Human papillomavirus genotypes and HPV16 E6/ E7 variants among patients with genital cancers in Vietnam

Trang Thi Thu Pham, Xiuqiong Bi, Huyen Thi Thanh Hoang, Azumi Ishizaki, Mai Thi Phuong Nguyen, Cuong Hung Nguyen, Hung Phi Nguyen, Thuc Van Pham, and Hiroshi Ichimura

Received: May 15, 2018. Accepted: June 12, 2018
Published online: June 29, 2018
DOI:10.7883/yoken.JJID.2018.206

Advance Publication articles have been accepted by JJID but have not been copyedited or formatted for publication.
Human papillomavirus genotypes and HPV16 E6/ E7 variants among patients with genital cancers in Vietnam

Trang Thi Thu Pham¹, Xiuqiong Bi¹, Huyen Thi Thanh Hoang², Azumi Ishizaki¹, Mai Thi Phuong Nguyen³, Cuong Hung Nguyen³, Nguyen Phi Hung⁴, Thuc Van Pham³,
Hiroshi Ichimura¹*  

¹ Department of Viral Infection and International Health, Graduate School of Medical Sciences/Advanced Preventive Medical Sciences, Kanazawa University, Kanazawa, Japan, 13-1, Takaramachi, Kanazawa, Ishikawa, 920-8640, Japan  
² National Hospital of Obstetrics and Gynecology, Hanoi, Vietnam, No. 43 Trang Thi street, Hoan Kiem district, Ha Noi city, Viet Nam  
³ Haiphong University of Medicine and Pharmacy, Haiphong, Vietnam, No. 72 Nguyen Binh Khiem street, Ngo Quyen district, Hai Phong city, Viet Nam  
⁴ Vietnam National Cancer Hospital, Hanoi, Vietnam, No. 43, Quan Su street, Hoan Kiem district, Ha Noi city, Viet Nam  

Correspondence: Hiroshi Ichimura, M.D., Ph.D.  
Department of Viral infection and International Health, Graduate school of Medical Sciences, and Advanced Preventive Medical Sciences, Kanazawa University, Japan.  
13-1, Takaramachi, Kanazawa, Ishikawa, 920-8640, Japan  
Tel: +81-76-265-2228, Fax: +81-76-234-4237  
E-mail: ichimura@med.kanazawa-u.ac.jp

KEYWORDS: HPV, HPV16 sublineages, HPV16 E6/E7 variations, genital cancer  
Running title: HPV infection in genital cancer patients
Trang Thi Thu Pham
金沢大学医薬保健学総合研究科医学系ウイルス感染症制御学
〒920-8640 石川県金沢市宝町 13 番 1 号
電話：076-265-2229、Fax：076-234-4237

Xiuqiong Bi 畢 袖晴
金沢大学医薬保健学総合研究科医学系ウイルス感染症制御学
〒920-8640 石川県金沢市宝町 13 番 1 号
電話：076-265-2229、Fax：076-234-4237

Azumi Ishizaki 石崎有澄美
金沢大学医薬保健学総合研究科医学系ウイルス感染症制御学
〒920-8640 石川県金沢市宝町 13 番 1 号
電話：076-265-2229、Fax：076-234-4237

