HHS Public Access

Author manuscript

Eur J Cancer. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

Eur J Cancer. 2015 July ; 51(11): 1415–1423. doi:10.1016/j.ejca.2015.04.016.

A functional variant at *miRNA*-122 binding site in *IL-1α* 3′ UTR predicts risk and HPV-positive tumors of oropharyngeal cancer

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Abstract

Background—Genetic polymorphisms in the 3′ UTRs targeted by miRNAs alter the strength of miRNA binding in a manner that affects the behavior of individual miRNAs. An insertion (Ins)/deletion (Del) polymorphism (rs3783553) in the 3′ UTR of *IL-1*α may disrupt a binding site for miRNA-122. *IL-1*α plays an important role in inflammation, immunity, and defense against infection. Thus, we hypothesized that the rs3783553 polymorphism affects individual susceptibility to HPV-associated oral squamous cell carcinoma (OSCC).

Methods—We genotyped the rs3783553 polymorphism; and determined HPV16 L1 serology, tumor HPV16 DNA, and serum *IL-1*α expression. Univariate/multivariable logistic regression models were used to calculate associations.

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Competing interests: The authors declare that they have no competing interests.

Results—We found that HPV16 L1 seropositivity alone was associated with an increased risk of OSCC (OR, 3.1; 95% CI, 2.1–4.6), and the risk of HPV16-associated OSCC was modified by the rs3783553 polymorphism. Patients with both HPV16 L1 seropositivity and Del/Del genotype for the rs3783553 had the highest risk of OSCC when using patients with HPV16 L1 seronegativity and Ins/Del + Ins/Ins genotypes as a comparison group. Notably, that effect modification was particularly pronounced in several subgroups (e.g., SCCOP, never-smokers, and never-drinkers). The patients with Del/Del genotype were approximately 3.0 times more likely to have HPV16-positive SCCOP tumors compared to those patients with Ins/Del + Ins/Ins genotypes. Additionally, functional relevance of this variant was characterized to explore the genotype-phenotype correlation.

Conclusion—These results suggest that *IL-1α* 3′ UTR rs3783553 polymorphism may be functional and influence susceptibility to HPV16-associated OSCC, particularly for SCCOP. Validation of our findings is warranted.

Keywords

IL-1α variant; HPV; oral cancer; SCCOP; cancer risk; biomarker; miRNA

Introduction

Oral squamous cell carcinoma (OSCC) comprises cancers arising from the oropharynx and oral cavity. In the United States, an estimated 42 440 new cases of OSCC and 8 390 deaths from OSCC are expected in 2014(1). Tobacco and alcohol are well-established risk factors for OSCC. Corresponding with the decrease in tobacco use in the United States, the incidence rate of OSCC has declined over the past two decades; however, the incidence of a subgroup of OSCC, squamous cell carcinomas of the oropharynx (SCCOP), has increased in recent years, particularly in young adults and never-smokers and never-drinkers. The rising incidence of SCCOP in the United States is likely a consequence of persistent infection with human papillomavirus (HPV), predominantly high-risk HPV type 16 (HPV-16)(2). The overall rise in SCCOP incidence from 1984 to 2004 is largely explained by the increasing incidence of HPV-positive cancers, whereas the incidence of HPV-negative cancers declined. Consequently, HPV prevalence in oropharyngeal tumors increased substantially, from 16.3% during the 1980s to 72.7% during the 2000s(3-5). The population-level incidence of HPV-positive SCCOP increased by 225% from 1988 to 2004 (from 0.8 per 100,000 to 2.6 per 100,000), while the incidence of HPV-negative SCCOP decreased by 50% (from 2.0 per 100,000 to 1.0 per 100,000)(3). However, only a small percentage of HPV-infected people actually develop OSCC. It is likely that other, as-yet-unknown genetic factors in inflammation and immune response pathways are associated with the risk of HPV-associated OSCC, particularly SCCOP.

