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Combined *p53*-related genetic variants together with HPV infection increase oral cancer risk

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Abstract

To explore the role of polymorphisms of *p53*-related genes in etiology of oral cancer, we investigated joint effects of seven putatively functional polymorphisms of *p53* (codon 72 Arg/Pro), *p73* (4/14 GC/AT), *MDM2* (A2164G and T2580G), and *MDM4* (rs11801299 G>A, rs10900598 G>T, and rs1380576 C>G) on risk of HPV16-associated oral cancer in a case-control study with 325 cases and 335 cancer-free controls. We found that HPV16 seropositivity alone was associated with an increased risk of oral cancer [adjusted odds ratio (OR), 3.1; 95% confidence interval (CI), 2.1–4.6]. After combining genotypes of seven polymorphisms and using the low-risk group (0–3 combined risk genotypes) and HPV16 seronegativity as the reference group, the medium-risk (4 combined risk genotypes) and high-risk groups (5–7 combined risk genotypes) and HPV16 seronegativity were associated with only an OR of 1.6 (95% CI, 1.1–2.5) and 1.2 (95% CI, 0.7–1.9) for oral cancer risk, respectively, while the low-risk, medium-risk, and high-risk groups and HPV16 seropositivity were significantly associated with a higher OR of 2.1 (95% CI, 1.2–3.6), 4.0 (95% CI, 1.8–9.1), and 19.1 (95% CI, 5.7–64.2), respectively. Notably, such effect modification by these combined risk genotypes was particularly pronounced in young subjects (aged < 50 years), never smokers, and patients with oropharyngeal cancer. Taken together, these findings suggest that the combined risk genotypes of *p53*-related genes may modify risk of HPV16-associated oral cancer, especially in young patients, never-smokers, and patients with oropharyngeal cancer. Larger studies are needed to validate our findings.

Keywords

p53; *p73*; *MDM2*; *MDM4*; HPV infection; oral cancer

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Introduction

There is a well-established link between tobacco consumption and oral cancer including squamous cell carcinoma of the oropharynx and oral cavity. Recently, the proportion of oral cancer has been increasing in young adult never-smokers. Oral cancer accounts for approximately 3% (35,000 cases) of new cancer cases in the United States with estimated 7,600 deaths in 2009.[1, 2] Epidemiological evidence suggests that human papillomavirus (HPV) is a major contributor to the incidence of oral cancer in the never-smoker population [3, 4]. Whereas low-risk HPV types cause benign epithelial hyperproliferations, high-risk HPV types are oncogenic, by encoding viral oncoproteins E6 and E7 that inhibit p53 and Rb cell cycle tumor suppressors. Moreover, high-risk HPV type 16 accounts for 90–95% of HPV-associated oral cancer, compared with approximately 70% of all cervical cancers [5–7]. While a majority of individuals are exposed to high-risk HPVs at some point in their lifetime, a minority develop persistent HPV infections and a very few will develop an HPV-associated malignancy [4, 5, 8]. It is likely that inherited genetic factors contribute to a range of susceptibility to HPV-associated cancers in the general population.

The p53 protein functions as the ‘guardian of the genome’ by regulating the cell cycle to conserve genomic stability and prevent mutation. The p53 protein blocks cell division, activates cell death, and inhibits tumor angiogenesis in response to DNA-damage including DNA breaks, UV exposure and oncogenes [9]. The HPV oncogenic protein E6 has a strong binding affinity for p53 leading to its ubiquitination and degradation, resulting in reduced protein function and loss of cell cycle control [10]. A member of the p53 gene family, the p73 protein, functionally and structurally resembles p53 and plays an important role in cell cycle control. When activated, p73 signals transcription of p53-responsive genes, thereby acting as a tumor suppressor. The *p73* gene is commonly deleted in neuroblastomas and other human cancers and contributes to tumorigenesis when deregulated [11, 12]. Through protein-protein binding interactions, HPV oncogene E6 inhibits p73 and reduces activation of downstream cell cycle modulators, particularly p21 which reduces the ability of p73 to inhibit the cell cycle [13].