Hiroshi Ichimura 市村 宏
金沢大学医薬保健学総合研究科医学系／先進予防医学研究科
ウイルス感染症制御学
〒920-8640 石川県金沢市宝町 13 番 1 号
SUMMARY: We previously reported human papillomavirus type 52 (HPV52) as the most prevalent high-risk genotype in non-cancer individuals in Vietnam. This study aimed to evaluate HPV genotypes and HPV16 E6 and E7 (E6/E7) gene variations in Vietnamese patients with genital cancers. Biopsy samples were collected from 124 Vietnamese patients with genital cancers (20 with vaginal, 50 with vulvar, and 54 with penile cancer). The HPV-DNA was amplified and genotyped, and HPV16 E6/E7 genes were compared with those previously reported for women with a normal cervical cytology (N=23). The HPV-DNA was detected in 80.6% (100/124) of the cancer patients (80.0% of vaginal, 82.0% of vulvar, and 79.6% of penile), with HPV16/18 in 86.0% (86/100) and HPV52 in 7.0% (7/100) of the HPV-positive samples. The HPV-DNA prevalence and HPV genotype distribution did not significantly differ among the genital cancer patients (both $P = 0.95$). Cancer patients harbored significantly less of the HPV16 A4 sublineage (34.8% vs. 82.6%, $P < 0.0001$) and HPV16 E7 29S (36.4% vs. 87.0%, $P = 0.0002$) than the women with normal cytology. Our results indicate HPV16/18 account for more than 85% of genital cancers in Vietnam, and the HPV16 sublineage A4 containing E7 29S may be less oncogenic.
INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted infection and it causes cancers, including cervical, anogenital, and oropharyngeal (1,2). Recently, it was reported that globally 100% of cervical, 78.0% of vaginal, 24.9% of vulvar, 88.0% of anal, 50.0% of penile, and 30.8% of oropharyngeal cancers are attributable to HPV infection (3).

HPV is a double-stranded DNA virus in the *Papillomaviridae* family, and more than 200 HPV genotypes have been identified based on the whole genome sequence (4). Twelve HPV genotypes, HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59, are designated as “high-risk HPV” based on their association with cancers (5,6). HPV16 and HPV18 account for about 70% of cervical cancers and 60–87% of non-cervical cancers, such as vaginal and anal cancers, worldwide (3,6). HPV16 is reported to be the most oncogenic and responsible for more than half of the papillomavirus-induced anogenital cancers (6,7).

The HPV16 E6 and E7 (E6/E7) gene products are oncoproteins. The strong relationship between HPV16 and cancers, especially cervical cancer, has led to studies on virological factors associated with cancer development as well as the oncogenic mechanisms of the HPV16 E6/E7 gene products. A variety of HPV16 E6/E7 variants were reported and classified as European, Asian, African 1 and 2, Asian-American, and North-American lineages (8-11). Recently, HPV16 variants were classified as A (A1–3: formerly European lineage and A4: Asian), B (African 1), C (African 2), and D (D1: Asian-American and D2: North-American) lineages based on the whole-genome sequences (1,12,13). The HPV16 sublineages A4 and D2 were reportedly associated with an increased risk of cervical cancer (11,14); whereas, another study found no significant difference in the distribution of HPV16 A1–3 and A4 sublineages among the cervical
intraepithelial neoplasia stages 1, 2/3, and cervical cancer (15). Moreover, the HPV16 E6/E7 variants, such as E6 L83V in Danish, Italian, and Swedish populations (16-18) and E6 D25E in Chinese, Thai, and Japanese populations (19-21), have been reportedly associated with an elevated-risk of cervical carcinoma; although, this association was not found in other studies (9,22-24).

In Vietnam, HPV52 was the most common high-risk genotype in commercial sex workers with a normal cervical cytology and men with symptoms of sexually transmitted infections (STI) (25,26), and the HPV16 Asian lineage was most common in the commercial sex workers (27). However, the prevalence of the HPV genotypes and HPV16 variants in cancer patients are not well known in Vietnam. Thus, we conducted this study to elucidate the HPV detection frequencies, genotype distribution, and the HPV16 E6/E7 variations in Vietnamese patients with vaginal, vulvar, and penile cancers.

**MATERIALS AND METHODS**

**Subjects and samples:** This was a cross-sectional study conducted at the National Cancer Hospital, Hanoi, Vietnam from March to December 2013. The subjects were 124 patients with genital cancers, including 20 with vaginal (median age 61.5 years, range: 35–88 years), 50 with vulvar (median age 58 years, range: 30–87 years), and 54 with penile cancer (median age 54 years, range: 28–83 years). There was no significant difference in the age distribution among the three cancer groups ($P = 0.169$). Two sections of cancer tissue were surgically collected from each patient and immediately frozen in liquid nitrogen for future use. One sample was used for
the histopathological diagnosis at the pathological department of the hospital and the other for genetic analysis of HPV infection at Kanazawa University in Japan. All participants provided written informed consent. This study was designed in accordance with the World Medical Association Declaration of Helsinki, the Japanese Ethics Guidelines for Human Genome/Gene Analysis Research, and the Vietnamese Ethics Guidelines. The protocol was approved by the Ethics Committees of Kanazawa University, Japan, and Hanoi Medical University, Hanoi, Vietnam.

