HPV DNA, E6/E7 mRNA, and p16^{INK4a} detection in head and neck cancers: a systematic review and meta-analysis

Cathy Ndiaye, Marisa Mena*, Laia Alemany, Marc Arbyn, Xavier Castellsagué, Louise Laporte, F Xavier Bosch, Silvia de Sanjosé, Helen Trottier*

Summary

Background We aimed to provide updated information about the global estimates of attributable fraction and type distribution of human papillomavirus (HPV) in head and neck squamous cell carcinomas by doing a systematic review and meta-analysis.

Methods We did a literature search on PubMed to identify studies that used PCR for detection of HPV DNA in head and neck squamous cell carcinomas with information about HPV genotype distribution. We included studies that tested 20 or more biopsies per cancer site and were published between July 15, 1990, and Feb 29, 2012. We collected information about sex, risk factors, HPV detection methods, and biomarkers of potentially HPV-induced carcinogenesis (E6/E7 mRNA and p16^{INK4a}). If it was not possible to abstract the required information directly from the paper, we contacted the authors. We did a meta-analysis to produce pooled prevalence estimates including a meta-regression to explore sources of heterogeneity.

Findings 148 studies were included, contributing data for 12 163 cases of head and neck squamous cell carcinoma from 44 countries. HPV DNA was detected in 3837 cases. HPV16 accounted for 82·2% (95% CI 77·7–86·4) of all HPV DNA positive cases. By cancer site, pooled HPV DNA prevalence estimates were 45·8% (95% CI 38·9–52·9) for oropharynx, 22·1% (16·4–28·3) for larynx (including hypopharynx), and 24·2% (18·7–30·2) for oral cavity. The percent positivity of p16^{INK4a} positive cases in HPV-positive oropharyngeal cancer cases was 86·7% (95% CI 79·2–92·9) and of E6/E7 mRNA positive cases was 86·9% (73·2–96·8). The estimate of HPV attributable fraction in oropharyngeal cancer defined by expression of positive cases of E6/E7 mRNA was 39·8% and of p16^{INK4a} was 39·7%. Of subsites, tonsils (53·9%, 95% CI 46·4–61·3) had the highest HPV DNA prevalence. HPV DNA prevalence varied significantly by anatomical site, geographic region, but not by sex or tobacco or alcohol consumption.

Interpretation The contribution of HPV prevalence in head and neck squamous cell carcinoma and in particular that of HPV16 in the oropharynx shows the potential benefit of prophylactic vaccines.

Funding European Commission.

Lancet Oncol 2014; 15: 1319–31

Published Online
October 16, 2014
http://dx.doi.org/10.1016/S1470-2045(14)70471-1

See Comment page 1282

*These authors contributed equally

Department of Social and Preventive Medicine, Université de Montréal, Montreal, QC, Canada (C Ndiaye PhD, H Trottier PhD); **Sainte-Justine Hospital Research Center, Montreal, QC, Canada** (C Ndiaye, L Laporte BSc, H Trottier); **Institut Català d'Oncologia (ICO) – Catalan Institute of Oncology, IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain** (M Mena PhD, L Alemany MD, X Castellsagué PhD, F X Bosch MD, S de Sanjosé PhD); **CIBER Epidemiología y Salud Pública, CIBERESP, Barcelona, Spain** (L Alemany, X Castellsagué, S de Sanjosé); **Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels, Belgium** (M Arbyn DrTMH); **and University of Antwerp, Antwerp, Belgium** (M Arbyn)

Correspondence to:
Dr Marisa Mena, Unit of Infections and Cancer, Cancer Epidemiology Research Program, Institut Català d'Oncologia (ICO), Catalan Institute of Oncology, IDIBELL, Gran Via de l'Hospitalet, L'Hospitalet de Llobregat, Barcelona, Spain
mmena@iconcologia.net

Introduction

In the past decade, an increasing amount of information about the role of human papillomavirus (HPV) in head and neck cancer has been amassed. Nowadays, it is widely accepted that HPV-related head and neck cancers differ substantially from those that are HPV-unrelated—which are mainly caused by tobacco and alcohol—at the genetic, molecular, epidemiological, and clinical level.[1] The resulting increased knowledge has raised some controversies about the accurate proportion of HPV-driven cases, the role of cofactors, and the heterogeneity of HPV prevalence in anatomical sites of the head and neck (oral cavity, oropharynx, and larynx) and across geographical regions. Studies have shown that patients with HPV-related oropharyngeal cancer respond better to treatment and have better survival than patients with HPV-unrelated oropharyngeal cancer.[2] Moreover, preliminary results from an ongoing randomised trial designed to assess the efficacy of the bivalent vaccine against cervical HPV infections and lesions have shown strong protection conferred by the vaccine against oral infection with HPV16 and HPV18.[3]

Worldwide, an estimated 599 637 new cases of and 324 794 deaths from head and neck cancer (excluding nasopharyngeal cancer) occur every year.[4] The epidemiology of head and neck cancer shows sex and regional diversities with most of the cases occurring in men in south central Asia, east Asia, North America, and central eastern Europe, typically attributed to greater tobacco and alcohol consumption.[4] A recent review estimated that the probability of an HPV-attributable cancer of the oral cavity, larynx, and hypopharynx is at least five times lower than that of oropharyngeal cancer.[5] An increase in the incidence of oropharyngeal cancer has been noted in several high-income countries during the past two decades[6,7] and also in the proportion of HPV-related oropharyngeal cancer.[8]

In 2005, a systematic review of HPV DNA detection in head and neck squamous cell carcinomas identified 5640 cases from 60 studies from 26 countries.[9] The review established that HPV16 accounted for most types of HPV detected in all sites, particularly in the oropharynx (86·7% of all HPV DNA positive cases). Substantial regional variations in HPV DNA prevalence were also reported.[9] However, major gaps remained including little data for Africa, Asia, and Oceania, and important covariates such as sex, tobacco, and alcohol

consumption were not taken into account. Differences between PCR methods were not considered. More recent reviews[8,10] provided updated information about HPV prevalence in head and neck squamous cell carcinomas, but analysed neither the distribution of HPV types nor the covariates mentioned above. To the best of our knowledge, no study so far has done a meta-analysis of HPV prevalence in head and neck subsites. Further, to our knowledge, no meta-analyses of HPV DNA prevalence and type distribution have been done presenting additional data for E6/E7 mRNA, a biomarker of HPV viral transcriptional activity, or $p16^{INK4a}$, a cell surrogate marker of HPV-induced carcinogenic transformation. The identification of HPV DNA in the tumour does not provide enough evidence for a causal viral association, because the technique cannot differentiate transitory versus oncogenically active infection. The identification of the transcripts of the viral oncogenes E6/E7, implicated in the oncogenic process, through mRNA techniques[11] is widely accepted as the present gold-standard test to elucidate the oncogenic role of HPV in the tumour. The use of inmunohistochemical techniques to detect $p16^{INK4a}$ followed by HPV DNA detection has been also validated to clinically detect an oncogenically active HPV infection in oropharyngeal cancer.[12]

We aim to provide updated information about the global prevalence and type distribution of HPV in head and neck squamous cell carcinomas, by cancer site and by continent, and to assess the proportion of HPV-driven head and neck squamous cell carcinomas by taking into account biomarkers of carcinogenesis such as $p16^{INK4a}$ and E6/E7 mRNA. Additionally, we explore the role of sex and other cofactors such as tobacco and alcohol consumption and HPV detection methods.

Methods

Search stratedy and selection criteria

CN and MM independently searched the National Institutes of Health PubMed electronic databases for relevant studies on the prevalence of HPV DNA in head and neck squamous cell carcinoma using the following MeSH terms: "papillomaviridae" and "head and neck neoplasms" in combination with keywords "polymerase chain reaction" or "PCR", published between Feb 1, 2004, and Feb 29, 2012. We also included all papers selected in Kreimer and colleagues' systematic review[9] (published from July 15, 1990 to Feb 20, 2004). MOOSE guidelines and the PRISMA statement were consulted to conduct the search.[13,14] We also reviewed reference lists of included studies to identify additional articles.

Study selection and data extraction

CN, MM, and LA independently assessed the studies for inclusion in the review. All articles reporting data for HPV DNA prevalence and type distribution in head and neck squamous cell carcinomas since Aug 15, 1990, were selected. An effort was made to exclude redundant articles including the same population from the final analysis. If articles from the same cohort were retrieved, the article analysing the largest sample was selected. For studies involving more than one geographic location, the data were divided into components from each continent if possible.

We included studies if they had HPV information about at least one of the following cancer sites or subsites: oral cavity, oropharynx, hypopharynx, and larynx; a minimum of 20 site-specific cases tested for HPV (for this criteria hypopharynx and larynx cases were combined); a clear description of DNA PCR-based testing methods; identification of the histological classification as squamous cell carcinoma; primary tumour; and diagnosis of a tumour confined in only one site. We excluded carcinomas in situ and nasopharyngeal carcinomas. Unless otherwise specified, we assumed that all cases were primary. We further excluded recurrent cases and purposively selected cases—eg, only stage III/IV.

