Detection of Human Papillomavirus Types 6 and 11 in Pubic and Perianal Hair from Patients with Genital Warts

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Genital human papillomavirus (HPV) types 6 and 11 are of clinical importance due to their role in the development of anogenital warts. A pilot study was performed to investigate whether DNAs from HPV types 6 and 11 are present in hairs plucked from the pubic and perianal regions and eyebrows of patients with genital warts at present and patients with a recent history of genital warts. Genital HPV DNA was detected in 9 of 25 (36%) pubic hair samples and in 11 of 22 (50%) perianal hair samples by the CPI/CPIfPCR. After sequencing of 17 of 20 samples, HPV type 6 or 11 was detected in 6 of 25 (24%) hair samples from the pubis and 8 of 22 (36%) hair samples from the perianal region. These types were not detected in plucked eyebrow hairs. In contrast, the HPV types associated with epidermodysplasia verruciformis were detected in similar proportions (62%) in both samples of pubic and eyebrow hairs. Moreover, HPV type 6 and 11 DNAs were detected in pubic hairs plucked from two patients who had been successfully treated and who did not have any lesions at the time of hair collection; this finding is an argument that HPV DNA may persist in this region. The presence of genital HPV types in plucked pubic and perianal hair suggests that there is an endogenous reservoir for HPV which may play a role in the recurrences of genital warts.

The genital human papillomavirus (HPV) types 6 and 11 are of clinical importance due to their role in the development of anogenital warts or condylomata acuminata. Genital warts are often refractory to treatment and tend to recur. The reason for the high recurrence rate is unclear; they may be caused either by reinfection or by activation or reactivation of HPV present in an endogenous reservoir.

Recently, we have demonstrated that DNA of a wide spectrum of epidermodysplasia verruciformis (EV)-associated HPV types (6) is present in hairs plucked from sun-exposed sites of both immunosuppressed and immunocompetent persons (5, 5a). These data strongly suggest that a considerable part of the general population is subclinically infected with one or more EV-associated HPV types.

In another study, transcripts of papillomavirus early genes E6 and E7 have been demonstrated in the hair follicles of the pelage after high-pressure injection of cottontail rabbit papillomavirus in rabbits (8). The primary target cells of the cottontail rabbit papillomavirus were reported to colocalize with clonogenic keratinocytes with stem cell properties, suggesting that the virus may be located in the stem cells of the skin.

We hypothesized that genital HPV types 6 and 11 are present in hair follicles of patients with genital warts or a history of genital warts. To test this hypothesis, we performed a pilot study to investigate whether DNAs from HPV types 6 and 11 are present in hair follicles of patients with genital warts or a history of genital warts. To test this hypothesis, we performed a pilot study to investigate whether DNAs from HPV types 6 and 11 are present in hair follicles of patients with genital warts or a history of genital warts. To test this hypothesis, we performed a pilot study to investigate whether DNAs from HPV types 6 and 11 are present in hair follicles of patients with genital warts or a history of genital warts. To test this hypothesis, we performed a pilot study to investigate whether DNAs from HPV types 6 and 11 are present in hair follicles of patients with genital warts or a history of genital warts. To test this hypothesis, we performed a pilot study to investigate whether DNAs from HPV types 6 and 11 are present in hair follicles of patients with genital warts or a history of genital warts. To test this hypothesis, we performed a pilot study to investigate whether DNAs from HPV types 6 and 11 are present in hair follicles of patients with genital warts or a history of genital warts.

**MATERIALS AND METHODS**

**Sampling.** Five to eight hairs were plucked per site from pubic and perianal regions and from the eyebrows of 25 patients with genital warts at the time of sampling (see Table 1 for details) by using new pairs of tweezers for each site. Only hairs with hair follicles were collected. They were snap frozen and were stored at 70°C until analysis. Informed consent was obtained prior to sampling.

**DNA isolation.** The snap-frozen hairs were put into L6 buffer containing guanidium isothiocyanate (Fluka, Buchs, Switzerland) and diatoms (Janssen Biochemica, Beerse, Belgium) (4), after which DNA was isolated by the guanidium isothiocyanate-diatom method (4). Parts of genital warts were digested in a solution containing 300 μg of proteinase K per ml, 0.5% (wt/vol) sodium dodecyl sulfate, 50 mM Tris-HCl, and 50 mM EDTA (pH 8.0) at 56°C overnight. After heat inactivation of the proteinase K (10 min at 95°C), 1 part was added to 9 parts of L6 lysis buffer containing diatoms (4), after which DNA was isolated by the guanidium isothiocyanate-diatom method (4). DNAs from hair samples and genital wart specimens were isolated and analyzed on separate days.

