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Prevalence of Human Papillomavirus (HPV) Types in Invasive Vulvar Cancers and VIN3 in the United States Before Vaccine Introduction

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Abstract

Objective—To determine the baseline prevalence of human papillomavirus (HPV) types in invasive vulvar cancers (IVC) and vulvar intraepithelial neoplasia 3 (VIN3) using data from 7 United States cancer registries.

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Materials and Methods—Registries identified eligible cases diagnosed in 1994–2005 and requested pathology laboratories to prepare one representative block for HPV testing on those selected. Hematoxylin and eosin stained (H&E) sections preceding and following those used for extraction were reviewed to confirm representation. HPV was detected using L1 consensus PCR with PGMY9/11 primers and type specific hybridization, with retesting of negative and inadequate samples with SPF10 primers. For IVC, the confirmatory H&E slides were re-evaluated to determine histologic type. Descriptive analyses were performed to examine distributions of HPV by histology and other factors.

Results—HPV was detected in 121/176 (68.8%) IVC and 66/68 (97.1%) VIN3 (p<.0001). IVC and VIN3 differed by median age (70 years vs. 55 years, p=.003). HPV16 was present in 48.6% of IVC and 80.9% of VIN3; other high-risk (HR) HPV was present in 19.2% of IVC and 13.2% of VIN3. HPV prevalence differed by squamous cell carcinoma (SCC) histologic subtype (p<.0001): keratinizing, 49.1% (n=55); non-keratinizing, 85.7% (n=14), basaloid, 92.3% (n=14), warty 78.2% (n=55), and mixed warty/basaloid, 100% (n=7).

Conclusions—Nearly all VIN3 and two-thirds of IVC were HR-HPV positive. HPV prevalence ranged from 49.1–100% across SCC histologic subtypes. Given the high prevalence of HPV in IVC and VIN3, prophylactic vaccines have the potential to decrease the incidence of vulvar neoplasia.

Keywords

Vulvar cancers; vulvar intraepithelial neoplasia; human papillomavirus; cancer registry; vaccine

Introduction

Vulvar cancer is rare, with about 3850 cases occurring annually in the United States, accounting for 0.6% of all cancers in women.(1) Incidence of vulvar cancer increases with age; the median ages at diagnosis of in situ and invasive vulvar cancers are 49 and 69 years, respectively.(2, 3) Rates of invasive vulvar cancer (IVC) and in situ vulvar cancer are highest among white women.(2, 3)

Although vulvar cancers can arise from other cell types, most are squamous cell carcinomas (SCC).(4, 5) Vulvar SCC are broadly classified according to presumed etiology: those typically associated with human papillomavirus (HPV), including warty and basaloid SCC, and types not typically associated with HPV, such as keratinizing and non-keratinizing SCC. (5) The warty and basaloid vulvar SCC often arise from precursor lesions of characteristic histologic types known as vulvar intraepithelial neoplasia (VIN) of “usual” or HPV-related type; the natural history of progression is similar to that of cervical cancer.(6) The keratinizing SCCs often have a different precursor, generally lichen sclerosis or epithelial hyperplasia; since these precursors are not generally considered to be neoplasias, they are infrequently collected in cancer registries until they become invasive. However, some keratinizing SCC are preceded or accompanied by differentiated VIN, which has a lower prevalence than usual VIN.(7) In part because differentiated VIN are seldom diagnosed, most in situ vulvar cancers collected in cancer registries are usual VIN(7). HPV-16 is by far the predominant genotype found in vulvar neoplasia.(8–11)

Licensed HPV vaccines, which provide immunity to HPV16 and HPV18, are efficacious against VIN2/3 endpoints, and therefore would be expected to influence the distribution of HPV types among VIN and invasive vulvar cancers.(12, 13) The aims of this study were to describe the HPV genotype distributions of invasive and in situ vulvar cancers in a multi-state cancer registry study, using tissues from women diagnosed before vaccine introduction.