Head and neck squamous cell carcinomas (with their associated International Classification of Diseases-10 code) were classified as follows: oral cavity: anterior two-thirds of the tongue (C02.0–3, C02.5–9), tongue unspecified (C02.9), lip (C00.0-9), gum (C03.0–9), floor of the mouth (C04.0-9), hard palate (C05.0), palate unspecified (C05.9), other oral cavity—including buccal mucosa and retromolar area (C06.0–8), oral cavity unspecified (C06.9); oropharynx: base of the tongue (C01), soft palate (C05.1), tonsil (C02.4, C09.0–9), uvula (C05.2), other parts of the oropharynx (C10.0-9), Waldeyer's ring (C14.2), and oropharynx unspecified (C10.9); or larynx: glottis (C32.0), supraglottis (C32.1), subglottis (C32.2), other and unspecified larynx subsites (C32.3–9). Hypopharynx cases (C13) were classified as belonging to the larynx, including pyriform sinus (C12). Tongue and palate unspecified cases were classified as belonging to the oral cavity. Maxilla, mandible, other bone, and cheek non-mucosal sites were excluded.

MM, LL, and LA extracted the data and CN and LA cross-checked the data extraction. CN, LA, and MM reviewed and solved discrepancies by consensus. The variables collected specifically for all anatomical sites when available included: first author, publication year, journal of publication, study country, study population, case selection methods, study period, mean age of study population, sex distribution, tobacco smoking (ever/never) and alcohol consumption (ever/never), method of specimen preservation, additional biomarkers other than HPV DNA detection, number of controls for contamination items mentioned in the paper, primers used for HPV DNA PCR testing and genotyping method, and length of the amplicon used in the PCR (taking HPV16 as a reference). Whenever the length of the amplicon was not directly reported in the paper, it was calculated with the HPV16 gene sequence and the forward and reverse primers obtained from the papers.

When the information was not available, the technique was classified as unspecified. In cases for which more than one type-specific primer was used, the shorter amplicon taking into account HPV16 as a reference was reported. Other variables collected were cancer site and subsite, site-specific sample size and number of HPV-positive samples, site-specific number of HPV types detected, and number of multiple infections. Data were collected for all detectable HPV types. The papers were assessed for quality through collection of eight quality criteria: use of broad spectrum PCR, use of human gene marker, whether the cases were histologically confirmed, control for contamination mentioned in the paper, whether the paper contained specific information about age and sex, whether the recruitment period was stated, whether sample size was at least 50 cases, and whether the cases were recruited randomly or consecutively or were incident cases. The quality score of all papers was calculated as the sum of the result (0=no/1=yes) for all quality control items. A new variable was created, HPV group, in which the different cancer sites or subsites were classified in view of their association with HPV according to the existing evidence:[15,16] HPV group 0 (weak association with HPV): oral cavity without subsite specification, lip, floor of the mouth, hard palate, gum, other oral cavity, larynx, and hypopharynx without subsite specification, glottis, supraglottis, subglottis, and pyriform sinus; HPV group 1 (moderate association with HPV): tongue and palate unspecified; or HPV group 2 (strong association with HPV): oropharynx without subsite specification, tonsil, soft palate, base of the tongue, and other parts of the oropharynx.

If it was not possible to extract the required information directly from the paper, authors were contacted a maximum of three times to provide clarifications or additional information.

Two additional datasets were also prepared, one with information about HPV DNA prevalence and type distribution stratified by sex and another with information about additional biomarkers E6/E7 mRNA and $p16^{INK4a}$. The variables collected specifically for the final dataset were type of cases tested for additional biomarkers, number of HPV DNA positive cases tested for $p16^{INK4a}$, E6/E7 mRNA, or both, antibody and staining cutoffs used in the $p16^{INK4a}$ assay, techniques used for the E6/E7 mRNA assay, and number of HPV DNA positive cases additionally positive for $p16^{INK4a}$ or E6/E7 mRNA.

Statistical analysis

HPV DNA prevalence was computed by dividing the number of HPV DNA positive cases by the total number of cases tested. HPV DNA type-specific prevalence was calculated by dividing the number of cases positive for a specific type (present in either single or multiple infections) by the total number of cases tested for that type. HPV DNA type-specific relative contribution was calculated by dividing the number of cases positive for a specific type (present in either single or multiple infections) by the total number of HPV DNA positive cases tested for the specific type. To pool prevalences and relative contributions, we used *metaprop*, statistical procedure in STATA (version 10.1), developed at the Unit Cancer Epidemiology (IPH, Brussels).[17,18] A random effects model including Freeman-Tukey arcsine transformation of the prevalence was used to normalise variance. 95% CIs were computed around study-specific and pooled prevalences based on the score-test statistic. Interstudy heterogeneity was assessed with Cochran's *Q*-test.[19] The percentage of total variation across studies because of heterogeneity was evaluated with the I^2 measure.[20] Prevalences were pooled by anatomical site, available subsites, worldwide, and by geographical region and sample size.

Relative risks (RR) for overall HPV DNA and HPV16 DNA prevalence in men compared with in women were pooled with *metan* in STATA (version 10.1).[21]

Pooled $p16^{INK4a}$ and E6/E7 mRNA percent positivities were computed with *metaprop* command.[17,18] $p16^{INK4a}$ and E6/E7 mRNA percent positivities refer to the percentage of HPV DNA positive samples that were also positive for $p16^{INK4a}$ or E6/E7 mRNA in relation to all the HPV DNA positive samples tested for the specific biomarker. Subgroup meta-analysis was done for the proportion of $p16^{INK4a}$ positivity by staining cutoffs and type of selection of patients with oropharyngeal cancer. The HPV attributable fraction was computed as the product of the HPV DNA prevalence and the percent positivities of the biomarker in HPV-positive cases.

The effect of covariates on HPV DNA prevalence in head and neck squamous cell carcinomas was explored with meta-regression.[22] The covariates were study country; geographical region; age; proportion of men; proportion of ever-smokers; proportion of ever-drinkers of alcohol; start, middle, and end year of the study period; method of specimen preservation; number of items mentioned related to control of contamination; primers used for HPV DNA amplification; length of the amplicon target of the PCR; the eight quality control measures, and the overall quality score. Only the covariates identified to be statistically significant ($p<0·05$) in a first-step bivariate analysis were included in the multivariate models.

Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We identified 355 abstracts from the PubMed search, in addition to the the 60 articles from Kreimer and colleagues' systematic review.[9] After duplicates were removed, 411 records were assessed for eligibility and

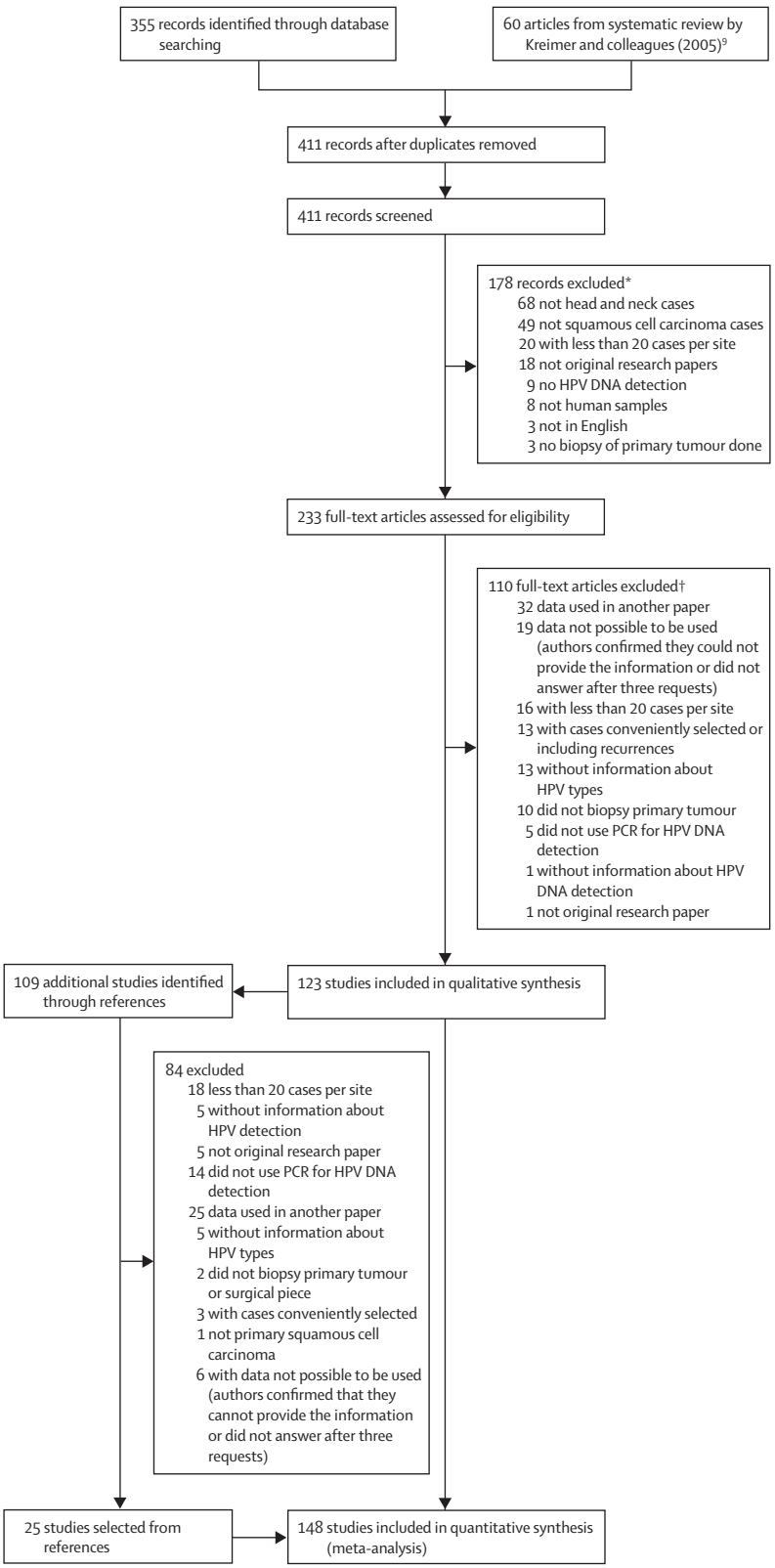

Figure 1: **Flow diagram of selected studies**
HPV=human papillomavirus. *Some records had more than one of the listed reasons for exclusion and were classified according to the following order of priority: not original research paper > not human beings > not head and neck localization > not primary squamous cell carcinoma > not biopsy in tumour > no information about HPV DNA detection > fewer than 20 cases > not in English language. †Some records had more than one of the listed reasons for exclusion and were classified according to the following order of priority: with data used in another paper > cases conveniently selected > PCR not used > no information about HPV types.

