HHS Public Access

Author manuscript

Int J Cancer. Author manuscript; available in PMC 2018 September 15.

Published in final edited form as:

Int J Cancer. 2018 September 15; 143(6): 1327–1334. doi:10.1002/ijc.31530.

A *TGF-β1* genetic variant at the miRNA187 binding site significantly modifies risk of HPV16-associated oropharyngeal cancer

Ye Tao[1,2], **Erich M. Sturgis**[1,3], **Zhigang Huang**[1,2], **Yan Sun**[1,4,*], **Kristina R. Dahlstrom**[1], **Qingyi Wei**[5], and **Guojun Li**[1,3,*]

[1]Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030

[2]Department of Otolaryngology-Head and Neck Surgery, Beijing Tongren Hospital, Capital Medical University, Key Laboratory of Otolaryngology Head and Neck Surgery, Beijing, China

[3]Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030

[4]Department of Otorhinolaryngology and Head and Neck Surgery, Yuhuangding Hospital of Qingdao University, Yantai, China

[5]Duke Cancer Institute, Duke University Medical Center, Durham, NC, USA.

Abstract

*TGF-β1*rs1982073 polymorphism at the miRNA-187 binding site may alter TGF-β1 expression and function, and thereby this polymorphism (genotype CT/CC) increases cancer susceptibility. HPV16 L1 seropositivity is associated with the risk of oral squamous cell carcinoma (OSCC), including oropharyngeal squamous cell carcinoma (OPSCC) and oral cavity squamous cell carcinoma (OCSCC). Thus, we hypothesized that *TGF-β1*rs1982073 polymorphism at the miRNA-187 binding site combined with HPV16 L1 seropositivity may have a joint effect on OSCC susceptibility. We determined the genotypes of *TGF-β1*rs1982073 and HPV16 status in 325 OSCC subjects and 335 cancer-free controls in the non-Hispanic white population, and used logistic regression models to evaluate the joint effects on OSCC susceptibility. *TGF-β1*rs1982073 polymorphism (CT/CC genotype) combined with HPV16 L1 seropositivity increased the risk of OSCC via joint effects, particularly in OPSCC subjects who were never-smokers (OR, 165.9; 95% CI, 28.6–960.4) or never-drinkers (OR, 196.0; 95% CI, 28.2–1000.0), respectively. Younger subjects had a higher risk of OPSCC than older subjects (OR, 23.5; 95% CI, 6.3–87.0 vs. OR, 6.0; 95% CI, 1.7–17.9, respectively). The significant associations between this polymorphism and HPV16-associated OSCC and OPSCC were also observed. However, OCSCC subjects did not have similar results. Our findings suggest that the joint effects of *TGF-β1*rs1982073 and HPV16 L1 seropositivity can increase risk of HPV16-associated oral cancer, particularly in OPSCC

[*]**Address for reprints and correspondence:** Guojun Li, MD, PhD, Department of Head and Neck Surgery, Unit 1445, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030; Phone: (713) 792-0227; Fax: (713) 794-4662; gli@mdanderson.org or Yan Sun, Department of Otorhinolaryngology and Head and Neck Surgery, Yuhuangding Hospital of Qingdao University, Yantai, China; Phone: 0118618660566677; lenards@163.com.

Disclosure of potential conflicts of interest: No potential conflicts of interest were disclosed.

subjects who are never-smokers, never-drinkers, and young. This result may help us understand the tumorigenesis process and improve early detection, which are critical for prevention and intervention strategies. However, larger studies are needed to validate our findings.

Keywords

TGF-β1; genetic variants; HPV; oropharyngeal cancer; susceptibility; biomarkers

Introduction

Oral squamous cell carcinoma (OSCC) consists of oral cavity squamous cell carcinoma (OCSCC) and oropharynx squamous cell carcinoma (OPSCC)[1,2]. Tobacco smoking and alcohol drinking are the major risk factors for OSCC in the U.S. Smoking cessation efforts have reduced the risk of OSCC sharply and decreased the incidence significantly[3]. In contrast to the declining trend for OCSCC, the incidence of OPSCC is increasing, particularly among young patients [1–5].This rising incidence trend for OPSCC is mainly attributed to human papillomavirus (HPV) infection [6]. The predominant virus type is HPV16, which has a more than 90% positive rate in HPV-positive OPSCC [7].

TGF-β1, a member of the TGF-β family, suppresses tumorigenesis in precancerous tissues and promotes invasiveness in advanced tumors, mainly owing to disequilibrium of TGF-β1 signaling, which features interwoven pathways with complex cross-talk and highly contextual dependence [8]. Since TGF-β1/Smad2–3 serves as a core pathway in homeostasis, its signaling disruption and disequilibrium is associated with several somatic mutations. The single nucleotide polymorphism (SNP) *TGF-β1*rs1982073 (merged into NCBI SNP rs1800470; T869C; codon 10 of exon 1; encoding Leu10Pro) can significantly affect the serum expression level of TGF-β1 and thereby increase the susceptibility to gastric and breast cancers [9–11]. Furthermore, *TGF-β1*rs1982073 located at the *miR-187* binding sites and the binding minimum free energy (MFE) were modified in the duplex of *miR-187*::*TGFB1*-mRNA by the T to C transition of *TGF-β1*rs1982073. Therefore, this binding modification can alter miRNA gene regulation and TGF-β1 expression and function and thereby affect the risk of cancer [12].

Polymorphism *TGF-β1*rs1982073 has been associated with increased susceptibility to breast cancer [13] and prostate cancer [14], and our previous study identified the potential effects of *TGF-β1*rs1982073 in OSCC subjects[15]. However, no case-control study has evaluated the effect of *TGF-β1*rs1982073 on susceptibility to HPV-associated OSCC. Since HPV16 infection is associated with risk of OSCC, and *TGF-β1*rs1982073 affects disequilibrium of TGF-β1/Smad2–3 signaling pathway, which facilitates HPV16 E6/7 carcinogenesis, we hypothesized that the *TGF-β1*rs1982073 polymorphism at the miRNA-187 binding site combined with HPV16 L1seropositivity has a joint effect on susceptibility to OSCC. To test this, we determined the *TGF-β1*rs1982073 polymorphism genotype and the HPV16 serological status of 325 non-Hispanic white OSCC patients and 335 cancer-free controls to assess the joint effects of these two factors on susceptibility to OSCC.

