Published in final edited form as:
Cancer. 2012 March 15; 118(6): 1684–1692. doi:10.1002/cncr.26423.

Modifying effect of *MDM4* variants on risk of HPV16-associated squamous cell carcinoma of oropharynx

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Abstract

BACKGROUND—The p53 pathway plays a critical role in maintaining genomic stability and preventing tumor formation. Given the roles of both MDM4 and HPV16 E6 oncoproteins in inhibition of p53 activity, we tested the hypothesis that *MDM4* polymorphisms are associated with the risk of HPV16-associated squamous cell carcinoma of head and neck (SCCHN).

METHODS—Genotyping was conducted on three tagging single nucleotide polymorphisms (rs11801299 G>A, rs10900598 G>T, and rs1380576 C>G) in *MDM4*, and serology was used to determine HPV 16 exposure in 380 cases and 335 cancer-free controls that were frequency-matched by age, sex, smoking, and drinking status.

RESULTS—None of three *MDM4* polymorphisms alone was significantly associated with risk of overall SCCHN. With further analysis stratified by HPV16 serology and tumor site, we found that each polymorphism individually modified the risk of HPV16-associated squamous cell carcinoma of the oropharynx (SCCOP), and such effect modification was particularly pronounced in never smokers and never drinkers.

CONCLUSION—The risk of HPV16-associated SCCOP could be modified by *MDM4* polymorphisms. Large and prospective studies are needed to validate our findings.

Keywords

MDM4 polymorphisms; genetic susceptibility; human papillomavirus; molecular epidemiology; squamous cell carcinoma of head and neck cancer; squamous cell carcinoma of the oropharynx

INTRODUCTION

Squamous cell carcinomas of the head and neck (SCCHN), which includes those of the oral cavity, pharynx, and larynx, is one of the most common malignancies worldwide with approximately 650,000 new cases reported annually [1]. It is estimated that approximately 49,260 new SCCHN cases will be diagnosed and that 11,480 deaths will occur from these patients in 2010 in the United States [2]. Both tobacco use and alcohol consumption are well-established etiologic factors for SCCHN, and at least 75% of all SCCHN are attributed to these exposures [3]. Although the overall smoking rate is declining in the United States in

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CONFLICT OF INTEREST STATEMENT: None declared.

recent years, the incidence of a specific subsite of SCCHN, the oropharyngeal cancer, is increasing, and this increase in the incidence appeared to be paralleled by an increase in human papillomavirus (HPV) associated squamous cell carcinoma of the oropharynx (SCCOP) [4–8]. HPV is another etiologic agent in addition to tobacco and alcohol for SCCHN; However, it would appear that only a small fraction of exposed individuals eventually develop SCCOP, indicating that inter-individual variationin genetic susceptibility may contribute to the variation in individual SCCOP risk.

The p53 tumor suppressor has been described as a major "guardian of the genome" [8]. It plays a key role in eliciting cellular responses to a wide variety of stress signals, including DNA damage, hypoxia, and oncogene activation. Following cellular stresses, the p53 protein is stabilized and activated to induce the transcription of genes involved in DNA repair, cell-cycle arrest, senescence, and apoptosis [9, 10]. Indeed, *p53* is mutated or deleted in nearly half of human cancers including SCCHN, demonstrating the crucial role of p53 in tumor suppression [11, 12]. Although defective p53 leads to increased cancer susceptibility, hyperactivation of p53 is also lethal. Therefore, the p53 activity must be stringently regulated to maintain normal tissue homeostasis [13].

As an MDM2-related protein, MDM4 (also known as MDMX) has emerged as a key negative regulator of p53. It has been demonstrated that MDM4 directly binds to the p53 transactivation domain, inhibits its transcriptional activity, and thus contributes to tumor formation. Studies in knock-out mice showed that mice lacking *MDM4* exhibited a p53 dependent embryonic lethality with defects in proliferation without apoptosis, which were completely rescued by the concomitant deletion of *p53*, suggesting that the major function of MDM4 during early development is to regulate p53 [14]. *MDM4* maps to the chromosomal region 1q32, which is frequently amplified in cancer tissues [15]. The amplification or overexpression of the human *MDM4* gene has been observed in both numerous tumor cell lines that retain the wild-type p53 and a large subset of human tumors including SCCHN [16–18]. It was also reported that over-expression of MDM4 was associated with not only tumor progression but also poor prognosis [19–22].