	Total studies	Cases	Overall HPV DNA prevalence (95% CI)	HPV16 DNA prevalence (95% CI)
Oral cavity	72	5478	24·2% (18·7–30·2)	14·9% (11·1–19·1)
Oropharynx	53	3946	45·8% (38·9–52·9)	40·6% (34·4–47·0)
Larynx*	54	2739	22·1% (16·4–28·3)	13·4% (9·1–18·4)
Overall	148	12 163	29·5% (25·5–33·6)	21·1% (17·8–24·6)

HPV=human papillomavirus. *Including hypopharynx cases.

Table 1: **HPV DNA prevalence in head and neck squamous cell carcinoma by anatomical site**

148 records were included in the final analysis (figure 1). The characteristics and variables collected for all studies are shown in the appendix (pp 1–8). 12 163 cases of head and neck squamous cell carcinomas were included from 44 countries.

Table 1 summarises the main results for each anatomical site, including the number of studies and cases tested for HPV DNA, which were detected in 3837 head and neck squamous cell carcinomas. By cancer site, pooled HPV DNA prevalence estimates were 45·8% (95% CI 38·9–52·9) for oropharynx, 22·1% (16·4–28·3) for larynx (including hypopharynx), and 24·2% (18·7–30·2) for oral cavity. No significant differences in HPV DNA prevalence were noted when larynx and hypopharynx were analysed separately (23·9% [95% CI 18·1–30·2] *vs* 21·9% [5·3–29·1]). Thus, the two anatomical sites were combined in further analyses.

In the analysis by anatomical site and geographical region (figure 2; appendix p 9), the highest HPV DNA prevalence in oral cavity squamous cell carcinoma was noted in Asia and South and Central America, whereas the lowest prevalence was noted in Africa. In oropharyngeal squamous cell carcinoma, HPV prevalence was significantly higher in North America than in Asia, Oceania, and Europe ($p<0·0001$). The lowest HPV DNA prevalence in oropharyngeal squamous cell carcinoma was noted in South and Central America. By contrast, this region showed the highest HPV DNA prevalence in laryngeal squamous cell carcinoma, whereas the lowest HPV DNA prevalence was detected in North America. Considerable heterogeneity was identified between regions and countries and also within regions and countries (figure 2). HPV DNA prevalence (overall and HPV16-specific) was also assessed by European subregions (Central and Eastern, Northern, Southern and Western,

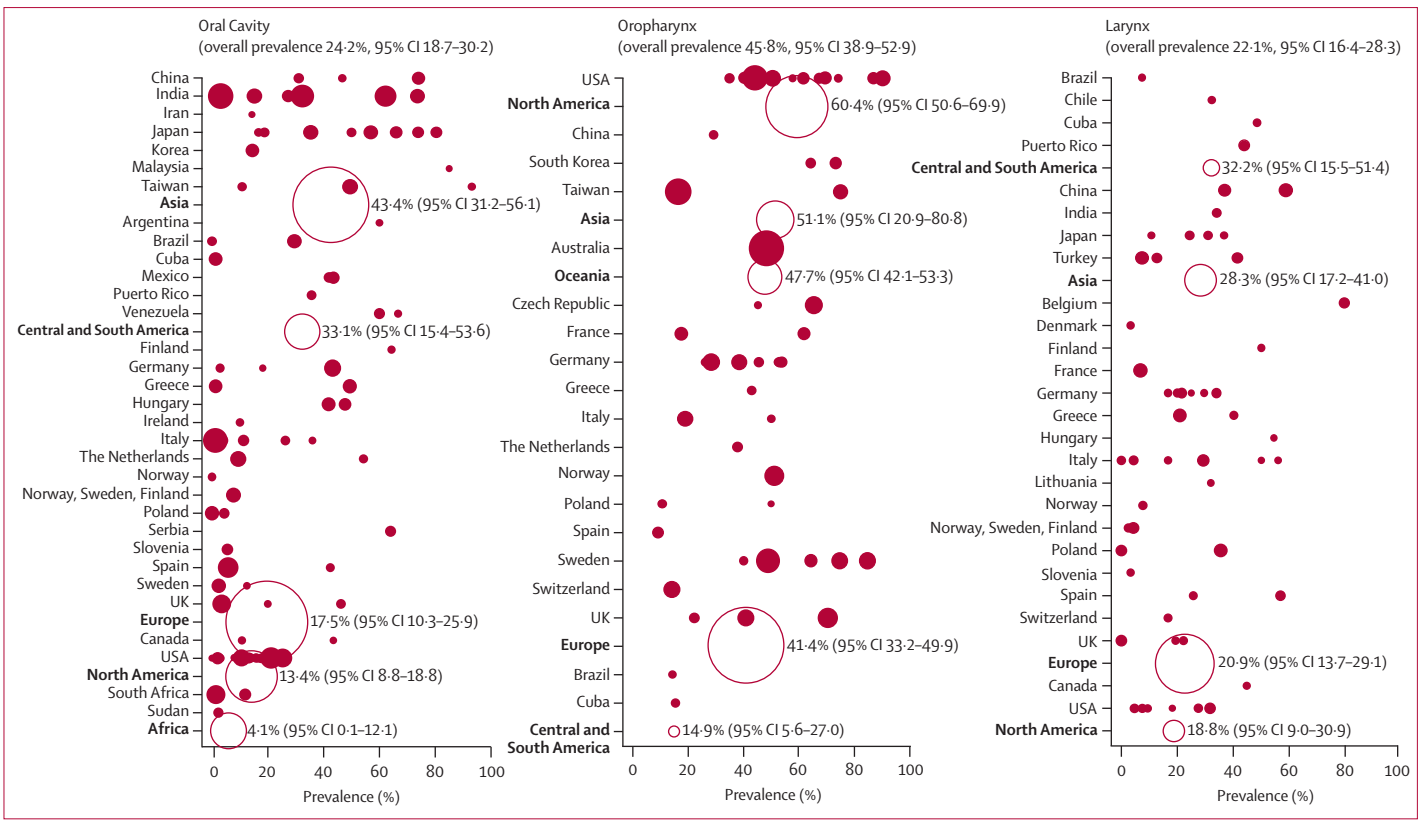

Figure 2: **HPV DNA prevalence in head and neck squamous cell carcinoma by anatomical site, study, country, and geographical region**
HPV=human papillomavirus. Larynx includes hypopharynx cases. Filled circles correspond to study-specific prevalence. Unfilled circles correspond to pooled estimated prevalence for corresponding region. Sizes of filled circles and unfilled circles are proportional to the number of cases. Two studies presented the information aggregated for Norway, Sweden, and Finland. One study with 507 cases was excluded for the regional analysis because data were presented aggregated for Central Europe and Central and South America.

as defined by GLOBOCAN)[4] and significant differences were noted for oropharyngeal squamous cell carcinoma. Overall HPV DNA prevalence for Northern Europe was 56·5% (95% CI 44·4–68·3), for Central and Eastern Europe was 41·8% (15·5–70·7), for Western Europe was 37·6% (27·4–48·3), and for Southern Europe was 24·2% (13·6–36·4; p value for heterogeneity between groups 0·004). The results were equivalent when pooling HPV16-specific prevalences: 51·1% (40·1–62·1) for Northern Europe, 32·6% (3·6–71·6) for Central and Eastern Europe, 32·5% (22·7–43·0) for Western Europe, and 21·9% (12·8–32·6) for Southern Europe (p value for heterogeneity between groups 0·003). No significant differences were noted across European subregions for oral cavity or laryngeal squamous cell carcinoma (appendix p 9).