Material and Methods

Study population

This case-control study included 325 newly diagnosed, untreated patients who had a clinical diagnosis and histopathological confirmation of OSCC. The patients had been recruited at The University of Texas MD Anderson Cancer Center as part of an ongoing molecular epidemiologic study, in which the patient eligibility criteria have been described previously[16, 17]. During that same period, 335 controls were recruited by the Kelsey-Seybold Foundation from a pool of healthy subjects, which included residents of metropolitan Houston, and by MD Anderson Cancer Center from a pool of healthy visitors who had accompanied cancer patients but were genetically unrelated to the patients. The 335 healthy controls were frequency matched to the 325 OSCC patients by sex, age (± 5 years), and smoking and drinking status. All study subjects recruited were non-Hispanic whites. The institutional review boards of both MD Anderson and Kelsey-Seybold approved this study, and every study subject signed an informed consent form. Smoking status was classified as "ever smokers" (those who had smoked more than 100 cigarettes in their lifetime) and "never smokers" (those who had smoked fewer than 100 cigarettes in their lifetime). Drinking status was classified as "ever drinkers" (those who had drunk more than one alcoholic beverage per day for at least 1 year during their lifetime) and "never drinkers" (those who never had such a pattern of drinking).

TGF-β1rs1982073 genotyping

At diagnosis or the recruitment, blood samples were collected and prepared for *TGF-β1* genotyping. The criteria for determination of *TGF-β1* polymorphism have been previously described[15, 18]. 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. The polymerase chain reaction restriction fragment length polymorphisms (PCR-RFLP) method was used for genotyping as previously described[18]. Genotyping was performed by laboratory personnel blinded to the case–control status. Repeated analysis was performed on a randomly selected subset of 10% of the samples, and the results were in 100% concordance with the initial analysis.

HPV16 L1 serologic detection

For serologic testing, we generated HPV16 L1 virus-like particles from insect cells infected with recombinant baculovirus to test for antibodies against the HPV16 L1 capsid protein in the plasma; this was done with the use of a standard enzyme-linked immunosorbent assay (ELISA), as described previously[17]. Two groups of control sera, one known to be positive and the other known to be negative, were tested in parallel with the study samples in duplicate on each plate. About 10% of the samples were randomly chosen for a repeat assay, and the results were 100% concordant with the results of the initial testing.

HPV16 detection in tumor specimens

The samples of DNA were extracted from paraffin-embedded tumor tissues of patients and determined for the presence of HPV16 using polymerase chain reaction and in situ

hybridization methods described in our previous studies[15, 17]. For some patients, tumor HPV16 status was determined by in situ hybridization and p16 immunohistochemical analysis from HPV data in the patient's clinical records, since the pathology laboratory at MD Anderson had begun classifying all OPSCC specimens as a standard clinical practice.

Statistical analysis

Statistical analyses were performed with SAS software (version 9.4; SAS Institute Inc., Cary, NC). We used χ^2 tests to test the differences in demographics, including smoking and drinking status, HPV16 L1 serology, and *TGF-β1*rs1982073 genotypes between the patients and controls. To evaluate the associations of HPV16 L1 serology and *TGF-β1*rs1982073 polymorphism with the risk of OSCC, we used both univariate and multivariable logistic regression analyses to compute odds ratios (ORs) and 95% confidence intervals (CIs). Both HPV16 serological status and *TGF-β1*rs1982073 genotypes (CT/CC or TT genotype) were evaluated individually or in joint effect in cases and controls. Stratified analyses of the joint effects of HPV16 serology and *TGF-β1*rs1982073 polymorphism were further performed by taking into account smoking and drinking status and age. In addition, logistic regression analysis was performed to evaluate the association between the *TGF-β1*rs1982073 polymorphism and HPV16 serological status and OSCC risk stratified by cancer site (OPSCC vs. OCSCC patients). All tests were two-sided, and $P < 0.05$ was considered significant.

Results

Demographics and risk factors

The demographic characteristics and risk factors of the 325 cancer-patient cases and 335 cancer-free controls are shown in Table 1. Age, sex, smoking status, and alcohol drinking status did not differ significantly between the cases and controls, and these results further confirmed the frequency-match validity. However, the frequency of HPV16 L1 seropositivity was significantly higher in the cases than in the controls ($P < 0.001$).

*Joint effect of TGF-β1*rs1982073 *polymorphism and HPV16 serology on risk of OSCC*

Regardless of HPV16 serology, subjects with the CT/CC genotype of *TGF-β1*rs1982073 had about a 4-fold higher risk of developing OSCC than those with the TT genotype of *TGF-β1*rs1982073 (Table 2). The subgroups of OPSCC and OCSCC had similar results (Table 2). When HPV16 serology was taken into account, and the effects were adjusted for age, sex, and smoking/drinking status, we found that HPV16 seronegative individuals carrying the TT genotype of *TGF-β1*rs1982073 had the lowest risk. Therefore, the combination of the TT genotype of *TGF-β1*rs1982073 and seronegativity of HPV16 was set as the reference group in further comparisons with the other three combinations of genotype (CT/CC or TT) and HPV16 serology (seropositivity or seronegativity).

The risk of OSCC was greatest among subjects with the CT/CC genotype and HPV16 L1 seropositivity (OR, 13.6; 95% CI, 7.1–26.2) (Table 2). This risk was even more pronounced in OPSCC among patients with the CT/CC genotype and HPV16 L1 seropositivity (OR,

26.4; 95% CI, 12.9–53.9). In contrast, the risk of OCSCC was highest among subjects with the CT/CC genotype and negative HPV16 serology (OR, 4.4; 95% CI, 2.8–7.0).

