The relationship between human papillomavirus and penile cancer over the past decade: a systematic review and meta-analysis

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Human papillomavirus (HPV) infection appears to play an important role in the development of penile cancer (PeCa), but their relationship remains unclear. Therefore, we performed a systematic review and meta-analysis to elucidate their relationship. We systematically searched Embase, PubMed, Cochrane Library, and Web of Science for case-control studies and cross-sectional studies using polymerase chain reaction (PCR) technology on formalin-fixed paraffin-embedded (FFPE) or paraffin-embedded (PE) PeCa tissues to detect HPV (published between January 1, 2007, and December 29, 2017; no language restrictions). Twenty-two studies were identified, and 1664 cases were available for analysis. The combined HPV infectious risk of PeCa is 51.0% (95% confidence interval [CI]: 43.0%–60.0%). The three most common subtypes of HPV were HPV16 (28.5%), HPV18 (2.3%), and HPV6 (2.3%). The virus was relevantly associated with basaloid (85.5%, 95% CI: 77.2%–93.8%) and warty (50.0%, 95% CI: 35.2%–64.8%) carcinomas. The invasiveness of PeCa was not associated with HPV ($\chi^2 = 0.181$, df = 1, $P < 0.671$). HPV infection in PeCa tended to be moderately differentiated (54.4%, 95% CI: 47.7%–61.1%). This study found that almost half of PeCa patients are associated with HPV. The most commonly associated genotype is HPV16, but several other genotypes were also detected. In addition to types 6 and 11, other single low-risk HPV infections have been found to contribute to PeCa to a lesser degree. HPV-positive tumors tend to exhibit warty and/or basaloid features, corresponding to a moderate histological grade. The role of HPV in PeCa should be revisited to provide evidence for the development of PeCa in the presence of HPV infection.

Asian Journal of Andrology (2019) 21, 375–380; doi: 10.4103/aja.aja_39_19; published online: 24 May 2019

Keywords: human papillomavirus; penile cancer; systematic review and meta-analysis

INTRODUCTION
Penile carcinoma is a rare malignant tumor, accounting for <1% of adult male cancers in Europe and North America.1 However, its incidence in South America, Africa, and some parts of Asia may be as high as 10%, and approximately 26,300 new cases are diagnosed each year in men who are older than 66 years.2 The disease is characterized by an increased incidence in older men, with an average age at diagnosis of 60 years. The peak incidence of penile cancer (PeCa) occurs at the age of 70 years.3,4 No consensus is available regarding the age distribution of PeCa cases.

Studies have identified several contributing factors for PeCa, including phimosis, smoking, and chronic inflammatory states.5 In addition, lesions on the glans are directly linked to poor hygiene.

Human papillomavirus (HPV) infection is associated with anogenital cancer (including cervical, vaginal, vulvar, penile, and anal cancers), oropharyngeal cancer, and genital warts. The HPV vaccination significantly reduces the incidence of anogenital cancer and genital warts.6 However, although the success of the quadrivalent vaccine against HPV has led to substantial decreases in HPV-associated infections and cancers in women, studies have not demonstrated similar success in men, specifically in relation to PeCa and penile precancerous lesions. Determining the pathogenesis of HPV infection may provide valuable therapeutic targets to treat this rare and difficult disease.7 The aim of this study was to evaluate the prevalence of HPV in penile malignant tumor samples in the most recent decade and to determine the relationship between histological types of PeCa and HPV to further understand the development of PeCa.

MATERIALS AND METHODS
Search strategy and selection criteria
This systematic review and meta-analysis is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement and was registered at the International Prospective Register of Systematic Reviews (No. CRD42018086094; available at: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=86094).

An extensive literature search was conducted by two independent authors to identify all relevant studies published between January 1, 2007, and December 29, 2017, by searching Embase, PubMed, Cochrane Library, and Web of Science. With no language restrictions, we used the following combined text and Medical Subject Headings (MeSH) terms: "penile neoplasms" and "human papillomavirus."
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RESULTS

Description of studies

A total of 22 studies,4,11–12 complied with the inclusion criteria, which included 1664 patients with penile carcinoma (Figure 1). Through AHRQ estimation, 7 articles4,7,24,26–28 were considered high quality, and 15 articles4,11,13–16,18–23,25,27,32,33 were considered moderate quality (Supplementary Table 1). The overall prevalence of HPV positivity in patients with penile tumors was approximately 51% (95% CI: 43%–60%; Figure 2). Overall, 73.8% of the penile carcinoma cases were squamous cell carcinoma (SCC). The most frequently used PCR primers were PCR GP5+6+ and PCR SPF-10. The data were analyzed for differences with respect to HPV DNA detection between PCR GP5+6+ and PCR SPF-10. The results were evaluated by Pearson’s Chi-squared test, and no significance ($\chi^2 = 2.938, df = 1, P > 0.05$) was detected (Supplementary Table 2).

