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Author(s)	Abiko, Satoshi; Shimizu, Yuichi; Miyamoto, Shuichi et al.
Citation	Journal of gastroenterology, 53(10), 1120-1130 https://doi.org/10.1007/s00535-018-1441-7
Issue Date	2018-10
Doc URL	https://hdl.handle.net/2115/75623
Rights	The final publication is available at link.springer.com
Type	journal article
File Information	JGastroenterol53_1120.pdf



Original article

Risk assessment of metachronous squamous cell carcinoma after endoscopic resection for esophageal carcinoma based on the genetic polymorphisms of alcohol dehydrogenase-1B and aldehyde dehydrogenase-2: Temperance reduces the risk

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Short title

Risk of metachronous SCC

Word count of the manuscript

4154 words

Abstract

Background Metachronous multiple squamous cell carcinoma (SCC) of the esophagus and the head and neck is commonly observed in patients who have previously undergone endoscopic resection (ER) for SCC of the esophagus (ESCC). We evaluated the risk for developing metachronous SCC following ER for ESCC based on the genetic polymorphisms for alcohol dehydrogenase-1B (ADH1B) and aldehyde dehydrogenase-2 (ALDH2) as well as the alcohol consumption and smoking habits.

Methods We studied 158 patients who underwent ER for ESCC (median follow-up 80 months). Genotyping of ADH1B/ALDH2 was performed using saliva sampling. The alcohol consumption and smoking histories of the patients before and after the ER were documented.

Results Multivariate analyses revealed that inactive heterozygous ALDH2 (hazard ratio [HR] = 2.25) and alcohol consumption after ER (HR = 1.94) were independently associated with the risk of developing secondary SCC. Moreover, inactive heterozygous ALDH2 (HR = 4.39) and alcohol consumption after the ER (HR = 2.82) were independently associated with the risk of a third SCC. We analyzed 110 patients who had a history of moderate or heavy alcohol consumption before the ER. The 3-year cumulative incidence rates of secondary SCC in the temperance (n = 65) and non-temperance groups

($n = 45$) were 14.0% and 42.1% ($p = 0.0002$). Further, the 5-year cumulative incidence rates of a third SCC in the temperance and non-temperance groups were 0% and 15.6% ($p = 0.0011$), respectively. In addition, the 7-year cumulative incidence rates of a fourth SCC in the temperance and non-temperance groups were 0% and 15.3% ($p = 0.0015$), respectively.

Conclusions Continued alcohol consumption is an important risk factor for the onset of metachronous SCC and is a risk factor for the third and subsequent SCCs. Strict advice in favor of temperance is crucial.

Keywords Alcohol dehydrogenase1B, Aldehyde dehydrogenase2, Squamous cell carcinoma, Esophageal cancer, Endoscopic resection

Abbreviations

SCC Squamous cell carcinoma

ER Endoscopic resection

ESCC Squamous cell carcinoma of the esophagus

HNSCC Squamous cell carcinoma of the head and neck

ADH1B Alcohol dehydrogenase-1B

ALDH2 Aldehyde dehydrogenase-2

HR Hazard ratio

PCR Polymerase chain reaction

RFLP Restriction fragment length polymorphisms

SD Standard deviation

IQR Interquartile range

QOL Quality of life

Introduction

Recent improvements in the endoscopic diagnosis and treatment technology have resulted in an increase in the number of cases of squamous cell carcinoma (SCC) of the esophagus (ESCC) that are detected early and an increase in the cases for which radical treatment is performed by endoscopic resection (ER). However, the esophagus can be preserved after treatment in such patients; therefore, the number of patients with metachronous multiple ESCC development has also increased [1-3]. Furthermore, patients with ESCC commonly present with synchronous or metachronous SCC of the head and neck (HNSCC). In fact, the reported incidence of metachronous ESCC that develops following ER is 14.6%–23.0% [1-3] and that of metachronous HNSCC is 5.1%–13% [4, 5]. Therefore, ER should not be considered a complete curative treatment for initial ESCC.

As demonstrated in the concept of field cancerization, the esophagus as well as head and neck are common sites for SCC onset [6]. This concept indicates that exposure to carcinogens, such as alcohol and cigarettes, leads to the onset of multiple cancers in the mucosa of the esophagus and that of the head and neck [6]. As per a study, the field cancerization phenomenon might be caused by the accumulation of acetaldehyde, the first

metabolite of ethanol, rather than alcohol itself [7]. Acetaldehyde is primarily metabolized by aldehyde dehydrogenase 2 (ALDH2); however, alcohol consumers with inactive heterozygous ALDH2, a genetic polymorphic type of ALDH2, are at a high risk of ESCC development [8-10]. A functional genetic polymorphism also exists in alcohol dehydrogenase 1B (ADH1B), the stage prior to ALDH2, and the homozygous slow-metabolizing type contributes to the ESCC risk [9, 10]. Japanese genome-related case-controlled studies found that habitual alcohol consumers and smokers who possess both inactive heterozygous ALDH2 and homozygous slow-metabolizing ADH1B are at an extremely high risk of ESCC development [11, 12]. In recent years, the genotyping of ADH1B and ALDH2 genes has become easier with saliva sampling, and Yokoyama et al. reported that the results obtained by using blood and saliva samples were consistent in 114 patients [13]. However, very few studies have reported on the risk of development of metachronous ESCC and metachronous HNSCC following ER according to ADH1B/ALDH2 genotypes; further, to our knowledge, no study has evaluated the risk for the development of a third (or more) SCC following ER for ESCC.

