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Oral Alpha, Beta and Gamma HPV Types and Risk of Incident Esophageal Cancer

Ilir Agalliu[1], **Zigui Chen**[2,#], **Tao Wang**[1], **Richard B. Hayes**[3], **Neal D. Freedman**[4], **Susan M. Gapstur**[5], and **Robert D. Burk**[1,2,6]

[1]Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, New York (NY), USA

[2]Department of Pediatrics (Genetics), Albert Einstein College of Medicine, Bronx, NY

[3]Department of Population Health and Environmental Medicine, New York University, New York, NY, USA

[4]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland (USA)

[5]American Cancer Society, Atlanta, Georgia (USA)

[6]Departments of Microbiology and Immunology; and Obstetrics, Gynecology and Women's Health, Albert Einstein College of Medicine, Bronx, NY (USA)

Abstract

Background—Several studies have examined association between human papillomaviruses (HPVs) and esophageal cancer, but results have been inconsistent. This is the first prospective study to investigate associations between alpha, beta and gamma HPV detection in the oral cavity and risk of esophageal cancer.

Methods—We conducted a nested case-control study among 96,650 cancer-free participants in the American Cancer Society Cancer Prevention Cohort and the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Incident esophageal cancer cases (n=125) were identified during an average 3.9 years of follow-up. Three controls per case (n=372) were selected and matched on age, sex, race/ethnicity and time since mouthwash collection. Alpha, beta and gamma HPV DNA in oral samples was detected using a next-generation sequencing assay. Conditional logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI), adjusting for smoking and alcohol consumption. Statistical significance was evaluated using permutation test.

To Whom Correspondence should be addressed: Ilir Agalliu MD., ScD., Department of Epidemiology and Population Health, Albert Einstein College of Medicine, 1300 Morris Park Ave., Belfer Bldg Room 1315-B, Bronx, NY 10461, Phone: (718) 430-2929; Fax: (718) 430-8780; ilir.agalliu@einstein.yu.edu. Robert D. Burk, M.D., Department of Pediatrics (Genetics), Albert Einstein College of Medicine, 1300 Morris Park Avenue, Ullmann Building, Room 515, Bronx, NY 10461, Phone: (718) 430-3720; Fax: (718) 430-8975, robert.burk@einstein.yu.edu.

[#]Current Address: Department of Microbiology, The Chinese University of Hong Kong, Hong Kong, China.

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Results—Prevalence of oral alpha, beta, and gamma HPV was 18.4%, 64.8%, and 42.4% in cases and 14.3%, 55.1%, and 33.6% in controls, respectively. Oral HPV16 detection was not associated with esophageal cancer (OR=0.54, 95%CI 0.1–4.84) and none of the esophageal squamous cell carcinoma cases (n=28) were HPV16 positive. Some oral HPV types were more common in cases than controls; however, none of the associations were statistically significant.

Conclusion—Although HPVs in the oral cavity are very common, this study showed no evidence of association between oral HPVs and esophageal cancer.

Impact—Oral HPVs may not contribute to risk of esophageal cancer.

Keywords

esophageal cancer; oral sample; oral HPV; alpha HPV; HPV16; beta HPV; gamma HPV; prospective study; nested case-control study; squamous cell carcinoma; adenocarcinoma

Introduction

Esophageal cancer is the eighth most commonly diagnosed cancer worldwide and is the sixth most common cause of cancer deaths (1–4). These figures include both adenocarcinoma and squamous cell carcinoma (ESCC) the two major histologic types of esophageal cancer. There is a large geographic variation in incidence and mortality rates of esophageal cancer, with the highest incidence rates reported in Iran, China, India and South Africa (1, 2, 4). Esophageal SCC is the most common type in these regions; however, in the last decade there has been an increase in the incidence rates of adenocarcinoma. By contrast, in the Western world including the U.S., incidence and mortality rates of esophageal cancer are much lower, with adenocarcinoma being the most predominant type (5–7). Nevertheless, the 5-year survival rate of esophageal cancer in the U.S. is low and has remained fairly constant over the past decade (5).

The main risk factors for esophageal cancer include increasing age, male sex, cigarette smoking and alcohol consumption especially for ESCC, whereas gender, cigarette smoking, gastroesophageal reflux disease and obesity are risk factors for adenocarcinoma (7–13). Infection with oncogenic HPV as a contributor to ESCC was hypothesized over three decades ago (14). However, the International Agency for Research on Cancer (IARC) in a recent review concluded that there was inadequate evidence for HPV carcinogenicity in association with ESCC (15). Several tissue-based studies, which have examined detection of alpha HPV16 and HPV18 in esophageal cancer versus adjacent normal tissue, have yielded conflicting results (16–20). The majority of studies originating from China reported positive associations, while studies from the Western countries reported no association (16–21). Serologic case-control studies also provide conflicting evidence, with a meta-analysis reporting an odds ratio (OR) of 1.89 (95% confidence interval [CI] = 1.09 to 3.29) for HPV16 E6 antibodies and ESCC, but no association for E7 antibodies (22). By contrast, a large cohort study demonstrated no association between HPV16 E6 or E7 antibody seropositivity and risk of esophageal cancer (23).

