Prevalence and type distribution of human papillomavirus infections in Danish patients diagnosed with vulvar squamous cell tumors and precursors

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ABSTRACT

Objective: To study the prevalence and type distribution of human papillomavirus (HPV) in patients with vulvar high-grade precancerous lesions and vulvar squamous cell carcinoma (VSCC).

Methods: Formalin-fixed and paraffin-embedded (FFPE) tissue samples from Danish patients diagnosed with vulvar precancerous lesions or VSCC in the period from 2010 to 2012 were obtained. HPV-DNA detection was carried out by the use of polymerase chain reaction (PCR) using GP5+/GP6+ primers and genotyped by sequencing. A systematic literature search on the PubMed database was performed to investigate the prevalence and genotype distribution worldwide.

Results: In the present study population (n = 149) 52 vulvar high-grade squamous intraepithelial lesions (HSIL), 2 differentiated vulvar intraepithelial neoplasia (dVIN), and 95 VSCC cases were identified. HPV was detected in 85 patients (57.0%). Overall, a higher proportion of the vulvar high-grade precancerous lesions were HPV positive compared to VSCC (83.6% vs. 42.1%, p < 0.001). Additionally, HSIL had a significantly higher HPV-positive rate compared to keratinizing VSCC (84.6% vs. 33.3%, p < 0.001). However, the HPV positivity was comparable between HSIL and non-keratinizing VSCC (84.6% vs. 82.4%, p = 0.825). One dVIN was HPV positive whereas the other was HPV negative. HPV-16 was the most common HPV type (68.2%), followed by HPV-33 (18.8%) and HPV-18 (8.2%).

Conclusions: Most vulvar HSIL and non-keratinizing VSCCs appear to be HPV associated. However, we find a high HPV association in keratinizing VSCC, which needs to be further studied. HPV-16 remains the predominant genotype, but HPV-33 also seems to play a role in the development of VSCC.

1. Introduction

Vulvar cancer is a relatively rare malignancy, accounting for approximately 5.5% of all gynecological cancers worldwide (Baandrup, 2011). However, vulvar squamous cell carcinoma (VSCC) is the most common cancer of the female external genitalia (Judson et al., 2006).

Moreover, in recent decades, the incidence of vulvar cancer and its precursor lesions has been reported to have increased, particularly among younger women (Judson et al., 2006; Van Dyne, 2018; Joura et al., 2000). Most vulvar cancers and precancerous lesions are of squamous cell origin (Tavassoli, 2003). For cancers of the cervix, high-risk HPV (HR-HPV) is detected in nearly 100% of all cases (Walboomers,
monitor potential carryover of viral DNA between samples, an empty paraffin block was processed for every 11th patient tissue sample and was subjected to DNA purification and PCR procedures identical to tissue-containing blocks.

2.2. Histopathological evaluation and diagnosis

Initial diagnosis and subdivision of patient material into VSCC, vulvar basocellular carcinoma (BSC), and severe dysplasia groups were performed by a gynecopathologist (Pathologist 1, ESH). Subsequently an additional pathologist (Pathologist 2, UB) classified the VSCC and severe dysplasia lesions into keratinizing or non-keratinizing (basoloid/warty) VSCC, and vulvar HSIL or dVIN dysplasias. This was carried out in accordance with the 2014 WHO classification and nomenclature system for vulvar cancers and vulvar dysplasias (Kurman et al., 2014).