The HPV genotype data obtained from 130 commercial sex workers with a normal cytology (25) and 48 male patients with STI (26) and HPV16 E6/E7 sequence data from 23 women with a normal cytology (27) were retrieved from the previous reports and used for comparative analyses.

**DNA extraction and HPV PCR:** Genomic DNA was extracted from the biopsy samples collected from the genital cancer patients using the GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma, St. Louis MO, USA) according to the manufacturer’s instructions. The HPV DNA was amplified by PCR with modified GP5+/6+ primers as described previously (27,28). The PCR was repeated for the HPV PCR-negative samples with original GP5+/GP6+ primers (29).

**HPV genotyping:** The HPV PCR-positive samples were genotyped using the 21 HPV GenoArray Diagnostic Kit (Hybribio limited, Hong Kong), which can detect 13 high-risk and eight low-risk HPV genotypes (26). We conducted population sequencing with Bigdye Terminator v1.1 on the 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) for the samples that were HPV-PCR positive, but genotyping negative. HPV genotypes were classified as high-risk (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), probably high-risk (HPV 68),
possibly high-risk (HPV 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, 97), or low-risk (HPV 6, 11, 40, 42, 43, 44, and so on) (5).

**HPV16 E6/E7 variant analysis:** The HPV16 E6/E7 genes were amplified with type-specific primers, as described previously (27,30). The amplified products were analyzed by population sequencing on an ABI PRISM 3130 and/or a 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA) with Big Dye Terminator v1.1 (Applied Biosystems, Foster City, CA). The obtained sequences were analyzed by GENETYX (Ver.9.1.0, GENETYX CORPORATION, Tokyo, Japan) and MEGA6. The HPV16 lineages and sublineages were determined by phylogenetic tree analysis using the neighbor-joining method with reference sequences retrieved from GenBank. The E6/E7 positions with two or more nucleotides at one site were considered variations.

**Statistical Analysis:** The Mann-Whitney U test, Fisher’s exact probability test or chi-squared test, and univariate and multivariate logistic regression analysis were used for statistical analyses (IBM SPSS Statistics V22.0). P values <0.05 were considered statistically significant.

**RESULTS**

**HPV genotypes detected in cancer patients:** HPV DNA was detected in 80.6% (100/124) of the genital cancer patients (80.0% [16/20] of vaginal, 82.0% [41/50] of vulvar, and 79.6% [43/54] of penile cancer patients) and the detection frequencies did not significantly differ among the three cancer groups (P = 0.95). Thirteen HPV genotypes were found in the 100 HPV DNA-positive patients (Fig. 1). HPV16 was most commonly found in all
three cancer patient groups, followed by HPV18. We found HPV16 and/or HPV18 (HPV16/18) in 86.0% (86/100) of the cancer patients (87.5% [14/16] of vaginal, 87.8% [36/41] of vulvar, and 83.7% [36/43] of penile cancer patients). In all the HPV DNA-positive cancer patients, at least one high-risk HPV was found; although we also found possibly high- or low-risk HPV genotypes in six of them (four penile and two vulvar cancer patients). HPV52 was found in seven (7.0%) of the cancer patients; however, it was found together with other high-risk HPV genotypes, such as HPV16/18 in four patients. The HPV genotype distribution did not significantly differ in the three cancer groups (P = 0.95).