To date, there has been no prospective study of oral HPV and risk of incident esophageal cancer. Moreover, recent data indicates that the oral cavity contains not only alpha HPVs,

including HPV16, but also a wide spectrum of other HPVs, namely beta and gamma HPV types (24, 25). We recently reported that in addition to HPV16, detection of several beta and gamma HPV species and types in the oral cavity were positively associated with risk of head and neck cancer independent of HPV16 (25). Therefore, we examined the association of alpha, beta and gamma HPV DNA detected in the oral cavity with subsequent risk of esophageal cancer, as well as with the risks of adenocarcinoma and ESCC types in a nested case-control study within two large prospective cohorts.

Materials and Methods

Study Cohorts and Data Collection

We conducted nested case-control studies amongst participants who provided mouthwash samples in two large prospective cohorts: the American Cancer Society Cancer Prevention Study-II Nutrition Cohort (CPS-II-NC) (26) and the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial (27). The CPS-II-NC cohort enrolled 184,192 men and women aged 50 to 79 years old residing in 21 U.S. states between 1992 and 1993; 53% were women and 97% were Caucasian (26). The PLCO trial enrolled 154,910 men and women aged 55 to 74 years old from 1993 through 2001 at 10 U.S. centers. Participants had no history of prostate, lung, colorectal or ovarian cancers at enrollment; 50% were women and 86% were Caucasian (27).

Participants in both cohorts completed self-administered baseline questionnaires, which collected information on demographics and social characteristics, previous cancer diagnoses, current and lifetime smoking history and alcohol consumption. Follow-up questionnaires were sent every two years to CPS-II-NC and annually to PLCO cohort members to update information on lifestyle exposures, health status, and to ascertain newly diagnosed cancers. Mouthwash samples were collected primarily for genomic DNA from 70,004 CPS-II-NC participants between 2001 and 2002 (26), and from 55,866 participants in the PLCO control arm between 1998 and 2005 (27, 28), who did not provide a blood sample.

Identification of incident cases of esophageal cancer and selection of controls

We designed parallel nested case-control studies among participants who provided informed consent, baseline questionnaire data, and a mouthwash sample. Of the 70,004 CPS-II-NC participants who provided a mouthwash sample, we excluded 16,664 who had a previous cancer diagnosis, 158 whose oral rinse specimens were inadequate, and two whose gender data were missing. Among the remaining 53,180 participants in the at-risk cohort, 51 were diagnosed with a primary incident esophageal cancer between the time of oral sample collection and the end of follow up (6/30/2009). Of the 55,866 PLCO control arm participants who provided a mouthwash sample, we excluded 5,526 who had a previous cancer diagnosis, and 6,870 whose oral rinse specimens were exhausted or unavailable. Among the remaining 43,470 participants in the at-risk cohort, 74 were diagnosed with a primary incident esophageal cancer between the time of oral sample collection and the end of follow up (7/31/2011). Thus, a total of 125 incident cases of esophageal cancer with available mouthwash samples were identified in both cohorts over an average 3.9 years of follow-up.

Three controls were selected for each case from the at-risk cohorts who were alive at the diagnosis date of the case and who had no prior history of cancer at that time. Controls were individually matched to cases on sex, race/ethnicity, date of birth (±6 months), and date of oral sample collection (±30 days for CPS-II-NC, and ±3 months for PLCO trial). A total of 372 controls with available mouthwash samples were used for the analysis (three cases had only two controls in their matched sets).

The present study was reviewed and deemed exempt by the institutional review board (IRB) of the Albert Einstein College of Medicine (Einstein). The original cohort studies received full IRB approval from both the American Cancer Society and the National Cancer Institute, and written informed consent was obtained from all study participants.

