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Worldwide human papillomavirus genotype attribution in over 2000 cases of intraepithelial and invasive lesions of the vulva

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Abstract ***Background:*** Human papillomavirus (HPV) contribution in vulvar intraepithelial lesions (VIN) and invasive vulvar cancer (IVC) is not clearly established. This study provides novel data on HPV markers in a large series of VIN and IVC lesions.

Methods: Histologically confirmed VIN and IVC from 39 countries were assembled at the Catalan Institute of Oncology (ICO). HPV-DNA detection was done by polymerase chain reaction using SPF-10 broad-spectrum primers and genotyping by reverse hybridisation line probe assay (LiPA$_{25}$) (version 1). IVC cases were tested for p16^{INK4a} by immunohistochemistry (CINtec histology kit, ROCHE).

An IVC was considered HPV driven if both HPV-DNA and p16^{INK4a} overexpression were observed simultaneously. Data analyses included algorithms allocating multiple infections to calculate type-specific contribution and logistic regression models to estimate adjusted prevalence (AP) and its 95% confidence intervals (CI).

Results: Of 2296 cases, 587 were VIN and 1709 IVC. HPV-DNA was detected in 86.7% and 28.6% of the cases respectively. Amongst IVC cases, 25.1% were both HPV-DNA and p16^{INK4a} positive. IVC cases were largely keratinising squamous cell carcinoma (KSCC) ($N = 1234$). Overall prevalence of HPV related IVC cases was highest in younger women for any histological subtype. SCC with warty or basaloid features (SCC_WB) ($N = 326$) were more likely to be HPV and p16^{INK4a} positive (AP = 69.5%, CI = 63.6–74.8) versus KSCC (AP = 11.5%, CI = 9.7–13.5). HPV 16 was the commonest type (72.5%) followed by HPV 33 (6.5%) and HPV 18 (4.6%). Enrichment from VIN to IVC was significantly high for HPV 45 (8.5-fold).

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[1] See Appendix for 'HPV VVAP study group for vulvar site'.

Conclusion: Combined data from HPV-DNA and p16^{INK4a} testing are likely to represent a closer estimate of the real fraction of IVC induced by HPV. Our results indicate that HPV contribution in invasive vulvar cancer has probably been overestimated. HPV 16 remains the major player worldwide.

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1. Introduction

Vulvar cancer is a rare entity with incidence rates ranging from 0.5 to 1.5 per 100,000 women, representing about 4% of all gynaecological malignancies.[1] Lower rates are observed in Asia and Africa than in other parts of the world. Over the past few decades, the incidence rates of invasive vulvar cancer (IVC) and vulvar intraepithelial neoplasia (VIN) have both been reported to increase, particularly amongst younger women.[2–5] Squamous cell carcinoma accounts for more than 90% of the malignant tumours of the vulva and several morphological variants have been described including keratinising, basaloid, warty and verrucous carcinoma. Basaloid and warty variants representing about 1/3 of cases, are commoner in younger women, and are often associated with human papillomavirus (HPV) DNA detection. These tumours share many risk factors with cervical cancer. By contrast, keratinising variants arise from chronic vulvar dermatosis, such as lichen sclerosus, are not associated with HPV and tend to occur in older women. HPV associated vulvar cancers do not seem to differ in prognosis from HPV negative cases although data are scanty and based on few observations.[6]

HPV-DNA is currently identified in the majority of VIN lesions (>80%), while HPV detection amongst invasive vulvar carcinomas is generally estimated to be around 40% for overall histological variants. Data derived from meta-analysis show that HPV-DNA detection in invasive warty/basaloid tumours is more frequent (69.4%) than in invasive keratinising types (13.3%).[7] HPV related vulvar neoplasia is mainly associated to HPV 16 contributing to a larger proportion compared to that observed for cervical cancer.[7–10] Previous reports have however been limited by the wide range of HPV assays used and by a potential publication bias towards HPV positive cases. A recent study on formalin fixed paraffin embedded cases (FFPE) retrieved from an archival in the US has reported a 36% HPV detection in IVC and 76% in VIN lesions.[11]

The objective of this international collaborative study was to evaluate the HPV contribution and genotype distribution in IVC and VIN lesions from pathological archives in 39 countries from five continents. The consortium included a common standard protocol and a sensitive assay was used for HPV-DNA detection (SPF10/DEIA/LiPA25 system). IVC cases were further largely tested for the cyclin-dependent kinase-4 inhibitor (p16^{INK4a}) which has shown to be overexpressed in at least 90% of VIN and HPV related IVC cases.[12–14] Cases in which HPV-DNA is detected but with no overexpression of p16^{INK4a} could represent a transient infection with no role in carcinogenesis. The use of p16^{INK4a} in combination with HPV-DNA detection is becoming a common recommended ancillary test for research and clinical studies when HPV is not a necessary cause.[15–17]