We compared the detection frequencies of representative, high-risk HPV genotypes, such as HPV16/18, with HPV52, which is most prevalent in non-cancer individuals in Vietnam (25, 26). We found HPV16/18 was present significantly more in cancer patients (86.0% vs. 32.0%, P < 0.0001) than in non-cancer individuals, especially HPV16 (69.0% vs. 14.6%, P < 0.0001); whereas HPV52 was found significantly less in cancer patients (7.0% vs. 25.2%, P = 0.0002) (Table 1).

**HPV16 lineages and sublineages:** Of the 69 HPV16-positive samples, 66 (95.6%) were successfully sequenced for both the E6/E7 genes (Table 2). Phylogenetic analyses based on the E6/E7 genes revealed that 57 (86.4%) belonged to lineage A, one (1.5%) to lineage C, and eight (12.1%) to lineage D (Fig. 2). Although HPV16 lineage A was found slightly less in the genital cancer patients than in women with normal cytology (86.4% vs. 95.7%, P = 0.285), the distribution of HPV16 lineages did not significantly differ between the two groups (A/C/D: 95.7%/4.3%/0% in normal cytology group, P = 0.155). However, as shown in Fig. 3, all cancer patient groups had significantly more A1–3 sublineages than the women with normal cytology (vaginal cancer 60.0%, vulvar cancer 58.6%, and penile cancer 40.7% vs. 13.0%, all P < 0.05),
but less A4 sublineage (vaginal cancer 20.0%, vulvar cancer 31.0%, and penile cancer 44.4% vs. 82.6%, all \( P < 0.01 \)). Overall, the cancer patients had more A1–3 sublineages than women with normal cytology (51.5% vs. 13.0%, \( P = 0.0013 \)), but less A4 sublineage (34.8% vs. 82.6%, \( P < 0.0001 \)).

**HPV16 E6/E7 variations:** As shown in Table 3, the HPV16 E6 and E7 genes have nucleotide variations in 16 and nine positions, respectively. Of the 16 E6 positions, three (94G/A, 178G/A/T, 350T/G) showed a significantly different proportion of each variation between the genital cancer patients and the control women with a normal cytology. The 94A and 350G were found significantly more in the cancer patients than the normal cytology controls (39.4% vs. 13.0%, \( P = 0.048 \); 18.1% vs. 0.0%, \( P = 0.032 \), respectively). The 178T (coding 25D, glutamic acid) and 178A (coding 25E, aspartic acid) were found significantly more in the cancer patients than in the women with a normal cytology (28.9% vs. 4.4%, \( P = 0.016 \); 36.4% vs. 13.0%, \( P = 0.036 \), respectively); whereas, the 178G (coding 25E) was found significantly less (34.8% vs. 82.6%, \( P < 0.0001 \)) in the cancer patients.

Of the nine E7 positions with nucleotide variations, three (647G/A, 666G/A, 846C/T) showed significantly different proportions between the cancer patients and normal cytology controls. The E7 647A/666A/846T was found significantly more in the cancer patients than in the women with normal cytology (63.6% vs. 13.0%, \( P < 0.0001 \); 42.4% vs. 13.0%, \( P = 0.012 \); 65.2% vs. 17.4%, \( P < 0.0001 \), respectively). Of these positions, 647G/A led to an amino acid change (29S/N, serine/asparagine). When the variations in E6/E7 genes were analyzed with multivariate regression, E7 647G/A (29S/N amino acid) was found to be an independent factor for genital cancer. The 647A (29N) occurred significantly more and the 647G (29S) significantly
less in the cancer patients compared with the normal cytology controls ($P = 0.0003$, 29S: OR 0.09, 95% confidence intervals 0.02–0.32).

**DISCUSSION**

In this study, we investigated the prevalence of HPV and its genotypes in 124 patients with genital cancers, including vaginal, vulvar, and penile cancers, for the first time in Vietnam. We found HPV DNA in 80.6% of the genital cancer patients, in 80.0% of vaginal, 82.0% of vulvar, and 79.6% of penile cancer patients, with no significant difference in the detection rate among the three cancer groups. This finding suggests that HPV accounts for the majority of these genital cancers in Vietnam. Furthermore, HPV DNA detection rates in the vulvar and penile cancer patients were much higher in the current study (82.0% and 79.6%, respectively) than previously reported (24.9% and 50.0%, respectively) (3). This discrepancy might be due to geographical differences and/or methodological differences, such as sample collection and the HPV detection methods. Further study is needed to clarify the reason(s).

