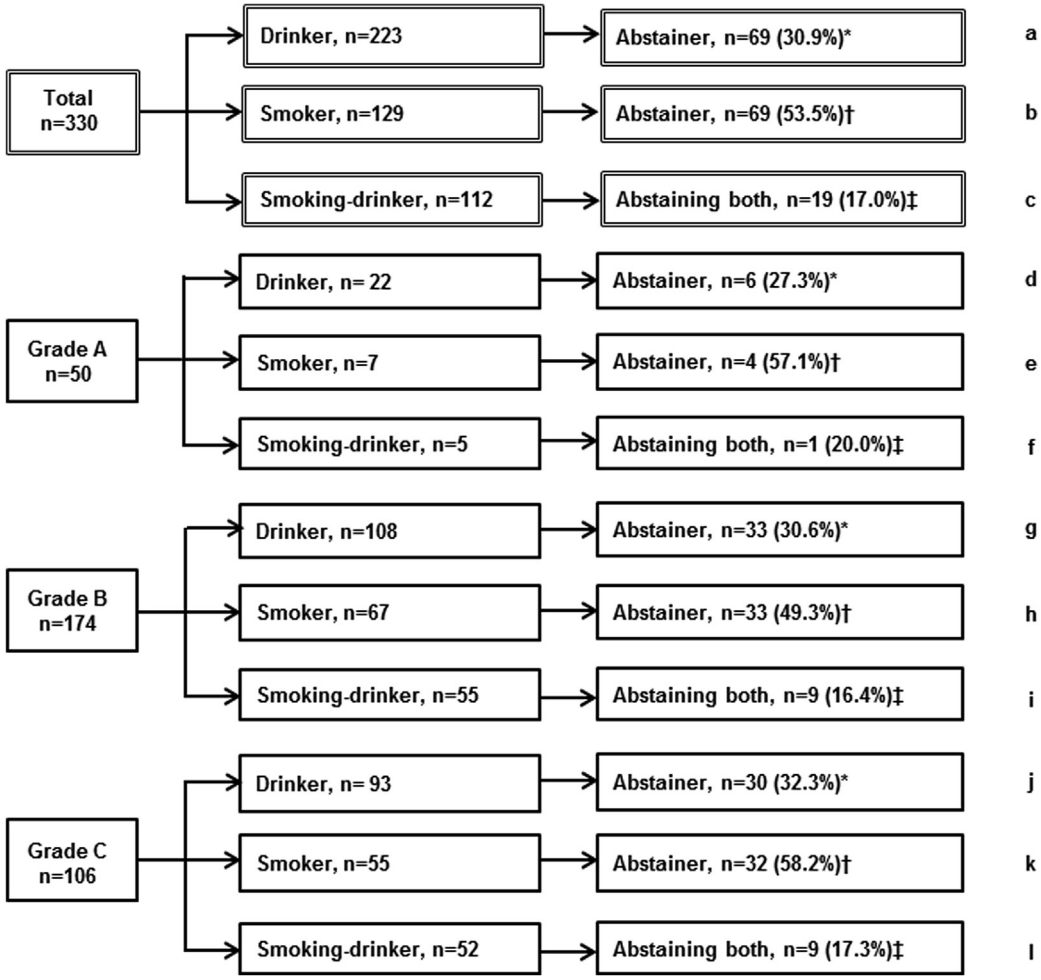
Supplementary Figure 1. A self-administered questionnaire for lifestyle surveys.



Supplementary Figure 2. *TP53* mutations in biopsy specimens of noncancerous background esophageal mucosa. Deep sequencing of the *TP53* gene amplicon was performed to detect small frequencies of mutations ($\geq$2% of total reads). A *rectangle* shows a biopsy specimen of each case. A *red plot* shows a mutation of *TP53*, and the plot size conforms to the mutation frequency. The 38 cases are divided according to their LVL grade (grade A, 7 cases; grade B, 9 cases; and grade C, 22 cases). ALDH2*1/*1 (ALDH2 wild-type homozygotes), ALDH2*1/*2 (ALDH2 heterozygotes), and ALDH2*2/*2 (ALDH2 mutant homozygotes) are shown with *blue rectangles*, *red rectangles*, and *yellow rectangles*, respectively.



Supplementary Figure 3. Comparison of the *TP53* mutation frequency between LVL grades. A *circle plot* shows a summation of *TP53* mutation frequency in each case. The *box height* represents the interquartile range showing the lower quartile (25th percentile values) and upper quartile (75th percentile values) values. The *line* in the box represents the median, and the *whiskers* represent the minimum and maximum *TP53* mutation frequencies. The *P* values were calculated by the Wilcoxon signed-rank test.



Supplementary Figure 4. Patient flow chart of alcohol and smoking abstinence according to the LVL grade. *$P = .90$, †$P = .61$, and ‡$P = .98$. (A) = a, (B) = j, (C) = b, (D) = k.