2. Material and methods

2.1. Study design and materials

The project was designed and coordinated by the Catalan Institute of Oncology (ICO) in Barcelona, Spain in collaboration with DDL Diagnostic Laboratory in Rijswijk, The Netherlands. The study started in 2007 as a retrospective cross-sectional survey to estimate the prevalence of HPV-DNA and type distribution in vulvar cancers and high grade pre-neoplastic lesions for the 30-year period 1980–2011. Case recruitment protocols were similar to the previously described in a similar study for cervical cancer.[18] Briefly, the study here presented includes 2296 paraffin embedded vulva specimens collected from pathology archives from 39 countries as follows: North and Latin America: Argentina, Brazil, Chile, Colombia, Ecuador, Guatemala, Honduras, Mexico, Paraguay, Uruguay, United States of America and Venezuela; in Africa: Mali, Mozambique, Nigeria, and Senegal; in Oceania: Australia and New Zealand; in Europe: Austria, Belarus, Bosnia-Herzegovina, Czech Republic, France, Germany, Greece, Italy, Poland, Portugal, Spain and United Kingdom and in Asia: Bangladesh, India, Israel, South Korea, Kuwait, Lebanon, Philippines, Taiwan and Turkey.

Participant centres provided cases diagnosed as IVC or VIN lesions with information on age at diagnosis, year at diagnosis and original histological diagnosis. Centres were requested to provide a non-selected series of IVC and/or VIN from their archives preferably consecutive in time.

2.2. Pathology and laboratory procedures: paraffin block processing, histological evaluation, HPV-DNA detection and genotyping, p16INK4a evaluation

Paraffin blocks were processed under strict conditions to avoid contamination. At least, four paraffin sections were systematically obtained for each block (sandwich method). Processing and pathology diagnosis were done

by the reference pathology laboratory at ICO. Pathology evaluation included a histological classification with the following categories: (1) Squamous cell carcinoma 100% warty, basaloid or warty/basaloid (SCC_WB), (2) SCC 100% non-warty/basaloid keratynising (KSCC), (3) mixed SCC with variable percentages of warty/basaloid and non-warty/basaloid features (SCC_mixed) and (4) other histological types (basal cell carcinoma, adenocarcinomas, etc.). A fifth FFPE section was also obtained for immunohistochemical staining for p16^{INK4a}. For VIN the histological classification includes (1) vulvar intraepithelial neoplasia usual type including VIN2, VIN 3, warty, basaloid or warty/basaloid (VIN usual or WB). Any case with diagnosis of either VIN1 or condiloma without any other more severe lesion was excluded, (2) vulvar intraepithelial neoplasia differentiated.[19]

For each specimen, a FFPE section was treated with 250 μl of freshly prepared Proteinase K solution to extract DNA. SPF-10 was performed using 10 μl of extracted DNA that was diluted 10 times in a final reaction volume of 50 μl. The amplified PCR products were tested for the detection of HPV-DNA through DNA enzyme immunoassay (DEIA) as previously described.[20] DEIA can recognise at least 54 HPV genotypes. Amplimers of HPV positive DNA by DEIA were used to perform the reverse hybridisation line probe assay (LiPA$_{25}$)[21] (version 1: produced at Laboratory Biomedical Products, Rijswijk, The Netherlands). The LiPA$_{25}$ detects 25 high-risk (HR) and low risk (LR) HPV types (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70 and 74). All detectable types belonged to nine species of the α-papillomavirus genus. The sequence variation within the SPF-10 inter-primer region allows the recognition of these different HPV genotypes, except for types 68 and 73, as their inter-primer regions are identical and cannot be distinguished on this test. Specimens that were HPV-DNA positive by DEIA but did not yield an HPV type by the LiPA$_{25}$ were further analysed by sequencing as previously described.[22] When no type could be assigned even after performing sequencing, HPV was labelled as *HPV undetermined*. Further, specimens that were HPV 68 or 73 or HPV 39 or HPV 68 or HPV 73 were also sequenced to discriminate the specific type.

p16^{INK4a} was detected using the CINtec histology kit (clone E6H4, ROCHE MTM Laboratories, Heidelberg, Germany), following the manufacturer's protocol in IVC cases with available material ($N = 1287$). In each series a negative and a positive control and an invasive cervical carcinoma, were included. As established in the uterine cervix,[23] only lesions showing strong and diffuse staining were considered positive for p16^{INK4a}.

In order to check the cellular DNA quality, a random sample of HPV-DNA negative specimens underwent Proteinase K digestion with modified conditions (70 °C instead of the 56 °C previously described and tested for human tubuline PCR amplification). Amongst 101 cases of IVC and 11 of VIN tubuline was not detected for 6.9% and 9.1%, respectively attributable to poor sample quality.

2.3. Statistical analysis

Data for analysis included: country, year at diagnosis, age at diagnosis, revised pathological data, HPV detection, HPV genotype and p16^{INK4a} overexpression.

