Manuscript title

Evaluation of the risk of metachronous multiple squamous cell carcinoma of the head and neck after transoral surgery based on the genetic polymorphisms of alcohol dehydrogenase 1B and aldehyde dehydrogenase 2

Short title

Head and neck cancer and alcohol metabolism

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Abstract

Patients with superficial head and neck squamous cell carcinoma (HNSCC) can be completely treated by techniques of transoral surgery (TOS). The aim of this study was to evaluate the risk of metachronous multiple HNSCC arising after TOS based on alcohol dehydrogenase 1B (ADH1B) and aldehyde dehydrogenase 2 (ALDH2). We registered patients who underwent TOS for superficial HNSCC. Buccal cell samples were obtained by using a cotton swab to examine two single nucleotide polymorphisms in ADH1B and ALDH2 genotyping. We used Cox proportional hazards models to examine the risk of metachronous HNSCC. A total of 198 patients who underwent TOS for HNSCC were evaluated. In multivariate analysis, risks for second HNSCC were ADH1B*1/*1 (hazard ratio (HR), 1.88; 95% confidence interval (CI), 1.11-3.19; *P* = 0.02), ALDH2*1/*2 (HR, 2.11; 95% CI, 1.00-5.16; *P* = 0.048), and alcohol consumption before TOS (HR, 1.17; 95% CI, 1.06-1.27; *P* = 0.01). The 5-year incidence rates of second primary HNSCC in the temperance group and the non-temperance group were 20.8% and 46.5%, respectively (HR, 0.54; 95% CI, 0.31-0.92; *P* = 0.02). Cumulative development rates of third HNSCC in the temperance group and non-temperance group at 10 years were 11.3% and 36.1%, respectively (HR, 0.19; 95% CI, 0.03-0.65; *P* = 0.006). ADH1B*1/*1, ALDH2*1/*2, and moderate or heavy alcohol consumption before treatment are independent risk factors of metachronous HNSCC. Since it was shown that temperance decreased the incidences of second and third metachronous HNSCC, advice to discontinue alcohol drinking is necessary.

Summary

Our study showed that ADH1B*1/*1, ALDH2*1/*2, and alcohol consumption before treatment were independent risk factors for metachronous head and neck squamous cell carcinoma (HNSCC). In addition, continuing to drink alcohol increased the incidence of metachronous HNSCC.

Introduction

Patients with head and neck squamous cell carcinoma (HNSCC) are often diagnosed in an advanced stage, and even if they can undergo curative treatment, an invasive surgical operation such as total laryngectomy that would result in the loss of important functions is usually chosen for treatment [1]. However, recent developments of optical imaging techniques have contributed to an increase in the role of detection of superficial HNSCC that is confined to the subepithelial layer (without muscle layer invasion) [2-4]. Patients with superficial HNSCC can be completely treated by techniques of transoral surgery (TOS), including endoscopic mucosal resection (EMR), endoscopic submucosal dissection (ESD), endoscopic laryngopharyngeal surgery (ELPS), and transoral video-assisted surgery (TOVS), and clinical outcomes after treatment have been reported to be excellent [5-8]. TOS enables preservation of the laryngopharynx and maintenance of the patients' quality of life in terms of avoidance of language disorders, dysphagia, and cosmetic deformities. However, metachronous multiple HNSCC sometimes arises from the preserved laryngopharynx after TOS. In a previous retrospective cohort study, Muto et al. reviewed long-term outcomes of 104 patients with superficial pharyngeal carcinoma after transoral surgery, and they showed that the cumulative development rate of metachronous multiple cancers in the pharynx at 5 years was 22% [6]. Hanaoka et al. conducted a prospective phase II trial including 54 patients with superficial pharyngeal cancer who underwent ESD and reported that the cumulative development rate of metachronous multiple cancers of the pharynx at 3 years was 18.4% [7]. TOS cannot be regarded as a complete curative treatment considering the possibility of metachronous HNSCC; however, there has been no report on evaluation of the risk for metachronous HNSCC after initial treatment.

It is well known that alcohol consumption and smoking habits are closely related to the risk for HNSCC [9, 10]. As for the risk of alcohol consumption, acetaldehyde, which is produced from alcohol, has been reported to have carcinogenicity [11, 12]. Acetaldehyde is metabolized by aldehyde dehydrogenase 2 (ALDH2). The allele A (ALDH2*2) of the rs671: G>A variant has a remarkable loss of acetaldehyde metabolizing activity compared with the allele G (ALDH2*1). Drinkers with inactive ALDH2*2 allele, which is one type of genetic polymorphism, are known to

be at a high risk for HNSCC [13]. Alcohol dehydrogenase 1B (ADH1B) metabolizes ethanol included in alcoholic drinks, and ADH1B*1/*1, which is one type of genetic polymorphism of ADH1B, was also reported to be associated with HNSCC [14]. These factors are thought to be related to the occurrence of not only initial HNSCC but also metachronous HNSCC. In this study, we evaluated the risk of metachronous multiple HNSCC arising after TOS for initial superficial HNSCC based on the genetic polymorphisms of ADH1B and ALDH2 along with alcohol consumption and smoking habits.