Molecular Detection of Oral HPV DNA

All HPV testing was performed at Einstein with the laboratory personnel blinded to case-control status of the mouthwash samples as previously described (25). Total DNA was purified from exfoliated oral cavity cells obtained from a Scope mouthwash rinse specimen (24, 25). As described previously (25) HPV DNA detection was performed using three different platforms: (1) The MY09/11 L1-targeted degenerate primer polymerase chain reaction (PCR) system using AmpliTaq Gold DNA Polymerase (Thermo Fisher Scientific, Waltham, MA), which preferentially detects alpha-HPV types (29); (2) a real time (RT)-PCR assay for HPV16 and (3) a multiplexed next-generation sequencing (NGS) method developed to detect and type the diverse and large number of alpha, beta and gamma HPVs present in the oral cavity (30). This method consisted of three separate PCR amplification assays that targeted primer-binding sites within the L1 (NG-S and NG-F assays) and E1 ORFs (NG-E1) (30). Each DNA sample was amplified using sample-specific barcoded primers. Successful amplification of predicted fragment sizes was verified by gel electrophoresis and PCR products were pooled and sequenced on an Illumina HiSeq 2000/2500 (Illumina Inc., San Diego, CA) at the Epigenomics Shared Facility at Einstein, using 150-bp paired-end reads. The reads were de-multiplexed, filtered for quality, and blasted against a PV reference database (30). We evaluated the sensitivity of the NG-S assay that was designed to specifically detect alpha-HPVs by serial dilution of an HPV16 plasmid and were able to detect this type at an input of plasmid DNA as low as 10 copies/μl. We evaluated specificity by comparing the NGS assays with MY09/FAP amplicon typing using oligonucleotide hybridization and the overall concordance rate and kappa value was 91.9% and 0.749, respectively.

Definition of HPV Type Positivity

Oral HPV type positivity was defined as previously described (25). Briefly, we considered a sample being positive for HPV16 if it scored positive in two out of three assays. For other alpha HPV types, we used both the MY09/11 PCR data and the NGS results, whereas for oral beta and gamma HPV types we relied on results of the NGS assays. Quality control analysis was carried out in 10% randomly selected oral samples for repeat testing; the agreement of the prevalence of HPV types between the two repeats was excellent (kappa ≥0.90).

Statistical Analyses

We examined association of alpha, beta and gamma HPV with incident esophageal cancer using conditional logistic regression models (CLR) for matched risk-sets to estimate odds ratios (OR) and 95% confidence intervals (CI) (31, 32). For alpha HPVs, we examined associations for the following exposures: HPV16; other high-risk (HR) oncogenic HPV types (15) i.e., HPV18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59; other non-HR alpha HPV types; and any alpha HPV type. For beta and gamma HPV species and types, we examined associations of any beta or of any gamma HPV type, different beta or gamma species groups, and specific beta or gamma HPV types with risk of esophageal cancer. The associations between the above HPV exposures and risk of esophageal cancer were adjusted for study cohort (CPS-II-NC vs. PLCO), smoking status (current or former smokers vs. never smokers), pack-years of smoking, and alcohol consumption (drinks/week). For the few participants with missing information on pack-years of smoking (3 cases and 9 controls from both cohorts) or alcohol consumption (22 cases and 46 controls from both cohorts), we imputed the missing data using the multiple imputation (MI) method in R-package (33). Since age, sex, race/ethnicity, and time since oral rinse collection were the matching variables, these were not included in CLR models.

A permutation procedure was also used to account for multiple comparisons of several HPV exposures and esophageal cancer (34, 35). For each replicate of 10,000 cycles, the matched pairs were permuted by shuffling the case-control status. For each permuted dataset, the CLR models were fit for HPV exposures and the minimum p-values were kept. This provided an empirical distribution of p-values under the null hypothesis of no association. The permutation p-value for an HPV exposure was obtained by comparing their observed p-values to this empirical distribution. Permutation p-values can be interpreted as the probability of observing a p-value less than or equal to what was observed under the null hypothesis of no associations of any of the HPV exposures and risk of esophageal cancer. After this procedure, an HPV exposure was considered to be statistically significantly associated with risk of esophageal cancer if the permuted p-value was <0.05 (two-sided).

We also examined the association of alpha, beta and gamma HPV with esophageal cancer by histologic type, i.e. ESCC and adenocarcinoma. To account for latency as well as potential for subclinical/undiagnosed cancer, we conducted a sensitivity analysis by excluding incident esophageal cancer cases (n=35) that were identified within the first two years of follow-up in both cohorts and their respectively matched controls (n=102). Lastly, we also examined the association between coinfection by multiple types of oral HPV and risk of esophageal cancer. All these statistical models were adjusted for the same variables as the models investigating the overall risk of esophageal cancer. All statistical analyses were carried out in STATA version 14 (Stata Corporation College Station, Texas).

Results

Demographic and lifestyle characteristics of incident cases of esophageal cancer and their matched controls are shown in Table 1. In the CPS-II-NC cohort, both cases and controls were on average 6 years older in comparison to the PLCO cohort. The majority of cases and controls in both cohorts were Caucasian males. Cases were more likely to be current

smokers in comparison to controls; however they were similar with respect to drinking habits. There were no major differences with regard to body mass index, education or marital status between the two groups (Table 1). With regard to tumor histology, the majority (64%) of esophageal cancers in both cohorts were adenocarcinoma (n=80), 22.4% (n=28) were ESCC and 13.6% were other histological types. The distribution of histological types was similar between the two cohorts.

In both cohorts, the prevalence of any oral HPV was 75.5% in cases vs. 69.4% in controls (p=0.26). The prevalence of any oral alpha, beta, and gamma HPV was 18.4%, 64.8%, and 42.4% among the cases and 14.3%, 55.1%, and 33.6% among the controls, respectively.