2.3. HPV detection and genotyping

DNA was extracted from FFPE tissue sections using the RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (FisherScientific) according to the manufacturer's recommendations. Presence of HPV DNA was detected using L1 consensus primers GP5+/GP6+ (De Roda Huroman et al., 1995) in a PCR reaction with the following conditions: 20 mM Tris-HCl pH 8.4, 50 mM KCl, 0.2 mM dNTP mix, 3.5 mM MgCl2, 50 pmol GP5+ primer (TTGTGACTGTGTTGATACATAC), 50 pmol GP6+ primer (GAAAAATACGTGAAATCATTTTC), 200 ng template DNA, 0.5 U Taq DNA polymerase (Thermo Fisher). PCR program: 94°C for 10 min, 50 X (94°C for 1 min, 40°C for 2 min, 72°C for 1.5 min), and 72°C for 4 min. PCR products were visualized on an agarose gel using the FlashGel™ DNA system (Lonza). A sample was only considered HPV positive if a positive PCR band appeared in two independent PCR reactions. The ACTB gene (beta-actin) was used as amplification control in a standard PCR reaction (20 mM Tris-HCl pH 8.4, 50 mM KCl, 0.2 mM dNTP mix, 1.5 mM MgCl2, 6 pmol forward beta-actin primer (ACTCGTCAT-TCTCGGCTT), 6 pmol reverse beta-actin primer (CCCTCTCAGATTCGCTGCTCTTCTC), 100 ng template DNA, 0.5 U Taq DNA polymerase (Thermo Fisher). PCR program: 95°C for 10 min, 40 X (95°C for 1 min, 95°C for 30 s, 72°C for 30 s), and 72°C for 4 min. In the case of beta-actin negative samples, an additional reaction was performed. In the case of two negative beta-actin PCR reactions, the sample was excluded from the study. HeLa cell DNA, containing integrated HPV-18 DNA, was used as a positive control and a no-template control (NTC) was used as a negative control. HPV genotyping was performed by direct sequencing of positive GP5+/GP6+ PCR products. Sequences were aligned against HPV sequences using BLAST from the NCBI homepage and a > 95% identity was considered a positive match.

2.4. Statistical analysis

Parameters such as age, histological subtype, HPV detection and genotyping from vulvar high-grade precancerous lesions and VSCC patient samples were analyzed and compared using IBM® SPSS Statistics 26. Median age and analyses of differences in median age with respect to histological subtypes of VSCC and precursors were investigated using chi-square and Fisher’s exact tests. The interquartile range (IQR) is displayed as the first (Q1) – third (Q3) quartiles. Differences in proportions of HPV positivity and genotyping between vulvar high-grade precancerous lesions and VSCC, and furthermore with respect to histological subtypes, were analyzed using chi-square and Fisher’s exact tests. A value of p < 0.05 was considered significant.

2.5. HPV prevalence in the literature – A systematic literature search

The PRISMA guidelines were used where applicable (Moher et al., 2009).

Search strategy: A systematic literature search was conducted in the
Pubmed database. The following search terms were used: (1) “human papillomavirus and vulvar intraepithelial neoplasia and prevalence”; (2) “human papillomavirus and vulvar high-grade squamous intraepithelial lesion” and “prevalence”; (3) “human papillomavirus and vulvar squamous cell carcinoma and prevalence”. The search was carried out on March 23, 2021.

Study selection: Titles, abstracts, and the methods of all articles were screened in order to identify relevant articles for whole-paper revision. Studies investigating HPV genotype prevalence in vulvar HSIL and/or VSCC samples using PCR, hybrid capture test, and/or in situ hybridization methods were eligible for inclusion. Case reports, duplicate papers, literature reviews, and non-English-language papers were excluded. Studies investigating only a few selected single HPV types (e.g., HPV-16 and HPV-18 only), or that grouped other HPV types together were excluded. Studies investigating <10 patients were excluded. In addition, articles not distinguishing between low-grade squamous intraepithelial lesions and vulvar HSIL were excluded from the study. However, if data on VSCC were present in the affected articles these were still included in the analysis. Study populations described in more than one paper were included only once, and the paper with the most detailed information was used. A flow diagram of the literature search is shown in Supplemental Figure SL. Articles were screened by two authors (ABV and SS).

Data analysis: Information on the number of participants, HPV prevalence, and identified HPV genotypes was recorded and used in the analyses. Data from all studies were combined and the overall HPV prevalence, as well as the prevalence of individual HPV types in vulvar HSIL and VSCC, were calculated. In the case of detection of multiple HPV types in one sample, each detected HPV type was included in the analysis.