Among the known HPV types, the high-risk HPV16 is the most common type, accounting for approximately 90% or more of the HPV-positive SCCOP [23–25]. The primary oncogenic effect of high-risk HPVs has been attributed to the E6 and E7 oncogenic proteins [26]. This is because HPV E6 oncoprotein binds to p53, resulting in p53 degradation through an ubiquitin-dependent pathway [27–29]. Taken together, these data indicated that both HPV E6 oncoprotein and MDM4 may play a critical role in HPV-associated SCCOP carcinogenesis.

Recently, Terizian et al. showed that haplo-insufficiency at the *MDM4* loci leaded to an increase in the p53 activity, exhibiting an increased sensitivity to DNA damage, a decreased transformation potential, and a reduced tumorigenesis, implying that genetic variants, which alter or influence MDM4 expression, may increase susceptibility to cancer [30]. It has also been reported that *MDM4* genetic variants are associated with increased risk in breast and ovarian cancers [31, 32]. However, no reported studies have investigated whether the common variants of *MDM4* play a role in the development of SCCOP associated with HPV16 seropositivity. In the present study, we hypothesize that common variants of *MDM4* are associated with risk of HPV-associated SCCOP. To test this hypothesis, we conducted an association study with the tagging polymorphisms of *MDM4* and evaluated their modification effects on risk of HPV-associated SCCOP.

MATERIALS AND METHODS

Patient and Control Samples

All patients with histopathologically confirmed SCCHN were consecutively recruited through the Head and Neck Surgery Clinic at The University of Texas MD Anderson Cancer Center between May 1996 and May 2002. Of patients initially contacted for participation, approximately 95% of eligible incident cases agreed to participate in the study. Excluded from participation were patients with second primary tumors; primary tumors of the sinonasal tract, and nasopharynx; primary tumors outside the upper aerodigestive tract; cervical metastases of unknown origin; and histopathologic diagnoses of tumors other than squamous cell carcinoma. In addition, patients with known immune suppression or who had received recent blood transfusions (in the last 6 months) or who were receiving immunosuppressive therapy were also excluded. Of the 432 patients included, serologic assessment for HPV16 was performed on 380 patients.

A pool of cancer-free subjects was recruited from the Kelsey-Seybold Foundation, a multispecialty physician practice with multiple clinics throughout the Houston metropolitan area, and from healthy visitors who accompanied cancer patients to outpatient clinics at MD Anderson Cancer Center but were genetically unrelated to the SCCHN patients. In this pool of cancer-free controls, each individual was first surveyed by means of a short questionnaire to determine his or her willingness to participate in the study and then interviewed. Each eligible subject provided demographic and epidemiologic information, such as age, sex, ethnicity, smoking history, and alcohol consumption. The overall proportion of responders was approximately 78%. Exclusion criteria for the control groups included receiving immunosuppressive therapy, having had previous cancer, and having received recent (in the last 6 months) blood transfusions.

In this study, 335 cancer-free control individuals were selected from the pool of potential controls that were frequency-matched by age (±5 years), gender, ethnicity, and smoking and alcohol drinking status. These variables were further adjusted for in later multivariable logistic regression analyses to control for any confounding effect. Those subjects who had smoked more than 100 cigarettes in their lifetime were defined as ‘ever smokers’ and the rest as ‘never smokers’. Individuals who drank alcoholic beverages at least once a week for more than one year were defined as ‘ever drinkers’ and the rest as ‘never drinkers’. After an informed written consent was given, each individual provided 30 mL of blood collected in heparinized tubes. The research protocol was approved by both the MD Anderson Cancer Center and Kelsey-Seybold Institutional Review Boards.

HPV16 Serologic Testing

We used HPV16 L1 virus-like particles generated from recombinant baculovirus-infected insect cells to test for antibody against the HPV16 L1 capsid protein in the plasma of study participants by using a standard enzyme-linked immunosorbent assay, as described previously [33, 34]. Briefly, control sera known to be positive and negative were also determined 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 that were within 15% of the cutoff level were tested twice more, and samples that were positive in all 3 runs were considered positive.10% of the samples were randomly selected to perform the repeated assay.

