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p73 G4C14-to-A4T14 Polymorphism and Risk of Human Papillomavirus Associated Squamous Cell Carcinoma of the Oropharynx in Never Smokers and Never Drinkers

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Abstract

BACKGROUND—p73 can be inactivated by oncoprotein E6 of human papillomavirus (HPV). It is possible that *p73* variation could alter the interaction between the E6 protein and p73, and thus alter the risk for HPV associated carcinogenesis. The *p73* G4C14-to-A4T14 polymorphism is thought to affect p73 function by altering gene expression, but whether this also alters the risk of HPV16 associated squamous cell carcinoma of the oropharynx (SCCOP) is unknown.

METHODS—This case-control study included 188 non-Hispanic white patients with newly diagnosed SCCOP and 349 healthy control subjects. Logistic regression analyses were used to calculate odds ratio (OR) and 95% confidence interval (CI) for cases and controls stratified by *p73* genotype, age, sex, smoking, drinking and HPV16 status. The effects of *p73* genotypes on risk of HPV16 associated SCCOP were explored with further stratification by smoking and drinking status.

RESULTS—HPV16 seropositivity was associated with an increased risk of SCCOP (adjusted OR, 5.98; 95% CI, 3.89-9.20), especially among never smokers (adjusted OR, 13.8; 95% CI, 5.91-32.1), never drinkers (adjusted OR, 14.9; 95% CI, 5.24-42.4), and subjects with *p73* variant genotypes (GC/AT + AT/AT) (adjusted OR, 7.96; 95% CI, 3.83-16.5). Moreover, the risk of HPV16 associated SCCOP for those with *p73* variant genotypes was particularly high in never smokers and never drinkers.

CONCLUSIONS—*p73* G4C14-to-A4T14 polymorphism may modulate the risk of HPV16 associated SCCOP and the *p73* variant genotypes may be a marker of genetic susceptibility to HPV16 associated SCCOP, particularly in never smokers and never drinkers.

Condensed abstract—*p73* G4C14-to-A4T14 polymorphism may modulate the risk of HPV16 associated squamous cell carcinoma of the oropharynx and the *p73* variant genotypes may be a marker of genetic susceptibility to HPV16 associated squamous cell carcinoma of the oropharynx, particularly in never smokers and never drinkers.

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Keywords

p73 polymorphism; cancer risk; genetic susceptibility; human papillomavirus (HPV); molecular epidemiology; oropharynx; squamous cell carcinoma

INTRODUCTION

Despite declining smoking prevalence in the United States, the incidence of squamous cell carcinoma of the oropharynx (SCCOP) has increased in recent decades, particularly in young adults.1 It is estimated that in the U.S., approximately 12,000 new cases of SCCOP will be diagnosed and 2,200 deaths will result from these cancers in 2008.2 Epidemiologic and experimental evidence clearly indicates that high-risk human papillomavirus (HPV), plays a causative role in the etiology of SCCOP, especially in the population lacking the known risk factors of tobacco and alcohol, accounting for the continually increasing incidence rate in the general population.3–7

Of the known HPV types, oncogenic HPV16 is the most frequent type, accounting for approximately 90% of HPV associated SCCOP.7–9 HPV16 antibodies, markers of past exposure to HPV16, have been found to be positively associated with increased risk of cervical cancer 10–13 and SCCOP 9,14,15. Although HPV infection may be a major risk factor for SCCOP 8,16, only a small fraction of population with a long period of high risk HPV exposure develop SCCOP, implying that host genetic variants in genes, which are involved in cell cycle control and apoptosis and interact with HPV oncoprotein E6 or E7, may contribute to inter-individual variation in susceptibility to HPV associated SCCOP.

Cell cycle-related genes play a role in modulating cellular DNA repair, cell cycle control, cell growth and apoptosis to maintain genomic stability by monitoring the order and integrity of cell division events, such as *p53*, one of the critical cell cycle regulatory tumor suppressor genes.17–20 The loss of p53 function may result in loss of cell cycle control and checkpoint integrity. The chiefly oncogenic E6 protein of HPV16 binds to the p53 protein of the host cell, ending in p53 degradation via the ubiquitination pathway.21–23 The polymorphism of *p53* at codon 72 was shown to alter the susceptibility of p53 to oncogenic HPV E6-mediated degradation,24 and was significantly associated with oncogenic HPV infection in cervical cancer.24,25 Furthermore, in case-control analyses, the Pro allele was observed to be associated with an increased risk of HPV associated SCCOP,26 and the risk appeared to be particularly high in never smokers.27