We previously reported that HPV52 was the most common high-risk genotype in commercial sex workers with a normal cervical cytology and men with symptoms of STI in Vietnam (25, 26). In the current study, however, we found HPV16/18 DNA in 86.0%, with HPV16 DNA in 69.0%, of the HPV DNA-positive cancer patients; whereas, HPV52 DNA was found only in 7.0% of them. Our results indicate that vaginal, vulvar, and penile cancers are mainly caused by HPV16/18 in Vietnam, and that the majority of these genital cancers can be prevented with the current vaccines targeting HPV16/18. Our results also indicate that HPV16 is
much more oncogenic than HPV52, which is supported by a previous study that found HPV52 failed to induce cell immortalization ex vivo (31).

In the current study, the HPV16 sublineages A1–3 (formerly European types) were found significantly more in the genital cancer patients than in the women with a normal cervical cytology (51.5% vs. 13.0%, $P < 0.01$), whereas sublineage A4 (formerly Asian type) was found significantly less in the cancer patients (34.8% vs. 82.6%, $P < 0.01$). These results suggest that HPV16 sublineages A1–3 may be more oncogenic than the sublineage A4 in inducing genital cancers in the Vietnamese population. However, these findings are inconsistent with the previous studies that reported the sublineages A4 and D2 were associated with an increased risk of cervical cancer (11,14), or that found no significant difference in the distribution of HPV16 sublineages A1–3 and A4 between samples from different stages of cervical intraepithelial neoplasia (15). The discrepancy in the oncogenicity of A4 sublineage between this study and others (11,14,15) may be attributed simply to the regional difference in the ethnic groups. However, it is noted that the distributions of the HPV16 sublineages A1–3 and A4 in the Vietnamese genital cancer patients (51.5% and 34.8%, respectively) are similar to those found in Chinese cervical cancer patients (54.0% and 45.6%, respectively) (14). In contrast, the distribution of HPV16 sublineages A1–3 and A4 in Vietnamese women with a normal cytology (13.0% and 82.6%, respectively) was the opposite of that in Chinese women with a normal cytology (67.1% and 32.9%, respectively) (14). Considering that it takes several decades for HPV to develop cancers, these findings suggest a possible chronological change of circulating HPV16 sublineages in Vietnam, such as increasing frequency of sublineage A4 and decreasing frequency of sublineages A1–3. This may be the reason why there is a discrepancy in the "oncogenicity" of the A1–3 and A4 sublineages between the studies in Vietnam and China. Thus,
the distribution of HPV16 A1–3 and A4 sublineages should have been compared in cancer patients vs. non-cancer individuals several decades ago; however, this may not be easy to do now.

Further analyses of the HPV16 E6/E7 variants revealed that only the E7 647A/G (29N/S) variation was an independent risk factor for genital cancers, as E7 29S was found significantly less in the genital cancer group than in the normal cytology group ($P = 0.0003$, OR: 0.09). In addition, all HPV16 strains that belonged to sublineage A4 in the current study had the E7 29S variation. These results suggest that the HPV16 strains with E7 29S may be less oncogenic. However, our result is inconsistent with previous studies that found the HPV16 E7 N29S variant more frequently in cancerous lesions of the uterine cervix than in noncancerous lesions in Taiwanese and Korean women (32,33), no significant difference in the frequency of E7 N29S between cancer and normal subjects (22,34), and the E7 29S variant had a transforming potential similar to the prototype E7 29N variation (35,36). As discussed above, we should be careful to choose the control group when investigating the oncogenicity of HPV16 lineages and sublineages by comparing their prevalence between cancer patients and non-cancer individuals. Besides, host factors such as human leukocyte antigen types may also play a role in these discordant observations. Further studies would be needed to clarify this.