Description of HPV detection

Analyses of type-specific HPV prevalence rates were limited to 16 studies,4,16–18,20–25,27–33 of SCC ($n = 1270$) with available data. Among these studies, approximately 19 HPV types were detected. Using all the cases tested as the denominator, the overall infection rate was 47.2% (95% CI: 31.0%–70.0%). The three most common types of HPV were HPV16 (28.5%), HPV18 (2.3%), and HPV6 (2.3%) (Supplementary Table 1). HPV18 and HPV 6 were detected in 34 (2.3%) of the 1465 cases that were tested for HPV subtypes. All other

| Records identified through database searches ($n = 500$) |
|--------------------------------------------------------|
| Additional records identified through other sources ($n = 352$) |
| Records after duplicates removed ($n = 264$) |
| Records screened ($n = 264$) |
| Records excluded ($n = 253$) |
| Full-text articles assessed for eligibility ($n = 31$) |
| Full-text articles excluded with reasons ($n = 11$) |
| Studies included in qualitative synthesis ($n = 20$) |
| Studies included in quantitative synthesis (meta-analysis) ($n = 20$) |

Figure 1: Flowchart of literature screening.
HPV subtypes had a prevalence of <2% and included the following: types 31, 33, 35, 42, 45, 52, 56, 53, 55, 58, 59, 62, 72, and 73.

**HPV infection and PeCa infiltration**

With respect to the correlation between HPV infection and invasive penile carcinoma, four studies\(^{15,19,22,33}\) were included, and no significant difference was found (Supplementary Table 3). This finding may be due to the small sample size.

**HPV infection in PeCa patients in various regions**

The risks of HPV infection in patients with PeCa in disparate regions were analyzed by subgroup, and the results revealed that HPV infection rates varied on different continents. Six articles from Latin America were included and revealed that the random-effects model risk difference (RD) was 0.60 (95% CI: 0.43–0.76; \(P < 0.00001\) for heterogeneity) (Figure 3a). The included studies consisted of 11 European articles, and the random-effects model RD was 0.50 (95% CI: 0.39–0.62; \(P < 0.00001\) for heterogeneity) (Figure 3b). The articles from Asia demonstrated that the random-effects model RD was 0.35 (95% CI: 0.09–0.60; \(P < 0.00001\) for heterogeneity) (Figure 3c). Significant heterogeneity was identified in the meta-analysis above. In addition, no sufficient studies from Africa or America were available for this meta-analysis. We also included three studies from Brazil, and the subgroup analysis revealed no significant change in HPV infection rates in Brazil from 2007 to 2017 (RD: 0.67, 95% CI: 0.61–0.72, \(\chi^2 = 1.68, P = 0.43\) (Figure 4), indicating a lack of heterogeneity and suggesting that the prevalence of HPV infection is stable and higher than the global average rate.

**Proportion of histotype types in penile carcinoma**

Data from the selected studies were classified according to histological type. The overall HPV prevalence was obtained from a total of 1026 penile carcinoma cases: 597 keratinizing SCC cases (58.2%, 95% CI: 54.2%–62.2%), 28 nonkeratinizing SCC cases (2.7%, 95% CI: −3.3%–8.7%), 48 verrucous SCC cases (4.7%, 95% CI: −1.3%–10.7%), 40 warty SCC cases (3.9%, 95% CI: −2.1%–9.9%), 84 basaloid SCC cases (8.2%, 95% CI: 2.3%–14.1%), 47 cases of SCC with mixed warty and basaloid features (4.6%, 95% CI: −1.4%–10.6%), 25 papillary SCC cases (2.4%, 95% CI: −3.6%–8.4%), and 112 cases of other SCC mixed forms (10.9%, 95% CI: 5.1%–16.7%) (Supplementary Table 4). The histological subtypes of the other 481 cases were not known in the primary studies.

**Relationship between HPV type and histology of penile carcinoma**

HPV infection in penile tumors is reportedly associated with various morphological changes, and determination of the subtype association can provide a better estimate of the HPV-related cancer burden and its preventable grade. The observed specific HPV contributions by histological type were as follows: basaloid SCC 85.5% (95% CI: 77.2%–93.8%); warty SCC 50.0% (95% CI: 35.2%–64.8%); nonkeratinizing/typical SCC 28.6% (95% CI: 11.9%–45.3%); keratinizing SCC 33.8% (95% CI: 29.9%–37.7%); and verrucous SCC 32.0% (95% CI: 16.7%–44.9%) (Supplementary Table 5).

**Relationship between HPV infection and patient age**

In our study, four studies\(^{22,25,26,33}\) had available information on patient age at diagnosis, which allowed us to observe the relationship between HPV infection and patient age. A total of 274 samples with HPV types detected from four studies were divided into two groups: older than 60 years and younger than 60 years (Supplementary Table 6). Pearson’s Chi-squared test was used to identify a correlation between these groups and the outcome (\(\chi^2 = 22.205, df = 1, P < 0.001\)). However, the significance was restricted by the limited sample size, and patients may delay a visit to the doctor, thus causing a delay in diagnosis.