3. Results

3.1. Patient samples – Histological classification and age distribution

The involvement of HPV was investigated in Danish vulvar high-grade precancerous lesions and VSCC patient samples. Initial quality control testing of the purified DNA from 156 FFPE patient tissue samples by beta-actin PCR identified three samples that contained poor DNA quality, and therefore led to the exclusion of these patients. Furthermore, the diagnosis of one sample could not be confirmed by pathologist two (UB), leading to the exclusion of an additional patient. Finally, two of the vulvar cancers were classified as basal cell carcinomas and one as a spindle cell and were excluded for the purposes of clarity. In total, 149 of the vulvar cancers were classified as basal cell carcinomas and one as dVIN (3.7%). Patient age ranged from 21 to 89 years with a median age of 69.0 years (IQR = 57.0–79.0). Patients with VSCC were significantly older than patients with vulvar high-grade precancerous lesions (69.0 versus 51.0 years, p < 0.001). There were no significant differences in median age between women with a non-keratinizing VSCC (median age of 74.0 years (IQR = 63.5–81.5)) compared to women with a keratinizing VSCC (median age of 68.0 years (IQR = 55.0–78.25)), p = 0.332 (Table 1).

3.2. HPV-16 and HPV-33 are the most common HPV-types in vulvar high-grade precancerous lesions and VSCC

Overall, 85 patients were found to be HPV positive, corresponding to 57.0% of all included patients (Table 2). Of these, a higher proportion of patients with vulvar precancerous lesions were HPV positive compared to the VSCC patients (83.3% vs. 42.1%; $X^2(1) = 23.88$, p = 0.001). Moreover, when further subdividing the VSCC group into keratinizing and non-keratinizing VSCCs, it was seen that a higher proportion of patients in the non-keratinizing group were HPV positive, compared to patients in the keratinizing group (82.4% vs. 33.3%; $X^2(1) = 13.76$, p < 0.001). There was no difference in HPV positivity between dVIN (one dVIN was HPV positive whereas the other was HPV negative, see Table 2) and vulvar HSIL (50.0% vs. 84.6%, p = 0.308); however, a higher proportion of patients diagnosed with vulvar HSIL were HPV positive compared to patients diagnosed with a keratinizing tumor (84.6% vs. 33.3%; $X^2(1) = 33.02$, p < 0.001). HPV prevalence in vulvar HSIL was comparable to non-keratinizing VSCC (84.6% vs. 82.4%, p = 0.825).

HPV genotype distribution is summarized in Table 3. Overall, HPV-16 was the most commonly detected HPV type, accounting for 68.2% of HPV positive samples, followed by HPV-33 (18.8%) and HPV-18 (8.2%). HPV-31, HPV-67, and HPV-81 were each detected once (1.2%). Among HPV-positive patients, HPV-16 accounted for 73.3% in vulvar high-grade precancerous lesions and 62.5% in VSCC; HPV-33 accounted for 17.8% in vulvar high-grade precancerous lesions and 20% in VSCC, and HPV-18 accounted for 4.4% in vulvar high-grade precancerous lesions and 12.5% in VSCC (Table 3). This makes HPV-33 the second most common HPV type found in both vulvar high-grade precancerous lesions and VSCC in Danish patients.