In this study, we retrospectively evaluated the risk of metachronous ESCC and HNSCC developing after ER for ESCC based on the genetic polymorphisms for ADH1B and ALDH2 along with the alcohol consumption and smoking histories.

Patients and Methods

This study was a retrospective study using newly obtained specimens and was approved by the institutional review board at the Hokkaido University Hospital. Written informed consent was obtained from the study subjects (UMIN Clinical Trials Registry ID UMIN000019852).

The study subjects were registered between July 2016 and August 2017. The study subjects included patients who underwent ER for ESCC at the Hokkaido University Hospital and were followed up using endoscopic examination for at least 2 years. The inclusion criteria of this study were as follows: 1) pathological diagnosis of ESCC in the resected specimens, 2) complete endoscopic resection by ER, 3) written informed consent obtained from the patient, and 4) the possibility of collecting saliva samples. Exclusion criteria were as follows: 1) surgical resection after ER, 2) unsuitability as subjects, 3) age < 20 years, and 4) current pregnancy. Total 158 patients were included in the study.

The physicians advised all subjects to exercise moderation in drinking and smoking after the ER. History regarding alcohol consumption and smoking before and after ER were carefully documented using a questionnaire. Some patients may have understated their alcohol consumption and smoking habits; therefore, we emphasized that the

questionnaires would not be used for the patient's personal health evaluation but for epidemiologic studies that would contribute to public health. To examine two single nucleotide polymorphisms (SNPs) in ADH1B and ALDH2 genotyping, we obtained approximately 1 mL saliva using a pipette or a cotton swab before the endoscopic examination. The subjects who consumed < 1 unit/week were classified as rare alcohol consumers, those who consumed 1 to 8.9 units/week were considered light alcohol consumers, those who consumed 9 to 17.9 units/week were classified as moderate alcohol consumers, and those who consumed alcohol $\geq$ 18 units/week were termed heavy alcohol consumers. One unit of alcohol = 22 g, the ethanol content of one serving of sake. Subjects who did not smoke or smoked rarely were classified as rare smokers (non smokers). The current smokers with a smoking history of < 30 pack years were termed light smokers, while those who had a smoking history of $\geq$ 30 pack years were classified as heavy smokers. Thirty pack years = one package of cigarettes (20 cigarettes) daily for 30 years [3, 14].

SNPs of the ADH1B and ALDH2 genes were genotyped using the TaqMan assay on an ABI 7300 Real-Time polymerase chain reaction (PCR) System (Applied Biosystems) [15]. 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 classified the ADH1B

and ALDH2 genotype combinations into five categories as per the classification proposed by Yokoyama et al. [16]: A group, *ADH1B**1/*1 + *ALDH2**1/*1; B group, *ADH1B**2 carrier + *ALDH2**1/*1; C group, *ADH1B**1/*1 + *ALDH2**1/*2; D group, *ADH1B**2 carrier + *ALDH2**1/*2; E group, *ADH1B* any + *ALDH2**2/*2.

Definitions of metachronous ESCC and HNSCC

All patients underwent endoscopic examination using iodine staining at 3 months, 6 months, and 1 year after the ER and every 6 months to 1 year thereafter. All the patients underwent annual physical examinations including laryngoscopy that was conducted by otolaryngologists. Metachronous ESCC was defined as a lesion located away from the scar of the initial ER and detected ≥ 1 year after the ER. The ER scar was identified endoscopically as the convergence of tiny folds. A lesion that occurred close to the scar was defined as local recurrence. Metachronous HNSCC was defined as a lesion detected ≥ 1 year after the ER. A lesion detected within 1 year of ER was defined as synchronous ESCC or HNSCC that was overlooked at the initial evaluation.

Statistical analyses

JMP® Pro 12.0.1 (SAS Institute, Inc., Cary, NC, USA) was used for data analyses. Age was expressed in terms of mean $\pm$ standard deviation (SD) values. Alcohol consumption and alcohol consumption after the ER are expressed as median values with 25 and 75 percentiles. T-tests (for continuous variables and parametric models), Wilcoxon signed-rank test (for continuous variables and non-parametric models), and Fisher's exact test (for categorical variables) were used to make comparisons. A cox proportional hazard model for univariate and multivariate analyses was used to examine the predictors of the development of metachronous SCC in the esophagus as well as in the head and neck region. Differences were considered statistically significant at $p < 0.05$. Variables that tended to be associated with the development of metachronous SCC in the esophagus as well as the head and neck region ($p < 0.05$) in the univariate analyses were included in the cox proportional hazard model for multivariate analyses for identification of the independent predictors for the development of metachronous SCC in the esophagus as well as the head and neck region. We defined the time to development of metachronous SCC from the day of the ER to the day of the endoscopic diagnosis of metachronous SCC. The Kaplan–Meier method and log-rank test were performed for analyzing the

development of metachronous SCC in the esophagus as well as the head and neck region.

A cox proportional hazards model was used for estimating the hazard ratio and 95% confidence interval (CI).

Results

Secondary SCC

During a median follow-up period of 80 months (range, 24–244 months), a secondary SCC was detected in 54 patients (34.2%). Table 1 shows the characteristics of patients with and without secondary SCC. Compared to patients without secondary SCC, patients those with secondary SCC were significantly younger (mean $\pm$ SD age: 63.5 $\pm$ 7.5 vs 67.2 $\pm$ 7.9 years, $p = 0.0059$), included a larger proportion of patients with inactive heterozygous ALDH2 (77.8% vs. 55.9%, $p = 0.0087$), had greater alcohol consumption (median and interquartile range [IQR] = 22.2 [13.2–32.6] vs. 11.3 [3.9–21] units/week, $p < 0.0001$), had greater alcohol consumption after ER (median [IQR] = 8.8 [0–21] vs. 0 [0–5.4] units/week, $p < 0.0001$), and included a larger proportion of heavy smokers (75.9% vs 50.0%, $p = 0.0024$).