Stratified analysis of the joint effect of HPV16 serology and TGF-β1rs1982073 polymorphism on risk of OSCC by smoking and drinking status

We evaluated the association between the *TGF-β1*rs1982073 genotype and the risk of HPV16-associated OSCC stratified by smoking or drinking status. Table 3 shows that the joint effect of *TGF-β1*rs1982073 polymorphism and HPV16 serology on risk of OSCC was more significant in never-smokers and never-drinkers than in ever-smokers and ever-drinkers, particularly for OPSCC patients. In fact, compared with the reference group, the risk of OSCC was 60.1-fold higher in the CT/CC genotype and HPV16 L1-seropositive never-smokers group and only 8.8-fold higher in the CT/CC genotype and HPV16 L1-seropositive ever-smokers group. Likewise, in the CT/CC genotype and HPV16 L1-seropositive never-drinkers group, the risk of OSCC was 59.9-fold higher, versus 10.1-fold higher in the ever-drinkers group. Similarly, these stratified results demonstrated that the joint effects of HPV16 serology and *TGF-β1*rs1982073 genotype were much more significant among OPSCC than OCSCC. Specifically, in OPSCC subjects, compared with the reference group, the CT/CC genotype and HPV16 L1 seropositivity resulted in 165.9-fold and 196.0-fold higher risks in never-smokers and never drinkers, respectively, compared with15.2-fold and 17.2-fold higher risks in ever-smokers and ever-drinkers, respectively.

Joint effect of TGF-β1rs1982073 polymorphism and HPV16 serology by age stratification

We evaluated the joint effects of the *TGF-β1*rs1982073 genotype and HPV16 serology stratified by younger age (<50 years) or older age (≥ 50 years), as shown in Table 4. The TT genotype of *TGF-β1*rs1982073 and HPV16 L1 seronegativity were set as the reference group. In the CT/CC genotype and HPV16 L1 seropositive OSCC younger group, the risk of OSCC was 12.9-fold higher, versus 3.2-fold higher in older subjects. Moreover, the risk of OPSCC was 23.5-fold higher in the CT/CC genotype and HPV16 seropositive younger group and only 6.0-fold higher in the older group. However, this pattern was not observed in OCSCC subjects.

Association of TGF-β1rs1982073 polymorphism with HPV16-associated OSCC

Among the 325 cases, there were 170 patients to either have tissue specimens available for tumor HPV determination or have existing tumor HPV status in clinical records. We also included another 40 patients, who were recruited at the same period as those in the current study and had tissue specimens available for tumor HPV determination. These 40 patients were also genotyped for *TGF-β1rs1982073* polymorphisms. Therefore, a total of 210 patients were included for this subgroup analysis. The association between *TGF-β1rs1982073* polymorphism with HPV16-associated OSCC is presented in Table 5. We found that compared with those with TT genotype, the carriers with CT/CC genotypes of *TGF-β1rs1982073* had approximately 3-fold significantly increased risk of OSCC (OR, 3.2; 95% CI, 1.4–7.4)) and OPSCC (OR, 3.3; 95% CI, 1.4–7.8) when our analysis was limited to only HPV16 L1 seropositive individuals. However, such a significantly increased risk was not found for OCSCC (OR, 1.3; 95% CI, 0.8–18.8). Furthermore, the genotype distribution of the *TGF-β1rs1982073* polymorphism differed significantly between tumor HPV16-

positive and tumor HPV16-negative patients ($P < 0.0001$). The patients with the CT/CC genotypes of *TGF-β1rs1982073* were almost 2 times more likely to have HPV16-positive tumors than those with the TT genotype among patients with OSCC (OR, 1.9; 95% CI, 1.1–3.4), OPSCC (OR, 2.0; 95% CI, 1.3–6.1) and OCSCC (OR, 1.1; 95% CI, 0.2–2.3), respectively.

Discussion

In our current study, we evaluated the association between the *TGF-β1*rs1982073 genotype and HPV16 L1 serology in 325 non-Hispanic white OSCC patients and 335 cancer-free controls, and found that *TGF-β1*rs1982073 polymorphism (CT/CC genotype) combined with HPV16 L1seropositivity increased the risk of OSCC via joint effects, most notably in OPSCC subjects who were never-smokers or never-drinkers, respectively. Younger patients with OPSCC had a higher risk than older OPSCC patients. Similar patterns were not observed in the OCSCC subjects. Moreover, the significant associations between this polymorphism and risk of OSCC and OPSCC among HPV16 L1 seropositive subjects only and between this polymorphism and tumor HPV16 status were also found. Taken together, our findings suggest that the joint effects of *TGF-β1*rs1982073 and HPV seropositivity can increase the risk of HPV16-associated oral cancer, particularly in OPSCC subjects who are never-smokers, never-drinkers, and young.

While HPV16 L1 seropositivity actually indicates that there has been prior HPV exposure, and this could and likely often occurred in other regions (e.g., anogenital sites, Etc), several recent studies have demonstrated that it is HPV16 E6/E7 that are associated with, or predictive of, HPV16-driven OPSCC[19–21]. Therefore, serum anti-HPV16 L1 antibody seems to be only in a proportion of HPV16-associated OSCC in contrast to antibodies to the proteins E6 and E7, which cooperate in neoplastic transformation and clearly are linked to HPV-associated OSCC. Thus, HPV seropositivity is only likely to indicate the humoral immune response following infection by HPV16 but not presence of a HPV-associated OSCC. To demonstrate a causal link between HPV L1 seropositivity and HPV16-associated OSCC and a link between *TGF-β1rs1982073* and HPV16-related OSCC, we further performed a subgroup analysis limited to only HPV16 L1 positive healthy controls and patients; and we found a significant association between *TGF-β1rs1982073* and risk of OSCC. Furthermore, another subgroup analysis also demonstrated that *TGF-β1rs1982073* was significantly associated with tumor HPV16 status. All these additional data might support a link of *TGF-β1rs1982073* and HPV16L1 seropositivity to HPV16-related OSCC, particularly for OPSCC, while future studies with large sample sizes are need to validate these findings.

The *TGF-β1*rs1982073 polymorphism increases the risk of HPV16 L1-seropositive OPSCC via *miR-187* regulation due to this target SNP being located in the miRNA binding sites [12, 22, 23]. The polymorphism of nucleotide C to T transition decreases the binding minimum free energy of the duplex of *miR-187*:: *TGF-β1*-mRNA, thereby increasing the binding strength of that duplex[22]. This suggests that the TT allele is protective, while the CT/CC allele increases the cancer risk. Since high expression of miR-187 was associated with a trend toward cancer progression in breast cancer [23] and prostate cancer [24], and the *TGF-*

*β1*rs1982073 CC/CT genotype could increase the binding strength of that duplex, we conclude that the *TGF-β1*rs1982073 CC/CT genotype could potentially increase the risk of and progression of HPV16 L1-seropositive OPSCC.

