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Prevalence of HPV infection in racial–ethnic subgroups of head and neck cancer patients

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Abstract

The landscape of human papillomavirus (HPV) infection in racial/ethnic subgroups of head and neck cancer (HNC) patients has not been evaluated carefully. In this study, a meta-analysis examined the prevalence of HPV in HNC patients of African ancestry. Additionally, a pooled analysis of subject-level data was also performed to investigate HPV prevalence and patterns of p16 (CDNK2A) expression amongst different racial groups. Eighteen publications ($N = 798$ Black HNC patients) were examined in the meta-analysis, and the pooled analysis included 29 datasets comprised of 3129 HNC patients of diverse racial/ethnic background. The meta-analysis revealed that the prevalence of HPV16 was higher among Blacks with oropharyngeal cancer than Blacks with non-oropharyngeal cancer. However, there was great heterogeneity observed among studies (Q test $P < 0.0001$). In the pooled analysis, after adjusting for each study, year of diagnosis, age, gender and smoking status, the prevalence of HPV16,18 in oropharyngeal cancer patients was highest in Whites (61.1%), followed by 58.0% in Blacks and 25.2% in Asians ($P < 0.0001$). There was no statistically significant difference in HPV16,18 prevalence in non-oropharyngeal cancer by race ($P = 0.682$). With regard to the pattern of HPV16,18 status and p16 expression, White patients had the highest proportion of HPV16,18+/p16+ oropharyngeal cancer (52.3%), while Asians and Blacks had significantly lower proportions (23.0 and 22.6%, respectively) [$P < 0.0001$]. Our findings suggest that the pattern of HPV16,18 status and p16 expression in oropharyngeal cancer appears to differ by race and this may contribute to survival disparities.

Abbreviations

FF	fresh frozen
FT	fresh tissue
FFPE	formalin-fixed paraffin-embedded
HPV	human papillomavirus
HNC	head and neck cancer
HR	hazard ratios
PCR	polymerase chain reaction

Introduction

Head and neck cancer (HNC) is the sixth most common cancer in the world, accounting for approximately 4% of all cancer cases (1). In 2012, there were an estimated 599 637 new cases of cancer of the oral cavity, larynx and oropharynx, and 324 794 deaths attributed to the disease worldwide (1). Although tobacco and alcohol use are the primary risk factors for developing HNC, human papillomavirus (HPV) is also an established risk factor for cancers arising in the oropharynx (2,3). Recently, HPV has also been reported to be associated with a subset of oral cavity cancers (4,5), but an etiological role has not been clearly demonstrated.

A recent review and meta-analysis from our group of HNC survival in relation to HPV demonstrated a survival advantage for all HPV-positive patients (6), but the survival advantage was only significant for patients with cancer of the oropharynx. Compared to patients with HPV-negative oropharyngeal cancer, the risk of death and risk of recurrence for patients with HPV-positive oropharyngeal cancer was reduced by ~28% and ~49%, respectively. In the USA, a clear disparity in HNC survival has been reported between Black and White patients, particularly for oropharyngeal cancers. Poor survival rates for Black Americans compared to White Americans have been observed (7), and some studies have suggested that this disparity may be explained at least partially by a difference in prevalence of HPV infection (8–10). Comparisons of HPV prevalence in cancer of the oral cavity and larynx between various racial/ethnic populations have been reported in a recent meta-analysis (11). However, a summary of HPV prevalence for Black patients was only reported for oral cavity cancer in this study (11). Furthermore, an assessment of attributed survival differences for oropharyngeal cancer between racial/ethnic populations was not conducted.

The goal of this study was to develop a more complete perspective of the landscape of HPV infection in ethnic subgroups of HNC patients by examining the published literature. We conducted a meta-analysis examining the prevalence of HPV in the Black population. We also performed a pooled analysis of cases reporting HNC and HPV status using subject-level data from the published literature to investigate HPV segregation and prevalence amongst different ethnic groups.

Materials and methods

This study was approved by the Fox Chase Cancer Center Institutional Review Committee.

Literature review and data collection

A PubMed search was conducted (from inception to December 2014) using the search terms, ['human papillomavirus' (All Fields) OR 'HPV' (All Fields)] AND ['squamous cell carcinoma' (All Fields) OR 'cancer' (All Fields)] AND ['oropharyngeal' (All Fields) OR 'oropharynx' (All Fields) OR 'head and neck' (All Fields) OR 'tonsil' (All Fields)]. All abstracts and full text of articles from the PubMed search were reviewed independently by two reviewers. When there was a discrepancy between reviewers, a third reviewer evaluated the article(s) to resolve the discrepancy. All studies that tested for the presence of HPV in HNC tissues from patients diagnosed with squamous cell carcinoma of the head and neck (oral cavity, oropharynx, larynx and hypopharynx) were eligible for inclusion in this analysis. The bibliographies of several review articles were also examined in order to identify additional publications that might have been missed by our PubMed search (11–15). This review identified 291 original articles that qualified conditionally for the analysis. Studies that used serology methods to detect HPV antibodies were excluded from the analysis, as this method does not identify which tissue is infected by HPV. Studies that primarily evaluated HPV in lip cancers were excluded from this analysis, with the exception of studies where it was impossible to distinguish lip cancer data from the other head and neck subsites. In addition, case reports and studies that included only HPV-positive HNC tumors/patients were excluded. Additional exclusion criteria includes studies of HNC patients who were co-infected with other diseases, such as HIV; studies in which the cancer tissues were sampled via cytobrushing and not biopsy or surgery; studies that classified HNC as HPV-related or HPV non-related tumors based on tumor site without directly testing that tissue for HPV; studies in which fewer than 80% of the eligible cases were tested for HPV; and studies that selected patient samples non-randomly, but applied pre-defined criteria for patient inclusion (e.g., patients with undifferentiated carcinoma only, metastasis only, positive lymph nodes only, advanced stage only, patients who underwent a specific treatment regimen, studies where smoking and drinking patient tissues were matched with nonsmoker and nondrinker patient tissues, etc.). For overlapping studies, the publication with the largest population and/or more complete information was included in this analysis. After accounting for these inclusion and exclusion criteria,

140 articles with data for all racial/ethnic populations were eligible for inclusion in this study. Of these, only 18 articles presented data that could be abstracted and were included in the meta-analysis of Black cancer patients. All 140 articles were eligible for inclusion in the pooled analysis. A flow diagram of study selection is illustrated in Figure 1.