As expected from the results shown in Table 1, secondary SCC was positively correlated with age, slow-metabolizing ADH1B, inactive heterozygous ALDH2, alcohol consumption, alcohol consumption after the ER, and cigarette smoking, as per the univariate analysis (Table 2). Multivariate analyses revealed inactive heterozygous ALDH2 (HR = 2.25, $p < 0.0183$) and alcohol consumption after the ER (HR = 1.94, $p < 0.0001$) to be independently associated with the risk of secondary SCC development (Table 2).

Third SCC

A third SCC was detected in 23 patients (14.6%). Table 3 depicts the characteristics of patients with and without a third SCC. Compared to the group of patients without a third SCC, that of those with a third SCC included a larger proportion of patients with inactive heterozygous ALDH2 (82.6% vs 60.0%, $p = 0.0591$) and those with greater alcohol consumption after the ER (median [IQR] = 12 [4.9–25] vs. 0.5 [0–8] units/week, $p < 0.0001$).

As expected based on the results shown in Table 3, a third SCC was positively correlated with slow-metabolizing ADH1B, inactive heterozygous ALDH2, and alcohol

consumption after the ER by univariate analysis (Table 4). Multivariate analyses revealed that inactive heterozygous ALDH2 (HR = 4.39, $p < 0.0247$) and alcohol consumption after the ER (HR = 2.68, $p < 0.0001$) was independently associated with the risk for the development of a third SCC after the ER (Table 4).

Fourth SCC

A fourth SCC was detected in seven patients (4.4%). Table 5 shows the characteristics of patients with and without the fourth SCC. Compared to patients without a fourth SCC, those with a fourth SCC were significantly younger (mean $\pm$ SD age: 59.6 ± 10.5 vs. 66.2 ± 7.7 years, $p = 0.0300$), had greater alcohol consumption (median [IQR] = $33.3 [16.5-35]$ vs. $14 [6-24]$ units/week, $p = 0.0192$), and had greater alcohol consumption after the ER (median [IQR] = $27 [12-25]$ vs. $1.5 [0-8.4]$ units/week, $p < 0.0001$).

Univariate and multivariate cox proportional hazards model analyses were not performed because of the small number of patients who had a fourth SCC.

Secondary esophageal SCC

Secondary ESCC was detected in 47 patients (29.8%).

Secondary ESCC was positively correlated with age, inactive heterozygous ALDH2, alcohol consumption, alcohol consumption after ER, and cigarette smoking by univariate analysis (Table 6). Multivariate analyses revealed that inactive heterozygous ALDH2 (HR = 2.77, $p < 0.0081$) and alcohol consumption after the ER (HR = 2.04, $p < 0.0001$) were independently associated with the risk for developing secondary ESCC after the ER (Table 6).

Secondary head and neck SCC

Secondary HNSCC was detected in 16 patients (10.1%).

Secondary HNSCC was positively correlated with slow-metabolizing ADH1B and alcohol consumption after ER by univariate analysis (Table 6). Multivariate analysis revealed that slow-metabolizing ADH1B (HR = 3.33, $p < 0.0223$) and alcohol consumption after ER (HR = 2.04, $p < 0.0002$) were independently associated with the risk for development of secondary HNSCC after ER (Table 6).

Evaluation of the effect of temperance in patients who consumed alcohol before the ER before the ER

We analyzed the temperance status of the 110 patients who were classified as moderate or heavy alcohol consumers before the ER based on their drinking habits after the ER and divided them into a temperance group ($n = 65$, $\leq$ light alcohol consumers after the ER) and a non-temperance group ($n = 45$). Cumulative incidence curves of the secondary SCC in the temperance and non-temperance groups revealed 3-year incidence rates of 14.0% and 42.1%, respectively ($p = 0.0002$) (hazard ratio [95% CI]: 2.82 [1.60–5.05], $p = 0.0003$) (Figure 1a). The cumulative incidence curves of a third SCC in the temperance and non-temperance groups revealed 5-year incidence rates of 0.0% and 15.6%, respectively ($p = 0.0011$) (hazard ratio [95% CI]: 4.62 [1.79–14.22], $p = 0.0012$) (Figure 1b), while the cumulative incidence curves of a fourth SCC in these groups revealed 7-year incidence rates of 0% and 15.3%, respectively ($p = 0.0015$) (hazard ratio not estimated because of no events in the temperance group, $p = 0.0003$) (Figure 1c).

Discussion

Genetic polymorphisms in alcohol metabolism have been found to vary considerably, depending on ethnicity. Further, inactive heterozygous ALDH2 has been found to affect < 5% of the Western population [17]; however, it affects 35% of the Japanese population [18]. By contrast, homozygous slow-metabolizing ADH1B affects up to 90% of the Western population but only up to 7% of the Japanese population [18]. This difference in the genetic polymorphisms in alcohol metabolism is assumed an underlying factor responsible for the differences in the incidence of the histological type of esophageal cancer between Japanese and Western populations.