The murine double minute 2 gene (*MDM2*) is another member of the *p53*-related genes that function as a negative modulator of *p53* by directly interacting with the p53 protein to repress transcriptional activation. DNA damage signals phosphorylation of MDM2 to cause protein structure changes that stabilize p53 resulting in progression through the cell cycle [14]. Mechanistically, MDM2 interacts with HPV E2 protein to synergistically activate the HPV16 promoter, demonstrating E2 can actively recruit MDM2 to the HPV promoter and supporting a role for MDM2 in the transcriptional activity of HPV [15]. As an MDM2-related protein, MDM4 has emerged as a key negative regulator of p53, which directly binds to the p53 transactivation domain, inhibits its transcriptional activity, and thus contributes to tumor formation and progression. Therefore, MDM4, together with p53, p73, MDM2, and HPV E6 oncoprotein, may play a critical role in HPV-associated oral carcinogenesis.

Of the identified *p53* variants, the polymorphism in codon 72 of exon 4 that encodes either a Proline (Pro) or Arginine (Arg) appears to influence individual susceptibility to cancer by functionally affecting the p53 protein. The two linked, non-coding exon 2 polymorphisms of *p73* at position 4 (G → A) and 14 (C → T) may functionally affect the p73 protein by affecting the efficiency of *p73* translation initiation [12]. Among the polymorphisms of *MDM2*, two polymorphisms in promoter, *MDM2*-A2164G and *MDM2*-T2580G, may lead to change of MDM2 transcription levels, resulting in altered p53-MDM2 binding affinity and regulation of cell cycle control [16]. Unlike p53, p73, and MDM2, few studies have investigated the role of *MDM4* variants in the risk of human cancers. We identified three common (minor allele frequency ≥ 0.10) tagging SNPs (rs11801299 G>A and rs1380576

C>G in 3′-untranslated region [3′-UTR] and rs10900598 G>T in 5′-UTR) of *MDM4* gene within an approximately 34 kb region on chromosome 1q32, implying that these genetic variants may alter or influence MDM4 expression and subsequently increase susceptibility to cancer.

Because these *p53*-related genes appeared to have interaction with HPV and might jointly alter susceptibility to HPV16-associated oral cancer risk, we evaluated joint effect between the variants in these genes and HPV16 infection on risk of oral cancer in a case-control study of 325 cases and 335 controls.

Materials and Methods

Study subjects

Patients with newly diagnosed, histopathologically confirmed, and untreated oral cancer were recruited between April 1996 and June 2002 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 oral cancers in this study are defined as squamous cell carcinomas arising in the oral tongue, floor of mouth, hard palate, gingivobuccal, retromolar trigone, base of tongue, tonsils, soft palate/uvula, and oropharyngeal wall (the ICD-9-CM codes for these anatomic sub-sites are 141.4, 144.0, 143.0, 141.0, 141.6, 145.3, 145.4, 146.0-3, and 146.5-9, respectively). The accrual rate was 81% for cases. The controls included two groups of cancer-free subjects. One group were 191 (57.0%) healthy controls 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 (overall response rate was approximately 75%). The other controls were 144 (43.0%) healthy visitors who were accompanying cancer patients to the outpatient clinics at M.D. Anderson Cancer Center but genetically unrelated to the cases (overall response rate was approximately 80%). Both control groups had no previous histories of any cancers (excluding non-melanoma skin cancer) and were frequency-matched to the cases on age, sex and smoking and drinking status. To avoid confounding due to ethnic characteristics, we included only non-Hispanic whites in both the case and the 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 one 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 for biological testing.

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, which has been previously described elsewhere [17]. We also retested a randomly chosen 10% of the samples and obtained 100% concordance on the repeat assays.

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 QIAGEN DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer’s instructions. The methods

for genotyping of these four polymorphisms have been previously described [16, 18–20]. The results evaluated without knowing of the subjects' case or control status. More than 10% of the samples were retested for each polymorphism randomly, and the results were 100% concordant.

