Prevalence of human papillomavirus DNA and p16^{INK4a} in penile cancer and penile intraepithelial neoplasia: a systematic review and meta-analysis

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Summary

Background Although previous meta-analyses have examined human papillomavirus (HPV) DNA prevalence in penile cancer, none, to our knowledge, have assessed pooled HPV DNA prevalence in penile intraepithelial neoplasia or p16^{INK4a} percent positivity in penile cancer and penile intraepithelial neoplasia. Therefore, we aimed to examine the prevalence of HPV DNA and p16^{INK4a} positivity in penile cancer and penile intraepithelial neoplasia worldwide.

Methods In this systematic review and meta-analysis, we searched PubMed, Embase, and the Cochrane Library until July 24, 2017, for English-language articles published from Jan 1, 1986, onwards reporting the prevalence of HPV DNA and p16^{INK4a} positivity, either alone or in combination, in at least five cases of penile cancer or penile intraepithelial neoplasia. Only studies that used PCR or hybrid capture for the detection of HPV DNA and immunohistochemical staining or methylation for the detection of p16^{INK4a} were included. Data were extracted and subsequently crosschecked, and inconsistencies were discussed to reach consensus. Using random-effects models, we estimated the pooled prevalence and 95% CI of HPV DNA and p16^{INK4a} positivity in penile cancer and penile intraepithelial neoplasia, stratifying by histological subtype and HPV DNA or p16^{INK4a} detection method. Type-specific prevalence of HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, and HPV45 in penile cancer was estimated.

Findings Our searches identified 1836 non-duplicate records, of which 73 relevant papers (71 studies) were found to be eligible. The pooled HPV DNA prevalence in penile cancer (52 studies; n=4199) was 50·8% (95% CI 44·8–56·7; I^2=92·6%, $p_{heterogeneity}$<0·0001). A high pooled HPV DNA prevalence was seen in basaloid squamous cell carcinomas (84·0%, 95% CI 71·0–93·6; I^2=48·0%, $p_{heterogeneity}$=0·0197) and in warty-basaloid carcinoma (75·7%, 70·1–81·0; I^2=0%, $p_{heterogeneity}$=0·52). The predominant oncogenic HPV type in penile cancer was HPV16 (68·3%, 95% CI 58·9–77·1), followed by HPV6 (8·1%, 4·0–13·7) and HPV18 (6·9%, 2·9–12·4). The pooled HPV DNA prevalence in penile intraepithelial neoplasia (19 studies; n=445) was 79·8% (95% CI 69·3–88·6; I^2=83·2%, $p_{heterogeneity}$<0·0001). The pooled p16^{INK4a} percent positivity in penile cancer (24 studies; n=2295) was 41·6% (95% CI 36·2–47·0; I^2=80·6%, $p_{heterogeneity}$<0·0001), with a high pooled p16^{INK4a} percent positivity in HPV-related squamous cell carcinoma (85·8%, 95% CI 72·1–95·4; I^2=56·4%, $p_{heterogeneity}$=0·0011) as compared with non-HPV-related squamous cell carcinoma (17·1%, 7·9–29·1; I^2=78·3%, $p_{heterogeneity}$<0·0001). Moreover, among HPV-positive cases of penile cancer, the p16^{INK4a} percent positivity was 79·6% (95% CI 65·7–90·7; I^2=89·9%, $p_{heterogeneity}$<0·0001), compared with 18·5% (9·6–29·6; I^2=89·3%, $p_{heterogeneity}$<0·0001) in HPV-negative penile cancers. The pooled p16^{INK4a} percent positivity in penile intraepithelial neoplasia (six studies; n=167) was 49·5% (95% CI 18·6–80·7).

Interpretation A large proportion of penile cancers and penile intraepithelial neoplasias are associated with infection with HPV DNA (predominantly HPV16), emphasising the possible benefits of HPV vaccination in men and boys.

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Introduction

Penile cancer is a rare cancer with 26 000 new cases occurring worldwide annually,[1] the majority of which occur in developing countries, particularly in Africa.[2] The underlying causes of penile cancer are not well understood; however, two major causative pathways have been suggested, one related to phimosis, inflammation, and lichen sclerosis, and one related to human papillomavirus (HPV) infection.[3]

In 2016, a new WHO classification of penile cancer was published.[4] In this classification, penile squamous cell carcinomas are categorised into non-HPV-related and HPV-related tumours, with HPV-related tumours comprising especially basaloid squamous cell carcinomas and warty carcinomas, whereas non-HPV-related squamous cell carcinomas comprise squamous cell carcinoma (usual type), verrucous carcinoma, and papillary squamous cell carcinomas. Penile cancer is preceded

Research in context

Evidence before this study

We searched PubMed for systematic reviews and meta-analyses published in English using the search terms "HPV" or "p16" and "penile cancer" or "PeIN". We did the search on July 24, 2017. 27 studies were identified, of these two systematic reviews and meta-analyses examined the prevalence of human papillomavirus (HPV) in penile cancer; however, these studies only included data up until 2008. Previous systematic reviews and meta-analyses, which included fewer than 1500 cases of penile carcinoma, have suggested an HPV DNA prevalence of approximately 47–48% in penile cancer. Since then, several studies have been published on this topic. By contrast, to our knowledge, no previous meta-analyses examined pooled $p16^{INK4a}$ percent positivity in penile cancer or penile intraepithelial neoplasia, and similarly no meta-analyses have assessed HPV DNA prevalence in penile intraepithelial neoplasia.

Added value of this study

On the basis of more than 4000 cases of penile cancer, which is to our knowledge one of the largest number of cases reported so far, we report a pooled HPV DNA prevalence of 50·8% in penile cancer. Basaloid squamous cell carcinomas (84·0%) and warty-basaloid carcinoma (75·7%) had a particularly high HPV DNA prevalence. To our knowledge, we also report the first estimate of HPV DNA prevalence in penile intraepithelial neoplasia. From our estimations of the pooled prevalence of HPV types 6, 11, 16, 18, 31, 33, and 45, we found that HPV16 is by far the most common HPV type in both penile cancer and penile intraepithelial neoplasia. To our knowledge, this meta-analysis is also the first to assess pooled $p16^{INK4a}$ percent positivity in penile cancer and penile intraepithelial neoplasia, and, based on more than 2000 cases of penile cancer and 167 cases of penile intraepithelial neoplasia, estimates of $p16^{INK4a}$ positivity amount to 41·6% for penile cancer and 49·5% for penile intraepithelial neoplasia.

Implications of all the available evidence

This meta-analysis points to a high prevalence of HPV DNA in both penile cancer (about 50%) and penile intraepithelial neoplasia (about 80%). The prevalence of HPV DNA in penile cancer of 50% is in line with the findings of two previous systematic reviews and meta-analyses. Given that our analysis also shows that HPV16 is the most common type of HPV in this setting, this result implies that prophylactic vaccines targeting HPV16 might prevent a considerable proportion of penile cancers and precancerous lesions.

by penile intraepithelial neoplasia,[4] and these are classified as either low grade or high grade.