HPV-DNA detection and p16^{INK4a} results were compared by means of kappa coefficient as a statistical measure of agreement between both assays. We considered that an IVC case was an HPV driven tumour if the sample was HPV-DNA positive and there was evidence of overexpression of p16^{INK4a}. Missing values of p16^{INK4a} were imputed in the data based on the results obtained for the 75% of the total samples on p16^{INK4a} stratified by age, country and histological diagnosis. Imputation was not performed for VIN as there were few cases contributing to p16^{INK4a} information.

Data are presented as HPV-DNA prevalence, for VIN lesions and for the combined positivity of HPV and p16^{INK4a} for IVC lesions. Unconditional logistic regression analysis was used to provide the estimates adjusted with 95%CI for the different geographical continents, age at and year of diagnosis. The best fitting model was selected based on the log likelihood ratio test.

HPV genotype distribution is provided as crude prevalence. The HPV type-specific relative contribution (RC) refers to the percent positive for a given type related to all HPV-DNA and p16^{INK4a} positive samples. HPV type-specific information always included information on multiple infections. Multiple infections were added to single types under a weighting attribution proportional to the detection found in cases with single types as previously described.[18]

Statistical significance for all analysis was set at the 2-sided 0.05 level.

Data analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 13.0 (SPSS Inc., Chicago, IL, United States of America (USA)) and with STATA version 10.0 (Stata Corporation, Computing Resource Center, College Station, Texas).

2.4. Ethical issues

FFPE were all anonymous. All protocols were approved by local and ICO ethics committees and all the study progress was overseen by an international steering committee.

3. Results

Table 1 describes the cases included in the study by histology categories. A total of 587 VIN cases and of 1709 IVC cases were included in the study. The majority of VIN was WB type (91.1%) and IVC cases were largely SCC keratinising (72.2%). IVC SCC_WB accounted for 19.1% of the cases (Table 1). IVC patients were, on average, significantly older than VIN cases (68.5 versus 49.5 years, $p < 0.05$).

VIN cases were more likely to originate from Europe, America and Australia and to be diagnosed in the most recent period 2000–2011 (Table 2). The overall HPV adjusted prevalence was 88.7%. VIN differentiated had lower prevalence (48.9%) compared to VIN WB (90.3%) (data not shown).

Half of the IVC cases (Table 3) were from Europe (49.8%) and the majority were diagnosed during the period 2000–2011. Overall positivity of both HPV and $p16^{INK4a}$ for IVC cases was 25.1% and the adjusted prevalence was 22.4%. Cases from Europe and those in the age group 67–74 and ⩾81 years old were significantly less likely than the average to be HPV and $p16^{INK4a}$ positive. Contrary, cases from North America and those younger than 56 years old were more likely to be HPV and $p16^{INK4a}$. Table 3 also describes the characteristics of the two most frequent histological categories of IVC cases, the SCC_WB and the KSCC that contributed 19.1% and 72.2%, respectively. Both histological groups were characterised by similar period distribution. SCC_WB were however significantly more frequent in Central South America, Africa and Oceania and were younger than KSCC cases. SCC_WB cases were more likely to be HPV and $p16^{INK4a}$ positive (69.5%) compared to KSCC cases (11.5%, p value < 0.001). No statistical variations in HPV and $p16^{INK4a}$ positivity were observed within the SCC_WB cases by region, period and age. Within KSCC cases those younger than 56 years old were more likely to be positive than the average. This difference was driven by the higher contribution of European samples.

Irrespective of the histological category of IVC cases, HPV and $p16^{INK4a}$ positivity was consistently higher amongst women younger than 67 years old compared to elder women (Fig. 1).

The majority of both VIN and IVC HPV positive cases had a single infection (91.6% and 93.5%, respectively); multiple HPV types were identified in 43 and 28 cases and undetermined types in six and eight cases respectively. Amongst VIN lesions, HPV 16 followed by HPV 33 were the two most common types, accounting for over 88% of all positive cases (Table 4). HPV 16 was the commonest type in IVC (311 out of 429 HPV and $p16^{INK4a}$ positive tumours; 72.5%). This was followed by HPV 33 (6.5%), HPV 18 (4.6%), HPV 45 (3.3%) and HPV 52 (1.9%).

Table 5 shows the agreement between HPV detection and $p16^{INK4a}$ (in cases with available material) results by

Table 1
Included cases of VIN and IVC by histological category.