Selection and Genotyping of Tagging SNPs

We used the public HapMap SNP database (http://www.hapmap.org/) to identify *MDM4* tagging SNPs by using tagger with a greedy algorithm [35], for which all SNPs either were directly genotyped or exceeded a threshold level of linkage disequilibrium (LD) value (r^2) with a genotyped SNP. We searched for the *MDM4* gene within an about 34-kb region on chromosome 1q32 (i.e., from 202,752,134 bp to 202,786,349 bp) among a European population (CEPH: Utah residents with ancestry from northern and western Europe). The tagging SNPs were selected on the basis of their pairwise LD with the r^2 threshold of 0.8 and minor allele frequency (MAF) ≥ 0.10. As a result, we identified three tagging SNPs (i.e., rs11801299, rs1380576, and rs10900598) in the 34-kb region, and the mean r^2 between the tagging SNPs and their covered but untyped SNPs was 0.98. Of the selected SNPs, both rs11801299 and rs10900598 are located in the 3′ untranslated region (3′ UTR) of the *MDM4* gene, while rs1380576 is located in the intron 1 of the gene.

The genotyping was performed using the Applied Biosystems TaqMan genotyping platform according to the manufacture's recommendations. Briefly, the reactions were prepared by using TaqMan Universal Master Mix, 80×SNP Genotyping Assay Mix, Dnase-free water, and 10-ng genomic DNA in a final volume of 5 μL per reaction. Both negative and positive controls and three repeated samples were included in each plate to ensure the accuracy of the genotyping. The PCR amplification was run, and the plate was read using a TaqMan 7900 HT sequence detection system (Applied Biosystems). The analyzed fluorescence results were then auto-called in to the genotypes using the built-in SDS2.3 software of the system.

Statistical Analysis

Differences between the patients and controls in the distributions of selected variables, including HPV16 serological status and *MDM4* genotypes, were examined using the χ^2 test. We estimated the association of HPV16 status and *MDM4* genotypes with cancer risk by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) using both univariate and multivariable logistic regression analyses. We also evaluated the joint effects of HPV16 serology and *MDM4* genotypes on cancer risk, and the joint effects were further stratified by smoking and drinking status. All tests were two-sided, and a $P < 0.05$ was considered the cutoff for statistical significance. All of the statistical analyses were performed with Statistical Analysis System software (Version 9.1; SAS Institute, Cary, NC).

RESULTS

Demographics and Risk Factors for Study Subjects

In this study, 380 SCCHN cases and 335 controls of non-Hispanic whites were recruited. Among the 380 SCCHN patients, 187 (49.2%) had cancers of the oropharynx, and 193 (50.8%) had cancers of non-oropharynx (i.e., oral cavity, hypopharynx, and larynx). The distribution of demographic characteristics and known SCCHN risk factors are summarized in Table 1. Because of frequency matching, there were no statistically differences in the distributions of age, sex, smoking status, and alcohol drinking status between cases and controls. However, we found that HPV16 seropositivity was significantly more common in patients than in controls ($P < 0.001$) and that HPV16 seropositivity was only associated with risk for SCCOP (adjusted OR = 5.6; 95% CI, 3.6–8.7) but not for non-oropharynx sites of SCCHN (adjusted OR = 0.8; 95% CI, 0.4–1.5).

Association of MDM4 Variants with the Risk of SCCHN

Among all the studied subjects, nine cases and fourteen controls failed to genotyping after repeated assays. Thus, the final analysis included 371 SCCHN cases and 321 controls. The distributions of *MDM4* genotypes among the controls were in agreement with the Hardy-

Weinberg equilibrium (P = 0.669 for rs10900598, P = 0.502 for rs380576, and P = 0.303 for rs11801299). When comparing genotype distribution for these three *MDM4* variants between cases and controls, no significant difference in the genotype distribution was found between the cases and controls (P = 0.619 for rs10900598, P = 0.969 for rs380576, and P = 0.996 for rs11801299, respectively). Overall, we did not find any association of the three *MDM4* polymorphisms with risk of SCCHN (Table 2).