Meta-analysis of Black HNC patients

From each of the 18 articles that included data from Black HNC patients (818 cases), information on the number of patients, HPV prevalence, HPV genotype, tumor subsite, mean age, year of cancer diagnosis, geographic location of the study, tissue source, HPV test methodology and HPV-infected cancer site were extracted and tabulated. All data were abstracted independently by two reviewers and cross-referenced to confirm that there were no data entry errors. Three studies that included data for fewer than 10 Black patients (16–18) were therefore excluded from the meta-analysis, leaving 15 studies including 798 cases.

Pooled analysis

All investigators from the 140 studies were invited to submit their subject-level data for this pooled analysis; data from 22 studies were obtained. The remaining study investigators either did not respond or did not wish to participate. Common data elements included in the pooled analysis were HPV test method, HPV status, HPV genotype, DNA source, geographic location of the study, age at diagnosis, gender, race/ethnicity, p16 status, tobacco and alcohol use, clinical variables (such as tumor site, histology and stage) and survival variables (such as vital status and follow-up time). Seven additional articles reported demographic, clinical, HPV results, tobacco, alcohol and survival data in the publications, which enabled us to create pseudo-datasets for inclusion in the pooled analysis. All patients included in this analysis were diagnosed with cancers of the oral cavity, oropharynx or larynx. Patients with hypopharyngeal cancers were grouped with the patients with cancers of the larynx. Patients with metastases or unknown primaries were excluded from this analysis. In total, there were 29 datasets including a total of 3129 HNC cases.

Statistical analysis

The Meta-proportion of any HPV and HPV16 only was calculated for all HNC subsites combined as well as separately for oropharynx and non-oropharynx data. All statistical analyses were performed using Intercooled STATA SE (version 10) software (StataCorp. LP, College Station, TX). Meta-analyses of the proportion of HPV-positive HNC were performed using the *metaprop* command in STATA. HPV proportions were calculated for each individual study and the reported confidence intervals were based on Clopper–Pearson exact binomial procedures (19). Pooled proportions of the multiple studies were estimated using a random effects model. The Meta-prevalence estimates were calculated by multiplying the Meta-proportion and confidence interval values by 100. The Q-statistics were used to test for heterogeneity between the studies included in the meta-analyses. The I^2 metric was also calculated to quantify variation between studies (20). Large between-study variation was observed when the I^2 values were ≥50% while moderate between-study heterogeneity was denoted by I^2 values between 25 and 50%. Evidence of publication bias or small study effects ($P < 0.05$) was assessed using the Egger's test (21).

For the pooled analysis, unequal variance in age was observed between categories of race. Therefore, a square root transformation of age at diagnosis was performed. Adjusted HPV prevalence and 95% confidence intervals for each racial/ethnic group was calculated from logistic regression estimates for HPV-positive status, adjusting for study, year of diagnosis, square root of age, sex, history of alcohol drinking and smoking history. The adjusted prevalence refers to the average HPV prevalence

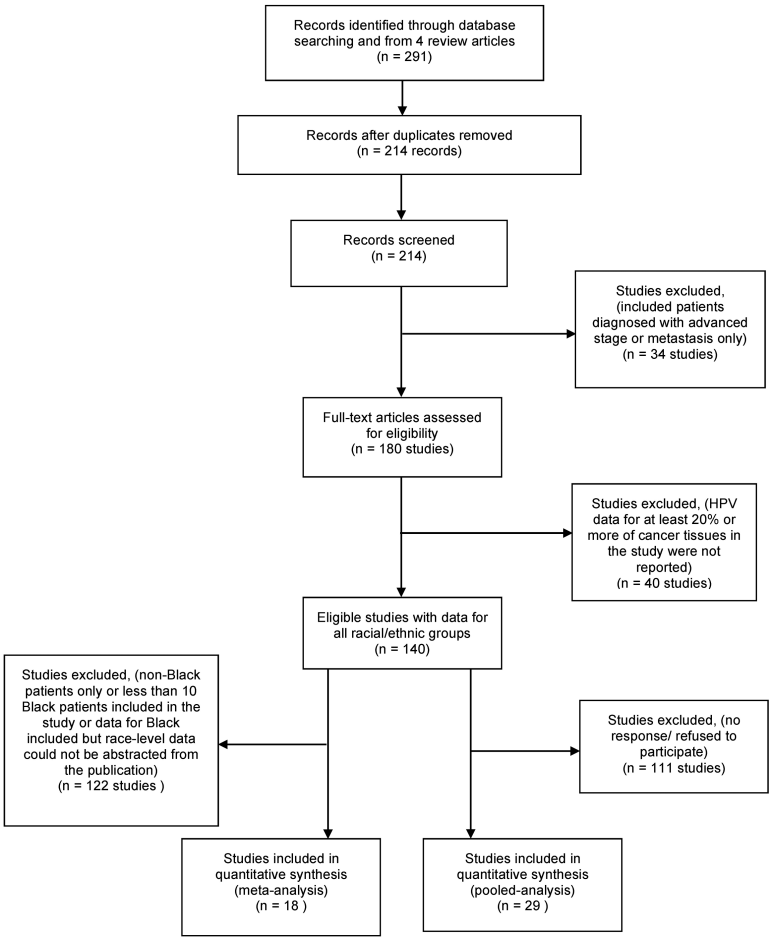

Figure 1. Flow diagram of study selection.

while averaging the values of the covariates in the regression model. The logistic coefficients and standard errors are provided in Supplementary Materials, available at *Carcinogenesis* Online. A Likelihood Ratio chi-square test was performed to evaluate differences between the adjusted prevalence according to race and an analysis of variance (ANOVA) was used to compare the mean square root of age at diagnosis between racial groups (*P* values for pairwise comparisons were Bonferroni adjusted). *P* values < 0.05 were considered statistically significant. Mean age at diagnosis for each stratum was back transformed and reported. Follow-up time for overall survival refers to the interval between date of diagnosis and the date of last contact (if the patient was alive) or date of death. Hazard ratios (HR) were calculated and adjusted for each study and other confounders for risk of death or risk of disease progression (i.e., disease persistence, recurrence and/or metastasis). HR < 1.0 represents an overall survival benefit and HR > 1.0 represents poor overall survival.

