Associations of Oral $\alpha$-, $\beta$-, and $\gamma$-Human Papillomavirus Types With Risk of Incident Head and Neck Cancer

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**IMPORTANCE**
Prospective studies are needed to examine the temporal relationship between oral human papillomavirus (HPV) detection and risk of head and neck squamous cell carcinoma (HNSCC). Moreover, the oral cavity contains a wide spectrum of $\alpha$-, $\beta$-, and $\gamma$-HPV types, but their association with risk of HNSCC is unknown.

**OBJECTIVE**
To prospectively examine associations between $\alpha$-, $\beta$-, and $\gamma$-HPV detection in the oral cavity and incident HNSCC.

**DESIGN**
A nested case-control study was carried out among 96,650 participants, cancer free at baseline, with available mouthwash samples in 2 prospective cohort studies: (1) the American Cancer Society Cancer Prevention Study II Nutrition Cohort and (2) the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Incident cases of HNSCC ($n = 132$) were identified during an average 3.9 years of follow-up in both cohorts. Three controls per case ($n = 396$) were selected through incidence density sampling and matched on age, sex, race/ethnicity, and time since mouthwash collection.

**METHODS**
Through a next-generation sequencing assay, DNA from $\alpha$-, $\beta$-, and $\gamma$-HPV types were detected. Conditional logistic regression models were used to estimate odds ratios (ORs) and 95% CIs, adjusting for smoking history, alcohol consumption, and detection of HPV-16 for $\beta$- and $\gamma$-HPVs.

**MAIN OUTCOMES AND MEASURES**
Incident HNSCC, which includes cancers of the oropharynx, oral cavity, and larynx.

**RESULTS**
A total of 132 participants developed HNSCC during the follow-up period (103 men and 29 women; average age at baseline, 66.5 years). Oral HPV-16 detection was associated with incident HNSCC (OR, 7.1; 95% CI, 2.2-22.6), with positive association for oropharyngeal SCC (OR, 22.4; 95% CI, 1.8-276.7), but not for oral cavity (OR, 4.5; 95% CI, 0.6-34.7) or laryngeal SCCs (OR, 0.11; 95% CI, 0.01-834.80). Detection of $\beta_1$-HPV-5 and $\beta_2$-HPV-38 types, as well as $\gamma_1$- and $\gamma_12$ species, had ORs for HNSCC that ranged from 2.64 to 5.45 ($P < .01$ for all comparisons). Detection of $\beta_1$-HPV-5 type was associated with oropharyngeal (OR, 7.42; 95% CI, 0.98-56.82; $P = .054$), oral cavity (OR, 5.34; 95% CI, 1.51-18.80; $P = .01$), and laryngeal SCCs (OR, 2.71; 95% CI, 1.00-7.43; $P = .05$), whereas $\gamma_1$- and $\gamma_12$-HPV species were associated with both oral cavity (OR, 7.47; 95% CI, 1.21-46.17; $P = .03$; and OR, 6.71; 95% CI, 1.47-30.75; $P = .01$ respectively) and laryngeal SCCs (OR, 7.49; 95% CI, 1.10-51.04; $P = .04$ and OR, 5.31; 95% CI, 1.13-24.95; $P = .03$, respectively).

**CONCLUSIONS AND RELEVANCE**
This study demonstrates that HPV-16 detection precedes the incidence of oropharyngeal SCC. Associations of other HPV species, including $\gamma_1$- and $\gamma_12$-HPV species and $\beta_1$-HPV-5 type suggest a broader role for HPVs in HNSCC etiology.
Human Papillomavirus Types and Incident Head and Neck Cancer

Key Points

Question What is the association between oral α-, β-, and γ-human papillomavirus (HPV) types and incident head and neck squamous cell carcinoma (HNSCC)?

Findings In a nested case-control study among nearly 100,000 participants, oral HPV-16 detection was associated with a 22.4-fold increased risk of incident oropharyngeal cancer, and detection of oral β1-HPV-5 type and γ1-HPV and γ12-HPV species was associated with a 3.3- to 5.5-fold higher risk of HNSCC after adjustment for smoking, alcohol consumption, and HPV-16 detection.

Meaning The association of β- and γ-HPV types with increased risk of HNSCC suggests a broader role for HPVs in HNSCC etiology.