Materials and Methods

The Centers for Disease Control Central Cancer Registries (CDC CCR) study was designed to describe the prevalence of HPV types in HPV-associated cancer cases prior to widespread HPV vaccine use from participating population-based cancer registries. Institutional review board approval was obtained from CDC and all seven participating registries. Four population-based cancer registries (Florida, Kentucky, Louisiana and Michigan) randomly sampled eligible cases, which were tracked back to the pathology laboratories where the tissue was stored. Additionally, three residual tissue repositories (RTRs) (Hawaii, Iowa, and Los Angeles County), which retain specimens that would otherwise be discarded, contributed additional targeted tissue types from eligible cases not provided to other ongoing studies of the same cancer. Pathology laboratories and RTRs were asked to submit one representative archived formalin fixed paraffin embedded (FFPE) tissue block or thin sections per protocol from each case. Cases of IVC diagnosed during 1995 to 2005 were sought by all registries (2004–2005 for Florida, Kentucky, Louisiana and Michigan; 2000–2004 for Hawaii RTR and 1995–1999 for Iowa and Los Angeles RTR). Cases of in situ vulvar cancer were only available from RTRs. Current International Society for the Study of Vulvovaginal Disease (ISSVD) terminology recognizes a single category of VIN, which encompasses the former VIN2 and VIN3(14); the cancer registries' classification of in situ vulvar cancer is generally consistent with the older VIN3 classification. Blocks were cut using precautions to prevent PCR contamination between cases, including single-use disposable microtome blades, cleaning microtome between cases, and direct transfer of sections for PCR from microtome to sterile tubes using clean single-use applicator (no contact with waterbath). The first and last sections were stained with Hematoxylin and Eosin (H&E). Intervening sections were transferred into 2 ml conical screw cap tubes with tether cap, one 10-micron section or two 5-mciron sections per tube (Simport, Beloeil, Canada).

A total of 320 IVC and 127 VIN3 cases were selected by registries. The CDC laboratory received eligible tissue specimens for 198 (61.8%) IVC and 68 (53.5%) VIN3. The most common reasons for lack of tissue submission were inadequate tissue, inability to locate tissue and facility refusal. The H&E sections were reviewed by a study pathologist (ERU) to confirm that histology was representative of the submitting diagnosis. Samples lacking representative material (n=16, all IVC) were not processed. However, for IVC cases, if the available material included an intraepithelial lesion but lacked the invasive focus, the sample was accepted (n=16). The first H&E slide was digitized using ScanScope XT (Aperio Technologies, Vista, CA) at 0.25 μm/pixel resolution, equal to 40X objective. For confirmed samples, DNA was extracted with the Chemagic Viral NA/gDNA Kit special (chemagen USA, Worcester MA) as previously described(15). Briefly, sections were heated for 20 min at 120°C in 180 μl tissue lysis buffer, then incubated with Proteinase K overnight at 65°C and then purified using Chemagic MSM1 (chemagen USA). The DNA was eluted in a final

volume of 100 μl. For every batch of 28 samples, a water blank was processed through all steps of extraction to serve as a “contamination control”.

All DNA extracts were tested with the Linear Array HPV Genotyping Test (LA, Roche Diagnostics, Indianapolis, IN). The test was performed according to the manufacturer’s protocol except for a template volume of 10 μl in the PCR reaction and the use of Beeblot instrument (Bee Robotics, Caernarfon, UK) for automated hybridization and washing of the reverse line blot. The LA detects 37 HPV types (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, XR(52), 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89, IS39). Samples positive for the XR probe that were also positive for HPV33, 35 and 58 were tested with an HPV52 type-specific quantitative PCR assay with a threshold of 50 copies to confirm detection of HPV52 (16).

Samples with negative or inadequate LA results were re-tested with the INNO-LiPA HPV Genotyping Assay (LiPA, Innogenetics, Gent, Belgium) following the manufacturer’s specifications. LiPA detects 29 HPV types (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 69, 70, 71, 73, 74, 81, 82) as well as detecting HPV not typed (HPV X). Samples failing both assays were considered inadequate and excluded from analysis (n=5). Overall, LA was used to generate 74.6% of HPV genotyping results and LiPA was used for 25.4%; assay used to report results did not differ significantly by sample type (i.e. invasive or in situ) or by histologic type in invasive cancers (not shown).