In the present study, multivariate analyses showed that inactive heterozygous ALDH2 and continued alcohol consumption after the ER for ESCC were significant risk factors of secondary SCC. Further, inactive heterozygous ALDH2 and continued alcohol consumption after the ER was a significant risk factor of a third SCC. Furthermore, factor analysis revealed that risk factors for a fourth SCC included younger age, alcohol consumption, and continued alcohol consumption after the ER. These results indicate that continued alcohol consumption, a factor that the patients can control using their will power, is a particularly important clinical risk factor.

To determine the risk of metachronous ESCC and metachronous HNSCC following ER for ESCC, Katada et al. conducted a multicenter prospective study involving 330 individuals and found that the patients who abstained from alcohol had a significantly lower incidence of metachronous multiple SCC onset than those who continued alcohol consumption [19]. Although the genes involved in alcohol metabolism were not examined in the study and the median observation period was relatively short (49.4 months), it included a large sample size; thus, and the conclusion was comparable to that based on the results of the present study. In contrast, Kagemoto et al. conducted a retrospective study involving 117 patients who had undergone ER for ESCC, and multivariate analyses revealed that the *ADH1B**1/*1 genotype (that results in the expression of slow-metabolizing ADH1B), inactive heterozygous ALDH2 and habitual smoking were risk factors for metachronous ESCC onset; further, alcohol consumption was not found to be a risk factor [20]. Although the reason for the contradictory results in our study is unclear, we believe this difference could be primarily attributable to the fact that habitual alcohol consumption and smoking were only examined prior to treatment in their study and their observation period was short (median 38.8 months). In our study with long-term observation (median 80.0 months), temperance following ER was found to be important for the prevention of metachronous SCC onset, especially in patients with inactive

heterozygous ALDH2. Furthermore, continued alcohol consumption was identified as the strongest risk factor for not only the second, but also the third and subsequent SCCs.

ESCC has been considered as a disease with a poor prognosis for a long time; however, reports indicate that a good prognosis can be expected with early detection. Previous studies have shown that the 5-year overall survival rate in patients with intramucosal ESCC who underwent ER was 89%–95% [2, 21, 22]; therefore, we believe that the prevention of metachronous multiple SCC is very important to ensure good quality of life (QOL) for the patient. The esophagus is an organ with a narrow lumen, wherein stricture and dysphagia are common problems following ER. Even if such adverse events do not occur with initial treatment, repeated ER causes multiple scars following resection, and this may consequently lead to dysphagia. While ER can also be performed for HNSCC if detected at an early stage, scars following resection can similarly cause dysphagia. We believe that patients with ESCC undergoing ER should be informed of the risks of detecting the development of metachronous multiple SCCs in an advanced stage and that repeated treatment carries not only a financial and social burden but also carries the risk of reduced QOL in the future. Such patients should also be strictly instructed to reduce or stop alcohol consumption.

When analysis was performed with metachronous SCC divided into ESCC and HNSCC,

inactive heterozygous ALDH2 and alcohol consumption after ER were identified as risk factors for ESCC and alcohol consumption after ER and slow-metabolizing ADH1B as those for HNSCC. Alcohol consumption in individuals with slow-metabolizing ADH1B is a risk factor for HNSCC [23-25], and the present study also confirmed this association in Japanese patients with ESCC undergoing ER. Further studies for personalized surveillance, for example, by shortening the intervals between endoscopic examinations for ESCC patients with inactive heterozygous ALDH2 who underwent ER and by shortening the intervals between the physical examinations by otolaryngologists for ESCC patients with slow-metabolizing ADH1B who underwent ER, are required.

A limitation of the present study is that it was a single-center retrospective study. However, it is challenging to perform long-term regular follow-ups for a cohort with one disease in prospective studies. We also believe that the retrospective nature of this study enabled us to perform long-term follow-up and examine the risk of third and subsequent SCCs onset. The higher incidence of metachronous ESCC in this study compared to that in earlier studies could also be attributed to the longer follow-up period.

In conclusion, for patients with ESCC who undergo ER, continued alcohol consumption, either at a moderate or at a higher level, is an important risk factor for metachronous multiple ESCC and HNSCC onset and represents a risk factor for third and subsequent

SCCs. Strict instruction in favor of temperance is important, and consultation with a specialist department, such as the psychiatry department is recommended for these patients.

Acknowledgments We thank Masayoshi Ono, Masakazu Takahashi, Yasuaki Mori, Manabu Nakagawa, Soichi Nakagawa, Katsuhiro Mabe, and Mototsugu Kato for general advice.

Compliance with ethical standards

Financial support The authors have no support or funding to report.

Conflict of interest The authors declare that they have no conflicts of interest.

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Figure legends

Figure 1a-c. Cumulative incidence curves of secondary, third and fourth SCC in the temperance and non-temperance groups.