Associations of HPV16 and other alpha HPVs with risk of incident esophageal cancer

Among controls from both cohorts, the prevalence of oral HPV16, other high-risk (HR) oncogenic HPVs, and non-HR alpha HPVs was 1.6%, 4.9% and 9.7%, respectively (Table 2). Detection of HPV16 DNA in the oral samples was not associated with risk of esophageal cancer (OR = 0.54, 95%CI 0.10 – 4.84). There were also no associations of other HR-oncogenic HPVs after excluding HPV16, as well as non-HR alpha HPVs with risk of esophageal cancer (Table 2).

In the stratified analyses by tumor histology, no oral HPV16 DNA was detected among the 28 cases of ESCC, whereas, one out of 83 (1.2%) matched controls was HPV16 positive (Table 2). Interestingly, oral DNA detection of other HR-HPVs (after excluding HPV16) was associated with a higher risk of ESCC (OR=10.5; 95%CI 1.01 – 108.5); however, this result is based on only three HPV positive cases and one HPV positive control and was not statistically significant after adjusting for multiple comparisons. There were no associations of other non-HR HPV types with risk of ESCC. Furthermore, there were no associations of oral HPV16, other HR-oncogenic HPVs or non-HR alpha HPV with risk of adenocarcinoma of the esophagus (Table 2).

Association of Beta and Gamma HPVs with risk of incident esophageal cancer

Among controls from both cohorts, the prevalence of any beta HPV was 55.1% and any gamma HPV was 33.6%. As shown in Table 3, there was a borderline statistically significant association between any oral beta HPV (OR=1.57; 95%CI 1.00 – 2.47) and risk of esophageal cancer in the multivariate-adjusted model. However, there were no association of specific beta HPV species or types and esophageal cancer. Similarly, no associations were observed for gamma HPV species (Table 3). After accounting for multiple comparisons, there was no association between any beta or gamma HPV and esophageal cancer (all permutated p-values were >0.05).

We also investigated the association of beta and gamma HPV with risk of histological type of esophageal cancer (Table 4). Neither oral beta nor gamma HPV species nor types were associated with risk of ESCC (Table 4A). By contrast, oral detection of any beta HPV (OR=1.84; 95%CI 1.05 – 3.23) and any beta1 HPV (OR=1.74; 95%CI 1.00 – 3.04) were associated with risk of adenocarcinoma of the esophagus (Table 4B), although results were no longer statistically significant after accounting for multiple comparisons (all permutated

p-values were >0.05). There were also no associations between gamma HPV species and type and risk of esophageal adenocarcinoma (Table 4B).

To account for latency as well as potential for subclinical/undiagnosed cancer, we excluded incident esophageal cancer cases (n=35) that were identified within the first two years of follow-up in both cohorts and their respectively matched controls (n=102). We did not observe any association of oral alpha, beta or gamma HPV types and esophageal cancer in this sensitivity analysis. There were also no statistically significant associations between oral HPV coinfection and risk of esophageal cancer.

Discussion

This is the first prospective study to examine the associations of molecularly detected alpha, beta and gamma HPVs in the oral cavity with risks of esophageal cancer overall and by histological type. Our results show that in general alpha, beta and gamma HPV detected in oral samples was neither associated with risk of overall esophageal cancer nor with risks of ESCC or adenocarcinoma types. Although, oral high-risk alpha HPVs (excluding HPV16) were associated with a 10-fold higher risk of ESCC, the number of cases and controls with positive test results was very small, the 95% confidence interval was wide and permutated p-values were not significant. In addition, the lack of association of any HPV type with esophageal cancer after excluding cases and their corresponding matched controls ascertained in the first two years of follow-up, further supports the null findings.

Among sampled controls from both cohorts, oral prevalence of HPV16, other high-risk oncogenic HPVs and any alpha HPV were 1.6%, 5.4% and 14.3%, respectively, with HPV16 being the most common alpha HPV type in the oral cavity. These prevalences were similar to those observed in our recent study of oral HPVs and head and neck cancers using a different sample of controls from the same cohorts (25). In the NHANES cross-sectional data, Gillison et al. (36) also reported oral prevalence of HPV16, high-risk oncogenic HPVs and any alpha HPVs of 1.0%, 3.7% and 6.9% among 5,579 men and women aged 14 to 69 years in the US (2009–2010). In that study, the prevalence of any alpha HPV was 11% and 4% among participants aged 55–64 and 65–69 years, respectively, and was higher in men in comparison to women, which is consistent with our data. Finally, oral HPV16 prevalence in our controls was also similar to the HPV16 prevalence reported in the HPV Infection in Men study (37) as well as to the pooled HPV16 prevalence of 1.3% reported among 4,581 healthy individuals from 18 different studies (38).