Supplementary Table 1. Clinical Characteristics of 38 Esophageal SCC Patients Analyzed With TP53 Deep Sequencing

Sample ID	LVL grade	ALDH2 genotype	Age, *y*	Sex	Alcohol drinking[a]	Smoking, pack-year
S1	A	*1/*1	74	Female	Never	35.25
S2	A	*1/*2	60	Female	Never	0
S3	A	*1/*1	85	Female	Light	0
S4	A	*1/*1	57	Male	Light	40
S5	A	*1/*1	64	Male	Light	40
S6	A	*1/*2	68	Male	Moderate	30
S7	A	*1/*1	60	Male	Moderate	40
S8	B	*1/*1	67	Male	Heavy	129
S9	B	*1/*2	69	Male	Moderate	60
S10	B	*1/*2	75	Male	Moderate	45
S11	B	*1/*2	72	Male	Moderate	2.5
S12	B	*1/*1	55	Female	Heavy	12.5
S13	B	*1/*1	68	Male	Moderate	45
S14	B	*1/*1	64	Female	Heavy	40
S15	B	*1/*2	75	Male	Moderate	87
S16	B	*1/*1	72	Male	Moderate	45
S17	C	*1/*2	75	Male	Heavy	5
S18	C	*1/*2	71	Male	Never	38
S19	C	*1/*2	53	Male	Heavy	70
S20	C	*1/*1	62	Female	Moderate	20
S21	C	*1/*2	67	Male	Heavy	70.5
S22	C	*1/*2	61	Male	Moderate	40
S23	C	*1/*2	64	Male	Moderate	34.5
S24	C	*1/*2	71	Male	Moderate	75
S25	C	*2/*2	58	Male	Heavy	40
S26	C	*1/*2	61	Male	Moderate	20
S27	C	*1/*1	69	Male	Heavy	21.5
S28	C	*1/*1	59	Female	Moderate	40
S29	C	*1/*2	67	Male	Moderate	100
S30	C	*1/*2	59	Male	Moderate	20
S31	C	*1/*2	59	Male	Heavy	0
S32	C	*1/*2	73	Male	Heavy	30
S33	C	*1/*2	64	Male	Heavy	39.6
S34	C	*1/*2	68	Male	Heavy	50
S35	C	*1/*2	71	Male	Heavy	75
S36	C	*1/*2	69	Male	Heavy	51
S37	C	*1/*2	66	Male	Heavy	86
S38	C	*1/*2	50	Male	Heavy	270

[a]Never/rare, $<$1 U/wk; light, 1–8.9 U/wk; moderate, 9–17.9 U/wk; heavy, $\geq$18 U/wk (1 U $=$ 22 g ethanol).

Supplementary Table 2. Clinical Characteristics of 38 Esophageal SCC Patients According to the LVL Grade

	LVL grade		
	A (n = 7)	B (n = 9)	C (n = 22)
Sex			
Male, %	57.1%	77.8%	91.0%
Age, *y*			
Means ± SD	67 ± 10	69 ± 6	64 ± 6
Drinking alcohol[a]			
Never/rare	28.6%	0.0%	4.5%
Light	42.9%	0.0%	0.0%
Moderate	28.6%	66.7%	36.4%
Heavy	0.0%	33.3%	59.1%
Heavy smoker, ≥30 pack-years	71.4%	77.8%	72.7%
ALDH2 genotype			
*1/*1	71.4%	55.6%	13.6%
*1/*2	28.6%	44.4%	81.8%
*2/*2	0.0%	0.0%	4.5%

[a]Never/rare, <1 U/wk; light, 1–8.9 U/wk; moderate, 9–17.9 U/wk; heavy, ≥18 U/wk (1 U = 22 g ethanol).