Because no significant associations of *MDM4* polymorphisms with risk of overall SCCHN were found and because SCCHN is a heterogeneous group in which the association of HPV16 with SCCHN risk is primarily limited to the oropharyngeal cancer subsite (SCCOP) [4, 5, 25, 34, 36, 37], we further evaluated the modifying effect of *MDM4* variants on the association between HPV16 serology and the risk of SCCHN stratified by tumor site. Table 3 shows that the associations between HPV16 serology and cancer risk were modified by these *MDM4* genetic variants only for SCCOP but not for non-oropharyngeal sites of SCCHN. Specifically, compared with individuals having rs10900598 GT or TT genotypes and HPV16 seronegativity, an increased risk of SCCOP was observed among those having the GT or TT genotypes and HPV16 seropositivity (OR, 4.9; 95% CI, 2.9–8.3), and the risk was more pronounced among those having both the GG genotype and HPV16 seropositivity (OR,11.4; 95% CI,4.9–26.4). Similarly, compared with individuals with the rs1380756 CC genotype and HPV16 seronegativity, an increased risk was also observed among those having the CC genotype and HPV16 seropositivity (OR, 4.4; 95% CI,2.2–8.8), and the risk was more pronounced among those having CG or GG genotypes and HPV16 seropositivity (OR,6.1; 95% CI,3.4–11.1). Similar results were found for *MDM4* rs11801299 polymorphism. Such effect modifications might suggest an interactive effect of *MDM4* polymorphisms and HPV16 seropositivity on risk of SCCOP. However, we did not find statistical evidence for the interaction between the genotypes of these *MDM4* variants and HPV16 seropositivity in the multivariable logistic regression model (data not shown), probably because of small sample size in each stratum that lacked sufficient statistical power.

Stratified Analysis of Joint Effects of HPV16 Seropositivity and MDM4 Variants on SCCOP Risk by Smoking/Drinking Status

To investigate the effects of other factors on the risk of HPV16 associated SCCOP, we stratified the effect modification between HPV16 serology and *MDM4* variants by smoking and drinking status (Table 4 and Table 5). Overall, we found that the modification effect of each polymorphism on the risk of HPV16-associated SCCOP risk was more pronounced in never smokers than in ever smokers (Table 4) and in never drinkers than in ever drinkers (Table 5), though such apparent interactions between HPV16 seropositivity and *MDM2* variants on the risk of SCCOP in each of these subgroups (including never smokers, ever smokers, never alcohol drinkers, and ever alcohol drinkers, respectively) were not statistically significant, likely, again, due to our limited study power in each of these subgroups (data not shown).

DISCUSSION

Although we did not find any significant main effect of each *MDM4* polymorphism on risk of SCCHN, we found that these *MDM4* polymorphisms modified the association between HPV16 serology and risk of SCCOP, and such effect modification were more prominent in never smokers and never drinkers than in ever smokers and ever drinkers, respectively. Our findings are consistent with the characteristics of SCCOP known to be caused by HPV infection, suggesting that *MDM4* polymorphisms may play a role in the development of HPV16-associated SCCOP.

To our knowledge, this is the first study that has examined the joint effect of *MDM4* genetic variants and HPV infection on the risk of SCCOP. Such joint effect of *MDM4* and HPV infection on risk of SCCOP is biologically plausible, because both HPV16 E6 and MDM4 oncoproteins may act synergistically in development of SCCOP through the common pathways that cause p53 degradation. It has been demonstrated that HPV E6 inactivates p53 by targeting it for proteasomal degradation [38], whereas the p53 pathway could also be inactivated through amplification or over-expression of MDM4 by directly binding to the p53 transactivation domain and thus inhibiting the p53 activity. Therefore, it is conceivable that the elevated level of MDM4 may inhibit the p53 functions, thus leading to oncogenesis. Indeed, MDM4 was found to be amplified or over-expressed in 10–20% of over 800 detected samples of diverse tumors including sarcoma, glioma, retinoblastoma, lung, colon, stomach, breast cancers, and head and neck cancer, and, strikingly, 65% of retinoblastomas [18, 39, 40].