Histology	Number (%)	Mean age	Standard deviation
Vulvar intraepithelial neoplasia (VIN)			
Warty/basaloid	535 (91.1)	48.5	14.5
Differentiated	48 (8.2)	60.0	16.5
Both	4 (0.7)	67.5	9.9
Total VIN	587 (100)	49.5	15.0
Invasive vulvar cancer (IVC)			
SCC keratinising	1234 (72.2)	70.2	14.5
SCC Warty/ basaloid	326 (19.1)	63.3	16.8
SCC Mixed	102 (5.9)	68.1	14.4
Other*	47 (2.7)	60.8	16.5
Total IVC	1709 (100)	68.5	15.3

SCC, squamous cell carcinoma.
* Other includes 16 basocellular carcinoma, 10 undifferentiated carcinoma, seven adenocarcinoma, four adenosquamous cell carcinoma, four paget disease, one cystic adenoid carcinoma, one neuroendocrine carcinoma, one carcinosarcoma, one malignant fusocellular carcinoma, one NOS – not otherwise specified carcinoma and one apocrine carcinoma.

Table 2
Characteristics of vulvar intraepithelial neoplasia (VIN) cases by period and age at diagnosis and HPV-DNA detection.

Characteristic	N VIN	Crude HPV-DNA prevalence (%)	Adjusted# HPV-DNA prevalence	
			(%)	95% Confidence interval
Geographical region*				
Europe	312	86.9	88.3	(84.1–91.5)
America	127	77.2	78.6	(70.3–85.1)
Africa	3	66.7	68.2	(15.2–96.3)
Asia	20	100.0	–&	–
Oceania	125	94.4	95.1	(89.7–97.7)
Period of diagnosis				
1990–1999	60	83.3	84.2	(71.3–91.9)
2000–2011	527	87.1	89.1	(85.8–91.7)
Age at diagnosis (years)*				
<37	115	93.0	93.7	(87.5–96.9)
37–44	113	92.0	92.6	(86.2–96.2)
45–50	102	88.2	88.9	(81.3–93.7)
51–61	127	89.0	89.0	(82.0–93.5)
⩾62	120	76.7	76.7	(68.1–83.6)
Missing	10	30.0	29.0	(9.3–62.0)
Total	587	86.7	88.7	(85.4–91.2)

N, number of cases; HPV-DNA, human papillomavirus DNA.
* Chi-squared test p-value < 0.05 when compared to the average estimate.
# Adjusted by geographical region, period of diagnosis and age at diagnosis; & Dropped from the estimations because model predicts success perfectly.

Table 3
HPV-DNA and p16^{INK4a} positive prevalence (%) in invasive vulvar cancer by geographical region, period and age at diagnosis by histological subtypes.

Characteristic	All invasive vulvar cancer				SCC Warty/basaloid				SCC keratinising			
	Number	Crude	Adjusted#	95%CI	Number	Crude	Adjusted#	95%CI	Number	Crude	Adjusted#	95%CI
Geographical region*												
Europe	903	18.3	15.0	(12.8–17.5)	119	57.1	57.6	(48.3–66.4)	723	9.4	8.6	(6.8–10.9)
North America	50	50.0	50.0	(36.2–63.8)	7	100.0	–&	–	38	42.1	41.9	(27.2–58.2)
Central-South America	324	35.2	34.2	(29.0–39.7)	98	62.2	62.9	(52.7–72.1)	182	23.1	22.0	(16.5–28.7)
Africa	24	70.8	71.9	(50.8–86.3)	7	100.0	–&	–	14	64.3	64.9	(37.6–85.0)
Asia	188	22.9	21.1	(15.8–27.6)	31	80.6	81.6	(64.2–91.7)	142	9.9	8.7	(5.1–14.4)
Oceania	220	37.7	36.7	(30.3–43.6)	64	85.9	87.0	(76.5–93.3)	135	15.6	14.1	(9.1–21.0)
Period of diagnosis*												
1980–1999	501	21.2	18.2	(15.0–22.0)	85	65.9	67.9	(56.3–77.6)	352	10.8	8.4	(5.9–11.8)
2000–2011	1208	26.7	24.3	(21.8–27.0)	241	69.3	70.0	(63.3–76.0)	882	15.0	13.0	(10.8–15.5)
Age at diagnosis*												
<56	312	48.1	48.1	(42.4–53.8)	103	80.8	82.7	(73.8–88.9)	176	29.0	27.7	(21.4–35.1)
56–66	304	28.3	27.3	(22.5–32.7)	69	72.5	72.6	(60.4–82.1)	202	15.3	14.0	(9.9–19.6)
67–74	333	15.0	14.0	(10.7–18.2)	31	58.1	58.9	(40.8–75.0)	272	9.2	8.3	(5.6–12.1)
75–80	309	17.2	16.1	(12.4–20.7)	46	52.2	52.6	(37.9–66.9)	239	10.0	9.2	(6.1–13.5)
⩾81	365	16.4	15.1	(11.8–19.2)	54	55.6	56.1	(42.5–68.9)	288	9.0	8.0	(5.4–11.7)
Missing	86	34.9	33.2	(23.6–44.3)	23	73.9	61.0	(35.0–82.0)	57	22.8	19.0	(10.5–31.8)
Total	**1709**	**25.1**	**22.4**	**(20.3–24.6)**	**326**	**68.4**	**69.5**	**(63.6–74.8)**	**1234**	**13.8**	**11.5**	**(9.7–13.5)**