Table 1. Characteristics of patients with and without secondary SCC

			All subjects (n = 158)	Secondary (n = 54, 34.2%)	Without secondary (n = 104)	<i>P</i>
Sex (male)			82.2%	88.9%	78.9%	0.1303
Age (years), mean $\pm$ SD			70.0 $\pm$ 8.0	63.5 $\pm$ 7.5	67.2 $\pm$ 7.9	0.0059
Genotype combinations						
	ADH1B	ALDH2				
A	*1/*1	*1/*1	3.8%	5.6%	2.9%	0.0173
B	*2 carrier	*1/*1	31.6%	16.7%	39.4%	
C	*1/*1	*1/*2	12.7%	18.5%	9.6%	
D	*2 carrier	*1/*2	51.3%	59.3%	47.1%	
E	Any	*2/*2	0.6%	0%	1.0%	
ADH1B genotype						0.0730
*1/*1			16.5%	24.1%	12.5%	
*2 carrier			83.5%	75.9%	87.5%	
ALDH2 genotype						0.0087
*1/*1 + *2/*2			36.7%	22.2%	44.2%	
*1/*2			63.2%	77.8%	55.9%	
Alcohol consumption (units/week)	Heavy		44.9%	57.4%	38.4%	0.0003
	Moderate		24.7%	33.3%	20.2%	
	Light		19.6%	7.4%	26.0%	
	Rare		10.8%	1.9%	15.4%	
	Median(IQR)		14(6–26.3)	22.2(13.2–32.6)	11.3(3.9–21)	
Alcohol consumption after ER (units/week)	Heavy		13.2%	31.5%	3.9%	<.0001
	Moderate		15.2%	20.4%	12.5%	
	Light		26.0%	22.2%	27.9%	
	Rare		45.6%	25.9%	55.8%	
	Median(IQR)		1.9(0–9.8)	8.8(0–21)	0(0–5.4)	

Cigarette smoking	Heavy	58.9%	75.9%	50.0%	0.0024
	Light	27.2%	20.4%	30.8%	
	Rare	14.0%	3.7%	19.2%	
Smoking after ER	Yes	20.9%	25.9%	18.3%	0.3039
	No	79.1%	74.1%	81.7%	

Rare, <1 unit/week; light, 1–8.9 units/week; moderate, 9–17.9 units/week;

heavy, ≥ 18 units/week (1 unit = 22 g ethanol).

Rare = non-smoker; Light, <30 pack years; Heavy, ≥ 30 pack years

30 pack years = one package of cigarettes (20 cigarettes) daily for 30 years.

Table 2. Univariate and multivariate cox proportional hazards model analyses demonstrating the relationships of secondary SCC with other variables

	Univariate		Multivariate	
	HR (95%CI)	<i>P</i>	HR (95%CI)	<i>P</i>
Age, per + 10 years	0.69(0.50–0.95)	0.0221	1.00(0.71–1.43)	0.9874
Sex				
Female	1(ref)	0.0754	-	-
Male	2.17(1.00–5.67)		-	-
ADH1B genotype				
*2 carrier	1(ref)	0.0383	1(ref)	0.1347
*1/*1	1.95(1.00–3.56)		1.64(0.83–3.10)	-
ALDH2 genotype				
*1/*1 + *2/*2	1(ref)	0.0079	1(ref)	0.0183
*1/*2	2.39(1.30–4.75)		2.25(1.18–4.59)	
Alcohol consumption per +10 U	1.23(1.07–1.38)	0.0014	1.17(0.95–1.38)	0.1021
Alcohol consumption after ER, per +10 U	2.17(1.71–2.72)	<.0001	1.94(1.49–2.52)	<.0001
Cigarette smoking				
Rare/light(<30)	1(ref)	0.0023	1(ref)	0.1197
Heavy(≥30)	2.64(1.45–5.14)		1.68(0.90–3.34)	
Smoking after ER				
No	1(ref)	0.2579	-	-
Yes	1.42(0.75–2.56)		-	-

Table 3. Characteristics of patients with and without a third SCC

			All subjects (n = 158)	Third (n = 23, 14.6%)	Without third (n = 135)	<i>P</i>
Sex (male)			82.2%	91.3%	80.7%	0.3738
Age (years), mean $\pm$ SD			70.0 $\pm$ 8.0	63.7 $\pm$ 7.9	66.3 $\pm$ 7.9	0.1478
Genotype combinations						
	ADH1B	ALDH2				
A	*1/*1	*1/*1	3.8%	4.4%	3.7%	0.1623
B	*2 carrier	*1/*1	31.6%	13.0%	34.8%	
C	*1/*1	*1/*2	12.7%	21.7%	9.5%	
D	*2 carrier	*1/*2	51.3%	60.9%	49.6%	
E	any	*2/*2	0.6%	0%	0.7%	
ADH1B genotype						0.2207
*1/*1			16.5%	26.1%	14.8%	
*2 carrier			83.5%	73.9%	85.2%	
ALDH2 genotype						0.0591
*1/*1 + *2/*2			36.7%	17.4%	40.0%	
*1/*2			63.2%	82.6%	60.0%	
Alcohol consumption (units/week)	Heavy		44.9%	56.5%	43.0%	0.0202
	Moderate		24.7%	39.1%	22.2%	
	Light		19.6%	4.4%	22.2%	
	Rare		10.8%	0%	12.6%	
	Median(IQR)		14(6–26.3)	22.7(15–33.3)	12.5(5–24)	0.0069
Alcohol consumption after ER (units/week)	Heavy		13.2%	39.1%	8.9%	0.0002
	Moderate		15.2%	26.1%	13.3%	
	Light		26.0%	17.4%	27.4%	
	Rare		45.6%	17.4%	50.4%	
	Median(IQR)		1.9(0–9.8)	12(4.9–25)	0.5(0–8)	<.0001

Cigarette smoking	Heavy	58.9%	73.9%	56.3%	0.2728
	Light	27.2%	21.7%	28.2%	
	Rare	14.0%	4.4%	15.6%	
Smoking after ER	Yes	20.9%	30.4%	19.3%	0.2665
	No	79.1%	69.6%	80.7%	

Table 4. Univariate and multivariate cox proportional hazards model analyses
demonstrating the relationships of a third SCC with other variables