**HPV infection and the location of PeCa**

Five articles\(^{15,22,26,33}\) containing 442 cases demonstrated the incidence rates of different sites of PeCa, with glans penis carcinoma being the most common, followed by foreskin carcinoma (Supplementary Table 7). Because studies examining the correlation between HPV infection and penile carcinoma locations are lacking, we could not define this relationship.

**HPV infection and differentiation of PeCa**

Various degrees of differentiation exist in cases of PeCa. We collected 408 cases from 6 articles\(^{16,17,24,26,33}\) containing the original tumor histological subtype and relevant statistics. Using Stata 15.0 for the Chi-squared test, we identified a significant statistical outcome (\(\chi^2 = 22.205, df = 2, P < 0.001\); Supplementary Table 8).

**DISCUSSION**

In the molecular evaluation, HPV infection was observed in 51% of lesions in the past 10 years, which is higher than the rate of 46.9% reported in earlier decades by Miralles-Guri et al.\(^{34}\) and the most common type found was HPV16. With respect to location, 45.50%
of the tumors were located in the glans, and the most common types were squamous cell carcinoma (73.8%). These results correspond with those found in the literature.

Apart from types 6 and 11, almost no other single low-risk HPV has been found to contribute to PeCa. Previous studies have reported no significant difference in age among patients with various subtypes of SCC. The presence of HPV and the distribution of HPV genotypes were not associated with any single age group. However, our study found that, on average, diagnosis predominates in patients of advanced age (>60 years), which may suggest that men seek health services very late in life and that young men are also affected but in smaller percentage.

Previous research has shown no correlation between HPV status and histological subtype (P = 0.51) or between HPV status and stage stratification. However, our findings indicated that the basaloid (85.5%, 95% CI: 77.2%–93.8%) and warty (50.0%, 95% CI: 35.2%–64.8%) subtypes are more likely to be HPV-positive than other subtypes. These findings are consistent with the WHO classification guidelines, indicating that HPV-related carcinomas are mostly basaloid and warty SCC. A higher proportion of basaloid cells correspond to a higher likelihood of HPV positivity in that tumor category. This cell type is morphologically similar to the predominant cell type observed in most invasive uterine cervical carcinomas, a known etiologically HPV-related cancer. HPV-positive tumors tend to exhibit warty and/or basaloid features and correspond to a moderate histological grade, whereas HPV-negative carcinomas usually correspond to well-differentiated tumors. Most reports validated the association of HPV with basaloid and warty carcinomas, which is consistent with our results. Verrucous carcinoma is defined as a non-HPV-related subtype of SCC, with carcinoma cuniculatum as a variant in the WHO classification guidelines. However, the incidence of HPV-positive verrucous carcinoma was calculated to be 32% in our studies. Similarly, in some studies, approximately one-third of usual and verrucous carcinomas were also reported to be HPV positive.

We consider that the differences in the prevalence of virus in penile carcinomas, either in general or special subtypes, are highly variable. In addition, non-HPV-related carcinomas may indicate no involvement of HPV in the pathogenesis, such as the P16\textsuperscript{ink4a} overexpression-negative carcinomas, but such cases may not include existing HPV infection, which has no role in the formation of cancer.

At present, the pathogenesis of PeCa is mostly related to overexpression of P16\textsuperscript{ink4a}. In addition, Sebastian et al. detected two...
genes related to the pathogenesis of PeCa by immunohistochemistry: P16\(^{ink4a}\) overexpression identifies HPV-HR-induced penile carcinogenesis independent of the HPV-HR genotype, and positive p53 expression with P16\(^{ink4a}\) negativity identifies HPV-negative cancers. In summary, the present study indicates that HPV plays an important role in the pathogenesis of PeCa.

The presence of metastatic disease in the inguinal lymph nodes is one of the most important prognostic factors in PeCa.\(^9\) Unfortunately, the included data regarding the relationship between lymph node metastasis and HPV were fairly limited and of little statistical value; therefore, we did not involve lymph node-related results. However, a study by Feber et al.\(^9\) in which methylation of penile oncogenes was first sequenced showed that a 4-gene epi-signature accurately predicted lymph node metastasis in an independent cohort (area under the curve [AUC] of 89%). When used as a predictive methylation index for each sample, the predictive accuracy of this signature (90% methylation array and 89% for quantitative methylation specific polymerase chain reaction [qMSP]) to identify the presence of lymph node metastasis is at least comparable to if not better than the sensitivity of sentinel lymph node biopsy. They also explored epigenetic alterations associated with PeCa-related HPV infection and defined a 30-loci lineage without an HPV-specific epi-signature or HPV16 signature that is an independent predictor of disease-free survival and suggests distinct HPV subtype-specific epigenetic alterations.