*TGF-β1*rs1982073 may also increase the risk of HPV16 L1seropositive OPSCC via the Leu10Pro signal peptide substitution that affects TGF-β1 secretion, structure, and function. In cytomegalovirus (CMV)-transfected HeLa cell lines, CMV-Pro10 (CC genotype) had a 2.8-fold increase in TGF-β1 secretion compared with CMV-Leu10 (TT genotype), and this increased secretion indicated that *TGF-β1*rs1982073 (Pro10 homozygosity CC genotype) was associated with an increased risk of breast cancer [9]. Likewise, in hepatoma cell lines (HepG2, SMMU7721, LX-2, and L02), cells transfected with CMV-Pro10 had higher capacity for TGF-β1 secretion, greater anti-apoptosis effects, and stronger enhancement of cell proliferation compared with those transfected with CMV-Leu10 *in vitro* [25]. *In vivo*, significantly higher serum levels of TGF-β1 have been identified in patients with gastric cancer [10, 26], hepatocellular carcinoma [25], and prostate cancer [27]; however, determining whether there is an association between *TGF-β1*rs1982073 serum levels and those specific cancers still requires further investigation.

The increased risk of OPSCC associated with *TGF-β1*rs1982073 depends on the constitutive and extensive crosstalk of TGF-β1 pathways with other signaling pathways (MAPK, PI3K/Akt, Wnt, etc.)[28]. The crosstalk between the TGF-β1 and HER2/Ras/MAPK pathways often leads to auto-induction of TGF-β1 itself and other growth factors, which in turn promotes epithelial-mesenchymal transition (EMT) and cell invasion [28–35]. Likewise, apoptosis and/or cell-cycle arrest were dysregulated by the crosstalk between the TGF-β1 and PI3K/Akt/mTOR pathways, which can enhance cell proliferation and induce carcinogenesis [30]. Moreover, TGF-β1/Smads and Wnt/β-catenin are key morphogen pathways that coordinate to influence cell division and cell differentiation so that stem cells can ultimately transform into differentiated cells; however, disrupted coordination in this process can lead to tissue-specific cancers [28]. E6/E7 can consistently activate MAPK, PI3K/Akt, Wnt, and many other pathways [36, 37], and in HPV16 L1 seropositive OPSCC, these activated pathways have pleiotropic crosstalk with TGF-β1 non-canonical pathways in which activation can promote EMT and tumorigenesis. Moreover, *TGF-β1*rs1982073 genotypes with higher TGF-β1 production can further aggravate that disrupted coordination and disequilibrium.

OPSCC risk increases with the loss of cellular control for apoptosis and homeostasis, and *TGF-β1*rs1982073 risk genotypes can aggravate that control loss and thereby induce tumorigenesis. p53-mediated cellular apoptosis due to DNA damage can be disrupted in HPV16 L1 seropositive cell lines, where the HPV E6 oncoprotein can bind wild-type p53 to stimulate p53 degradation while E7 can inhibit apoptosis and enhance proliferation via Rb pathway inhibition[38]. Furthermore, E6/E7 can constitutively activate PI3K signaling; and PI3K activation can inhibit p53 apoptosis via Hdm2 and notch1 to sustain cellular transformation, which was induced by HPV16 E6/E7 in Hacat-Neo cells [30]. Therefore, the cellular control of apoptosis and homeostasis is lost in HPV-infected cells in which the effector arm for proliferation (TGF-β1/non-canonical pathways [i.e., PI3K]) was enhanced and the suppressor arm (TGF-β1 canonical pathway) was not strengthened [39]. Since unbalanced arms and loss of apoptosis have constructed the disequilibrium context, and this

disequilibrium cannot be compensated for by a higher serum level of TGF-β1 (caused by *TGF-β1*rs1982073 risk genotypes), that increased level can aggravate the disequilibrium and facilitate tumorigenesis.

Decreased immunity associated with HPV infection increases the risk of OPSCC [36]. For example, HIV/AIDS patients are at higher risk for all HPV-related cancers, including oropharyngeal cancers [40,41]. In a large population-based study, Chaturvedi et al found a standardized incidence ratio of 1.6 (95% CI, 1.2–2.1) for oropharyngeal cancer among individuals with AIDS compared with the general population during the period of 1980–2004 [41].

Thus *TGF-β1*rs1982073 can further devastate the immune microenvironment and facilitate cancer immunoediting and promote tumor evasion. During virus-host interactions, HPV16 infected epithelial cells and depended on epithelial differentiation to complete the virus life cycle. High-risk HPV E6 and E7 can drive epithelial cells into the S-phase and thereby created an environment that is conducive for viral genome replication and cell proliferation [36]. Furthermore, High-risk HPV E7 can blunt or inhibit interferon regulatory factor 1(IRF-1) in a concentration-dependent manner and thereby decrease production of interferon-γ (INF-γ), which is known for antivirus and antitumor immunity [37]. In contrast, TGF-β1, at high concentrations in the tumor microenvironment, can promote either Th17 or Treg cell lineage differentiation to generate more growth factors, including TGF-β1 itself, to promote tumor invasion[42]. Moreover, growth factor generation can be promoted by *TGF-β1*rs1982073, and thereby *TGF-β1*rs1982073 can dampen the microenvironment and promote tumor-infiltrating lymphocytes [43] that result in chronic inflammatory conditions [44], which enhance the risk of malignancy [45] and disrupt the immune system's equilibrium to facilitate cancer immunoediting [46].

Genetic alterations caused by *TGF-β1*rs1982073 polymorphism and HPV16 may jointly facilitated OPSCC tumorigenesis [37]. Our results show that HPV16 infection plays a major and independent role in OPSCC susceptibility, whereas OCSCC etiology is mainly associated with tobacco exposure and alcohol use [47–49]. In our current study, we found that the significant joint effects of *TGF-β1*rs1982073 and HPV16 L1 seropositivity significantly increased the risk of cancer, particularly OPSCC, in patients who were never-smokers or never-drinkers or young (age <50). Since the incidence of HPV-seropositive OPSCC has grown in recent decades among subjects without high risk due to advanced age or exposure to tobacco and alcohol, this increased risk may be caused by prevalent oral HPV16 infection in young adults with hereditary susceptibility to cancer development. However, this question still requires further study.

