



HPV Subtypes in Oropharyngeal Squamous Cell Cancer

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Abstract

Title

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Educational Objective

At the conclusion of this presentation, the participants should appreciate the differences between HPV types and subtypes present in cervical and oropharyngeal cancer.

Objectives

To explore the differences, specifically E6 genomic polymorphisms, in the HPV 16 present in cervical versus oropharyngeal carcinoma in a southeast Texas community.

Study Design

Archival tissue cohort assessment

Methods

130 patients with HPV positive cervical cancer and 89 patients with HPV positive oropharyngeal cancer were identified retrospectively. For each patient, formalin-fixed paraffin embedded biopsy or surgical specimen tissue was obtained. DNA was extracted from each tissue sample and was amplified using PCR. The PCR products were then sequenced and used in analysis. Two different primer sets were utilized. The gp5+/gp6+ primer set was used to identify HPV type present and an E6-specific primer set was used to identify the HPV16 subtype and to determine the E6 DNA sequence.

Results

The predominate HPV types in the cervical cancer group were identified as 16, 18, and 45. In the oropharyngeal cancer population HPV type 16 was the overwhelming majority (91%). With respect to HPV 16 subtypes, the cervical cancer group had 70% belong to the European subtype and 26% were of the Asian-American subtype. This is in contrast to the oropharyngeal cancer group in which 91% were of the European subtype with no instances of the Asian-American subtype.

Conclusions

Significant differences exist in the genetic makeup of HPV positive cervical and oropharyngeal cancers.

Introduction

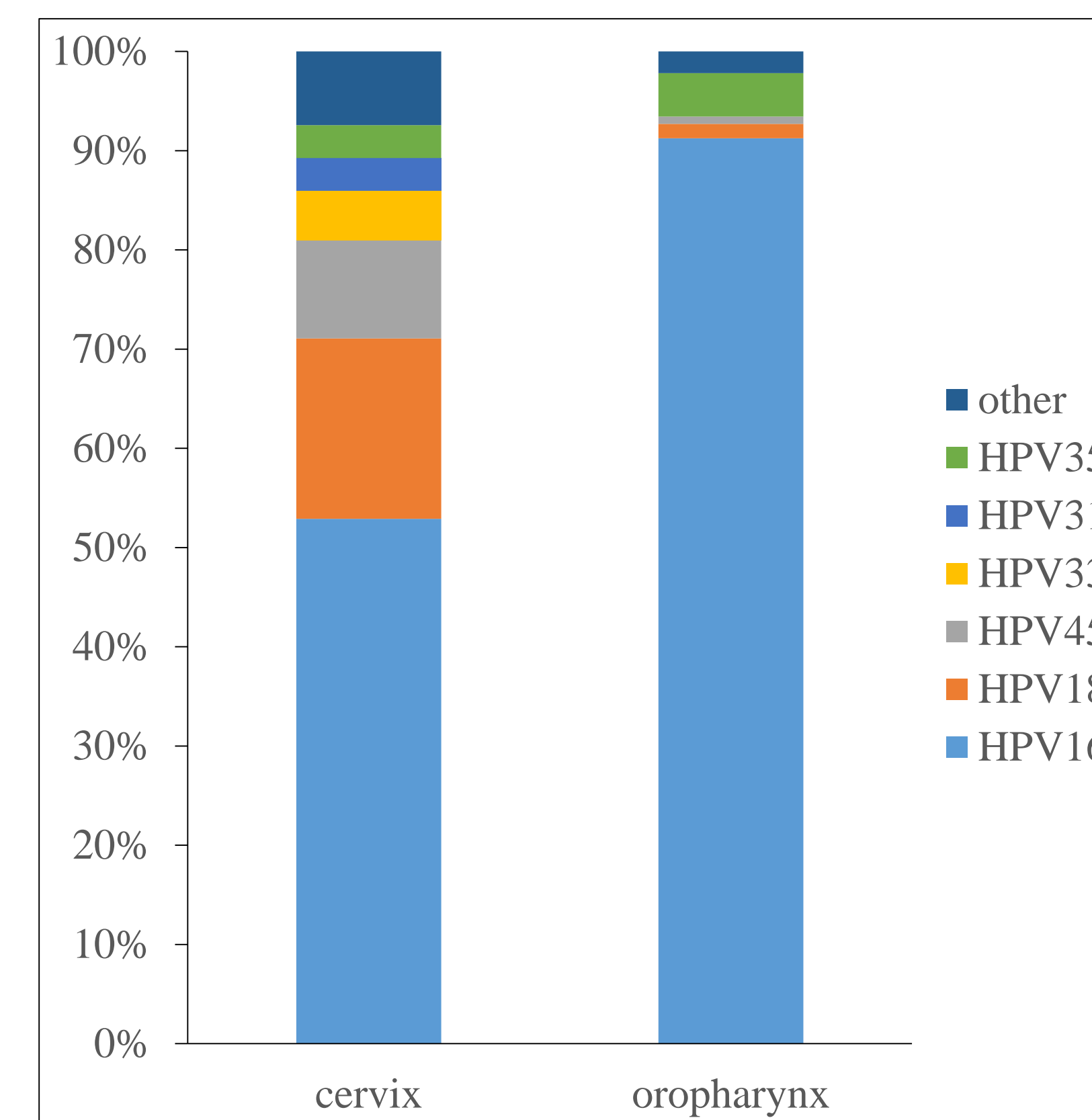
Beginning in the 1970s, HPV related oropharyngeal squamous cell carcinoma (OSCC) incidence in the US increased while non-HPV related OSCC incidence declined.^{1,2,3} The decline in non-HPV related OSCC is often linked to the corresponding decrease in smoking prevalence^{4,5}, while the increase in HPV related OSCC is usually attributed to increases in orogenital sexual contact.¹ An alternative explanation for the increase in HPV related oropharyngeal cancer incidence is genetic drift in the HPV genome that leads to higher infectivity or carcinogenesis in the oropharynx.