Materials and methods

Patients

This study was an observational retrospective multicenter study using newly obtained specimens (buccal cell samples) in Japan. We registered patients who underwent TOS for superficial HNSCC. The study subjects had been followed up by annual physical examinations, otolaryngologists' laryngoscopy, and endoscopic examination for at least two years at seven Japanese institutes. The registration was carried out from January 2019 to March 2020. Inclusion criteria for this study were (1) pathological diagnosis of HNSCC in the first resected specimens, (2) complete resection by TOS, (3) written informed consent obtained from the patient, and (4) possibility of collecting buccal cell samples. Exclusion criteria were (1) after total laryngectomy (2) additional surgical resection after TOS, (3) with muscle layer invasion, (4) unsuitability as subjects, (5) age < 20 years, and (6) current pregnancy.

Information on past treatment of HNSCC by TOS and histological diagnosis was obtained from medical records. After confirmation of consent, buccal cell samples were obtained by using a cotton swab to examine two single nucleotide polymorphisms in ADH1B and ALDH2 genotyping.

This study was carried out according to the principles of the Declaration of Helsinki and was approved by the Medical Ethics and Human Clinical Trial Committee of Hokkaido University Hospital and all participant institutes. The study was registered with the Japanese University Hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN: 000034980). Written informed consent was obtained from the study subjects at the registration.

Alcohol consumption and smoking habits

The physicians in the participant institutes had advised, in principle, all of the patients to stop or reduce drinking and smoking after treatment. Information on alcohol consumption and smoking before and after TOS was obtained by using a questionnaire. Subjects whose alcohol consumption was less than 1 unit/week were classified as rare drinkers, those whose alcohol consumption was 1 to 8.9 units/week were classified as light drinkers, those whose alcohol consumption was 9 to 17.9 units/week were classified as moderate drinkers, and those whose alcohol consumption was 18 or more units/week were classified as heavy drinkers. We defined 1 unit of alcohol as 22 g of ethanol, which is contained in 500 mL of beer or two glasses (200 mL) of wine. Subjects who did not smoke or smoked rarely were classified as rare smokers (nonsmokers). Habitual smokers with a smoking history of < 30 pack years were classified as light smokers, while subjects who had a smoking history of ≥ 30 pack years were classified as heavy smokers. Thirty pack years = one package of cigarettes (20 cigarettes) daily for 30 years [15, 16]. If alcohol consumption and smoking habits varied during the period for evaluation, we registered the average amount of them for statistical analysis.

ADH1B/ALDH2 polymorphisms

SNPs of ADH1B and ALDH2 genes were genotyped using the TaqMan assay on an ABI 7300 real-time polymerase chain reaction (PCR) System (Applied Biosystems) [17]. The slow-metabolizing form of ADH1B is encoded by the ADH1B*1 allele, while the inactive form of ALDH2 is encoded by the ALDH2*2 allele. We compared ADH1B*1/*1 genotype carriers with ADH1B*2 allele carriers and ALDH2*2 allele carriers with ALDH2*1/*1 genotype carriers.

Definitions

Metachronous HNSCC was defined as new HNSCC that was diagnosed more than one year after TOS for the primary HNSCC (New HNSCC that was diagnosed within one year from treatment of the primary HNSCC was defined as synchronous HNSCC.). Second primary HNSCC was metachronous cancer arising after the first primary HNSCC, and third primary HNSCC was metachronous cancer arising after the second primary HNSCC. New HNSCC that

was adjacent to a scar of surgical margin-positive HNSCC was defined as local recurrence. In order to evaluate the effectiveness of temperance and smoking cessation for preventing metachronous carcinoma, habitual drinkers and smokers were each divided into two groups. Patients in the non-temperance group were patients who continued to drink alcohol with heavy or moderate consumption after the first TOS, and patients in the temperance group were patients who reduced consumption of alcohol from heavy or moderate consumption to light or rare consumption after the first TOS. Patients in the smoking group were patients who continued with heavy or light smoking after the first TOS, and patients in the nonsmoking group were patients who had been heavy or light smokers and stopped smoking after the first TOS.

Statistical analysis

We examined the risk of metachronous HNSCC. Age is expressed as mean ± standard deviation (SD). Drinking volume is shown as median and interquartile range (IQR). Differences in frequency distributions were tested using the chi-square test or Fisher's exact test, and quantitative data were examined with Mann-Whitney's U test. Differences were considered statistically significant at $P < 0.05$. The time to development of metachronous HNSCC was defined as the period from the day of TOS to the day of diagnosis of metachronous HNSCC. The Kaplan–Meier method and log-rank test were used for analyzing the development of metachronous HNSCC. We used Cox proportional hazards models on the scale of the time from the first TOS to metachronous HNSCC to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). We conducted univariate analysis, and significant items from univariate analysis were used in multivariate analysis. JMP® Pro 14.0.1 (SAS Institute, Inc., Cary, NC, USA) was used for data analyses.

Results

Subjects

We enrolled 200 patients who underwent TOS for HNSCC in the study period. One patient was excluded because of a genetic examination error. Another patient was excluded because

additional surgical resection was performed in the head and neck region after TOS. A total of 198 patients were evaluated.

Characteristics of the patients and the first primary HNSCC are shown in Table 1. The median follow-up period at the registration was 54 months (range, 24-184 months). The primary HNSCC locations were the hypopharynx in 157 patients (79.3%), oropharynx in 27 patients (13.6%), and larynx in 14 patients (7.1%). As for methods of TOS, ELPS was performed for 142 patients (71.7%), EMR was performed for 31 (15.7%), ESD was performed for 23 (11.6%), and TOVS was performed for 2 (1.0%). According to the clinical practice guideline committee of Japan Society for Head and Neck Cancer, TOS was performed for patients who were diagnosed to be in clinical N0, M0 stage. No patients died of primary HNSCC during the follow-up period.