Therefore, in the context of HPV16 infection, E6/E7 dose-dependent carcinogenic effects, and the oropharyngeal infection prevalence, TGF-β1 loss of pathway regulations causes systemic disequilibrium of cell differentiation [36, 45, 46]. This disequilibrium would dysregulate hundreds of genes [39] and increase the risk of OPSCC. However, our results have 3 main limitations: 1) the exact molecular mechanisms for *TGF-β1*rs1982073 binding at the miRNA site and its effects on the protein structure of TGF-β1, canonical and noncanonical pathways, the immune system, and tumorigenesis have not been evaluated; 2) crosstalk and

effects between TGF-β1 and many other pathways require bioinformatics analysis with big data; and 3) in this case-control study, a possible selection bias was generated due to the design limit, with only non-Hispanic whites included in the study so that our results cannot be extrapolated to other ethnic groups.

Taken together, our results suggest that the *TGF-β1*rs1982073 polymorphism at the miRNA-187 binding site increases OSCC susceptibility, and this polymorphism (genotype CT/CC) combined with HPV16 L1 seropositivity can jointly increase the risk of OSCC, particularly in OPSCC subjects who are never-smokers, never-drinkers, and young. Therefore, this result can help us understand the tumorigenesis process and improve early detection which are critical for prevention and intervention strategies. However, to confirm our findings and elucidate the underlying mechanisms, additional larger population or functional studies are warranted for further validation.

Acknowledgments:

The authors gratefully thank Ms. Yingdong Li for laboratory support.

Grant Support: NIEHS R01 ES-11740 (to Q.W.), N.I.H. CA 135679 (to G.L.), CA133099 (to G.L.), and CA186261–01A1 (to G.L.)

Abbreviations:

TGF-β1/*TGFB1*	transforming growth factor-β1
OPSCC	oropharyngeal squamous cell carcinoma
HPV	human papillomavirus
OR	odds ratio
CI	confidence intervals
HR	hazard ratio
OSCC	oral squamous cell carcinoma
OCSCC	oral cavity squamous cell carcinoma
SNP	single nucleotide polymorphism

References

1. Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. J Clin Oncol 2008;26: 612–9. [PubMed: 18235120]
2. Ward MJ, Mellows T, Harris S, Webb A, Patel NN, Cox HJ, Piper K, Ottensmeier CH, Thomas GJ, King EV. Staging and treatment of oropharyngeal cancer in the human papillomavirus era. Head Neck 2015;37:1002–13. [PubMed: 24753272]
3. Blot WJ, McLaughlin JK, Winn DM, Austin DF, Greenberg RS, Preston-Martin S, Bernstein L, Schoenberg JB, Stemhagen A, Fraumeni JF. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer research 1988;48: 3282–7. [PubMed: 3365707]

4. Carvalho AL, Nishimoto IN, Califano JA, Kowalski LP. Trends in incidence and prognosis for head and neck cancer in the United States: a site-specific analysis of the SEER database. Int J Cancer 2005;114: 806–16. [PubMed: 15609302]

5. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66: 7–30. [PubMed: 26742998]

6. Boscolo-Rizzo P, Del Mistro A, Bussu F, Lupato V, Baboci L, Almadori G, Da Mosto M, Paludetti G. New insights into human papillomavirus-associated head and neck squamous cell carcinoma. Acta Otorhinolaryngologica Italica 2013;33: 77. [PubMed: 23853396]

7. Pytynia KB, Dahlstrom KR, Sturgis EM. Epidemiology of HPV-associated oropharyngeal cancer. Oral oncology 2014;50: 380–6. [PubMed: 24461628]

8. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. Nature 2004;432: 332. [PubMed: 15549095]

9. Dunning AM, Ellis PD, McBride S, Kirschenlohr HL, Healey CS, Kemp PR, Luben RN, Chang-Claude J, Mannermaa A, Kataja V. A transforming growth factorβ1 signal peptide variant increases secretion in vitro and is associated with increased incidence of invasive breast cancer. Cancer research 2003;63: 2610–5. [PubMed: 12750287]

10. Li X, Yue ZC, Zhang YY, Bai J, Meng Xn, Geng JS, Fu SB. Elevated serum level and gene polymorphisms of TGF-β1 in gastric cancer. Journal of clinical laboratory analysis 2008;22: 164–71. [PubMed: 18484655]

11. Scollen S, Luccarini C, Baynes C, Driver K, Humphreys MK, Garcia-Closas M, Figueroa J, Lissowska J, Pharoah PD, Easton DF, Hesketh R, Metcalfe JC, et al. TGF-beta signaling pathway and breast cancer susceptibility. Cancer Epidemiol Biomarkers Prev 2011;20: 1112–9. [PubMed: 21527583]

12. Nicoloso MS, Sun H, Spizzo R, Kim H, Wickramasinghe P, Shimizu M, Wojcik SE, Ferdin J, Kunej T, Xiao L, Manoukian S, Secreto G, et al. Single-nucleotide polymorphisms inside microRNA target sites influence tumor susceptibility. Cancer Res 2010;70: 2789–98. [PubMed: 20332227]

13. Reeves GK, Travis RC, Green J, Bull D, Tipper S, Baker K, Beral V, Peto R, Bell J, Zelenika D. Incidence of breast cancer and its subtypes in relation to individual and multiple low-penetrance genetic susceptibility loci. Jama 2010;304: 426–34. [PubMed: 20664043]

14. Teixeira A, Ribeiro R, Morais A, Lobo F, Fraga A, Pina F, Medeiros R. Combined analysis of EGF + 61G> A and TGFB1+ 869T> C functional polymorphisms in the time to androgen independence and prostate cancer susceptibility. The pharmacogenomics journal 2009;9: 341. [PubMed: 19488063]

15. Guan X, Sturgis EM, Lei D, Liu Z, Dahlstrom KR, Wei Q, Li G. Association of TGF-β1 genetic variants with HPV16-positive oropharyngeal cancer. Clinical Cancer Research 2010;16: 1416–22. [PubMed: 20179236]

16. Jin L, Sturgis EM, Zhang Y, Huang Z, Song X, Li C, Wei Q, Li G. Association of tumor necrosis factor-alpha promoter variants with risk of HPV-associated oral squamous cell carcinoma. Mol Cancer 2013;12: 80. [PubMed: 23870134]

