Geographic heterogeneity in the prevalence of human papillomavirus in head and neck cancer

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Abstract (197 words)

Human papillomavirus (HPV) causes oropharyngeal squamous cell carcinoma (OPSCC), although strongly divergent results have been reported regarding the prevalence of HPV16 in different countries, whether this represents important differences in etiology remains unclear. Applying rigorous protocols for sample processing, we centrally evaluated 1420 head and neck tumors (533 oropharynx, 395 oral cavity and 482 larynx) from studies conducted in the US, Europe and Brazil for mucosal HPV DNA and $p16^{INK4a}$ expression to evaluate regional heterogeneity in the proportion of HPV16-associated OPSCC and other head and neck cancer, and to assess covariates associated with the risk of HPV16-positive OPSCC. While majority of OPSCC in the US (60%) were HPV16-positive, this proportion was 31% in Europe and only 4% in Brazil (p<0.01). Similar differences were observed for other head and neck tumors, ranging from 7% in the US and 5% in Europe, to 0% in South America. The odds of HPV16-positive OPSCC declined with increasing pack years of smoking (OR: 0.75; 95% CI: 0.64 -0.87) and drink years of alcohol use (OR: 0.64; 95% CI: 0.54-0.76). These results suggest that while the contribution of HPV16 is substantial for the oropharynx, it remains limited for oral cavity and laryngeal cancers.

Novelty and Impact

Whether geographic heterogeneity regarding the extent of HPV16 infection in HNC represents etiologic differences remains unclear. Here authors show that the proportion of HPV16-positive oropharynx cancer was ~60% in the U.S., 31% in Europe and 4% in Brazil. Less than 4% of laryngeal and oral cavity cancers were HPV16-positive. These results suggest that the U.S. would likely experience the largest benefit from prophylactic HPV vaccination and Brazil the least, with Europe being intermediate.

Keywords: HPV16, oropharynx, head and neck cancer, smoking, alcohol, prevalence

Abbreviations: HPV16- human papillomavirus type 16; OPSCC- Oropharyngeal squamous cell carcinoma; HNC: Head and neck cancer; FFPE: formalin-fixed paraffin-embedded; ISH: in situ hybridization

Introduction

Head and neck cancers (HNC) contribute nearly 600,000 new cases diagnosed, and over 300,000 deaths[1] each year. Alongside traditional risk factors such as smoking and excessive alcohol consumption[2, 3], human papillomavirus type 16 (HPV16) infection has been recognized to cause a subset of these cancers[4, 5].

Strongly divergent results have been reported regarding the extent of HPV16 infection in HNC in different countries[6-8]. Studies in the U.S. suggest that the majority of oropharyngeal squamous cell carcinoma (OPSCC) are now caused by HPV16[9-11], although proportions of less than 10% have been reported in the few studies completed in South America[12-14], with European estimates being in between[15-17]. Further, the role of HPV16 in HNC outside the oropharynx remains unclear[8]. A recent review has estimated that the probability of an HPV-attributable cancer of the oral cavity, larynx, and hypopharynx could be up to five times lower than that of oropharyngeal cancer[18]. Whether these divergent geographic results represent important differences in the etiology of HNC or whether they are explained by differences in laboratory practices is unknown. The recent publication on global HPV prevalence in HNC is of note where, based on a comparison of over 3000 tumors tested for HPV DNA and subsequently, following triage for HPV16 E6*mRNA and p16^{INK4a}, the authors report that nearly 22% of OPSCC could be attributed to HPV infection, while fewer than 5% of oral cavity and laryngeal cancers were HPV-positive[19]. The study also reported strongly divergent results for the South and Central America (OPSCC HPV prevalence of 37%), and did not provide estimates for North America. Although the largest study to date, this report was unable to explore lifestyle factors associated with HPV-positivity.

We centrally tested a large series of HNCs from three different continents using a combination of HPV16 DNA detection and p16^{INK4a} expression, our aims were to (i) evaluate regional heterogeneity in the proportion of HPV16-associated OPSCC and other HNC following rigorous protocols of sample processing; and (ii) evaluate covariates associated with the risk of HPV16-positive OPSCC.