The presence of HPV in tumour tissue can be detected using various methods, such as PCR amplification for the detection of HPV DNA. PCR is a highly sensitive method that can detect both transient and persistent HPV infections, as well as deposition of HPV.[5] On the basis of the strong correlation between transcriptionally active HPV and $p16^{INK4a}$ overexpression in tumour cells, $p16^{INK4a}$ expression has been used as a surrogate marker of transforming HPV infections.[6]

In 2008, two systematic reviews and meta-analyses examined the prevalence of HPV DNA in penile cancer.[7,8] In this updated meta-analysis, we were able to add almost 3000 new cases of penile cancer, thus allowing for more robust estimates of the pooled prevalence of HPV DNA and type distribution in penile cancer; moreover, we stratified by histological subtype as defined by WHO[4] and other variables, such as geographical region and HPV or $p16^{INK4a}$ test. Additionally, we were able to assess HPV DNA prevalence and type distribution in penile intraepithelial neoplasia and to examine pooled $p16^{INK4a}$ percent positivity in both penile cancer and penile intraepithelial neoplasia.

Methods

Search strategy and selection criteria

We did a systematic review and meta-analysis of the literature complying with PRISMA guidelines[9] to identify English language studies published from Jan 1, 1986, to July 24, 2017, that examined the prevalence of HPV DNA and $p16^{INK4a}$ positivity, either alone or in combination, in penile cancer and penile intraepithelial neoplasia worldwide. PubMed, Embase, and the Cochrane Library were searched up until July 24, 2017. Full details of the search strategy for PubMed are in the appendix (p 1). Titles and abstracts were reviewed independently by two authors (TBO and FLS), and likewise, full-text articles were reviewed independently by two authors (TBO and CLR). Inconsistencies were discussed by three authors (TBO, FLS, and CM) to reach consensus. Reference lists were reviewed to identify additional relevant papers.

See Online for appendix

We included studies reporting the prevalence of HPV DNA and $p16^{INK4a}$ positivity, either alone or in combination, in histological material from at least five cases of penile cancer or penile intraepithelial neoplasia. Only studies that made use of PCR or hybrid capture for the detection of HPV DNA and immunohistochemical staining or methylation for the detection of $p16^{INK4a}$ were included. In each category, study populations could only be included once, and if we were in doubt whether two studies were overlapping, the authors were contacted. The study protocol is available in the appendix (p 14).

Data extraction

We extracted data on the following variables: author, year of publication, country, age at diagnosis (mean, median, and range), year of sample collection, type of tissue, type of lesion (penile cancer or penile intraepithelial

neoplasia), histological type (for penile cancer), sub-diagnosis (penile intraepithelial neoplasia), HPV DNA detection method, PCR primers, detectable HPV types, DNA control, sample size, number of HPV-positive cases, and HPV type-specific prevalence for single and multiple infections. We also extracted data for $p16^{INK4a}$ detection method; sample size; number of $p16^{INK4a}$-positive samples among all samples, among HPV-positive samples, and among HPV-negative samples; definition of $p16^{INK4a}$ positivity; and number of evaluators of immunohistochemical staining. Data were extracted independently by two authors (TBO and CLR) and subsequently crosschecked (CM), and inconsistencies were discussed to reach consensus. A molecular biologist (BN) reviewed the data extraction and full-text papers for correct classification of HPV DNA detection method and PCR primers. In case of uncertainty of the histological type of penile cancer, a pathologist (BGT) was consulted. The quality of the studies was assessed by only including studies published from 1986 onwards and studies that used PCR or Hybrid Capture 2 for the detection of HPV DNA. Furthermore, studies with fewer than five cases were excluded. Patient-level data were not sought.

Data analysis

We used random-effects models to estimate the overall pooled prevalence of HPV DNA in penile cancer and $p16^{INK4a}$ percent positivity in penile intraepithelial neoplasia. The variance between studies was established with the DerSimonian-Laird estimator and we applied the arcsine transformation to raw proportions. The HPV DNA prevalence and $p16^{INK4a}$ percent positivity for the individual studies were presented with exact binomial 95% CIs. Tests of across study heterogeneity (I^2 statistic) were done, and the Cochran's Q test was used to establish the significance of heterogeneity.

Source of heterogeneity was explored by stratified analyses and meta-regression. For studies examining HPV DNA prevalence in penile cancer and penile intraepithelial neoplasia, we did a stratified analysis for different histological types of penile cancer according to WHO classification[4] and a subdivision of penile intraepithelial neoplasia (low-grade penile intraepithelial neoplasia or high-grade penile intraepithelial neoplasia). Low-grade penile intraepithelial neoplasia comprised penile intraepithelial neoplasia grade 1 and dysplastic condyloma, whereas high-grade penile intraepithelial neoplasia comprised penile intraepithelial neoplasia grades 2 and 3 and carcinoma in situ. A priori explanatory variables for the meta-regression analysis were geographical region, HPV DNA detection method and PCR primer, and type of tissue. For studies examining $p16^{INK4a}$ positivity in penile cancer, stratified analysis for histological types was done, and for the meta-regression analysis, a priori variables were selected (geographical region and $p16^{INK4a}$ detection method). In the meta-regression, each variable was initially evaluated in a univariable test of a moderator variable, a test of the overall significance of a variable based on the χ^2 test. Then, each variable was mutually adjusted by including all variables, and the multivariable models were compared using likelihood ratio tests.

In studies using both $p16^{INK4a}$ immunohistochemistry and methylation detection methods,[10–12] only the estimates from immunohistochemistry were included in the analyses. For studies using $p16^{INK4a}$ immunohistochemistry, additional analyses were done, stratifying the pooled $p16^{INK4a}$ percent positivity according to the definitions of $p16^{INK4a}$ positivity used. Furthermore, separate analyses were done by calculating the pooled percentage of