p73, a member of the p53 family, activates the promoters of several p53-responsive genes participating in DNA repair, cell cycle control and apoptosis, and it inhibits cell growth in a p53-like manner by inducing apoptosis or G_1 cell cycle arrest.28–31 Inactivation of p73 by oncogenic HPV E6 appeared to be analogous to that of p53 without modulating the DNA binding activity.32 Therefore, p73 may act as a tumor suppressor with some of the same functions as p53 and may compensate for the loss of p53 function. However, p73, unlike p53, is resistant to degradation by HPV16 E6 and can suppress cell growth and induce apoptosis in HPV16 E6-expressing cells.33 It is possible that *p73* variation could alter the interaction between E6 protein and p73, and thus alter the risk of HPV16 associated carcinomas.

The two linked non-coding exon 2 polymorphisms of *p73* at positions 4 (G→A) and 14 (C→T) (termed *p73* G4C14-to-A4T14 polymorphism) are thought to affect p73 function by altering gene expression, perhaps by altering the efficiency of translational initiation.30 It is plausible that genetic variation of *p73* may lead to inter-individual variation in susceptibility

to HPV associated SCCOP. To the best of our knowledge, the association of this polymorphism with the risk of HPV16 associated SCCOP has not been investigated, particularly among never smokers and never drinkers.

To test the hypothesis, we evaluated the relationship between the *p73* G4C14-to-A4T14 polymorphism and HPV16 serological status for risk of SCCOP, and explored the joint effects of *p73* variant genotypes and HPV16 serological status in subgroups of subjects stratified by smoking and drinking status in a case-control study of 188 case subjects newly diagnosed with SCCOP and 349 cancer-free control subjects.

MATERIALS AND METHODS

Study Subjects

Consecutive patients with newly diagnosed, histopathologically confirmed, and untreated SCCOP were recruited between May 1996 and January 2001 through the Head and Neck Center at The University of Texas M. D. Anderson Cancer Center in Houston, Texas, as part of a molecular epidemiologic study of squamous cell carcinoma of the head and neck. The accrual rate was 81% for the cases. The controls included two groups of cancer-free subjects. One group were 160 (45.8%) healthy controls who were selected from a control pool of enrollees at the Kelsey-Seybold Clinic, a multi-specialty physician practice with multiple clinics throughout the Houston metropolitan area. The overall response rate was approximately 75%. The other controls were 189 (54.2%) healthy visitors who were accompanying cancer patients to the outpatient clinics at M. D. Anderson Cancer Center but genetically unrelated to the cases. The response rate for this M. D. Anderson Cancer Center control group was approximately 80%. Both control groups had no previous histories of any cancers, and were not on therapies or treatment for any diseases, and were frequency-matched to the cases on age (± 5 years), gender, smoking and drinking status. To avoid confounding due to ethnic characteristics, we included only non-Hispanic whites in both the case and control groups.

Participants who had smoked more than 100 cigarettes in their lifetimes were categorized as “ever smokers” and the rest as “never smokers”. Participants who had drunk alcoholic beverages at least once a week for more than 1 year were categorized as “ever drinkers” and the rest as “never drinkers”. After signing informed consent forms, which had been approved by the institutional review boards of both M. D. Anderson Cancer Center and Kelsey-Seybold, study participants completed a questionnaire regarding demographic and relevant risk factors and donated 30 ml of blood.

HPV16 Serological Testing

HPV16 L1 virus-like particles generated from recombinant baculovirus-infected insect cells were used to test for antibodies against HPV16 in the plasma of study subjects by using a standard enzyme-linked immunosorbent assay, as described previously.[10,34] Control sera known to be positive and negative were also tested in parallel with the study samples in duplicate on each plate. The cutoff level, above which optical density (OD) values were considered positive and below which OD values were considered negative for HPV16, was based on the absorbance value of a standard pooled serum known to be at the threshold of detection. Samples which were in 15% of the cutoff were tested twice more, and those positive in all three runs were considered positive. We also randomly selected 10% of the samples to retest for confirmation of the original findings. To eliminate potential binding interference by heparin, we treated the plasma samples with 43 U/ml heparinase I (Sigma, St. Louis, MO) before testing.[35] We tested heparinized plasma, as well as serum, obtained

from three individuals and did not detect discernible difference between the reactions of the serum samples and the heparinized plasma samples treated with heparinase.