Methods

The study was designed to include at least 400 HNCs, including 110 OPSCCs, from three HNC case-control studies conducted in the U.S., Europe and Brazil. All tumors were histologically confirmed diagnoses, and squamous cell in origin. HNC included cancers arising at the oral cavity (International Classification of Diseases for Oncology [ICD-O] C00.3–C00.9, C02.0–C06.9, C14.0-C14.9, excluding C02.4, C05.1, C05.2), oropharynx (ICD-O: C01, C02.4, C09, C10), hypopharynx and larynx (ICD-O: C13, C32), and non-specified and overlapping sites (ICD-O: C05.1-C05.2, C14.0, C014.2, C014.8).

The Carolina Head and Neck Cancer (CHANCE, North Carolina, U.S.) study recruited population-based incident HNCs and matched controls between 2002 and 2006[20]. This analysis included 477 tumors comprising 123 oral cavity, 243 oropharynx, 107 laryngeal and 4 tumors of overlapping sites. The European Alcohol Related Cancers and Genetic susceptibility in Europe (ARCAGE) study was conducted across 10 countries in Europe[21]. Tumor samples from 539 HNCs from Germany (n=181), Italy (n=289) and the UK (n=69) were included that consisted of 165 oral cavity, 119 oropharynx, 251 larynx and 4 cancers of overlapping sites. The Brazilian Head and Neck Genome Project (GENCAPO, São Paulo, Brazil) study recruited incident HNC patients and matched controls during 2002 to 2015[22, 23]. Four hundred and four HNCs included 107 oral cavity, 171 oropharyngeal, 124 larynx and 2 cancers of overlapping sites. Risk factor and lifestyle data were combined; briefly, tobacco use was categorized as ever or never smokers; ever smokers were defined as individuals who smoked any tobacco product (U.S.), at least once a week for a year (Europe), or daily for a year (Brazil). Pack years were calculated for all types of tobacco smoking based on cigarette equivalents, where 1 cigar equaled 5 cigarettes, 1 pipe equaled 4 cigarettes and 1 hand-rolled cigarette equaled 5 store-bought cigarettes (Brazil only). Ever drinkers were those who reported ever consumption of any alcoholic beverage (US, and Europe) or at least once a month (Brazil). A drink-year was defined as two drinks per week for one year, where a drink equaled 330mL of beer, 125mL of wine, and 50mL of liquor.

A tumor sectioning protocol was established as described previously[24] that was applied to tumors from Brazil and Europe, while in the U.S., pre-sectioned slides generated using a similar scheme were available and included. All tumors were subjected to pathology assessment completed centrally at IARC. Briefly, pathology evaluation determined 3.7% of all cases as ineligible due to insufficient tumor tissue or necrotic tissue, of which 0.8% were from the U.S., 5.4% from Europe and 4.4% from Brazil. The $p16^{INK4a}$ expression was evaluated using the CINtec Histology $P16^{INK4a}$ Kit (9511, mtmlabs) following manufacturer's instructions. Staining and scoring was performed blinded with respect to tumor subsite and HPV DNA status. Expression was scored based on the percentage and intensity of nuclear and/or cytoplasmic staining, a composite score of 4 or greater was considered positive for $p16^{INK4a}$ overexpression[25]. However, the proportion of p16 invalid tumors was under 1% and did not vary by study. DNA was extracted from tumor tissues[26] and HPV genotyping was performed using the Type-Specific E7 PCR bead-based multiplex assay (TS-E7-MPG, IARC, Lyon, France)[27-29]. Briefly, reporter fluorescence was quantified using Luminex reader 200 (Luminex Corporation, Austin, TX) and cutoffs were computed by adding 5 to 1.1 x the median background value expressed as median fluorescence intensity (MFI). HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV58, HPV59 were considered carcinogenic[5, 30]. Since HPV16 accounts for over 95% of all HPV-positive HNC[8, 12, 31, 32], the main results described in this manuscript are focused on this type. The largest proportion of betaglobin negative samples were noted in the Brazilian study (14%), compared to the U.S. (2.4%) or European studies (4.6%). We further included several quality indicators at each stage of HPV testing including: (i) sectioning empty paraffin block every 10 tumors, (ii) one DNA extraction control per batch, (iii) one PCR control per amplification plate, (iv) one negative water hybridisation control per batch, and (v) randomly selected tumors for retesting from each of the three studies (~5%). HPV testing was completed centrally at the International Agency for Research on Cancer (IARC). Twenty nine of 188 empty paraffin sections yielded a positive result for the betaglobin control gene (all were HPV DNA negative) reflecting a contamination rate of 15%. None of the other technical controls were positive for either the beta globin or HPV DNA. Among the 208 HNCs randomly retested, 8 tumors (3 each from Brazil and Europe and 2 from the U.S.) were discordant for either HPV DNA (n=6, 4 for HPV16 DNA) or betaglobin status (n=2). The discordance rate in the duplicate testing was under 4%, and did not vary by region.