Chronic inflammation and host immune responses have been shown to be biologically important risk factors for HPV-associated carcinogenesis. However, the host's immune reaction against HPV infection remains poorly understood. Nonetheless, inflammatory cytokines undoubtedly play a key role through activating and coordinating the immune response(6). Interleukin (IL) -1 is a pleiotropic cytokine that plays an important role in the regulation of immune response and the defense against viral infections by activating genes

encoding multiple cytokines, receptors, and adhesion molecules(7). Several studies have also suggested that IL-1 either promotes or blocks the processes of tumorigenesis(8-12), tumor proliferation, angiogenesis, invasion, and metastasis(13, 14). Merrick et al showed that the HPV-18 immortalized keratinocyte cell line, accompanied by a loss of IL-1α and IL-1β expression, had the ability to form tumors spontaneously in nude mice; re-establishing IL-1 expression in most IL-1-expressing lines showed complete inhibition of tumor formation(15). Woodworth et al found that IL-1α inhibited the proliferation of normal epithelial cells cultured from human cervix tissue(16). In contrast, IL-1α significantly stimulated the proliferation of cervical cell lines immortalized by transfection with HPV-16 or HPV-18 DNA. However, Hu et al and Manavi et al found that gene expression for IL-1α was decreased in HPV-16- or HPV-18-associated cervical squamous cell carcinoma samples and HPV-infected cells(17, 18). Given the crucial and conflicting roles of IL-1α in immune regulation(7, 15-19), its genetic variants may affect the host immune system and, subsequently, associated HPV-associated cancer development and progression(11, 12).

MicroRNA (miRNA) are small, noncoding, single-stranded RNA 18–24 nucleotides long, which can regulate gene expression post-transcriptionally through the degradation of targeted mRNA and inhibition of their translation(20-26). A growing body of evidence suggests that miRNA have important roles in a broad range of biological processes, such as embryonic development, cellular differentiation, proliferation, apoptosis, and cancer development(27-29). To regulate mRNA level and protein expression, miRNAs bind to targeted mRNA in the 3′ UTR. Thus, polymorphisms in the 3′ UTR targeted by miRNAs can either abolish existing binding sites or create illegitimate binding sites, which results in the regulation of target genes that can affect an individual's cancer risk(22, 26, 30-35). Recently, an insertion/deletion polymorphism (rs3783553, an insertion or deletion of TTCA bases) at the miRNA-122 binding site, which is located in the *IL-1*α 3′ UTR, was shown to be associated with gastric, hepatocellular, nasopharyngeal, and thyroid carcinomas, as well as alopecia areata(12-14, 27, 28, 34). Individuals carrying the homozygote genotype (Insertion [Ins]/Insertion [Ins]) had significantly decreased susceptibility to cancers, possibly owing to regulation of IL-1α expression levels. To the best of our knowledge, no study has been performed to examine the relationship between the *IL-1*α rs3783553 polymorphism and OSCC risk. Therefore, we hypothesized that the rs3783553 polymorphism is associated with risk of HPV-associated OSCC, particularly SCCOP. In the present study, we genotyped the *IL-1*α rs3783553 polymorphism and evaluated its association with risk of HPV16-associated OSCC in 325 OSCC patients and 335 cancer-free controls, all of whom were non-Hispanic whites.

Methods

Study participants

In this case control study, the 325 cases were patients with newly diagnosed, histopathologically confirmed and untreated OSCC. The details of recruitment and the inclusion criteria for these cases were described previously(36). Briefly, these cases had been consecutively recruited at The University of Texas MD Anderson Cancer Center as part of an ongoing molecular epidemiologic study of head and neck cancers. During that

same period, the controls had been selected from a pool of cancer-free subjects recruited from the Kelsey-Seybold Foundation, a multispecialty physician practice with multiple clinics throughout the Houston metropolitan area, as well as from healthy visitors who had accompanied cancer patients to outpatient clinics at MD Anderson Cancer Center but who were genetically unrelated to the patients. The 335 controls were frequency-matched to the patients by age (±5 years), sex, and smoking and drinking status. Only non-Hispanic whites were included as controls because most of the cancer patients recruited were non-Hispanic whites. Approximately 95% of eligible incident cases and 78% of eligible controls agreed to participate in the study. For tumor HPV status analysis, we also genotyped the polymorphism for another cohort of 552 SCCOP patients, whose tumors HPV status and genomic DNA samples from the blood were available. The study received approval from the institutional review boards of both MD Anderson and Kelsey-Seybold, and all study subjects signed an informed consent form when approached for recruitment. Subjects who had smoked more than 100 cigarettes in their lifetimes were categorized as “ever-smokers” and others as “never-smokers.” Subjects who had consumed alcoholic beverages at least once a week for more than 1 year previously were categorized as “ever-drinkers” and others as “never-drinkers.”