HPV, human papillomavirus; IVC, invasive vulvar cancer; CI, confidence interval; SCC, squamous cell carcinoma.
Adjusted by geographical region, period of diagnosis and age at diagnosis in quintiles. *Chi-squared test p-value < 0.05 when compared to the column average values; & Dropped from the estimations because model predicts success perfectly.

histological subtypes. Overall agreement was high (kappa 0.71 p value < 0.001). Agreement was highest for KSCC (kappa 0.64, p value < 0.001).

4. Discussion

This is the largest study on HPV contribution and type distribution in IVC using standardised protocols and highly sensitive HPV testing technology. It provides a robust HPV genotype worldwide reference data and, probably, the best available HPV driven estimates for this relatively rare cancer. This analysis confirms the predominant contribution of HPV 16 to the aetiology of HPV related vulvar cancer and VIN and suggests that other HPV types, such as HPV 33, HPV 18 and HPV 45, which are common in cervical cancer, are also important to vulvar carcinogenesis although to a lesser extent.

Our results indicate that HPV contribution in IVC has probably been overestimated in previous evaluations done through meta-analysis or systematic review of published studies[7,8] which estimated the HPV contribution to VIN lesions at around 84% and at 40% to IVC.

Within VIN lesions, the low proportion of HPV negative lesions correlates with the lower proportion of differentiated or usual histological type. Evidence is however being accumulated showing that as well as IVC, VIN lesions can also have two different etiopathogenesis, one associated to HPV in the usual-WB type, and a second independent of HPV infection in the differentiated type.[24]

A major contribution of this study is that p16^{INK4a} positivity was included in the criteria to consider a tumour to be HPV driven. While almost all cervical cancer cases are reported to be p16^{INK4a} positive,[17] 87.9% of the HPV-DNA positive IVC cases were also p16^{INK4a} positive resulting in an overall adjusted estimate of HPV attribution of the tumour of 22.4%. Neglected the results of p16^{INK4a} analysis and relying only on HPV-DNA data would suggest that 28.6% of IVC cases were HPV driven. Our overall estimate for IVC is lower than the one recently reported for the US using a test with similar sensitivity to detect HPV-DNA.[11] Gargano et al. observed an overall HPV-DNA positivity of 68.8% but when the data were stratified by the presence of WB characteristics their series and ours showed a similar contribution even when we were more restrictive and considered both HPV and p16^{INK4a} detection (69.5%). Thus, the observed variation could be explained by the different histology contribution of tumours of warty/basaloid type (50%) in the US study as compared to that seen in our series (19.1%).

The data presented here show an important inverse association of age with HPV prevalence in vulvar cancer tissue. HPV was significantly more common in tissue amongst women below age 56 than amongst older women. The reduction of HPV positivity with age, although larger for WB, was also observed for types that did not include WB features. Although a cohort effect cannot be totally discarded, as the same effect was observed for cases diagnosed before and after the year

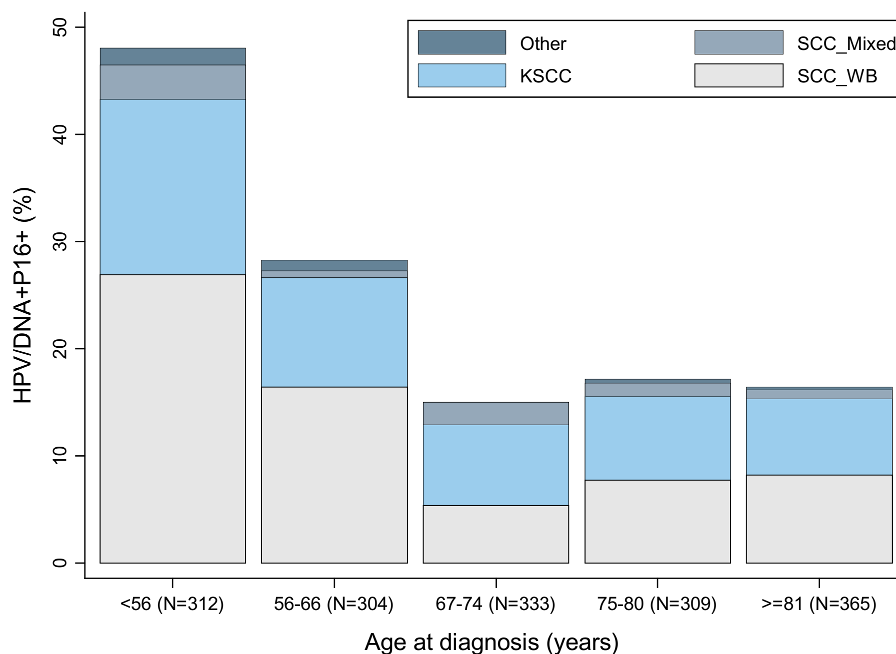

Fig. 1. HPV-DNA and p16^{INK4a} positivity (%) by histological categories and age at diagnosis amongst invasive vulvar cancer.