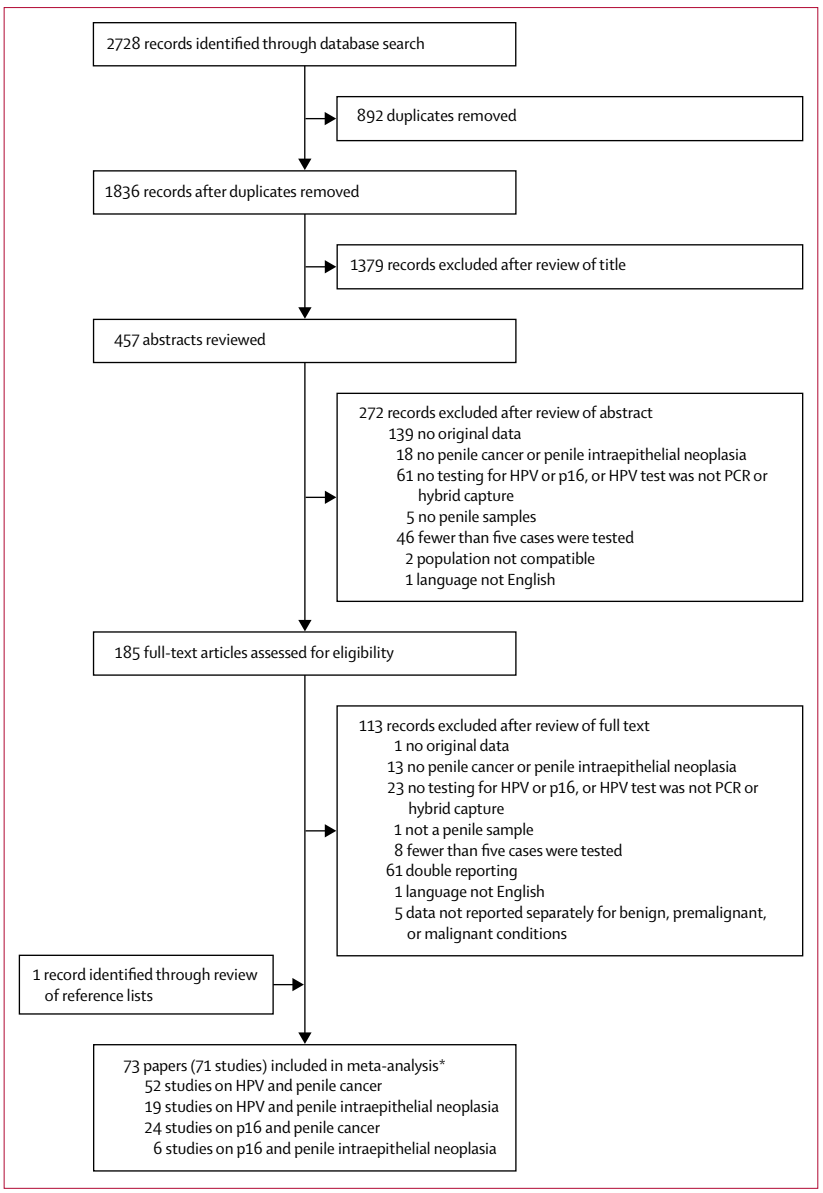

Figure 1: **Flow diagram of study selection**
HPV=human papillomavirus. *Some studies were included in more than one of the categories listed.

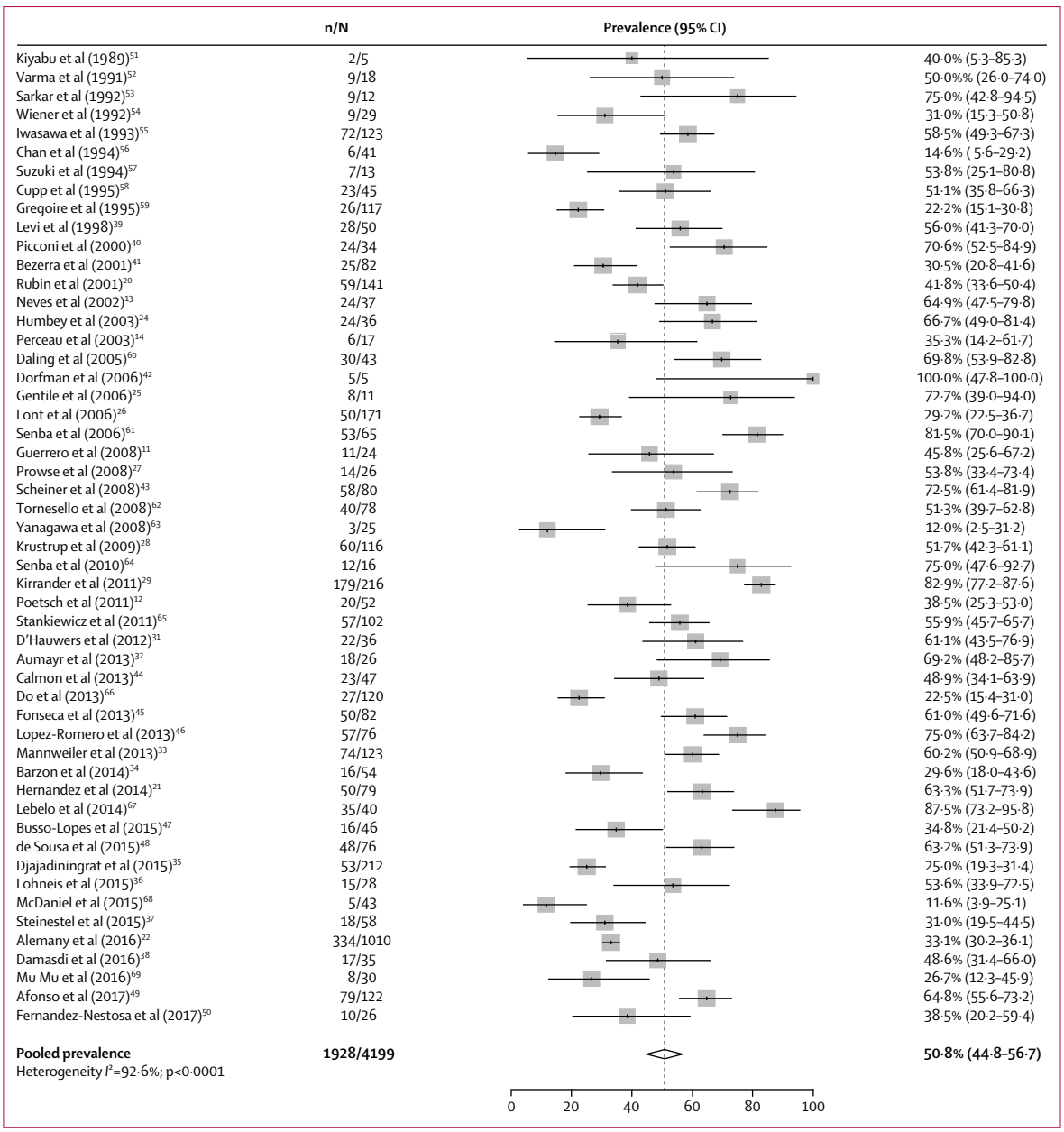

Figure 2: **Pooled prevalence of HPV DNA in penile cancer worldwide**
HPV=human papillomavirus. n=number of HPV-positive cases. N=total number of cases.

p16^{INK4a} positivity in all cases, in HPV-positive cases, and in HPV-negative cases.

We also did analyses examining the pooled prevalence of HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, and HPV45, stratifying the analyses to either single or multiple HPV infections. The denominator used was the total number of HPV-positive cases when examining multiple HPV infections and the number of single HPV-positive cases when examining single HPV infections. Studies that provided a combined estimate for HPV6 and HPV11 were not included in the analysis.

Sensitivity analyses were done excluding studies of high-risk or possible high-risk populations[13–19] (eg, men with HIV) and, furthermore, excluding studies with a possible overlap of cases.[20–22] All analyses were done using R version 3.4.1 (package meta).[23]

Role of the funding source

There was no funding source for this study. The corresponding author had full access to all the data and the final responsibility for the decision to submit for publication.