Characteristics of cases were compared using Pearson's chi-squared statistic for categorical and Kruskal-Wallis test for continuous variables. The primary outcome was HPV16-positive tumors defined as being HPV16 DNA and $P16^{INK4a}$-positive, with cases that were HPV16 DNA or $P16^{INK4A}$-negative considered HPV16-negative. Unconditional logistic regression was used to estimate odds ratios (OR) and corresponding 95% confidence intervals (95% CI) of HPV16-positive OPSCC using covariates of age, sex, education level, stage, region, smoking (10 pack-year intervals) and alcohol consumption (10 drink-year intervals). The incidence rates of HPV16-positive and –negative OPSCC and Non-OPSCC were estimated by multiplying the incidence tae per 100,000 with the corresponding HPV16 prevalence observed in the present study. All statistical analyses were performed using STATA statistical software, version 11 (StataCorp, College Station, TX), and all reported *P* values are two sided. Statistical significance was set at *P* less than .05.

Results

This analysis included 1420 HNCs, including 477 from the U.S., 539 from Europe and 404 from Brazil (Table 1). European subjects were only marginally older (median age of 60) than the U.S. (median age=56) and Brazilian subjects (median ages= 57) (p<0.01), current smoking and drinking were high in all three regions. More than two thirds of the HNC cases were diagnosed at late stages of the disease in Brazil (69% stage IV) compared to the less than half of cases in the U.S. and Europe (~47% stage IV).

Majority of the tumors were positive for a single HPV type (~95%), with HPV16 DNA contributing 92% of all HPV DNA positivity (Supplementary table 1), and HPV35 (3.5%), HPV18 (2.7%) and HPV33 (1.5%) were also observed. The proportion of HPV16-positive OPSCC differed dramatically by continent, U.S. had the highest proportion (59.3%), Brazil the lowest (4.1%), and Europe reflected intermediate HPV16 prevalence (31.1%) (Table 2). The proportion of HPV16-positive tumors did not vary among the three European countries. Regarding oral cavity cancer, 10.6% of tumors in the U.S. and 6.1% in Europe were HPV16-positive (p=0.20), whereas 2.8% of larynx tumors in the U.S. and 5.2% in Europe were HPV16-positive (p=0.30). None of the 231 oral or larynx cancer cases in Brazil were HPV16-positive. In order to clarify whether subsite misclassification could contribute to some HPV16-positive oral cavity cancers, we reassessed the sub-anatomic classification of these tumors based on ICD-O codes. Twelve of the 23 HPV16-positive oral cavity tumors were lesions overlapping the tongue, HPV16 prevalence when excluding these was 2.8% (95% CI: 1.19- 5.55). When we examined the marginal incidence of HPV16-positive OPSCC, similar results were observed; the highest incidence of HPV-positive OPC are reflected in the U.S. (2 per 100,000), followed by Europe (~1 per 100,000) and the least in Brazil (~0.2 per 100,000). Similarly, HPV16-positive non-OPSCC incident fraction was consistently under 1% in the U.S and Europe, while it was 0% in Brazil.