Study participants in both cohorts completed self-administered baseline questionnaires, which collected information on demographic data (including sex and race/ethnicity), social characteristics, previous cancer diagnoses, and current and lifetime smoking history and alcohol consumption. Follow-up questionnaires were sent every 2 years to CPS-II-NC participants and annually to PLCO cohort members to update information on lifestyle exposures and health status, and to ascertain newly diagnosed cancers. Mouthwash samples were collected primarily as a source for genomic DNA from 70,004 CPS-II-NC participants between 2001 and 2002,21 and 55,866 participants in the PLCO control arm between 1998 and 2005,22,23 who did not provide a blood sample.

Identification of Incident Cases of HNSCC and Selection of Controls

We designed parallel nested case-control studies among participants who provided informed consent, baseline questionnaire data, and a mouthwash sample in each cohort. The definition of HNSCC included cancers of the following sites: oral cavity (excluding salivary glands), tongue, oropharynx (including tonsils and base of the tongue), hypopharynx, and larynx. A detailed description of inclusion criteria for each cohort is provided in eMethods in the Supplement. A total of 132 incident HNSCC cases were identified in both cohorts during an average 3.9 years of follow-up. In each cohort, 3 controls were selected for each case using incidence density sampling, and controls were individually matched to cases by sex, race/ethnicity, date of birth (±6 months), and date of oral rinse collection (±30 days for the CPS-II-NC, and ±3 months for the PLCO trial). The present study was reviewed and deemed exempt by the institutional review board of Albert Einstein College of Medicine. The original cohort studies received full institutional review board approval from both the American Cancer Society and the National Cancer Institute, and written informed consent was obtained from all study participants for the original data collection.

Molecular Detection of Oral HPV DNA

All HPV testing was performed at the Albert Einstein College of Medicine, and all laboratory personnel were blinded to the case-control status of the mouthwash samples. Total DNA was purified from exfoliated oral cavity epithelial cells obtained from

Methods

Study Cohorts and Data Collection

We conducted nested case-control studies among participants who provided mouthwash samples in 2 large prospective cohorts: the American Cancer Society Cancer Prevention Study II Nutrition Cohort (CPS-II-NC)21 and the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO).22 The CPS-II-NC enrolled 184,192 men and women residing in 21 US states, mostly (98%) aged 50 to 79 years at baseline (53% women and 97% white). The PLCO trial enrolled 154,910 men and women aged 55 to 74 years from 1993 through 2001 at 10 US centers. Participants had no history of prostate, lung, colorectal, or ovarian cancers; 50% were women; and 86% were non-Hispanic white.

In 2015, approximately 60,000 people were diagnosed with head and neck cancer (HNC) in the United States, and 12,000 died of this cancer.1,2 These cancers are predominately (>90%) squamous cell carcinomas (SCCs) arising from a variety of epithelial sites in the upper aerodigestive system, including the oral cavity, nasopharynx, oropharynx, hypopharynx, and larynx. The main risk factors for HNC are increasing age, male sex, cigarette smoking, and alcohol consumption.3-7 More recently, alpha species human papillomavirus (a-HPV) infection has been identified as an important risk factor for head and neck squamous cell carcinoma (HNSCC), although there is considerable heterogeneity by tumor site.6,10 Primarily, HPV-positive HNSCCs are due to HPV-16 infection, with an estimated prevalence of 41% in oropharyngeal SCCs and 13% to 15% in oral cavity and larynx SCCs.10 Two meta-analyses of case-control studies8,16 showed strong associations of HPV-16 detection at the time of diagnosis with tonsillar cancer (odds ratios [OR], 15.1; 95% CI, 6.8-33.7) and oropharyngeal cancer (OR, 4.3; 95% CI, 2.1-8.9), whereas the associations with laryngeal cancer (OR, 2.0; 95% CI, 1.0-4.2) and oral cavity cancers (OR, 2.0; 95% CI, 1.2-3.4) were lower.6 In addition, recent serologic data from large case-control and prospective cohort studies have demonstrated a strong association between HPV-16 E6 or E7 antibody positivity and risk of HNSCC, in particular for HPV-16 E6 and oropharyngeal SCCs.17-19

However, to our knowledge, there have been no prospective studies examining associations between oral HPVs and risk of incident HNSCC. Moreover, recent data indicate that the oral cavity contains not only α-HPVs, but a wide spectrum of other HPVs, including β- and γ-HPV types, although their association with HNSCC is unknown.20 Therefore, we examined associations of α-, β-, and γ-HPV DNA detection in the oral cavity with incident HNSCC in a case-control study nested within 2 large prospective cohorts.