p73 Genotyping

We extracted genomic DNA from a leukocyte cell pellet, which was obtained from the buffy coat by centrifugation of 1 ml of whole blood, by using the QIAGEN DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions. We typed for the *p73* G4C14-to-A4T14 genotypes by polymerase chain reaction (PCR) with confronting two-pair primers, which makes genotyping possible by electrophoresis without restriction digestion.36 The A4T14 allele was amplified with primers F1 [5′-CCACGGATGGGTCTGATCC-3′] and R1 [5′-GGCCTCCAAGGGCAGCTT-3′], which produced a 270-bp fragment, and the G4C14 allele was amplified with primers F2 [5′-CCTTCCTTCCTGCAGAGCG-3′] and R2 [5′-TTAGCCCAGCGAAGGTGG-3′], which amplified a 193-bp fragment. F1 and R2 also produced a common 428-bp fragment in each PCR. The PCR reaction was performed in a 10 μl volume containing approximately 20 ng of genomic DNA, 0.1 mM each dNTP, 1×PCR buffer (50 mM KCl, 10 mM Tris HCl and 0.1% Triton X-100), 1.5 mM $MgCl_2$, 0.5 U of Taq polymerase (Sigma-Aldrich Biotechnology, Saint Louis, MO), and 2 pmol of each of four primers. The amplification conditions included 10 min of initial denaturation at 95°C, 35 cycles of 1 min at 95 °C, 45 s at 62 °C and 1 min at 72 °C, and a final 5-min extension at 72 °C. All PCR products were visualized on a 2% agarose gel containing a 0.25 mg/ml of ethidium bromide. More than 10% of the samples were randomly selected to perform the repeated assays, and the results were 100% concordant.

Statistical Analysis

The differences in the distributions of selected demographic variables, tobacco smoking, alcohol use and *p73* allele and genotype frequencies between cases and controls were evaluated using the χ^2 test of association. Both univariate and multivariable logistic regression analyses were used to calculate odds ratio (OR) and 95% confidence interval (CI) for cases and controls stratified by age, sex, *p73* genotype, smoking, drinking and HPV16 status. In the multivariable logistic regression model, OR and 95% CI were adjusted by age, sex, smoking and alcohol use. Because only a small number of individuals (14 cases and 20 controls) were homozygous for the AT allele, which may preclude meaningful subgroup analyses, *p73* genotype data were dichotomized according to a dominant model, in which homozygosity for GC/GC was coded as 0, and both the heterozygosity for GC/AT and the homozygosity for AT/AT were coded as 1. All tests were two-sided, and a *P* value of 0.05 was considered the cutoff for statistical significance. All the statistical analyses were performed with Statistical Analysis System software (Version 9.1; SAS Institute, Cary, NC).

RESULTS

Table 1 lists the distributions of demographic variables and risk factors of the study population with 188 cases and the 349 cancer-free controls. All study subjects were non-Hispanic whites, and the cases and controls appeared to be adequately frequency-matched for age, sex, smoking and alcohol consumption, but these factors were also further adjusted in later analyses to control for any residual effects. The distributions of *p73* genotypes among the controls were in agreement with Hardy-Weinberg equilibrium ($P = 0.349$). When comparing *p73* genotype distributions between cases and controls, no significant differences were found ($P = 0.738$). The *p73* AT allele was present in 39.4% of the cases and 38.4% of the cancer-free controls ($P = 0.893$), and the *p73* AT allele frequency was 23.4% in the cases and 22.1% in the controls ($P = 0.682$). We also found that the distribution of *p73* AT carriers and non-carriers between the cases and the controls was not statistically significant (*P* =

0.826), but the HPV16 seropositivity was significantly more common in the cases than in the controls ($P < 0.001$).

Table 2 summarizes the distributions of *p73* genotypes, age, sex, smoking and alcohol use stratified by HPV16 status and their association with SCCOP risk. Overall, HPV16 seropositivity was associated with an approximately 6-fold risk of SCCOP (adjusted OR = 5.98; 95% CI = 3.89-9.20) after adjusting for age, sex, smoking status and alcohol use. HPV16 seropositivity among those with the *p73* homozygous wild-type genotype (GC/GC) was associated with an elevated risk of SCCOP (adjusted OR = 5.47; 95% CI = 3.14-9.54). While among individuals with the *p73* variant genotypes (GC/AT + AT/AT), HPV16 seropositivity was associated with an even higher risk of SCCOP (adjusted OR = 7.96; 95% CI = 3.83-16.5).