	Univariate		Multivariate	
	HR (95%CI)	<i>P</i>	HR (95%CI)	<i>P</i>
Age, per + 10 years	0.76(0.46–1.31)	0.2947	-	-
Sex				
Female	1(ref)	0.1983	-	-
Male	2.61(0.75–16.4)		-	-
ADH1B genotype				
*2 carrier	1(ref)	0.0178	1(ref)	0.0544
*1/*1	3.23(1.13–8.15)		2.81(0.92–7.82)	
ALDH2 genotype				
*1/*1+*2/*2	1(ref)	0.0276	1(ref)	0.0247
*1/*2	3.98(1.34–17.07)		4.39(1.89–3.86)	
Alcohol consumption per + 10 U	1.21(0.95–1.48)	0.0836	-	-
Alcohol consumption after ER, per + 10 U	2.48(1.77–3.49)	<.0001	2.68(1.89–3.86)	<.0001
Cigarette smoking				
Rare/light(<30)	1(ref)	0.2171	-	-
Heavy(≥30)	1.83(0.73–5.16)		-	-
Smoking after ER				
No	1(ref)	0.0816	-	-
Yes	2.26(0.85–5.53)		-	-

Table 5. Characteristics of patients with and without a fourth SCC

			All subjects (n = 158)	Fourth (n = 7, 4.4%)	Without fourth (n = 151)	<i>P</i>
Sex (male)			82.2%	85.7%	82.1%	1.0000
Age (years), mean $\pm$ SD			70.0 $\pm$ 8.0	59.6 $\pm$ 10.5	66.2 $\pm$ 7.7	0.0300
Genotype combinations						
	ADH1B	ALDH2				
A	*1/*1	*1/*1	3.8%	0.0%	4.0%	0.4849
B	*2 carrier	*1/*1	31.6%	14.3%	32.5%	
C	*1/*1	*1/*2	12.7%	28.6%	11.9%	
D	*2 carrier	*1/*2	51.3%	57.1%	51.0%	
E	any	*2/*2	0.6%	0%	0.7%	
ADH1B genotype						0.3241
*1/*1			16.5%	28.6%	15.9%	
*2 carrier			83.5%	71.4%	84.1%	
ALDH2 genotype						0.4241
*1/*1 + *2/*2			36.7%	14.3%	37.8%	
*1/*2			63.2%	85.7%	62.3%	
Alcohol consumption (units/week)	Heavy		44.9%	71.4%	43.7%	0.5054
	Moderate		24.7%	28.6%	24.5%	
	Light		19.6%	0%	20.5%	
	Rare		10.8%	0%	11.3%	
	Median(IQR)		14(6–26.3)	33.3(16.5–35)	14(6–24)	0.0192
Alcohol consumption after ER (units/week)	Heavy		13.2%	71.4%	10.6%	<.0001
	Moderate		15.2%	28.6%	14.6%	
	Light		26.0%	0%	27.2%	
	Rare		45.6%	0%	47.7%	
	Median(IQR)		1.9(0–9.8)	27(12–25)	1.5(0–8.4)	<.0001

Cigarette Smoking	Heavy	58.9%	71.4%	58.3%	0.7486
	Light	27.2%	28.6%	27.2%	
	Rare	14.0%	0.0%	14.6%	
Smoking after ER	Yes	20.9%	28.6%	20.5%	0.6368
	No	79.1%	71.4%	79.5%	

Table 6. Univariate and multivariate cox proportional hazards model analyses demonstrating the relationships of secondary ESCC and HNSCC with other variables

Univariate and multivariate cox proportional hazards model analyses demonstrating the relationships of a secondary ESCC with other variables				
	Univariate		Multivariate	
	HR (95%CI)	<i>P</i>	HR (95%CI)	<i>P</i>
Age, per + 10 years	0.69(0.49–0.99)	0.0381	1.03(1.36–6.24)	0.8850
Sex				
Female	1(ref)	0.0954	-	-
Male	2.21(0.96–6.40)		-	-
ADH1B genotype				
*2 carrier	1(ref)	0.1913	-	-
*1/*1	1.60(0.75–3.11)		-	-
ALDH2 genotype				
*1/*1 + *2/*2	1(ref)	0.0056	1(ref)	0.0081
*1/*2	2.80(1.42–6.18)		2.77(1.36–6.24)	
Alcohol consumption per + 10 U	1.22(1.06–1.39)	0.0035	1.14(0.91–1.37)	0.1989
Alcohol consumption after ER, per + 10 U	2.27(1.76–2.88)	<.0001	2.04(1.54–2.69)	<.0001
Cigarette smoking				
Rare/light(<30)	1(ref)	0.0042	1(ref)	0.1490
Heavy(≥30)	2.68(1.41–5.53)		1.68(0.85–3.56)	
Smoking after ER				
No	1(ref)	0.3336	-	-
Yes	1.38(0.69–2.59)		-	-

Univariate and multivariate cox proportional hazards model analyses demonstrating the relationships of secondary HNSCC with other variables				
	Univariate		Multivariate	
	HR (95%CI)	<i>P</i>	HR (95%CI)	<i>P</i>
Age, per + 10 years	0.88(0.50–1.66)	0.6862	-	-
Sex				
Female	1(ref)	0.1679	-	-
Male	4.16(0.84–75.3)		-	-
ADH1B genotype				
*2 carrier	1(ref)	0.0135	1(ref)	0.0223
*1/*1	3.61(1.22–9.78)		3.33(1.11–9.17)	
ALDH2 genotype				
*1/*1 + *2/*2	1(ref)	0.0971	-	-
*1/*2	2.90(0.93–12.66)		-	-
Alcohol consumption				
per + 10 U	1.19(0.92–1.45)	0.1200	-	-
Alcohol consumption after ER, per + 10 U	2.11(1.42–3.06)	0.0001	2.04(1.39–2.94)	0.0002
Cigarette smoking				
Rare/light(<30)	1(ref)	0.1505	-	-
Heavy(≥30)	2.29(0.80–8.21)		-	-
Smoking after ER				
No	1(ref)	0.2676	-	-
Yes	1.82(0.57–5.00)		-	-