This article identified genotype-specific HPV cases from studies using more sensitive PCR measures to allow investigation of HPV type distributions in PeCa in a large sample. These data also allowed us to investigate the differences between histological subtypes that are usually limited in the number of individual publications.

The included articles all included comparisons of cross-sectional studies, which may not be of high value. Studies on PeCa are limited, and most samples rely on FFPE tissue for HPV detection. Moreover, persuading healthy people to participate in HPV detection is extremely difficult, complicating the establishment of a control group.RCTs for related research have not been found.

Because the specific phenotype of mixed HPV infection was not clear in the included original literature, the analysis effect of the data may not be optimal. Fortunately, unknown mixed HPV infections accounted for only a small proportion of the overall sample. Due to the research type, the assessment of multiple infectious contributions is limited. Our results are based on cross-sectional data, which may not reflect the natural history of the disease. However, because the incidence of PeCa is relatively low, conducting a better longitudinal study to examine disease progression is difficult. HPV testing alone is not sufficient to prove cause and effect. However, HPV has been recognized as a tumor pathogen, and HPV infection may therefore have the same effect on penile tumors, given the similar histopathological features between men and women. Merck announced the completion of an initial study, demonstrating that Gardasil has 90% efficacy in preventing external genital lesions caused by HPV types 6, 11, 16, and 18 in men aged 16–26 years.\(^9\) More extensive and effective vaccinations should be applied to prevent HPV-related malignancies. As in cervical cancer, hHRPV is also a high-risk factor for PeCa; therefore, the countries and regions with high rates of PeCa and HPV infection, such as South Africa and Brazil, should promote HPV vaccination. HPV vaccines can even be considered for infertile men with HPV infection, spouses who are HPV positive, and gay people, especially with the development of therapeutic vaccines. This study may provide a reference for clinical diagnosis and treatment and suggests that the available HPV vaccine is urgently needed in high-risk populations.

AUTHOR CONTRIBUTIONS

YBY and YHW conceived the study, participated in its design, and coordinated and drafted the manuscript. XCY and MLW collected the data. YZ performed the statistical analysis. YL and HTN participated in critical revision of the manuscript and approved the manuscript. All authors read and approved the final manuscript and agreed with the order of presentation of the authors.

COMPETING INTERESTS

All authors declared no competing interests.

ACKNOWLEDGMENTS

We would like to thank the authors who provided the data. This study was financially supported by the National Natural Science Foundation of China (Grant No. 81772713, No. 81472411, No. 81401899, and No. 81372752), the Taishan Scholar Program of Shandong Province (No. tsqn20161077), the Key Research and Development Program of Shandong Province (No. 2018GSF118197), the China Postdoctoral Science Foundation (No. 2017M622144), the Natural Science Foundation of Shandong Province (No. ZR2014HM088), and the Qingdao Postdoctoral Application Research Project and Qingdao Young Scientist Applied Basic Research Fund (No. 15-9-1-51-jh and No.15-9-1-105-jh).

Supplementary Information is linked to the online version of the paper on the Asian Journal of Andrology website.

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Supplementary Table 1: Type-specific prevalence of human papillomavirus in penile carcinomas by study-relevant items and histological type