Regarding the discordance between $p16^{INK4a}$ and HPV16 DNA, there were 10.1% OPSCC (n=54) and 14.5% non-OPSCC tumors (n=127) that were $p16^{INK4a}$-positive but HPV16 DNA negative with no regional difference (p>0.20) (Supplementary table 2). Similarly, 9.8% of OPSCC and 14.5% of non-OPSCC were p16^{INK4a}-negative but HPV16 DNA-positive. The highest proportion was observed in Europe compared to U.S. and Brazil (p<0.01). Concurrent overexpression of P16^{INK4a} with oncogenic HPV types other HPV16 was rare (14 of 4120, <1%), nearly all were from the U.S. and were more often observed in OPSCC (11/529) compared to the oral cavity or larynx (3/887) (Supplementary table 3).

In multivariable analyses, the odds of being a HPV16-positive OPSCC was significantly associated with college education level (OR (95%CI): 3.60 (1.77 -7.30)), pack years of ever smoking (OR: 0.75; 95% CI: 0.64 -0.87), drink years of ever alcohol use (OR: 0.64; 95% CI: 0.54-0.76), and geographic region (Europe vs. U.S.: OR=0.42; 95% CI: 0.22-0.82; Brazil vs. U.S.: OR: 0.04; 95% CI: 0.01-0.09) (Table 3). Similar results were observed in univariable analysis (Supplementary table 4). We observed that ever smokers were less likely to be HPV16-positive (OR: 0.09; 95% CI: 0.04- 0.18, Supplementary table 4), and the prevalence of HPV16 declined with increasing pack years (Supplementary table 5), similar results were noted for alcohol use. The observed inverse associations with smoking and alcohol were robust across geographic regions (p-heterogeneity: 0.10 and 0.66, respectively), while education was only significant in the U.S..

Discussion

In this study of 1420 HNCs from three world regions, we demonstrate dramatic differences in the prevalence of HPV16-positive HNC. While nearly 60% of OPSCCs in the U.S. are HPV16-positive, this proportion is only about 4% in Brazil, and OPSCCs in Europe have intermediate HPV16 prevalence (31%). Less than 4% of laryngeal and oral cavity cancers are HPV16-positive. That similar results were noted for the marginal incident fractions of HPV16-positive OPSCC and non-OPSCC lends support to these conclusions.

We show that ever smokers and ever drinkers are less likely to be HPV16-positive, and the prevalence of HPV16-positive OPSCC declines with increasing pack years of smoking and drink years of alcohol use. It is noteworthy that these conclusions are based on case comparisons, therefore, a higher HPV attributable fraction is expected among never smokers/ drinkers as these individuals developed the disease in the absence of the most common risk factors- smoking and alcohol. It also remains to be noted that these results do not represent incidence rate interpretations.

We show that ever smokers and ever drinkers are less likely to be HPV16-positive, and the prevalence of HPV16-positive OPSCC declines with increasing pack years of smoking and drink years of alcohol use.

Our results are consistent with the few studies that have examined both HPV DNA and $p16^{INK4a}$ expression in OPSCC, both for estimates in the U.S.[32-34] as well as Europe[15-17, 35]. Fewer studies have been reported from South America, our observations are similar to the earlier report describing HPV16 prevalence in OPSCC as 4.4%[14]. In this context, the recent pooled analysis on HPV prevalence in HNC is of note where, based on 158 tumors from South and Central America tested for HPV DNA and subsequently, following triage for HPV16 E6*mRNA and $p16^{INK4a}$, the authors report a prevalence of 37% for HPV-positive OPSCC[19]. Our estimates are substantially lower than this report even though the number of OPSCC included is comparable. Similarly, our estimates for HPV16-positive OPSCC in Europe are higher than the reported 16%. It is possible that these differences could reflect distinct geographic locations of sampling; while majority of the tumors in the published pooled analysis were drawn from southern Europe, nearly 70% of our European OPSCC subjects were from western and northern Europe. Similarly, South and Central America were represented by nine countries of the region (Argentina, Chile, Colombia, Ecuador, Guatemala, Honduras, Mexico, Paraguay and Venezuela), it is noteworthy that Brazil was not among these. It is also possible that the differences could be due to differing periods of sampling, the pooled analysis retrieved tumors diagnosed over a longer period (1990-2012), with almost 60% of the cases diagnosed after 2005 while most OPSCC tumors included in this analysis were recruited before 2005. Our study consistently showed that a low proportion (~4%) of non-OPSCC were HPV-positive across all three continents. A marginally higher proportion of HPV16-positive tumors were observed in the oral cavity (5.8%) compared to the larynx (3.3%). Closer inspection of the subanatomic sites revealed that almost half all the HPV16-positive oral cavity tumors may have been potentially misclassified and suggest that the true prevalence of HPV16 in oral cavity cancer is likely to be much lower (~3%), and similar to that of laryngeal tumors.

