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Polymorphisms of the *DNMT3b* gene and risk of squamous cell carcinoma of the head and neck: a case-control study

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Abstract

DNA-methyltransferase-3B (DNMT3B) may play an oncogenic role during tumorigenesis, and its genetic variants have been reportedly to be associated with risk of several cancers, but few studies have investigated their roles in head and neck cancer. Here we report a hospital-based case-control study with 832 SCCHN patients and 843 cancer-free controls of non-Hispanic whites that evaluated the association between two *DNMT3B* single nucleotide polymorphisms (SNPs) *DNMT3B*-149C>T (rs2424913) and *DNMT3B*-579G>T (rs2424909) in the promoter region and risk of squamous cell carcinoma of the head and neck (SCCHN). We found that compared with C-allele carriers, the *DNMT3B*-149 TT genotype was statistically significantly associated with increased risk of SCCHN (adjusted OR, 1.35, 95% CI, 1.01-1.80, $P = 0.043$), whereas the *DNMT3B*-579 TT genotype showed only a non-statistically significant risk compared with G-allele carriers. Further analysis of the effects of combined genotypes suggested that subjects with either *DNMT3B*-149 TT or *DNMT3B*-579 TT homozygous genotypes had statistically significantly increased risk of SCCHN (adjusted OR = 1.36, 95% CI = 1.07-1.73, $P = 0.013$). Stratification analysis showed a more profound risk in the subgroups of the young (≤ 57 years, the median age of the controls), males, current smokers, current drinkers, and patients with primary tumor sites of pharynx and larynx. This large study provides reliable risk estimates for associations between *DNMT3B* variants and SCCHN risk in non-Hispanic whites, and our findings are consistent with that of previously reported cancer case-control studies of other cancers. Further mechanistic studies are needed to unravel the underlying molecular mechanisms.

Keywords

Polymorphism; Methylation; DNMT3B; Molecular epidemiology; Cancer risk

1. Introduction

Cancers in the oral cavity, pharynx, and larynx account for >90% of squamous cell carcinoma of the head and neck (SCCHN), and smoking and alcohol use are known risk factors for SCCHN [1]. A number of studies have suggested that genetic variation modulates cancer risk associated with environmental risk factors and that genetic variation may serve as a biomarker to identify

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individuals at risk of developing cancer [2]. Cumulative genetic alterations have been shown to be associated with phenotypic progression of SCCHN, resulting in inactivation of multiple tumor suppressor genes and activation of proto-oncogenes [3]. Among them, *p16INK4A* at chromosome 9p and *RASSF1A* at chromosome 3p are two major tumor suppressor genes epigenetically inactivated at the early stage from normal mucosa to dysplasia [4,5]. Interestingly, over-expression of DNA-methyltransferase-3B (DNMT3B) has been also associated with the inactivation of both *p16INK4A* and *RASSF1A* [6], suggesting an oncogenic role of DNMT3B during tumorigenesis.

DNA methylation, mediated by DNA methyltransferases (DNMTs, including DNMT1, DNMT2, DNMT3A, DNMT3B and DNMT3L), is considered one of important epigenetic mechanisms that regulates chromosomal stability and gene expression [7], and their dysregulation contributes to cancer development [8,9]. Three DNMTs (DNMT1, DNMT3A and DNMT3B) that have been identified in humans are catalytically active [10]. In general, DNMT1 is responsible for maintaining pre-existing methylation patterns after DNA replication, whereas DNMT3A and DNMT3B are required for de novo methylation that is involved in carcinogenesis [7]. The *DNMT3A* and *DNMT3B* genes are highly polymorphic. For example, DNMT3A has 303 reported variants, whereas *DNMT3B* has 345 reported polymorphisms (<http://ncbi.nlm.nih.gov/SNP/>), but only two for *DNMT3A* and four for *DNMT3B* are non-synonymous single nucleotide polymorphisms (nsSNPs) that cause an amino acid change, but these are rare SNPs [i.e. minor allele frequency (MAF) < 0.05] in European descents. It is reported that a single nucleotide polymorphism (SNP) -149C>T or C46359T (rs2424914) was significantly associated with both high promoter activity of *DNMT3B* and risk for several cancers in white populations [11-14]. However, another SNP -579G>T (rs2235758) was also associated with a higher promoter activity of *DNMT3B*, and it was associated with risk of lung cancer in an Asian population [15]. Therefore, frequency distribution of the different *DNMT3B* polymorphisms may present ethnic difference in cancer risk.