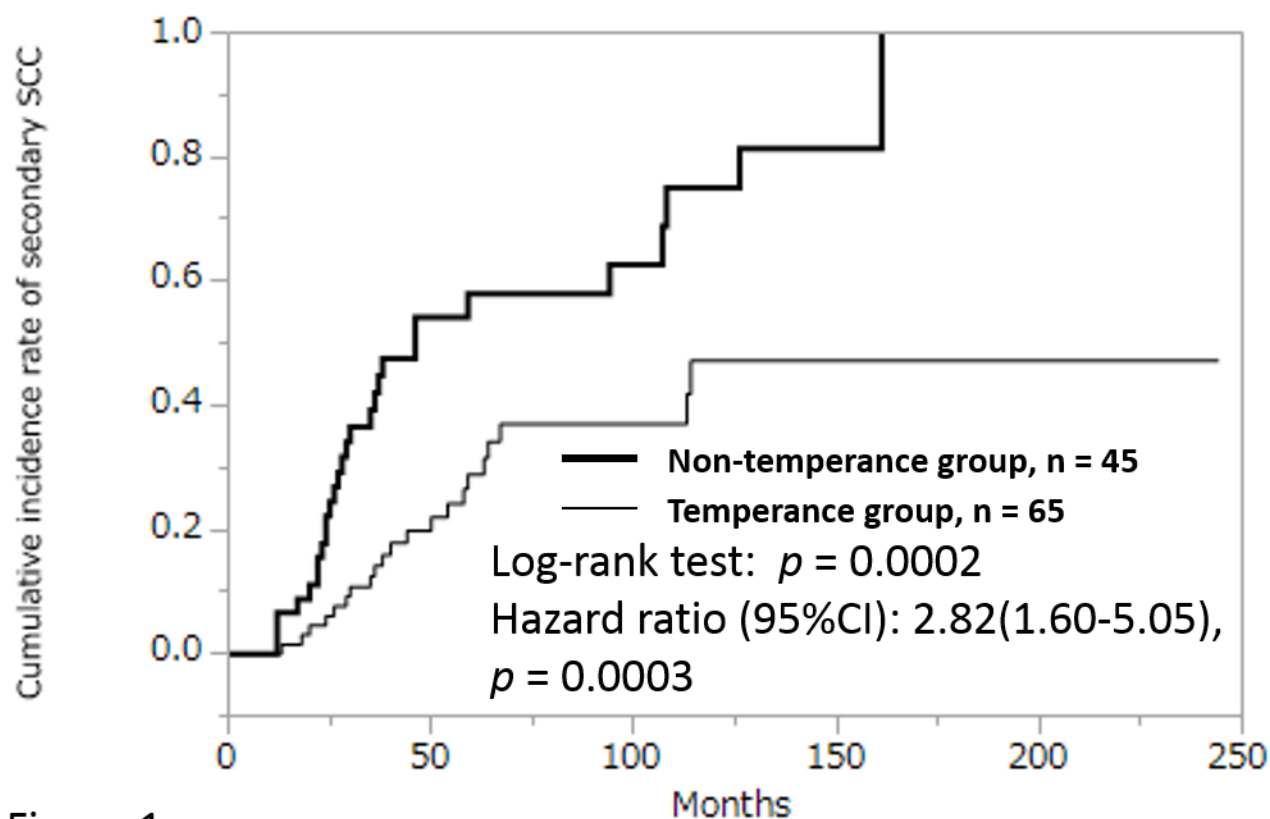


Figure 1a

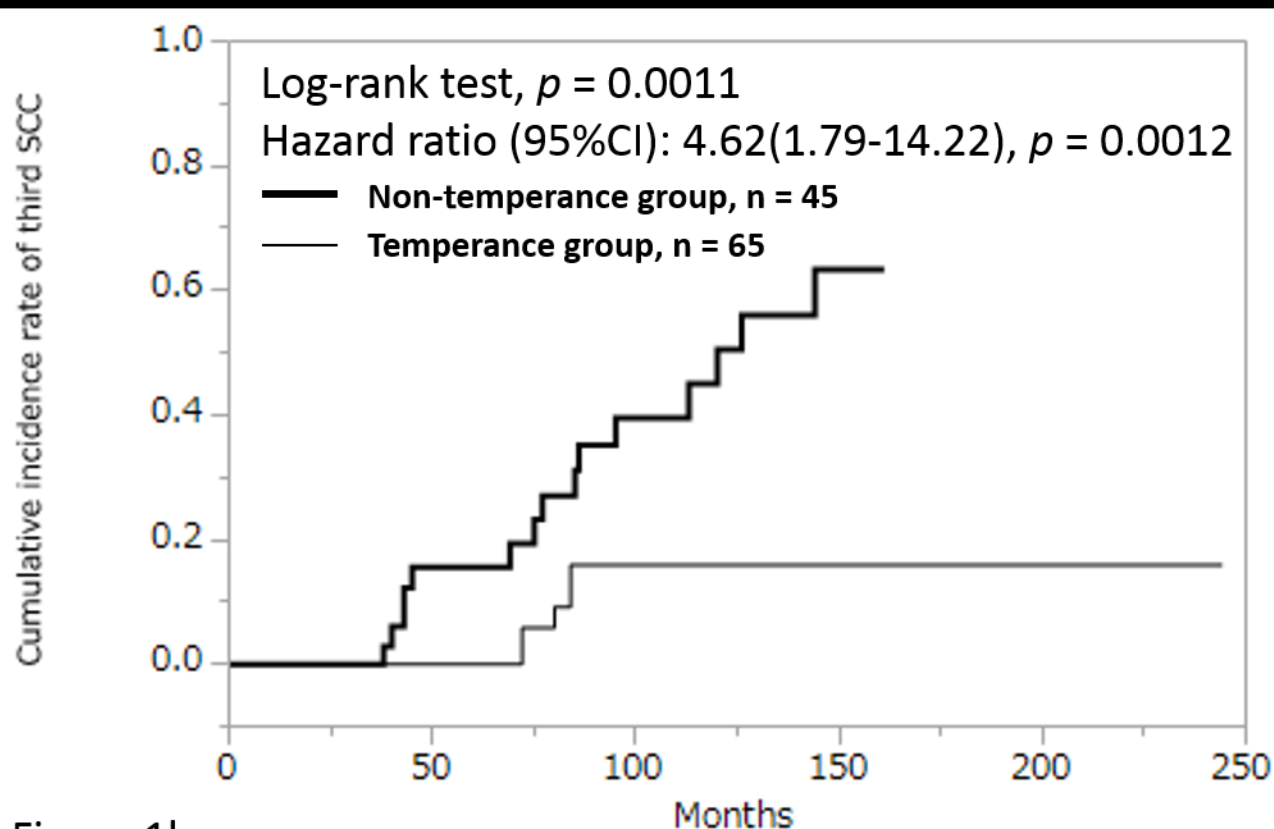