HPV16 DNA accounted for 82·2% (95% CI 77·7–86·4) of all HPV DNA positive cases. HPV16 DNA prevalence was higher in oropharyngeal squamous cell carcinoma (40·6%, 95% CI 34·4–47·0) than in oral cavity squamous cell carcinoma (14·9%, 11·1–19·1) or in laryngeal squamous cell carcinoma (13·4%, 9·1–18·4; p<0·0001). Figure 3 shows the HPV type distribution of the six most common types for anatomical sites, overall, and by region. HPV16 was the most common type in all sites and regions with the exception of in oral cavity squamous cell carcinoma in Africa where HPV18 was the most common type. Nevertheless, the prevalence of HPV16 DNA varied substantially between regions and sites, with the highest observed in oropharyngeal squamous cell carcinoma in North America (54·7%, 95% CI 47·0–62·4) and the lowest in oral cavity squamous cell carcinoma in Africa (0·5%, 0·0–1·2). Worldwide, HPV18 was the second most prevalent type in oral cavity squamous cell carcinoma (5·9%, 95% CI 3·4–9·0) and laryngeal squamous cell carcinoma (1·6%, 0·6–3·1). However, this ranking showed marked heterogeneity across regions. In oropharyngeal squamous cell carcinoma, the prevalence of HPV types other than HPV16 was much lower than in oral cavity and laryngeal squamous cell carcinoma, with HPV33 being the second most frequent type worldwide (0·7%, 95% CI 0·3–1·1) followed by HPV18 (0·2%, 0·0–0·5). The proportion of cases with multiple infections was highest in oral cavity squamous cell carcinoma (2·1%, 95% CI 1·0–3·6) followed by laryngeal squamous cell carcinoma (1·0%, 0·3–2·1), and oropharyngeal squamous cell carcinoma (0·3%, 0·1–0·6). More detailed information about HPV

See Online for appendix

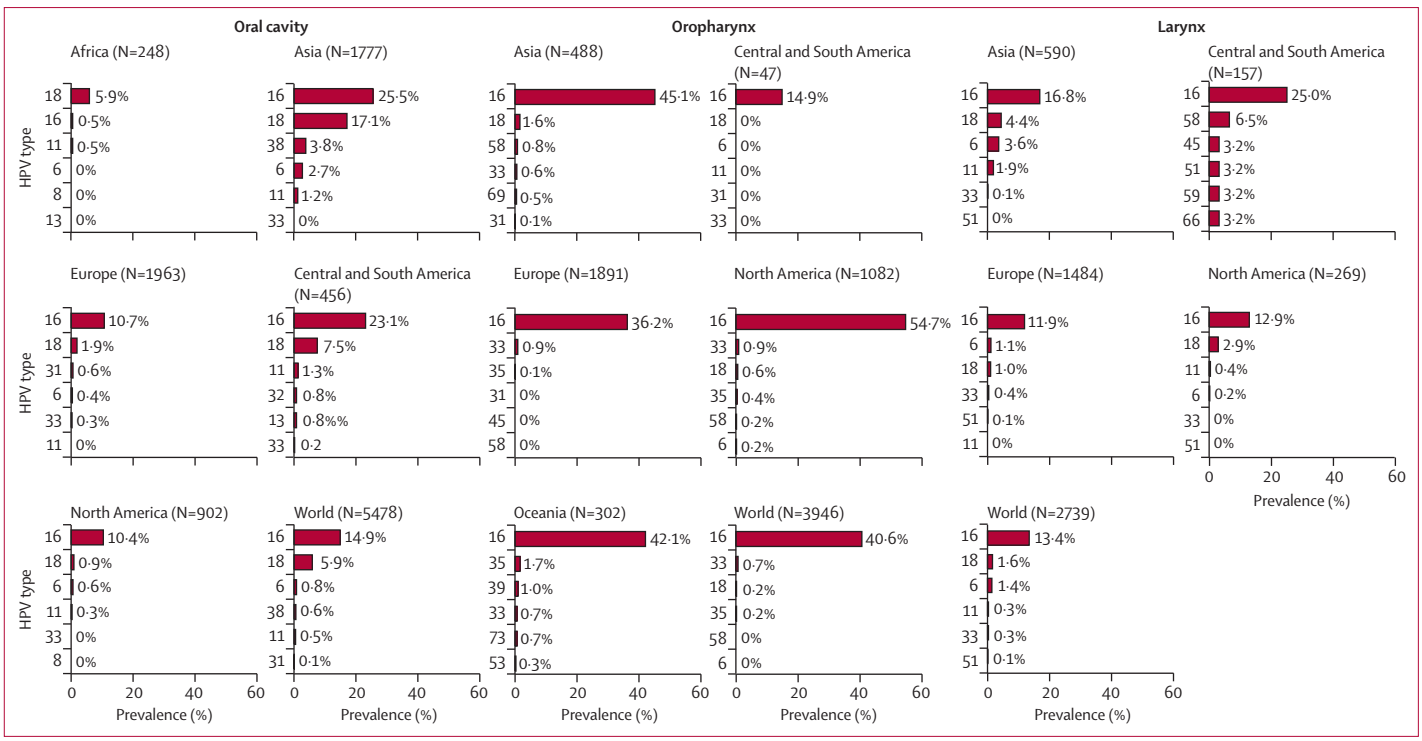

Figure 3: **Prevalence of the six most common HPV types in head and neck squamous cell carcinoma by anatomical site and geographical region**
HPV=human papillomavirus. Larynx cancer includes hypopharynx cases. One study with 507 cases was excluded for the regional analysis because data were presented aggregated for Central Europe and Central and South America.

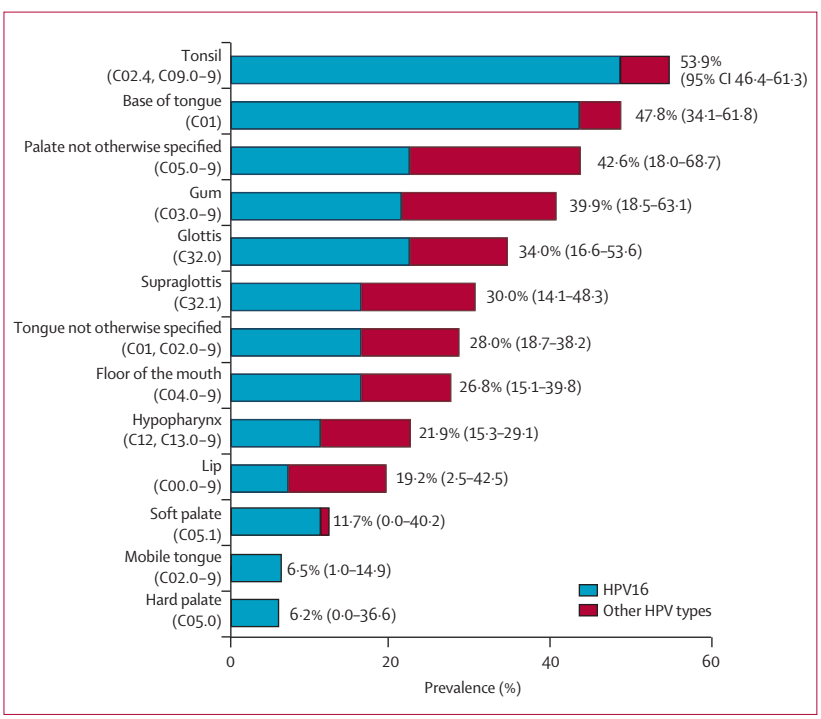

Figure 4: **HPV prevalence in head and neck squamous cell carcinoma by anatomical subsite**
HPV=human papillomavirus. Anatomical subsites classified according to the 10th edition of the International Classification of Diseases-10.

type distribution by anatomical site is shown in the appendix (p 10).

Although HPV DNA prevalence in oropharyngeal squamous cell carcinoma was not affected by study size, a tendency towards lower prevalence in larger studies was noted in oral cavity and laryngeal squamous cell carcinoma (appendix p 16).

Of 148 studies, 52 contained information disaggregated by subsites. Authors of 14 studies who presented incomplete information provided subsite-specific data upon request, which resulted in 66 studies contributing 4928 cases of head and neck squamous cell carcinomas (356 for larynx, 2193 for oral cavity, and 2379 for oropharynx). HPV detection was highest in the tonsil and base of the tongue, and lowest in subsites of the oral cavity such as hard palate and mobile tongue (figure 4). In laryngeal subsites, HPV DNA prevalence was 30·0% (95% CI 14·1–48·3) in supraglottis and 34·0% (16·6–53·6) in glottis. HPV16 DNA contributed to most HPV-positive squamous cell carcinoma of the tonsils (93·8%, 95% CI 91·0–96·2), base of the tongue (97·9%, 90·6–100·0), and soft palate (95·7%, 69·0–100·0), confirming the predominant role of HPV16 in oropharyngeal squamous cell carcinoma. The relative contribution of HPV16 was more than 50% in all subsites, with the lowest relative contribution of HPV16 obtained for squamous cell carcinoma of the lip (60·4%, 16·5–97·2) and the supraglottis (63·1%, 27·9–93·3). Figure 4 shows HPV

DNA prevalence by subsite, overall, and HPV16-specific; further estimations by geographical region are shown in the appendix (pp 11–14).

57 studies presented information about HPV DNA prevalence stratified by sex, contributing a total of 5080 head and neck squamous cell carcinomas (3667 men and 1413 women). No significant differences in HPV prevalence between men and women (in both all HPV types and HPV16-specific analyses) were noted for any anatomical site, whether overall or stratified by geographical region (appendix pp 17–22). There was a suggestion that overall HPV DNA prevalence in men was higher than that in women for oropharyngeal squamous cell carcinoma in North America, although this difference was not significant (RR 1·26, 95% CI 0·99–1·60).