The comparison of a large number of tumors tested for HPV16 DNA and $p16^{INK4a}$ shows that the methods are discordant in 8-13% of the cases. The lack of specificity of $p16^{INK4a}$ appears to vary by subsite but not by region and the specificity of $p16^{INK4a}$ outside the OPSCC appears to be low[18, 36, 37]. Some authors have suggested the use of $p16^{INK4a}$ testing as replacement for HPV DNA[38, 39], however our results argue against this approach. For oncogenic HPV types other than HPV16, concurrent $P16^{INK4A}$ expression occurred more often in OPSCC compared to other HNC sites. Whether this reflects oncogenicity awaits further functional data.

Geographic differences in the proportion of HPV16-positive OPSCC may in part be explained by differences in tobacco use (i.e. the varying proportion of smoking-related OPC in each region). This hypothesis is supported by higher age-standardized incidence rates of OPSCC in Brazil ($ASR_{São\ Paulo}$: 4.0) compared to other regions ($ASR_{North\ Carolina}$, U.S.: 3.4 and $ASR_{Germany,\ Italy,\ Scotland}$: 2.4)[4040]. However, data on smoking prevalence in the general population of these regions does not provide clear support. While tobacco smoking was lower among controls in the U.S. study compared to Europe (median smoking pack-years: 6.5 and 7.6 in U.S. and Europe), Brazil had the lowest smoking prevalence (median smoking pack-years: 2.6), suggesting the differences in tobacco use do not fully explain the observed HPV prevalence differences in OPSCC. As performing oral sex is the primary risk factor for HPV-positive OPSCC, differences in oral sexual behavior likely contribute to differences in incidence, but unfortunately sexual data was not available in two of the studies. It is important to note however that these risk factors are subject to strong birth cohort effects. In addition, smoking and alcohol are additionally impacted by strong interaction effects.

Despite the dramatic differences in HPV prevalence, the three studies were similar in terms of the risk factor profiles, which was reflected in the consistency of the factors associated with HPV-positivity. The limitations of our study arise from utilizing previously collected survey data from studies that did not collect identical lifestyle data. For instance, sexual behavior questions were not included in the European and South American study, while the data collected in the U.S. study was limited. In addition, although we present results for three world regions, it must be noted that the U.S. is represented by a single study conducted in North Carolina where smoking prevalence is among the highest in the country. Similarly, Europe is represented by three countries only while South America is represented by a single study that was conducted in Brazil with all cases being recruited from public hospitals in São Paulo.

We conclude that the proportion of OPSCC now caused by HPV16 varies by geographic region with low proportions in Brazil, moderate proportions in Western Europe, and the majority of OPSCC diagnosed in the U.S. being HPV16-positive. Our results suggest that prophylactic HPV vaccination would have the largest benefit in the US, intermediate benefit in Europe, and low benefit in Brazil. This geographic heterogeneity in HPV prevalence may not be entirely explained by the varying proportion of smoking in these regions.