Recently, apolipoprotein B editing complex (APOBEC3) family members that are cytidine deaminases reportedly induced G-to-A and/or C-to-T hypermutations in HPV16 DNA in a cell-culture model, suggesting a possible role of APOBEC3 family in HPV-related oncogenesis (37). In this study, HPV16 E6 G94A and E7 G666A/C846T variations were found significantly more in the genital cancer patients than in the women with a normal cervical cytology. Considering that APOBEC3G prefers a dinucleotide context of CpC for editing and APOBEC3 A/B/C/D/F/H prefer TpC dinucleotide context (38), and that the HPV16 E6 94G and E7
666G/846C are at the APOBEC3 family's preferring dinucleotide contexts for editing, these variations may be the traces of APOBEC3 family's function on the HPV16 E6/E7 genes during the development of the genital cancers, although these variations were not independent risk factors for the cancers (Table 3).

Previously, HPV16 E6 D25E variation was reportedly found more in cervical cancer patients than non-cancer individuals in Japan and Thailand (20,21). In this study, however, the HPV16 E6 D25E variation was found less in the genital cancer patients than in the women with a normal cytology (71.2% vs. 95.6%, \( P = 0.016 \)). HPV16 E6 25E is encoded by both HPV16 E6 178A and 178G. In Vietnam, HPV16 E6 178A-encoding E6 25E variation that belongs to sublineages A1–3 was found significantly more in the cancer patients than in the women with a normal cytology (36.4% vs. 13.0%, \( P = 0.036 \)), which is consistent with the previous studies (20,21). On the other hand, the 178G-encoding E6 25E variation that belongs to sublineage A4, was found significantly less in the cancer patients than the normal cytology women (34.8% vs. 82.6%, \( P < 0.0001 \)). Thus, the discordant observation between the previous studies (20,21) and the current study may be due to the difference in the proportion of HPV16 E6 178A and 178G variants circulating in Vietnam, Japan (20) and Thailand (21).

In conclusion, 80.6% of vaginal/vulvar/penile cancer patients were positive for HPV DNA, and 86% of them had the HPV16/18 genotypes. The HPV16 strains with E7 647A (29N, mainly sublineages A1–3) may be more oncogenic, whereas those with E7 647G (29S, mainly A4 sublineage) may be less oncogenic, at least for vaginal, vulvar, and penile cancers in Vietnam.
Acknowledgements: The authors are grateful to the patients who participated in this study for their invaluable support through allowing the use of their samples, to the staff of the National Cancer Hospital, Hanoi, Vietnam, who made enormous contributions to this work. This study was supported in part by the Japan Society for the Promotion of Science [Grant-in-Aid for Scientific Research (C) 25461509 and 26460743].

Conflict of Interest

There are no potential conflicts of interests related to this article.
REFERENCES


FIGURE LEGENDS

Fig. 1. The HPV genotypes detected in vaginal, vulvar, and penile cancer patients positive for HPV-DNA (n=100). The HPV genotype distribution did not differ among the three cancer groups (P = 0.95).

Fig. 2. HPV16 lineages and sublineages based on E6 and E7 sequences from genital cancer patients and women with a normal cytology. The GenBank accession numbers of the reference sequences used are shown in this figure. The bootstrap values > 700 are shown.

Fig. 3. Comparison of HPV16 lineages and sublineages found in patients with genital cancers and women with a normal cytology. The detection frequencies of HPV16 A1–3 and A4 differed between the cancer patients and women with normal cytology, but not among the cancer groups. * P < 0.05, ** P < 0.01 for HPV 16 sublineages in cancer patients compared with women with a normal cytology.
Table 1. Frequencies of HPV16, 18 and 52 in HPV-DNA-positive cancer and non-cancer individuals