35 studies did additional tests for $p16^{INK4a}$ or E6/E7 mRNA, which were most often done in oropharyngeal squamous cell carcinoma. Only four studies (one from Hungary, one from the Netherlands, and two from the USA) tested oral cavity squamous cell carcinoma for $p16^{INK4a}$. The pooled $p16^{INK4a}$ percent positivity in oral cavity squamous cell carcinoma positive for HPV DNA was 28·1% (95% CI 1·6–65·1). The $p16^{INK4a}$ percent positivity in laryngeal squamous cell carcinoma pooled from two studies (USA and Belgium) was 86·3% (95% CI 56·8–100·0). For oropharyngeal squamous cell carcinoma, 18 studies (eleven from Europe, one from Oceania, two from North America, and four from Asia) tested for $p16^{INK4a}$, providing an overall pooled percent positivity of 86·7% (95% CI 79·2–92·9). The percent positivity for $p16^{INK4a}$ did change by type of cases tested (p value for heterogeneity between groups <0·0001), ranging from 75·8% (95% CI 62·8–87·0) when all study cases were tested for $p16^{INK4a}$ to 96·6% (82·8–99·4) when only a subgroup of HPV DNA positive cases were tested for $p16^{INK4a}$; figure 5A). $p16^{INK4a}$ positivity also changed by p16 test cutoff (p value for heterogeneity between groups <0·0001) although no trend was noted (appendix p 23).

Only three studies tested for E6/E7 mRNA in the oral cavity and larynx. In oral cavity squamous cell carcinoma, studies were from the Netherlands, Italy, and the USA and the pooled E6/E7 mRNA percent positivity was 67·3% (95% CI 39·4–90·9). For laryngeal squamous cell carcinoma, the studies were from Italy (two studies) and the USA and the pooled E6/E7 mRNA percent positivity was 39·1% (95% CI 0·0–93·1). As for $p16^{INK4a}$, most of the

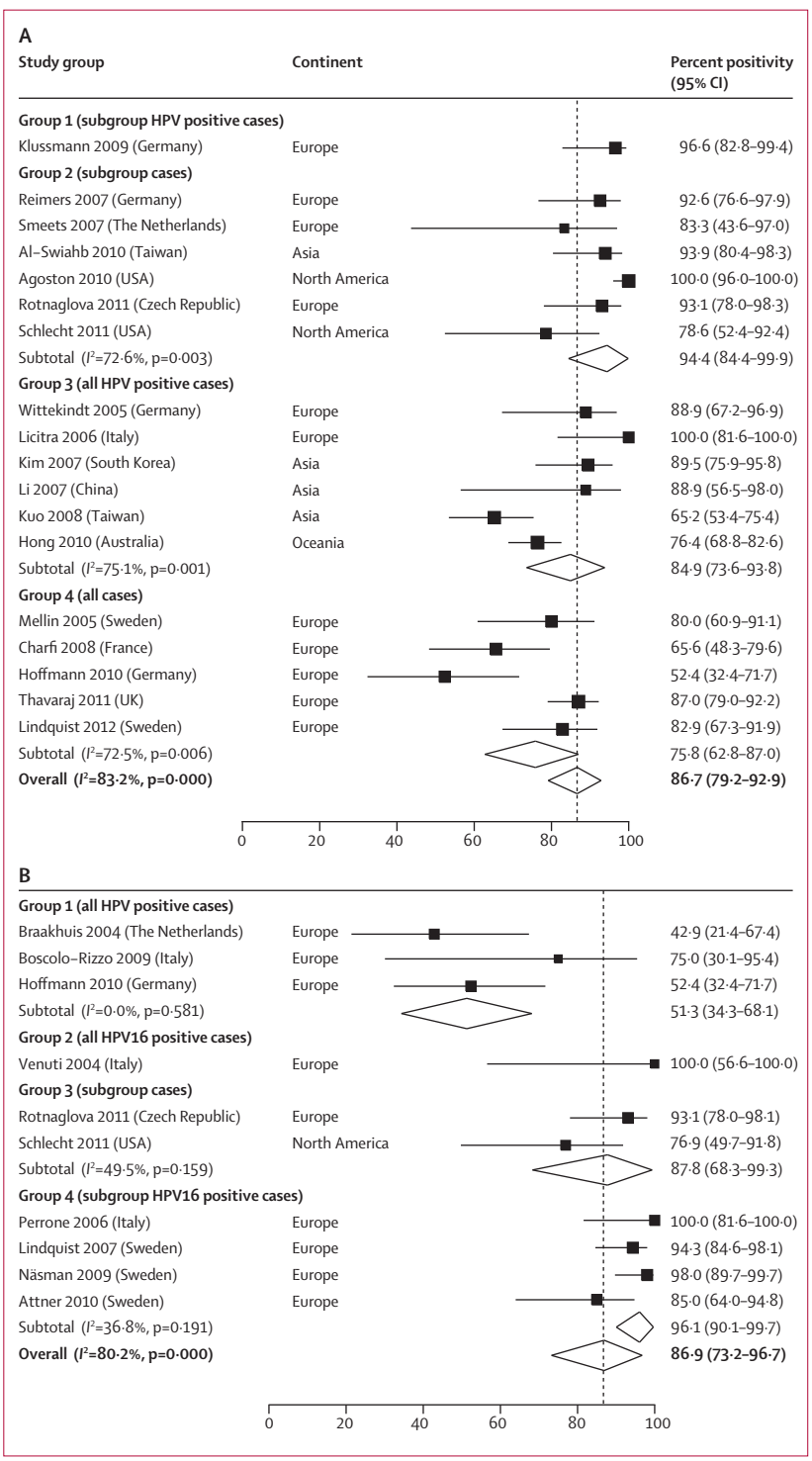

Figure 5: **$p16^{INK4a}$ (A) and E6/E7 mRNA (B) percent positivity in HPV DNA positive oropharyngeal squamous cell carcinoma by type of cases tested for the additional biomarker**

Oropharynx includes the following subsites classified according to the 10th edition of the International Classification of Diseases-10: C01, C02.4, C05.1, C09.0–9, C10.0–9. (A) Group 1: studies in which only a subgroup of HPV DNA positive cases were tested for p16; Group 2: studies in which only a subgroup of cases (both HPV DNA positive and negative cases) were tested for p16; Group 3: studies in which all HPV DNA positive cases were tested for p16 (HPV DNA negative cases were not tested for p16); Group 4: studies in which all cases were tested for p16 (both HPV-DNA positive and negative cases). For all groups, only results on HPV DNA positive cases are herein analysed. (B) Group 1: studies in which all HPV DNA positive cases were tested for E6/E7 mRNA (HPV DNA negative cases were not tested for E6/E7 mRNA); Group 2: studies in which all HPV16 positive cases were tested for E6/E7 mRNA (HPV DNA positive for other types than HPV16 and HPV DNA negative cases were not tested for E6/E7 mRNA); Group 3: studies in which only a subgroup of cases (both HPV DNA positive and negative cases) were tested for E6/E7 mRNA; Group 4: studies in which only a subgroup of HPV16 positive cases were tested for E6/E7 mRNA. For all groups, only results on HPV DNA positive cases are analysed.

studies testing for E6/E7 mRNA were done in oropharyngeal squamous cell carcinoma (nine from Europe and one from the USA), providing a pooled E6/E7 mRNA percent positivity of 86·9% (95% CI 73·2–96·8). Highest E6/E7 mRNA percent positivity were noted in studies in which only a subgroup of HPV16-positive cases were tested for E6/E7 mRNA (p value for heterogeneity between groups <0·0001; figure 5B).

HPV attributable fractions in oropharyngeal squamous cell carcinoma, when considering $p16^{INK4a}$ positive results in addition to HPV DNA, was assessed by pooling the prevalence of cases positive for both HPV DNA and $p16^{INK4a}$ (appendix p 24). The analysis was restricted to studies testing all cases for both $p16^{INK4a}$ and HPV DNA (five studies, all from Europe) and the pooled prevalence was 45·0% (95% CI 32·9–57·3). The simple product of pooled HPV DNA positivity in oropharyngeal squamous cell carcinoma (45·8%) and $p16^{INK4a}$ percent positivity (86·7%) yielded an estimated HPV attributable fraction of 39·7%. When considering E6/E7 mRNA in addition to HPV DNA, the corresponding estimated HPV attributable fraction was 39·8%. Table 2 summarises the results of the analysis on the estimates of HPV attributable fraction in oropharyngeal squamous cell carcinoma.

In multivariate meta-regression analyses, the prevalence of overall HPV and HPV16 DNA in oral cavity squamous cell carcinoma was significantly higher in South and Central America and Asia than in North America (table 3). HPV and HPV16 DNA prevalence in oropharyngeal squamous cell carcinoma was significantly higher in studies including more recent cancers and in North American studies than in European studies. For laryngeal squamous cell carcinoma, some effect of certain covariates was noted in bivariate analyses. However, none of these (with the exception of increased prevalence in Belgium, China, and Hungary compared with the USA) were confirmed in the multivariate analysis, either for HPV overall or HPV16-specific DNA prevalence.

Although not statistically significant, a suggestion of an inverse correlation between HPV DNA prevalence (overall and HPV16-specific) and prevalence of ever tobacco smoking was noted for oropharyngeal squamous cell carcinoma in bivariate meta-regression analyses; the same trend was noted for ever alcohol drinking (appendix pp 25–26). With adjustment for the end year of diagnosis, the observed inverse correlation was significant for alcohol drinking (appendix p 15).