17. Ji X, Sturgis EM, Zhao C, Etzel CJ, Wei Q, Li G. Association of p73 G4C14-to-A4T14 polymorphism with human papillomavirus type 16 status in squamous cell carcinoma of the head and neck in non-Hispanic whites. Cancer 2009;115: 1660–8. [PubMed: 19197996]

18. Yuan X, Liao Z, Liu Z, Wang L-E, Tucker SL, Mao L, Wang XS, Martel M, Komaki R, Cox JD. Single Nucleotide Polymorphism at rs1982073: T869C of the TGF β 1 Gene Is Associated With the Risk of Radiation Pneumonitis in Patients With Non–Small-Cell Lung Cancer Treated With Definitive Radiotherapy. Journal of clinical oncology 2009;27: 3370–8. [PubMed: 19380441]

19. Kreimer AR, Clifford GM, Snijders PJ, Castellsagué X, Meijer CJ, Pawlita M, Viscidi R, Herrero R, Franceschi S; International Agency for Research on Cancer (IARC) Multicenter Oral Cancer Study Group. HPV16 semiquantitative viral load and serologic biomarkers in oral and oropharyngeal squamous cell carcinomas. Int J Cancer 2005;115: 329–32. [PubMed: 15688391]

20. Holzinger D, Wichmann G, Baboci L, Michel A, Höfler D, Wiesenfarth M, Schroeder L, Boscolo-Rizzo P, Herold-Mende C, Dyckhoff G, Boehm A, Del Mistro A, Bosch FX, Dietz A, Pawlita M, Waterboer T. Sensitivity and specificity of antibodies against HPV16 E6 and other early proteins

for the detection of HPV16-driven oropharyngeal squamous cell carcinoma. Int J Cancer 2017;140: 2748–57. [PubMed: 28316084]

21. Anderson KS, Dahlstrom KR, Cheng JN, Alam R, Li G, Wei Q, Gross ND, Chowell D, Posner M, Sturgis EM. HPV16 antibodies as risk factors for oropharyngeal cancer and their association with tumor HPV and smoking status. Oral Oncol 2015;51: 662–7. [PubMed: 25957822]
22. Mulrane L, Madden SF, Brennan DJ, Gremel G, McGee SF, McNally S, Martin F, Crown JP, Jirstrom K, Higgins DG, Gallagher WM, O'Connor DP. miR-187 is an independent prognostic factor in breast cancer and confers increased invasive potential in vitro. Clin Cancer Res 2012;18: 6702–13. [PubMed: 23060431]
23. Blitzblau RC, Weidhaas JB. MicroRNA binding-site polymorphisms as potential biomarkers of cancer risk. Molecular diagnosis & therapy 2010;14: 335–42. [PubMed: 21275451]
24. Casanova-Salas I, Rubio-Briones J, Calatrava A, Mancarella C, Masia E, Casanova J, Fernandez-Serra A, Rubio L, Ramirez-Backhaus M, Arminan A, Dominguez-Escrig J, Martinez F, et al. Identification of miR-187 and miR-182 as biomarkers of early diagnosis and prognosis in patients with prostate cancer treated with radical prostatectomy. J Urol 2014;192: 252–9. [PubMed: 24518785]
25. Gu X, Ji X, Shi L-H, Yi C-H, Zhao Y-P, Wang A-H, Lu L-G, Yu W-B, Gao C-F. Transforming growth factor beta1 gene variation Leu10Pro affects secretion and function in hepatic cells. Digestive diseases and sciences 2012;57: 2901–9. [PubMed: 22615019]
26. Shim KS, Kim KH, Han WS, Park EB. Elevated serum levels of transforming growth factor-β1 in patients with colorectal carcinoma. Cancer 1999;85: 554–61. [PubMed: 10091729]
27. Shariat SF, Shalev M, Menesses-Diaz A, Kim IY, Kattan MW, Wheeler TM, Slawin KM. Preoperative plasma levels of transforming growth factor beta1 (TGF-β1) strongly predict progression in patients undergoing radical prostatectomy. Journal of Clinical Oncology 2001;19: 2856–64. [PubMed: 11387358]
28. Guo X, Wang XF. Signaling cross-talk between TGF-beta/BMP and other pathways. Cell Res 2009;19: 71–88. [PubMed: 19002158]
29. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-β family signalling. Nature 2003;425: 577–84. [PubMed: 14534577]
30. Zhang L, Wu J, Ling MT, Zhao L, Zhao KN. The role of the PI3K/Akt/mTOR signalling pathway in human cancers induced by infection with human papillomaviruses. Mol Cancer 2015;14: 87. [PubMed: 26022660]
31. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med 2004;10: 789–99. [PubMed: 15286780]
32. Schmierer B, Hill CS. TGFbeta-SMAD signal transduction: molecular specificity and functional flexibility. Nat Rev Mol Cell Biol 2007;8: 970–82. [PubMed: 18000526]
33. Nair P, Somasundaram K, Krishna S. Activated Notch1 Inhibits p53-Induced Apoptosis and Sustains Transformation by Human Papillomavirus Type 16 E6 and E7 Oncogenes through a PI3K-PKB/Akt-Dependent Pathway. Journal of Virology 2003;77: 7106–12. [PubMed: 12768030]
34. Massague J TGFbeta signalling in context. Nat Rev Mol Cell Biol 2012;13: 616–30. [PubMed: 22992590]
35. Zhang YE. Non-Smad pathways in TGF-beta signaling. Cell Res 2009;19: 128–39. [PubMed: 19114990]
36. Doorbar J The papillomavirus life cycle. Journal of clinical virology 2005;32: 7–15. [PubMed: 15572000]
37. Rampias T, Sasaki C, Psyrri A. Molecular mechanisms of HPV induced carcinogenesis in head and neck. Oral oncology 2014;50: 356–63. [PubMed: 23953776]
38. Wang X, Wang H-K, McCoy JP, Banerjee NS, Rader JS, Broker TR, Meyers C, Chow LT, Zheng Z-M. Oncogenic HPV infection interrupts the expression of tumor-suppressive miR-34a through viral oncoprotein E6. Rna 2009;15: 637–47. [PubMed: 19258450]
39. Massague J TGFbeta in Cancer. Cell 2008;134: 215–30. [PubMed: 18662538]
40. Shiels MS, Pfeiffer RM, Gail MH, et al. Cancer burden in the HIV-infected population in the United States. J Natl Cancer Inst 2011;103:753–62. [PubMed: 21483021]