Second primary HNSCC

Clinical characteristics of patients with and those without second HNSCC are shown in Table 2. Second HNSCC was detected in 64 patients (32.3%). Patients with second HNSCC were significantly younger than patients without second HNSCC (mean ± SD age, 61.8 ± 9.1 and 67.4 ± 7.0 years, respectively; $P < 0.001$). Patients with second HNSCC included a significantly larger proportion of patients with ADH1B*1/*1 (51.6% and 25.4%, respectively; $P < 0.001$) and a significantly larger proportion of patients with ALDH1*1/*2 + *2/*2 (89.1% and 71.6%, respectively; $P = 0.006$). The amount of alcohol consumption before TOS was significantly larger in patients with second HNSCC (median and IQR, 32.3 (23.9-47.2) and 23.4 (14.7-37.3) units/week, respectively; $P = 0.011$), and the amount of alcohol consumption after TOS was also significantly larger in patients with second HNSCC (median and IQR, 15.3 (2.0-31.0) and 7.8 (0-21.5) units/week, respectively; $P = 0.007$). There was no significant difference between the two groups in smoking before and after TOS. The results obtained by using univariate and multivariate Cox proportional hazards models are summarized in Table 3. In multivariate analysis, risks for second HNSCC were ADH1B*1/*1 (HR, 1.82; 95% CI, 1.08-3.10; $P = 0.026$), ALDH2*1/*2 + *2/*2 (HR, 2.11; 95% CI, 1.00-5.17; $P = 0.049$), and alcohol consumption before TOS (HR, 1.15; 95% CI, 1.04-1.28; $P = 0.014$).

Third primary HNSCC

The clinical characteristics of patients with third HNSCC are summarized in Table 4. Third HNSCC was detected in 20 patients (10.1%). Patients with third HNSCC were significantly younger than patients without third HNSCC (mean ± SD age, 59.0 ± 6.9 and 66.3 ± 8.0 years, respectively; $P < 0.001$). Patients with third HNSCC included a significantly larger proportion of patients with ADH1B*1/*1 (65.0% and 30.3%, respectively; $P = 0.002$) and a significantly larger proportion of patients with ALDH1*1/*2 + *2/*2 (95.0% and 75.3%, respectively; $P = 0.049$). The amount of alcohol consumption after TOS was significant larger in patients with third HNSCC (median and IQR, 17.9 (11.8-33.0) and 8.0 (0-23.1) units/week, respectively; $P = 0.005$). Smoking before and after TOS was also not significant for patients with third HNSCC. The results using a univariate Cox proportional hazards model are summarized in Table 5. In univariate analysis, risks for third HNSCC were young age (HR, 0.48; 95% CI, 0.27-0.84; $P = 0.010$), ADH1B*1/*1 (HR, 3.81; 1.56-10.16; $P = 0.003$), ALDH2*1/*2 + *2/*2 (HR, 4.84; 95% CI, 1.00-87.05; $P = 0.049$), and alcohol consumption after the first TOS (HR, 1.36; 95% CI, 1.05-1.70; $P = 0.020$). There were no differences in sex, alcohol consumption before the primary TOS, and smoking before and after the primary TOS. The number of third primary HNSCC was not sufficient for multivariate analysis.

Effect of temperance for habitual drinkers and effect of smoking cessation for habitual smokers.

Figure 1A and 1B show cumulative incidence curves according to the effect of temperance and effect of smoking cessation based on the development of second primary HNSCC. The 5-year incidence rates of second primary HNSCC after initial TOS in the temperance group and the non-temperance group were 20.8% and 46.5%, respectively (HR, 0.54; 95% CI, 0.31-0.92; $P = 0.02$). Even when the hazard ratio was adjusted for age, sex, ADH1B, ALDH2 and alcohol consumption before TOS, temperance decreased the incidence of second HNSCC (adjusted HR, 0.54; 95% CI, 0.29-0.96; $P = 0.04$). The rate of development of second HNSCC in the nonsmoking group was not significantly different from that in the continuous smoking group. Figure 1C shows cumulative incidence curves according to the effect of temperance based on the development of

third primary HNSCC. Cumulative development rates of third HNSCC in the temperance group and non-temperance group at 10 years were 11.3% and 36.1%, respectively (HR, 0.19; 95% CI, 0.03-0.65; *P* = 0.006).

Discussion

The phenomenon of multiple squamous cell carcinomas arising in the esophagus and head and neck region has been known as the “field cancerization” phenomenon since more than a half a decade ago [18]. Field cancerization is a concept of multiple cancers developing in the mucosal epithelium of the esophagus and head and neck region due to exposure to carcinogens produced by drinking or smoking [18]. Multiple cancers arise not only synchronously but also metachronously, and the risks of their development, especially the risk for metachronous multiple esophageal squamous cell carcinoma (ESCC) after endoscopic resection, have been reported [19, 20]. As for the relationship between alcohol metabolism gene and field cancerization, Abiko et al. reviewed 158 patients with ESCC who underwent endoscopic resection according to the relationship between metachronous cancer and polymorphisms of ADH1B/ALDH2. They concluded that inactive heterozygous ALDH2 and alcohol consumption after treatment were independent risk factors for metachronous ESCC and HNSCC [21]. Our study including patients with HNSCC who underwent TOS showed that ADH1B*1/*1, ALDH2*1/*2, and alcohol consumption before treatment were independent risk factors for metachronous HNSCC. These differences might be explained by direct genetic damage due to exposure of the pharyngolaryngeal mucosa to alcohol. In fact, the IARC Working Group concluded that there is sufficient evidence for the carcinogenicity of ethanol itself [22, 23].