Results

Description of studies: meta-analysis

Table 1 summarizes all published studies from which data were available to estimate HPV (any HPV or HPV16) prevalence in Black populations. Study size ranged from 13 to 161 patients. The majority (13/15, 87%) of studies included polymerase chain reaction (PCR)-based methods to test for the presence of HPV DNA. For all site strata (all head and neck, oropharynx and non-oropharynx), large heterogeneity was observed between the studies (Q test *P*-value range from 0.000 to 0.048; I^2 values range from 62.1 to 94.6%). Nevertheless, as expected, the prevalence of any HPV or HPV16 was higher among oropharyngeal cancer patients (any HPV: 31.5%, 95% CI = 17.7–47.1; HPV16: 45.7%, 95% CI = 25.5–66.6) in comparison to non-oropharyngeal cancer patients (any HPV: 14.5%, 95% CI = 1.4–36.0; HPV16: 1.1%, 95% CI = 0.0–6.0). There was no evidence of publication bias or small study effect. The reasons for underlying heterogeneity were explored by stratifying the dataset according to geographic region (Sub-Saharan Africa versus USA) as well as HPV test methods (ISH versus PCR/RT-PCR). Large heterogeneity remained when stratified by HPV test method (data not shown). When stratified by geographic region (see Supplementary Table 1, available at *Carcinogenesis* Online), large heterogeneity was still observed except when data were limited to HPV16 infections only. For all head and neck subsites combined, the meta-prevalence of HPV16 in patients from Sub-Saharan Africa (*N* = 4 studies) was 1.0% (95% CI = 0.0–3.9), Q test *P*-value was 0.129, I^2 was 47.0%. Large heterogeneity was still observed between the remaining eight studies that included patients from the USA (Q test *P* <0.0001, I^2 = 89%). Further stratification of the Sub-Saharan Africa studies according to head and neck subsite resulted in a meta-prevalence of HPV16 in non-oropharyngeal cancers at 0.1% (95% CI = 0.0–1.8, Q test *P* value = 0.768, I^2 = 0.0%). The only study in the USA that reported HPV16 data for non-oropharyngeal cancer showed a higher prevalence (13.6%, 95% CI = 1.9–31.7) than that of patients in Sub-Saharan Africa. There were no studies in Sub-Saharan Africa that reported data for HPV16 in oropharyngeal cancer patients and the large heterogeneity remained for the USA studies that reported HPV16 data in Black oropharyngeal cancer patients.

Description of studies: pooled analysis

There were a total of 3129 patients included in this analysis (Table 2). Variations among the 29 studies were noted with regard to study size, the geographic region where the study was conducted, tumor site and the tissue source. Studies varied in size from 15 to 489 patients and were conducted mostly in Europe (48%, 14/29 studies), followed by the USA (31%, 9/29), Asia (17%, 5/29) and a single study in Australia. Most of the studies (65%, 19/29) involved patients diagnosed with cancers at both oropharyngeal and non-oropharyngeal sites (oral cavity, larynx, hypopharynx and non-oropharyngeal sites not otherwise specified). The remaining studies included patients diagnosed with oropharyngeal cancers only. Formalin-fixed paraffin-embedded (FFPE) tissues were examined in 66% of studies to test for the presence of HPV, rather than Fresh Frozen (FF) or Fresh Tissue (FT). All except for four studies used PCR methodology to detect HPV DNA, using either consensus or type-specific primers, and of these, five also evaluated HPV status using DNA *in situ* hybridization combined with PCR. Two studies detected HPV RNA using only RT-PCR and the other two detected both HPV RNA and DNA using RT-PCR and PCR. CDKN2A (p16) expression was evaluated in 16 studies using immunohistochemistry. With regard to race/ethnicity, the pooled dataset was diverse with patients representing African, African American, Asian and White populations. There was one study that included Aboriginal Australian patients. These patients were combined with the African and African American patients and classified as Black. There were 82 patients classified as other race (for 63 patients race was unknown and 19 patients included Pacific Islander, Middle Eastern, Indian, Hispanic or other not otherwise specified). These patients were grouped and classified as other race. Follow-up time was available for 19 studies and ranged from 0.03 to 244.5 months with a mean follow-up of 41.7 months and a median follow-up of 30.6 months.

Prevalence of HPV16 and HPV18 according to race and head and neck subsite

The prevalence of HPV16 and/or HPV18 (HPV16,18) stratified by race was calculated for all HNCs, oropharyngeal cancers only and non-oropharyngeal cancers only after adjusting for study, year of diagnosis, age, gender, alcohol drinking, and smoking status (Table 3, and Supplementary Table 2, available at *Carcinogenesis* Online, which summarizes the logistic coefficients and standard errors). As expected, the overall mean age for HPV-positive patients diagnosed with oropharyngeal cancers was lower than the mean age of HPV-positive patients diagnosed with non-oropharyngeal cancers irrespective of whether the patient carried HPV16 or HPV18 in their tumor. The mean age at diagnosis was 56.3 years for HPV16,18+ oropharyngeal cancer patients, and 60.1 years for HPV16,18+ non-oropharyngeal HNC patients (*P* < 0.0001). There was no statistically significant difference in the mean age at diagnosis of HPV16,18+ oropharyngeal cancer patients according to race. However, for non-oropharyngeal HNC patients, a Bonferroni *post hoc* test shows that Asians were statistically significantly older compared to Whites (HPV16,18: Asians, 64.1 years versus Whites, 54.9 years, *P* = 0.038).

As expected, the prevalence of HPV16,18 was higher in oropharyngeal cancer tissues compared to non-oropharyngeal cancer tissues (HPV16,18: 48.7% versus 18.2%). HPV16 was the predominant genotype carried in all patient tissues, 46.6% of oropharyngeal cancer patients and 13.4% of non-oropharyngeal HNC patients were positive for this genotype. In contrast, only approximately 1–2% of patients carried HPV18, irrespective of whether the cancer was diagnosed in the oropharynx or at a non-oropharyngeal head and neck site.