Statistical analysis

The differences between cases and controls in the distributions of selected demographic variables (age and sex), tobacco smoking, alcohol use, and HPV16 status were evaluated using the χ^2 test. In the univariate logistic regression analysis, we estimated the association of selected demographic variables, tobacco smoking, alcohol use, and HPV16 status with the risk of oral cancer by computing the odds ratios (ORs) and their 95% confidence intervals (CIs). In the multivariable logistic regression models with adjustment for age, sex, smoking, and alcohol use, we evaluated the joint effects of HPV16 serology and each polymorphism of *p53*-related genes on the risk of oral cancer and tumor subsite: oropharyngeal cancer. We also evaluated the joint effects of HPV16 serology and the combined risk genotypes of *p53*-related genes on the risk of oral cancer: the joint effects were further stratified by smoking status and tumor site. For the combined analysis of the four polymorphisms, we categorized subjects into three combined risk groups based on main effect of each polymorphism on oral cancer risk in our previously published or unpublished results from our large molecular epidemiologic study of squamous cell carcinoma of the head and neck. Therefore, we dichotomized the genotype data, in which genotypes for *p53* Arg/Arg, *p73* 4/14 GC/GC, *MDM2*-A2164G AA, *MDM2*-T2580G TG/GG, *MDM4* rs10900598 GT/TT, *MDM4* rs1380576 CC, and *MDM4* rs11801299 GG genotypes were coded as 0, and the *p53* Arg/Pro + Pro/Pro, *p73* 4/14 GC/AT + AT/AT, *MDM2*-A2164G AG/GG, *MDM2*-T2580G TT, *MDM4* rs10900598 GG, *MDM4* rs1380576 CG/GG, and *MDM4* rs11801299 AG/AA genotypes were coded as 1. All tests were 2-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

All cases and controls were self-reported non-Hispanic whites. The demographic variables and risk factors for 325 cases and 335 cancer-free controls and their association with oral cancer risk are summarized in Table 1. The cases and controls appeared to be adequately frequency-matched for age, sex, smoking, and alcohol status ($P = 0.100$ for age, $P = 0.100$ for sex, $P = 0.673$ for smoking status, $P = 0.121$ for alcohol status). We did not observe a significant association of age, sex, smoking, and alcohol status with risk of oral cancer. These variables were further adjusted in later logistic regression analyses to control for residual effects. However, we found that HPV16 seropositivity was more common in cases than in controls ($P < 0.001$) and was significantly associated with an increased risk of oral cancer (OR, 3.1, 95% CI, 2.1–4.6).

Among all the studied subjects, five cases and fourteen controls failed to genotyping for MDM4 after repeated assays. Thus, the final analysis for genetic data included 320 oral cancer cases and 321 controls. As we previously reported [18, 20–22], within the control group, the distributions of the p53, p73, MDM2, and MDM4 genotypes were in agreement with Hardy-Weinberg equilibrium [18, 20–22]. As shown in Table 2, overall, compared with individuals having corresponding common homozygous genotypes and HPV16 seronegativity, HPV16 seropositivity among those with p53 codon 72, p73 4/14 GC/AT, MDM2-A2164G, MDM4 rs1380576 C>G, and MDM4 rs11801299 G>A variant genotypes was associated with a higher risk of oral cancer than among those with common homozygous genotypes after adjusting for age, sex, smoking and alcohol status [18, 20–22]. Conversely, compared with those with *MDM2*-T2580G TG/GG and *MDM4* rs10900598

GT/TT variant genotypes and HPV16 seronegativity, the risk for HPV16-associated oral cancer was higher among individuals with *MDM2*-T2580G TT and MDM4 rs10900598 GG common homozygous genotypes than among those with TG/GG and GT/TT variant genotypes, respectively [20, 22].