2000, this pattern suggests that HPV driven IVC cases can occur at any age. In absolute numbers HPV would be mainly related to the cases presented at an earlier age while the majority of cases would be diagnosed in late adulthood (approximately 60 years old) and are largely HPV unrelated.

HPV 16 was the commonest viral type detected in both HPV associated IVC and VIN with a borderline significant decreased ratio in favour of VIN contrary to what is generally observed in cervical cancer neoplasia where HPV 16 increases its contribution in more advanced lesions.[25] HPV 33 was the second commonest viral type accounting for 10.6% of VIN lesions and 6.5% of IVC cases. HPV 18 was the third most common type with slightly higher presence in IVC as compared to VIN. HPV 45 ranked 4th in IVC but was almost nonexistent in VIN. It is possible that the difference in the contribution of HPV 45 to various HPV-associated malignancies might result from the rapid progression and integration of HPV 45. HPV45-associated cases were, however, not younger than other cases associated with other HPV types as it was observed in invasive cervical cancer.[18] HPV 52 was also enriched in IVC compared to VIN. Other non-significant differences in viral prevalence in IVC compared to VIN were observed for HPV 56 and HPV 58. Numbers were, however, small but altogether are suggestive that HPV type distribution in VIN and IVC are very close.

When interpreting our results several potential limitations should be considered: (1) are we estimating HPV in a biased case sample? We believe that this is unlikely as the cases included were obtained from large pathology laboratories some of which served as the unique national laboratory for the country. Sampling bias might also occur if the centre or the local researcher selected cases based on some specific histological types more likely related HPV (i.e. WB). To reduce this source of potential bias, we requested that selection of consecutive cases be based on overall diagnosis of VIN or invasive vulvar cancer or in the availability of tissue in a given period without any additional selection criteria. (2) How can we estimate the true proportion of negative samples? The proportion of negative samples was particularly high amongst the KSCC histologies, but not in VIN WB and the SCC_WB histologies. Tissue quality is always a concern particularly when we identified that samples from earlier than the year 2000 were significantly more likely to be negative. A cohort effect possible could explain an increased proportion of HPV related cases as it is observed for orophrayngeal cancers.[26] Data on time trends of HPV related IVC are, however, very limited.

There remains the possibility that a certain fraction of HPV negative samples were false negatives. However, a random sample of the negative samples were also tested for cellular DNA quality through the detection of human tubuline and about 10% of the samples were tubuline negative. Thus, the estimated prevalence is likely to be only slightly underestimated.

The strengths of the study include the international network of collaborating centres, the use of a common protocol for specimen collection, histological confirmation and classification of tumour specimens, HPV testing centralised in two laboratories with common

Table 4
HPV genotype distribution amongst HPV-DNA VIN positive cases and invasive vulvar cancer cases that were both HPV-DNA and p16^{INK4A} positive.

HPV type	VIN $N = 509$ HPV positive		IVC $N = 429$ HPV and p16^{INK4A} positive	
	N	(%)	*N*	(%)
HPV6	4	(0.9)	3	(0.7)
HPV11	2	(0.5)	1	(0.2)
HPV16	393	(77.3)	311	(72.5)
HPV18	13	(2.5)	20	(4.6)
HPV26	1	(0.2)	1	(0.2)
HPV30	0	(0.0)	1	(0.2)
HPV31	6	(1.2)	4	(1.0)
HPV33	54	(10.6)	28	(6.5)
HPV35	2	(0.5)	0	(0.0)
HPV39	0	(0.0)	3	(0.7)
HPV42	2	(0.4)	0	(0.0)
HPV44	1	(0.2)	0	(0.0)
HPV45	2	(0.4)	14	(3.3)
HPV51	4	(0.8)	0	(0.0)
HPV52	3	(0.6)	8	(1.9)
HPV53	0	(0.0)	1	(0.2)
HPV55	2	(0.4)	0	(0.0)
HPV56	2	(0.5)	5	(1.2)
HPV58	1	(0.2)	4	(1.0)
HPV61	0	(0.0)	0	(0.0)
HPV66	0	(0.0)	3	(0.7)
HPV67	2	(0.4)	1	(0.2)
HPV68	0	(0.0)	2	(0.5)
HPV68or73	1	(0.2)	0	(0.0)
HPV69	0	(0.0)	2	(0.5)
HPV70	1	(0.2)	2	(0.5)
HPV73	2	(0.4)	1	(0.2)
HPV74	0	(0.0)	4	(1.0)
HPV83	1	(0.2)	0	(0.0)
HPV102	0	(0.0)	0	(0.0)
HPV114	1	(0.2)	0	(0.0)
HPVUndetermined	6	(1.2)	8	(1.9)