41. Chaturvedi AK, Madeleine MM, Biggar RJ, Engels EA. Risk of Human Papillomavirus–Associated Cancers Among Persons With AIDS. JNCI: Journal of the National Cancer Institute 2009;101: 1120–30 [PubMed: 19648510]

42. Lu P, Cao Y, Wang M, Zheng P, Hou J, Zhu C, Hu J. Mature dendritic cells cause Th17/Treg imbalance by secreting TGF-β1 and IL-6 in the pathogenesis of experimental autoimmune encephalomyelitis. Cent Eur J Immunol 2016;41:143–52. [PubMed: 27536199]

43. Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, Shen Y, Du J, Rubtsov YP, Rudensky AY. TGF-β-induced Foxp3 inhibits TH17 cell differentiation by antagonizing RORγt function. Nature 2008;453: 236. [PubMed: 18368049]

44. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420: 860. [PubMed: 12490959]

45. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-β. Annual review of immunology 1998;16: 137–61.

46. Quezada SA, Peggs KS, Simpson TR, Allison JP. Shifting the equilibrium in cancer immunoediting: from tumor tolerance to eradication. Immunological reviews 2011;241: 104–18. [PubMed: 21488893]

47. Lubin JH, Purdue M, Kelsey K, Zhang Z-F, Winn D, Wei Q, Talamini R, Szeszenia-Dabrowska N, Sturgis EM, Smith E. Total exposure and exposure rate effects for alcohol and smoking and risk of head and neck cancer: a pooled analysis of case-control studies. American journal of epidemiology 2009;170: 937–47. [PubMed: 19745021]

48. Lingen MW, Xiao W, Schmitt A. Low etiologic fraction for high-risk human papillomavirus in oral cavity squamous cell carcinomas. Oral Oncol 2013;49:1–8. [PubMed: 22841678]

49. Zafereo ME, Xu L, Dahlstrom KR, Viamonte CA, El-Naggar AK, Wei Q, Li G, Sturgis EM. Squamous cell carcinoma of the oral cavity often overexpresses p16 but is rarely driven by human papillomavirus. Oral Oncol 2016;56:47–53. [PubMed: 27086486]

Novelty and Impact statements:

*TGF-β1*rs1982073 polymorphism at the miRNA-187 binding site combined with HPV16 L1 seropositivity may have a joint effect on oral cancer susceptibility, particularly in never smokers and never drinkers, young subjects, and patients with oropharyngeal cancer, suggesting that this genetic variant may be a susceptible biomarker for risk of HPV16-associated oral cancer.

Table 1.

Demographics and risk factors of OSCC patients and controls

Variables	Patients (n =325)			Controls (n = 335) n (%)	*P* value[1]
	OPSCC (n =188) n (%)	OCSCC (n=137) n (%)	All (OSCC) (n =325) n (%)		
Age (year)					0.183
< 50	54 (28.7)	33 (24.1)	87 (26.8)	87 (26.0)	
≥ 50	134 (71.3)	104 (75.9)	238 (73.2)	248 (74.0)	
Sex					0.100
Male	155 (82.5)	86 (62.8)	241 (74.2)	269 (80.3)	
Female	33 (17.5)	51 (37.2)	84 (25.8)	66 (19.7)	
Smoking					0.673
Ever	125 (66.6)	102 (74.5)	227 (69.8)	239 (71.3)	
Never	63 (33.5)	35 (25.5)	98 (30.2)	96 (28.7)	
Alcohol drinking					0.121
Ever	150 (79.8)	100 (73.0)	250 (76.9)	240 (71.6)	
Never	38 (20.2)	37 (27.0)	75 (23.1)	95 (28.4)	
HPV16 serology					< 0.001
Positive	87 (46.3)	13 (9.5)	100 (30.8)	42 (12.5)	
Negative	101(53.7)	124 (90.5)	225 (69.2)	293 (87.5)	

[1] *P* values of two-sided $\chi 2$ test between OSCC patients and controls.

Table 2.

Joint effect of *TGF-β1*rs1982073 polymorphism and HPV16 L1 serology on risk of OSCC

Variables	HPV16 serology	Patients (n= 325)			Controls(335)	Adjusted OR (95% CI)[1]		
		OPSCC (n =188) n (%)	OCSCC (n=137) n (%)	All (OSCC) n =325) n (%)	*n* (%)	All (OSCC)	OPSCC	OCSCC
Overall								
TT		61(32.4)	30 (21.9)	115 (35.4)	238 (71.0)	1.0	1.0	1.0
CT/CC		127 (67.6)	107 (78.1)	210 (64.6)	97 (29.0)	4.6 (3.3–6.4)	4.9 (3.3–7.3)	4.4 (2.8–6.8)
By HPV16 L1 serology								
TT	-	31 (16.5)	26 (19.0)	80 (24.6)	210 (62.7)	1.0	1.0	1.0
CT/CC	-	70 (37.2)	98 (71.5)	145 (44.6)	83 (24.8)	3.4 (1.9–6.0)	5.6 (3.4–9.2)	4.4 (2.8–7.0)
TT	+	30 (16.0)	4 (3.0)	35 (10.8)	28 (8.3)	4.7 (3.2–6.9)	7.2 (3.8–13.6)	0.7 (0.3–2.1)
CT/CC	+	57 (30.3)	9 (6.5)	65 (20.0)	14 (4.2)	13.6 (7.1–26.2)	26.4 (12.9–53.9)	3.1 (1.2–8.1)

[1] Adjusted for age, sex, and smoking and drinking status.

Table 3.