Figure 1b

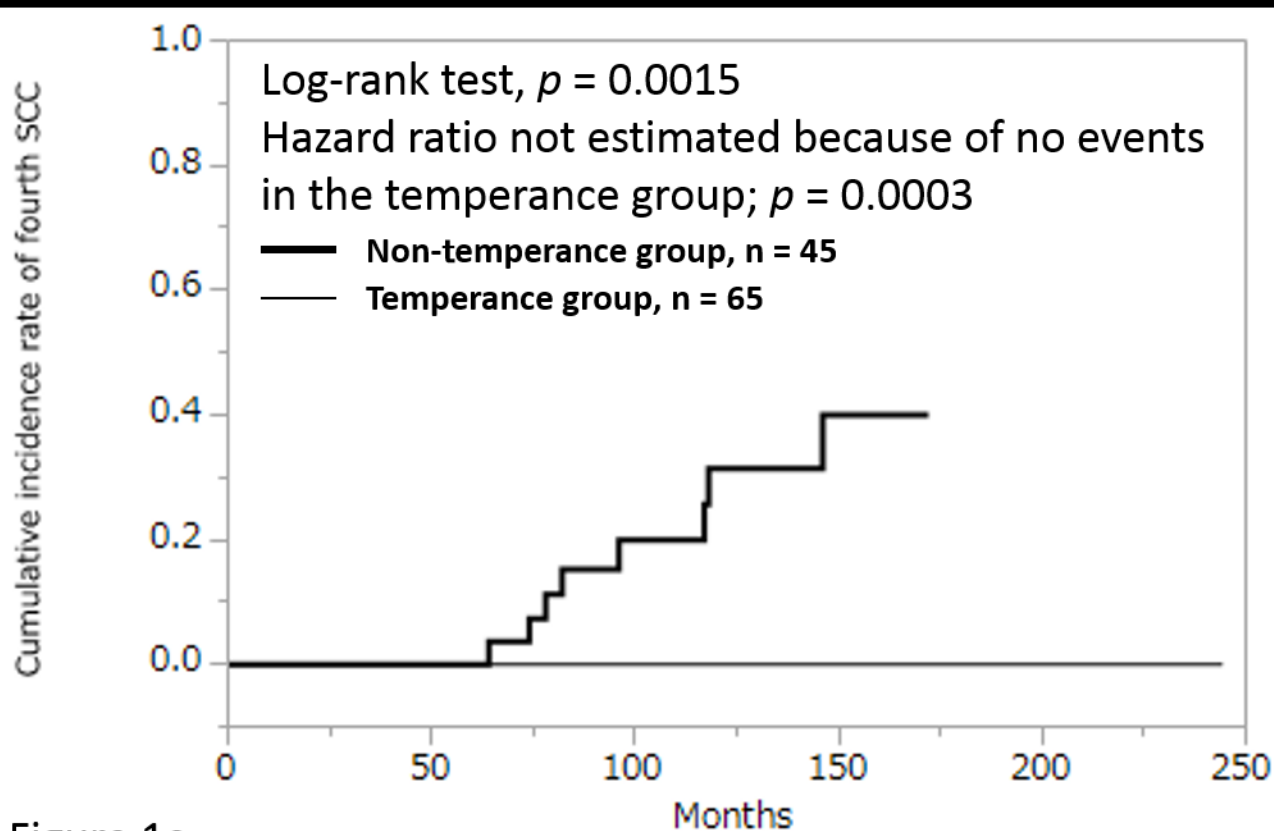


Figure 1c