Results of several tissue-based studies that examined the relationship between HPV16 and HPV18 DNA detection in esophageal cancer versus adjacent normal tissue, or tissues from individuals without cancer (controls) have been inconsistent (16–18, 20, 21). A number of studies from China have reported a positive association between oncogenic HPVs and esophageal cancer (17, 18). For example, Zhang and colleagues (17) in their meta-analysis of 10 studies, which included 1,442 esophageal cancer cases and 1,602 controls, reported a pooled OR of 6.36 (95%CI: 4.46, 9.07) for HPV16 DNA detection in cancer vs. adjacent normal tissue. In another meta-analysis of tissue DNA detection of HPV16 and HPV18 and risk of esophageal cancer, Wang et al. (18) reported a pooled OR=1.62 (95%CI 1.33–1.98).

Methodological issues of some of the studies included in these meta-analyses were that HPV16 DNA detection in paraffin-embedded tissues was examined in cancer vs adjacent normal tissues in the same subject, and there was substantial heterogeneity with respect to geographic region, control group selection, and various HPV detection methods. By contrast similar studies that examined the relationship between HPV16 and other high-risk HPVs DNA detection in tissue with ESCC in the Western countries reported modest or no association (16, 20, 21). A meta-analysis of serologic case-control studies reported an OR of 1.89 (95%CI, 1.09 – 3.29) between HPV16 E6 antibodies and risk of ESCC, but there was no association for E7 antibodies (22). However, a recent large prospective cohort study, where antibodies were measured in the blood before cancer, demonstrated no association between HPV16 E6 or E7 antibody seropositivity and risk of esophageal cancers (23).

To our knowledge, no prior study to-date has provided information on the potential temporal association between oral HPV detection and subsequent incidence of esophageal cancer (i.e., evidence that oral 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, particularly ESCC in the US, requiring large sample sizes for prospective collection of mouthwash samples and subsequent follow-up for cancer incidence. We utilized the collection of oral samples originally intended for isolation of genomic DNA in two large prospective cohorts with verified cancer endpoints that provided the opportunity to efficiently determine whether HPV DNA detection in the oral cavity precedes cancer development. This is a critical component to determine whether oral HPV is associated with incident esophageal cancer. Indeed, the temporal relationships of HPV infection with risk of cervical (39) and oropharyngeal cancers (25) are well established.

In addition to alpha HPV types, the oral cavity contains a large number of beta and gamma HPV species and types (24) (25), and our study is the first to examine associations of beta and gamma oral HPV types with risk of esophageal cancer. There was a modest signal of an association between any beta HPV and any beta1 HPV and risk of esophageal adenocarcinoma, but we could not identify a specific type responsible for this association, unlike the type-specific associations we previously reported for beta1 HPV5 and gamma-11 and 12 species with risk of head and neck cancers in the same cohorts (25). Moreover, results were no longer statistically significant after accounting for multiple comparisons. In addition, we did not observe any association of HPV DNA detection of other oral beta and gamma types and species with risk of esophageal cancer in this study.

A major strength of this study is the prospective design to examine associations of incident esophageal cancer with HPV DNA detection of alpha, beta and gamma types in oral specimens collected prior to cancer diagnosis. This is also the first study to examine the full spectrum of beta and gamma HPVs that might contribute to risk of esophageal cancer after adjusting for smoking and alcohol consumption. Limitations of this study include the modest number of cases, particularly for ESCC, which is a relatively rare type of esophageal cancer in the U.S. In addition, sequential oral mouthwash samples were unavailable to evaluate the risk of new infection and/or persistent HPV infections associated with esophageal cancer. Finally, the majority of participants in this study were Caucasian, and therefore it is unclear if the results can be generalized to other race/ethnicities.

In conclusion, this study demonstrates that HPV16 and other oral alpha, beta and gamma HPVs are not associated with risk of esophageal cancer.

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Abbreviations

CPS-II-NC	American Cancer Society Cancer Prevention Study-II Nutrition Cohort
PLCO	Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial
HPV	human papilloma virus
SCC	squamous cell carcinoma
OR	odds ratio
CI	confidence interval,

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Table 1

Selected characteristics of incident cases of esophageal cancer and their matched controls in each cohort study