**PCR primers and amplification.** All primers were purchased from Perkin-Elmer, Nieuwerkerk aan de IJssel, The Netherlands. For the detection of mucosal HPV types with degenerate primers, CPI/CPIfPCR was performed as described earlier (9). DNA from an HPV type 16 (HPV-16)-containing cervical cell line (SiHa) was used as a positive control. For the detection of EV-associated HPV types, the nested M′H′PCR with the degenerate primer pairs M′ and H′ specific for EV-associated HPV types was performed (5). The 3′ primer CP70 was modified into CP70′ 5′-AA(C/T) TTT (G/T) CC(C/T) A(A/G) A(G/A) G(T/C) TGA TC 3′. The annealing site of the CP70′ primer corresponds to positions 7273 to 7298 in the HPV-8 genome. To determine whether the samples were adequate for PCR analysis, a PCR for an α-myb gene fragment was performed (5).

Five to 10% of the isolated DNA was used as input in each PCR. Negative controls (water instead of DNA) were included between every two samples and were subjected to the same procedure to which the samples were subjected. These samples remained negative in all three PCRs.

**Sequence analysis.** The amplimers were cloned with the TA Cloning Kit (Invitrogen, San Diego, Calif.) and were processed in accordance with the procedure described by the manufacturer of the kit. Independent clones were analyzed on an automatic sequencer as described earlier (5). The PCR and HPV typing were performed blindly.

**Putative new EV-associated HPV types.** Novel putative new types were detected by the nested PCR for EV-associated HPV types. For reference, all putative new types found in the present study are listed in the NCBI GenBank database under the indicated accession numbers: HPV X1, L38918 (2); HPV X2, L38922 (2); HPV X7, U35660 (1); and HPV X13, AF054873. Others had previously submitted sequences with 96 to 100% homologies with putative new types X16 and X19. The accession numbers of the homologous types for HPV types X16 and X19 are L38388 (10) and AF042006, respectively.
TABLE 1. HPV DNA in plucked hairs from patients with genital warts or a history of genital warts

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Note</th>
<th>PCR (EV-associated HPV)</th>
<th>HPV type detected by PCR</th>
<th>HPV type detected in:</th>
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<tbody>
<tr>
<td>GW tissue</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>M</td>
<td>26</td>
<td>10</td>
<td>—</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>24</td>
<td>11</td>
<td>—</td>
<td>Neg</td>
<td></td>
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<tr>
<td></td>
<td>F</td>
<td>55</td>
<td>6</td>
<td>—</td>
<td>Pos</td>
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<tr>
<td></td>
<td>M</td>
<td>57</td>
<td>6</td>
<td>—</td>
<td>Neg</td>
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<tr>
<td></td>
<td>F</td>
<td>26</td>
<td>6</td>
<td>—</td>
<td>Pos</td>
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<td></td>
<td>M</td>
<td>34</td>
<td>1</td>
<td>—</td>
<td>Neg</td>
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<tr>
<td></td>
<td>M</td>
<td>64</td>
<td>6</td>
<td>—</td>
<td>Neg</td>
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<td></td>
<td>F</td>
<td>25</td>
<td>6</td>
<td>—</td>
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<td>M</td>
<td>34</td>
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<td></td>
<td>M</td>
<td>34</td>
<td>1</td>
<td>—</td>
<td>Neg</td>
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</tr>
<tr>
<td></td>
<td>M</td>
<td>34</td>
<td>1</td>
<td>—</td>
<td>Neg</td>
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Patients with GW or a history of GW in plucked hairs

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (yr)</th>
<th>Note</th>
<th>PCR (EV-associated HPV)</th>
<th>HPV type detected by PCR</th>
<th>HPV type detected in:</th>
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Patients with GW but no genital HPV in plucked hairs (n = 12)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (yr)</th>
<th>Note</th>
<th>PCR (EV-associated HPV)</th>
<th>HPV type detected by PCR</th>
<th>HPV type detected in:</th>
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Abbreviations and symbols: GW, genital warts; M, male; F, female; Pos, positive by PCR; Neg, negative by PCR; HIV, human immunodeficiency virus; RTR, renal transplant recipient; NA, not available for more PCR; —, not collected; ND, not done.

Superscript roman numerals indicate the number of sequenced clones.

Mean ± standard deviation.

The age range for the group was 19 to 57 years.

EV-associated HPV results indicate the number of sequenced clones.

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RESULTS

HPV-6 and -11 DNAs in plucked genital hairs from patients with genital warts. Hairs were collected from pubic and perianal sites and from the eyebrows of patients with genital warts. Subsequently, the samples were analyzed for HPV DNA by PCR. HPV was detected by the CPI/CPIIg PCR in 9 of 25 (36%) hair samples plucked from the pubic region and 11 of 22 (50%) hair samples plucked from the perianal region. After sequencing of DNAs from 17 of 20 samples, HPV type 6 or 11 was detected in 6 of 25 (24%) samples from the pubic region and 8 of 22 (36%) samples from the perianal region. Frequently, the same HPV type was found in both the pubic and perianal hair samples within one individual. For the three patients tested, the same HPV type was found in the genital hair samples and the genital warts. In some patients, other or additional HPV types were detected: genital HPV types 16, 35, and 59 and cutaneous HPV type 10. In contrast, no genital HPV types were detected in the eyebrow hair samples (n = 24) by the CPI/CPIIg PCR except in that from one immunosuppressed renal transplant patient, whose eyebrow hair sample contained HPV-45 DNA. The HPV-36 DNA detected in this sample is one of the EV-associated HPV types.