<table>
<thead>
<tr>
<th>HPV types</th>
<th>Females</th>
<th>Males</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaginal/vulvar Cancer n = 57</td>
<td>Normal cytology n = 130</td>
<td>Penile cancer n = 43</td>
</tr>
<tr>
<td></td>
<td>n = 57</td>
<td>n = 130</td>
<td>n = 43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P values</td>
</tr>
<tr>
<td>16 and/or 18</td>
<td>50 (87.7)*</td>
<td>43 (33.1) &lt; 0.0001</td>
<td>36 (83.7)</td>
</tr>
<tr>
<td>16 only</td>
<td>40 (70.2)</td>
<td>23 (17.7) &lt; 0.0001</td>
<td>29 (67.4)</td>
</tr>
<tr>
<td>16+52</td>
<td>2</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>16+others**</td>
<td>1</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>16+18</td>
<td>4</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>16+18+52</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>16+18+others</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>18 only</td>
<td>14 (24.6)</td>
<td>23 (17.7) 0.277</td>
<td>16 (37.2)</td>
</tr>
<tr>
<td>18+others</td>
<td>0</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>18+52</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>52 only</td>
<td>6 (10.5)</td>
<td>36 (27.7) 0.010</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>52+others</td>
<td>2</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>13</td>
<td>0</td>
</tr>
</tbody>
</table>

*: number of individuals (%); **: high-risk HPV types other than HPV16, 18, and 52; Normal cytology: female sex workers with a normal cervical cytology (25); STI: male patients with symptoms of sexually transmitted infections (26); Cancer: patients with vaginal, vulvar, or penile cancer; Non-cancer: female sex workers with a normal cervical cytology and male patients with symptoms of sexually transmitted infections.
Table 2. Variations in the HPV16 E6/E7 genes in patients with vaginal/vulvar/penile cancers (n = 66)

<table>
<thead>
<tr>
<th>Nucleotides</th>
<th>AA</th>
<th>E6 region</th>
<th>Nucleotides</th>
<th>AA</th>
<th>E7 region</th>
<th>Overall No. (Sub) % lineage</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 9 1 1 1 1 1 1 1 2 2 2 3 3 4 5</td>
<td>6 6 7 7 7 7 8 8 8 8</td>
<td>a l l l l l l l</td>
<td>2</td>
<td>A1-3 (n = 34)</td>
<td>51.5</td>
<td></td>
</tr>
<tr>
<td>4 5 0 3 4 4 7 8 7 8 8 8 9 3 5 0 3</td>
<td>6 6 3 4 8 9 2 4 4 4</td>
<td>a l l l l l l l</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 5 0 3 4 4 7 8 7 8 8 8 9 3 5 0 3</td>
<td>6 6 3 4 8 9 2 4 4 4</td>
<td>a l l l l l l l</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g a l g c g t t a t a g c t a a</td>
<td>a g l l l l l l l</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a g</td>
<td>a</td>
<td>25E</td>
<td>a</td>
<td>a</td>
<td>63A</td>
<td>2</td>
</tr>
<tr>
<td>a g</td>
<td>A</td>
<td>25E</td>
<td>a</td>
<td>G</td>
<td>58S</td>
<td>3</td>
</tr>
<tr>
<td>a g</td>
<td>A</td>
<td>25E</td>
<td>a</td>
<td>G</td>
<td>29S</td>
<td>1</td>
</tr>
<tr>
<td>g</td>
<td>a</td>
<td>25E</td>
<td>c</td>
<td>29S</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>A</td>
<td>25E/27R/64N</td>
<td>c</td>
<td>29S</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>c T G T</td>
<td>a</td>
<td>T</td>
<td>g</td>
<td>G</td>
<td>c</td>
<td>29S</td>
</tr>
<tr>
<td>c T</td>
<td>a</td>
<td>g</td>
<td>T</td>
<td>G</td>
<td>c</td>
<td>29S</td>
</tr>
<tr>
<td>10I/14D/78Y</td>
<td>e</td>
<td>g</td>
<td>29S</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14H/78Y/83V</td>
<td>c</td>
<td>c</td>
<td>29S</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| AA: amino acid; Nucleotides: capital letters indicate nonsynonymous variations; small letters indicate synonymous variations; No.: number of cancer patients.
Table 3. Comparison of HPV16 E6/E7 variations between the genital cancer patients and the women with a normal cytology.