Characteristics Matching Variables	ACS CPS-II NC Cohort			PLCO Cohort		
	Cases N= 51	Controls N= 153	p	Cases N= 74	Controls N = 219	p
Age at mouthwash collection; mean (SD)	71.4 (6.1)	71.4 (6.0)	0.97	65.2 (5.6)	65.3 (5.5)	0.82
Months from mouthwash collection to Dx; mean (SD)	35.3 (24.5)	35.3 (24.3)	1.00	52.6 (30.1)	53.3 (29.9)	0.85
Gender; n %			1.00			0.97
Female	8 (15.7)	24 (15.7)		15 (20.3)	44 (20.1)	
Male	43 (84.3)	129 (84.3)		59 (79.7)	175 (79.9)	
Race/Ethnicity; n %			1.00			0.99
Caucasian	50 (98.0)	150 (98.0)		67 (90.5)	198 (90.4)	
African-American/Other	1 (2.0)	3 (2.0)		7 (9.5)	21 (9.6)	
Unmatched Variables						
BMI group (kg/m2); n (%)			0.38			0.89
< 25	22 (43.1)	50 (32.7)		18 (24.3)	55 (25.1)	
25–29.9	18 (35.3)	63 (41.2)		37 (50.0)	113 (51.6)	
≥ 30	5 (9.8)	26 (17.0)		17 (23.0)	48 (21.9)	
Missing	6 (11.8)	14 (9.1)		2 (2.7)	3 (1.4)	
Smoking status; n (%)			0.01			0.01
Never	11 (21.6)	60 (39.2)		20 (27.0)	99 (45.2)	
Former	32 (62.7)	86 (56.2)		44 (59.5)	108 (49.3)	
Current	8 (15.7)	7 (4.6)		10 (13.5)	12 (5.5)	
Pack-years, former and current smokers; mean (SD)	31.5 (26.7)	31.9 (31.9)	0.94	54.0 (30.4)	36.2 (28.3)	0.0002
Alcohol consumption; n (%)						
None	16 (31.4)	55 (35.9)	0.55	23 (31.1)	73 (33.3)	0.72
Drinker	35 (68.6)	98 (64.1)		51 (68.9)	146 (66.7)	

Characteristics Matching Variables	ACS CPS-II NC Cohort			PLCO Cohort		
	Cases N= 51	Controls N= 153	p	Cases N= 74	Controls N = 219	p
Drinks/week; median (IQR)	5.7 (2.1 – 18.1)	3.4 (1.0 – 9.1)	0.10	5.8 (1.1 – 14.6)	2.4 (1.1 – 7.0)	0.13
Education; n %			0.94			0.41
<12 grade	3 (5.9)	12 (7.8)		2 (2.7)	17 (7.8)	
High School/Vocational	15 (29.4)	39 (25.5)		30 (40.5)	77 (35.2)	
Some College	10 (19.6)	33 (21.5)		10 (13.5)	38 (17.4)	
College Graduate	10 (19.6)	29 (19.0)		13 (17.6)	39 (17.8)	
Graduate Degree	12 (23.5)	39 (25.5)		18 (24.3)	48 (21.9)	
Missing	1 (2.0)	1 (0.7)		1 (1.4)	-	
Marital Status; n %			0.48			0.85
Married	40 (78.4)	113 (73.9)		60 (81.1)	175 (79.9)	
Separated/Divorced	-	2 (1.3)		7 (9.5)	20 (9.1)	
Widowed	2 (3.9)	12 (7.8)		4 (5.4)	18 (8.2)	
Never Married	-	-		2 (2.7)	6 (2.7)	
Missing	9 (17.6)	26 (17.0)		1 (1.4)	-	
Tumor Histology; n (%)						
Adenocarcinoma	31 (60.8)	N/A		49 (66.2)	N/A	
Squamous cell carcinoma	12 (23.5)	N/A		16 (21.6)	N/A	
Other	8 (15.7)	N/A		9 (12.2)	N/A	

BMI – body mass index, SD – standard deviation, IQR – inter-quartile range

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Table 2

Associations of HPV16, high-risk (HR) oncogenic HPV and other alpha HPV types with risks of esophageal cancer and histological type

All Esophageal Cancers						
Alpha HPV Type	**Cases (n=125)**	**Controls (n=371)**[†]	**Adjusted Model**[*]			**Permutated P**[ξ]
	N (%)	**N (%)**	**OR**[†]	**95% CI**	**p**	
HPV16	1 (0.8)	6 (1.6)	0.54	0.10 – 4.84	0.58	>0.999
HR-HPVs[‡] excluding HPV16	8 (6.4)	18 (4.9)	1.21	0.49 – 2.99	0.68	>0.999
Non-HR HPV types	16 (12.8)	36 (9.7)	1.47	0.77 – 2.81	0.24	0.801
Any Alpha HPV	23 (18.4)	53 (14.3)	1.39	0.78 – 2.48	0.26	0.823
Esophageal Squamous Cell Carcinoma (ESCC)						
Alpha HPV Type	Cases (n=28)	Controls (n=83)	OR[†]	95% CI	p	Permutated **P**[ξ]
HPV16	0 (0)	1 (1.2)	-	-	-	>0.999
HR-HPVs[‡] excluding HPV16	3 (10.7)	1 (1.2)	10.52	1.01 – 108.5	0.048	0.236
Non-HR HPV types	4 (14.3)	11 (13.3)	1.68	0.41 – 6.91	0.48	>0.999
Any Alpha HPV	6 (21.4)	13 (15.7)	2.21	0.61 – 8.05	0.23	0.772
Esophageal Cancer: Adeno-Carcinoma						
Alpha HPV Type	Cases (n=80)	Controls (n=238)	OR[†]	95% CI	p	Permutated **P**[ξ]
HPV16	1 (1.3)	5 (2.1)	0.60	0.10 – 5.85	0.66	>0.999
HR-HPVs[‡] excluding HPV16	3 (3.8)	14 (5.9)	0.52	0.14 – 1.96	0.33	>0.999
Non-HR HPV types	8 (10.0)	21 (8.8)	1.21	0.51 – 2.86	0.66	>0.999
Any Alpha HPV	12 (15.0)	35 (14.6)	0.98	0.46 – 2.08	0.96	>0.999