The HPV E2 oncoprotein has been known as a major regulator of viral DNA replication and gene expression. Recently, it has been demonstrated that E2 can actively recruit the MDM2 ubiquitin ligase to the HPV promoter, which, together with MDM2, acts synergistically to activate the transcriptional activity of HPV16 E2 [41]. It has been also found that MDM4 is a new member of the RING finger family of ubiquitin ligases and that the RING finger domain of MDM4 is indispensable for its activity *in vitro* experiments [42]. Because *MDM4* shows a high similarity to *MDM2* at the level of gene sequence and structure, a structural homolog of *MDM2*, *MDM4* shares several regions of homology with *MDM2*, including the p53 binding domain, a zinc finger motif, and a C-terminal RING finger domain [43]. Therefore, it is tempting to speculate that, like MDM2, MDM4 would also interact with HPV E2 to further increase the transcriptional activity of HPV16 E2. However, this hypothesis needs to be tested in future studies.

There is increasing evidence that the 3′-untranslated (3′-UTR) region and intron1 of gene have very important gene-regulatory functions, involving in regulation of gene expression, especially through regulation of the mRNA stability and translational efficiency or localization, thus affecting gene expression anddisease susceptibility [44–50]. Among the three studied SNPs, two of which (i.e., rs10900598 and rs11801299) are located in the 3′UTR region and another one, rs1380576, in the intron 1 of the *MDM4* gene. With current knowledge of MDM4 function, it is possible that these *MDM4* variants may affect *MDM4* gene expression and therefore contributes to susceptibility to HPV16-associated SCCOP. In addition, it is possible that these *MDM4* polymorphisms may be in LD with other loci having functional and disease-causing effects. Unfortunately, due to lack of tumor tissue specimens, we were unable to explore the functional relevance of these polymorphisms in *MDM4*, such as the genotype-phenotype correlation by determining the MDM4 mRNA or protein expression in these tumor samples. Therefore, the exact mechanism by which *MDM4* polymorphisms are involved in the development of SCCHN warrants further *in vitro* and *in vivo* studies.

In the present study, further stratified analysis by smoking and drinking status for each polymorphism showed that the joint effect of *MDM4* polymorphisms and HPV16 seropositivity on the risk of SCCOP was higher in never smokers (or never drinkers) than ever smokers (or ever drinkers), respectively. These data further support that risk genotypes of the three polymorphisms of *MDM4* may be involved in the development of SCCOP associated with HPV16 among never smokers and never drinkers in the general population. However, the modification effects of *MDM4* polymorphisms on the risk of SCCOP associated with HPV16 was not statistically significant in each subgroup. This lack of significance could be either because there was no such interaction in these subgroups or because the small sample size in each subgroup limited the statistical power to detect such a

significant interaction. Therefore, our findings should be interpreted with caution. Further studies with larger sample sizes are needed to validate these potential interactions in each subgroup. Our findings also suggest that when evaluating the modification effects of *MDM4* variants on the SCCOP risk associated with HPV seropositivity, smoking and alcohol drinking status should be taken into account.

Some of limitations of our study should be considered. First of all, our study was hospital-based case-control study with inherent limitations that could introduce bias in the selection of subjects. Secondly, stratified analyses had a limited number of individuals in each subgroup, and thus our results require confirmation. Thirdly, because our study only included non-Hispanic white subjects, it is uncertain whether these results are generalizable to other ethnic populations. Finally, since a serologic assay is not site-specific, HPV16 seropositivity may not reflect the actual tumor HPV16 status, leading to possible misclassifications. For example, some patients may have been classified as seronegative, although their tumors actually may have been HPV16 positive or vice verse. However, an early multicenter case-control study also confirmed a reasonable concordance between HPV16 seropositivity and HPV16 DNA positivity in tumor tissues [51]. In addition, a nested case-control study showed that the risk of SCCHN that contained HPV16 DNA in HPV16 seropositive subjects was significant (OR = 37.5, 95% CI: 4.0–348.8), whereas the risk of SCCHN that did not carry the viral genome was much lower (OR = 2.1; 95% CI: 1.1–3.8), indicating that the risk of SCCHN associated with HPV16 seropositivity was largely attributable to infection at the site of the tumor [52]. Therefore, with this uncertainty applied to both the cases and controls, possible false-negative HPV16 cases might result in misclassification of HPV16 status. Thus, we will closely monitor the tumor HPV status (i.e, p16 immunohistochemical staining) and interaction among HPVseropositivity, smoking, and *MDM4* polymorphisms in the development of SCCHN, particularly in oropharyngeal cancer, in our future studies when a much larger patient cohort with HPV-associated tumor becomes available. Although testing for HPV DNA in tumors is an effective method for measuring exposure, it should be noted, however, that using the serologic status allows for the inclusion of a cancer-free control group and the present case-control study design.