HPV in plucked genital hairs after treatment of the genital warts. After collection of the hairs (time zero), the genital warts were locally treated. To study whether HPV types 6 and 11 persist in the hairs of the genital region after treatment, hairs were collected 4 to 6 months after treatment from 7 patients (Table 1, patients 1 to 7) who had been found to be positive for HPV type 6 or 11 DNA (Table 1, time zero). Five of seven patients had been successfully treated and did not show any genital warts, whereas two patients still had perianal lesions. The latter two patients (patients 2 and 5) still had HPV DNA (type 6 and/or type 11) in their genital hairs. Samples from three patients who had been successfully treated for genital warts were negative by the PCR. Most remarkably, however, samples of plucked pubic hairs from two patients (patients 3 and 7) who had been successfully treated and who did not show any genital warts contained HPV types (HPV-6 or HPV-11) identical to those detected when the patients had such lesions (time zero).

EV-associated HPV DNA in hairs plucked from the genital region. Previously, we have demonstrated that EV-associated HPV DNA can be detected in eyebrow hairs (5) by a nested PCR with degenerate primers specific for the EV-associated HPV types. This finding was confirmed by the detection of EV-associated HPV DNA in 8 of 13 (62%) eyebrow hair samples derived from patients with condylomata in the present study. As mentioned above, no genital HPV types were detected in eyebrow hairs by the CPI/CPIIg PCR with the exception of the sample from the immunosuppressed, renal transplant recipient patient (Table 1, patient 7).

In the present study, EV-associated HPV DNA was also demonstrated in genital hair samples. Eight of 13 (62%) hair samples from the pubic region were positive for EV-associated HPV DNA, whereas EV-associated HPV DNA could be detected in only 1 of 12 (8%) hair samples from the perianal region. Furthermore, EV-associated HPV DNA was not found in the three genital wart samples.

DISCUSSION

HPV types 6 and 11 are frequently detected in genital warts, and they are believed to cause such lesions. After treatment, genital warts frequently recur. In this study, we have demonstrated the presence of genital HPV types 6 and 11 in plucked hairs from the pubic and/or perianal regions of 9 of 25 patients (36%) with genital warts at the time of sampling. Moreover, HPV type 6 and 11 DNAs were detected in pubic hairs plucked from two patients who had been treated successfully and who had no clinical evidence of genital warts at the time of hair collection. The detection of HPV DNA in pubic and/or perianal hairs of patients who have been successfully treated for genital warts is an argument for the persistence of HPV DNA. Genital warts may occur by reactivation of HPV that persists in hair follicles but may also be induced by reinfection.

To compare the presence of the genital HPV types in plucked genital hairs with the presence of HPV types in hair samples from other locations, eyebrow hairs were collected simultaneously with the genital hairs. Genital HPV types were rarely detected in the eyebrow hair samples. The only exception was the finding of HPV-45 DNA in the eyebrow hair sample from a renal transplant recipient, but this may have been related to immunosuppressive therapy. The apparent absence of genital HPV types in plucked eyebrow hairs suggests that the genital types are mainly restricted to the genital area, although some studies have suggested that genital HPV types are present in skin lesions (7). By contrast, in the present study, EV-associated HPV DNA was detected not only in the eyebrow hairs but also in similar proportions in the pubic hair samples. Similar EV-associated HPV types (HPV types 38 and X7) were found in two of four patients whose genital and eyebrow hairs contained EV-associated HPV DNA.

In this study, we demonstrate that genital HPV types can be detected in plucked pubic and perianal hairs from patients with genital warts, suggesting that there is an endogenous reservoir for HPV which may play a role in the recurrence of genital warts in these patients. Even though all patients had genital warts at the time of entrance into the study, HPV type 6 and 11 DNAs were found only in the genital hair samples from 9 of 25 (36%) patients. This finding might be an underestimation, because only five to eight hairs were sampled from each site. To establish the correct prevalence, more elaborate studies are warranted.

It would be interesting to use this technique to monitor the presence of HPV in these patients after treatment for genital warts or even to study the natural history of HPV infection in the general population. To fully understand the consequences of our finding, the prevalence and incidence of HPV types 6 and 11 should be estimated in the general population. Moreover, the incidence of genital warts must be studied in a cohort with and without HPV type 6 and 11 DNAs in their pubic and perianal hairs. The outcomes of such a study may change current opinions on the occurrence of recurrences, the routes of transmission, and methods for prevention.

ACKNOWLEDGMENTS

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REFERENCES