Registries supplied a range of demographic and clinical data about each case, including age at diagnosis, race/ethnicity, and information on submitting histology. The digital images were reviewed by one author (EJW) masked to all registry data including submitting diagnosis. Based on this review, information was gathered on presence of invasive focus, and histologic type of invasive lesions and any associated intraepithelial lesions.(5) For cases identified as IVC in the registry which had no invasive focus evident in the reviewed slide, the histology was assigned as the observed VIN type when possible (e.g., warty VIN classified as SCC: Warty). We present IVC as classified by the reviewer, although for selected sub-analyses, we combine warty, basaloid, and mixed warty and basaloid SCC into “Warty/Basaloid SCC,” keratinizing and non-keratinizing SCC into “Usual SCC,” and all other histologies into “Others.” Likewise, intraepithelial lesion types are presented as classified by the reviewer, although for some analyses warty, basaloid, and mixed VIN are combined as “Warty/Basaloid VIN” and VIN—unable to classify, intraepithelial basal cell carcinoma, and no intraepithelial lesions are combined as “Other/None” for some analyses.

Descriptive analyses combined registry and genotyping data. We described prevalence of any HPV, HR-HPV (defined as HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, or 68) and individual HPV types. We also described the proportion of cases with single and multiple genotypes present. We compared median age at diagnosis between groups using the non-parametric Wilcoxon two-sample test or Kruskal-Wallis test. We compared proportions between categorical variables using chi-square or Fisher’s exact test, depending on cell counts. For time trend analysis, we used the Cochran-Mantel-Haenszel test. All statistical analyses were performed using SAS 9.2 (Statistical Analysis Software, Cary, NC).

Results

Table 1 provides distributions of tissue sources and patient characteristics for 176 IVC and 68 VIN3 cases with complete genotyping data, stratified by HR-HPV. Nearly 80% of IVC cases were obtained from the four population-based registries; two of the RTRs contributed the remaining 20%, and one RTR did not contribute any IVC cases because of a competing study. The vast majority of cancer cases (84.7%) were diagnosed in 2004 or 2005. The median age at diagnosis for IVC was 70 years (range 35–98). Over three-quarters of the women with IVC were non-Hispanic white, 7.4% were non-Hispanic black, 6.3% were Hispanic white, 7.9% were Asian/Pacific Islander, and 1.1% belonged to another race/ethnicity category. Two-thirds (67.6%) of IVC had HR-HPV detected. The median age at diagnosis of women with HR-HPV positive cancers was younger than those with HR-HPV negative cancers (61 vs. 75 years, p=.002). Although numbers in some race/ethnicity categories were small, HR-HPV detection was associated with race/ethnicity: a larger proportion of HPV-negative cancers were in white women, and all cancers in non-Hispanic black women were HR-HPV positive.

All VIN3 cases were contributed by the three RTRs; 27 (39.7%) were diagnosed before the year 2000 (Table 1). The median age at VIN3 diagnosis was 55 years, significantly lower than the median age at diagnosis for invasive cases (p=.003). Most (94.1%) VIN3 samples were positive for HR-HPV, thus stratification by HR-HPV status was uninformative.

More detailed HPV genotyping results for IVC and VIN3 are presented in Table 2. Overall, significantly fewer IVC than VIN3 had HPV detected (68.3% vs. 97.1%, respectively). HPV16 was, by far, the most prevalent genotype in both sample types, present in nearly half of IVC and in 80.9% of VIN3. At least one of the other 13 HR-HPV types was detected in 17.1% of IVC and 11.8% of VIN3 (not shown). Among HPV positive cases, a smaller proportion of IVC than VIN3 had HPV16 (70.2% vs. 83.3%, p=.049, not shown). After HPV16, the most prevalent HPV types among IVC were HPV33 (n=18, 10.2%), HPV52 (n=5, 2.8%) and HPV18 (n=3, 1.7%). After HPV16, the most prevalent HPV types among VIN3 were HPV33 (n=6, 8.8%) and HPV59 (n=2, 2.9%). All other HPV types were present in less than 2% of cases (not shown). Eleven IVC had multiple genotypes detected; of these, seven included HPV16 (two 16/18, two 16/33, one each of 16/68, 16/70, and 16/44/56). The remaining four IVC included one or two HR-HPV types (52/56, 51/52, 33/35, and 52/62). Four VIN3 cases had multiple genotypes detected, and all included HPV16 (16/72, 16/18/68, 16/59/84, and 16/51/59).