Study participants in both cohorts completed self-administered baseline questionnaires, which collected information on demographic data (including sex and race/ethnicity), social characteristics, previous cancer diagnoses, and current and lifetime smoking history and alcohol consumption. Follow-up questionnaires were sent every 2 years to CPS-II-NC participants and annually to PLCO cohort members to update information on lifestyle exposures and health status, and to ascertain newly diagnosed cancers. Mouthwash samples were collected primarily as a source for genomic DNA from 70,004 CPS-II-NC participants between 2001 and 2002,21 and 55,866 participants in the PLCO control arm between 1998 and 2005,22,23 who did not provide a blood sample.

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a mouthwash rinse specimen, as described previously.\textsuperscript{20} Testing for HPV DNA detection was performed using 3 different platforms: (1) The MY09/11 L1-targeted degenerate primer polymerase chain reaction system using AmpliTaq Gold DNA Polymerase (ThermoFisher Scientific Inc), which preferentially detects α-HPV types (including HPV-16);\textsuperscript{24} (2) a multiplexed next-generation sequencing method (see eMethods in the Supplement) developed to detect and type the diverse and large number of α-, β-, and γ-HPV isolates present in the oral cavity;\textsuperscript{25} and (3) a real-time polymerase chain reaction assay for HPV-16.

### Definition of HPV Type Positivity

We considered a sample to be positive for HPV-16 if it scored positive in 2 of 3 assays. For other α-HPV types, we used both the MY09/11 polymerase chain reaction data and next-generation sequencing results. To detect β- and γ-HPV DNA in oral samples, we relied strictly on the next-generation sequencing assays (see eMethods in the Supplement). Quality control analysis was carried out in 10% of oral wash samples randomly selected for repeat testing. The concordance of the prevalence of HPV genotypes between the 2 quality control sets was excellent (κ ≥ 0.90).

### Statistical Analysis

We examined associations of α-, β-, and γ-HPVs with incident HNSCC using conditional logistic regression models for matched risk sets to estimate ORs and 95% CIs.\textsuperscript{26,27} For α-HPVs, we examined associations for HPV-16; other high-risk oncogenic HPV types that are associated with cervical cancer (ie, HPV-18, -31, -33, -35, -39, -45, -51, -52, -56, -58, and -59);\textsuperscript{28} other non-high-risk α-HPV types; and any α-HPV type. For β-HPV types, we examined associations of any β-HPV type, different species groups, and specific β-HPV types with risk of HNSCC. We also examined associations of any γ-HPV type, different γ-species groups, and specific γ-HPV types with risk of HNSCC.

The associations between various HPV types and species and risk of HNSCC were adjusted for study cohort (CPS-II-NC vs PLCO trial), smoking status (current or former smokers vs never smokers), pack-years of smoking (as continuous), and number of alcoholic drinks per week (as continuous). For the few participants with missing information on pack-years of smoking (3 cases and 9 controls from both cohorts) or alcoholic drinks per week (22 cases and 46 controls from both cohorts), their missing data were imputed as described in eMethods in the Supplement. Since age, sex, race/ethnicity, and time since oral rinse collection were the matching variables, these were not included in conditional logistic regression models. Cigarette smoking and alcohol consumption were independently associated with risk of HNSCC and also changed the coefficient of association between various HPV types (eg, HPV-16) and HNSCC by more than 10%. The conditional logistic regression models, which examined associations of β- and γ-HPVs with HNSCC risk were also adjusted for HPV-16 DNA detection. We also tested for interactions between covariates in conditional logistic regression models, but there were no statistically significant interactions. A permutation procedure was used to account for the effect of multiple comparisons of several types of HPV detection and risk of HNSCC (see eMethods in the Supplement). After this procedure, an HPV type or species was considered significantly associated with risk of HNSCC at a permuted 2-sided \( P < .05 \).