To date, few published studies that have evaluated the effects of genetic variation of *DNMT3B* on risk of SCCHN, particularly for non-Hispanic whites. We hypothesize that the *DNMT3B* polymorphisms are associated with risk of SCCHN. To test this hypothesis, we conducted a hospital-based case-control study of these two *DNMT3B* promoter SNPs in 832 SCCHN cases and 843 cancer-free controls in a non-Hispanic white population.

2. Materials and Methods

2.1. Study subjects

The recruitment of subjects for our ongoing SCCHN study has been described previously [16]. Briefly, all new patients had newly diagnosed, untreated SCCHN that was histologically confirmed at The University of Texas M D Anderson Cancer Center from May 1995 through May 2005. Patients with second SCCHN primary tumors, primary tumors of the nasopharynx or sinonasal tract, primary tumors outside the upper aerodigestive tract, cervical metastases of unknown origin, or any histopathological diagnosis other than SCCHN were excluded. Because genotype frequencies can vary between ethnic groups, the patients enrolled who were not white were also excluded from this analysis. Of the eligible cases, the response rate was approximately 93%. As a result, this study included 832 non-Hispanic white subjects with primary tumors of the oral cavity (n = 250; 30.0%), oro-/hypo-pharynx (n = 430; 51.7%) or larynx (n = 152; 18.3%).

The 843 cancer-free control subjects were recruited from hospital visitors, genetically unrelated to the enrolled case subjects or each other, which accompanied patients to the clinics but were not seeking medical care. We first surveyed potential control subjects at the clinics by using a

short questionnaire to determine their willingness to participate in research studies and to obtain demographic information for frequency matching to the cases by age (± 5 years) and sex. The purpose of frequency matching was to control confounding in order to evaluate the main effect of the *DNMT3B* polymorphisms. Of the eligible controls, the response rate was ~85%. Having obtained informed consent, we interviewed each eligible subject to collect additional information about risk factors, such as tobacco smoking and alcohol use. Those who had smoked <100 cigarettes in their lifetime were considered “never smokers”; all others were considered “ever smokers.” Among ever smokers, those who had quit and had not smoked for >1 year were considered “former smokers” and the others were considered “current smokers.” Similarly, subjects who had drunk alcoholic beverages at least once a week for more than 1 year previously were defined as ever drinkers. Ever drinkers who had quit drinking more than 1 year previously were defined as former drinkers and the others as current drinkers. After we obtained informed consent, each subject provided 30 ml of blood for biomarker tests. The research protocol was approved by the M. D. Anderson Cancer Center institutional review board.

2.2 Selection of DNMT3B SNPs and genotyping

The *DNMT3B* gene is located on chromosome 20q11.2. It consists of 23 exons and 22 introns and spans approximately 47kb of the genomic DNA. It is reported that the variant in the alternative promoter region -579G>T (rs2424909) from the exon 1B transcription start site most probably has an effect on the gene functions [15]. We previously identified a C-T single-base transition in a novel promoter of *DNMT3B*, -149 C>T (rs2424913) from the transcription start site, and we found that this polymorphism can significantly increase the promoter activity of the *DNMT3B* gene and is also associated with increased risk for lung cancer and decreased post surgical survival in patients with small cell carcinoma of the lung [13,17,18]. Therefore, we examined the associations between these two variants (*DNMT3B* -149 C>T and -579 G>T) in the alternative promoter regions and risk of SCCHN.

A leukocyte cell pellet was obtained from the buffy coat by centrifugation of 1 ml of whole blood. The cell pellet was used for DNA extraction. The Qiagen DNA Blood Mini Kit (Qiagen, Valencia, CA) was used according to the manufacture’s instructions to obtain genomic DNA. The DNA purity and concentration were determined by spectrophotometric measurement of absorbance at 260 and 280 nm. Genotyping of *DNMT3B* -149C>T and -579G>T was followed the PCR-RFLP assays described previously [13,15]. About 10% of the samples were repeated for the genotyping assays and the results were 100% concordant.