HPV16 serological testing

For the current study, serum samples from each subject were tested for anti-HPV16 (antibody against HPV16) by a standard enzyme-linked immunosorbent assay with HPV16 L1 virus-like particles generated from recombinant baculovirus-infected insect cells, as described previously(37). Ten percent of the samples were randomly chosen for re-testing, and the results were in 100% concordance with those of the initial assays.

IL-1α 3′ UTR rs3783553 genotyping

For this study, we extracted genomic DNA from a leukocyte cell pellet using the QIAamp DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA) in accordance with the manufacturer's instructions. Genotyping using a polymerase chain reaction (PCR) assay was performed by laboratory personnel blinded to the case–control status(28). DNA fragments containing the polymorphism were amplified with the forward primer 5′-ATTGGTCCGATCTTTGACTC-3′ and reverse primer 5′-TGATAA CAGTGGTCTCATGG-3′. The PCR products were analyzed by 6% non-denaturing polyacrylamide gel electrophoresis and visualized by silver staining. The genotypes were determined by the numbers and the lengths of the bands in the gels. Repeat analysis was performed on a randomly selected subset of 10% of the samples, and the results were in 100% concordance with the initial analysis.

Tumor HPV16 determination

Paraffin-embedded tissues were tested for HPV16 DNA using polymerase chain reaction (PCR)-based, type-specific assays with modification and quality control for the E6 and E7 regions(4, 38). Assays of the samples were run in triplicate, with positive and negative controls (Siha and TPC-1 cell lines, respectively). β-Actin was used as a DNA quality control. Specificity for HPV16 E6 and E7 was confirmed by Southern blot analysis of paraffin-embedded tissue samples using a Roche Diagnostics labeling and hybridization

system (Roche Applied Science, Indianapolis, IN). HPV16 E6 and E7 specificity was confirmed by retesting 10% of the samples using restriction digestion of the PCR products with Ban*II* and Msp*I* to verify the presence of E6- and E7-specific fragments. The results of both methods were 100% concordant.

Serum IL-1α determination

Plasma was stored at -80°C until use. Plasma level of *IL-1α* was measured using eBioscience Human Th1/Th2 11plex FlowCytomix Kit (eBioscience, San Diego, CA) following manufacturing instruction for sample collection, storage and assay procedure. Each sample was tested in duplicate and the mean of tests was used for analysis. Furthermore, 10% of samples were randomly chosen and tested again for quality assurance.

Statistical analysis

Statistical analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC). All tests were two-sided, and a P value of < 0.05 was considered the cutoff for statistical significance. We used χ^2 tests to examine differences between the patients and controls in the distributions of demographic variables, smoking status, drinking status, serological and tumor HPV16 status, and genotypes. The t-test was used to compare the expression level of *IL-1α* between the groups with different genotypes of *rs3783553* polymorphism and tumor HPV status. We evaluated the associations of both HPV16 status and *IL-1α* genotypes, individually and in combination, with the risk of OSCC by computing ORs and their 95% CIs, using both univariate and multivariable logistic regression analyses. The analyses of joint effects were further stratified by tumor site, smoking status, and drinking status.

Results

Demographics and risk factors for the study population

The demographics and OSCC risk factors for the 325 patients and 335 controls are shown in Table 1. Among the 325 patients, 188 (57.8%) had SCCOP and 137 (42.2%) had oral cavity cancers. Age, sex, and smoking and drinking status did not differ significantly between the patients and controls as a result of frequency matching. However, HPV16 L1 seropositivity was more common in patients than in controls ($P< 0.001$) and was associated with a 3.1 - times higher risk of OSCC in patients than in controls (odds ratios [OR], 3.1; 95% confidence interval [CI], 2.1−4.6).