Registry histology was non-specific when compared with reviewed histology (not shown). For example, the registries classified 111 cases as “other” SCC; upon review, these were classified as 20 basaloid SCC, 38 warty SCC, six mixed basaloid/warty SCC, 32 keratinizing SCC, 11 non-keratinizing SCC, one other SCC, and three unclassifiable. Table 3 shows the distribution of HPV by reviewed histology classification. Median age at diagnosis differed non-significantly across the three major histologic categories (p=.09): usual (keratinizing/non-keratinizing) SCC, 73 years (range 35–98 years); warty, basaloid or mixed SCC, 66 years (range 36–91 years); all other histology, 76 years (range 40–88 years). HPV prevalence differed across the three major histologic categories (p<.0001): 56.5% in usual

(keratinizing/non-keratinizing) SCC; 84.1% in warty, basaloid, or mixed SCC; and 42.1% in all other IVC. After histologic subclassification, HPV prevalence was significantly higher in non-keratinizing SCC than keratinizing SCC (p=.02), but there was no significant difference in prevalence among warty, basaloid and mixed SCC (p=.22).

Many IVC had associated intraepithelial lesions (Table 4). Not surprisingly, warty and basaloid VIN were most often observed with warty and basaloid SCC, respectively. Notably, 33 of 55 keratinizing SCC cases had differentiated VIN present, while six other keratinizing SCC had warty, basaloid, or mixed VIN. In addition, the majority of non-keratinizing SCC (i.e., 8/14 cases) had some form of VIN present.

We further explored differences between HPV-positive and HPV-negative IVC by examining distributions of VIN groupings and age by histology type and HPV status (Table 5). Across all invasive histology types, those with warty/basaloid VIN had the highest proportion HPV-positive (86.5%) compared to those with differentiated VIN (44.4%) and those with other/no VIN (55.3%). In keratinizing SCC, 42.4% of the 33 cases with differentiated VIN and 50% of 16 cases with other/no VIN were HPV-positive. The only invasive histologic group that had a significant age difference by HPV status was warty/basaloid SCC (median age 59 years for HPV-positive and 81 years for HPV-negative, p=.001).

Discussion

This study supports evidence of the important role of HPV, especially HPV16, in VIN3 and IVC, as well as the marked differences in HPV prevalence by SCC histologic type. To the best of our knowledge, this is the largest US genotyping study of IVC to date, and one of the largest studies of VIN3.

Our observed HPV-positivity in IVC (68.8%) across all histologic types is slightly higher than a US data pooling study of HPV in IVC (65.3% based on 4 studies)(10) and a meta-analysis of North American studies (63.2% based on 18 studies)(8). However, the prevalence we observed was considerably higher than several international estimates (8, 9, 11); the reasons for the discrepancy in published prevalence estimates between the US and other countries are not clear.

We observed a high prevalence (97.1%) of HPV in cases identified by registries as in situ vulvar cancer (i.e., VIN3), as expected based on published literature that implicates HPV in the vast majority of VIN – particularly warty and basaloid (usual) VIN(8, 10, 11, 17–20). We did not review the histology of in situ (VIN3) cases submitted by the registries. However, during the histologic review of IVC, the pathologist noted accompanying VIN types. Although PCR methods do not enable the separation of HPV from VIN or adjacent invasive cancers, HPV was about twice as common in cancers associated with warty/basaloid VIN than in those accompanying differentiated VIN (i.e., 91.0% vs. 46.9%). Based on this and the expectation that differentiated VIN is infrequently diagnosed(7), we suspect most cases of VIN submitted as in situ vulvar cancers were of the warty/basaloid (usual) type.

Many studies have found that HPV prevalence and age differ by IVC histologic type(21–24), which has led to a hypothesis that distinct pathways lead to HPV-associated and other tumors(25). The HPV prevalence we observed in reviewed warty and basaloid cancers (84.1%), the cancers often attributed to HPV, was in the expected range based on recent meta-analyses that incorporated histology data(8, 11). We also observed a similarly high prevalence of HPV in the 14 non-keratinizing SCC in our study (85.7%), suggesting that these tumors might have a similar etiology to warty and basaloid SCC; we have not identified previous published reports on HPV prevalence in non-keratinizing SCC. While the HPV prevalence we observed for keratinizing SCC (49.1%) was lower than for non-keratinizing and warty/basaloid cancers (78.2 – 100% depending on histologic subtype), it is higher than many(8, 9, 11, 22, 23), but not all (24), previous studies have reported. A recent HPV genotyping study of 116 vulvar cancers which incorporated histologic review by a surgical pathologist identified a high HPV prevalence (62%) among SCC not otherwise specified (i.e., not warty or basaloid); although this prevalence was somewhat lower than that observed in the other types of SCC in the same study (i.e., warty 86%; basaloid 70%), the authors suggested the HPV difference by histologic type may actually not be as great as previously reported(24). On the other hand, a recent study suggested that HPV prevalence overestimates the causal association of the virus, because in differentiated VIN-associated SCC (i.e., generally keratinizing SCC), the detected HPV was not integrated or activated. (26) Potential differences in histologic classification across studies pose challenges for cross-study comparisons.