Because each of the seven *p53*-related genes appeared to have interaction with HPV and because each of the seven polymorphisms of these genes appeared to have an effect on risk of HPV16-associated oral cancer[18, 20–22], we then performed combined modifying effect of all seven polymorphisms on risk of HPV16-associated oral cancer (Table 3). In the study subjects who had data available on all seven polymorphisms, we categorized all putative risk (ORs > 1.0) genotypes of each polymorphism into a new variable according to the number of risk genotypes carried by an individual for each of the seven polymorphisms in a dominant model (for the *MDM2*-T2580G and *MDM4* rs10900598 G>T genotypes, we reversed the reference group to reflect the protective effects of the variant genotypes *MDM2*-T2580G TG/GG and *MDM4* rs10900598 GT/TT). Therefore, according to the level of HPV16-associated oral cancer risk linked to the risk genotypes of each individual polymorphism, we categorized the individuals into three combined genotype groups to evaluate the collective effects of the *p53*, *p73*, *MDM2*, and *MDM4* polymorphisms on the risk of HPV16-associated oral cancer. As shown in Table 3, when we used the individuals in the low-risk group (0–3 combined risk genotypes) with HPV16 seronegativity as the comparison group, we found that the risk of oral cancer increased among individuals in the medium-risk group (4 combined risk genotypes) with HPV16 seronegativity (OR, 1.6; 95% CI, 1.1–2.5), the high-risk group (5–7 combined risk genotypes) with HPV16 seronegativity (OR, 1.2; 95% CI, 0.7–1.9), the low-risk group with HPV16 seropositivity (OR, 2.1; 95% CI, 1.2–3.6), the medium-risk group with HPV16 seropositivity (OR, 4.0; 95% CI, 1.8–9.1), and the high-risk group with HPV16 seropositivity (OR, 19.1; 95% CI, 5.7–64.2), respectively. These risks were even higher for oropharyngeal cancer (OR, 1.8, 1.3, 3.9, 7.7, and 36.8, respectively). Such modification effect may suggest a more-than-multiplicative interaction.

To further investigate this association, we evaluated the association of the combined risk genotypes with risk of HPV16-associated oral cancer stratified by age and smoking status, summarized in Table 4 and Table 5. We found that for all combined risk groups, young subjects (aged < 50 years) had a greater risk for HPV16-associated oral cancer than older subjects (aged ≥ 50 years) in the corresponding combined risk group. For example, the young subjects in high-risk group exhibited an approximately 32-fold increased risk for HPV16-associated oral cancer, while the older subjects in high-risk group had an approximately 17-fold increased risk for HPV16-associated oral cancer (Table 4). Similarly, never smokers had a greater risk for HPV16-associated oral cancer than ever smokers in the corresponding combined risk group. The subjects in never smokers in high-risk group exhibited an approximately 69-fold increased risk for HPV16-associated oral cancer, while the ever smokers in high-risk group only a 9-fold increased risk for HPV16-associated oral cancer (Table 5). Moreover, these results were even more dramatic for oropharyngeal cancer (Table 4 and Table 5).

Discussion

As we reported previously, the risk for HPV16-associated oral cancer was modified by each of seven *p53*-related genetic polymorphisms[18, 20–22]. In this study, after combining the seven polymorphisms, as the number of risk genotypes carried by individuals increased, the risk for HPV16-associated oral cancer also increased, and this risk was more pronounced in young subjects, never smokers, and patients with oropharyngeal cancer. These findings

suggest that these polymorphisms of *p53*-related genes may individually, and more likely collectively, contribute to development of HPV16-associated oral cancer.

The p53, p73, MDM2, and MDM4 proteins act to modify the cell cycle through direct regulation and modulation of other cell cycle proteins [9, 11, 12, 14]. In addition to interactions among each other, they all interact directly or indirectly with HPV oncogenic proteins to influence cellular activities such as cell cycle and apoptosis. Essentially, p73 acts to transactivate p53 and its target genes, including MDM2 and MDM4, and MDM2 and MDM4 act as negative modulators of p53 [23, 24]. p53 and p73 are structurally similar and play analogous roles in cell cycle control, DNA repair, and apoptosis [9, 11, 13]. Additionally, both interact directly with, and are bound and inactivated by, the HPV16 E6 oncoprotein [13] while MDM2 and MDM4 together with HPV E2 may act synergistically to activate the HPV16 promoter [15]. Therefore, these numerous interactions may support the biological plausibility that the combination of all variants of the *p53*-related genes in the p53-dependent pathways could result in more comprehensive and accurate estimates of risk for HPV-associated oral cancer than that from a single variant.