Furthermore, it appears that HPV-33 seems to be overrepresented in the non-keratinizing VSCC patient group compared to the others (28.6% vs. 15.9% (vulvar HSIL, p = 0.31) and 15.4% (keratinizing VSCC, p = 0.32)) and HPV-18 is overrepresented in the keratinizing VSCC group compared to the other groups (19.2% vs. 4.5% (vulvar HSIL, p = 0.52), and 0% (non-keratinizing VSCC, p = 0.79)). However, these differences were not statistically significant.

| Diagnosis                          | Number of patients | HPV positive |
|-----------------------------------|-------------------|--------------|
|                                   | n (%              |              |              |
| Vulvar high-grade precancerous lesions | 54 (83.3)     |              |
| dVIN                              | 2 (1)            |              |
| Vulvar HSIL                       | 52 (44)          |              |
| VSCC                              | 95 (40)          |              |
| Keratinizing                      | 78 (26)          |              |
| Non-keratinizing                  | 78 (33)          |              |
| Total                             | 149 (85)         |              |

Table 2

HPV prevalence in vulvar high-grade precancerous lesions and VSCC patient samples.

| Diagnosis                          | Number of patients | HPV positive |
|-----------------------------------|-------------------|--------------|
|                                   | n (%              |              |              |
| Vulvar high-grade precancerous lesions | 54 (83.3)     |              |
| dVIN                              | 2 (1)            |              |
| Vulvar HSIL                       | 52 (44)          |              |
| VSCC                              | 95 (40)          |              |
| Keratinizing                      | 78 (26)          |              |
| Non-keratinizing                  | 78 (33)          |              |
| Total                             | 149 (85)         |              |

Table 1

Histology and age distribution of FFPE tissue samples.

| Histology                        | Number of patients | Median age (years) | Age range (years) |
|----------------------------------|--------------------|--------------------|-------------------|
|                                  | n (%)              |                    |                   |
| Vulvar high-grade precancerous lesions | 54 (36)     | 51.0               | 21–89             |
| dVIN                              | 2 (3.7)            | 59.5               | 51–68             |
| Vulvar HSIL                       | 52 (96.3)          | 51.0               | 21–89             |
| VSCC                              | 95 (64)            | 69.0               | 19–97             |
| Keratinizing                      | 78 (82.1)          | 68.0               | 19–90             |
| Non-keratinizing                  | 17 (17.9)          | 74.0               | 47–97             |
| Total                             | 149 (100)          | 62.0               | 19–97             |
3.3. HPV-33 may play an important role in vulvar squamous cell carcinoma development

The study performed on Danish vulvar high-grade precancerous lesions and VSCC patients found that HPV-33 was the second most common HPV type. This distribution of HPV types is different to what is observed in cervical cancer, where HPV-18 is the second most frequently detected HPV type (Clifford et al., 2003). Therefore, we conducted an updated systematic literature search on the prevalence of individual HPV types in vulvar HSIL and VSCC in the PubMed database to support the tendency on a global scale.

The screening procedure is visualized in Supplemental Figure S1. In total, 42 articles fulfilled the inclusion criteria and were included in the analysis. These articles investigated 1286 patients with vulvar HSIL and 3908 patients with VSCC (Table 4). Of these, HPV was present in 1140 (88.6%) vulvar HSIL and 1370 (35.1%) VSCC patients. Among HPV-positive patients, HPV-16 was the most frequently found HPV type accounting for 77.5% in vulvar HSIL and 74.9% in VSCC. This was followed by HPV-33, which was found in 10.2% and 9.3% of HPV-positive vulvar HSIL and VSCC, respectively. Other frequently detected HPV types were HR-HPV types HPV-18 (2.4% in vulvar HSIL; 4.2% in VSCC), HPV-31 (2.8% in vulvar HSIL; 0.9% in VSCC), HPV-45 (0.5% in vulvar HSIL; 1.9% in VSCC), HPV-52 (0.6% in vulvar HSIL; 1.8% in VSCC) and low-risk HPV-6 (2.8% in vulvar HSIL; 0.9% in VSCC).

4. Discussion

This study confirms the important role of HPV, predominantly HPV-16 and to a lesser extent HPV-33 and HPV-18, in the development of vulvar high-grade precancerous lesions and VSCC, and the noticeable differences in HPV positivity in VSCC histological subtypes.