2.3 Statistical analysis

Differences in select demographic variables, smoking, and alcohol consumption between SCCHN cancer cases and controls were evaluated by using the χ^2 -test. The associations between SCCHN and genotypes of *DNMT3B* -149C>T and -579G>T were estimated by computing the odds ratio (ORs) and their 95% confidence intervals (CIs) from both univariate and multivariate logistic regression analysis. The odds ratios (ORs) and their 95% confidence intervals (CIs) for the *DNMT3B* genotype were calculated by logistic regression analysis, with adjustment for age (in years), sex, smoking status and alcohol use. For logistic regression analysis the *DNMT3B* genotype was recorded as a dummy variable. Considering the potential interaction of the two *DNMT3B* polymorphisms on SCCHN risk, we evaluated the association between the SCCHN risk and the combined genotypes of these two polymorphisms. Stratification analysis was used to estimate risk for subgroups by age, sex, smoking status, drinking status and tumor histology. All tests were two-sided, and all statistical analyses were performed with the SAS software (version 8.2; SAS Institute, Inc., Cary, NC).

3. Results

Case patients and control subjects were adequately matched by age and sex (Table 1). Compared with the controls, the cases had more smokers (current smokers: 34.8% versus 15.9%; former smokers: 39.4% versus 36.9%) and more drinkers (current drinkers: 51.0% vs. 40.0%; former drinkers: 25.6% vs. 19.4%) ($P < 0.001$ for both smoking and drinking status). However, all these variables were further adjusted for any residue confounding effect in later multivariate logistic regression analyses.

As shown in Table 2, genotype distributions of the selected two polymorphisms in control subjects were consistent with those expected from the Hardy-Weinberg equilibrium ($P = 0.153$ for *DNMT3B* -149C>T and 0.787 for *DNMT3B* -579 G>T), further suggesting that the genotype distributions were not biased by our control selection. The *DNMT3B* -149C>T variant T allele frequency was 0.458 among cases and 0.428 among controls ($P = 0.075$), whereas the *DNMT3B* -579G>T variant T allele frequency was 0.415 among cases and 0.400 among controls ($P = 0.399$) (Table 2).

When the *DNMT3B* -149 CC genotype was used as the reference group, the CT genotype was not associated with risk (adjusted OR, 0.91, 95% CI, 0.72-1.14; $P = 0.409$), but the TT genotype was associated with significantly increased risk for SCCHN (adjusted OR = 1.35, 95% CI = 1.01-1.80; $P = 0.043$). Under the recessive model of inheritance, the TT genotype was associated with significantly increased risk for SCCHN (adjusted OR = 1.43, 95% CI = 1.11-1.84; $P = 0.006$), compared with other genotypes, after adjustment for age, sex, smoking and alcohol use in the multivariate logistic regression analysis, and these risks were not observed for the *DNMT3B* -579 G>T (Table 2).

When the two polymorphisms were evaluated together by the number of variants and the combined -149C and -579G genotypes were used as the reference group, carriers of -149C/-579TT and -149TT/-579TT had a significantly increased risk of SCCHN (adjusted OR = 1.81, 95% CI = 1.13-2.91, $P = 0.014$ and adjusted OR = 1.32, 95% CI = 0.99-1.75, $P = 0.058$, respectively); a borderline increased risk was observed in carriers of -149TT/-579G+-149C/-579TT and -149TT/-579TT (adjusted OR = 1.44, 95% CI = 0.98-2.11, $P = 0.063$ and adjusted OR = 1.32, 95% CI = 0.99-1.75, $P = 0.058$, respectively). When the combined -149C and -579G genotypes were used as the reference group, the carriers of -149TT or -579TT homozygous genotypes had a significantly increased risk of SCCHN (adjusted OR = 1.36, 95% CI = 1.07-1.73, $P = 0.013$) (Table 3).

In further stratification analysis, compared with the *DNMT3B* -149C and -579G carriers, the *DNMT3B* variant homozygotes had a significantly increased risk of SCCHN in the subgroups of subjects ≤ 57 years (adjusted OR = 1.42, 95% CI = 1.02-1.97, $P = 0.037$), males (adjusted OR = 1.33, 95% CI = 1.01-1.75, $P = 0.040$), current smokers (adjusted OR = 2.04, 95% CI = 1.19-3.50, $P = 0.010$), current drinkers (adjusted OR = 1.59, 95% CI = 1.09-2.32), and subjects with cancer of the pharynx (adjusted OR = 1.34, 95% CI = 1.01-1.78, $P = 0.016$) and larynx (adjusted OR = 1.58, 95% CI = 1.02-2.46, $P = 0.040$) (Table 4). However, we did not find any evidence for gene-gene and gene-environmental interactions in this study population (data not shown).