The *p53* codon 72 polymorphism alters the susceptibility of p53 to E6-mediated degradation and it is likely that genetic polymorphisms in *p73* alter its interaction with E6 as well, thereby contributing to the risk of HPV16-associated oral cancer. MDM2 is a suppressor of both p53 and p73; however, while the interaction between p53 and MDM2 results in degradation of p53, MDM2 associates with, but does not degrade, p73 [24–26]. It has been hypothesized that the MDM2-p73 interaction acts as a competitor to the MDM2-p53 complex and thus protects p53 from MDM2-mediated degradation [26]. It is possible that polymorphisms in these genes result in functional changes of these proteins that affect the interaction of either among these proteins or their interaction with HPV16 oncoprotein E6 and subsequently interfere with cell cycle control, DNA repair and apoptosis [10, 13].

MDM4, a structural homolog of *MDM2,* is a new member of the RING finger family of ubiquitin ligases, and the RING finger domain of MDM4 is indispensable for its activity *in vitro* experiments [27]. Because *MDM4* shows a high similarity to *MDM2* at the level of gene sequence and structure and shares several regions of homology with *MDM2*, including the p53 binding domain, a zinc finger motif, and a C-terminal RING finger domain [28]. 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. Studies in knock-out mice showed that the major function of MDM4 during early development is to regulate p53 [29], and *MDM4* is frequently amplified in cancer tissues and tumor cell lines with the wild-type p53 [30, 31]. Therefore, the intimate relationship between these proteins and the overlap in their biological functions suggest that *p53*, *p73, MDM2,* and *MDM4* polymorphisms may cooperatively contribute to the development of HPV16-associated oral cancer.

Several studies have reported the association between the *p53* codon 72 polymorphism and the risk for HPV-associated cancers [18, 32–34], but the results are inconsistent. The inconsistent results of these studies may be attributed to differences in anatomical cancer sites, different genetic background in different ethnic groups studied, sample sizes, HPV types, and study design. There are fewer epidemiological studies to examine the association between *p73*, *MDM2* and *MDM4* polymorphisms and HPV16-associated cancer risk. A Portuguese study reported a significant association between the *p73* 4/14 GC/AT polymorphism and cervical cancer [35] and a Japanese study reported a borderline significant association between this *p73* polymorphism and risk for cervical cancer [36]. In our previous case-control study among non-Hispanic whites, we found that these polymorphisms have been shown to be individually associated with risk for subsites of squamous cell carcinomas

of the head and neck, especially in never-smokers [20–22], while there are no studies that simultaneously investigate the combined effects of these polymorphisms on risk of HPV16-associated oral cancer.

We evaluated the combined effect of these seven polymorphisms on risk of HPV16-associated oral cancer; as the number of risk genotypes carried by an individual increases, so does the risk for HPV16-associated oral cancer, particularly in never-smokers. This finding suggests that exposure to tobacco might not modify the relationship between the polymorphisms of *p53*-related genes and susceptibility to HPV16-associated oral cancer, suggesting that HPV16-associated oral cancer differs etiologically from smoking related oral cancer, and that smoking does not further increase risk of HPV16-associated oral cancer [37]. Another finding in our study was that the association between HPV16 seropositivity and the combined risk genotypes was significantly higher for oropharyngeal cancer, but not for oral cavity cancer, likely reflecting the different sites may result from different etiologies for oropharyngeal and oral cavity cancers. This finding is in agreement with those in previous studies, in which most non-oropharyngeal head and neck cancers were associated with smoking and drinking while many oropharyngeal cancers were associated with HPV [38]. Ragin et al also indicated that p53 mutations were less likely to occur in the HPV-positive patients, and oropharyngeal cancer patients were more than twice as likely to have an HPV-positive16-positive tumor (OR, 2.4; 95% CI, 1.0–5.8) as patients with oral cavity cancers[39].