HNSCC had been considered as a disease with a poor prognosis, but it has been revealed that a favorable prognosis can be expected if HNSCC is found at an early stage. It was reported that 5-year disease-specific survival rates for patients with superficial HNSCC who underwent TOS were 97%-100% [6, 24]. We therefore consider that early detection of HNSCC and prevention of

metachronous HNSCC are very important for maintaining the patient's quality of life after treatment. The head and neck region, especially the hypopharyngeal region, has important roles in swallowing and vocalization. Since the pharynx originally has a narrow lumen, multiple scars caused by repeated TOS would induce swallowing difficulty and aspiration pneumonia. We therefore consider that detection of metachronous HNSCC as early as possible and when its size is small is very important. We recommend that patients who have two or three of the above-mentioned risk factors should undergo detailed follow-up examinations. In addition, our study revealed that continuing to drink alcohol, even in moderate amounts, increased the incidence of not only second primary HNSCC but also third primary HNSCC. Patients would be able to control the amount of drinking by themselves unlike the alcohol metabolism gene. If patients with HNSCC who underwent TOS continue to drink alcohol after treatment, they should be encouraged to reduce alcohol consumption by warning them that continuous drinking leads to economic, social, and physical burdens caused by repeated treatment under general anesthesia, hospital treatment, and deterioration in the quality of life due to organ dysfunctions such as swallowing difficulty. On the other hand, an unexpected result in the present study was that smoking cessation did not influence the development of metachronous HNSCC. As for the effectiveness of smoking cessation for preventing metachronous carcinoma, Katada et al. conducted a prospective study including 330 patients with ESCC who underwent endoscopic resection and their results showed that smoking cessation did not reduce the incidences of metachronous ESCC and HNSCC [25]. Although it is obvious that smoking is one of the most important risk factors, our results suggested that genetic damage when initial HNSCC developed might remain after treatment. Elucidation of the mechanism would require further molecular-biological investigation.

Several limitations of this study should be mentioned. First, our study was a retrospective study. We therefore could not evaluate patients who died of other diseases and who dropped out from the follow-up examination. However, since detection of superficial HNSCC requires an advanced diagnostic technique, it would be difficult to collect a sufficient number of cases as a prospective study design and to carry out regular long-term follow-up. Second, in this study, we did not

investigate human papillomavirus (HPV) infection, which is known as a significant carcinogenic factor for oropharyngeal cancer [26]. However, HPV-positive oropharyngeal squamous cell carcinoma was reported to be mainly associated with the lateral wall of the oropharynx [27]. In this study, the proportion of patients with cancer in the lateral wall of the oropharynx was only 1.5%, and it was therefore considered that the influence of HPV infection was extremely limited. Third, because we simply classified the registered patients according to the average amount of alcohol consumption and smoking habits, detailed variation of them during the period for evaluation (for example, heavy drinking in the former period and abstinence in a later period after treatment) was not evaluated. Fourth, all of the subjects in our study were Japanese. Genetic polymorphisms of alcohol metabolism vary in different ethnic groups and are very different in East Asians and Westerners. It was reported that the frequency of inactive ALDH2*1/*2 allele was less than 5% in Westerners and was 35% in Japanese, whereas the frequency of ADH1B*1/*1 was more than 90% in Westerners and was only 7% in Japanese [28, 29]. Thus, our results regarding the alcohol metabolism gene polymorphism may not be able to be completely applied to other ethnic groups. Further study may therefore be required.

In conclusion, ADH1B *1/*1, ALDH2 *1/*2, and amount of alcohol consumption before treatment are independent risk factors of metachronous HNSCC for patients with HNSCC who have undergone TOS. A follow-up examination schedule should be planned on the basis of the above risk factors. Regarding habitual drinkers, since it was shown that temperance decreased the incidences of second and third metachronous HNSCC, advice to discontinue alcohol drinking is necessary.

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Conflict of Interest Statement: None declared.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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TABLE AND FIGURES LEGENDS

Table 1. Abbreviations: HNSCC, head and neck squamous cell carcinoma; ADH1B, alcohol dehydrogenase 1B; ALDH2, aldehyde dehydrogenase 2; ELPS, endoscopic laryngopharyngeal surgery; EMR, endoscopic mucosal resection; ESD, endoscopic submucosal dissection; TOVS,

transoral video-assisted surgery; PW, posterior wall; AW, anterior wall; LW, lateral wall; UW, upper wall; SEP, subepithelial invasion.

Table 2. Abbreviations: HNSCC, head and neck squamous cell carcinoma; SD, standard deviation; ADH1B, alcohol dehydrogenase 1B; ALDH2, aldehyde dehydrogenase 2; TOS, transoral surgery; IQR, interquartile range. Heavy, ≥18 units/week; Moderate, 9-17.9 units/week; Light, 1-8.9 units/week; Rare, <1 unit/week (1 unit = 22 g ethanol).