HPV, human papillomavirus; VIN, vulvar intraepithelial neoplasia; IVC, invasive vulvar carcinoma; numbers do not add up because of rounding decimals after computing the proportional contribution of each type in presences of multiple HPV infections or because there are few times that we found that all the HPV types detected in a case are not identified as single types.

protocols and quality control procedures and the inclusion of p16^{INK4a} testing for IVC cases. Further, a highly sensitive assay was used for HPV detection when analysing paraffin embedded specimens. Finally, robust statistical methods were used to adjust prevalence rates for the variables that strongly influence the results available in the literature including age and imputation to undetermined the effect of missing values.

Existing HPV vaccines may reduce a considerable amount of VIN lesions and a quarter of IVC based on the trial reported efficacy.[27] Further, next generation vaccines such as the nine-valent HPV vaccine could almost eradicate a major part of IVC HPV driven cases by about 37%[28,29] and almost all of the HPV positive VIN lesions.

To conclude, worldwide HPV contributes to a quarter of invasive vulvar cancers and a large part of VIN. HPV 16 is present in about three-quarters of all HPV positive cases. This international effort adds relevant information and reinforces the rationale for HPV-related cancer prevention through wide range HPV vaccines. Geographical variation of the proportion of HPV associated IVC should be further evaluated.

Sponsorship

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Table 5
p16^{INK4a} overexpression in cases of invasive vulvar cancer, by HPV-DNA detection, stratified by histological type.

	Overall IVC			Histological type											
				SCC Warty/basaloid			SCC Keratynising			SCC Mixed			Other		
	HPV			HPV			HPV			HPV			HPV		
p16INK4a detection	*N*	Negative *N* (%)	Positive *N* (%)	*N*	Negative *N* (%)	Positive *N* (%)	*N*	Negative *N* (%)	Positive *N* (%)	*N*	Negative *N* (%)	Positive *N* (%)	*N*	Negative *N* (%)	Positive *N* (%)
Negative	909	850 (90.6)	59 (16.9)	65	53 (63.9)	12 (6.2)	765	728 (95.3)	37 (31.1)	59	51 (81.0)	8 (28.6)	20	18 (64.3)	2 (22.2)
Positive	378	88 (9.4)	290 (83.1)	211	30 (36.1)	181 (93.8)	118	36 (4.7)	82 (68.9)	32	12 (19.0)	20 (71.4)	17	10 (35.7)	7 (77.8)
Total	1,287	938	349	276	83	193	883	764	119	91	63	28	37	28	9
Kappa index; *p*-value		0.7184; <0.001			0.6143; <0.001			0.6442; <0.001			0.5038; <0.001			0.3232; 0.0138	

IVC, invasive vulvar cancer; HPV, human papillomavirus; SCC, squamous cell carcinoma. Included cases: IVC cases with information on both markers.

who had no role in the data collection, analysis or interpretation of the results.

Role of the funding source

The sponsors did not have any role in the study design and collection, analysis or interpretation of the data. None of the sponsors had access to the row data. The corresponding author had full access to all the data, and had the final responsibility to submit for publication.

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Conflict of interest statement

The corresponding author has received occasional travel grants from Merck and Qiagen and holds a restricted grant from Qiagen and from Merck not related to the study presented. The senior author F. Xavier Bosch is in the Advisory Board (Merck and Co., Inc.); Speakers Bureau (GlaxoSmithKline); has received Institutional Research Grants (Merck and Co., Inc., Sanofi Pasteur MSD, GlaxoSmithKline).