Our observations of more pronounced modifying effects in younger subjects than in older subjects might be partly explained by the increased oral HPV16 prevalence in young adults perhaps owing to distinct changing in sexual behaviors or that susceptible groups develop cancers at a younger age [40]. Therefore, future studies on genetic susceptibility to oropharyngeal cancer should take HPV status as a risk confounder into consideration because the *p53*-related genes may play different roles in HPV16-positive and HPV16-negative oropharyngeal cancer.

Although this study may add to the current literature of gene-gene and gene-virus interactions on risk for oral cancer, it has limitations. Firstly, the possible selection bias could not be ruled out due to the possible selection of hospital-based cases and controls. Secondly, due to restricting the study to a non-Hispanic white population, the results may not be applicable to all ethnic populations. In addition, stratified analyses included a limited number of individuals in each subgroup, so our results could be a chance finding and should be confirmed in larger studies. Thirdly, in current study, the absence of HPV tumor status did not allow us to evaluate its potential influence on risk of oral cancer, while using HPV serologic status allows for the inclusion of a cancer-free control group for this case-control study design. 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 misclassification could result in a major selection bias for the estimates of the association. Thus, we will closely monitor the role of HPV in oral cancer in our future studies when a much larger patient cohort with HPV tumor status becomes available. In addition, because this is the first study concerning the combined effects of *p53*-related genetic variants on the risk of HPV16-associated oral cancer, it is likely that some associations we presented here could be chance findings. Finally, the width of some of the confidence intervals suggests that the numbers of cases and controls in some of the categories are very small. Thus, this exploratory analysis serves as hypothesis generating and other independent epidemiologic and functional studies with larger sample sizes are needed to validate these results.

In conclusion, this study provides support for the multigenetic effects of the genetic variants from *p53* exon 4, *p73* promoter, *MDM2* promoter, and *MDM4* 3′- or 5′-UTR resulting in a

significantly increased risk for HPV16-associated oral cancer in a non–Hispanic white population. We also noted that the multigenetic effects were most evident in young subjects, never smokers, and patients with oropharyngeal cancer. This is the first study to examine association of multiple polymorphisms from the *p53*-related genes with risk of HPV16-associated oral cancer. This approach highlights the value of examining multiple polymorphisms in genes in common pathways to improve the precision of risk estimates.

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Abbreviations

CI	confidence interval
HPV	human papillomavirus
OR	odds ratio
PCR	polymerase chain reaction
SNP	single nucleotide polymorphism

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Table 1

Frequency distribution of demographic and risk factors in cases and controls and their association with risk of oral cancer

Variables	Cases (n = 325)		Controls (n = 335)		OR[a], 95% CI
	No.	%	No.	%	
Age (years)					
≥ 40	31	9.5	27	8.1	1.0
41 – 55	126	38.8	105	31.3	1.1 (0.6–1.9)
56 – 70	119	36.7	154	46.0	0.7 (0.4–1.2)
> 70	49	15.0	49	14.6	0.9 (0.5–1.7)
Sex					
Male	241	74.2	269	80.3	1.0
Female	84	25.8	66	19.7	1.4 (0.9–2.1)
Tobacco smoking					
Ever	227	69.8	239	71.3	1.0
Never	98	30.2	96	28.7	1.1 (0.8–1.5)
Alcohol drinking					
Ever	250	76.9	240	71.6	1.0
Never	75	23.1	95	28.4	0.8 (0.5–1.1)
HPV16					
Negative	225	69.2	293	87.5	1.0
Positive	100	30.8	42	12.5	3.1 (2.1–4.6)

[a]Univariate OR and 95% CI.