We also examined associations of α-, β-, and γ-HPVs with HNSCC tumor site (oral cavity, oropharynx, and larynx/hypopharynx) in separate conditional logistic regression models. All statistical models were adjusted for the same variables as the models investigating the overall risk of incident HNSCC. An exception was for models that assessed associations of β- and γ-HPVs with risks of oral cavity and larynx SCCs; these models were not adjusted for HPV-16 because these sites are infrequently associated with HPV-16 detection.\textsuperscript{29} All statistical analyses were carried out in STATA, version 13 (StataCorp LP).

### Results

Participant demographic and lifestyle characteristics of incident cases of HNSCC and their matched controls are listed in Table 1. In the CPS-II-NC, both cases and controls, were on average 8 years older than their counterparts in the PLCO cohort. Most HNSCC cases and controls in both cohorts were male and white. Cases were more likely than controls to be current smokers and alcohol drinkers. However, there were no major differences with regard to body mass index, education, or marital status between the 2 groups (see eTable 1 in Supplement). The anatomic distribution of HNSCC tumors was 32.6% in the oral cavity (\( n = 43 \)), 18.9% in the oropharynx (\( n = 25 \)), 3.8% in the hypopharynx (\( n = 5 \)), and 44.7% in the larynx (\( n = 59 \)) and was similar between the 2 cohorts (Table 1).

### Associations of HPV-16 and Other α-HPV Types With Risk of Incident HNSCC

Among controls from both cohorts, the detection prevalence of oral HPV-16, other high-risk oncogenic HPVs, and non–high-risk α-HPVs were 1.8%, 5.1%, and 9.6%, respectively. Detection of HPV-16 DNA in oral rinse specimens was associated with a 7.1-fold higher odds of incident HNSCC (95% CI, 2.2–22.6) after adjusting for smoking history and alcohol consumption (Table 2). However, oral DNA detection of other high-risk oncogenic HPV types (excluding HPV-16) as well as non–high-risk α-HPVs was not associated with risk of HNSCC.

In stratified analyses by tumor anatomical site, oral HPV-16 DNA detection was associated with a 22.4-fold (95% CI, 1.8–276.7) higher odds of incident oropharyngeal SCC in multivariate models adjusted for smoking and alcohol consumption (Table 2). Oral HPV-16 was also identified in 50% (2 of 4) of tonsillar SCC cases. However, there were no associations between other α-HPVs and oropharyngeal SCC. There were no associations between detection of HPV-16, other high-risk oncogenic HPVs, or non–high-risk α-HPVs and cancers of the oral cavity or larynx/hypopharynx (Table 2).

### Association of β- and γ-HPVs With Risk of Incident HNSCC

Among controls from both cohorts, the prevalence of any β-HPV was 58.8%, and the prevalence of any γ-HPV was 35.4%. As summarized in Table 3, there were statistically significant positive associations between detection of any β-HPV (OR, 1.74;
### Table 1. Characteristics of Incident Cases of Head and Neck Squamous Cell Carcinoma and Matched Controls in the 2 Cohorts*