	Studies*	Cases	HPV DNA-positive cases	Pooled HPV DNA prevalence (95% CI)	Heterogeneity	
					I^2	p value
Total penile cancers	52	4199	1928	50·8% (44·8–56·7)	92·6%	<0·0001
Histology						
SCC	47	3772	1628	49·0% (43·1–54·9)	91·5%	<0·0001
HPV-related SCC	21	429	309	72·7% (63·2–81·3)	60·3%	<0·0001
Basaloid SCC	15	89	71	84·0% (71·0–93·6)	48·0%	0·0197
Warty-basaloid carcinoma	3	238	180	75·7% (70·1–81·0)	0%	0·52
Warty carcinoma	15	70	39	58·7% (38·0–78·0)	65·0%	0·0003
Other HPV-related subtypes†	3	32	19	62·1% (34·7–85·8)	49·1%	0·14
Non-HPV-related SCC	28	1272	322	19·4% (12·1–28·0)	85·3%	<0·0001
SCC, usual type	11	438	185	32·2% (16·1–50·7)	93·7%	<0·0001
Verrucous carcinoma	24	124	33	21·7% (7·9–40·0)	77·5%	<0·0001
Papillary SCC	9	35	6	15·5% (1·4–40·5)	57·8%	0·0151
Sarcomatoid (spindle-cell) carcinoma	10	18	4	10·5% (0·0–35·8)	38·4%	0·10
Other non-HPV-related subtypes‡	6	657	94	3·1% (0·0–15·3)	37·8%	0·14
Total penile intraepithelial neoplasia	19	445	367	79·8% (69·3–88·6)	83·2%	<0·0001
Low-grade penile intraepithelial neoplasia§	5	41	39	98·6% (89·5–100·0)	38·5%	0·17
High-grade penile intraepithelial neoplasia¶	15	340	275	80·5% (68·7–90·1)	81·4%	<0·0001

HPV=human papillomavirus. SCC=squamous cell carcinoma. *Studies could have contributed cases to more than one histological subtype of SCC and more than one subgroup of penile intraepithelial neoplasia. Moreover, some studies only contributed to the analysis of all penile intraepithelial neoplasia, given that they did not specify the severity (grade) of the neoplasia. †Clear-cell squamous carcinomas, basaloid or warty SCC, warty or basaloid SCC, lymphothelioma-like carcinoma, and mixed basaloid-warty SCC. ‡SCC without warty-basaloid features, carcinoma cuniculatum SCC, pseudohyperplastic SCC, mixed verrucous or usual-type SCC, adenosquamous SCC, and mixed SCC. §Includes penile intraepithelial neoplasia grade 1 and dysplastic condyloma. ¶Includes penile intraepithelial neoplasia grades 2 and 3 and carcinoma in situ.

***Table 1*: Pooled HPV DNA prevalence in penile cancer and penile intraepithelial neoplasia by histological subtypes and by grade of penile intraepithelial neoplasia**

Results

Our searches identified 1836 non-duplicate records. After review of titles (n=1836), abstracts (n=457), and full-text articles (n=185), 72 relevant papers were found. One additional relevant paper was identified after review of reference lists (figure 1). These 73 papers originated from 71 studies (two studies contributed their data in two separate papers). Characteristics of the included studies are in the appendix p 2–12. We identified 52 studies examining the prevalence of HPV DNA in penile cancer, which together included a total of 4199 cases of penile cancer. The studies were published between 1989 and 2017. Most of the studies originated from Europe (n=18)[11,12,14,24–38] and South America (n=13).[13,39–50] All studies used a PCR-based test for the detection of HPV DNA (appendix p 2–7). The overall pooled HPV DNA prevalence in penile cancer based on these 52 studies was 50·8% (95% CI 44·8–56·7) ranging from 11·6% to 100%, and with a large between-study heterogeneity (I^2=92·6%; p<0·0001; figure 2). A sensitivity analysis, excluding seven studies[13–19] of high-risk populations (eg, men with HIV and men who attended a sexual health clinic), did not change the pooled HPV DNA prevalence (50·8%, 95% CI 44·7–56·9). When we excluded studies[20,21] with a small possible overlap of cases with a large international study[22] (four cases[20] and 17 cases),[21] the results were unchanged. In the 47 studies with information about histological types of squamous cell carcinoma, HPV-related squamous cell carcinoma, as defined by WHO, showed a higher pooled HPV DNA prevalence than non-HPV-related squamous cell carcinoma (table 1). In particular, basaloid squamous cell carcinoma and warty-basaloid carcinoma had a high pooled HPV DNA prevalence (table 1).

Table 2 shows the HPV DNA prevalence in penile cancer according to geographical region, HPV DNA detection method and primers, and type of tissue. When stratified by geographical region, a high HPV DNA prevalence in penile cancer was seen in studies from South America and in the one study from Africa, with a lower HPV DNA prevalence in the multicountry studies and in studies from Asia. The HPV DNA prevalence in penile cancer was generally similar for the different types of primers. In the multivariable metaregression, none of the included variables explained the heterogeneity.

In total, 19 studies assessed the prevalence of HPV DNA in penile intraepithelial neoplasia, comprising a total of 445 cases. These studies were published between 1991 and 2017 and approximately half of the studies were from Europe (n=9;[15,16,28,31,33,34,70–72] appendix p 8–9). The pooled HPV DNA prevalence in penile intraepithelial neoplasia was 79·8% (95% CI 69·3–88·6; figure 3), with a higher prevalence in low-grade (98·6%, 95% CI 89·5–100·0) than in high-grade penile intraepithelial neoplasia

	Penile cancer								Penile intraepithelial neoplasia							
	Studies	Cases	HPV DNA-positive cases	Pooled HPV DNA prevalence (95% CI)	p value (univariable test of moderator variable)	p value (multivariable test of moderator variable)	Heterogeneity		Studies	Cases	HPV DNA-positive cases	Pooled HPV DNA prevalence (95% CI)	p value (univariable test of moderator variable)	Heterogeneity	
							I^2	p value						I^2	p value
Geographical region					0·0805	0·22							0·27		
Europe	18	1343	662	50·3% (39·8–60·9)	..	..	93·0%	<0·0001	9	189	149	71·2% (49·7–88·6)	..	88·6%	<0·0001
North America	8	274	137	48·4% (31·4–65·6)	..	..	86·9%	<0·0001	5	101	81	74·7% (52·8–91·4)	..	75·9%	0·0023
South America	13	763	447	59·3% (50·1–68·2)	..	..	84·1%	<0·0001	2	27	23	93·2% (52·6–100·0)	..	84·5%	0·0110
Asia	8	433	188	41·9% (22·6–62·5)	..	..	94·2%	<0·0001	0	..	..	..	..	..	..
Africa	1	40	35	87·5% (75·6–95·8)	..	..	..	..	0	..	..	..	..	..	..
Oceania	0	..	..	..	..	..	..	..	0	..	..	..	..	..	..
Multicountry	4	1346	459	36·3% (27·1–46·0)	..	..	86·3%	<0·0001	3	128	114	93·1% (81·5–99·2)	..	67·3%	0·0472
HPV test and primers					0·43	0·70							0·052		
PCR, consensus primers	25	2614	1068	47·0% (39·9–54·1)	..	..	90·9%	<0·0001	9	283	241	86·0% (78·8–91·8)	..	54·5%	0·0247
PCR, type-specific primers	9	520	343	56·9% (38·5–74·3)	..	..	93·1%	<0·0001	5	62	43	68·7% (48·0–86·1)	..	61·2%	0·0354
PCR, combination of consensus primers	3	124	80	64·5% (55·9–72·7)	..	..	0%	0·82	2	57	43	37·9% (0·0–100·0)	..	98·4%	<0·0001
PCR, combination of consensus and type-specific primers	13	878	414	49·2% (37·3–61·1)	..	..	92·0%	<0·0001	3	43	40	95·0% (83·5–99·9)	..	33·1%	0·23
PCR, unknown primers	2	63	23	75·9% (1·0–100·0)	..	..	94·3%	<0·0001	0	..	..	..	..	..	..
Type of tissue					0·74	1·00							0·89		
Frozen	3	176	102	55·0% (33·0–76·1)			88·7%	0·0001	2	51	45	73·9% (16·8–100·0)		85·9%	0·0077
Fixed	48	3901	1747	50·2% (44·0–56·4)	..	..	92·7%	<0·0001	17	394	322	80·0% (68·7–89·3)	..	83·7%	<0·0001
Unknown	1	122	79	64·8% (56·1–73·0)	..	..	..	..	0	..	..	..	..	..	..