For oropharyngeal cancers, there was a statistically significant difference in the prevalence of HPV16,18 according to race. White patients had the highest prevalence of HPV16,18+ cancers followed by Blacks then Asians, however, only the prevalence in Asian patients was statistically significantly lower (61.1 versus

Table 1. Meta-analysis of HPV prevalence in populations of African descent

Study	HPV test method	HPV types detected	N	Any HPV N%, 95% CI	HPV 16 N%, 95% CI
All head and neck					
Van Rensburg *et al.* (22)	ISH		66	0.0% (0.0–5.4)	0.0% (0.0–5.4)
Gillison *et al.* (23)	PCR	16, 18, 33, 31	48	20.8% (10.5–35.0)	
Boy *et al.* (24)	PCR	16,18	21	9.5% (1.2–30.4)	0.0% (0.0–16.1)
Agrawal *et al.* (25)	ISH	16	13	0.0% (0.0–24.7)	0.0% (0.0–24.7)
Lewis *et al.* (26)	ISH, PCR	16, 33	26	11.5% (2.4–30.1)	
Jalouli *et al.* (27)	PCR	X	20	65.0% (40.8–84.6)	
Jiron *et al.* (28)	PCR	6,33,11,16,18,31,52,35,45,51,56	161	24.8% (18.4–32.3)	20.5% (14.5–27.6)
Stephen *et al.* (29)	qRT-PCR	16	31	16.1% (5.4–33.7)	16.1% (5.4–33.7)
Babiker *et al.* (30)	PCR	16, 18, 33, 31	100	8.0% (3.5–15.2)	5.0% (1.6–11.3)
Isayeva *et al.* (31)[a]	qRT-PCR	16, 18	30	60.0% (40.6–77.3)	43.3% (25.5–62.6)
Ndiaye *et al.* (32)	PCR	16, 35, 45	110	3.6% (1.0–9.0)	0.9% (0.0–5.0)
Salazar *et al.* (33)	PCR, RT-PCR	16	57	15.8% (7.5–27.9)	15.8% (7.5–27.9)
Worsham *et al.* (10)	q-PCR	NR	49	30.6% (18.2–45.4)	30.6% (18.2–45.4)
Isayeva *et al.* (34)[a]	qRT-PCR	16, 18	22	22.7% (7.8–45.4)	13.6% (2.9–34.9)
Liu *et al.* (35)[a]	PCR	16	44		72.7% (57.2–85.0)
Total			798	17% (8.8–27.0)	13.7% (1.5–26.4)
P value, Q test				0.000	0.000
I^2 test				89.8%	93.8%
P value, Egger's test				0.419	0.643
Oropharynx					
Lewis *et al.* (26)	ISH, PCR	16, 33	26	11.5% (2.4–30.1)	
Jiron *et al.* (28)	PCR	6,33,11,16,18,31,52,35,45,51,56	36	25.0% (12.1–42.2)	
Isayeva *et al.* (31)[a]	qRT-PCR	16, 18	30	60.0% (40.6–77.3)	43.3% (25.5–62.6)
Salazar *et al.* (33)	PCR, RT-PCR	16	23	34.8 (16.4–57.3)	34.8% (16.4–52.3)
Worsham *et al.* (10)	q-PCR	NR	49	30.6% (18.2–45.4)	30.6% (18.2–45.4)
Liu *et al.* (35)[a]	PCR	16	44		72.7% (57.2–85.0)
Total			146	31.5% (17.7–47.1)	45.7% (25.5–66.6)
P value, Q test				0.003	0.000
I^2 test				75.5%	84.1%
P value, Egger's test				0.997	0.807
Non-oropharynx					
Van Rensburg *et al.* (22)	ISH		66	0.0% (0.0–5.4)	0.0% (0.0–5.4)
Boy *et al.* (24)	PCR	16, 18	21	9.5% (1.2–30.4)	0.0% (0.0–16.1)
Jalouli *et al.* (27)	PCR	X	20	65.0% (40.8–84.6)	
Jiron *et al.* (28)	PCR	6,33,11,16,18,31,52,35,45,51,56	125	24.8% (17.5–33.3)	
Ndiaye *et al.* (32)	PCR	16,35,45	105	3.8% (1.0–9.5)	1.0% (0.0–5.2)
Isayeva *et al.* (34)[a]	qRT-PCR	16,18	22		13.6% (2.9–34.9)
Total			337	14.5% (1.4–36.0)	1.1% (0.0–6.0)
P value, Q test				0.000	0.048
I^2 test				94.6%	62.1%
P value, Egger's test				0.685	0.424

NR, not reported; X, HPV genotype unknown.
[a]Studies included in the pooled analysis.

58.0% and 25.2%, respectively; $P < 0.0001$). A similar pattern was observed for the prevalence of HPV16 infections. However, for HPV18, Black patients had the highest prevalence (14.8%) compared to Asians (1.6%) and Whites (1.1%) and this difference was statistically significant ($P = 0.0025$). For the non-oropharyngeal cancer patients, there was no statistically significant difference in HPV16 and/or 18 prevalence according to race.

Expression of p16 and HPV16,18 DNA according to race in oropharynx cancer patients

Twelve studies (1397 patients) presented with both HPV16,18 and p16 data. Among oropharyngeal cancer patients, the pattern of combined HPV16,18 and p16 status differed according to race, and this difference was statistically significant (Figure 2A, $P < 0.0001$). White patients had the highest proportion of cancers that were HPV16,18+/p16+ (52.3%). In contrast, Asian and Black patients had lower proportions of tumors with HPV16,18+/p16+ cancers (23.0 and 22.6%, respectively). In addition, Black patients had a higher proportion of cancers that were HPV16,18+, but p16– compared to Asian and White patients (31.1 versus 10.5% and 4.7%, respectively). The proportion of patients with HPV16,18–/p16– disease also differed significantly by race. Asian patients had the highest proportion of HPV16,18–/p16– cancers, in contrast to Black and White patients (66.8 versus 37.7% and 29.6%, respectively).

When the oropharyngeal cancer patients were stratified according to smoking history, the pattern of combined HPV16,18 and p16 status according to race co-segregated with the fraction of patients that were ever smokers (Figure 2B). Among never smokers (Figure 2C), as expected, patients with HPV16,18+/p16+ cancers comprised the predominant fraction among Asian, Black and White patients. However, White patients still