4. Discussion

The present study investigated the associations between *DNMT3B* -149C>T and -579G>T polymorphisms and SCCHN risk. When we evaluated each polymorphism separately, a significant increased risk of SCCHN was found to be associated with the *DNMT3B* -149 TT genotype. Although *DNMT3B* -579G>T genotypes were not associated with SCCHN risk, the two polymorphisms jointly contributed to the risk of SCCHN. In stratification analysis, this

significant association was confined to subgroups of age ≤ 57 years, males, current smokers and current drinkers, and for primary tumor sites of the pharynx and larynx. These findings are biologically plausible.

DNMT3B plays an important role in cancer development. Over-expression of DNMT3B has been observed in some cancer types, and it contributes to the generation of aberrant methylation in cancer [19-26]. As we previously reported, the *DNMT3B* contains a C-T transition polymorphism (C46359T) at a novel alternative promoter region, -149 base pairs from the transcription start site, which in *in vitro* assays confers a 30% increase in the promoter activity of *DNMT3B* [13]. Heterozygous carriers of the *DNMT3B* polymorphism have been shown to have decreased survival following surgical resection in patients with SCCHN [17]. Although the mechanism for this association was not clear, it was postulated that the C-T transition increases the *DNMT3B* promoter activity and may up-regulate DNMT3B expression that involves an aberrant *de novo* methylation of CpG islands in some tumor suppressor genes [24].

Several case-control studies have been reported in which the association between the *DNMT3B* -149C>T polymorphism and risk of cancer was assessed [11-14,27,28]. However, the results from these molecular epidemiological studies are conflicting. We previously genotyped for *DNMT3B* -149C>T polymorphism in 319 patients with lung cancer and 340 cancer-free controls in non-Hispanic whites and found that the heterozygous CT genotype was associated with a significantly increased risk for lung cancer as compared with the homozygous CC genotype [13]. In a much smaller study of 81 patients with prostate cancer and 42 controls selected from patients with benign prostatic hypertrophy, Singal *et al.* also found that the TT genotype may be associated with an increased risk of prostate cancer [14]. Similarly, in 146 mismatch repair mutation carriers from 72 families, Jones *et al.* reported that hereditary non-polyposis colorectal cancer patients carrying one or two copies of the *DNMT3B* variant T allele developed the cancer significantly earlier than those patients who were homozygous for the wild-type *DNMT3B* allele [11]. Our present results are consistent with these reports, although the TT genotype, but not the CT genotype, was associated with significantly increased risk for SCCHN, when the *DNMT3B* -149 C>T C allele carriers were used as the reference group.

In contrast, in a case-control study of 352 cases and 258 controls in a British population, it was found that the *DNMT3B* -149 C alleles had a nominally significant increased risk of breast cancer compared with TT homozygotes [12]. In a Japanese study of 152 gastric cancer patients and 247 controls, it was reported that only the T/T genotype was detected in all case and control subjects [27]. Although this variation among different ethnic groups may be due to ethnically specific genetic differences. It is also possible that other factors such as small sample size, inclusion of a single polymorphism or different ethnic groups (population stratification) in a single study, or inadequate adjustment for confounding factors could cause the inconsistent results. However, this speculation needs to be tested further in studies with different cancer sites and larger sample sizes including different ethnic groups.

Two studies have investigated the association between the *DNMT3B* -579G>T promoter polymorphism and the risk of lung cancer and colon cancer in Korea populations. In one study (432 cases and 432 controls), although individuals with at least one -579 G allele were at a significantly decreased risk of adenocarcinoma and small cell carcinoma of lung cancer, the -579G>T polymorphism did not have an affect on the *DNMT3B* promoter activity in a Korea population [15]. In the other study (248 cases and 248 controls), the combined GT and GG genotypes were significantly associated with reduced risk of adenocarcinoma of the colon in younger and male subjects[29].