| Reference | Journal (year) | Study area | Mean age (year) | Method of detection and primers | Sample | Sample Number of case | Overall HPV positive rate Number of cases (%) | Histology vegetative and number of cases | Relative HPV Prevalence, n (%) |
|-----------|----------------|------------|----------------|-------------------------------|--------|----------------------|-----------------------------------------------|----------------------------------------|-----------------------------|
| LA        | J Med Virol (2017) | Brazil     | 58 (26–92)     | PCR MY09/11                   | TFTE   | 122                  | 79 (64.8)                                     | –                                      | –                           |
| Damásdi   | Pathol Oncol Res (2015) | Hungary    | 61.2 (44–87)   | PCR SPF10                     | FFPE   | 35                   | 14 (40.0)                                     | –                                      | –                           |
| Isaura    | BMC Urol (2015) | Brazil     | 66±17.10       | PCR/Nested automated sequencing | FFPE   | 76                   | 48 (63.2)                                     | –                                      | –                           |
| Hernandez | Front Oncol (2014) | USA        | 66±17.10       | PCR-based Linear Array and INNO-LiPA assays | FFPE   | 79                   | 50 (63.3)                                     | Keratinizing (53) Basaloid or warty-SCC (11) Other SCC (15) SCC (183) Verrucous (5) Warty (5) Basaloid (5) Basaloid-SCC (2) Papillar (9) Others (3) Keratinizing SCC (4) Verrucous (9) Papillary SCC (2) Others (20) Basaloid (3) Keratinising (83) Non-keratinising (20) Verrucous (8) Warty (2) Undetermined (4) No warty/basaloid –features (53) Warty/basaloid-features (36) Basaloid (-) Warty-basaloid (-) Warty (-) Usual (-) Papillary (-) Mixed (-) Keratinizing (68) Basaloid (33) Warty (11) Verrucous (16) Giant cell (3) Combined types (12) |
| Dajadiningrat | J Urol (2014) | Netherlands | 63 (54–71) | PCR GP5+6+ | FFPE | 212 | 53 (24.1) | – | – |
| Lebelo    | J Med Virol (2014) | South Africa | 49.8 (18–87) | TaqMan-based qPCR | FFPE | 44 | 35 (79.5) | Keratinizing SCC (4) Verrucous (9) Papillary SCC (2) Others (20) Basaloid (3) Keratinising (83) Non-keratinising (20) Verrucous (8) Warty (2) Undetermined (4) No warty/basaloid –features (53) Warty/basaloid-features (36) |
| Do        | Br J Cancer (2013) | Vietnam    | 53 (51–57)     | PCR SPF10 and HPV-16 E6 | PE | 120 | 27 (22.5) | Basaloid (3) Keratinising (83) Non-keratinising (20) Verrucous (8) Warty (2) Undetermined (4) No warty/basaloid –features (53) Warty/basaloid-features (36) |
| Chaux     | World J Urol (2013) | Paraguay   | 62             | PCR SPF-10                   | Tumor tissue | 61 | 29 (47.5) | Keratinizing (68) Basaloid (33) Warty (11) Verrucous (16) Giant cell (3) Combined types (12) |
| D’Hauwers | Vaccine (2012) | Belgium    | –             | Real-time quantitative PCR | FFPE | 55 | 24 (43.6) | Basaloid (-) Warty-basaloid (-) Warty (-) Usual (-) Papillary (-) Mixed (-) Keratinizing (68) Basaloid (33) Warty (11) Verrucous (16) Giant cell (3) Combined types (12) |
| Cubilla   | Am J Surg Pathol (2010) | Paraguay | 61 (16–95) | PCR SPF-10 | FFPE | 202 | 64 (31.7) | Basaloid (-) Warty-basaloid (-) Warty (-) Usual (-) Papillary (-) Mixed (-) Keratinizing (68) Basaloid (33) Warty (11) Verrucous (16) Giant cell (3) Combined types (12) |
| Krustrup  | Int J Exp Pathol (2009) | Denmark   | –             | PCR GP5+6+                   | FFPE | 145 | 89 (61.4) | Basaloid (-) Warty-basaloid (-) Warty (-) Usual (-) Papillary (-) Mixed (-) Keratinizing (68) Basaloid (33) Warty (11) Verrucous (16) Giant cell (3) Combined types (12) |