Table 2. Description of studies included in the pooled analysis

Author (Ref)	Study size	Tissue source	HPV testing method	p16 expression	Geographic region	Race/ ethnicity	Tumor site	FU, months (median)
Cruz *et al.* (36)[a]	35	FF	PCR	—	Europe	W	NO	—
Tsuhako *et al.* (37)[a]	88	FFPE	PCR	—	Asia	AS	NO, O	—
Koskinen *et al.* (38)[a]	61	FF	PCR	—	Europe	W	NO, O	—
De Petrini *et al.* (39)	70	FF	PCR	—	Europe	W	NO, O	30.4
Ragin *et al.* (16)	125	FFPE	PCR	IHC	USA	W	NO, O	48.4
Armas *et al.* (40,41)	280	FFPE	PCR	IHC	Asia	AS	NO, O	18.6
Cohen *et al.* (42)[a]	35	FFPE	PCR	—	USA	UNK	O	—
Worden *et al.* (43)	70	FFPE	PCR	—	USA	AA, W	NO, O	13.5
Szarka *et al.* (44)	33	FF	PCR	—	Europe	W	NO, O	25.4
Szarka *et al.* (45)	55	FF	PCR	—	Europe	W	NO, O	77.4
Straetmans *et al.* (46)	81	FFPE	PCR/ISH	IHC	Europe	W	O	—
Tachezy *et al.* (47)	135	FFPE	PCR	—	Europe	W	NO, O	47.7
D'Souza *et al.* (48)	246	FFPE	PCR/ISH	—	USA	AA, AS, W	NO, O	31.0
Eng *et al.* (49)	15	FFPE	PCR	IHC	USA	W	NO, O	69.8
Chernock *et al.* (8)	266	FFPE	PCR/ISH	IHC	USA	AA, AS, W	O	—
Kabeya *et al.* (50)[a]	31	FF	PCR	IHC	Asia	AS	NO	—
Hoffman *et al.* (51)[a]	78	FF	PCR	IHC	Europe	W	NO, O	—
Park *et al.* (52)	89	FFPE	PCR	IHC	Asia	AS	O	20.9
Heusinkveld *et al.* (53)[a]	41	FFPE	PCR	—	Europe	W	NO, O	—
Bussu *et al.* (54,55)	136	FT	RT-PCR/HC2	IHC	Europe	A, W	NO, O	12.5
Isayeva *et al.* (31,34)	315	FFPE	RT-PCR	IHC	USA	AA, AS, W	NO, O	27.5
Deng *et al.* (56,57)	131	FF	PCR	—	Asia	AS	NO, O	25.1
Morbini *et al.* (58)	52	FFPE	PCR/ISH	IHC	Europe	W	NO, O	50.5
Hong *et al.* (59)	489	FFPE	PCR	IHC	Australia	W, AB, AS	O	49.0
Kruger *et al.* (12)	88	FF	PCR	—	Europe	W	NO	—
Liu (35)	44	FFPE	PCR	IHC	USA	AA	O	18.9
Morbini *et al.* (60)	41	FFPE	PCR/ISH	IHC	Europe	W	O	21.2
Total	3129							30.6

A, African; AA, African American; AB, Aboriginal Australian; AS, Asian; FU, follow-up; HC2, hybrid capture 2; ISH, *in situ* hybridization; IHC, immunohistochemistry; NO, non-oropharynx; O, Oropharynx; PCR, polymerase chain reaction; RT-PCR, real-time PCR (mRNA); UNK, unknown race; UNKP, unknown primary; W, White.
[a]Pseudo datasets created from publication data.

had the highest proportion, and Asian patients had the lowest (White: 80.1%, Black: 62.5% Asian: 39.6%, $P < 0.0001$). Even among never smokers, Asians continued to have the largest proportion of patients with HPV16,18–/p16– cancers (37.4%), which was almost equal to the proportion of HPV16,18+/p16+ cancers (39.6%) observed in this subgroup.

Predictors of overall survival for oropharyngeal cancer patients according to race

Independent predictors of overall survival for oropharyngeal cancer patients were age at diagnosis, smoking history, late stage (III/IV) at diagnosis, and combined HPV16,18 and p16 status (Table 4). Patients with HPV16,18–/p16+ cancers had an increased risk of death compared to patients with HPV16,18+/p16+ oropharyngeal cancers. There was also an even greater increased risk of death for patients with p16– cancers irrespective of HPV status. When stratified according to smoking history, among never smokers, HPV16,18–/p16– patients were the only group with a statistically significantly increased risk of death compared to HPV16,18+/p16+ patients (Hazard Ratio[HR]: 2.70, 95% Confidence Interval [CI] 1.12–6.51). Patients with HPV16,18+/p16– or HPV16,18–/p16+ oropharyngeal cancers also had an increased risk of death compared to patients with HPV16,18+/p16+ oropharyngeal cancers, but the hazard ratios were not statistically significant. When stratified according to race, non-White patients differed in comparison to White patients regarding risk of death based on HPV16,18/p16 status. Table 4 shows that p16 status rather than HPV DNA status appeared to be a predictor of overall survival for non-White patients, but not for White patients. For non-Whites, the risk of death was statistically significantly increased for patients with p16-negative oropharyngeal cancers, irrespective of HPV16,18 status (HPV16,18+/p16–: HR = 2.95, 95% CI = 1.60–5.42, HPV16,18–/p16–: HR = 3.11, 95% CI = 1.97–4.92 versus HPV16,18–/p16+: HR = 0.69, 95% CI = 0.24–2.01). In contrast, the risk of death for White patients with p16+ cancers was dependent upon HPV16,18 status. White patients with HPV16,18-/p16+ oropharyngeal cancers had an increased risk of death (HR = 2.91, 95% CI = 1.72–4.92) in comparison to White patients with HPV16,18+/p16+ oropharyngeal cancers.

The risk of disease persistence, recurrence or metastasis based on HPV16,18/p16 status differed between White and non-White oropharyngeal cancer patients and is presented in Table 5. White patients that did not have HPV16,18+/p16+ disease had an increased risk of disease persistence and/or recurrence in comparison to patients diagnosed with HPV16,18+/p16+ disease. In contrast non-white patients with HPV16,18–/p16– were the only subgroup with a greater risk of disease persistence and/or recurrence in comparison to HPV16,18+/p16+ disease (HR = 2.70, 95% CI = 1.52–4.82). The risk of metastasis was only associated with non-White patients carrying HPV16,18–/p16– oropharyngeal cancers.