To investigate the effects of other factors on the risk of HPV16 associated SCCOP, we further stratified the associations between the HPV16 status and the cancer risk by age, sex, smoking status and alcohol use. We found that the risk of SCCOP associated with HPV16 seropositivity was evident for all of the subgroups, particularly among individuals less than 56 years old (adjusted OR = 7.94; 95% CI = 4.26-14.8), men (adjusted OR = 6.65; 95% CI = 4.09-10.8), never smokers (adjusted OR = 13.8; 95% CI = 5.91-32.1), and never drinkers (adjusted OR = 14.9; 95% CI = 5.24-42.4). However, the interaction between HPV16 status (seropositive vs seronegative) and *p73* polymorphism (AT carriers vs AT non-carriers), age, and sex was not statistically significant ($P = 0.408$ for *p73* polymorphism; $P = 0.169$ for age; and $P = 0.386$ for sex).

Because we found a significant interaction between HPV16 status (seropositive vs seronegative) and smoking status (ever smoking vs never smoking) on the risk of SCCOP ($P = 0.029$), we further investigated the effects of *p73* genotypes on risk of SCCOP stratified by HPV16 serological status and smoking status (with adjustment for age, sex and alcohol use) (Table 3). Those never smokers who were HPV16 seropositive and had *p73* variant genotypes exhibited an approximately 29-fold greater risk of SCCOP (adjusted OR = 28.6; 95% CI = 5.53-148.2) than those never smokers who were HPV16 seronegative and had the wild-type genotype. However, HPV16 seropositivity in never smokers with the wild-type genotype conferred an approximately 15-fold increased risk of SCCOP (adjusted OR = 14.6; 95% CI = 5.24-40.5). Ever smokers who had *p73* variant genotypes and were HPV16 seropositive exhibited only a 4-fold higher risk of SCCOP (adjusted OR = 4.43; 95% CI = 2.09-9.42) compared with ever smokers who had the wild-type genotype and were HPV16 seronegative. Ever smokers with the wild-type genotype who were HPV16 seropositive exhibited a less increased risk of SCCOP (adjusted OR = 3.35; 95% CI = 1.71-6.55) compared with ever smokers with the wild-type genotype who were HPV16 seronegative.

Similarly, because a borderline significant interaction between HPV16 status (seropositive vs seronegative) and drinking status (ever drinking vs never drinking) was found on the risk of SCCOP (P = 0.068), we further investigated the effects of *p73* genotypes on risk of SCCOP stratified by HPV16 serological status and drinking status (with adjustment for age, sex and smoking status) (Table 3). Those never drinkers who were HPV16 seropositive and had *p73* variant genotypes exhibited an approximately 23-fold greater risk of SCCOP (adjusted OR = 23.1; 95% CI = 2.01-265.4) than those never drinkers who were HPV16 negative and had the wild-type genotype. However, HPV16 seropositivity in never drinkers with the wild-type genotype conferred an approximately14-fold increased risk of SCCOP (adjusted OR = 13.7; 95% CI = 4.27-43.8). Ever drinkers who had *p73* variant genotypes and were HPV16 seropositive exhibited only a 5.5-fold higher risk of SCCOP (adjusted OR = 5.55; 95% CI = 2.73-11.3) compared with ever drinkers who had the wild-type genotype and were HPV16 seronegative. Ever drinkers with the wild-type genotype who were HPV16

seropositive exhibited a less increased risk of SCCOP (adjusted OR = 3.83; 95% CI = 2.04-7.21) compared with ever drinkers with the wild-type genotype who were HPV16 seronegative. Although the ORs we observed above were different in one group than another, the 95% CIs overlapped widely and the differences were not statistically different ($P > 0.05$). The estimates of association could be biased due, in part, to small sample sizes within the strata of each stratification variables, misclassification of HPV serological status, and other confounding factors. The findings from this study need to be confirmed in future larger and well-designed studies.