Summarily, we found that *MDM4* polymorphisms may modify the SCCOP risk associated with HPV16 infection, and such effect modification was particularly pronounced in never smokers and never drinkers. However, further prospectively studies with larger sample sizes are necessary to verify our findings.

Acknowledgments

We thank Ana Neumann, Margaret Lung, Kathryn Tipton, and Jessica Fiske for their assistance in recruiting the subjects and gathering the questionnaire information, Yawei Qiao, Jianzhong He, Kejing Xu and Min Zhao for laboratory assistance, and Dakai Zhu for his technical support.

Financial Disclosures: This work was partly supported by the National Institute of Health grants R01 CA131274 and R01 ES011740 (Q. Wei), ES015587 (D.G. Johnson), P50 CA097007 (S. Lippman), P30 CA016672 (The University of Texas M. D. Anderson Cancer Center), and NIH grants (CA135679; G. Li) and (CA133099; G. Li). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

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Table 1

Frequency Distribution of Demographic and Risk Factors in SCCHN Patients and Controls

Characteristics	Controls[a] (n=335)		Cases (n=380)		*P* value
	No.	%	No.	%	
Age (years)					0.526
≤ 40	27	8.1	32	8.4	
41 – 55	109	32.5	142	37.4	
56 – 70	157	46.9	159	41.8	
> 70	42	12.5	47	12.4	
Sex					0.091
Male	269	80.3	285	75.0	
Female	66	19.7	95	25.0	
Tobacco smoking					0.588
Ever	239	71.3	278	73.2	
Never	96	28.7	102	26.8	
Alcohol drinking					
Ever	240	71.6	84	22.1	0.054
Never	95	28.4	296	77.9	
HPV16 serostatus					
Positive	42	12.5	103	27.1	0.000
Negative	293	87.5	277	72.9	
Tumor site					
Oropharynx cancer	-	-	187	49.2	-
Non-Oropharynx cancer	-	-	193	50.8	-

[a]The controls were selected by frequency matching to the patients on the factors shown in this table.

Table 2

Association between *MDM4* Polymorphisms and SCCHN Risk by Tumor Sites

Genotypes	Controls [a] n =321 (%)	Overall SCCHN		Oropharynx		Non-Oropharynx	
		Cases n=371 (%)	OR (95% CI) [b]	Cases n = 186 (%)	OR (95% CI) [b]	Cases n = 185 (%)	OR (95% CI) [b]
rs10900598							
GG[c]	93 (29.0)	233 (62.8)	1.0	67 (36.0)	1.00	51 (27.6)	1.00
GT	156 (48.6)	126 (34.0)	1.0 (0.7–1.4)	83 (44.6)	0.6 (0.4–1.0)	96 (51.9)	1.3 (0.8–2.0)
TT	72 (22.4)	12 (3.2)	1.1 (0.5–2.6)	36 (19.4)	0.7 (0.4–1.2)	38 (20.5)	1.1 (0.7–2.0)
GT+TT	228 (71.0)	138 (37.2)	1.0 (0.7–1.4)	119 (64.0)	0.7 (0.4–1.0)	134 (72.4)	1.2 (0.8–1.9)
rs1380576							
CC	150 (45.8)	170 (46.3)	1.0	76 (40.9)	1.0	94 (50.8)	1.0
CG	135 (42.6)	158 (42.1)	0.9 (0.7–1.3)	89 (47.8)	1.1 (0.7–1.6)	69 (37.3)	0.8 (0.5–1.2)
GG	36 (11.6	43 (11.6)	1.0 (0.6–1.7)	21 (11.3)	1.0 (0.5–2.0)	22 (11.9)	0.9 (0.5–1.7)
CG+GG[c]	171 (54.2)	201 (54.7)	1.0 (0.7–1.3)	110 (59.1)	1.1 (0.7–1.6)	91 (49.2)	0.8 (0.6–1.2)
rs11801299							
GG	202 (62.9)	118 (31.8)	1.0	122 (65.6)	1.0	111 (60.0)	1.0
AG	109 (34.0)	179 (48.2)	0.9 (0.6–1.3)	59 (31.7)	1.0 (0.6–1.5)	67 (36.2)	1.1 (0.7–1.6)
AA	10 (3.1)	74 (20.0)	0.9 (0.6–1.4)	5 (2.7)	1.5 (0.5–4.8)	7 (3.1)	1.0 (0.4–2.9)
AG+AA[c]	119 (37.1)	253 (68.2)	0.9 (0.7–1.3)	64 (34.4)	1.0 (0.7–1.5)	74 (40.0)	1.1 (0.7–1.6)