Joint effect of HPV-16 L1 seropositivity and rs3783553 polymorphism on the risk of OSCC

Table 2 shows the association between *IL-1α* genotype and the risk of HPV16-associated OSCC. HPV16-seronegative individuals carrying the Ins/Ins or Ins/Deletion (Del) genotypes of rs3783553 had the lowest risk of OSCC after adjustment for age, sex, and smoking and drinking status. Using those individuals with both HPV16 seronegativity and the Ins/Ins or Ins/Del genotypes as the reference group, the risk of OSCC progressively increased among individuals with the Del/Del genotype and HPV16 seronegativity (OR, 1.5; 95% CI, 1.1–2.2), the Ins/Ins or Ins/Del genotype and HPV16 seropositivity (OR, 2.8; 95% CI, 1.7–4.7), and the Del/Del genotype and HPV16 seropositivity (OR, 5.6; 95% CI, 2.9–10.6),

respectively. In all groups, that effect modification was particularly pronounced for oropharyngeal as opposed to oral cavity cancer.

Stratification analysis of the joint effect of HPV-16 serology and rs3783553 polymorphism on risk of OSCC

We further evaluated the association between the rs3783553 genotype and the risk of HPV16-associated OSCC, stratified by smoking or drinking status. As shown in Table 3, the joint effect of positive HPV16 serology and rs3783553 polymorphism on risk of OSCC was greater in never-smokers than in ever-smokers. Specifically, the risk of OSCC was 25.2 times higher in HPV16-seropositive never-smokers and 3.9 times higher in HPV16-seropositive ever-smokers in the Del/Del genotype groups compared with the Ins/Del and Ins/Ins genotype groups. Similarly, as shown in Table 4, never-drinkers were at greater risk of OSCC than were ever-drinkers. Specifically, in the Del/Del genotype group, HPV16-seropositive never-drinkers had an OR of 13.0 versus an OR of 4.5 in ever-drinkers. Moreover, such risk estimates stratified by smoking and drinking status were even more pronounced for SCCOP, as opposed to oral cavity cancers (Tables 3 and 4).

Association of rs3783553 polymorphism with HPV16-positive tumors among SCCOP patients

The genotype distribution for rs3783553 polymorphism among another cohort of 552 SCCOP patients with tumor HPV status available is shown in Table 5. The genotype distribution of rs3783553 polymorphism indicated that HPV16-positive patients were more likely to have the Del/Del genotype than the HPV16-negative patients (44.2% vs. 20.4%). The genotype distribution of rs3783553 polymorphism varied significantly between HPV16-positive and HPV16-negative patients ($P < 0.0001$). The patients with rs3783553 Del/Del genotype were approximately 3.0 times more likely to have HPV16-positive tumors than the patients with rs3783553 Ins/Del + Ins/Ins genotypes (OR, 3.2, 95% CI, 1.9-5.7).

Characterization of genotype-phenotype correlation

To further characterize the potentially functional relevance of this polymorphism in *IL-1*α 3′UTR, we determined serum expression levels of *IL-1*α in 200 incident SCCOP patients, who were recently recruited and whose serum and tumor tissue samples were available. We conducted a correlation analysis between tumor HPV16 status/genotypes of this 3′UTR polymorphism and the circulating expression levels of *IL-1*α. As shown in Table 6, we found that the expression of *IL-1*α was significantly higher in tumor HPV16-positive patients than the HPV16-negative cases ($P = 0.0262$). Furthermore, the expression of *IL-1*α was significantly higher in the patients with Del/Del genotype than the patients with the corresponding Ins/Del + Ins/Ins genotypes ($P = 0.0033$) (Table 6).

Discussion

Growing evidences have suggested that the polymorphisms in the miRNA target site may influence the strength of miRNA binding, regulation of target genes and affecting the individual's cancer risk (33, 39). The rs3783553 lies within a predicted binding site (seed region) for human miR-122, which is a liver specific miRNA comprising up to 70% of all

hepatic miRNA which mostly regulates lipid homeostasis(21, 23, 25). MiR-122 was found to be downregulated in hepatocellular carcinoma with a function of tumor suppressor (40). Furthermore, Gao et al. reported the rs3783553 polymorphism affects the transcription of IL-1α by altering the binding strength of miRNA-122(28). Subsequently, this polymorphism has been identified to be associated with decreased risks for developing hepatocellular carcinoma, nasopharyngeal carcinoma, gastric cancer, papillary thyroid carcinoma, and cervical cancer (12, 14, 27, 28, 41).