Table 3. Abbreviations: HR, hazard ratio; CI, confidence interval; ref, reference; ADH1B, alcohol dehydrogenase 1B; ALDH2, aldehyde dehydrogenase 2; TOS, transoral surgery

Table 4. Abbreviations: HNSCC, head and neck squamous cell carcinoma; SD, standard deviation; ADH1B, alcohol dehydrogenase 1B; ALDH2, aldehyde dehydrogenase 2; TOS, transoral surgery; IQR, interquartile range. Heavy, ≥18 units/week; Moderate, 9-17.9 units/week; Light, 1-8.9 units/week; Rare, <1 unit/week (1 unit = 22 g ethanol).

Table 5. Abbreviations: HR, hazard ratio; CI, confidence interval; ref, reference; ADH1B, alcohol dehydrogenase 1B; ALDH2, aldehyde dehydrogenase 2; TOS, transoral surgery

Fig. 1. (**A**) Cumulative incidence curves according to the effect of temperance based on the development of second primary HNSCC. (**B**) Cumulative incidence curves according to the effect of smoking cessation based on the development of second primary HNSCC. (**C**) Cumulative incidence curves according to the effect of temperance based on the development of third primary HNSCC

Abbreviations: HR, hazard ratio; CI, confidence interval; non-temperance group, patients who continued to drink alcohol with heavy consumption (≥18 units/week) or moderate consumption (9-17.9 units/week) after transoral surgery; temperance group, patients who reduced drinking alcohol from heavy or moderate to light (1-8.9 units/week) or rare (<1 unit/week) consumption after transoral surgery; smoking group, patients who continued heavy smoking (≥30 pack years)

or light smoking (1-29 pack years) after transoral surgery; nonsmoking group, patients who had been heavy or light smokers and who stopped smoking after transoral surgery.

Tables

Table 1 Characteristics of patients at the registration and first primary HNSCC

	All cases (n = 198) n (%)
Age (years), median (range)	65.5 (42-86)
Sex (male)	180 (90.9)
Alcohol consumption	
Heavy	141 (71.2)
Moderate	41 (20.7)
Light	11 (5.6)
Rare	5 (2.5)
Smoking	
Heavy	148 (74.7)
Light	40 (20.2)
Rare	10 (5.1)
ADH1B genotype	
*1/*1	67 (33.8)

*1/*2		56 (28.3)
*2/*2		75 (37.9)
ALDH2 genotype		
*1/*1		45 (22.7)
*1/*2		153 (77.3)
*2/*2		0 (0.0)
Treatment methods		
ELPS		142 (71.7)
EMR		31 (15.7)
ESD		23 (11.6)
TOVS		2 (1.0)
Tumor subsites		
Hypopharynx	Postcricoid area	8 (4.0)
	Pyriform sinus	117 (59.1)
	PW	32 (16.2)
Oropharynx	AW	5 (2.5)
	LW	3 (1.5)
	PW	11 (5.6)

	UW	8 (4.0)
Larynx	Epiglottis	6 (3.0)
	Arytenoid	8 (4.0)
Depth of invasion		
Carcinoma in situ		113 (57.1)
SEP		85 (42.9)
Local recurrence		0 (0)

Table 2. Clinical characteristics of patients according to second HNSCC development.

		Second HNSCC (n = 64)	Without second HNSCC (n = 134)	*P*
Sex (male), n (%)		56 (88.0)	124 (92.5)	0.29
Age (years), means (SD)		61.8 (9.1)	67.4 (7.0)	<0.001
ADH1B genotype, n (%)	*1/*1	33 (51.6)	34 (25.4)	<0.001
	*1/*2 + *2/*2	31 (48.4)	100 (74.6)	
ALDH2 genotype, n (%)	*1/*2 + *2/*2	57 (89.1)	96 (71.6)	0.006
	*1/*1	7 (10.9)	38 (28.4)	
Alcohol consumption before TOS	Heavy, n (%)	55 (85.9)	86 (64.2)	0.011
	Moderate, n (%)	8 (12.5)	33 (24.6)	
	Light, n (%)	1 (1.6)	10 (7.5)	
	Rare, n (%)	0 (0.0)	5 (3.7)	
	units/week, median (IQR)	32.3 (23.4)	23.4 (22.5)	0.011

	Heavy, n (%)	27 (42.2)	41 (30.6)	
Alcohol				
	Moderate, n (%)	17 (26.6)	20 (14.9)	
consumption				0.010
	Light, n (%)	5 (7.8)	31 (23.1)	
after TOS				
	Rare, n (%)	15 (23.4)	42 (31.3)	
	units/week, median (IQR)	15.3 (29.0)	7.8 (21.5)	0.007
	Heavy, ≥30, n (%)	52 (81.3)	96 (71.6)	
Smoking, pack				
	Light, 1-29, n (%)	11 (17.2)	29 (21.6)	0.198
years				
	Rare, 0, n (%)	1 (1.6)	9 (6.7)	
Smoking after				
	Yes, n (%)	19 (29.7)	29 (21.6)	0.22
TOS				

Table 3. Risk factors of second HNSCC by a Cox proportional hazards model.