So far, only one published study has examined the association between the *DNMT3B* -579G>T polymorphism and the risk of SCCHN in 226 SCCHN patients and 249 controls in a Chinese population [30], but no significant association with cancer risk was found. In the present study, no significant positive association was found between the *DNMT3B* -579G>T polymorphism and risk of SCCHN in the single locus analysis. However, when the *DNMT3B* -149C>T and *DNMT3B* -579G>T polymorphisms were evaluated together, the carriers of both -149C allele and -579TT, -149TT and -579TT had a significant increase in risk of SCCHN compared with -149C and -579G allele carriers. This risk was more pronounced in younger subjects (≤ 57 years), suggesting that the -149TT and -579TT genotypes may cause an early age of onset and contribute to genetic susceptibility to SCCHN in this study population. Another possibility is that we had included more subjects ≤ 57 years, which provided an improved power to detect such a risk. We also found a significantly higher risk in men than women, and for primary tumor sites of the pharynx and larynx. More importantly, we also observed a significantly higher risk in current smokers and drinkers, consistent with genetic susceptibility and effects of a gene-environment interaction.

In summary, in this case-control study, we found that the carriers of *DNMT3B*-149 TT genotype had a significantly increased risk of SCCHN compared with *DNMT3B* -149 CC genotype carriers, although *DNMT3B* -579 G>T variants alone were not associated with SCCHN risk. Our results support the hypothesis that *DNMT3B* polymorphisms may be associated with an increased risk of SCCHN in a non-Hispanic Caucasian population. This large study provides reliable risk estimates for associations between *DNMT3B* variants and SCCHN risk in non-Hispanic whites. It is possible that these SNPs may alter a transcription factor binding site in the alternative promoter, that the variant genotype(s) may be in linkage disequilibrium with other untyped susceptibility loci, and that the variants may alter the binding of a regulatory miRNA or contribute to the differential expression of alternatively spliced *DNMT3B* variants. Future mechanistic studies are needed to unravel the underlying molecular mechanisms of the variants investigated and larger association studies are needed to confirm our results.

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Abbreviations

DNMT3B	DNA-methyltransferase-3B
SNP	single nucleotide polymorphism
SCCHN	squamous cell carcinoma of the head and neck
OR	odds ratio
CI	confidence interval

Table 1
Frequency distributions of selected variables in SCCHN cases and cancer-free controls

Variables	Cases (n = 832)		Controls (n = 843)		p ^a
	n	%	n	%	
Age (years) ^b					
≤ 57	444	53.4	449	53.3	0.966
> 57	388	46.6	394	46.7	
Sex					
Female	204	24.5	195	23.1	0.505
Male	628	75.5	648	76.9	
Smoking status					
Never	215	25.8	398	47.2	< 0.001
Former	328	39.4	311	36.9	
Current	289	34.8	134	15.9	
Alcohol use					
Never	195	23.4	342	40.6	< 0.001
Former	213	25.6	164	19.4	
Current	424	51.0	327	40.0	
Tumor site					
Oral cavity	250	30.0			
Pharynx ^c	430	51.7			
Larynx	152	18.3			

^aTwo-sided χ^2 test.

^bThe median age of the controls.

^cIncluded both oropharyngeal and hypopharyngeal cancer cases.

Table 2
Frequency distribution of *DNMT3b-149C>T* and *DNMT3b-579G>T* genotypes and their associations with risk of SCCHN

DNMT3b Genotype	No of cases (%)	No of controls (%)^a	<i>P</i>^b	Adjusted OR (95% CI)^c	<i>P</i>^c
All subjects	832 (100.0)	843 (100.0)			
<i>DNMT3b-149C>T</i>					
CC	259 (31.1)	266 (31.6)	0.011	referent	0.409
CT	384 (46.2)	433 (51.4)		0.91 (0.72-1.14)	
TT	189 (22.7)	144 (17.1)		1.35 (1.01-1.80)	0.043
C-allele carriers	643 (77.3)	699 (82.9)		referent	
TT	189 (22.7)	144 (17.1)	0.004	1.43 (1.11-1.84)	0.006
<i>DNMT3b-579 G>T</i>					
GG	299 (35.9)	305 (36.2)	0.341	referent	
GT	376 (45.2)	401 (47.6)		0.90 (0.72-1.13)	0.368
TT	157 (18.9)	137 (16.2)		1.14 (0.85-1.53)	0.380
G-allele carriers	675 (81.1)	706 (83.7)	0.159	referent	
TT	157 (18.9)	137 (16.3)		1.21 (0.93-1.57)	0.163

^aThe observed genotype frequency among the control subjects was in agreement with the Hardy-Weinberg equilibrium ($p^2+2pq+q^2=1$) ($\chi^2=2.045$, $P=0.153$ for *DNMT3B-149C>T* and $\chi^2=0.073$, $P=0.787$ for *DNMT3B-579G>T*).