To our knowledge, this is the first epidemiological study to assess the association between the rs3783553 polymorphism and HPV-associated OSCC risk. In this hospital-based case-control study of 325 OSCC patients and 335 cancer-free controls, we investigated the association between the rs3783553 polymorphism within 3′ UTR of the *IL-1*α gene and the risk of OSCC in non-Hispanic whites and showed that the risk of HPV16-associated OSCC, and SCCOP in particular, was increased by the rs3783553 Del/Del genotype, which suggests a joint effect of the *IL-1*α polymorphism and HPV16 seropositivity on risk of OSCC. Head and neck cancer risk associated with tobacco smoking, alcohol drinking, and HPV16 infection has been shown to differ by tumor site, with HPV16 infection being the strongest risk factor for SCCOP and smoking and drinking being the strongest risk factors for oral cavity cancers(42). These findings are in accordance with our findings in the present study that the joint effect of HPV16 infection and the *IL-1*α polymorphism was much more pronounced for SCCOP than for oral cavity cancers. Moreover, this joint effect was more evident in never-smokers and never-drinkers, which suggests that smoking and drinking may not play a major role in HPV-associated OSCC and that it is modulated instead by genetic factors such as the *IL-1α* polymorphism. This hypothesis is further supported by the evidence that HPV16 is an independent risk factor for SCCOP regardless of smoking or drinking status(42).

Our current study showed that *IL-1α* 3′ UTR rs3783553 polymorphism was significantly associated with HPV16-positive tumors among SCCOP patients. Although we do not know how this *IL-1α* 3′ UTR variant influences the HPV16-positive tumors, it is biologically plausible that this variant may be either functional or in linkage disequilibrium with other functional variants of *IL-1α*, thereby altering the function of *IL-1α*, or with alleles at other nearby susceptibility loci. Such functional variants could increase or reduce *IL-1α* expression levels and thus affect the regulation of the immune and inflammation as well as apoptotic responses. For example, *IL-1α* 3′ UTR rs3783553 Del/Del genotype might alter regulation in these pathways which might enable many HPV-infected cells to escape or counterattack against the immune system and might not enhance apoptotic response, leading to more likely to be HPV-positive tumors and subsequently better response to chemoradiotherapy.

So far, no studies on functional relevance of *IL-1α* 3′ UTR rs3783553 polymorphism have been reported. Since this polymorphism is within the functional region of the gene's 3′UTR of *IL-1α*, we speculated that this *IL-1α* genetic variant may have potentially functional effect on expression levels of *IL-1α* by altering the efficiency of translational initiation, leading to inter-individual differences in susceptibility to HPV16-associated SCCOP. Indeed, in this study, we found that the Del/Del genotype of this polymorphism is significantly correlated

with increased expression of *IL-1α* in serum. While the functional relevance of this polymorphism has not yet been elucidated, our results might partially suggest a functional correlation between this polymorphism and expression of *IL-1α*, which may provide preliminary evidence of biological plausibility for the observed association in the current study.

The current study has some limitations. Although we matched the cases and controls according to smoking and drinking status, it was necessary to further adjust for the residual effects of these risk factors to reduce the bias in the estimates of the association between *IL-1α* polymorphism and HPV16-associated OSCC. Also, there is a possible selection bias in this case–control study owing to the nature of its hospital-based design. We only included non-Hispanic whites in the study, so our results cannot be generalized to other ethnic groups. Because there were limited numbers of individuals in some subgroups in our stratification analysis, the results need to be confirmed in future studies with larger sample sizes. In addition, because HPV serological status might not fully reflect actual HPV tumor status, future studies will be needed to establish the correlation of HPV status between sera and tumor tissue. Finally, as we observed, some of the confidence intervals were very wide, indicating a lack of precision and reduced study power owing to the small numbers of individuals in the subgroups. This can be improved by a future patient cohort with a larger sample size and tumor HPV data.