Table 2

Associations of *p53*-related genetic variants with risk of HPV16 associated oral cancer

		Cases (n = 320)		Controls (n = 321)		
Variables	**HPV16 Status**	**No.**	**%**	**No.**	**%**	**Adj. OR (95% CI)***
P53						
Arg/Arg	−	130	40.6	144	44.8	1.0
Arg/Pro + Pro/Pro	−	91	28.4	136	42.4	0.7 (0.5–1.1)
Arg/Arg	+	43	13.7	24	7.5	2.0 (1.1–3.6)
Arg/Pro + Pro/Pro	+	56	17.5	17	5.3	3.6 (2.0–6.6)
P73						
GC/GC	−	132	41.2	171	53.3	1.0
GC/AT + AT/AT	−	89	27.8	109	34.0	1.0 (0.7–1.4)
GC/GC	+	54	16.9	26	8.1	2.7 (1.6–4.6)
GC/AT + AT/AT	+	45	14.1	15	4.8	3.7 (2.0–7.0)
MDM2-rs2279744						
GT + GG	−	125	39.1	188	58.6	1.0
TT	−	96	30.0	92	28.7	1.6 (1.1–2.3)
GT + GG	+	50	15.6	27	8.4	2.8 (1.7–4.8)
TT	+	49	15.3	14	4.3	5.5 (2.9–10.5)
MDM2-rs937283						
AA	−	49	15.3	94	29.3	1.0
AG + GG	−	172	53.7	186	57.9	1.8 (1.2–2.8)
AA	+	20	6.3	17	5.3	2.2 (1.0–4.6)
AG + GG	+	79	24.7	24	7.5	6.8 (3.8–12.2)
MDM4-rs10900598						
GT + TT	−	153	47.8	195	60.7	1.0
GG	−	68	21.3	85	26.5	1.0 (0.7–1.5)
GT + TT	+	60	18.7	32	10.0	2.4 (1.5–3.8)
GG	+	39	12.2	9	2.8	5.9 (2.7–12.7)
MDM4-rs1380576						
CC	−	109	34.1	133	41.4	1.0

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		Cases (n = 320)		Controls (n = 321)		
Variables	**HPV16 Status**	**No.**	**%**	**No.**	**%**	**Adj. OR (95% CI)***
CG + GG	−	112	35.0	147	45.8	0.9 (0.6–1.3)
CC	+	32	10.0	16	5.0	2.5 (1.3–4.9)
CG + GG	+	67	20.9	25	7.8	3.2 (1.9–5.6)
MDM4-rs11801299						
GG	−	137	42.8	173	53.9	1.0
AG + AA	−	84	26.3	107	33.3	1.0 (0.7–1.4)
GG	+	58	18.1	28	8.7	2.6 (1.6–4.3)
AG + AA	+	41	12.8	13	4.1	4.2 (2.1–8.3)

*ORs were adjusted for age, sex, smoking and alcohol drinking.

Table 3

Joint effect of HPV16 serology and the combined risk genotypes of *p53*-related genes on risk of oral cancer

Risk groups*	HPV16 status	Cases (*n* = 320)		Controls (*n* = 321)		Adj. OR (95% CI)[a]	
		No.	%	No.	%	Overall	Oropharynx
Low-risk group	−	123	38.4	179	55.8	1.0	1.0
Medium-risk group	−	63	19.7	57	17.8	1.6 (1.1–2.5)	1.8 (1.1–3.2)
High-risk group	−	35	10.9	44	13.7	1.2 (0.7–1.9)	1.3 (0.7–2.5)
Low-risk group	+	41	12.8	29	9.0	2.1 (1.2–3.6)	3.9 (2.2–7.0)
Medium-risk group	+	22	6.9	9	2.8	4.0 (1.8–9.1)	7.7 (3.3–18.3)
High-risk group	+	36	11.3	3	0.9	19.1 (5.7–64.2)	36.8 (10.6–126.9)

*Low-risk group: individuals carrying 0–3 risk genotype; medium-risk group: individuals carrying 4 risk genotypes; and high-risk group: individuals carrying 5–7 risk genotypes.

[a]odds ratios (ORs) were adjusted for age, sex, smoking and drinking status in logistic regression models.