	Oral cavity	Oropharynx	Larynx*
Cases tested for HPV DNA	5478	3946	2739
HPV DNA positive cases	1360	1828	649
HPV DNA prevalence	24·2% (18·7–30·2)	45·8% (38·9–52·9)	22·1% (16·4–28·3)
HPV DNA positive cases tested for p16	47	738	57
p16 and HPV DNA positive cases	12	617	51
Percent p16-positive cases in HPV DNA positive cases	28·1% (1·6–65·1)	86·7% (79·2–92·9)	86·3% (56·8–100·0)
Attributable fraction of p16 and HPV DNA positive cases	6·8%	39·7%	19·1%
HPV DNA positive cases tested for E6/E7 mRNA	17	227	23
E6/E7 mRNA and HPV DNA positive cases	11	196	10
Percent E6/E7 mRNA positive cases in HPV DNA positive cases	67·3% (39·4–90·9)	86·9% (73·2–96·8)	39·1% (0·0–93·1)
Attributable fraction of E6/E7 mRNA and HPV DNA positive cases	16·3%	39·8%	8·6%

Data are n, %, or % (95% CI). HPV=human papillomavirus. *Including hypopharynx.

***Table 2*: HPV attributable fractions of HPV infection in head and neck squamous cell carcinoma according to different biomarkers**

Discussion

We present updated global estimates of HPV attributable fractions in head and neck squamous cell carcinomas with consideration of—in addition to HPV DNA prevalence—more accurate biomarkers of oncogenic activity of the virus such as E6/E7 mRNA or $p16^{INK4a}$. Notably, some authors do not judge a head and neck squamous cell carcinoma to be HPV-positive from the biological point of view unless it is also positive for E6/E7 mRNA or $p16^{INK4a}$.[23] Estimates of HPV attributable fractions in oropharyngeal squamous cell carcinoma taking either E6/E7 mRNA or $p16^{INK4a}$ positive results into account, in addition to HPV DNA, were as high as 40%. Nevertheless, some selection bias (increased testing of those cases that were HPV DNA positive) might exist regarding the type of cases tested for the additional biomarkers. Moreover, studies reporting E6/E7 mRNA or $p16^{INK4a}$ originated mainly from Europe with regions such as Africa or Oceania being under-represented. HPV attributable fractions that took into account either E6/E7 mRNA or $p16^{INK4a}$ were much lower in oral cavity squamous cell carcinoma and laryngeal squamous cell carcinoma, but less consistent than those noted in oropharyngeal squamous cell carcinoma. Addition of $p16^{INK4a}$ to HPV DNA resulted in an HPV attributable fraction of 6·8% in oral cavity squamous cell carcinoma and 19·1% in laryngeal squamous cell carcinoma. For E6/E7 mRNA, the estimates were 16·3% for oral cavity squamous cell carcinoma and 8·6% for laryngeal squamous cell carcinoma. Nevertheless, very few studies tested for the additional biomarkers in these sites, showing the need to further explore the fraction of HPV-driven cancers in head and neck sites other than oropharynx with standardised procedures.

Compared with a previous review, our study shows a substantially higher proportion of HPV DNA positive oropharyngeal squamous cell carcinoma (45·8% *vs* 35·6%[9]). Meta-regression analysis confirmed a significant effect of time, with increased prevalence noted in studies including more recent oropharyngeal cancers, which is in agreement with a previous meta-analysis,[8] and with observations from several high-income

	Oral cavity		Oropharynx		Larynx*	
	Coefficients of linear metaregression for HPV prevalence (95% CI)	Coefficients of linear metaregression for HPV16 prevalence (95% CI)	Coefficients of linear metaregression for HPV prevalence (95% CI)	Coefficients of linear metaregression for HPV16 prevalence (95% CI)	Coefficients of linear metaregression for HPV prevalence (95% CI)	Coefficients of linear metaregression for HPV16 prevalence (95% CI)
Last year of diagnosis†	NI	NI	0·027 (0·011 to 0·043)	0·023 (0·010 to 0·037)	NI	NI
Geographical region						
Africa	−0·175 (−0·453 to 0·104)	−0·149 (−0·360 to 0·052)	..	..	NI	NI
Latin America	0·208 (0·258 to 0·352)	0·138 (0·001 to 0·276)	−0·251 (−0·659 to 0·158)	−0·296 (−0·658 to 0·066)	NI	NI
Asia	0·263 (0·109 to 0·412)	0·140 (0·029 to 0·252)	−0·205 (−0·429 to 0·188)	0·179 (−0·377 to 0·018)	NI	NI
Europe	0·692 (−0·720 to 0·210)	0·011 (−0·092 to 0·115)	−0·195 (−0·329 to −0·060)	−0·161 (−0·281 to −0·041)	NI	NI
Oceania	..	..	−0·264 (−0·634 to 0·107)	−0·192 (−0·529 to 0·145)	NI	NI
North America	Reference	Reference	Reference	Reference	..	..
Number of contamination items mentioned†	NI	NI	0·041 (−0·021 to 0·105)	NI	NI	NI
Preservation method						
Paraffin embedded and fixed formalin	..	..	−0·219 (−0·598 to 0·159)	..	..	..
Fixed formalin	..	..	−0·061 (−0·245 to 0·122)	..	..	..
Paraffin embedded	NI	NI	Reference	NI	NI	NI
Length of the amplicon†	NI	NI	NI	NI	NI	−0·000 (−0·001 to 0·000)
Quality criteria 1: broad spectrum PCR						
Yes	0·230 (−0·231 to 0·692)	NI	NI	NI	−0·083 (−0·208 to 0·042)	−0·030 (−0·251 to 0·190)
No	Reference	NI	NI	NI	Reference	Reference
Quality criteria 2: information about age and sex						
Yes	−0·089 (−0·230 to 0·051)	−0·078 (−0·175 to 0·019)	NI	NI	NI	NI
No	Reference	Reference	NI	NI	NI	NI
Quality criteria 5: cases recruited consecutively or randomly or incident cases						
Yes	−0·056 (−0·181 to 0·689)	NI	NI	NI	NI	NI
No	Reference	NI	NI	NI	NI	NI
Quality criteria 6: human quality controls done						
Yes	NI	NI	NI	NI	−0·109 (−0·235 to 0·174)	NI
No	NI	NI	NI	NI	Reference	NI
Quality score (from 0 to 8)†	−0·004 (−0·576 to 0·049)	−0·007 (−0·034 to 0·020)	NI	NI	NI	NI

Results from multivariate analysis. Only the covariates identified to be statistically significant (p<0·05) in a previous-step bivariate analyses were included in the multivariate models. Negative values correspond to the units of decrease of HPV prevalence for each category with regard to the reference one. Positive values correspond to the units of increase of HPV prevalence for each category with regard to the reference one. NI=not-included in multivariate models because non-significant predictor of HPV prevalence in a previous-step bivariate analysis. Quality criteria 3, 4, 7, and 8 were not shown to be statistically significant in a previous-step bivariate analyses and thus were not included in the multivariate model. HPV=human papillomavirus. *Including hypopharynx. †Continuous variable. Negative values correspond to the units of decrease of HPV prevalence for each additional unit of the covariate. Positive values correspond to the units of increase of HPV prevalence for each additional unit of the covariate.

Table 3: **Effect of selected study covariates on the HPV DNA prevalence in head and neck squamous cell carcinoma**

countries in the past two decades,[6,7,16] particularly in younger individuals. This increase might be attributed to changes in tobacco and alcohol consumption and in sexual behaviour, which includes an increased number of sexual partners[24,25] and orogenital sex practices.[26–28] The increase in HPV DNA prevalence with time was not noted in oral cavity squamous cell carcinoma or laryngeal squamous cell carcinoma.

Significant heterogeneity of HPV DNA prevalence was noted when analysed by geographical location. Our findings indicate higher detection of oral cavity squamous cell carcinoma and laryngeal squamous cell carcinoma in

South and Central America and Asia than in North America and Europe. Meta-regression analysis confirmed the observed regional differences for oral cavity squamous cell carcinoma but not for laryngeal squamous cell carcinoma. These variations could be explained by issues with the methods including number of cases tested, type of specimen collected, techniques used for DNA detection, or control for contamination or biased sample collection methods. Reduced HPV DNA prevalence estimates were noted with increased sample sizes for oral cavity and laryngeal squamous cell carcinoma, which could suggest a potential publication bias.[29] However, study size was not statistically significant covariate in meta-regression analyses for any anatomical site. Meta-regression analysis confirmed a lower HPV prevalence of oropharyngeal squamous cell carcinoma in Europe than in North America. Nevertheless, after subregional analysis considering only North Europe, the observed increase of HPV prevalence in North America (22·0%, 95% CI 10·6–33·4) and North Europe (18·0%, 4·3–31·7) compared with the rest of the world were much the same. Our estimates of HPV DNA prevalence by geographical region for oropharyngeal squamous cell carcinoma showed the same pattern but with slightly increased prevalences for all regions than those obtained by de Martel and colleagues,[30] in which the estimations were also based on PCR of HPV DNA detection in tumour tissue, but with some of them including HPV E6/E7 mRNA detection.

In terms of genotype distribution, HPV16 was by far the most important oncogenic HPV type accounting for more than 80% of all HPV-positive head and neck squamous cell carcinomas. The highest relative contribution of HPV16 was noted in oropharyngeal squamous cell carcinoma, with nine of ten positive cases being HPV16 positive. The oncogenic role of HPV16 in oropharyngeal cancer has been established by the International Agency for Research on Cancer.[31] Significant HPV16 positivity was also reported in laryngeal and oral cavity squamous cell carcinomas. The second most common high-risk HPV type, HPV18, was detected in 2·5% of all head and neck squamous cell carcinomas. Therefore, the types targeted in the present HPV vaccines, HPV16 and HPV18, were detected in 23·6% of head and neck squamous cell carcinoma cases, with a total relative contribution of 89·9% in HPV DNA positive cases. The proportion of cases with multiple HPV infections (1·1%) was ten times lower than in cervical cancer (11·2%).[32] This difference could be explained by the increased exposure of the cervix to HPV.