Discussion

This study expands on our prior reported meta-analysis of HPV and HNC (6). In that study, we showed that the presence of HPV infection, specifically in the oropharynx had a significant effect

Table 3. Adjusted prevalence of HPV16 and HPV18 according to race stratified by head and neck subsite

	N	HPV16+ mean age[a] (years ± SD)	HPV16 prevalence[b] % (95% CI)	*N*	HPV18+ mean age[a] (years ± SD)	HPV18 prevalence[b]	*N*	HPV16,18+ mean age[a] (years ± SD)	HPV16,18 prevalence[b]
All HNC									
	Number of studies = 28			Number of studies = 24			Number of studies = 28		
Asian	634	58.9 ± 0.64	28.4% (23.8–33.4)	631	61.9 ± 0.90	1.6% (0.7–3.9)	632	59.0 ± 0.68	26.0% (21.6–30.9)
Black	158	56.6 ± 0.55	43.7% (34.2–53.8)	85	56.7 ± 0.20	9.8% (4.0–21.9)	131	56.7 ± 0.51	56.2% (45.1–66.7)
White	2.123	56.5 ± 0.47	36.9% (34.0–40.0)	1778	54.5 ± 0.50	1.6% (0.9–2.8)	1915	56.6 ± 0.48	44.0% (40.8–47.3)
Other[c]	58	55.3 ± 0.52	34.3% (16.2–58.5)	54	—	7.7% (1.0–39.6)	58	55.3 ± 0.51	44.9% (23.1–68.8)
			$P = 0.0123^{¥}$			$P = 0.0077^{¥}$			$P < 0.0001^{¥}$
Total	2973	56.8 ± 0.50	35.0% (32.8–37.2)	2548	58.2 ± 0.70	1.9% (1.2–2.8)	2736	56.9 ± 0.51	39.3% (37.1–41.7)
Oropharynx									
	Number of studies = 25			Number of studies = 21			Number of studies = 25		
Asian	433	56.8 ± 0.63	25.9% (21.1–31.4)	431	55.8 ± 1.02	1.6% (0.5–4.7)	432	56.7 ± 0.63	25.2% (20.5–30.7)
Black	120	56.7 ± 0.58	51.1% (39.0–63.0)	65	57.0 ± 0.12	14.8% (5.6–33.7)	110	56.9 ± 0.54	58.0% (45.0–70.0)
White	1317	56.2 ± 0.46	57.3% (53.1–61.4)	1100	56.5 ± 0.30	1.1% (0.4–2.5)	1229	56.2 ± 0.46	61.1% (56.8–65.3)
Other[c]	44	55.9 ± 0.49	74.1% (35.4–93.7)	42	—	—	44	55.9 ± 0.48	74.1% (35.5–93.7)
			$P < 0.0001^{¥}$			$P = 0.0025^{¥}$			$P < 0.0001^{¥}$
Total	1914	56.3 ± 0.49	46.6% (43.7–49.4)	1638	56.4 ± 0.46	1.6% (1.0–2.7)	1815	56.3 ± 0.48	48.7% (45.9–51.6)
Non-oropharynx									
	Number of studies = 21			Number of studies = 19			Number of studies = 21		
Asian	201	65.0 ± 0.50	27.1% (16.4–41.4)	200	64.6 ± 0.80	—	200	64.1 ± 0.64	20.9% (11.9–34.0)
Black	38	54.6 ± 0.22	13.3% (5.0–30.8)	20	55.5 ± 0.90	3.2% (0.4–22.6)[d]	21	54.9 ± 0.31	30.2% (12.9–55.7)
White	806	59.2 ± 0.52	11.3% (8.7–14.6)	678	51.9 ± 0.76	1.5% (0.6–3.8)[d]	686	58.7 ± 0.59	17.2% (13.5–21.5)
Other[c]	14	—	6.7% (0.9–37.0)	12	—	11.6% (1.3–56.3)[d]	14	—	18.4% (4.2–53.3)
			$P = 0.0553^{¥}$			$P = 0.1434^{¥}$			$P = 0.6344^{¥}$
Total	1059	60.5 ± 0.55	13.4% (11.0–16.3)	910	59.9 ± 0.88	1.4% (0.6–3.2)[d]	921	60.1 ± 0.63	18.2% (15.2–21.8)

[a]Age at diagnosis was back transformed after ANOVA using square root transformation.
[b]Adjusted for each study, year of diagnosis, square root age, gender, alcohol and smoking status.
[c]Other includes other race/ethnic groups and unknown race.
[d]Smoking status predicted HPV18 perfectly and was excluded as a covariate.
[¥]Chi-square *P* value for the differences between the four race/ethnic group categories.

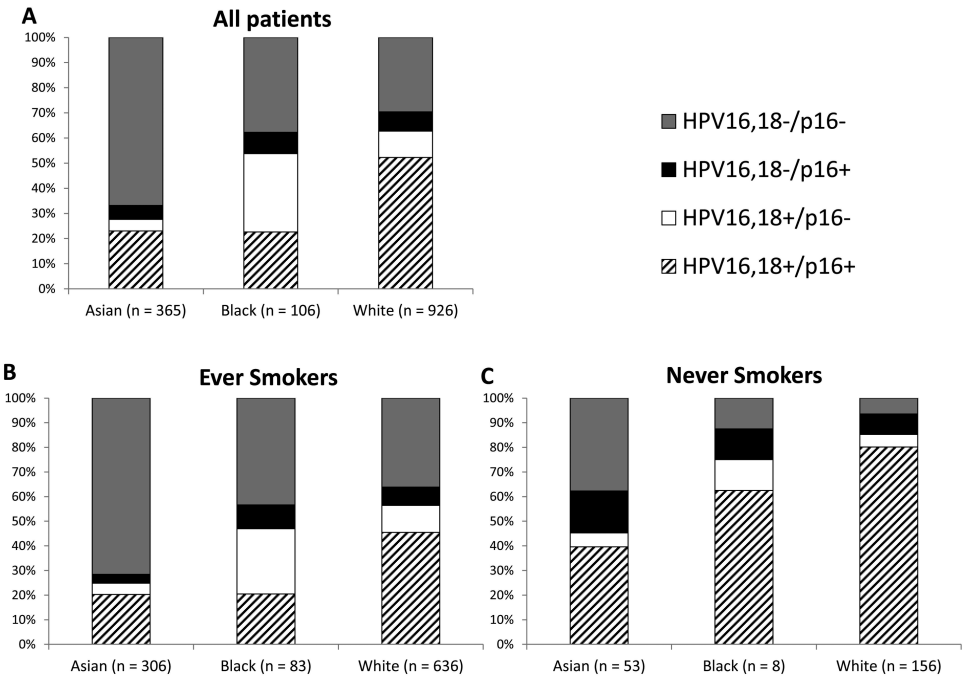

Figure 2. Proportions of combined HPV16,18 and p16 status among all (**A**), ever smoker (**B**) and never smoker (**C**) oropharynx cancer patients stratified by race.

on disease-free survival and overall survival. Since the time of that publication, HPV-positive squamous cell carcinoma of the oropharynx has been well described and reported as a distinct clinical entity. Oropharyngeal cancer patients are often non-smokers, male, younger and White compared to traditional substance abuse-related (tobacco and alcohol) HNC. A dramatic increase in oropharyngeal cancer prevalence has been identified over the last decade (2,61,62). The number of cases of oropharyngeal cancer exceeded the number of cervical cancer cases in 2010 in the United States, and the number of HPV+ oropharyngeal cancer is expected to exceed the incidence of cervical cancer by 2020 (2). In addition, the more favorable outcome of HPV+ oropharyngeal cancer is well-documented and has been confirmed in multiple studies (63,64). These tumors appear to be HPV-related, and a hallmark of favorable tumors is p16 positivity.