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Declaration of Interests[1]

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Table I: Description of the study group

Description		U.S.	Europe	Brazil	Total
N cases		**477**	**539**	**404**	**1420**
		N (%)	**N (%)**	**N (%)**	**N (%)**
Years of diagnosis		2002-2006	2002-2005	2002-2011	
Age	IQR	64-50	68-54	64-50	66-51
(in years)	Median	56	60	57	58
Sex	Men	365 (76.5)	426 (79.0)	356 (88.1)	1147 (80.8)
	Women	112 (23.5)	113 (21.0)	48 (11.9)	273 (19.2)
Education	≤Primary school	144 (30.2)	222 (46.5)	314 (77.9)	680 (50.1)
level[‡]	Secondary school	142 (29.8)	219 (45.8)	61 (15.1)	422 (31.1)
	Any college	191 (40.0)	37 (7.7)	88 (7.0)	256 (18.8)
Race[ǂ]	Caucasian	346 (72.5)	539 (100.0)	290 (72.5)	1175 (83.0)
	Black	120 (25.2)	0	39 (9.7)	159 (11.2)
	Asian	5 (1.1)	0	3 (0.8)	8 (0.6)
	Mixed	2 (0.4)	0	64 (16.0)	66 (4.6)
	Other	4 (0.8)	0	4 (1.0)	8 (0.6)
Smoking[*]	Never	58 (12.2)	55 (10.2)	11 (2.7)	124 (8.8)
	Former	129 (27.2)	173 (32.2)	89 (22.2)	391 (27.7)
	Current	288 (60.6)	310 (57.6)	301 (75.1)	899 (63.6)
	Median pack years	43.0	39.0	43.7	
Alcohol[ϕ]	Never	46 (9.7)	43 (8.1)	42 (10.5)	131 (9.3)
	Former	149 (31.3)	63 (11.9)	163 (40.7)	375 (26.7)
	Current	281 (59.0)	423 (80.0)	196 (48.9)	900 (64.0)
	Median drink years	49.6	23.3	100.3	
Stage[¥]	I	70 (15.3)	69 (16.9)	18 (4.5)	157 (12.4)
	II	80 (17.5)	71 (17.4)	49 (12.3)	200 (15.8)
	III	89 (19.5)	77 (18.8)	57 (14.4)	223 (17.7)
	IV	218 (47.7)	192 (46.9)	273 (68.8)	683 (54.1)
Cancer site	Oral cavity	123 (25.8)	165 (30.6)	107 (26.5)	395 (27.8)
	Oropharynx	243 (50.9)	119 (22.1)	171 (42.3)	533 (37.5)
	Larynx[§]	107 (22.4)	251 (46.6)	124 (30.7)	482 (33.9)
	Overlapping[¥]	4 (0.8)	4 (0.7)	2 (0.5)	10 (0.7)

[‡] 62 cases missing education information; 61 from Europe, 1 case from Brazil

[ǂ] missing information for 4 cases (Brazil)

[*] data missing for 6 cases (2 each from the US,1 from Europe and 3 from Brazil). Median estimated among ever smokers

[ϕ] data missing for 14 cases (1 US, 10 Europe, 3 from Brazil). Median estimated among ever alcohol users

[¥] missing for 130 cases from Europe, 20 from the US and 7 from Brazil

[§] includes hypopharynx and larynx cases

[¥] includes cancers of overlapping sites and non-specified sites

Table II: Prevalence of HPV16-related cancer‡: by region

Cancer site	HPV16 DNA & p16 positives							
Region	Overall*		U.S.		Europe		Brazil	
	N	Prevalence% (95% CI)	N	Prevalence% (95% CI)	N	Prevalence% (95% CI)	N	Prevalence% (95% CI)
Oropharyngeal cancer‡	533	35.3 (31.2- 39.3)	243	59.3 (53.1- 65.5)	119	31.1 (22.7- 39.5)	171	4.1 (1.1- 7.1)
Non-oropharyngeal cancers	887	4.4 (3.0- 5.7)	234	6.8 (3.6- 10.1)	420	5.5 (3.3- 7.7)	233	0
Oral cavity ¥	395	5.8 (3.5- 8.1)	123	10.6 (5.1- 16.0)	165	6.1 (2.4- 9.7)	107	0
Larynx/ hypopharynx§	482	3.3 (1.7- 4.9)	107	2.8 (0.0- 6.0)	251	5.2 (2.4- 7.9)	124	0