DISCUSSION

In this hospital-based case-control study of a non-Hispanic white population, we found that HPV16 seropositivity was associated with an increased risk of SCCOP. HPV16 seropositivity among patients with *p73* variant genotypes (GC/AT + AT/AT) exhibited a slightly greater risk for developing SCCOP than those with *p73* wild-type genotype (GC/GC), but the risk was relatively greater in never smokers and never drinkers. This finding suggests that the *p73* G4C14-to-A4T14 polymorphism may modulate the genetic susceptibility to HPV16 associated SCCOP.

Although the precise mechanism by which the *p73* G4C14-to-A4T14 polymorphism plays a role in the development of HPV16 associated SCCOP has not yet been clarified to date, these findings are biologically plausible. p73 shares structural and functional similarities to p53 and can be functionally inactivated through physical interaction with E6 oncoprotein, possibly analogous to that of p53.[28-32] Currently, no reported studies have investigated the association between this *p73* polymorphism and risk of HPV16 associated SCCOP, but the *p53* codon 72 polymorphism has been shown to alter the susceptibility of p53 to oncogenic HPV E6-mediated degradation [24] and be associated with an increased risk of HPV associated cancers.[24-27] These suggest that p73 may have a similar effect to that of p53 on the development of HPV16 associated SCCOP.

Genetic variation may lead to different splice transcripts and have functional consequences, which may contribute to inter-individual difference and disease susceptibility.[37,38] The *p73* G4C14-to-A4T14 polymorphism lies upstream of the initiating AUG of exon 2 and may affect the differential usage of *p73* promoters, leading to different functional transcripts with variable 5′-untranslated regions. This *p73* polymorphism may also form a stem-loop structure, which may result in alteration of gene expression, possibly by altering the efficiency of translational initiation.[30] Additionally, this *p73* polymorphism could also be in linkage disequilibrium with other functional polymorphisms or adjacent susceptibility loci of the gene, thereby affecting *p73* gene expression and activity, leading to altered interaction between E6 protein and p73, and thus modulate the risk of HPV associated carcinogenesis.[37] But more studies are needed to further confirm these hypotheses.

In this study, a higher risk of HPV16 associated SCCOP was found among never smokers than among ever smokers, as well as a greater risk among never drinkers than among ever drinkers. Moreover, a significant interaction was found between the HPV16 status and smoking status, and also a near significant interaction between HPV16 status and drinking status. For instance, in patients with the variant *p73* genotypes (GC/AT + AT/AT) and HPV seropositivity, there is an approximately 7-fold increased risk of SCCOP in never smokers whereas among the patients with wild-type *p73* genotypes (GC/GC) and HPV seropositivity, there is an approximately 4-fold increased risk of SCCOP, compared with the patients with HPV16 seronegativity and the wild-type *p73* genotype (GC/GC). These findings imply that this *p73* polymorphism may have a stronger interaction with HPV16 among never smokers and never drinkers, than among ever smokers and ever drinkers or that this *p73*

polymorphism may be less important in smokers and alcohol drinkers because non-HPV SCCOP already have a heavy carcinogen/mutation burden driving SCCOP development. Therefore, the *p73* G4C14-to-A4T14 polymorphism may play a role in the development of HPV16 associated SCCOP among never smokers and never drinkers in the general population. However, smoking or drinking and *p73* variants may not be co-factors in the etiology of HPV16 associated SCCOP, suggesting that it is imperative to control for the confounding effects of smoking and alcohol use while assessing the role of this *p73* polymorphism as a risk factor for HPV16 associated SCCOP. However, these findings need to be further tested in studies with larger sample sizes.

In addition, we found that the risk of HPV16 associated SCCOP was more evident among younger subjects (less than 56 years old) and men, but there was no evidence of significant interaction between HPV16 status and age and sex. These findings are consistent with previous studies that showed HPV associated carcinoma had a trend of younger age onset and might be relative to sexual practice (i.e., orogenital sexual contact).[6,39,40] Overall, squamous cell carcinomas of the head and neck occur more frequently in men (approximately 70%) than in women.[41]

This study is the first one to investigate the relationship between the *p73* G4C14-to-A4T14 polymorphism and the risk of HPV16 associated SCCOP. A Japanese study[42] with 112 cases and 320 healthy women and 122 non-cancer female outpatients investigated the role of this *p73* polymorphism in the risk of cervical cancer, which is chiefly caused by HPV16 infection. *p73* variant genotypes were found to be associated with a borderline increased risk of cervical cancer (adjusted OR, 1.51; CI, 0.98-2.35). Our previous study[43] explored the association of this *p73* polymorphism with the risk of squamous cell carcinoma of the head and neck, and found no significant association with the risk of SCCOP after stratified analysis by cancer sites. We expected that the estimated risk in these two studies could be confounded by HPV16 status, smoking, and drinking status. Therefore, it is necessary to further stratify the data by HPV16 status, smoking and drinking status in future studies that aim at assessing the effect of this *p73* polymorphism on the risk of HPV associated cancers.