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References

1. IARC: Cancer incidence in 5 continents. <http://ci5iarcfr/CI5i-ix/ci5i-ix.htm>; 2012 [accessed 1/8/2012].
2. Jones RW, Baranyai J, Stables S. Trends in squamous cell carcinoma of the vulva: the influence of vulvar intraepithelial neoplasia. *Obstet Gynecol* 1997;**90**(3):448–52.
3. Baandrup L, Varbo A, Munk C, Johansen C, Frisch M, Kjaer SK. In situ and invasive squamous cell carcinoma of the vulva in Denmark 1978–2007-a nationwide population-based study. *Gynecol Oncol* 2011;**122**(1):45–9.
4. Judson PL, Habermann EB, Baxter NN, Durham SB, Virnig BA. Trends in the incidence of invasive and in situ vulvar carcinoma. *Obstet Gynecol* 2006;**107**(5):1018–22.
5. Joura EA, Losch A, Haider-Angeler MG, Breitenecker G, Leodolter S. Trends in vulvar neoplasia. Increasing incidence of vulvar intraepithelial neoplasia and squamous cell carcinoma of the vulva in young women. *J Reprod Med* 2000;**45**(8):613–5.
6. Alonso I, Fuste V, del Pino M, et al. Does human papillomavirus infection imply a different prognosis in vulvar squamous cell carcinoma? *Gynecol Oncol* 2011;**122**(3):509–14.
7. De Vuyst H, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S. Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. *Int J Cancer* 2009;**124**(7):1626–36.
8. International Agency for Research on Cancer. *IARC monographs on the evaluation of carcinogenic risks to humans*. Lyon; 2007.
9. Parkin DM, Bray F. Chapter 2: The burden of HPV-related cancers. *Vaccine* 2006;**24**(Suppl. 3), S3-11-S3/25.
10. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens – Part B: Biological agents. *Lancet Oncol* 2009;**10**(4):321–2.
11. Gargano JW, Wilkinson EJ, Unger ER, et al. Prevalence of human papillomavirus types in invasive vulvar cancers and vulvar intraepithelial neoplasia 3 in the United States before vaccine introduction. *J Low Genit Tract Dis* 2012;**16**(4):471–9.
12. Santos M, Landolfi S, Olivella A, et al. P16 overexpression identifies HPV-positive vulvar squamous cell carcinomas. *Am J Surg Pathol* 2006;**30**(11):1347–56.
13. Hampl M, Deckers-Figiel S, Hampl JA, Rein D, Bender HG. New aspects of vulvar cancer: changes in localization and age of onset. *Gynecol Oncol* 2008;**109**(3):340–5.
14. van de Nieuwenhof HP, Bulten J, Hollema H, et al. Differentiated vulvar intraepithelial neoplasia is often found in lesions, previously diagnosed as lichen sclerosus, which have progressed to vulvar squamous cell carcinoma. *Mod Pathol* 2011;**24**(2):297–305.
15. Chaux A, Cubilla AL, Haffner MC, et al. Combining routine morphology, p16 immunohistochemistry, and in situ hybridization for the detection of human papillomavirus infection in penile carcinomas: A tissue microarray study using classifier performance analyses. *Urol Oncol* 2013. http://dx.doi.org/10.1016/j.urolonc.2012.04.017.
16. Smeets SJ, Hesselink AT, Speel EJ, et al. A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. *Int J Cancer* 2007;**121**(11):2465–72.
17. Halec G, Alemany L, Lloveras B, Schmitt M, Alejo M, Bosch FX et al. Pathogenic role of Human Papillomavirus (HPV) Types 26,53, 66, 67, 68, 70, 73 and 82 in cervical cancer. Submitted.
18. de Sanjose S, Quint WG, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol* 2010;**11**(11):1048–56.
19. Sideri M, Jones RW, Wilkinson EJ, et al. Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISSVD Vulvar Oncology Subcommittee. *J Reprod Med* 2005;**50**(11):807–10.
20. Kleter B, van Doorn LJ, ter Schegget J, et al. Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. *Am J Pathol* 1998;**153**(6):1731–9.
21. Kleter B, van Doorn LJ, Schrauwen L, et al. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. *J Clin Microbiol* 1999;**37**(8):2508–17.
22. Geraets D, Alemany L, Guimera N, et al. Detection of rare and possibly carcinogenic human papillomavirus genotypes as single infections in invasive cervical cancer. *J Pathol* 2012;**228**:534–43.
23. Bergeron C, Ordi J, Schmidt D, Trunk MJ, Keller T, Ridder R. Conjunctive p16INK4a testing significantly increases accuracy in diagnosing high-grade cervical intraepithelial neoplasia. *Am J Clin Pathol* 2010;**133**(3):395–406.
24. Del PM, Rodriguez-Carunchio L, Ordi J. Pathways of vulvar intraepithelial neoplasia and squamous cell carcinoma. *Histopathology* 2013;**62**(1):161–75.
25. Guan P, Howell-Jones R, Li N, et al. Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. *Int J Cancer* 2012;**131**(10):2349–59.
26. Gillison ML, Alemany L, Snijders PJ, et al. Human papillomavirus and diseases of the upper airway: head and neck cancer and respiratory papillomatosis. *Vaccine* 2012;**30**(Suppl. 5):F34–54.
27. Joura EA, Leodolter S, Hernandez-Avila M, et al. Efficacy of a quadrivalent prophylactic human papillomavirus (types 6, 11, 16,

and 18) L1 virus-like-particle vaccine against high-grade vulval and vaginal lesions: a combined analysis of three randomised clinical trials. *Lancet* 2007;**369**(9574):1693–702.
28. Serrano B, Alemany L, Tous S, et al. Potential impact of a nine-valent vaccine in human papillomavirus related cervical disease. *Infect Agent Cancer* 2012;**7**(1):38.
29. De MC, Ferlay J, Franceschi S, et al. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol* 2012;**13**(6):607–15.