Overall, the HPV prevalence was significantly higher in vulvar high-grade precancerous lesions (83.3%) than VSCC (42.1%) patient samples, matching the findings in former studies (de Sanjose, 2013; De Vuyst et al., 2009; Smith et al., 2009; Gillison et al., 2008; Koh et al., 2017). However, the prevalence of HPV was the same in vulvar HSIL and non-keratinizing VSCC (84.6% vs. 82.4%), underscoring that HSIL could be HPV-dependent precursors for non-keratinizing VSCC because of their similar HPV positive rates. Interestingly, we and others find a high proportion of the keratinizing tumors (33.3%) to be HPV positive (in our study with a tendency towards HPV-18 overrepresentation and HPV-33 underrepresentation), compared to the non-keratinizing VSCCs (Rakislova, 2017; Alonso, 2011; Santos, 2006; Siriauungkul, 2014; Gargano, 2012; Sutton et al., 2008). This challenges the two-pathway model; suggesting a potential overlap between the histotypos of VSCC or that HPV might play a role in both pathways. This notable amount of keratinizing tumors that are HPV positive stands in contrast to previous meta-analysis by De Vuyst et al. from 2009 (De Vuyst et al., 2009) and Faber et al. from 2017 (Faber, 2017), in which only 13.2% were HPV positive. The reason for the higher level of HPV-positive keratinizing tumors and the divergence between the histotypos of VSCC and the association of HPV is unknown. However, geographical, cultural, or ethnic differences could possibly explain this, since, for instance in a large-scale study by de Sanjose et al. (de Sanjose, 2013) the contribution of European samples resulted in a higher level of HPV-positive keratinizing tumors in younger patients. Furthermore, this

### Table 3
Prevalence and genotype distribution of HPV infections in vulvar high-grade precancerous lesions and VSCC patient samples.

| HPV type | Total vulvar high-grade precancerous lesions | dVIN | Vulvar HSIL | Total VSCC | Keratinizing | Non-keratinizing | Total |
|----------|---------------------------------------------|------|------------|------------|-------------|----------------|-------|
|          | n (% )                                      | n (%)| n (%)      | n (%)      | n (%)       | n (%)          | n (%) |
| Total    | 54 (46)                                     | 2 (1) | 52 (48)    | 95 (81)    | 78 (68)     | 17 (15)        | 149 (121) |
| HPV positive | 45 (83)                                    | 1 (50) | 44 (86)   | 40 (82)    | 26 (53)     | 14 (28)        | 85 (57) |
| HPV-16   | 33 (73)                                     | 0 (0) | 33 (75)    | 25 (50)    | 16 (32)     | 9 (18)         | 58 (32) |
| HPV-18   | 2 (4.4)                                     | 0 (0) | 2 (4.5)    | 5 (12.5)   | 5 (12.5)    | 0 (0)          | 8 (2.8) |
| HPV-31   | 1 (2.2)                                     | 0 (0) | 1 (2.3)    | 0 (0)      | 0 (0)       | 0 (0)          | 1 (1.2) |
| HPV-33   | 8 (17.8)                                    | 1 (100) | 7 (15.9)  | 8 (20)     | 4 (10.0)    | 4 (10.0)       | 18 (8.8) |
| HPV-67   | 0 (0)                                       | 0 (0) | 0 (0)      | 1 (25)     | 0 (0)       | 1 (25)         | 1 (1.2) |
| HPV-81   | 0 (0)                                       | 0 (0) | 0 (0)      | 1 (25)     | 1 (38)      | 0 (0)          | 1 (1.2) |
| Risk     | Undetermined                                | 1 (2.2) | 0 (0)    | 1 (2.3)    | 0 (0)      | 0 (0)          | 1 (1.2) |