While our observed HPV prevalence in VIN3 and IVC are not out of range of previous studies, they are on the high side of prior estimates, particularly for keratinizing SCC. We speculate that the improved digestion methods and the use of two genotyping assays may have increased the sensitivity of HPV genotyping. If we had relied only on the LA assay, we would have reported an HPV prevalence in vulvar cancers as low as 46.6% (i.e. 82/176). In at least one published study, LiPA was demonstrated to have greater analytic sensitivity than LA for HPV DNA detection and genotyping of archival vulvar tissue(27). Other possible explanations for finding a higher than expected HPV prevalence include false positives or contamination. The HPV genotyping laboratory routinely uses methods to guard against contamination, including the use of water blanks in all steps. While we cannot rule out the possibility of contamination by HPV during tissue handling prior to specimen arrival at the CDC, we consider this is unlikely to be a major factor in differences between our studies and others using archival tissue.

Like previous vulvar disease genotyping efforts, we found that HPV16 is by far the most important genotype in both IVC and VIN3(8, 10, 11). The second most prevalent genotype in both sample types was HPV33 (found in 10.2% of IVC and 8.8% of VIN3 overall); large U.S. and international data pooling studies have found HPV33 to be the second or third most prevalent HPV type in IVC and VIN3(8, 10, 11). We note that HPV18 was only found in 4 patients in the entire study, and only one of these occurred in a patient who did not also have HPV16.

This study is among the largest case series to perform a detailed histology review on invasive cancers and evaluate HPV DNA by histologic type. A previous medical record reabstraction

study for common cancer sites identified histology as a common area of data inaccuracies(28); in the case of vulvar cancer it may be particularly important to review histology because SCC subtypes may not be identified during the diagnostic process. In addition, this study encompasses more diverse geographic coverage than other U.S. vulvar cancer genotyping studies. HPV genotyping was conducted in a state-of-the-art laboratory, and incorporated recently improved methods for DNA extraction from FFPE tissues(15) and use of two genotyping assays to increase sensitivity for HPV detection.

We also acknowledge several limitations. Detailed lifestyle data is not routinely collected from cancer registries, thus we cannot further explore etiologic hypotheses for HPV-negative vulvar cancers. Additionally, no conclusions can be drawn regarding race differences in IVC vs. VIN3, because the two sample types were obtained from different registries that covered populations with different demographic make-ups. Although generalizability to the U.S. population is unknown, preliminary analyses of the four population-based sampled registries show that typed cases are similar to cases diagnosed in the registries in terms of age, race, stage, and histology distribution (Meg Watson, CDC, personal communication). Like all PCR-based studies, we cannot be sure that the HPV DNA was present in the lesion of interest. In some cases, the HPV may have been an active infection in nearby tissue rather than in the neoplasm; however, given the expected low prevalence of HPV infections among older women in the general population (e.g., 19.6% among women aged 50–59 in a nationally representative sample(29)), we think it is more likely that the HPV prevalence observed reflects the neoplastic tissue.

Our findings extend the literature on the potential role of HPV in the etiology of histologic subtypes of vulvar cancer. We found HPV DNA in the vast majority of VIN3 and in two-thirds of invasive vulvar cancers. Although the HPV prevalence differed by SCC subtype, it was present in ≥49% of all SCC subtypes. Our results indicate that if HPV is causally related to the neoplastic vulvar tissues in which it is found, the virus may play more of a role in keratinizing and non-keratinizing SCC of the vulva than previously thought, raising the possibility that HPV vaccines might prevent some of these cancers.