Contd...
| Reference | Journal (year) | Study area | Mean age (year) | Method of detection and primers | Sample | Sample Number of case | Overall HPV positive rate Number of cases (%) | Histology vegetative and number of cases | Relative HPV Prevalence, n (%) |
|-----------|----------------|------------|----------------|--------------------------------|--------|----------------------|-----------------------------------------------|----------------------------------------|----------------------------------|
| SENBA     | Oncol Lett (2009) | Japan     | –              | PCR SPF10                      | FFPE   | 16                   | 12 (75.0)                                     | Keratinizing (10)                       | 8 (80.0)                          |
|           |                |           |                |                                |        |                      |                                               | Nonkeratinizing (4)                      | 3 (75.0)                          |
|           |                |           |                |                                |        |                      |                                               | Verrucous (2)                           | 1 (50.0)                          |
| Scheiner  | Int Braz J Urol (2008) | Brazil | –              | PCR MY09/MY11                  | FFPE   | 80                   | 58 (72.5)                                     | –                                      | –                                |
| Guerrero  | B J U Int (2008)  | Spain     | 67.6           | PCR GP5+/-GP6+biotinylated primers | FFPE   | 24                   | 11 (45.8)                                     | Squamous (17)                          | –                                |
|           |                |           |                |                                |        |                      |                                               | Warty (4)                              | –                                |
|           |                |           |                |                                |        |                      |                                               | Verrucous (1)                           | –                                |
|           |                |           |                |                                |        |                      |                                               | Basaloid (2)                           | –                                |
| Yanagawa  | Pathology (2008) | Japan     | 67.6 (46-87)   | PCR-RFLP                       | FFPE   | 26                   | 3 (11.5)                                      | Keratinizing (37)                      | –                                |
| Tornesello| Int J Cancer (2008) | Italy     | –              | PCR MY09/MY11 and GP5/GP61    | FFPE   | 41                   | 19 (46.3)                                     | Verrucous (2)                          | –                                |
| Madsen    | Am Assoc Cancer Res (2008) | Denmark | –              | PCR GP5 +/6 +                 | FFPE   | 71                   | 25 (35.2)                                     | Basaloid (1)                           | –                                |
|           |                |           |                |                                |        |                      |                                               | Sarcomatoid (1)                        | –                                |
| Prowse    | Br J Dermatol (2007) | UK       | –              | PCR SPF10                      | FFPE   | 26                   | 14 (53.8)                                     | Keratinizing SCC (15)                  | –                                |
| Tornesello ML | Cancer Let (2008) | Uganda | 60.6±11.1      | semi-nested PCR                | PE     | 17                   | 11 (64.7)                                     | Basaloid SCC (1)                      | –                                |
|           |                |           |                |                                |        |                      |                                               | Verrucous SCC (1)                      | –                                |
|           |                |           |                |                                |        |                      |                                               | Warty SCC (0)                          | –                                |
|           |                |           |                |                                |        |                      |                                               | Sarcomatoid (0)                        | –                                |
|           |                | Italy     | 61±12.6        | Semi-nested PCR                | PE     | 61                   | 29 (47.5)                                     | Keratinizing (54)                      | –                                |
|           |                |           |                |                                |        |                      |                                               | Basaloid SCC (3)                       | –                                |
|           |                |           |                |                                |        |                      |                                               | Verrucous (2)                          | –                                |
|           |                |           |                |                                |        |                      |                                               | Warty (1)                              | –                                |
|           |                |           |                |                                |        |                      |                                               | Sarcomatoid (1)                        | –                                |
| Heideman  | J Clin Oncol (2007) | Germany   | 65 (27-92)     | PCR GP5 +/6 +                 | FFPE   | 83                   | 46 (55.4)                                     | Not-Specified (72)                     | 40 (55.6)                         |
|           |                |           |                |                                |        |                      |                                               | Verrucous (7)                          | 4 (57.1)                          |
|           |                |           |                |                                |        |                      |                                               | Warty (2)                              | 1 (50.0)                          |
|           |                |           |                |                                |        |                      |                                               | Sarcomatoid (2)                        | 1 (50.0)                          |
| Proetzl   | Cellular Molecular Biol (2007) | Germany | 69.4 (35-89)   | High Pure PCR Template         | FE     | 19                   | 7 (36.8)                                      | Nonkeratinizing (4)                    | 2 (50.0)                          |
|           |                |           |                |                                |        |                      |                                               | Keratinizing (9)                       | 2 (22.2)                          |
|           |                |           |                |                                |        |                      |                                               | Papillary (1)                          | 0 (0)                             |
|           |                |           |                |                                |        |                      |                                               | Verrucous (1)                          | 0 (0)                             |
|           |                |           |                |                                |        |                      |                                               | Condylomatous (1)                      | 0 (0)                             |
|           |                |           |                |                                |        |                      |                                               | Basaloid (3)                           | 3 (100.0)                         |
| Pascual   | Cellular Molecular Biol (2007) | Spain    | –              | PCR My09/My11 and GP5 +/-GP6 + | FFPE   | 49                   | 38 (77.5)                                     | –                                      | –                                |
| Total     |                |           |                |                                |        |                      |                                               |                                        | 1465                              |

*Contd...*
### Supplementary Table 1: Contd...