In this hospital-based case-control study, there might have been some selection bias, because our cases and controls may have different population bases. SCCOP cases were enrolled from the M. D. Anderson Cancer Center outpatients, and the controls were recruited from both outpatient clinic visitors at M. D. Anderson Cancer Center and a control pool of enrollees at the Kelsey-Seybold Clinic through the Houston metropolitan area. However, there was no statistically significant difference in the frequency of *p73* genotypes between cases and controls as we previously reported for the same control populations.[43] In addition, stratified analyses included a limited number of individuals in some subgroups, so our results could be a chance finding and should be confirmed in larger studies. Moreover, because our study included only non-Hispanic white subjects, it is uncertain whether these results are generalizable to other ethnic populations. However, the cases and controls were frequency-matched for age, sex, smoking status and drinking status, and the effects of any confounding demographic factors might have been minimized. Finally, HPV16 seropositivity might not reflect actual tumor HPV16 status, leading to some misclassification, i.e., some patients might be classified as serologically negative while their tumors were actually HPV16 DNA positive. This is because individual patients may have differences in immune response to HPV16 infection or the responsive antibody instability. This misclassification could result in a major selection bias for the estimates of the association. We may assess the HPV16 specificity and sensitivity for SCCOP patients to explore the discordance between HPV16 serological and tumor status in our future study with larger sample sizes when the tumor tissues become available. However, an early study confirmed a reasonable concordance between HPV16 seropositivity and HPV16 DNA

positivity of tumor tissues,9 and more importantly, the use of serologic status allows for the inclusion of a cancer-free control group.

In conclusion, our findings suggest that *p73* G4C14-to-A4T14 polymorphism may modulate the risk of HPV16 associated SCCOP, and the *p73* variant genotypes may be a marker of genetic susceptibility to HPV16 associated SCCOP, particularly in never smokers and never drinkers. Advanced biological studies are needed to validate these results. Further investigations with larger sample sizes of different populations are also warranted. To advance these findings, we are currently testing other functional polymorphisms of genes involved in cell cycle and apoptosis pathways to elucidate the roles of these genetic variants in the development of HPV16 associated SCCOP.

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Abbreviations

CI	confidence interval
HPV	human papillomavirus
OR	odds ratio
PCR	polymerase chain reaction
SCCOP	squamous cell carcinoma of the oropharynx

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TABLE 1

Frequency Distribution of Demographic and Risk Factors in SCCOP Cases and Cancer-Free Controls

Characteristic	Cases (n = 188)		Controls (n = 349)		*P*-value*
	No.	(%)	No.	(%)	
Age (years)					.259
≤ 40	17	9.1	37	10.6	
41 - 55	86	45.7	129	37.0	
56 - 70	64	34.0	141	40.4	
> 70	21	11.2	42	12.0	
Sex					.217
Male	155	82.4	272	77.9	
Female	33	17.6	77	22.1	
Smoking					.498
Ever	125	66.5	242	69.3	
Never	63	33.5	107	30.7	
Alcohol use					.129
Ever	150	79.8	258	73.9	
Never	38	20.2	91	26.1	
p73 genotype ◇					.738
GC/GC	114	60.6	215	61.6	
GC/AT	60	31.9	114	32.7	
AT/AT	14	7.5	20	5.7	
GC/AT+AT/AT	74	39.4	134	38.4	.826
HPV16 status					<.001
Positive	87	46.3	43	12.3	
Negative	101	53.7	306	87.7	

*Two-sided χ^2 test.

◇*P* = 0.738 for genotype distributions; *P* = 0.682 for allele frequency; the observed genotype frequencies among controls were in agreement with Hardy-Weinberg equilibrium ($p^2+2pq+q^2 = 1$) (*P* = 0.349).