For unclear reasons, the prevalence and favorable outcome of HPV+ oropharyngeal cancer is seen mostly in Whites. Variations in the prevalence of HPV have been noted previously in studies of Black patients with oropharyngeal cancer, where some report lower prevalence and others report a prevalence that is higher and/or comparable to White oropharyngeal cancer patients (9,10,35). In the first part of this study, the meta-analysis of published HPV prevalence and HNC in Black patients echoes these findings. Consistent with what is expected when comparing HPV prevalence in oropharyngeal and non-oropharyngeal cancer subsites, we show that for Black patients, cancers in the oropharynx have a higher prevalence of HPV16 (45.7%), than non-oropharyngeal sites (14.5%). There was large heterogeneity between the studies included in our meta-analysis. It is possible that differences in the HPV detection methods used in different studies may have influenced HPV positivity rates. For example, DNA ISH assays lack sensitivity and in general, PCR may lack specificity for transcriptionally active virus. Nevertheless, we observed that the meta-prevalence of HPV16 among Black patients is similar to the prevalence reported in our pooled analysis (i.e., higher in the oropharynx and lower in non-oropharyngeal sites).

We performed a pooled analysis of published HPV and HNC data in racial/ethnic subgroups in order to obtain a broader perspective. HPV status was obtained predominantly by PCR on FFPE tissues. Evaluation of HPV16, HPV18 and HPV16,18 prevalence by subsite and race yielded multiple findings. First, it is clear that HPV, specifically HPV16 or HPV18 within the oropharynx is most common in Whites (61%). There is a similar yet lower rate of HPV16,18+ disease in Blacks (58%) and a significant difference in the rate of HPV16,18+ disease in Asians (25%). This highlights the major HPV prevalence difference between Whites and Asians. This finding is curious, since the prevalence of HPV in Black patients has been reported to be statistically significantly lower than what has been reported for White patients in the literature (61,65). However, our pooled analysis reflects data from multiple institutions which is more reliable than a single study. The observed differences in HPV prevalence between Asians and Whites is also interesting and is not consistent with the previously reported meta-analysis (11). This inconsistency might be explained by differences in the type of Asian populations included in our study. This pooled analysis only included Asians from Taiwan (China) and Japan while the previously published meta-analysis included Asian populations from China and Korea. Significantly higher HPV prevalence was observed in Korean patients compared to Chinese patients and could explain the higher prevalence of HPV+ oropharyngeal cancer in Asians in that review (11).

An unexpected finding was the higher prevalence of HPV18 amongst Blacks. While HPV18 is rarely reported at either oropharyngeal (1.1%) or non-oropharyngeal cancer sites (1.5%) in Whites, HPV18 is nearly 15 times more frequently detected in Black oropharyngeal cancer patients. This major difference was unexpected. It is unclear if this is due to a higher rate of HPV18 infection in HNC in Blacks or a lower rate of HPV16+ oropharyngeal cancer in Blacks, thereby unmasking HPV18.

To better characterize oropharyngeal cancers, we evaluated by both HPV and p16 status. Canonical HPV oropharyngeal cancer is characterized by a HPV+/p16+ signature and p16 status

Table 4. Predictors of overall survival (all-cause mortality) for oropharyngeal cancer patients according to smoking status and race

All-cause mortality HR, 95% CI[a]							
		All races	All races				
Oropharynx	All races (N = 880)	Ever smokers (n = 746)	Never smokers (n = 134)	White (n = 475)	Non-White (n = 401)	Stages 0/I/II (n = 149)	Stages III/IV (n = 731)
Race							
White	Ref (1.00)	Ref (1.00)	Ref (1.00)			Ref (1.00)	Ref (1.00)
Asian	0.87 (0.56–1.37)	0.80 (0.50–1.28)	1.27 (0.27–5.91)			2.54 (0.73–8.78)	0.67 (0.41–1.11)
Black	0.85 (0.50–1.45)	0.78 (0.45–1.37)	2.14 (0.19–24.08)			1.47 (0.27–7.82)	0.71 (0.40–1.28)
Other	0.57 (0.08–4.15)	—	3.99 (0.46–34.18)			—	—
Age at diagnosis	1.25 (1.09–1.44)	1.23 (1.06–1.42)	1.47 (0.90–2.39)	1.75 (1.39–2.21)	1.00 (0.89–1.26)	1.45 (0.93–2.26)	1.24 (1.07–1.43)
Smoking							
Never smoker	Ref (1.00)			Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
Ever smoker	1.95 (1.34–2.83)			1.70 (0.99–2.93)	1.87 (1.11–3.17)	2.58 (0.79–8.46)	2.08 (1.39–3.11)
Alcohol							
Never drinker	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
Ever drinker	1.00 (0.80–1.26)	0.96 (0.76–1.22)	1.45 (0.65–3.23)	1.46 (0.92–2.34)	0.89 (0.67–1.20)	1.06 (0.51–2.22)	1.00 (0.79–1.28)
Sex							
Male	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
Female	0.74 (0.54–1.01)	0.74 (0.53–1.05)	0.69 (0.30–1.61)	0.70 (0.49–1.01)	0.87 (0.48–1.59)	0.36 (0.16–0.81)	0.87 (0.62–1.23)
Stage							
0/I/II	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)		
III/IV	2.08 (1.56–2.77)	2.15 (1.59–2.90)	1.83 (0.64–5.23)	2.20 (1.53–3.16)	2.13 (1.27–3.55)		
HPV/p16 status							
HPV16,18+/p16+	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
HPV16,18–/p16+	1.88 (1.19–2.97)	1.82 (1.09–3.03)	2.29 (0.74–7.08)	2.91 (1.72–4.92)	0.69 (0.24–2.01)	7.96 (2.08–30.43)	1.58 (0.95–2.63)
HPV16,18+/p16–	3.24 (2.25–4.66)	3.41 (2.32–5.01)	2.19 (0.57–8.44)	3.30 (2.09–5.21)	2.95 (1.60–5.42)	7.22 (2.26–23.11)	2.85 (1.91–4.25)
HPV16,18–/p16–	3.17 (2.39–4.20)	3.30 (2.43–4.47)	2.70 (1.12–6.51)	2.82 (1.94–4.10)	3.11 (1.97–4.92)	4.36 (1.51–12.60)	3.07 (2.28–4.13)

[a]Covariates included: square root age, year of diagnosis, race, sex, smoking, alcohol, stage at diagnosis combined HPV16,18 and p16 status and study.