Polymorphisms in the HPV 16 genome leading to higher infectivity in the cervix have been described in previous studies.^{6,7} Specifically, several polymorphisms have been described in the E6 as well as LCR genes of the high risk HPV 16 virus in the cervix and potential roles in tumor biology for some of these polymorphisms have been suggested.^{6,7} The HPV protein product E6 is essential to the malignant potential of the virus. It is able to drive degradation of the p53 tumor suppressor by activation of the ubiquitin pathway. This leads to low p53 levels in HPV infected cells with consequent tumor cell growth, since without the p53 induced apoptosis of DNA damaged cells, tumor growth is able to proceed unchecked.^{8,9}

While extensive research has been done regarding the connection between HPV and cervical cancer, the role of HPV genomic polymorphisms in oncogenesis is a generally unexplored area for oropharyngeal cancer.

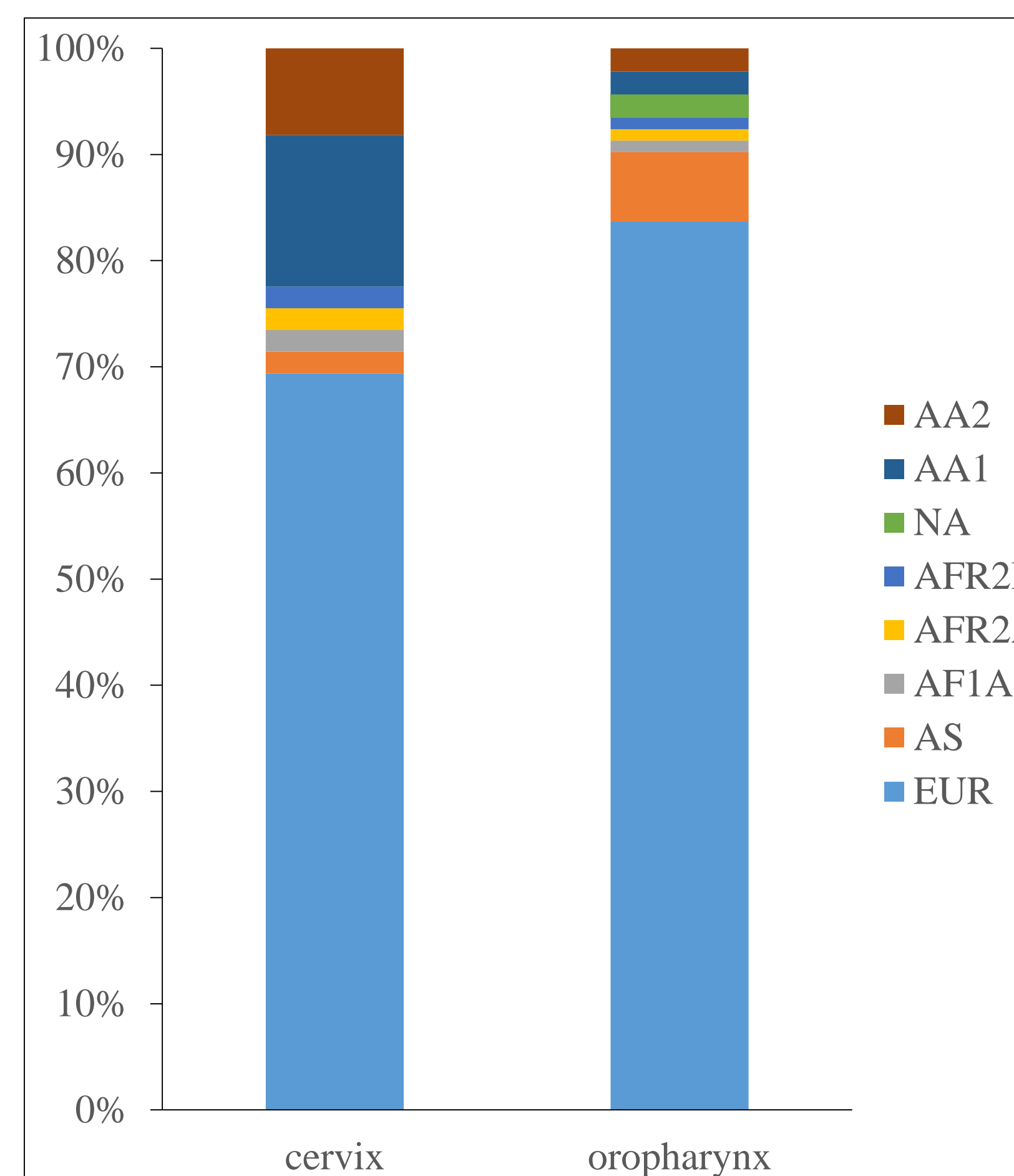
The purpose of this study is to explore the differences, specifically E6 genomic polymorphisms, in the HPV present in cervical versus oropharyngeal carcinoma in a southeast Texas community. We hypothesize that important genetic differences exist between E6 sequences within HPV-related cervical cancers and HPV-related oropharyngeal cancers.