[a] The observed genotype frequency among the control subjects was in agreement with the Hardy-Weinberg equilibrium (chi-square = 0.183, $P = 0.669$ for rs10900598, chi-square = 0.451, $P = 0.502$ for rs1380576, and chi-square = 1.06, $P = 0.303$ for rs11801299).

[b] ORs were adjusted for age, sex, smoking status, and alcohol use age, sex, smoking, drinking, and HPV16 serostatus in a logistic regression model.

[c] Assumed risk genotypes; the risk genotypes used for the calculation were *MDM4* rs10900598 GG, rs1380576 CG+GG, and rs11801299AG+AA, genotypes.

Table 3

Joint Effects of HPV16 Seropositivity and *MDM4* Genotypes on Risk of SCCHN by Tumor Sites

HPV16 status	Genotypes		Overall SCCHN		Oropharynx		Non-oropharynx	
		Controls n=321 (%)	Cases n=371 (%)	OR (95%CI) [a]	Cases n=186 (%)	OR (95%CI) [a]	Cases n=185 (%)	OR (95%CI) [a]
	rs10900598							
−	GT+TT(Ref.)	195 (60.8)	101 (27.2)	1.0	64 (34.4)	1.0	125 (67.6)	1.0
−	GG	85 (26.5)	167 (45.0)	1.1 (0.8–1.5)	36 (19.4)	1.4 (0.8–2.2)	43 (23.2)	0.7 (0.4–1.1)
+	GT+TT	33 (10.3)	37 (10.0)	3.5 (1.8–7.7)	55 (29.6)	4.9 (2.9–8.3)	9 (4.9)	0.4 (0.2–1.0)
+	GG	8 (2.5)	66 (17.8)	2.5 (1.5–4.2)	31 (16.7)	11.4 (4.9–26.4)	8 (4.3)	1.7 (0.6–5.0)
	rs1380576							
−	CC (Ref.)	133 (41.4)	135 (36.4)	1.0	48 (25.8)	1.0	87 (47.0)	1.0
−	CG+GG	147 (45.8)	133 (35.8)	0.9 (0.6–1.3)	52 (28.0)	1.0 (0.6–1.5)	81 (43.8)	0.8 (0.5–1.2)
+	CC	17 (5.3)	35 (9.4)	2.2 (1.2–4.2)	28 (15.0)	4.4 (2.2–8.8)	7 (3.8)	0.7 (0.3–1.8)
+	CG+GG	24 (7.5)	68 (18.3)	2.9 (1.7–4.9)	58 (31.2)	6.1 (3.4–11.1)	10 (5.4)	0.7 (0.3–1.5)
	rs11801299							
−	GG (Ref.)	107 (33.3)	189 (50.9)	1.0	36 (19.3)	1.0	65 (35.1)	1.0
−	AG+AA	173 (53.9)	79 (21.3)	0.9 (0.6–1.3)	64 (34.4)	1.0 (0.6–1.6)	103 (55.7)	1.0 (0.6–1.5)
+	GG	12 (3.7)	64 (17.3)	2.1 (1.3–3.3)	28 (15.1)	5.3 (3.1–9.0)	9 (4.9)	0.5 (0.2–1.1)
+	AG+AA	29 (9.0)	39 (10.5)	5.3 (2.4–11.6)	58 (31.2)	6.1 (2.9–12.9)	8 (4.3)	1.4 (0.6–3.5)

[a] ORs were adjusted for age, sex, smoking, drinking.