[†]One control had missing alpha HPV exposure and thus was excluded from these analyses

[*]OR and 95% CI were estimated from conditional logistic regression models adjusted for smoking, alcohol consumption and study cohort.

[‡]High-risk oncogenic (HR) HPVs include HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59

[ξ]Permutated P-values were calculated to account for multiple comparisons (see methods)

Table 3

Associations of beta and gamma HPV species and types with risk of esophageal cancer

Beta HPV Species†	Cases (n=125)		Controls (n= 372)		Adjusted Model*			Permutated P§
	N	%	N	%	OR*	95% CI	p	
Any Beta HPV	81	64.8	205	55.1	1.57	1.00 – 2.47	0.048	0.538
Any Beta1 HPV	59	47.2	146	39.3	1.49	0.96 – 2.31	0.08	0.716
Any Beta2 HPV	64	51.2	160	43.0	1.34	0.88 – 2.05	0.17	0.951
Any Beta3 HPV	24	19.2	60	16.1	1.26	0.73 – 2.18	0.41	>0.999
Specific Beta HPV Types†								
Beta1 HPV5	22	17.6	42	11.3	1.75	0.97 – 3.16	0.07	0.655
Beta1 HPV12	11	8.8	27	7.3	1.24	0.58 – 2.65	0.58	>0.999
Beta1 HPV20	22	17.6	46	12.4	1.53	0.87 – 2.70	0.14	0.908
Beta1 HPV36	15	12.0	33	8.9	1.20	0.61 – 2.35	0.60	>0.999
Clade of Beta1 HPVs 5, 36, 47, & 143	26	20.8	59	15.9	1.34	0.79 – 2.27	0.28	0.995
Beta1 HPV105	14	11.2	29	7.8	1.31	0.65 – 2.63	0.45	>0.999
Beta1 HPV124	10	8.0	28	7.5	1.01	0.46 – 2.21	0.99	>0.999
Beta2 HPV23	12	9.6	26	7.0	1.34	0.64 – 2.81	0.44	>0.999
Beta2 HPV37	8	6.4	28	7.5	0.97	0.42 – 2.27	0.95	>0.999
Beta2 HPV38	24	19.2	57	15.3	1.28	0.75 – 2.18	0.37	>0.999
Beta2 HPV107	17	13.6	29	7.8	1.85	0.94 – 3.62	0.08	0.703
Gamma HPV Species†								
Any Gamma HPV	53	42.4	125	33.6	1.38	0.89 – 2.13	0.15	0.924
Any Gamma7 HPV	20	16.0	49	13.2	1.11	0.62 – 2.01	0.72	>0.999
Any Gamma8 HPV	13	10.4	38	10.2	1.02	0.52 – 2.01	0.95	>0.999
Any Gamma10 HPV	13	10.4	28	7.5	1.33	0.66 – 2.71	0.42	>0.999
Any Gamma12 HPV	8	6.4	19	5.1	1.34	0.56 – 3.20	0.51	>0.999
Any Gamma15 HPV	13	10.4	28	7.5	1.39	0.66 – 2.89	0.39	>0.999

†Beta and gamma HPV species and types presented have a prevalence of 5% or higher either in cases or controls

*OR and 95% CI were estimated from conditional logistic regression models adjusted for smoking, alcohol consumption and study cohort.

ξPermutated P-values were calculated to account for multiple comparisons (see methods)

Table 4

Associations of beta and gamma HPVs species and types with risk of histological type of esophageal cancer