	Univariate		Multivariate	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age, per +10 years	0.60 (0.44-0.81)	<0.001	0.85 (0.59-1.22)	0.37
Sex				
Female	1 (ref)	0.052	1 (ref)	0.27
Male	0.44 (0.22-1.01)		0.62 (0.29-1.49)	
ADH1B genotype				
*1/*2 + *2/*2	1 (ref)	<0.001	1 (ref)	0.026
*1/*1	2.44 (1.49-4.00)		1.82 (1.08-3.10)	
ALDH2 genotype				
*1/*1	1 (ref)	0.007	1 (ref)	0.049
*1/*2 + *2/*2	2.62 (1.28-6.31)		2.11 (1.00-5.17)	
Alcohol consumption before TOS, per +10 units/week	1.17 (1.06-1.28)	0.002	1.15 (1.04-1.28)	0.014
Alcohol consumption after TOS, per +10 units/week	1.23 (1.07-1.41)	0.005	1.11 (0.96-1.28)	0.173

Smoking, pack years	1.00 (0.99-1.01)	0.46	-	-
Smoking after TOS				
No	1 (ref)	0.120	-	-
Yes	1.55 (0.89-2.62)		-	

Table 4. Clinical characteristics of patients according to third HNSCC development.

		Third HNSCC (n = 20)	Without third HNSCC (n = 178)	*P*
Sex (male), n (%)		17 (85.0)	163 (91.6)	0.40
Age (y), means (SD)		59.0 (6.9)	66.3 (8.0)	<0.001
ADH1B genotype, n (%)	*1/*1	13 (65.0)	54 (30.3)	0.002
	*1/*2 + *2/*2	7 (35.0)	124 (69.7)	
ALDH2 genotype, n (%)	*1/*2 +*2/*2	19 (95.0)	134 (75.3)	0.049
	*1/*1	1 (5.0)	44 (24.7)	
Alcohol consumption before TOS	Heavy, n (%)	17 (85.0)	124 (69.7)	0.69
	Moderate, n (%)	3 (15.0)	38 (21.4)	
	Light, n (%)	0 (0.0)	11 (6.2)	
	Rare, n (%)	0 (0.0)	5 (2.8)	
	units/week, median (IQR)	32.9 (22.7)	25.8 (23.3)	0.48

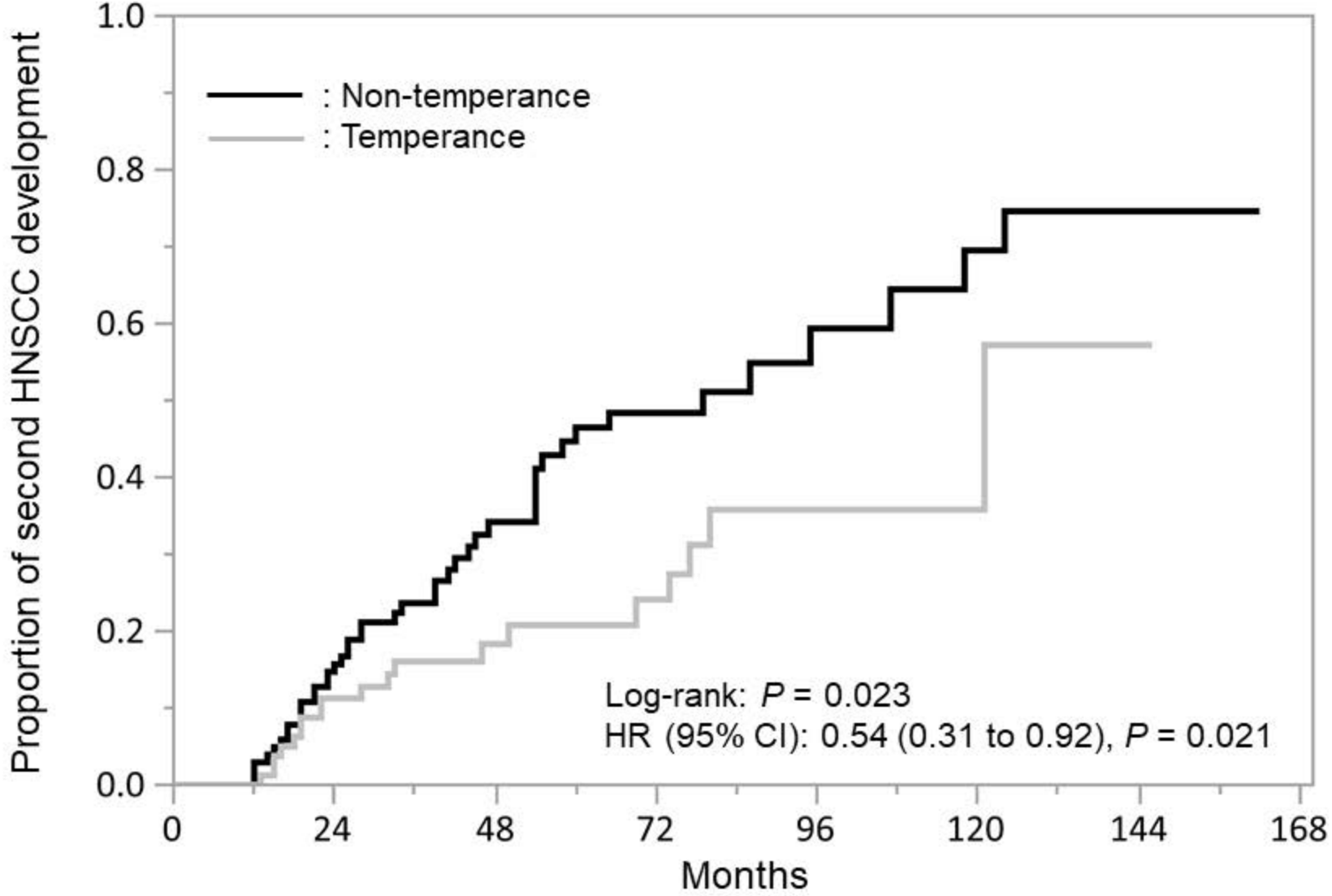
	Heavy, n (%)	10 (50.0)	58 (32.6)	
Alcohol				
	Moderate, n (%)	8 (40.0)	29 (16.3)	
consumption				0.002
	Light, n (%)	0 (0.0)	36 (20.2)	
after TOS				
	Rare, n (%)	2 (10.0)	55 (30.9)	
	units/week, median (IQR)	17.9 (21.2)	8.0 (23.1)	0.005
	Heavy, ≥30, n (%)	17 (85.0)	131 (73.6)	
Smoking, pack				
	Light, 1-29, n (%)	3 (15.0)	37 (20.8)	0.61
years				
	Rare, 0, n (%)	0 (0.0)	10 (5.6)	
Smoking after				
	Yes, n (%)	5 (25.0)	43 (24.2)	0.93
TOS				