| Characteristic                             | CPS-ii-NC |                      | PLCO Trial |                      |
|-------------------------------------------|-----------|----------------------|------------|----------------------|
|                                           | HNSCC     | Controls             | HNSCC      | Controls             |
|                                           | (n = 60)  | (n = 180)            | (n = 72)   | (n = 216)            |
| **Matching Variables**                    |           |                      |            |                      |
| Age at mouthwash collection, mean (SD), y | 70.9 (6.4)| 70.9 (6.3)           | 63.0 (4.9) | 63.2 (5.0)           |
| Months from mouthwash collection to diagnosis, mean (SD) | 42.1 (24.9) | 42.1 (24.8) | 52.8 (29.4) | 53.1 (29.7) |
| Sex                                       |           |                      |            |                      |
| Male                                      | 44 (73.3) | 132 (73.3)           | 59 (81.9)  | 175 (81.0)           |
| Female                                    | 16 (26.7) | 48 (26.7)            | 13 (18.1)  | 41 (19.0)            |
| Race/ethnicity                            |           |                      |            |                      |
| White                                     | 60 (100)  | 180 (100)            | 68 (94.4)  | 204 (94.4)           |
| African American or other                 | 0         | 0                    | 4 (5.6)    | 12 (5.6)             |
| **Unmatched Variables**                   |           |                      |            |                      |
| Smoking status                            |           |                      |            |                      |
| Never                                     | 10 (16.7) | 83 (46.1)            | 8 (11.1)   | 101 (46.8)           |
| Former                                    | 33 (55.0) | 94 (52.2)            | 39 (54.2)  | 101 (46.8)           |
| Current                                   | 17 (28.3) | 3 (1.7)              | 25 (34.7)  | 14 (6.5)             |
| Pack-years, former and current smokers, mean (SD) | 43.9 (29.9) | 24.5 (25.8) | 56.3 (8.6) | 34.3 (27.1) |
| Alcohol consumption                       |           |                      |            |                      |
| None                                      | 12 (20.0) | 40 (22.2)            | 7 (9.7)    | 60 (27.8)            |
| Drinker                                   | 48 (80.0) | 140 (77.8)           | 65 (90.3)  | 156 (72.2)           |
| Drinks/wk, median (IQR)                   | 5.7 (4.4-12.3) | 1.3 (0.8-7.4) | 5.7 (3.1-13.3) | 1.6 (0.9-7.1) |
| Tumor site                                |           |                      |            |                      |
| Oral cavity                               | 19 (31.7) | NA                   | 24 (33.3)  | NA                   |
| Oropharynx                                | 12 (20.0) | NA                   | 13 (18.1)  | NA                   |
| Hypopharynx                               | 2 (3.3)   | NA                   | 3 (4.2)    | NA                   |
| Larynx                                    | 27 (45.0) | NA                   | 32 (44.4)  | NA                   |

* Unless otherwise indicated, data are reported as number (percentage) of participants.

Abbreviations: CPS-ii-NC, The American Cancer Society Cancer Prevention Study II Nutrition Cohort; HNSCC, head and neck squamous cell carcinoma; IQR, interquartile range; NA, not applicable; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial.

We also investigated association between β- and γ-HPVs and risk of HNSCC by anatomic site (eTable 2 in the Supplement). Oropharyngeal SCC was associated with detection of β1-HPV-5 (OR, 7.42; 95% CI, 0.98-56.82; P = .054), or β2-HPV-38 (OR, 7.28; 95% CI, 1.33-39.72) after adjusting for smoking, alcohol consumption, and HPV-16 detection (eTable 2A in the Supplement). For oral cavity SCC, we observed statistically significant associations with several β-HPV types, with ORs ranging from 3.75 to 8.09 in models adjusted for smoking and alcohol consumption (eTable 2B in the Supplement).

### Discussion

This is the first study to our knowledge to examine and demonstrate the temporal association between HPV DNA detection in mouthwash specimens and risk of incident HNSCC using a nested case-control design among 96 650 participants, cancer free at baseline, with available specimens from 2 large cohort studies. Our results show that HPV-16 detection in the oral...
cavity, which preceded cancer diagnosis for an average of 3.9 years, was associated with a 22.4-fold increased risk of incidental oropharyngeal SCC (95% CI, 1.8-276.7) after adjusting for smoking history and alcohol consumption, but not with risks of oral cavity or larynx SCCs. This association is consistent with results of prior case-control studies of oral HPV-16 and preva-
lent HNC,8,16,29-33 although such studies do not provide evidence for a temporal relationship. In addition, the observed associations of oral β- and γ-HPVs with risk of incident HNSCC is novel and suggests that the role of HPV in HNSCC may be more important than currently recognized.

Among sampled controls from both cohorts, oral prevalence of HPV-16, other high-risk oncogenic HPVs, and any α-HPV were 1.8%, 5.1%, and 13.9%, respectively, with HPV-16 being the most common α-HPV type in the oral cavity. In the NHANES cross-sectional data, Gillison et al34 reported oral prevalence of HPV-16, high-risk oncogenic HPVs, and any α-HPVs of 1.0%, 3.7%, and 6.9%, respectively, among 3579 men and women aged 14 to 69 years in the United States (2009-2010). In that study, the prevalence of any α-HPV was 11% and 4% among participants aged 55 to 64 and 65 to 69 years, respectively, and was higher in men than in women (10.1% vs 3.6%). Taking into consideration that the average ages of controls in our study were 71 and 63 years old in the CPS-II-NC and PLCO cohorts, respectively, and the majority were men, the NHANES HPV data are consistent with our findings. Oral HPV-16 prevalence in controls was also similar to the HPV-16 prevalence reported in the HPV Infection in Men study35 and to the pooled HPV-16 prevalence (1.3%) reported among 4581 healthy individuals from 18 different studies.36 Nevertheless, there is variability in HPV-16 prevalence by country of origin and at-risk populations.