| Reference | Hr HPV | Subtotal (%) | Lr HPV | Subtotal (%) | AHRQ score |
|-----------|--------|--------------|--------|--------------|-------------|
|           | 16     | 18           | 31     | 33           | 35          | 45          | 56          | Others      | 6           | 11          | Others |          |
| LA        | 32 (40.5%) | 7 (8.9%)     | 7 (8.9%) | 0            | 0            | 9 (11.4%)   | –           | 1 (1.3%)    | 56 (70.9)   | 13 (16.5%) | 6 (7.6%)   | 4 (5.1%) | 23 (29.1) |
| Damási    | 11 (78.6%) | 0            | 0            | 0            | 0            | 0            | 2 (14.3%)   | 13 (92.9)   | 0            | 0            | 1 (7.1%)  | 1 (7.1%) | 6           |
| Isaura    | 10 (20.8%) | 4 (8.3%)     | –            | –            | –            | 1 (2.1%)    | –           | –           | –           | 6 (12.5%)   | –          | –          | 6           |
| Hernandez | 36 (72.0%) | 2 (4.0%)     | 0            | 3 (6.0%)     | 1 (2.0%)     | 2 (4.0%)    | 0           | 5 (10.0%)   | 49 (98.0)   | 1 (2.0%)    | 0          | 0          | 1 (2.0)    |
| D'ajdiningrat | 42 (79.2%) | 3 (5.7%)     | 1 (1.9%)    | 4 (7.5%)     | 0            | 2 (3.8%)    | 1 (1.9%)    | 0           | 53 (100)    | –           | –          | –          | 6           |
| Lebelo    | 2 (5.7%)  | 0            | 0            | 0            | 1 (2.8%)     | 0            | 0           | 3 (8.6)     | 0            | 0          | 0          | 0           | 8           |
|           | 4 (11.4%) | 4 (11.4%)    | 0            | 0            | 0            | 0            | 0           | 8 (22.8)    | 1 (2.8%)    | 7 (20.0%)   | 0           | 8 (22.8)   |              |
|           | 0        | 0            | 0            | 0            | 0            | 0            | 0           | 1 (2.8%)    | 0           | 1 (2.8)    | 0          | 1 (2.8)    |              |
| Do       | 24 (88.9%) | –            | –            | –            | –            | –            | –           | –           | –           | –          | –          | –          | 5           |
| Chaux     | –        | –            | –            | –            | –            | –            | –           | –           | –           | –          | –          | –          | 7           |
| D'Hauwers | 17       | 1            | –            | 1            | 3           | 22 (91.7)   | 1           | 1           | 0           | 2 (8.3)    | 4           |              |
| Cubilla   | 13       | 1            | –            | 1            | 0            | 4           | 19          | 0           | 0           | 0           | 0           | 0           | 5           |
|           | 4        | 0            | –            | 0            | 0            | 3           | 7           | 2           | 0           | 0           | 2           |              |
|           | 3        | 1            | –            | 1            | 0            | 2           | 7           | 0           | 0           | 1           | 1           |              |
|           | 10       | 1            | –            | –            | 0            | 3           | 16          | 1           | 1           | 0           | 2           |              |
|           | 5        | 0            | –            | 0            | 0            | 1           | 6           | 0           | 0           | 0           | 0           |              |
|           | 1        | 0            | –            | 0            | 0            | 3           | 6           | 0           | 0           | 0           | 0           |              |
| Krusstrup | 78       | 87 (6.6%)    | 0           | 1 (1.1%)     | 3 (3.4%)     | 1 (1.1%)    | 1 (1.1%)    | 1 (1.1%)    | 1 (1.1%)    | 86 (96.6)   | 5 (5.6%)    | 1 (1.1%)    | 1 (1.1%)    | 7 (7.9)    |
| SENBA     | 0        | 1 (8.3%)     | –            | –            | –            | –           | –           | 10 (83.3%)  | –           | –          | –          | –          | 7           |
| Scheiner  | 12       | 20 (7.7%)    | 1 (1.7%)    | 1 (1.7%)     | 1 (1.7%)     | 0           | 1 (1.7%)    | 0           | 0           | 16 (27.6)   | 4 (6.9%)    | 42 (72.4)   |              |
| Guerrero  | 10       | 90 (9.9%)    | 0           | 0            | 0            | 0           | 1 (9.1%)    | 11 (100.0)  | 0           | 0          | 0           | 0           |              |
| Yanagawa  | –        | –            | –            | –            | –            | –           | –           | –           | –          | –          | –          | 8           |
| Tornesello| –        | –            | –            | –            | –            | –           | –           | –           | –          | –          | –          | 5           |
| Madsen    | –        | –            | –            | –            | –            | –           | –           | –           | –          | –          | –          | 4 (4.0)    |
| Prowse    | 10       | 71 (7.1%)    | 1 (7.1%)    | 0           | 0            | 0           | 0           | 1 (7.1%)    | 12 (85.7)   | 1 (7.1%)    | 0           | 1 (7.1%)    | 2 (14.3)   |
| Tornesello ML | 76 (63.6%) | 0            | 0            | 0            | 0            | 0           | 3 (27.3%)   | 10 (90.9)   | 1 (9.1%)    | 0           | 0          | 1 (9.1)    | 9           |
| Heideman  | 22       | 47 (8.6%)    | 2 (4.3%)    | 0           | 0            | 2 (4.3%)    | 1 (2.2%)    | 15 (32.6%)  | 44 (95.6)   | 1 (2.2%)    | 0           | 0          | 2 (4.3)    |
|           | 2 (4.3%) | 0            | 0            | 0            | 0           | 2 (4.3%)    | 0           | 0           | 1 (100%)    | 0           | 0          |              |
|           | 0        | 0            | 0            | 0            | 0            | 0           | 0           | 0           | 0           | 0          | 0          |              |
| Protzel   | 1 (1.4%) | –            | –            | –            | –            | –           | –           | 1 (14.3)    | 1 (14.3%)   | 1 (14.3)    |              |
|           | 2 (28.6%) | –            | –            | –            | –            | –           | –           | 2 (28.6)    |              |              |              |
|           | 0        | –            | –            | –            | –            | –           | –           | 0           |              |              |              |
|           | 0        | –            | –            | –            | –            | –           | –           | 0           |              |              |              |
|           | 0        | –            | –            | –            | –            | –           | –           | 0           |              |              |              |
|           | 2 (28.6%) | –            | –            | –            | –            | –           | –           | 2 (28.6)    | 1 (14.3%)   | 1 (14.3)    |              |
| Pascual   | 32       | 84 (2.2%)    | 4 (10.5%)   | –            | –            | –           | –           | 2 (5.3%)    | 38 (100.0)  | –           | –          | –          | 6           |
| Total     | 418      | (28.5%)     | 34 (2.3%)   | 10 (0.7%)    | 12 (0.8%)    | 5 (0.3%)    | 21 (0.1%)   | 4 (0.2%)    | 59 (4.0%)   | 539 (36.8)  | 34 (2.3%)   | 23 (1.6%)   | 8 (0.5%)    | 97 (6.6)   |