In summary, we conclude that the Del/Del genotype of the *IL-1α* 3′ UTR polymorphism rs3783553 may be individually or, more likely, jointly associated with risk of HPV16-associated OSCC in the non-Hispanic white population. Furthermore, we found that the joint effects of the rs3783553 Del/Del genotype and HPV16 seropositivity were particularly pronounced among never-smokers or never-drinkers and for SCCOP as opposed to oral cavity cancers. Although this is the first study investigating the association between this *IL-1α* polymorphism and risk of HPV16-associated OSCC, future studies with larger sample sizes and more accurate HPV tumor status information are needed to validate these findings.

Acknowledgments

We thank Ms. Margaret Lung and Ms. Jenny Vo for patient recruitment, Ms. Dawn Chalaire for article editing, Ms. Yingdong Li for laboratory support, and funding support [NIEHS R01 ES-11740 (to Q.W.) and N.I.H. CA 135679 and CA133099 (to G.L.)].

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Abbreviations

IL-1α	Interleukin-1α
UTR	Untranslated regions
CI	Confidence interval
OR	Odds ratio
PCR	Polymerase chain reaction
OSCC	Oral squamous cell carcinoma
SCCOP	Squamous cell carcinoma of the oropharynx
HPV	Human papillomavirus

Highlights

- HPV seropositivity synergizes with the *IL-1α* 3′ UTR variant to increase risk of SCCOP.
- The effect modification is particularly pronounced in never-smokers/never-drinkers.
- The *IL-1α* 3′ UTR variant is significantly associated with tumor HPV status of SCCOP.
- Functional relevance of *IL-1α* 3′ UTR variant shows a genotype-phenotype correlation.

Table 1

Demographic characteristics and risk factors of patients and controls

Variables	Patients (n=325)		Controls (n=335)		
	No.	%	No.	%	*P*
Age (years)					0.183
≤40	31	9.5	27	8.1	
41-55	126	38.8	105	31.3	
56-70	119	36.7	154	46.0	
>70	49	15.0	49	14.6	
Sex					0.100
Male	241	74.2	269	80.3	
Female	84	25.8	66	19.7	
Tobacco smoking					0.673
Ever	227	69.8	239	71.3	
Never	98	30.2	96	28.7	
Alcohol drinking					0.121
Ever	250	76.9	240	71.6	
Never	75	23.1	95	28.4	
HPV16 serology					< 0.001[a]
Negative	225	69.2	293	87.5	
Positive	100	30.8	42	12.5	

[a]Significant difference in HPV16 serological status between cases and controls

Table 2

Joint effect of rs3783553 polymorphism and HPV16 serology on risk of OSCC

Variables	HPV16 status	Patients		Controls		Adjusted OR (95% CI)*		
		No.	%	No.	%	OSCC	SCCOP	OC
Overall								
Ins/Del + Ins/Ins		179	55.1	223	66.6	1.0	1.0	1.0
Del/Del		146	44.9	112	33.4	1.6 (1.2-2.2)	2.1 (1.4-3.2)	1.2 (0.8-1.8)
By HPV serology								
Ins/Del + Ins/Ins	-	128	39.4	195	58.2	1.0	1.0	1.0
Del/Del	-	97	29.8	98	29.2	1.5 (1.1-2.2)	2.1 (1.3-3.3)	1.2 (0.8-1.9)
Ins/Del + Ins/Ins	+	51	15.7	28	8.4	2.8 (1.7-4.7)	5.4 (3.0-9.6)	0.8 (0.4-1.8)
Del/Del	+	49	15.1	14	4.2	5.6 (2.9-10.6)	12.6(6.4-25.1)	0.8 (0.2-2.5)

*Adjusted for age, sex, and smoking and alcohol drinking status

Table 3

Joint effect of rs3783553 polymorphism and HPV16 serology on risk of OSCC, stratified by smoking status