Joint effect of *TGF-β1*rs1982073 polymorphism and HPV16 L1 serology on risk of OSCC, stratified by smoking and drinking status

Risk groups	HPV16 serology	Patients			Controls n (%)	Adjusted OR (95% CI)[1]		
		OPSCC n (%)	OCSCC n (%)	All (OSCC) n (%)		All (OSCC)	OPSCC	OCSCC
Never-smoker								
TT	-	6 (9.5)	14 (40.0)	20 (20.4)	58 (60.4)	1.0	1.0	1.0
CT/CC	-	21(33.3)	18 (51.4)	39 (39.8)	30 (31.2)	4.5 (2.1–9.7)	7.8 (2.6–23.0)	3.4 (1.2–9.2)
TT	+	10 (15.9)	1 (2.9)	11 (11.2)	6 (6.3)	8.5 (2.6–28.0)	24.7 (5.9–103.0)	0.7 (0.1–7.5)
CT/CC	+	26 (41.3)	2 (5.7)	28 (28.6)	2 (2.1)	60.1 (12.3–293.3)	165.9 (28.6–960.4)	5.0 (0.4–65.6)
Ever-smoker								
TT	-	25 (20.0)	35 (34.3)	60 (26.4)	152 (63.6)	1.0	1.0	1.0
CT/CC	-	49 (39.2)	57 (55.9)	106 (46.7)	53 (22.2)	5.2 (3.3–8.2)	5.6 (3.1–10.1)	5.0 (2.8–8.6)
TT	+	20 (16.0)	4 (3.9)	24 (10.6)	22 (9.2)	2.9 (1.5–5.5)	5.6 (2.7–11.9)	0.9 (0.3–2.8)
CT/CC	+	31 (24.8)	6 (5.9)	37 (16.3)	12 (5.0)	8.8 (4.2–18.5)	15.2 (6.8–34.2)	2.6 (0.9–7.8)
Never-drinker								
TT	-	4 (10.5)	14 (37.8)	18 (24.0)	60 (63.2)	1.0	1.0	1.0
CT/CC	-	14 36.8)	18 (48.6)	32 (42.7)	26 (27.4)	5.8 (2.5–13.7)	10.5 (2.9–38.4)	4.8 (1.6–14.1)
TT	+	5 (13.2)	3 (8.1)	8 (10.7)	7 (7.4)	4.9 (1.4–16.7)	12.5 (2.5–61.9)	2.2 (0.4–11.8)
CT/CC	+	15 39.5)	2 (5.5)	17 (22.6)	2 (2.1)	59.9 (10.8–332.9)	196.0 (28.2–1000.0)	6.5 (0.4–112.6)
Ever-drinker								
TT	-	27 (18.0)	35 (35.0)	62 (24.8)	150 (62.5)	1.0	1.0	1.0
CT/CC	-	56 (37.3)	57 (57.0)	113 (45.2)	57 (23.7)	4.8 (3.1–7.4)	5.2 (3.0–9.1)	4.7 (2.7–8.1)
TT	+	25 (16.7)	2 (2.0)	27 (10.8)	21 (8.8)	3.2 (1.7–6.1)	6.6 (3.2–13.5)	0.4 (0.1–1.9)
CT/CC	+	42 (28.0)	6 (6.0)	48 (19.2)	12 (5.0)	10.1 (4.9–20.9)	17.2 (7.9–37.6)	2.3 (0.8–6.8)

[1]Adjusted for age, sex, and smoking and drinking status.

Table 4.

Joint effect of HPV16 L1 serology and the combined risk genotypes of *TGF-β1*rs1982073 on risk of OSCC, stratified by age

Risk groups	HPV16 status	Patients (n = 325)			Controls (n = 335)		Adjusted OR (95% CI)[1]		
		OPSCC	OCSCC	All (OSCC)					
		n (%)	n (%)	n (%)	n (%)		All (OSCC)	OPSCC	OCSCC
Young[*]									
TT[2]	−	9 (16.7)	10 (30.3)	19 (21.8)	43	(49.4)	1.0	1.0	1.0
CT/CC	−	16 (29.6)	20 (60.6)	36 (41.4)	34	(39.1)	2.5 (1.1−5.4)	2.6 (0.9–6.9)	3.2 (1.2−8.8)
TT	+	7 (13.0)	1 (3.0)	8 (9.2)	5	(5.7)	5.2 (1.4−19.9)	9.0 (2.1–38.3)	1.5 (0.1−17.0)
CT/CC	+	22 (40.7)	2 (6.1)	24 (27.6)	5	(5.8)	12.9 (4.0−41.5)	23.5 (6.3–87.0)	4.1 (0.6−30.7)
Older[†]									
TT[2]	−	22 (16.4)	39 (37.5)	61 (25.6)	167	(67.3)	1.0	1.0	1.0
CT/CC	−	54 (40.3)	55 (52.9)	109 (45.8)	49	(19.8)	6.1 (3.9−9.6)	8.0 (4.4–14.6)	5.1 (2.9−8.7)
TT	+	23 (17.2)	4 (3.8)	27 (11.3)	23	(9.3)	3.3 (1.7−6.2)	7.7 (3.6–16.2)	0.7 (0.2−2.3)
CT/CC	+	35 (26.1)	6 (5.8)	41 (17.2)	9	(3.6)	3.2 (1.7−7.1)	6.0 (1.7–17.9)	3.3 (1.0−10.2)

[1] Adjusted for age, sex, and smoking and drinking status.

[*] Age < 50 years

[†] Age ≥ 50 years.

[2] Reference group.

Table 5.

Associations between *TGF-β1*rs1982073 polymorphism and HPV16-associated OSCC

Genetic variants	HPV16 L1 serology	Patients (n =100)			Controls(n = 42)	Adjusted OR (95% CI)[1]		
		All (OSCC) n (%)	OPSCC n (%)	OCSCC n (%)	*n* (%)	All (OSCC)	OPSCC	OCSCC
TT	+	35 (35.0)	30 (34.5)	8 (61.5)	28 (66.7)	1.0	1.0	1.0
CT/CC	+	65 (65.0)	57 (65.5)	5 (38.5)	14 (33.3)	3.2 (1.4–7.4)	3.3 (1.4–7.8)	1.3 (0.8–18.8)
		Tumor HPV16(+) patients (n = 156)			Tumor HPV16(−) patients (n = 54)			
		All (OSCC) n (%)	OPSCC n (%)	OCSCC n (%)	n (%)	All (OSCC)	OPSCC	OCSCC
TT		53 (34.0)	46 (33.0)	7 (44.0)	26 (48.4)	1.0	1.0	1.0
CT/CC		103 (66.0)	94 (67.0)	9 (56.0)	28 (51.6)	1.9 (1.1–3.4)	2.0 (1.3–6.1)	1.1 (0.2–2.3)

[1]Adjusted for age, sex, and smoking and drinking status.