‡defined as joint positivity to HPV16 DNA and $p16^{INK4a}$ overexpression

*None of the 10 tumors of overlapping sites were HPV16-positive

‡Chi-squared p-value for the difference in prevalence between the three regions was <0.01

¥ Chi-squared p-value for the difference in prevalence between the U.S. and Europe was 0.20, U.S. and Brazil: <0.01 and Europe and Brazil was 0.01

§Chi-squared p-value for the difference in prevalence between the U.S. and Europe or Brazil was >0.10, Europe and Brazil was 0.01

Table III: Multivariable factors associated with HPV16-positive oropharyngeal cancer

	Overall (N=533)			U.S. (N=243)	Europe (N=119)	Brazil (N=171)	P-heterogeneity[¥]
Risk factor	N	% HPV16 positive[‡]	OR (95% CI)[§]	OR (95% CI)[§]	OR (95% CI)[§]	OR (95% CI)[§]	
Age							0.64
(10 year increase)	533	--	0.95 (0.71 -1.27)	0.75 (0.49 -1.16)	0.98 (0.53 -1.81)	1.21 (0.39 -3.78)	
Sex							
Men	447	33.8	1.0	1.0	1.0	1.0	
Women	86	43.0	0.56 (0.27 -1.15)	0.65 (0.22 -1.96)	0.34 (0.07 -1.68)	0.42 (0.02 -8.19)	0.79
Education level							
≤Primary school	252	15.9	1.0	1.0	1.0	1.0	
Secondary school	144	40.3	**2.31 (1.23 -4.35)**	2.68 (0.88 -8.13)	1.76 (0.54 -5.72)	17.59 (0.56 -555.11)	0.45
Any college	131	67.9	**3.60 (1.77 -7.30)**	2.23 (0.82 -6.06)	0.29 (0.01 -7.69)	25.24 (0.45 -1410.25)	0.24
Race							
Caucasian	--	--	--	1.0	--	--	
Black	--	--	--	**0.04 (0.01 -0.13)**	--	--	
Mixed	--	--	--	0.15 (0.01 -3.16)	--	--	
Lifetime sex partners							
0-3	--	--	--	1.0	--	--	
4-5	--	--	--	**4.21 (1.36 -13.0)**	--	--	
Stage[‡]							
I	25	32.0	1.0	1.0	1.0	1.0	
II	44	27.3	0.79 (0.20 -3.11)	0.91 (0.16 -5.25)	--	--	
III	35	39.8	1.62 (0.49 -5.32)	1.93 (0.38 -9.77)	1.21 (0.11 -13.78)	--	0.75
IV	120	35.7	2.49 (0.83 -7.45)	1.73 (0.41 -7.37)	3.20 (0.35 -28.89)	--	0.65
Smoking status							
Never	57	82.5	1.0	1.0	1.0	1.0	
Pack years[*]	472	29.8	**0.75 (0.64 -0.87)**	0.84 (0.66 -1.06)	**0.59 (0.41 -0.84)**	**0.36 (0.13 -0.99)**	0.10
Alcohol status							

Never	39	53.8	1.0	1.0	1.0	1.0	
Drink years*	478	33.9	**0.64 (0.54 -0.76)**	**0.64 (0.48 -0.84)**	**0.62 (0.44 -0.87)**	**0.40 (0.15 -1.05)**	0.66
Region							
US	243	59.3	1.0	--	--	--	
Europe	119	31.1	**0.42 (0.22 -0.82)**	--	--	--	
Brazil	171	4.1	**0.04 (0.01 -0.09)**	--	--	--	

‡HPV16 positivity was define as tumor positive for HPV16 DNA and overexpressing P16INK4a protein

§ estimates were adjusted for age, sex, education level, stage, region, smoking (10 pack year intervals) and alcohol consumption (10 drink year intervals) as appropriate

*proportion of HPV16 positives among ever smokers or ever drinkers as appropriate. Denotes increments of 10 pack years or drink years until 40 or more as appropriate.

‡ Seven HPV16 positives in Brazil were all of advanced stage

¥ chi-squared test for heterogeneity across the studies