Hr HPV: high-risk Human Papillomavirus; Lr HPV: low-risk Human Papillomavirus; –: no involve; PCR: polymerase chain reaction; TFE: Tumor fragments stored in TE solution (10 mM Tris hydrochloride [pH 7.5], 1 mM ethylenediaminetetraacetic acid (EDTA)); FFPE: formalin-fixed paraffin-embedded; PE: paraffin embedded; AHRQ: The Agency for Healthcare Research and Quality; SCC: squamous cell carcinoma
Supplementary Table 2: The number of human papillomavirus-positive samples detected by two different primers

| Group          | PCR G5+G6+ | PCR SPF10 | Total |
|----------------|------------|-----------|-------|
| HPV Positive   | 213        | 146       | 359   |
| HPV Negative   | 323        | 279       | 602   |
| Total          | 536        | 425       | 961   |
\[\chi^2=2.938, \text{df}=1, P=0.087. \text{HPV: human papillomavirus; PCR: polymerase chain reaction}\]

Supplementary Table 3: The invasiveness of penile carcinoma and the potential relationship with human papillomavirus infection

| Group | HPV infection | Total |
|-------|---------------|-------|
|       | Positive      | Negative |
| In situ | 106          | 131     | 237   |
| Invasive | 124          | 142     | 266   |
| Total  | 230          | 273     | 503   |
\[\chi^2=0.181, \text{df}=1, P=0.671. \text{HPV: human papillomavirus}\]

Supplementary Table 4: The constituent ratio of different histological types of penile cancer

| Histological type     | Constituent ratio (%) | Number of cases | 95% CI |
|-----------------------|-----------------------|-----------------|-------|
| Keratinizing SCC      | 58.2                  | 597             | 54.2–62.2 |
| Basaloid SCC          | 8.2                   | 84              | 2.3–14.1 |
| Verrucous SCC         | 4.7                   | 48              | -1.3–10.7 |
| Non-keratinizing      | 2.7                   | 28              | -3.3–8.7 |
| Warty                 | 3.9                   | 40              | -2.1–9.9 |
| Warty-basaloid        | 2.4                   | 25              | -3.6–8.4 |
| Others                | 10.9                  | 112             | 5.1–16.7 |
| Total                 | 100                   | 1026            | -4.3–26.6 |

Supplementary Table 5: The relationship between histological type and human papillomavirus infection

| Histological type     | HPV positive (%) | Total | 95% CI % |
|-----------------------|------------------|-------|----------|
| Keratinizing SCC      | 33.8             | 574   | 29.9–37.7 |
| Non-keratinizing      | 28.6             | 28    | 11.9–45.3 |
| Basaloid SCC          | 85.5             | 69    | 77.2–93.8 |
| Verrucous SCC         | 32.0             | 50    | 16.7–44.9 |
| Warty                 | 50.0             | 44    | 35.2–64.8 |
| Papillary             | 16.7             | 24    | 1.8–31.6 |
| Combined types        | 28.3             | 53    | 16.2–40.4 |

Supplementary Table 6: Age at penile cancer diagnosis and the relationship with human papillomavirus infection

| Age (year) | HPV infection | Total |
|------------|---------------|-------|
|            | Positive      | Negative |
| <60        | 47            | 24     | 71     |
| >60        | 102           | 101    | 203    |
| Total      | 149           | 125    | 274    |
\[\chi^2=22.205, \text{df}=1, P<0.001. \text{HPV: human papillomavirus}\]

Supplementary Table 7: Distribution of tumor sites among penile cancers

| Region               | Number of cases | Component ratio (%) |
|----------------------|-----------------|--------------------|
| Glans                | 201             | 45.50              |
| Foreskin             | 87              | 19.70              |
| Corpus               | 5               | 1.10               |
| Glans and foreskin   | 29              | 6.60               |
| Non-evaluable        | 120             | 27.10              |
| Total                | 442             | 100                |

Supplementary Table 8: Constituent ratio of histological differentiation in penile tumors with human papillomavirus infection

| Differentiation | HPV-positive | Total | HPV-positive rate (%) |
|----------------|--------------|-------|-----------------------|
| Well           | 48           | 160   | 30.0                  |
| Moderately     | 117          | 215   | 54.4                  |
| Poorly         | 27           | 60    | 45.0                  |
\[\chi^2=22.205, \text{df}=2, P<0.001. \text{HPV: human papillomavirus}\]