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Table 1

Comparison between high-risk human papillomavirus (HR-HPV)* positive and negative invasive vulvar cancers (IVC) and in situ vulvar cancers (VIN3)

	Total		HR-HPV* Positive		HR-HPV* Negative		P-value
	No.	%	No.	%	No.	%	
IVC	**176**	**100**	**119**	**67.6**	**57**	**32.4**	
Tissue Source							
Population-Based Registries (Florida, Kentucky, Louisiana, and Michigan)	140	79.6	98	82.4	42	73.7	0.182
Residual Tissue Repositories (Hawaii and Iowa)	36	20.4	21	17.7	15	26.3	
Year of diagnosis							
1995–1999†	14	8.0	6	5.0	8	14.0	0.080
2000–2003‡	13	7.4	8	6.7	5	8.8	
2004	82	46.6	64	53.8	18	31.6	
2005	67	38.1	41	34.5	26	45.6	
Age at diagnosis							
Median	70		61		75		0.002
[Range]	[35–98]		[36–95]		[35–98]		
Race/ethnicity							
White non-Hispanic	136	77.3	87	73.1	49	86.0	0.046
Black non-Hispanic	13	7.4	13	10.9	0	0.0	
Hispanic	11	6.3	8	6.7	3	5.3	
Asian/Pacific Islander	14	7.9	9	7.6	5	8.8	
Other	2	1.1	2	1.7	0	0.0	
In Situ (VIN3)	**68**	**100.0**	**64**	**94.1**	**4**	**5.9**	
Tissue Source							
Population-Based Registries (N/A)	0						
Residual Tissue Repositories (Hawaii, Iowa, and Los Angeles)	68	100.0	64	100.0	4	100.0	
Year of diagnosis							
1994–1999§	27	39.7	24	37.5	3	75.0	
2000–2003‡	31	45.6	30	46.9	1	25.0	
2004‡	10	14.7	10	15.6	0	0.0	

	Total		HR-HPV* Positive		HR-HPV* Negative		P-value
	No.	%	No.	%	No.	%	
Age at diagnosis							
Median	55		55		52		
Range	19–85		19–85		35–85		
Race/ethnicity							
White non-Hispanic	37	54.4	35	54.7	2	50.0	
Black non-Hispanic	0	0.0	0	0.0	0	0.0	
Hispanic	5	7.4	3	4.7	2	50.0	
Asian/P.I.	14	20.6	14	21.9	0	0.0	
Other	12	17.7	12	18.8	0	0.0	

*HR-HPV defined as types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68

†Only Iowa submitted cases for this time period.

‡Only Hawaii submitted cases for this time period.

§Only Los Angeles and Iowa submitted cases for this time period.

Table 2

HPV status and genotype distribution in invasive vulvar cancer and in situ vulvar cancer (VIN3) cases, based on registry classification

	Invasive (n=176)		In Situ (VIN3) (n=68)		P-value
	N	%	N	%	
HPV positive	121	68.8	66	97.1	<.0001
HR-HPV * positive	119	67.6	64	94.1	<.0001
Individual HPV genotypes †					
16	85	48.3	55	80.9	
18	3	1.7	1	1.5	
33	18	10.2	6	8.8	
52	5	2.8	0	0.0	
59	0	0.0	2	2.9	
Multiple genotype, among all cases	11	6.3	4	5.9	1.00
Multiple genotypes, among HPV-positive samples	11	9.1	4	6.1	0.58

* HR-HPV defined as types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

† The following additional genotypes were tested and identified in <2% of all cases: 6, 11, 26, 31, 35, 39, 40, 42, 43, 44, 45, 51, 53, 54, 55, 56, 58, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 74, 81, 82, 83, 84, IS39 and X.

Table 3

Prevalence of HPV by reviewed pathology, invasive vulvar cancers (n=176 *)