^bTwo-sided χ^2 test for either genotype distribution or allele frequency.

^cAdjusted for age, sex, smoking status, and alcohol use in a logistic regression model.

Table 3
Frequency distribution of combined *DNMT3b*-149C>T and *DNMT3b*-579G>T genotypes and the association with SCCHN risk

<i>DNMT3b</i> genotype carriers	No of cases (%)	No of controls (%)	<i>P</i> ^a	Adjusted OR (95% CI) ^b	<i>P</i> ^b
Total	832	843			
-149C/-579G	623 (74.9)	675 (80.1)	0.019	referent	
-149TT/-579G	20 (2.4)	24 (2.8)		0.94 (0.50-1.76)	0.845
-149C/-579TT	52 (6.2)	31 (3.7)		1.81 (1.13-2.91)	0.014
-149TT/-579TT	137 (16.5)	113 (13.4)		1.32 (0.99-1.75)	0.058
-149C/-579G	623 (74.9)	675 (80.1)	0.037	referent	
-149TT/-579G+/-149C/579TT	72 (8.7)	55 (6.5)		1.44 (0.98-2.11)	0.063
-149TT/-579TT	137 (16.4)	113 (13.4)		1.32 (0.99-1.75)	0.058
				<i>P</i> _{trend} = 0.025	
-149C/-579G	623 (74.9)	675 (80.1)	0.011	referent	
-149TT or -579TT	209 (25.1)	168 (19.9)		1.36 (1.07-1.73)	0.013

^aTwo-sided χ^2 test for either genotype distribution or allele frequency.

^bAdjusted for age, sex, smoking status, and alcohol use in a logistic regression model.

Table 4
Stratification analysis of SCCHN risk associated with the *DNMT3b-149C>T* and *DNMT3b-579G>T* combined genotypes

Variables	No of cases (%)		No of controls (%)		Adjusted OR (95% CI) ^a		P
	-149C/-579G	-149TT or -579TT	-149C/-579G	-149TT or -579TT	-149C/-579G	-149TT or -579TT	
Age (years)							
≤ 57	329 (74.1)	115 (25.9)	362 (80.6)	87 (19.4)	referent	1.42 (1.02-1.97)	0.037
> 57	294 (75.8)	94 (24.2)	313 (79.4)	81 (20.6)	referent	1.31 (0.91-1.89)	0.145
Sex							
Female	154 (75.5)	50 (24.5)	160 (82.0)	35 (18.0)	referent	1.41 (0.84-2.37)	0.190
Male	469 (74.7)	159 (25.3)	515 (79.5)	133 (20.5)	referent	1.33 (1.01-1.75)	0.040
Smoking status							
Never	165 (76.7)	50 (23.3)	318 (79.9)	80 (20.1)	referent	1.24 (0.83-1.85)	0.305
Former	253 (77.1)	75 (22.9)	246 (79.1)	65 (20.9)	referent	1.18 (0.81-1.72)	0.401
Current	205 (70.9)	84 (29.1)	111 (82.8)	23 (17.2)	referent	2.04 (1.19-3.50)	0.010
Drinking status							
Never	141 (72.3)	54 (27.7)	266 (77.8)	76 (22.2)	referent	1.36 (0.90-2.05)	0.143
Former	166 (77.9)	47 (22.1)	128 (78.1)	36 (21.9)	referent	0.97 (0.59-1.61)	0.916
Current	316 (74.5)	108 (25.5)	281 (83.4)	56 (16.6)	referent	1.59 (1.09-2.32)	0.016
Tumor site							
Oral cavity	189 (75.6)	61 (24.4)	675 (80.1)	168 (19.9)	referent	1.37 (0.95-1.96)	0.089
Pharynx	322 (74.9)	108 (25.1)	675 (80.1)	168 (19.9)	referent	1.34 (1.01-1.78)	0.046
Larynx	112 (73.7)	40 (26.3)	675 (80.1)	168 (19.9)	referent	1.58 (1.02-2.46)	0.040

^a Adjusted for age, sex, smoking status, and alcohol use in a logistic regression model, where it was appropriate.