If only one study contributed to the analysis, then no pooled estimate was provided, merely the actual proportion reported in the study. HPV=human papillomavirus.

Table 2: **Pooled HPV DNA prevalence in penile cancer and penile intraepithelial neoplasia by stratification variables**

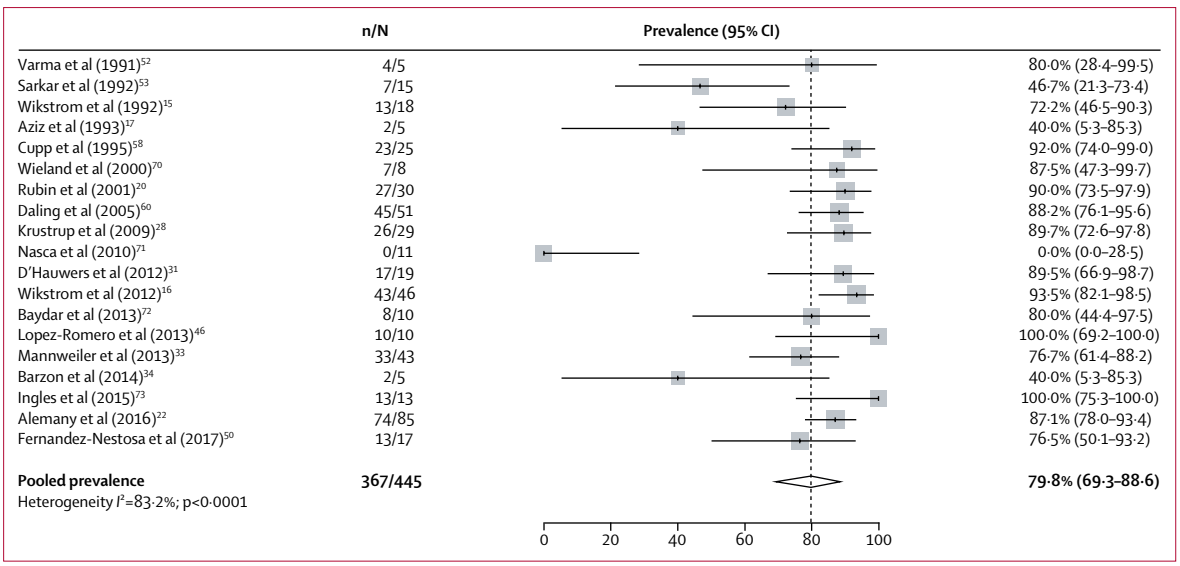

Figure 3: **Pooled prevalence of HPV DNA in penile intraepithelial neoplasia worldwide**
n=number of HPV-positive cases. N=total number of cases. HPV=human papillomavirus.

(80·5%, 68·7–90·1), although these results were based on a small number of cases (table 1).

We also estimated the pooled prevalence of HPV DNA according to specific HPV types (HPV types 6, 11, 16, 18, 31, 33, and 45) in penile cancer and in penile intraepithelial neoplasia. Among HPV DNA-positive penile cancers, HPV16 was the most common type (68·3%, 95% CI 58·9–77·1), followed by HPV6 (8·1%, 4·0–13·7) and HPV18 (6·9%, 2·9–12·4). HPV16 was also by far the most common HPV type in HPV-positive penile intraepithelial neoplasia (69·8%, 53·3–83·9), followed by HPV6 (18·4%, 4·0–39·9) and HPV11 (6·8%, 1·3–16·0). In both penile cancer and penile intraepithelial neoplasia, HPV16 was the most prevalent HPV subtype in cases with infection with just one HPV type (figure 4).

We included 24 studies published between 2003 and 2017 in which the percentage of $p16^{INK4a}$ positivity in penile cancer was examined in a total of 2295 penile cancer cases. The majority were European (n=11)[10–12,27,30,32–34,36,37,74] or North American studies (n=6),[75–79] and most studies used fixed histological material (n=23). Two studies[49,63] used methylation for the detection of $p16^{INK4a}$, whereas the remainder used immunohistochemistry (n=22; appendix pp 10–11). The overall pooled percentage of $p16^{INK4a}$-positive penile cancer was 41·6% (95% CI 36·2–47·0) with a large degree of heterogeneity (I^2=80·6%; p<0·0001; figure 5). We found that HPV-related squamous cell carcinoma, as defined by WHO, including basaloid squamous cell carcinoma, showed a higher pooled $p16^{INK4a}$ percent positivity than non-HPV-related squamous cell carcinoma (table 3). In the meta-regression, we found that the $p16^{INK4a}$ percent positivity was lower in the studies from South America (9·1%; 95% CI 0–56·8) than in other regions (42–50%); however, this result was based on few studies. Moreover, the estimates were similar irrespective of the $p16^{INK4a}$ test used (table 4). Additionally, we found that $p16^{INK4a}$ percent positivity did not differ significantly according to the cutoff used to define $p16^{INK4a}$ positivity (appendix p 13).

When examining the pooled $p16^{INK4a}$ percent positivity in relation to the HPV status of penile cancers, we found that HPV-positive penile cancers had a much higher pooled $p16^{INK4a}$ percent positivity (79·6%, 95% CI 65·7–90·7) than HPV-negative penile cancers (18·5%, 9·6–29·6; figure 5).

We identified six studies[18,19,22,33,34,72] (n=167) that examined $p16^{INK4a}$ positivity in penile intraepithelial neoplasia (appendix p 12) from which we calculated a pooled $p16^{INK4a}$ percent positivity of 49·5% (95% CI 18·6–80·7; table 3).

Discussion

In this systematic review and meta-analysis, we included 71 studies examining the prevalence of HPV DNA and $p16^{INK4a}$ positivity, either alone or in combination, in penile cancer and penile intraepithelial neoplasia, worldwide.