4A. Esophageal Squamous Cell Carcinoma (ESCC)								
	Cases (n=28)		**Controls (n=83)**		**Adjusted Model***			**Permutated P$^{\xi}$**
Beta HPV Species†	**N**	**%**	**N**	**%**	**OR***	**95% CI**	**P**	
Any Beta HPV	14	50.0	41	49.4	1.05	0.37 – 2.97	0.93	>0.999
Any Beta1 HPV	10	35.7	27	32.5	1.19	0.42 – 3.35	0.75	>0.999
Any Beta2 HPV	11	39.3	33	39.8	0.99	0.36 – 2.72	0.99	>0.999
Any Beta3 HPV	3	10.7	19	22.9	0.35	0.1 – 1.48	0.15	0.792
Specific Beta HPV Types†								
Beta1 HPV5	5	17.9	10	12.1	1.56	0.44 – 5.53	0.49	>0.999
Beta1 HPV12	3	10.7	9	10.8	1.24	0.26 – 5.79	0.79	>0.999
Beta1 HPV20	3	10.7	8	9.6	1.10	0.25 – 4.81	0.89	>0.999
Beta1 HPV36	4	14.3	6	7.2	1.72	0.36 – 8.14	0.50	>0.999
Clade of Beta1 HPVs 5, 36, 47, & 143	6	21.4	12	14.5	1.31	0.39 – 4.39	0.66	>0.999
Beta2 HPV23	2	7.1	6	7.2	1.31	0.20 – 8.38	0.78	>0.999
Beta2 HPV38	4	14.3	16	19.3	0.62	0.18 – 2.11	0.45	>0.999
Gamma HPV Species†								
Any Gamma HPV	12	42.9	26	31.3	1.31	0.51 – 3.31	0.57	>0.999
Any Gamma7 HPV	3	10.7	6	7.2	0.81	0.15 – 4.48	0.81	>0.999
Any Gamma8 HPV	3	10.7	9	10.8	0.61	0.12 – 3.11	0.55	>0.999
Any Gamma10 HPV	4	14.3	8	9.6	1.46	0.37 – 5.82	0.59	>0.999
Any Gamma15 HPV	3	10.7	8	9.6	1.22	0.29 – 5.14	0.79	>0.999

4B. Esophageal Adenocarcinoma								
	Cases (n=80)		**Controls (n=239)**		**Adjusted Model***			**Permutated P$^{\xi}$**
Beta HPV Species†	**N**	**%**	**N**	**%**	**OR***	**95% CI**	**p**	
Any Beta HPV	56	70.0	134	56.1	1.84	1.05 – 3.23	0.04	0.199
Any Beta1 HPV	41	51.3	94	39.3	1.74	1.00 – 3.04	0.05	0.296
Any Beta2 HPV	42	52.5	105	43.9	1.34	0.80 – 2.25	0.27	>0.999

4B. Esophageal Adenocarcinoma								
	Cases (n=80)		Controls (n=239)		Adjusted Model*			Permutated P$^{\xi}$
Beta HPV Species†	**N**	**%**	**N**	**%**	**OR***	**95% CI**	**p**	
Any Beta3 HPV	18	22.5	34	14.2	1.86	0.95 – 3.63	0.07	0.363
Specific Beta HPV Types†								
Beta1 HPV5	14	17.5	27	11.3	1.78	0.85 – 3.72	0.13	0.605
Beta1 HPV12	6	7.5	16	6.7	1.11	0.42 – 2.93	0.83	>0.999
Beta1 HPV20	14	17.5	31	13.0	1.42	0.70 – 2.91	0.33	>0.999
Beta1 HPV36	10	12.5	23	9.6	1.13	0.50 – 2.55	0.77	>0.999
Clade of Beta1 HPVs 5, 36, 47, & 143	17	21.3	39	16.3	1.36	0.71 – 2.58	0.35	>0.999
Beta1 HPV105	11	13.8	20	8.4	1.62	0.72 – 3.63	0.24	>0.999
Beta1 HPV124	7	8.8	20	8.4	1.06	0.42 – 2.67	0.90	>0.999
Beta2 HPV23	9	11.3	17	7.1	1.67	0.70 – 3.97	0.24	>0.999
Beta2 HPV37	8	10.0	21	8.8	1.21	0.49 – 2.97	0.68	>0.999
Beta2 HPV38	15	18.8	34	14.2	1.48	0.75 – 2.92	0.26	>0.999
Beta2 HPV107	10	12.5	19	8.0	1.69	0.72 – 3.98	0.23	>0.999
Gamma HPV Species†								
Any Gamma HPV	32	40.0	77	32.2	1.53	0.86 – 2.70	0.15	0.668
Any Gamma7 HPV	13	16.3	34	14.2	1.12	0.54 – 2.34	0.76	>0.999
Any Gamma8 HPV	4	5.0	21	8.8	0.43	0.14 – 1.36	0.15	>0.999
Any Gamma10 HPV	7	8.8	17	7.1	1.38	0.52 – 3.66	0.52	>0.999
Any Gamma12 HPV	7	8.8	13	5.4	1.92	0.72 – 5.14	0.19	>0.999
Any Gamma15 HPV	8	10.0	14	5.9	1.93	0.69 – 5.42	0.21	>0.999

†Beta and gamma HPV species and types presented have a prevalence of 5% or higher either in cases or controls

*OR and 95% CI were estimated from conditional logistic regression models adjusted for smoking, alcohol consumption, and study cohort

$^{\xi}$Permutated P-values were calculated to account for multiple comparisons (see methods)