Figure 1: HPV Types in Cervical and Oropharyngeal Squamous Cell Cancer



The HPV present in the oropharynx was predominately HPV16 with little variation, whereas in the cervix HPV 18 and 45 made significant contributions in addition to HPV16.

Figure 2: HPV16 Subtypes in Cervical and Oropharyngeal Squamous Cell Cancer



The HPV16 subtype predominately found in both the cervix and the oropharynx was the European subtype. Asian American subtypes were seen more frequently in the cervix, whereas the Asian subtype was seen more often in the oropharynx.

Methods and Materials

Samples to be included in the study consisted of patients diagnosed by histopathology with squamous cell carcinoma of the oropharynx, tonsils, or base of tongue between 2003 and 2016 at UTMBHealth in Galveston, Texas. 270 cases fitting the criteria were identified and 226 formalin fixed paraffin embedded (FFPE) samples were available. Samples for comparison consisted of patients diagnosed by histopathology with squamous cell carcinoma of the cervix between 2011 and 2015 at UTMBHealth, with 154 FFPE samples available. A pathologist evaluated the samples and regions containing tumor cells were marked. DNA was extracted using the QIAamp DNA minikit (Qiagen) following the manufacturer's instructions. HPV DNA was detected and amplified by PCR using the GP5+/GP6+ primer set under the published conditions. Additionally, the E6 region was amplified with additional primer pairs. The PCR products were sequenced and the sequences were aligned to the reference HPV genome to identify the type, subtype, and mutations in the E6 gene. Alterations were multiply and independently verified.

Figure 3: Percentage of HPV Positive Oropharyngeal Squamous Cell Cancer Samples by Year