Several case-control studies8,16,29-33 have examined associations between oncogenic α-HPV DNA detection in the oral cavity at the time of diagnosis of head and neck cancers; however, ORs have varied among the studies. In a hospital-based case-control study (201 cases, 333 controls) in Iowa City, Smith and colleagues29 reported ORs of 2.6 (95% CI, 1.5-4.2) and 3.6 (95% CI, 1.8-7.1) for associations between oral high-risk HPVs and HNC and oropharyngeal cancers, adjusting for smoking and alcohol consumption. In that study, HPV-16 was the most frequently detected oncogenic HPV type in oral exfoliated cells in both cases and controls, with prevalences of 19% and 10%, respectively.29 However, it should be noted that these reported prevalences are considerably higher than those reported in other studies. In another hospital-based, case-control study (72 HNC cases and 129 controls), Pintos et al30 reported an OR of 4.8 (95% CI, 1.2-19.4) for the association between oral high-risk HPV detection and HNC after adjustment for tobacco and alcohol intake, and an adjusted OR of 19.3 (95% CI, 2.3-159.5) when analysis was restricted to tonsillar cancers. Oral HPV-16 was not detected in controls but was present in 13 of 72 (18%) cases.30 Since HPV is differentially associated with anatomic sites of cancer development in the head and neck region, D’Souza et al14 carried out a hospital-based, case-control study of 100 patients with newly diagnosed oropharyngeal cancer and 200 control patients. The prevalence of oral HPV-16 was 32% in cases and 4% in controls, yielding an OR of 14.6 (95% CI, 6.3-36.6) after adjusting for smoking and alcohol consumption.34 Finally, the International Agency for Research on Cancer multicenter case-control study of oral cancer23 tested oral exfoliated cells among 90 oropharyngeal cancers, 511 oral cavity cancers, and 613 controls but did not observe HPV as a risk factor. The prevalence of HPV DNA in exfoliated oral cells was 8.9% and 4.7% among patients with oropharyngeal and oral cavity cancer, respectively, and 6.9% in controls.32

Only 1 study has shown that anti-E6 antibodies for HPV-16 detected in prospectively collected serum samples is positively associated with HNSCC incidence, in particular oropharyngeal cancer.18 However, to our knowledge, no study to date has provided information on the temporal association between oral HPV detection and the incidence of HNSCC or oropharyngeal SCC specifically (ie, evidence that HPV infection preceded the development of cancer). The lack of data on this issue is due, in part, to the relative rarity of these cancers, requiring large sample sizes for longitudinal prospective data and collection of mouthwash samples. We used the collection of oral mouthwash samples originally intended to isolate genomic DNA in 2 large prospective cohort studies with verified cancer end points to efficiently determine whether HPV detection in the oral cavity precedes cancer development.

In addition to α-HPV types, the oral cavity contains a plethora of β- and γ-HPV species and types,20 but their association with risk of HNSCC has not been adequately tested in prior studies. Our study is the first to examine prospectively the associations between oral detection of β- and γ-HPV types and risk of incident HNSCC. Oral DNA detection of any β- or γ-HPV was much higher than that of α-HPVs and was associated with a nearly 2-fold increased risk of HNSCC. After multiple comparisons were accounted for, the associations between β-HPV-5 type, any y11-HPV species, and y12-HPV species and increased risk of HNSCC remained statistically significant. The increased risk of HNSCC associated with β1-HPV-5 detection, previously implicated in epidermodysplasia verruciformis and cutaneous SCC,37-39 as well as other HPV types from γ11- and γ12-HPV species, implies a broader role for HPVs in HNSCC etiology.