Supplementary Table 3. Summary of TP53 Mutations in 38 Esophageal SCC Patients

Sample ID	Mutation type	Coverage	Frequency	Start position	End position	Reference allele	Mutated allele	Amino acid change	Cosmic occurrence
S5	Nonsynonymous SNV	891	2.5	7578446	7578446	T	A	I162F	10
S6	Nonsynonymous SNV	1243	2.7	7578532	7578532	A	C	M133R	6
S6	Frameshift deletion	1030	2.0	7577572	7577572	T	–		2
S6	Nonsynonymous SNV	649	2.3	7579358	7579358	C	A	R110L	25
S7	Nonsynonymous SNV	1571	2.2	7578394	7578394	T	C	H179R	114
S7	Nonsynonymous SNV	1280	3.6	7578203	7578203	C	A	V216L	7
S7	Nonsynonymous SNV	1280	2.7	7578191	7578191	A	G	Y220H	12
S7	Nonsynonymous SNV	944	2.9	7577524	7577524	T	G	T253P	3
S12	Nonsynonymous SNV	197	2.0	7579362	7579362	A	G	F70L	2
S13	Nonsynonymous SNV	522	2.1	7579358	7579358	C	G	R110P	9
S14	Nonsynonymous SNV	967	5.5	7578190	7578190	T	C	Y220C	231
S15	Splicing	1055	2.2	7574035	7574035	T	G		0
S15	Nonframeshift deletion	986	2.9	7577580	7577585	TAGTGG	–		0
S15	Nonsynonymous SNV	987	3.7	7577530	7577530	T	A	I251F	8
S15	Nonsynonymous SNV	989	2.2	7577547	7577547	C	T	G254D	110
S16	Nonsynonymous SNV	1445	13.2	7578522	7578522	T	A	Q136H	1
S16	Nonsynonymous SNV	880	2.6	7578396	7578396	G	T	H178Q	4
S16	Nonsynonymous SNV	646	2.8	7577139	7577139	G	A	R267W	26
S22	Nonsynonymous SNV	869	2.2	7577539	7577539	G	A	R248W	495
S23	Nonsynonymous SNV	810	2.5	7577139	7577139	G	A	R267W	26
S24	Nonsynonymous SNV	345	2.6	7579358	7579358	C	A	R71L	25
S25	Frameshift deletion	1844	3.0	7579879	7579879	G	–		0
S26	Nonsynonymous SNV	936	4.1	7578260	7578260	C	T	V197M	10
S26	Stopgain SNV	825	3.5	7574003	7574003	G	A	R342*	74
S27	Splicing	950	9.8	7577610	7577610	T	A		10
S28	Nonsynonymous SNV	485	2.5	7578448	7578448	G	T	A29D	9
S28	Nonsynonymous SNV	800	2.9	7578190	7578190	T	C	Y220	231
S28	Stopgain SNV	372	5.1	7577100	7577100	T	A	R148X	10
S29	Nonsynonymous SNV	1504	2.3	7578266	7578266	T	A	I195F	21
S29	Nonsynonymous SNV	748	3.6	7578235	7578235	T	C	Y205C	66
S29	Nonsynonymous SNV	877	6.6	7577565	7577565	T	C	N239S	21
S29	Nonsynonymous SNV	879	4.9	7577539	7577539	G	A	R248W	495
S30	Nonsynonymous SNV	356	11.2	7577121	7577121	G	A	R141C	474
S30	Stopgain SNV	1091	5.7	7574021	7574021	C	A	E336*	4
S31	Nonsynonymous SNV	1225	5.7	7578526	7578526	C	T	C135Y	52
S31	Frameshift deletion	694	3.7	7577599	7577599	C	–		0
S31	Nonsynonymous SNV	702	5.0	7577536	7577536	T	A	R249W	34
S31	Nonsynonymous SNV	502	2.4	7577124	7577124	C	A	V272L	23
S32	Nonsynonymous SNV	1079	2.5	7577559	7577559	G	A	S241F	73
S32	Nonsynonymous SNV	1080	4.8	7577570	7577570	C	T	M237I	68
S32	Nonsynonymous SNV	537	4.8	7577098	7577098	T	A	R280S	11
S32	Nonsynonymous SNV	538	3.0	7577094	7577094	G	A	R282W	395
S32	Nonsynonymous SNV	1638	2.6	7578538	7578538	T	C	N131S	4
S33	Splicing	1416	4.5	7578370	7578370	C	T		21

Supplementary Table 3. Continued

Sample ID	Mutation type	Coverage	Frequency	Start position	End position	Reference allele	Mutated allele	Amino acid change	Cosmic occurrence
S33	Nonsynonymous SNV	1393	4.5	7578406	7578406	C	T	R175H	818
S33	Nonsynonymous SNV	339	5.0	7577124	7577124	C	A	V140L	23
S33	Stopgain SNV	339	6.8	7577058	7577058	C	A	E162X	43
S34	Nonsynonymous SNV	1394	13.8	7578190	7578190	T	C	Y220C	231
S34	Nonsynonymous SNV	1346	9.2	7578536	7578536	T	G	K132Q	10
S35	Nonsynonymous SNV	679	23.4	7577559	7577559	G	A	S241F	73
S36	Nonsynonymous SNV	2181	2.5	7578395	7578395	G	A	H179Y	85
S36	Nonsynonymous SNV	2181	4.4	7578393	7578393	A	T	H179Q	12
S36	Nonsynonymous SNV	1147	4.3	7578271	7578271	T	C	H193R	81
S36	Nonsynonymous SNV	674	9.5	7577538	7577538	C	T	R248Q	565
S36	Nonsynonymous SNV	563	2.7	7577121	7577121	G	A	R273C	474
S36	Frameshift deletion	583	2.2	7579391	7579391	G	–		0
S37	Nonsynonymous SNV	717	4.0	7578454	7578454	G	A	A159V	37
S37	Nonsynonymous SNV	691	8.7	7577547	7577547	C	A	G245V	62
S37	Nonsynonymous SNV	607	14.5	7577120	7577120	C	T	R273H	513
S37	Nonsynonymous SNV	645	3.7	7579358	7579358	C	A	R110L	25
S38	Splicing	896	6.5	7577609	7577609	C	T		13
S38	Frameshift insertion	899	6.3	7577530	7577530	–	T	I119fs	0
S38	Nonsynonymous SNV	899	2.6	7577528	7577528	G	C	I251M	0
S38	Nonsynonymous SNV	454	26.7	7577098	7577098	T	G	R148S	5

NOTE. The asterisks indicate the termination of translation.
SNV, single nucleotide variant.