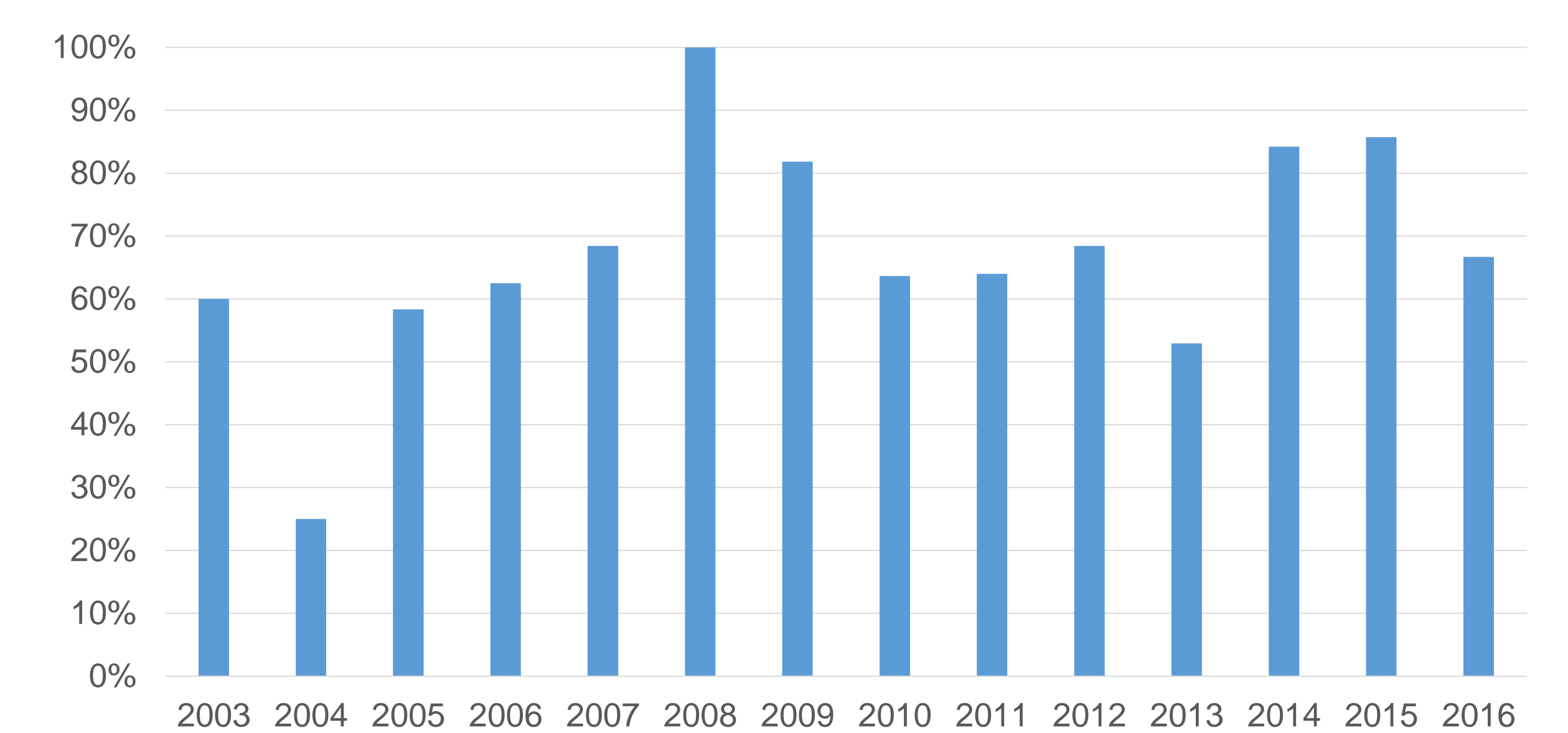


Figure 4: Non-Silent Mutations in HPV Present in Cervical and Oropharyngeal Squamous Cell Cancer

tumor site	DNA mutation	protein change
oropharynx	315G	S78C
	315G+350G	S78C+L90V
	173A	H31N
	188A	E36K
	293A	D71N
	395C +350G	D105H +L90V
	375G	Q98R
	395T	D105Y
	93C+245T+350G	K4T+R55W+L90V
	350G+442C	E120D+L90V
	93A,94G,178A	R5G+D32E
	95-427	DELETION R5-K115
	256T+350G	silent +L90V
	454G	silent
	178G*+360A	D32E*+T93K
	178G*+474C	D32E*+R131T
	*As subtype marker	
cervix	365G	L95V
	187G	silent

The numbering for DNA mutations is based on the HPV16 reference genome, NC_001526.3. Note that in Nov 2016, the NCBI introduced a new version of this sequence, NC_001526.4, which radically changed the numbering of the bases. At this time, we have chosen to retain the old numbers to facilitate comparisons with previously published data. The numbering for the amino acids has remained unchanged.

Results and Discussion

This study demonstrates that HPV16 is the predominant HPV type found in OPSCC (91%) with other types making up at a maximum 4%. This is in contrast to cervical squamous cell carcinoma, in which HPV16 only makes up 53% with HPV 18 and 45 making up a significant portion (18% and 10%, respectively). See Figure 1. Furthermore, when looking at HPV16 subtypes OPSCC has 84% European versus 70% European in the cervix. Additionally, in the cervix Asian American 1 and 2 make up 14% and 8% of the HPV16 subtypes versus only 2% for each in the head and neck. Lastly in the head and neck, the Asian subtype makes up 7% of the HPV16 versus only 2% in the cervix. See Figure 2. These results highlight the fact that not only are there different HPV types present in the cervix versus the oropharynx, but there are also different subtypes.

In contrast to published data, our population did not show an increasing trend of HPV positive OPSCC. See Figure 3.

Functional mutations in E6 have been described in the cervix, but have not been evaluated in the oropharynx. This study demonstrates that there are more and different mutations in E6 found in the oropharynx than are found in the cervix. See Figure 4. The next step is to determine if the mutations are causing a functional difference. If they are, this could explain an increase in infectivity or carcinogenesis of the HPV16 virus in the oropharynx leading to an increase in the incidence of OPSCC in recent years.

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