On the basis of 4199 cases of penile cancer, we found that around half of the cases were associated with infection with HPV DNA. Contrary to previous meta-analyses,[7,8] we found a higher pooled HPV DNA prevalence in studies from South America and a lower pooled HPV DNA prevalence in studies from Asia than in the studies from other geographical regions. The differing pooled estimates for the geographical regions could reflect true differences or could be influenced by differences in the distribution of the histological subtypes of squamous cell carcinoma in the studies from the respective geographical regions. We should emphasise that these subset analyses are based on a small number of cases, which are therefore more prone to random

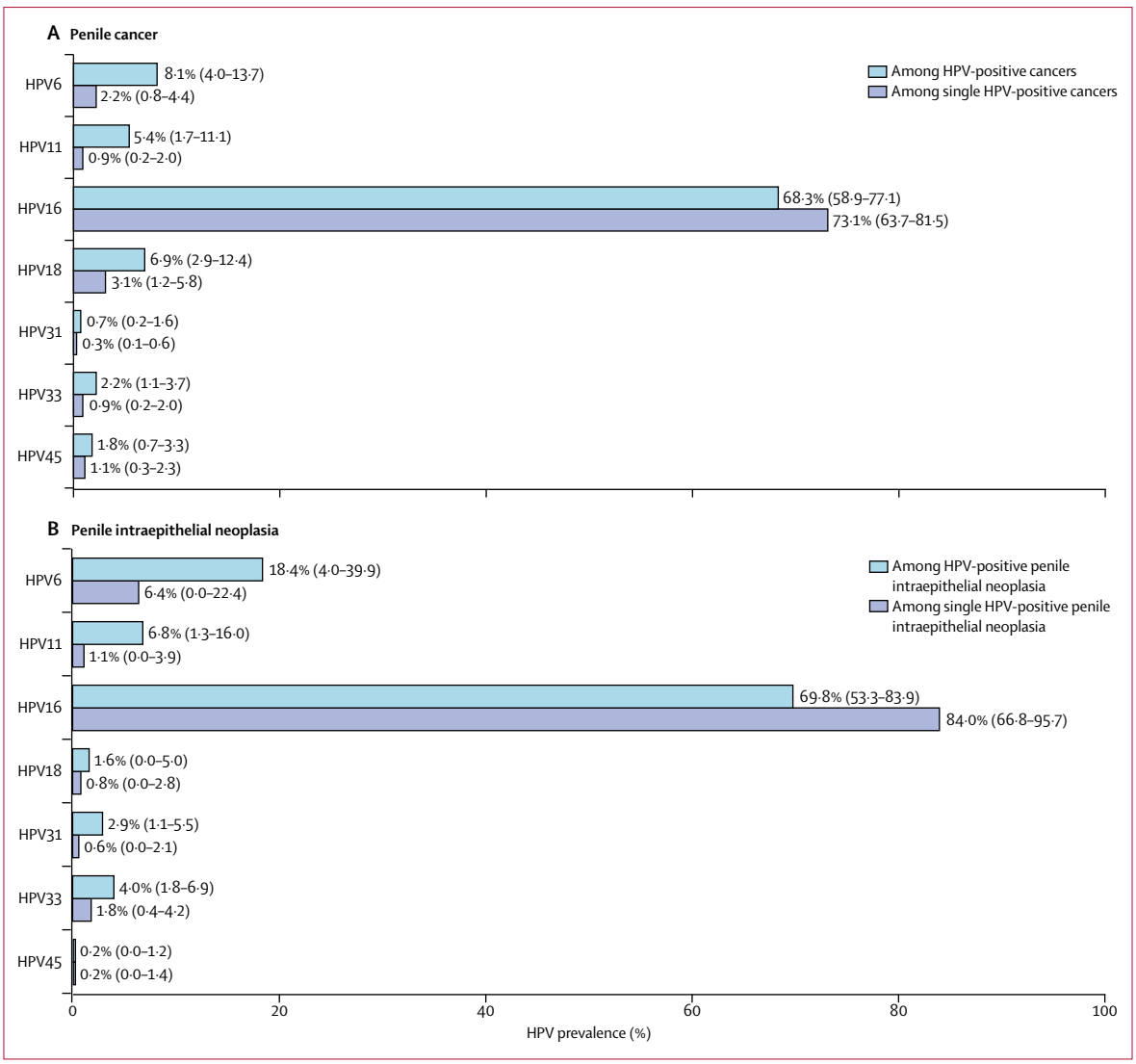

Figure 4: **Pooled prevalence of HPV subtypes in penile cancer and penile intraepithelial neoplasia worldwide**
The pooled prevalence of HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, and HPV45 in penile cancer (A) and penile intraepithelial neoplasia (B), according to whether the cancer or penile intraepithelial neoplasia was positive for multiple HPV types or for a single HPV type, worldwide. Ranges are 95% CIs. HPV=human papillomavirus.

variation. Additionally, we noted a high pooled HPV DNA prevalence in the studies using a combination of consensus primers or with unknown primers, although the estimates were based on small numbers. These findings, however, might highlight the importance of stratifying results by the HPV DNA detection method used. The categorisation of primers was different in previously published meta-analyses and the results are thus difficult to compare.

In the analyses stratified according to the histological subtypes of squamous cell carcinoma, the pooled HPV DNA prevalence was higher in cases of HPV-related squamous cell carcinoma than non-HPV-related squamous cell carcinoma. Our estimates of the pooled HPV DNA prevalence in basaloid squamous cell carcinoma and warty-basaloid carcinomas are higher than those of a previous meta-analysis;[8] however, we included more cases in these groups of histological subtypes, which might have resulted in more reliable estimates than in previous analyses. The pooled HPV DNA prevalence in verrucous carcinoma was similar to the estimates previously reported.[7,8] Moreover, usual-type squamous cell carcinoma, which is classified as non-HPV-related according to the latest WHO classification, displayed a high pooled HPV DNA prevalence of 32·2%. This estimate is based on 438 cases and the CI is wide, so to confirm our finding, additional studies are needed. However, this result could indicate that the histological classification of tumours into HPV-related and non-HPV-related squamous cell carcinoma should be interpreted with caution. Another explanation could be misclassification of tumours, although in about half of