Table 4

Joint Effects of HPV16 Seropositivity and *MDM4* Genotypes on Risk of SCCOP Stratified by Smoking Status

HPV16 status	Genotypes	Never smokers		Ever smokers		Adjusted OR (95% CI) [a]	
		Controls (92)	Cases (63)	Controls (229)	Cases (123)	Never smokers	Ever smokers
	rs10900598						
−	GT+TT(Ref.)	61	19	134	45	1.0	1.0
−	GG	23	8	62	28	1.0 (0.4–2.7)	1.5 (0.8–2.7)
+	GT+TT	7	24	26	31	13.1 (4.6–37.3)	3.6 (1.9–6.8)
+	GG	1	12	7	19	43.0 (5.0–367.4)	7.9 (3.1–20.4)
HPV16 status	Genotypes	Never smokers		Ever smokers		Adjusted OR (95% CI) [a]	
		Controls (92)	Cases (63)	Controls (229)	Cases (123)	Never smokers	Ever smokers
	rs1380576						
−	CC (Ref.)	38	13	95	35	1.0	1.0
−	CG+GG	46	14	101	38	0.8 (0.3–2.0)	1.0 (0.6–1.8)
+	CC	5	14	12	14	9.4 (2.7–33.2)	3.2 (1.3–7.8)
+	CG+GG	3	22	21	36	25.0 (6.0–104.6)	4.4 (2.2–8.6)
HPV16 status	Genotypes	Never smokers		Ever smokers		Adjusted OR (95% CI) [a]	
		Controls (92)	Cases (63)	Controls (229)	Cases (123)	Never smokers	Ever smokers
	rs11801299						
−	GG (Ref.)	54	21	119	43	1.0	1.0
−	AG+AA	30	6	77	30	0.5 (0.2–1.5)	1.2 (0.7–2.1)
+	GG	5	22	24	36	13.6 (4.3–43.4)	4.1 (2.2–7.8)
+	AG+AA	3	14	9	14	14.6 (3.5–60.8)	4.5 (1.7–11.3)

[a] ORs were adjusted for age, sex, and alcohol drinking.

Table 5

Joint Effect of HPV16 Seropositivity and *MDM4* Genotypes on Risk of SCCOP Stratified by Drinking Status

HPV16 status	Genotypes	Never drinker		Ever drinkers		Adjusted OR (95% CI) [a]	
		Controls (93)	Cases (37)	Controls (228)	Cases (149)	Never drinkers	Ever drinkers
	rs10900598						
−	GT+TT(Ref.)	58	9	137	55	1.0	1.0
−	GG	26	8	59	28	2.1 (0.7–6.3)	1.3 (0.7–2.3)
+	GT+TT	7	14	26	41	15.6 (4.7–52.1)	3.8 (2.1–6.8)
+	GG	2	6	6	25	25.4 (4.0–162.5)	9.9 (3.8–25.9)
HPV16 status	Genotypes	Never drinker		Ever drinkers		Adjusted OR (95% CI) [a]	
		Controls (93)	Cases (37)	Controls (228)	Cases (149)	Never drinkers	Ever drinkers
	rs1380576						
−	CC (Ref.)	44	6	89	42	1.0	1.0
−	CG+GG	40	11	107	41	2.0 (0.7–6.0)	0.8 (0.5–1.3)
+	CC	5	8	12	20	13.6 (3.1–58.9)	3.0 (1.3–6.8)
+	CG+GG	4	12	20	46	26.5 (6.0–116.3)	4.5 (2.4–8.7)
HPV16 status	Genotypes	Never drinker		Ever drinkers		Adjusted OR (95% CI) [a]	
		Controls (93)	Cases (37)	Controls (228)	Cases (149)	Never drinkers	Ever drinkers
	rs11801299						
−	GG (Ref.)	47	14	126	50	1.0	1.0
−	AG+AA	37	3	70	33	0.3 (0.1–1.0)	1.3 (0.8–2.3)
+	GG	5	12	24	46	9.2 (2.7–32.1)	4.9 (2.7–8.9)
+	AG+AA	4	8	8	20	8.3 (2.0–34.6)	5.4 (2.2–13.4)

[a] ORs were adjusted for age, sex, and smoking.