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>Cancer patients (n = 66)</th>
<th>Normal cytology (n = 23)</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>E6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>94 G/ A</td>
<td>K</td>
<td>65.1/ 34.9</td>
<td>87.0/ 13.0</td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td>95 G/ A</td>
<td>R</td>
<td>22.7/ 77.3</td>
<td>13.0/ 87.0</td>
<td>0.383</td>
<td></td>
</tr>
<tr>
<td>109 C/ T</td>
<td>2 F</td>
<td>1.5/ 98.5</td>
<td>4.3/ 95.7</td>
<td>0.452</td>
<td></td>
</tr>
<tr>
<td>132 G/ T</td>
<td>10 R/ I</td>
<td>98.5/ 1.5</td>
<td>95.7/ 4.3</td>
<td>0.452</td>
<td></td>
</tr>
<tr>
<td>143 C/ G</td>
<td>14 Q/ D</td>
<td>98.5/ 1.5</td>
<td>95.7/ 4.3</td>
<td>0.452</td>
<td></td>
</tr>
<tr>
<td>145 G/ T</td>
<td>14 Q/ H</td>
<td>86.3/ 13.7</td>
<td>95.7/ 4.3</td>
<td>0.285</td>
<td></td>
</tr>
<tr>
<td>178 G/A/ T</td>
<td>25 E/ E/ D</td>
<td>34.8/ 36.4/ 28.9</td>
<td>82.6/ 13.0/ 4.4</td>
<td><strong>0.0004</strong></td>
<td></td>
</tr>
<tr>
<td>E7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>647 G/ A</td>
<td>29 S/ N</td>
<td>36.4/ 63.6</td>
<td>87.0/ 13.0</td>
<td><strong>&lt; 0.0001</strong></td>
<td>0.0003</td>
</tr>
<tr>
<td>666 G/ A</td>
<td>35 E</td>
<td>57.6/ 42.4</td>
<td>87.0/ 13.0</td>
<td><strong>0.012</strong></td>
<td></td>
</tr>
<tr>
<td>732 C/ T</td>
<td>57 F</td>
<td>11.9/ 88.1</td>
<td>0.0/ 100.0</td>
<td>0.106</td>
<td></td>
</tr>
<tr>
<td>748 G/ T</td>
<td>63 A/ S</td>
<td>3.0/ 97.0</td>
<td>0.0/ 100.0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>E7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>789 C/ T</td>
<td>76 I</td>
<td>13.6/ 86.4</td>
<td>4.3/ 95.7</td>
<td>0.285</td>
<td></td>
</tr>
<tr>
<td>795 G/ T</td>
<td>78 T</td>
<td>13.6/ 86.4</td>
<td>4.3/ 95.7</td>
<td>0.285</td>
<td></td>
</tr>
<tr>
<td>822 G/ A</td>
<td>87 L</td>
<td>3.0/ 97.0</td>
<td>0.0/ 100.0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>843 C/ T</td>
<td>94 C</td>
<td>11.9/ 88.1</td>
<td>21.7/ 78.3</td>
<td>0.307</td>
<td></td>
</tr>
<tr>
<td>846 C/T</td>
<td>95 S</td>
<td>34.8/ 65.2</td>
<td>82.6/ 17.4</td>
<td><strong>&lt; 0.0001</strong></td>
<td></td>
</tr>
</tbody>
</table>

OR (95%CI): odds ratio (95% confidence intervals); Normal cytology: female sex workers with a normal cervical cytology who were tested HPV16 positive and successfully analyzed the HPV16 E6/E7 genes (27).
Fig. 1
Fig. 2
Fig. 3

- HPV16 sublineages
- Vaginal cancer
- Vulvar cancer
- Penile cancer
- All cancers
- Normal cytology

%