We do not anticipate that the relationship between β- and γ-HPVs and HNSCC will involve similar mechanisms and/or follow the same molecular pathways that have been described for α-HPV-16 and other high-risk HPV types associated with cervical and HNSCC cancers.40,41 We anticipate that the association of β- and γ-HPVs with HNSCC might be more similar to the elusive role these types play in skin cancer.42-43 Howley and Pfister43 report that β-E6 and β-E7 proteins can influence DNA damage and apoptotic processes and inactivate pRB proteins, respectively. These papillomaviruses are anticipated to play a role in the initiation of the tumorigenic process since neither the viral genomes nor transcripts are detected in skin cancers. Based on this current understanding, it is unlikely that β- and γ-HPVs would be active in the tumor tissue itself.44

We were able to detect a relationship between β- and γ-HPV types and HNSCC for the first time based on the prospective design of the study and the sampling from an anatomic site (oral cavity) contiguous with the regions at risk for cancer development combined with the innovative detection and characterization of HPV. Such a study is complicated for the skin, given the high prevalence of HPV in the skin and hair follicles37-38 and the difficulty of sampling a specific anatomic site that will in the future develop skin cancer. Nevertheless, HPV vaccines to prevent skin cancer are under consideration.45
This study has strengths and limitations. A major strength is that it prospectively examined associations between DNA detection of \( \alpha \), \( \beta \), and \( \gamma \)-HPVs in mouthwash specimens collected prior to cancer diagnosis of HNSCC. This study also examined the full spectrum of \( \beta \)- and \( \gamma \)-HPVs that might contribute to risk of HNSCC after adjusting for smoking, alcohol consumption, and \( \alpha \)-HPV-16 detection. The use of a next-generation sequencing assay to detect known and unknown \( \beta \)- and \( \gamma \)-HPV types combined with a prospective study design allowed us to identify additional HPV types besides \( \alpha \)-HPV-16 that are associated with HNSCC.

Limitations of this study include the limited sample size of cases, reflecting the rarity of HNSCC and its anatomic subtypes. In addition, sequential oral mouthwash samples were unavailable to evaluate the risk of HNSCC associated with new and/or persistent HPV infections.

Conclusions

This study is the first to our knowledge to demonstrate that \( \alpha \)-HPV-16 detection precedes the incidence of oropharyngeal cancers. Risk of HNSCC identified with \( \gamma11 \) and \( \gamma12 \)-HPV species and \( \beta1 \)-HPV-5 type, previously associated with skin cancer, suggests a broader role for HPVs in HNSCC etiology. The use of easily collected oral mouthwash samples can provide a prospective marker for risk of HNSCC and oropharyngeal SCC.

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**Research Original Investigation**

**Human Papillomavirus Types and Incident Head and Neck Cancer**

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**Invited Commentary**

The Alpha, Beta, Gammas of Oral Human Papillomavirus Infection and Head and Neck Cancer Risk

Dana E. Rollison, PhD; Maura L. Gillison, MD, PhD

**Alpha human papillomavirus** (α-HPV) infection (overwhelmingly HPV-16) is an established cause of head and neck squamous cell carcinoma (HNSCC), predominantly oropharyngeal cancer (OPC). Although this is a widely accepted causal association, a critical piece of evidence has been missing: a link between oral HPV infection and subsequent risk of HNSCC. In this issue of *JAMA Oncology*, Agalliu et al re-report findings from a prospective investigation of oral HPV infection (i.e., HPV DNA detection in oral samples) and HNSCC risk. After analyzing 132 HNSCC cases and 396 controls nested within 2 prospective cohorts, the authors found that oral HPV-16 DNA detection was associated with a 22-fold increase in OPC risk. These data are remarkably consistent with risk estimates from numerous case-control studies. However, the study by Agalliu et al contributes to the existing body of evidence by supporting a temporal association between HPV exposure and HNSCC. Previous nested case-control studies have observed serological measures of HPV exposure (eg, seropositivity to HPV-16 viral capsid or E6 and/or E7 oncoproteins) to increase risk of OPC. By linking oral HPV detection directly to HNSCC risk, the Agalliu data have important implications for the use of oral HPV detection for OPC screening.

While the association between HPV-16 and OPC is well-established, a role for β- and γ-HPV types is less so and the subject of ongoing research. Notably, the study by Agalliu et al is the first to report a positive association between infection with β- and/or γ-HPV types and HNSCC. However, β-HPV infection has been previously implicated as a risk factor for cutaneous squamous cell carcinoma (cSCC). Characterized prior to γ-HPV types, β-HPV types have been studied more extensively and...