Analyses by anatomical subsites showed that the tonsils and the base of the tongue have the highest HPV prevalence, in particular by HPV16 infection. HPV prevalence in the soft palate was low. However, this estimate was based on small numbers and potential subsite misclassification could play a part if we take into account the greater number of cases classified as "palate non-specified" than as "soft palate". HPV prevalence in palate non-specified was high, ranking third after tonsils and base of the tongue. In future studies, one should take into account that ethiopathogenic agents are different according to anatomical site and we recommend the systematic use of the codes of the International Classification of Diseases to avoid misclassification and to provide a better definition of sites and subsites.

Prevalence of HPV types other than HPV16 was increased in non-oropharyngeal sites (figures 3, 4). Preliminary results from an international retrospective study of HPV prevalence and type distribution analysing around 4000 head and neck cancers also noted this trend,[33] however there is no clear explanation for this variability in type distribution. The variability could be due to differences in tissue tropism of the HPV type or in inmunological profiles between tissues. Of note, we included non-oncogenic types such as HPV6 and HPV11 when reporting overall HPV prevalences. We have previously observed the presence of these types in anogenital tumour tissue after laser capture microdissection[34,35] and we consider interesting the assessment of HPV6 and HPV11 prevalence in terms of assessing the effect of the tetravalent vaccine and of an HPV nine-valent vaccine (V503) under development that includes HPV6 and HPV11 antigens.[36]

Analysis by sex did not show any significant differences in HPV DNA prevalence, even though men have a twice the incidence of head and neck squamous cell carcinomas than do women. A non-significant increased overall HPV DNA prevalence in men compared with women was noted for oropharyngeal squamous cell carcinoma in North America. This finding is in agreement with recent studies estimating prevalence of oral HPV in a healthy population in North America, which showed a higher HPV prevalence in men than in women.[37]

The role of covariates such as age, sex, alcohol or tobacco consumption, geographical region, time of diagnosis, PCR methods, study size, method of specimen preservation, and quality of the papers reporting on HPV DNA prevalence in head and neck squamous cell carcinomas was explored jointly for the first time in our study through multivariate meta-regression analyses. Of all covariates explored, only time of diagnosis and geographical region were identified as statistically significant covariates. However, the failure to find significant associations for covariates such as tobacco and alcohol consumption may not be surprising because high heterogeneity was identified between studies regarding classification of smokers and drinkers. If studies do not categorise smoking or alcohol drinking in the same way and at the same level of detail, the power of simple meta-regression is restricted and the prevalence of smoking in studies is affected. Description of the prevalence described for non-smokers and smokers (multiple, uniformly defined strata) for many studies would increase the

discriminative power. To obtain robust global estimates on the role of risk factors of head and neck squamous cell carcinomas other than HPV infection, information combining both risk factors and with the use of standardised indicators (eg, packs smoked per years) are needed. Our finding that covariates related to PCR detection methods or quality of the studies or cancer specimens were not significant could also be explained by the heterogeneity of indicators of studies or under-reporting of some of them.

Our study has several limitations. Heterogeneity of studies could have remained even though we stratified by cancer site and geographical region. However, as pointed out by Greenland[38] when critically assessing the use of meta-analyses, the main strength of the method lies on their use as a comparative rather than a synthetic exercise, because no statistical technique can compensate for fundamental limitations of the input data, but they can help to identify patterns in study results and sources of heterogeneity. Misclassification of anatomical subsites and even sites could occur because head and neck squamous cell carcinomas are frequently diagnosed at advanced stages and in several anatomical sites. Cases classified as head and neck not-otherwise-specified were not extracted or included and, because such cases might vary across study or geographic regions, misclassification of tumour site could be an important explanatory factor for some of the heterogeneity observed in studies and regions. Additionally, even if an underlying increase in HPV DNA prevalence with time in oropharyngeal sites was noted, almost 40% of the studies did not specify any study period. Surrogate measures such as publication date could not be as precise as necessary to fully assess time trends. Information about age was also insufficient in many studies and therefore it was difficult to assess its association with HPV prevalence. Because of the absence of information about sexual behaviour in most studies, it was not possible to assess the role of this important covariate. Similarly, the absence of robustness of data for tobacco and alcohol consumption hampered full assessment of the role of these risk factors. Inclusion of only significant covariates in the meta-regression analysis also has limitations; it would have been interesting to do multivariate regression including non-significant covariates such as age, alcohol, tobacco, and timeperiod, and to look for interactions. However, we were restricted by available data and those available did not contain the required level of detail. Other limitations include the variability of the sensitivity of the PCR protocols, the number of HPV types genotyped, and insufficient standardisation for histopathological assessment. Some regions such as Africa or Oceania were still under-represented in the study and the number of cases was small in several analyses. The more accurate biomarkers of causality than HPV DNA detection used here—E6/E7 mRNA or $p16^{INK4a}$—are also not exempt from limitations.[1,39] Even if E6/E7 mRNA detection is accepted as the present gold-standard test to elucidate the oncogenic role of HPV in the tumour, the sensitivity of the test could be affected by the tissue preservation methods used because paraffin-embedded tissue blocks can suffer from nucleic acids degradation during the fixation and storage process. The specificity has also to be ensured with use of reverse transcriptase PCR methods to clearly differentiate complementary DNA from HPV DNA to avoid false-positive hybridisations. With respect to $p16^{INK4a}$, the technique has shown a high sensitivity, but moderate to high specificity. About 10% of the oropharyngeal cases are HPV DNA negative, $p16^{INK4a}$ positive, which could be due to either misclassification as HPV DNA negative, because of insufficient sensitivity of the HPV DNA test or, alternatively, the cases represent tumours in which $p16^{INK4a}$ overexpression has been triggered by other cellular signalling pathways. This percentage does not notably vary across head and neck anatomical sites according to preliminary results of the international study analysing around 4000 head and neck cancers.[40] Notably, the reported attributable fractions reflect maximum estimates, because some of tumours that were positive for $p16^{INK4a}$ or E6/E7 mRNA might not have been caused by HPV infection. Nevertheless, we expect those to be very few. Another limitation is that because our chosen method was quite restrictive in terms of selection criteria and did not include studies with only $p16^{INK4a}$ as an HPV detection method in head and neck cancer, we might have missed some key large clinically oriented studies which only, or predominantly, used $p16^{INK4a}$ to assess HPV positivity. However, in most of the clinical trials, the cases are selected according to their stage (eg, only stages III/IV) and studies with cases purposively selected were excluded from the present analysis because HPV-related tumours could affect stage presentation. Finally, our estimates of the HPV attributable fractions for oral cavity and laryngeal squamous cell carcinoma are weak and imprecise in view of the little data available for E6/E7 mRNA or $p16^{INK4a}$ for these cancer sites. Although some new data has appeared after our last literature search was done, these still do not contain much new information about E6/E7 mRNA or $p16^{INK4a}$ for these cancer sites. A recent review has assessed the role of HPV in non-oropharyngeal head and neck cancers,[5] which included relevant papers published up to April, 2013. Their results are in agreement with ours in terms of HPV attributable fractions in oropharyngeal squamous cell carcinoma according to different biomarkers (HPV DNA PCR, in-situ hybridisation, E6/E7 mRNA, and $p16^{INK4a}$), and also regarding the little information about non-oropharyngeal sites and the higher differences of HPV attributable fractions noted between the different biomarkers for these sites.

In the past 20 years, growing evidence for the involvement of HPV in head and neck squamous cell carcinomas has

been collected. This meta-analysis suggests that prophylactic vaccines targeting HPV16 and HPV18 have the potential to prevent a substantial fraction of head and neck squamous cell carcinomas worldwide, in particular oropharyngeal cancers. Moreover, even if the HPV attributable fractions in non-oropharyngeal sites are much lower than in the oropharyngeal cancers, the number of incident oral cavity and laryngeal cancers worldwide far exceeds that of oropharyngeal cancers, therefore even small HPV attributable fractions for these sites could potentially translate to a high number of HPV-positive oral or laryngeal cancers versus HPV-positive oropharyngeal cancers. Because of the absence of identifiable cancer precursor lesions, no screening instruments are available at present for head and neck cases. If vaccination was proven efficacious against HPV-driven cancers, as preliminary results indicate for oral HPV infection,[3] a substantial proportion of head and neck cancers could be prevented.

Contributors

CN, MM, LA, XC, SS, and HT designed the study. CN, MM, and LA did the literature searches and designed the data extraction form. MM, LA, and LL extracted the data. CN and LA crosschecked the data extraction. CN, MM, and MA did the statistical analyses. MA supervised the statistical analyses. CN and MM wrote the paper. CN, MM, LA, XC, SS, MM, FXB, and HT critically revised subsequent drafts. All authors read and approved the submitted version.