Table 5. Risk of disease progression for oropharyngeal cancer patients according to HPV/p16 status and race

	Disease persistence and/or recurrence HR, 95% CI[a]	
HPV/p16 status	White N = 475	Non-White N = 401
HPV16,18+/p16+	Ref (1.00)	Ref (1.00)
HPV16,18–/p16+	2.33 (1.22–4.45)	0.52 (0.12–2.27)
HPV16,18+/p16–	3.62 (2.21–5.95)	1.44 (0.58–3.61)
HPV16,18–/p16–	3.23 (2.14–4.88)	2.70 (1.52–4.82)
	Metastasis HR, 95% CI[a]	
HPV16,18+/p16+	Ref (1.00)	Ref (1.00)
HPV16,18–/p16+	1.84 (0.49–6.90)	0.81 (0.35–1.88)
HPV16,18+/p16–	2.61 (0.90–7.51)	1.08 (0.48–2.42)
HPV16,18–/p16–	2.08 (0.88–4.91)	1.94 (1.26–2.99)

[a]Adjusted for year of diagnosis, square root age, sex, race, stage, smoking, alcohol and study.

has been reported previously as the best prognostic marker for this disease (63,66). Oropharyngeal cancer that develops in White nonsmokers is mostly likely to be HPV-associated. Our study confirmed this finding; nearly 80% of White nonsmokers were HPV+/p16+ (Figure 2C). As p16 loss is associated with smoking (67), amongst ever smokers, a much higher incidence of p16– disease was reported in all races. Although approximately 45% of ever smokers continue to be HPV+/p16+, only half that frequency of HPV+/p16+ is reported in non-Whites. Amongst Blacks and especially Asians, HPV–/p16+ disease comprises the majority of oropharyngeal disease, in distinction to Whites, where HPV+/p16+ disease is the predominant disease.

While it is not surprising that patients with HPV–/p16+ oropharyngeal cancer have a higher risk of death compared to patients with HPV+/p16+ oropharyngeal cancer, it was interesting to note that among non-Whites, the risk of death for patients with HPV–/p16+ oropharyngeal cancer was not different from patients diagnosed with HPV+/p16+ oropharyngeal cancer (HPV16,18–/p16+ HR: 0.69, 0.24–2.01). Unlike Whites (HPV16,18–/p16+ HR: 2.91, 1.72–4.92), the survival benefit among non-Whites appears to be attributed to p16 status rather than HPV. In Whites, the survival benefit appears to be attributed to HPV status rather than p16 status. However, it is possible that HPV16,18–/p16+ oropharyngeal cancers in non-Whites may be attributed to other high-risk HPV types. Further investigation of the possible role of high-risk HPV types other than HPV16,18 in non-White oropharyngeal cancer patients is needed. Overall, our findings suggest that the difference in HPV/p16 patterns according to race may impact survival differently. Given the multifactorial cause of racial survival disparities, such as poor socioeconomic status and poor access to care, the effect of HPV/p16 patterns on racial disparities in survival is not easily identified and further investigations are needed.

A limitation of this study is the use of publications as the source of patient data. Unlike database data, like SEER or The National Cancer Database, published data represent a sampling of the true population. A major assumption of our pooled analysis is that the landscape of the published literature is representative of the population as a whole. Given the dramatic differences noted in survival here between Whites and non-Whites, we feel it is highly unlikely that an error in sampling of the literature can explain these differences. A high fraction of cells with expression of p16 in both the nucleus and cytoplasm is the only good correlation with prognosis and with high-risk HPV mRNA. For each of the studies included in the pooled analysis, we did not have detailed information on the cutoffs used to define p16 status (i.e., fraction of p16 expression in nuclei versus cytoplasm). This is also a limitation of our study, as this detail may have provided more accurate correlations of p16 expression and outcome according to race.

The reasons for this difference in patterns of HPV/p16 in oropharyngeal cancer are unclear. While smoking status has predicted p16 status (67), even amongst never smokers in this study, the prevalence of HPV+/p16+ disease is lower in non-Whites. Possible explanations include genetic and environmental causes. The development of HPV+ oropharyngeal cancer has been associated with differences in sexual behavior patterns and marijuana use (68). Differential sexual and behavior patterns amongst Whites versus non-Whites have not been studied well. While the number of oral sex partners has been identified in the risk of developing HPV+ oropharyngeal cancer (68). The percentage difference in ever oral sex partners in individuals 45–60 years old between Whites and Blacks appears modest (about 15% difference in prevalence) from a few major studies (69,70), but this remains an area of active research. Other potential explanations are genetic differences between races and differences in the host response to HPV infection, which merit further investigation. Intratypic variation of HPV16 is associated with geographical distribution and may contribute to differences in outcome (71–76). For example, African and Asian-American intratypic variants of HPV16 show higher transforming potential in tumors of the anogenital tract. Therefore, in HNC, differential infection by HPV variants between races may also be an important area for investigation.

At this time, we do not have sufficient understanding to offer a clear recommendation as to how to reduce oropharyngeal HPV infection or the risk of developing HPV+ oropharyngeal cancer. This appears to be a problem of environment and biology, without a reversible modifiable factor to reduce risk. We hope that greater adoption of HPV vaccination will alter the incidence curve within about 20 years. Our study has examined HPV and HNC, with a focus on oropharyngeal cancer. This study demonstrates that while HPV-related oropharyngeal cancer (HPV+/p16+) represents the majority cause among White patients, Blacks and Asians have lower rates. Because HPV-related oropharyngeal cancer has a more favorable outcome regardless of race, the differential HPV prevalence amongst Blacks and Asians is expected to cause a significant outcome disparity in oropharyngeal cancer treatment. Further studies specifically examining racial differences in HPV+ oropharyngeal cancer are needed to corroborate these findings. However, this comprehensive pooled analysis of the published literature strongly supports a prevalence disparity in HPV+ oropharyngeal cancer that would predict an outcome/survival disparity.

Supplementary material

Supplementary data are available at *Carcinogenesis* online.

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