	SCC, Usual Type n=69, 39.2%		SCC, Warty/Basaloid Type† n=88, 50.0%			Others n=19, 10.8%	
	Keratinizing	Non-Keratinizing	Basaloid	Warty	Warty & Basaloid	Basal Cell Carcinoma	Paget's Disease
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Total	55	14	26	55	7	7	7
Age, median [range]	73 [35–98]	69 [39–90]	58 [38–90]	68 [36–91]	61 [45–86]	78 [40–81]	76 [66–82]
HPV-positive	27 (49.1)	12 (85.7)	24 (92.3)	43 (78.2)	7 (100)	3 (42.9)	1 (14.3)
HR-HPV positive	27 (49.1)	10 (71.4)	23 (88.5)	40 (72.7)	7 (100)	3 (42.9)	1 (14.3)
HPV 16	18 (35.3)	7 (50.0)	14 (53.9)	33 (60.0)	5 (71.4)	3 (42.9)	1 (14.3)
HPV 18	0	0	1 (3.9)	2 (3.6)	0	0	0
HPV 31	1 (1.8)	1 (7.1)	0	0	0	0	0
HPV 33	4 (7.3)	2 (14.3)	6 (23.1)	4 (7.3)	2 (28.6)	0	0
HPV 45	0	0	0	1 (1.8)	0	0	0
HPV 52	1 (1.8)	0	0	2 (3.6)	1 (14.3)	0	0
Multiple genotypes	1 (1.8)	0	1 (4.6)	6 (10.9)	1 (14.3)	0	0

* Total includes all cases classified as invasive vulvar cancer by the submitting registries. However, 5 cases are not shown in the subclassification, including 3 cases that lacked sufficient material in slide for classification, 1 adenocarcinoma, and 1 SCC of other type.

† Cases that lacked an invasive focus on the reviewed slide were classified according to the type of VIN present. These include 1 basaloid VIN, 11 warty VIN, and 4 warty and basaloid VIN.

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Table 4

Types of vulvar intraepithelial lesions associated with invasive vulvar cancers*

Invasive histology	TOTAL	Intraepithelial Lesions						
		Differ-entiated VIN	Warty VIN	Basaloid VIN	Mixed Warty & Basaloid VIN	VIN, unable to classify	Intraepithelial basal cell carcinoma	No VIN
SCC: Keratinizing	55	33	2	1	3	–	–	16
SCC: Non-keratinizing	14	3	2	1	1	1	–	6
SCC: Warty	44	–	38	–	3	–	–	3
SCC: Basaloid	23	–	–	18	2	–	–	3
SCC: Mixed Warty & Basaloid	3	–	–	1	2	–	–	–
SCC: Other	1	–	–	–	–	–	–	1
Basal Cell Carcinoma	7	–	–	–	–	–	6	1
Adeno-carcinoma	1	–	–	–	–	–	–	1
Total	**148**	**36**	**42**	**21**	**11**	**1**	**6**	**31**

Note: SCC=squamous cell carcinoma; VIN=vulvar intraepithelial neoplasia; – combination not found in reviewed samples.

*Restricted to 148 cases with invasive focus present on reviewed slide.

Table 5

Comparison between HPV-positive and HPV-negative invasive vulvar cancers*: associated VIN types and age at diagnosis

	Total	VIN Type				Age at Diagnosis (years)		
	No. (%)	Differentiated No. (%)	Warty/Basaloid No. (%)	Other/None No. (%)	P-value	Median	Range	P-value
Total	148 (100.0)	36 (24.3)	74 (50.0)	38 (25.7)		70	35–98	
All Invasive Histology	148 (100.0							
HPV-positive	101 (68.2)	16 (44.4)	64 (86.5)	21 (55.3)	<.0001	64	36–95	0.005
HPV-negative	47 (31.8)	20 (55.6)	10 (13.5)	17 (44.7)		75	35–98	
SCC: Keratinizing	55 (37.2)							
HPV-positive	27 (49.1)	14 (42.4)	5 (83.3)	8 (50.0)	0.20	73	41–95	0.93
HPV-negative	28 (50.9)	19 (57.6)	1 (16.7)	8 (50.0)		73	35–98	
SCC: Non-keratinizing	14 (10.2)							
HPV-positive	12 (85.7)	2 (66.7)	4 (100.0)	6 (85.7)	0.69	68	39–90	0.47
HPV-negative	2 (14.3)	1 (33.3)	0	1 (14.3)		75	65–85	
SCC: Warty/Basaloid	70 (46.0)							
HPV-positive	58 (82.9)	0	55 (85.9)	3 (50.0)	0.06	59	36–88	0.001
HPV-negative	12 (17.1)	0	9 (14.1)	3 (50.0)		81	64–91	
Other Histology	9 (6.6)							
HPV-positive	4 (44.4)	0	0	4 (44.4)	–	76	58–81	0.61
HPV-negative	5 (55.6)	0	0	5 (55.6)		61	40–80	

*Restricted to 148 cases with invasive focus present on reviewed slide.