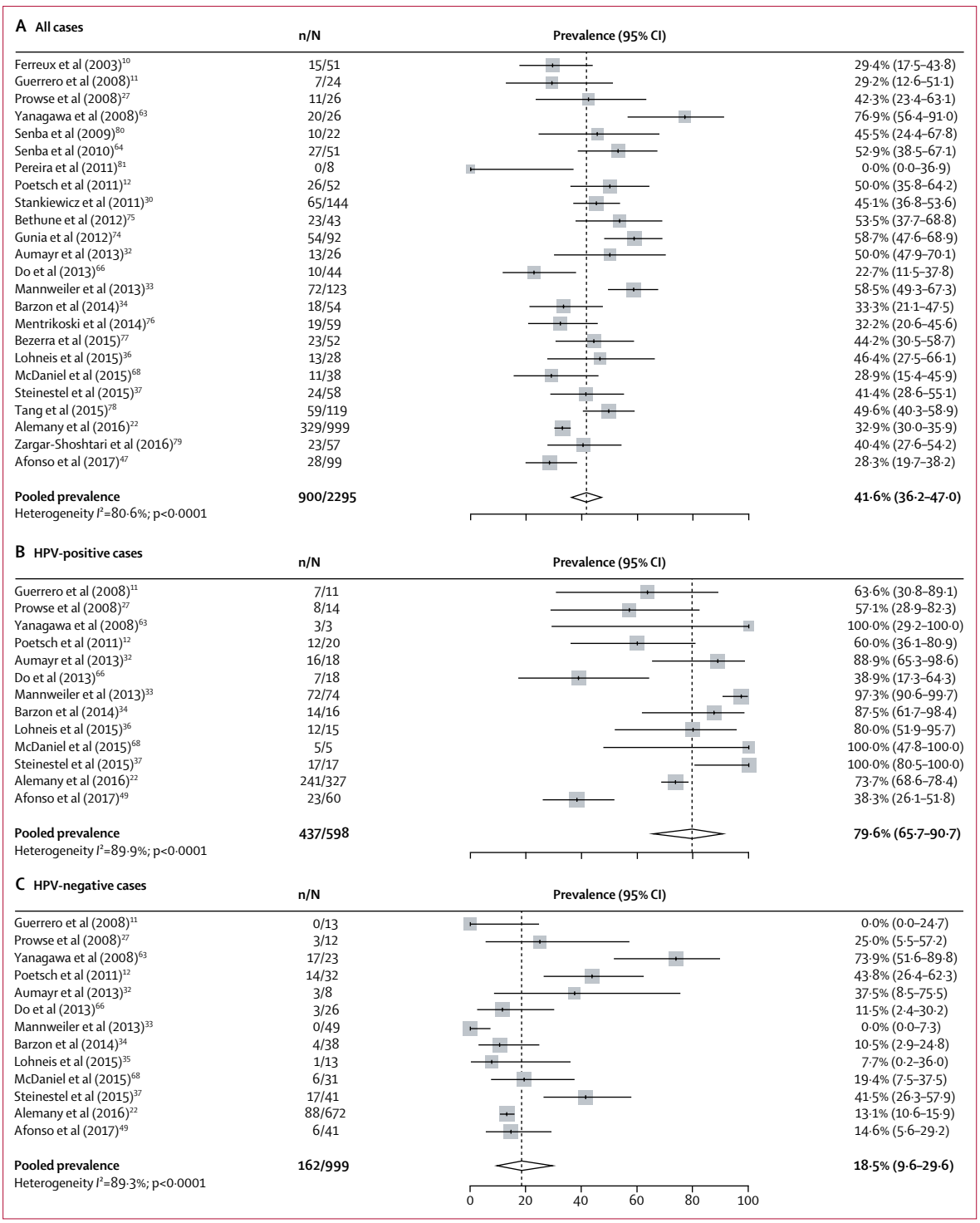

Figure 5: **Pooled p16^{INK4a} percent positivity in penile cancer worldwide in all cases (A), HPV-positive cases (B), and HPV-negative cases (C)**
n=number of HPV-positive cases. N=total number of cases. HPV=human papillomavirus.

the studies the histological diagnoses were reviewed by one or more pathologists. Vulvar cancer is morphologically similar to penile cancer, and in vulvar cancer, non-HPV-related tumours (eg, keratinising squamous cell carcinoma) also show a somewhat high pooled HPV DNA prevalence (13·2%[82] and 17·0%[83]) and combined HPV DNA and p16^{INK4a} percent positivity (11·5%),[84] and another study concluded that histological classification

	Studies*	Cases	p16^{INK4a}-positive cases	Pooled p16^{INK4a} percent positivity (95% CI)	Heterogeneity	
					I^2	p value
Total penile cancers	24	2295	900	41·6% (36·2–47·0)	80·6%	<0·0001
Histology						
SCC	23	1296	571	42·1% (36·4–47·8)	75·8%	<0·0001
HPV-related SCC	11	89	72	85·8% (72·1–95·4)	56·4%	0·0011
Basaloid SCC	8	44	40	94·9% (84·4–99·8)	21·8%	0·26
Warty-basaloid carcinoma	2	3	3	100·0% (71·3–100·0)	0·0%	1·00
Warty carcinoma	7	27	15	55·2% (24·4–84·0)	62·8%	0·0132
Other HPV-related subtypes†	2	15	14	94·6% (78·1–100·0)	0·0%	0·66
Non-HPV-related SCC	10	351	126	17·1% (7·9–29·1)	78·3%	<0·0001
SCC, usual type	8	305	122	36·9% (22·5–52·7)	86·7%	<0·0001
Verrucous carcinoma	7	29	3	4·8% (0·0–20·7)	31·0%	0·19
Papillary SCC	4	8	0	0·0% (0·0–11·5)	0·0%	1·00
Sarcomatoid (spindle-cell) carcinoma	4	7	0	0·0% (0·0–13·1)	0·0%	1·00
Other non-HPV-related subtypes‡	2	2	1	50·0% (0·0–100·0)	79·7%	0·026
Total penile intraepithelial neoplasia	6	167	100	49·5% (18·6–80·7)	93·5%	<0·0001

SCC=squamous cell carcinoma. HPV=human papillomavirus. *Studies could have contributed cases to more than one histological subtype of SCC. †Clear-cell squamous carcinomas, basaloid or warty SCC, warty or basaloid SCC, lymphothelioma-like carcinoma, and mixed basaloid-warty SCC. ‡SCC without warty-basaloid features, carcinoma cuniculatum SCC, pseudohyperplastic SCC, mixed verrucous or usual-type SCC, adenosquamous SCC, and mixed SCC.

***Table 3*: Pooled p16^{INK4a} percent positivity in penile cancer and penile intraepithelial neoplasia by histological subtype**

does not allow differentiation between HPV-related and HPV-independent squamous cell vulvar cancers.[83]

Our study is the first, to our knowledge, to provide a pooled measure of the HPV DNA prevalence and type distribution in penile intraepithelial neoplasia. On the basis of 19 studies including 445 cases of penile intraepithelial neoplasia, we also found a high pooled HPV DNA prevalence in these lesions (79·8%). This prevalence is similar to a meta-analysis of vulvar intraepithelial neoplasia, which reported a pooled HPV DNA prevalence of 76·3%.[82]

We examined the type distribution of five high-risk HPV types (HPV16, HPV18, HPV31, HPV33, and HPV45) and two low-risk HPV types (HPV6 and HPV11) in penile intraepithelial neoplasia and penile cancer. By far the most important oncogenic HPV genotype was HPV16, which accounted for more than 70% of the single infections in penile cancer. This subtype was followed by HPV18 and HPV6. In penile intraepithelial neoplasia lesions, HPV16 accounted for more than 80% of single-infection-positive penile intraepithelial neoplasia, followed by HPV6. Our data highlight the predominance of HPV16 in penile cancer and penile intraepithelial neoplasia, underlining the potential preventive effect of available HPV vaccines. In most countries, HPV vaccination is only targeted at girls; however, mounting evidence suggests that HPV causes a range of different cancers in men too, including penile and oropharyngeal cancer. Although penile cancer is a rare disease, HPV vaccination of men and boys should be considered to prevent this burden of disease and other HPV-related diseases in men.