TABLE 2

Adjusted Odds Ratio and 95% CI for Associations between Selected Variables and Risk of HPV16 Associated SCCOP

Characteristic	HPV16 status	Cases (n = 188)		Controls (n = 349)		Adj. OR* (95% CI)
		No.	(%)	No.	(%)	
All subjects	-	101	53.7	306	87.7	1.0
	+	87	46.3	43	12.3	5.98 (3.89-9.20)
p73 genotype						
GC/GC	-	64	56.1	187	87.0	1.0
	+	50	43.9	28	13.0	5.47 (3.14-9.54)
GC/AT+AT/AT	-	37	50.0	119	88.8	1.0
	+	37	50.0	15	11.2	7.96 (3.83-16.5)
Age (years)						
≤ 55	-	50	47.6	152	88.4	1.0
	+	55	52.4	20	11.6	7.94 (4.26-14.8)
> 55	-	51	61.5	154	87.0	1.0
	+	32	38.5	23	13.0	4.21 (2.25-7.88)
Sex						
Male	-	80	51.6	239	87.9	1.0
	+	75	48.4	33	12.1	6.65 (4.09-10.8)
Female	-	21	63.6	67	87.0	1.0
	+	12	36.4	10	13.0	4.06 (1.51-10.9)
Smoking						
Never	-	27	42.9	97	90.6	1.0
	+	36	57.1	10	9.4	13.8 (5.91-32.1)
Ever	-	74	59.2	209	86.4	1.0
	+	51	40.8	33	13.6	4.34 (2.59-7.27)
Alcohol use						
Never	-	18	47.4	84	90.3	1.0
	+	20	52.6	9	9.7	14.9 (5.24-42.4)
Ever	-	83	55.3	222	86.7	1.0

Characteristic	HPV16 status	Cases (n = 188)		Controls (n = 349)		Adj. OR* (95% CI)
		No.	(%)	No.	(%)	
	+	67	44.7	34	13.3	4.79 (2.96-7.75)

*HPV positive versus HPV negative in each stratum; OR were adjusted for age, sex, smoking and alcohol use accordingly in logistic regression models.

TABLE 3

Stratification of Risk of SCCOP in Never and Ever Smokers/Drinkers by *p73* Genotype and HPV16 Status

Smoking & drinking status & *p73* genotype	HPV16 status	Cases	Controls	Crude OR (95%CI)	Adj.* OR (95%CI)
		No. (%)	No. (%)		
Never smokers		(n = 63)	(n = 107)		
GC/GC	-	12 (19.1)	57 (53.3)	1.0	1.0
	+	24 (38.0)	8 (7.4)	14.3 (5.17-39.3)	14.6 (5.24-40.5)
GC/AT+AT/AT	-	15 (23.8)	40 (37.4)	1.78 (0.75-4.21)	1.66 (0.68-4.08)
	+	12 (19.1)	2 (1.9)	28.5 (5.63-144.2)	28.6 (5.53-148.2)
Ever smokers		(n = 125)	(n = 242)		
GC/GC	-	52 (41.6)	130 (53.7)	1.0	1.0
	+	26 (20.8)	20 (8.3)	3.25 (1.67-6.32)	3.35 (1.71-6.55)
GC/AT+AT/AT	-	22 (17.6)	79 (32.6)	0.70 (0.39-1.23)	0.67 (0.38-1.19)
	+	25 (20.0)	13 (5.4)	4.81 (2.29-10.1)	4.43 (2.09-9.42)
Never drinkers		(n = 38)	(n = 91)		
GC/GC	-	12 (31.6)	55 (60.4)	1.0	1.0
	+	16 (42.1)	6 (6.6)	12.2 (3.96-37.7)	13.7 (4.27-43.8)
GC/AT+AT/AT	-	6 (15.8)	29 (31.9)	0.95 (0.32-2.79)	0.98 (0.32-3.00)
	+	4 (10.5)	1 (1.1)	18.3 (1.88-179.0)	23.1 (2.01-265.4)
Ever drinkers		(n = 150)	(n = 258)		
GC/GC	-	52 (34.7)	132 (51.2)	1.0	1.0
	+	34 (22.7)	22 (8.5)	3.92 (2.10-7.33)	3.83 (2.04-7.21)
GC/AT+AT/AT	-	31 (20.6)	90 (34.9)	0.87 (0.52-1.47)	0.86 (0.50-1.45)
	+	33 (22.0)	14 (5.4)	5.98 (2.96-12.1)	5.55 (2.73-11.3)

*Adjusted for age, sex, and drinking/smoking in logistic regression models.