### Table 4
HPV genotype distribution in vulvar HSIL and VSCC from a systematic literature search.

| HPV type | Vulvar HSIL | VSCC |
|----------|-------------|------|
|          | (n = 1286)  | (n = 3908) |
| HPV positive | 1140 (88.6) | 1370 (35.1) |
| HPV negative | 146 (11.4)  | 2538 (64.9) |
| HPV6 | 32 (2.5)     | 12 (0.3)    |
| HPV11 | 10 (0.8)     | 2 (0.1)     |
| HPV16 | 883 (67.8)   | 1026 (26.3) |
| HPV18 | 27 (2.1)     | 58 (1.5)    |
| HPV31 | 32 (2.5)     | 12 (0.3)    |
| HPV33 | 116 (9.0)    | 128 (3.2)   |
| HPV35 | 3 (0.2)      | 10 (0.3)    |
| HPV45 | 6 (0.5)      | 26 (0.7)    |
| HPV52 | 7 (0.5)      | 24 (0.6)    |
| HPV56 | 8 (0.6)      | 13 (0.3)    |
| HPV58 | 5 (0.4)      | 12 (0.3)    |
| HPV59 | 10 (0.8)     | 1 (0.0)     |
| Other HPV types | 53 (4.1) | 65 (1.7) |
| -HR | 11 (0.8)      | 13 (0.3)    |
| -Probably HR | 22 (1.7) | 29 (0.7) |
| -LR | 17 (1.3)      | 18 (0.5)    |
| -Unclassified | 3 (0.2) | 5 (0.1) |
| Undetermined | 22 (1.7) | 27 (0.7) |

%: the percentage of single HPV types of all included vulvar HSIL or VSCC patients, (%): the percentage of single HPV types of HPV-positive patients. Numbers do not add up because of multiple infections. HR: high risk; LR: low risk. HR is defined as HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 68. Probable HR is defined as HPV types 26, 30, 53, 66, 67, 69, 70, 73, and 82. LR is defined as HPV types 2, 6, 11, 27, 42, 43, 44, 54, 61, 62, 72, 74, 83, 84, and 89, and unclassified are types 1, 55, 114, and 120. The classification of HPV types is based on (de Villiers et al., 2004; Doorbar, 2012). In this literature search, “other HPV types” includes (n vulvar HSIL + VSCC) HR: HPV-39 (2.6%), HPV-51 (8.4%), Probable HR: HPV-5 (0.1%), HPV-26 (3.3%), HPV-30 (1.1%), HPV-53 (2.5%), HPV-66 (2.7%), HPV-67 (4.1%), HPV-68 (1.3%), HPV-69 (0.2%), HPV-70 (2.4%), HPV-73 (4.3%), HPV-82 (3.2%), and HPV-68 or73 (1%), LR: HPV-2 (0.1%), HPV-27 (0.1%), HPV-40 (1.0%), HPV-42 (4.2%), HPV-43 (1.0%), HPV-44 (1.1%), HPV-54 (2.3%), HPV-61 (2.0%), HPV-62 (0.1%), HPV-72 (2.0%), HPV-74 (0.7%), HPV-83 (3.0%), HPV-84 (1.0%), and HPV-89 (0.2%); Unclassified: HPV-1 (0.1%), HPV-55 (0.2%), HPV-64 (0.2%), HPV-114 (1.0%), and HPV-120 (0.2%).
difference might also be explained by age (de Sanjose, 2013).

The HPV genotype distribution is different in vulvar cancer compared to cervical carcinoma (Clifford et al., 2003) and to a smaller degree other anogenital cancers (De Vuyst et al., 2009). Like the previous studies, we confirm that HPV-16 is the predominant HPV type in both vulvar high-grade precancerous lesions and VSCC, and that other HPV types such as HPV-33, HPV-18, and HPV-31 also play a role in the carcinogenesis, although to a lesser extent (De Vuyst et al., 2009; Smith et al., 2009; Pils, 2017; Gargano, 2012; Insigna et al., 2008). While the summarized HPV-33 prevalence in the literature search is lower compared to our observation (10.2% in other studies vs. 17.8% in our study for vulvar high-grade precancerous lesions and 9.3% in other studies vs. 20% in our study for VSCC), the literature search supports the idea that HPV-33 appears to play a notable role in vulvar high-grade precancerous lesions and VSCC development. This is in contrast to the carcinogenic importance that has previously been ascribed to HPV-33 based on its association with cervical cancer (Clifford et al., 2003). The same tendency has been observed in oropharyngeal cancer, where HPV-33 is the second most commonly detected HPV type, after HPV-16, which is found in the majority of the cases (Castellsaguet, 2016). The special tropism of HPV-33 for vulvar tissue and the role of the different HPV types in the malignant transformation in vulva cancer and precursors are however not known. Potential factors such as interaction between proteins expressed by each HPV genotype, alternative splicing of the HPV genome, the nature of the epithelium and perhaps local immunity might explain the HPV type tropism (Egawa et al., 2015).