Declaration of interests

CN, LL, MA, HT, and MM declare no competing interests. LA received travel grants to attend conferences occasionally granted by Merck and Sanofi Pasteur MSD. XC received travel grants for scientific meetings and honorarium for consultancy occasionally granted by Vianex and Sanofi Pasteur MSD, and institutional grants for HPV research from Merck & Co, GlaxoSmithKline, Sanofi Pasteur MSD, and Genticel. FXB received travel grants to conferences, symposia, meetings, and honorarium for consultancy occasionally granted by GlaxoSmithKline, Merck, Sanofi Pasteur MSD, and Qiagen, and institutional grants for HPV research from GlaxoSmithKline, Merck & Co. Sanofi Pasteur MSD, Qiagen, and Roche. SS received travel grants to conferences, symposia, and meetings occasionally granted by Merck & Co, Sanofi Pasteur MSD or Qiagen, and institutional grants for HPV research from GlaxoSmithKline, Merck & Co, and Qiagen.

Acknowledgments

Work by MM, LA, SS, XC, and FXB has been supported by European Commission (7th Framework Programme Grant HEALTH-F2-2011-282562 HPV AHEAD); Spanish grants from Instituto de Salud Carlos III (FIS PI08/1535, FIS PI11/02096, FIS PI11/02104, RCESP C03/09, RTICESP C03/10, RTIC RD06/0020/0095, RD12/0036/0056 and CIBERESP); and LA has received personal funding through the Programa Avanzado de Formación en Oncología-2012, Asociación Española Contra el Cáncer. Work by CN, LL, and HT has been supported by Canadian Institutes of Health Research, Fonds de Recherche du Québec en Santé. MA received funding from the 7th Framework Programme of the European Commission through the COHEAHR Network (Grant number 603019) and the HPV AHEAD Network (Grant number HEALTH-F2-2011-282562); the Belgian Cancer Centre; and the SEHIB project (Surveillance of effects of HPV Immunisation in Belgium, through the University of Gent, Belgium). This work was also made possible with the support of the ICO Information Centre on HPV and Cancer. We thank Sara Tous and Claudie Laprise for their assistance in data analysis; acknowledge all the authors whose papers have been included in this meta-analysis for their work, which made this analysis possible; and offer special thanks to authors who provided additional information (appendix) and to Dana Holzinger and Michael Pawlita for general suggestions and their specific assistance in the classification of the PCR methods.

References

1 Gillison M, Alemany L, Snijders PJF, et al. Human papillomavirus and diseases of the upper airway: head and neck cancer and respiratory papillomatosis. *Vaccine* 2012; **30:** F34–54.
2 O'Rorke MA, Ellison MV, Murray LJ, Moran M, James J, Anderson LA. Human papillomavirus related head and neck cancer survival: a systematic review and meta-analysis. *Oral Oncol* 2012; **48:** 1191–201.
3 Herrero R, Quint W, Hildesheim A, et al. Reduced prevalence of oral human papillomavirus (HPV) 4 years after bivalent HPV vaccination in a randomized clinical trial in Costa Rica. *PLoS One* 2013; **8:** e68329.
4 Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11. Lyon, France: International Agency for Research on Cancer; 2013. http:// http://globocan.iarc.fr (accessed Aug 25, 2014).
5 Combes JD, Franceschi S. Role of human papillomavirus in non-oropharyngeal head and neck cancers. *Oral Oncol* 2014; **50:** 370–79.
6 Chaturvedi AK, Engels EA, Pfeiffer RM et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol* 2011; **29:** 4294–301.
7 Simard EP, Torre LA, Jemal A. International trends in head and neck cancer incidence rates: Differences by country, sex and anatomic site. *Oral Oncol* 2014; **50:** 387–403.
8 Mehanna H, Beech T, Nicholson T, et al. The prevalence of human papillomavirus in oropharyngeal and non-oropharyngeal head and neck cancer—a systematic review and metaanalysis of trends by time and region. *Head Neck* 2013; **35:** 747–55.
9 Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2005; **14:** 467–75.
10 Termine N, Panzarella V, Falaschini S, et al. HPV in oral squamous cell carcinoma vs head and neck squamous cell carcinoma biopsies: a meta-analysis (1988–2007). *Ann Oncol* 2008; **19:** 1681–90.
11 Holzinger D, Schmitt M, Dyckhoff G, Benner A, Pawlita M, Bosch FX. Viral RNA patterns and high viral load reliably define oropharynx carcinomas with active HPV16 involvement. *Cancer Res* 2012; **72:** 4993–5003.
12 Rietbergen MM, Leemans CR, Bloemena E, et al. Increasing prevalence rates of HPV attributable oropharyngeal squamous cell carcinomas in the Netherlands as assessed by a validated test algorithm. *Int J Cancer* 2013; **132:** 1565–71.
13 Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol* 2009; **62:** 1006–12.
14 Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008–12.
15 Blomberg M, Nielsen A, Munk C, Kjaer SK. Trends in head and neck cancer incidence in Denmark, 1978-2007: focus on human papillomavirus associated sites. *Int J Cancer* 2011; **129:** 733–41.
16 Chaturvedi AK, Anderson WF, Lortet-Tieulent J, et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. *J Clin Oncol* 2013; **31:** 4550–59.
17 Nyaga VN, Arbyn M, Aerts M. Metaprop: a Stata command to perform meta-analysis of binomial data. *Arch Public Health* (in press).
18 Nyaga VN, Arbyn M, Aerts M. METAPROP: Stata module to perform fixed and random effects meta-analysis of proportions. *Statistical Software Components* 2014; S457781.
19 Cochran WG. The combination of estimates from different experiments. *Biometrics* 1954; **10:** 101–29.
20 Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327:** 557–60.
21 Harris R, Bradburn M, Deeks J, Harbord RM, Altman DG, Sterne JAC. metan: fixed- and random-effects meta-analysis. *Stata J* 2008; **8:** 3–26.
22 Harbord R, Higgins JP. Meta-regression in Stata. *Stata J* 2013; **8:** 493–519.

23 Braakhuis BJM, Snijders PJF, Keune W-JH, et al. Genetic patterns in head and neck cancers that contain or lack transcriptionally active human papillomavirus. *J Natl Cancer Inst* 2004; **96:** 998–1006.

24 Schwartz SM, Daling JR, Doody DR, et al. Oral cancer risk in relation to sexual history and evidence of human papillomavirus infection. *J Natl Cancer Inst* 1998; **90:** 1626–36.

25 D'Souza G, Kreimer AR, Viscidi R, et al. Case–control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* 2007; **356:** 1944–56.

26 Ernster JA, Sciotto CG, O'Brien MM, et al. Rising Incidence of Oropharyngeal Cancer and the Role of Oncogenic Human Papilloma Virus. *Laryngoscope* 2007; **117:** 2115–28.

27 D'Souza G, Zhang HH, D'Souza WD, Meyer RR, Gillison ML. Moderate predictive value of demographic and behavioral characteristics for a diagnosis of HPV16-positive and HPV16-negative head and neck cancer. *Oral Oncol* 2010; **46:** 100–04.

28 Jung AC, Briolat J, Millon R, et al. Biological and clinical relevance of transcriptionally active human papillomavirus (HPV) infection in oropharynx squamous cell carcinoma. *Int J Cancer* 2010; **126:** 1882–94.

29 Dickersin K. The existence of publication bias and risk factors for its occurrence. *JAMA* 1990; **263:** 1385–89.

30 De Martel C, Ferlay J, Franceschi S, et al. The global burden of cancers attributable to infections in the year 2008: a review and synthetic analysis. *Lancet Oncol* 2012; **13:** 607–15.

31 IARC. IARC monographs on the evaluation of carcinogenic risks to humans: Biological agents. Vol. 100B, 2009.

32 Li N, Franceschi S, Howell-Jones R, Snijders PJF, Clifford GM. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. *Int J Cancer* 2011; **128:** 927–35.

33 Castellsagué X, Alemany L, Holzinger D, et al, on behalf of the ICO Head and Neck Cancer Study group. Estimation of the HPV etiological fraction in over 4000 head and neck cancers worldwide. 29th International Papillomavirus Conference; Seattle, WA, USA; Aug 1–25, 2014. Oral poster session PH.PD03.01.

34 Guimerà N, Lloveras B, Lindeman J, et al. The occasional role of low-risk human papillomaviruses 6, 11, 42, 44, and 70 in anogenital carcinoma defined by laser capture microdissection/PCR methodology: results from a global study. *Am J Surg Pathol* 2013; **37:** 1299–310.

35 Guimerà N, Lloveras B, Alemany L, et al. Laser capture microdissection shows HPV11 as both a causal and a coincidental infection in cervical cancer specimens with multiple HPV types. *Histopathology* 2013; **63:** 287–92.

36 Joura E, on behalf of the V503-001 study team. Immunogenicity of a novel 9-valent HPV L1 virus-like particle vaccine in 16 to 26-year-old women. Eurogin; Florence, Italy; Nov 3–6, 2013. Oral communication SS 8–4.

37 Gillison ML, Broutian T, Pickard RK, et al. Prevalence of oral HPV infection in the United States, 2009–2010. *JAMA* 2012; **307:** 693–703.

38 Greenland S. Can meta-analyses be salvaged? *Am J Epidemiol* 1994; **140:** 783–87.

39 Robinson M, Schache A, Sloan P, Thavaraj S. HPV specific testing: a requirement for oropharyngeal squamous cell carcinoma patients. *Head Neck Pathol* 2012; **6:** S83–90.

40 Alemany L, Castellsagué X, Alejo M, et al, on behalf of the ICO Head and Neck Cancer Study group. The ICO international study on head and neck cancers: preliminary results on immunohistochemistry analysis. 28th International Papillomavirus Conference; San Juan de Puerto Rico, Puerto Rico; Dec 2–6, 2012. Oral poster session EPO3-390.