Table 4

Joint effect of HPV16 serology and combined risk genotypes of *p53*-related genes on risk of oral cancer stratified by age

Risk groups*	HPV16 status	Cases (*n* = 320)		Controls (*n* = 321)		Adj. OR (95% CI) [a]	
		No.	%	No.	%	Overall	Oropharynx
Young (aged < 50 years)		(*n* = 86)		(*n* = 83)			
Low-risk group	−	28	32.6	46	55.4	1.0	1.0
Medium-risk group	−	18	20.9	16	19.3	2.6 (1.0–6.6)	3.6 (1.0–12.7)
High-risk group	−	9	10.5	11	13.2	1.8 (0.6–5.2)	3.9 (1.1–13.8)
Low-risk group	+	14	16.3	8	9.6	4.5 (1.5–13.1)	9.1 (2.7–30.9)
Medium-risk group	+	6	7.0	1	1.2	12.8 (1.3–123.6)	32.3 (3.1–341.2)
High-risk group	+	11	12.8	1	1.2	32.0 (3.5–290.9)	87.0 (8.5–890.4)
Older (aged > 50 years)		(n = 234)		(n = 238)			
Low-risk group	−	95	40.6	133	55.9	1.0	1.0
Medium-risk group	−	45	19.2	41	17.2	1.5 (0.9–2.5)	1.6 (0.9–3.1)
High-risk group	−	26	11.1	33	13.9	1.1 (0.6–1.9)	1.0 (0.4–2.1)
Low-risk group	+	27	11.5	21	8.8	1.8 (1.0–3.5)	3.4 (1.7–6.9)
Medium-risk group	+	16	6.8	8	3.4	3.0 (1.2–7.3)	4.8 (1.8–12.6)
High-risk group	+	25	10.7	2	0.8	17.2 (3.9–75.2)	29.8 (6.6–134.7)

*Low-risk group: individuals carrying 0–3 risk genotype; medium-risk group: individuals carrying 4 risk genotypes; and high-risk group: individuals carrying 5–7 risk genotypes.

[a]ORs were adjusted for age, sex, smoking and drinking status in logistic regression models.

Table 5

Joint effect of HPV16 serology and combined risk genotypes of *p53*-related genes on risk of oral cancer stratified by smoking status

Risk groups*	HPV16 status	Cases (*n* = 320)		Controls (*n* = 321)		Adj. OR (95% CI)[a]	
		No.	%	No.	%	Overall	Oropharynx
Never-smokers		(*n* = 98)		(*n* = 92)			
Low-risk group	−	30	30.6	55	59.8	1.0	1.0
Medium-risk group	−	14	14.3	19	20.6	1.4 (0.6–3.4)	1.0 (0.3–3.3)
High-risk group	−	8	8.2	10	10.9	1.4 (0.5–4.2)	1.2 (0.3–5.4)
Low-risk group	+	13	13.3	6	6.5	4.8 (1.6–14.5)	7.4 (2.2–24.8)
Medium-risk group	+	8	8.2	1	1.1	21.2 (2.4–189.4)	60.0 (6.2–584.3)
High-risk group	+	25	25.5	1	1.1	68.8 (8.5–555.1)	110.0 (13.1–915.4)
Ever-smokers		(n = 222)		(n = 229)			
Low-risk group	−	93	41.9	124	54.2	1.0	1.0
Medium-risk group	−	49	22.1	38	16.6	1.7 (1.0–2.8)	2.2 (1.2–4.2)
High-risk group	−	27	12.1	34	14.8	1.1 (0.6–2.0)	1.3 (0.6–2.7)
Low-risk group	+	28	12.6	23	10.0	1.7 (0.9–3.1)	3.2 (1.6–6.3)
Medium-risk group	+	14	6.3	8	3.5	2.6 (1.0–6.5)	4.2 (1.6–11.3)
High-risk group	+	11	5.0	2	0.9	8.8 (1.9–41.6)	13.4 (2.8–65.2)

*Low-risk group: individuals carrying 0–3 risk genotype; medium-risk group: individuals carrying 4 risk genotypes; and high-risk group: individuals carrying 5–7 risk genotypes.

[a]ORs were adjusted for age, sex, smoking and drinking status in logistic regression models.