Risk groups	HPV16 status	Patients (n=325)		Controls (n=335)		Adjusted OR (95% CI)*		
		No.	%	No.	%	OSCC	SCCOP	OC
Never-smokers								
Ins/Del + Ins/Ins	-	29	29.6	54	56.3	1.0	1.0	1.0
Del/Del	-	30	30.6	34	35.4	1.8(0.9-3.6)	2.7(1.1-7.0)	1.2(0.5-3.0)
Ins/Del + Ins/Ins	+	21	21.4	6	6.3	8.3(2.8-24.0)	18.6(5.5-62.7)	1.0(0.1-6.3)
Del/Del	+	18	18.4	2	2.1	25.2(5.2-121.7)	69.3(12.7-379.8)	1.3(0.1-20.8)
Ever-smokers								
Ins/Del + Ins/Ins	-	99	43.6	141	59.0	1.0	1.0	1.0
Del/Del	-	67	29.5	64	26.8	1.5(0.9-2.4)	2.0(1.1-3.5)	1.3(0.7-2.1)
Ins/Del + Ins/Ins	+	30	13.2	22	9.2	2.0(1.1-3.7)	3.6(1.8-7.3)	0.8(0.3-2.1)
Del/Del	+	31	13.7	12	5.0	3.9(1.8-8.1)	8.3(3.8-18.1)	0.7(0.2-2.7)

*Adjusted for age, sex, and alcohol drinking status

Table 4

Joint effect of rs3783553 polymorphism and HPV16 serology on risk of OSCC, stratified by drinking status

Risk groups	HPV16 status	Patients (n=325)		Controls (n=335)		Adjusted OR (95% CI)*		
		No.	%	No.	%	OSCC	SCCOP	OC
Never-drinkers								
Ins/Del + Ins/Ins	-	28	37.3	53	55.8	1.0	1.0	1.0
Del/Del	-	22	29.3	33	34.7	1.4(0.6-2.9)	1.7(0.6-4.8)	1.3(0.5-3.2)
Ins/Del + Ins/Ins	+	11	14.7	6	6.3	4.0(1.3-12.8)	8.9(2.4-33.1)	1.5(0.3-8.8)
Del/Del	+	14	18.7	3	3.2	13.0(3.2-52.9)	29.1(6.5-131.1)	2.3(0.3-19.3)
Ever-drinkers								
Ins/Del + Ins/Ins	-	100	40.0	142	59.2	1.0	1.0	1.0
Del/Del	-	75	30.0	65	27.1	1.7(1.1-2.5)	2.3(1.3-4.0)	1.3(0.7-2.2)
Ins/Del + Ins/Ins	+	40	16.0	22	9.1	2.6(1.4-4.7)	4.9(2.5-9.4)	0.7(0.3-1.8)
Del/Del	+	35	14.0	11	4.6	4.5(2.2-9.4)	10.4(4.8-22.6)	0.4(0.1-2.0)

*Adjusted for age, sex, and tobacco smoking status

Table 5

Association of IL-1α rs3783553 genotypes with tumor HPV16 status in 552 SCCOP patients

Genotypes	HPV16-positive SCCOP cases (N = 439)		HPV16-negative SCCOP cases (N = 113)		*P*	Adj.OR (95% CI)*
	No.	%	No.	%		
IL-1α rs3783553						
Ins/Del + Ins/Ins[a]	245	55.8	90	79.6	< 0.0001	1.0
Del/Del	194	44.2	23	20.4		3.2 (1.9-5.7)

P value for χ^2 test for genotype distribution.

*Adjusted for age, sex, ethnicity, smoking and alcohol status in a logistic regression model.

[a]Ref. = reference group.

Table 6

Correlation of *IL-1α* expression level in serum with different genotypes of *IL-1α* rs3783553 variant and tumor HPV status in 200 SCCOP patients

Variables	No. SCCOP patients	Serum *IL-1α* level (Mean ± SD, pg/ml)	*p* (unpaired *t* test)
Tumor HPV16 status			0.0262
Negative	51	5.1 ± 3.9	
Positive	149	6.6 ± 4.2	
*IL-1*α rs3783553			0.0033
Ins/Del + Ins/Ins	110	5.3 ± 3.5	
Del/Del	90	6.9 ± 4.1	