Table 5. Risk factors of third HNSCC by a Cox proportional hazards model

	Univariate	
	HR (95% CI)	*P*
Age, per +10 years	0.48 (0.27-0.84)	0.010
Sex		
Female	1 (ref)	0.130
Male	0.34 (0.11-1.46)	
ADH1B genotype		
*1/*2 + *2/*2	1 (ref)	0.003
*1/*1	3.81 (1.56-10.16)	
ALDH2 genotype		
*1/*1	1 (ref)	0.049
*1/*2 + *2/*2	4.84 (1.00-87.05)	
Alcohol consumption before TOS, per +10 units/week	1.09 (0.90-1.30)	0.36
Alcohol consumption after TOS, per +10 units/week	1.36 (1.05-1.70)	0.020

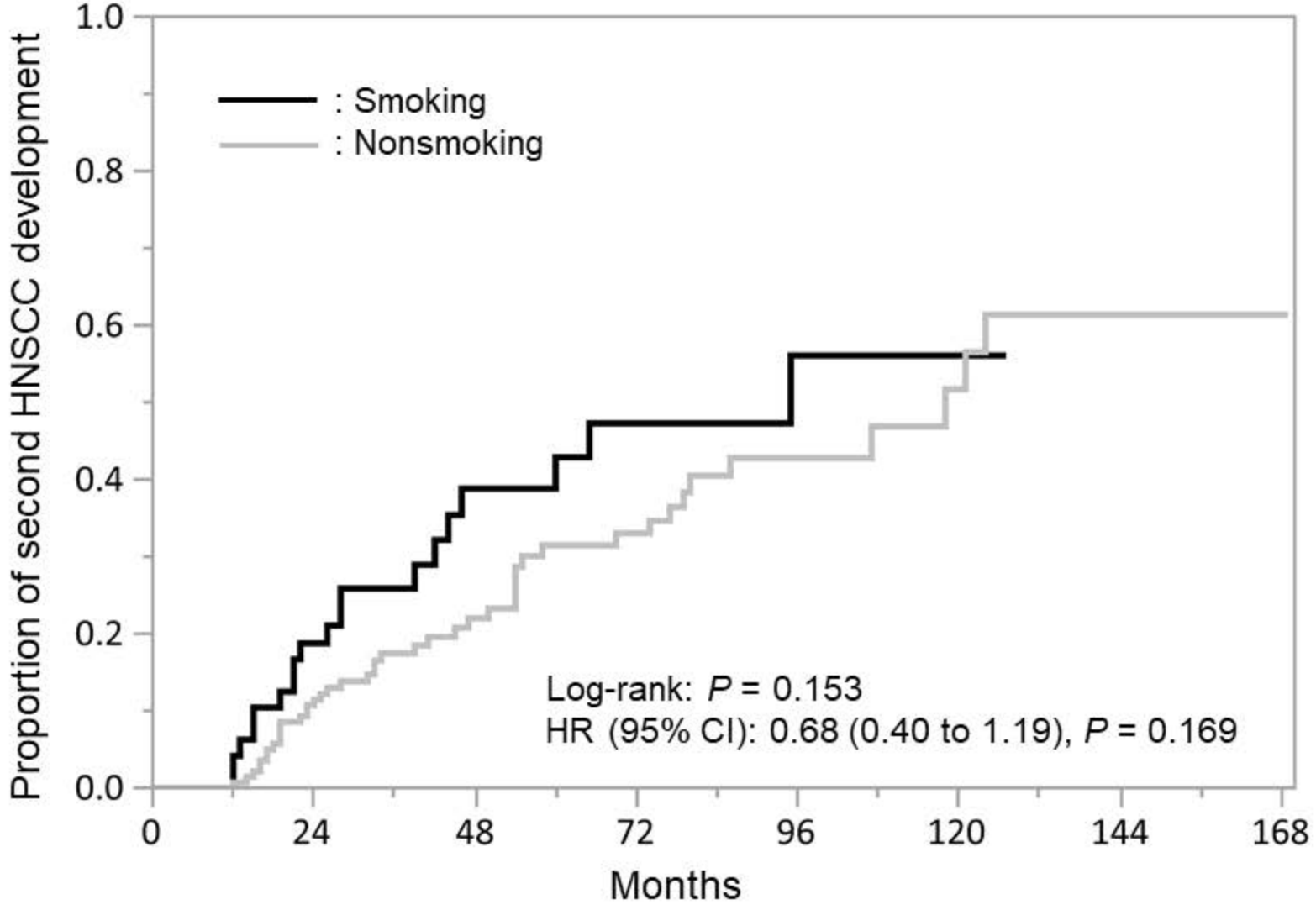
Smoking, pack years	1.00 (0.98-1.01)	0.49
Smoking after TOS		
No	1 (ref)	0.90
Yes	1.07 (0.35-2.80)	