The pooled percent positivity of the tumour suppressor protein p16^{INK4a} was 41·6% in all penile cancers irrespective of the HPV status of the cancer. A significantly higher pooled p16^{INK4a} percent positivity was seen in HPV-positive cancers (79·6%) than in HPV-negative cancers (18·5%), which was expected, given that p16^{INK4a} is upregulated by the HPV oncogene. From a biological point of view, some authors suggest that cancers of other sites (eg, head and neck squamous cell carcinoma) should only be considered HPV positive if the tumour is positive for both HPV DNA and p16^{INK4a}.[85] This so-called double positivity might be clinically relevant, given that individuals with tumours at other sites (eg, tonsillar and base of tongue) have shown improved survival when their tumours are positive for both HPV DNA and p16^{INK4a}.[86] Even though the definition of p16^{INK4a} positivity varied between studies, we did not detect any difference in the pooled p16^{INK4a} percent positivity in relation to the cutoff used to define p16^{INK4a} positivity. In line with this finding, Prigge and colleagues[87] did not detect any difference in the sensitivity or specificity of the p16^{INK4a} immunohistochemistry test in oropharyngeal cancers, irrespective of the definition of p16^{INK4a} positivity. Ndiaye and colleagues[88] found that p16^{INK4a} positivity changed by p16^{INK4a} test cutoff in oropharyngeal squamous cell carcinoma, although the difference was small.

We found that HPV-related squamous cell carcinomas had a higher pooled p16^{INK4a} percent positivity (85·8%) than did non-HPV-related squamous cell carcinomas (17·1%). Moreover, a pooled p16^{INK4a} percent positivity of 36·9% was seen in usual-type squamous cell carcinoma

	Studies	Cases	p16^{INK4a}-positive cases	Pooled p16^{INK4a} percent positivity (95% CI)	p value (univariable test of moderator variable)	Heterogeneity	
						I^2	p value
Geographical region					0·10		
Europe	11	678	318	44·9% (38·4–51·5)	..	63·5%	0·0022
North America	6	368	158	41·9% (34·5–49·4)	..	51·9%	0·0646
South America	2	107	28	9·1% (0·0–56·8)	..	89·3%	0·0023
Asia	3	121	57	50·4% (21·6–79·1)	..	91·2%	<0·0001
Africa	1	22	10	45·5% (25·6–66·1)	..		
Oceania	0	..	..	..	..	..	..
Multicountry	1	999	329	32·9% (30·1–35·9)	..		
p16^{INK4a} test					0·46		
p16^{INK4a} immunohistochemistry	22	2170	852	41·1% (35·8–46·4)	..	78·5%	<0·0001
p16^{INK4a} methylation	2	125	48	52·3% (9·3–93·2)	..	95·3%	<0·0001

If only one study contributed to the analysis, then no pooled estimate was provided, merely the actual proportion of the study.

***Table 4*: Pooled p16^{INK4a} percent positivity in penile cancer by geographical region and p16^{INK4a} detection method**

despite this type being considered non-HPV-related. The estimate is based on 305 cases and this finding needs to be confirmed in future studies. These results are similar to the analysis of the HPV DNA prevalence, and indicate caution when dividing squamous cell carcinoma into HPV-related and non-HPV-related squamous cell carcinoma; however, the results could also be due to misclassification of tumours. Among the 167 penile intraepithelial neoplasia lesions, a pooled p16^{INK4a} percent positivity of 49·5% was seen. Unfortunately, because of the small number of cases, examining high-grade and low-grade penile intraepithelial neoplasia separately or stratifying by HPV status was not possible.

The strengths of this meta-analysis include the large number of studies and hence the large number of cases, resulting in more robust estimates than previous meta-analyses. Moreover, the pooled HPV DNA prevalence and p16^{INK4a} percent positivity was assessed according to the latest WHO classification of penile tumours,[4] which to our knowledge has not been done before. Additionally, we examined the prevalence of five high-risk and two low-risk HPV types in penile intraepithelial neoplasia and penile cancer. This meta-analysis is the first, to our knowledge, to examine pooled p16^{INK4a} percent positivity in penile cancer stratified by histological subtype and HPV status, and the first to assess pooled p16^{INK4a} percent positivity in penile epithelial neoplasia.

Limitations of the study must also be acknowledged. A large degree of between-study heterogeneity was present, and even though we examined the sources of heterogeneity in subgroup analyses and meta-regression, heterogeneity could not be explained by the factors included in the analysis. The heterogeneity could also affect the generalisability of the study results. Additionally, data about risk factors (eg, circumcision status or HIV status) were only rarely available from the included studies. Such factors might potentially be responsible for some of the heterogeneity. Notably, in a sensitivity analysis excluding seven studies comprising high-risk or potential high-risk populations (eg, men with HIV, and men who attended a sexual health clinic), the pooled prevalence was virtually identical. We included English-language articles only; however, language restrictions do not seem to affect overall estimates in meta-analyses.[89] Importantly, some of the estimates (eg, histological subtypes and differences by geography) were based on small numbers and should therefore be interpreted with caution.

In conclusion, we found that HPV is associated with more than three-quarters of penile intraepithelial neoplasia lesions and half of penile cancers, with the highest pooled prevalence observed in basaloid squamous cell carcinoma and warty-basaloid squamous cell carcinoma. HPV16 was the most predominant HPV type in both penile cancer and penile intraepithelial neoplasia. p16^{INK4a} positivity was found in more than 40% of penile cancers and, in particular, in nearly 80% of the HPV-positive penile cancers. Our meta-analysis supports the suggestion that prophylactic vaccines targeting HPV16 have the potential to prevent a substantial proportion of penile cancers and penile intraepithelial neoplasia lesions, adding to the possible benefits of HPV vaccination in men and boys.

Contributors

SKK designed the study. TBO and FLS reviewed titles and abstracts. TBO and CLR reviewed full-text articles. TBO and FLS designed the data extraction form. TBO and CLR extracted the data. CM crosschecked the data extraction. BN reviewed the data regarding human papillomavirus DNA detection methods and PCR primers, and BGT reviewed the data regarding histology. TBO and FLS wrote the analysis plan. VA did the statistical analyses. TBO, FLS, and SKK drafted the manuscript. CLR, VA,

BGT, BN, and CM critically revised subsequent drafts. All authors read and approved the submitted version.

Declaration of interests

FLS has received speaker's fees and support for conference participation from Becton, Dickinson and Company. CM has received speaker's fees and support for conference participation from Sanofi Pasteur MSD. SKK has received speaker's fee from Sanofi Pasteur MSD and Merck, scientific advisory board fees from Merck, and an unrestricted research grant through her institution from Merck. The other authors declare no competing interests.

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