Since HPV type distributions and prevalence differ between cervical and vulvar cancers, and the current HPV vaccines are mainly designed and developed to protect against cervical cancers, it is essential to monitor how HPV vaccines perform in protecting against other HPV-related cancer types, including vulvar cancers. Moreover, it is important to determine the etiology behind the different histological subtypes of VSCC to see the effect of the HPV vaccines. Several studies have demonstrated that the current HPV vaccines very efficiently protect against cervical lesions (Group, 2007; Ault, 2007; Villa, 2005; Huh, 2017). It may be expected that the 9-valent (Gardasil 9, Merck & Co., USA) vaccine should protect against most HPV-associated vulvar HSIL and VSCC lesions (Garland, 2018). In contrast, the other two, the quadrivalent (Gardasil and Silgard, Merck & Co., USA) and the bivalent (Cervarix GSK, UK) vaccines, may display poorer protection since HPV-33 is not targeted (Garland, 2018). However, since the majority of HPV positive vulvar HSIL and VSCC are positive for HPV-16 and HPV-18 (~85%) all HPV vaccines may decrease the burden (Gillison et al., 2008). A reduction in the risk of genital warts and vulvar precancerous lesions after quadrivalent HPV vaccination has been found (Joura, 2012). Additionally, a Danish study by Rasmussen et al. (2020) found that after HPV-vaccine introduction, the incidence of vulvar precancerous lesions in young women decreased, indicating a protective role. The prevention was not seen among patients with VSCC, however, as the median age at diagnosis of VSCC in our study is 69 years, the effect in VSCC will be delayed (Rasmussen et al., 2020). Based on the controversial origin of the HPV-negative VSCC tumors, it will be highly interesting to see if the prevalence of these remains unaffected in the post-vaccination era, or if declines, thus indicating an HPV-associated etiology.

Moreover, we narrowed our search by including “prevalence”, which entails a risk of missing some studies. However, we believe that our literature search, despite these limitations, gives a representative image of the current prevalence of HPV in vulvar high-grade precancerous lesions and VSCC, which is in line with the findings by the review by Faber et al. from 2017 (Faber, 2017). The strengths of our study are that we include a relatively high number of patient samples, thus increasing the power of the current study. Moreover, the confirmation of diagnosis was performed by two independent pathologists. Finally, the findings were supported on a global scale by a systematic literature search.

5. Conclusion

In conclusion, HPV is implicated in approximately 40% of VSCC and 80% of vulvar high-grade precancerous lesions in Danish patient samples, which is in line with other studies. We find, however, a large proportion of keratinizing tumors, normally considered HPV independent, to be HPV positive. Finally, HPV-16 is found in the majority of cases followed by HPV-33, which is the second most common HPV type. This was confirmed on a global scale by a literature search. Based on the controversial origin of the HPV-negative VSCC tumors, it will be highly interesting to see if the prevalence of these remains unaffected in the post-vaccination era, or if declines, thus indicating an HPV-associated etiology.

CRediT authorship contribution statement

Annemarie Brusen Villadsen: Formal analysis, Funding acquisition, Investigation, Writing – original draft, Writing – review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gore.2021.100828.

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