A



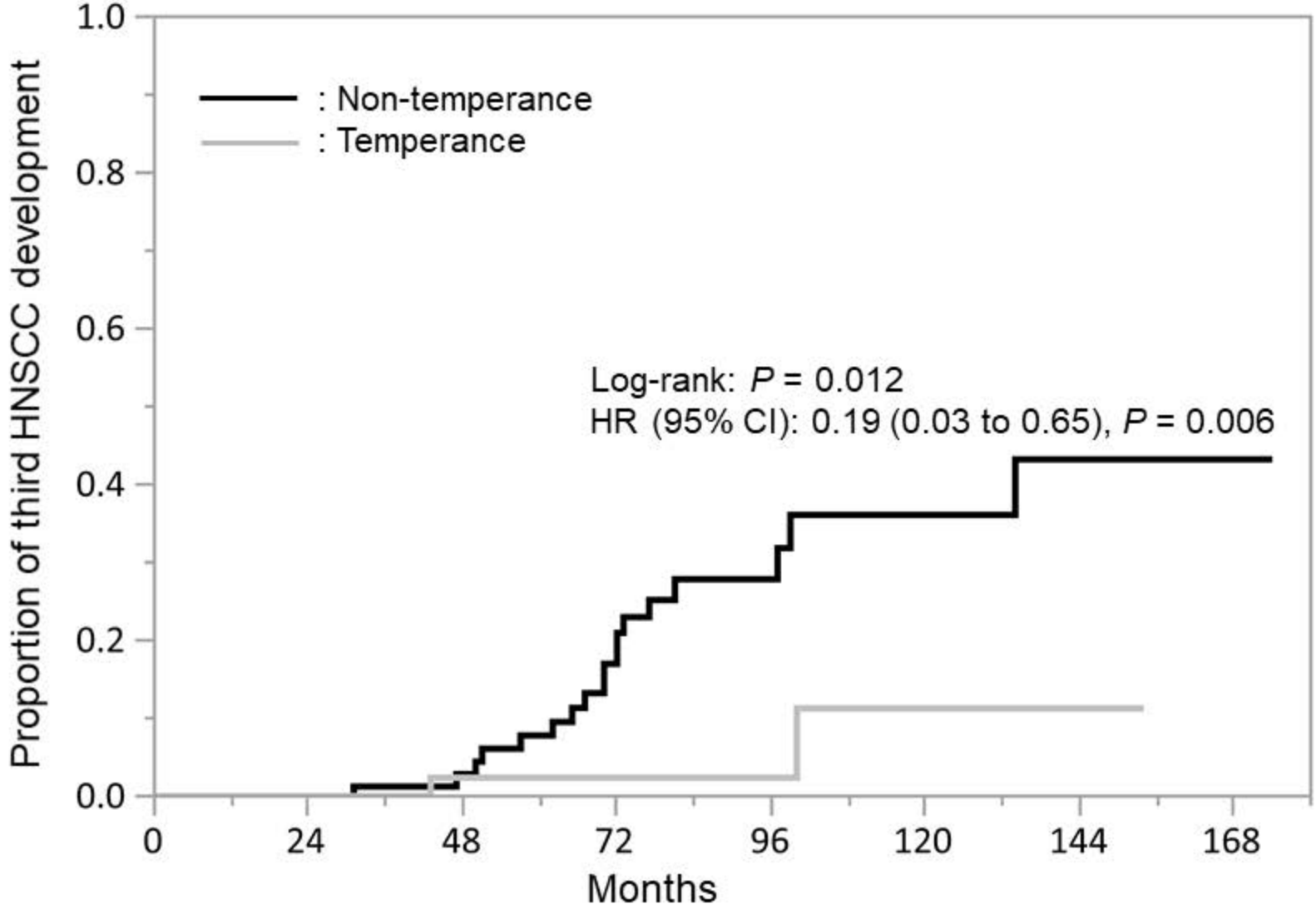
No. non-temperance	102	87	40	24	9	6	2	0
No. temperance	80	71	34	23	8	3	1	0

B



No. smoking	48	39	16	11	5	2	0	0
No. nonsmoking	140	125	63	42	17	11	5	1

C



No. non-temperance	102	102	61	42	18	13	7	1
No. temperance	80	80	36	25	12	5	3	0