Genomic distribution of beta papillomaviruses in single eyebrow hair samples and pools of eyebrow hair samples

K. Fujs Komloš, B. J. Kocjan, A. Šterbenc, M. M. Jelen, K. Seme, P. Košorok, M. Poljak

KEY WORDS

HPV, beta papillomaviruses, hair follicle, genotype distribution

ABSTRACT

Human beta papillomaviruses (beta-HPVs) are frequently detected in hairs and the majority of people are infected with multiple beta-HPV genotypes. This study was conducted to investigate for the first time the distribution of beta-HPV genotypes in single hair specimens and to estimate the contribution of a single hair to the beta-HPV profile obtained from a specimen made of multiple hairs pooled together. A total of 85 eyebrow hair specimens, representing 64 single hairs and 21 pools of hairs, obtained from 21 immunocompetent individuals, were tested using a reverse-line blot-based beta-HPV genotyping assay that allows identification of 25 different beta-HPVs. Overall, beta-HPV DNA was detected in 82/84 (97.6%) samples. The great majority of hair pools (19/21; 90.5%) contained multiple beta-HPVs, the mean number of identified beta-HPV genotypes per hair pool was 5.2 (ranging from 1 to 12). In individual hairs, the great majority of individual hairs (43/63; 68.3%) contained multiple beta-HPVs, the mean number of identified beta-HPV genotypes was 4 (ranging from 1 to 12). Overall, HPV-23 was the most prevalent genotype, followed by HPV-24 and HPV-38. A comparison of beta-HPV genotype distribution in pooled hair specimens and in at least one individual hair within a single patient revealed that 5/20 patients had a complete match between the number and profile of identified genotypes, 2/20 patients had the same/similar number of HPV genotypes but different genotype profile, 9/20 patients had more HPV genotypes identified in pools than in the majority of individual hairs and 4/20 patients had at least one individual hair with more HPV genotypes identified than in the corresponding pool. Our results suggest that beta-HPVs are unevenly distributed over the eyebrows and even pools made of several hairs do not necessarily provide information on the whole spectrum of HPV genotypes present in eyebrows.

Introduction

Cutaneous human papillomaviruses (HPV) belonging to genus *Betas* (beta-HPV) were originally associated with the development of cutaneous squamous cell carcinoma in patients suffering from a rare autosomal recessive hereditary disorder called *epidermodysplasia verruciformis* (1). An etiological role in the

development of precancerous lesions and squamous cell carcinoma of the skin in organ-transplant patients, as well as in the immunocompetent population, has also subsequently been suggested (1-6). In addition, several studies have shown that beta-HPV can be detected in both immunocompetent and immunosuppressed individuals, not only in various cutaneous lesions but also in hairs plucked from different parts of the body, suggesting that hair follicles are an important endogenous reservoir of cutaneous HPVs (6-9). Interestingly, it has recently been shown with the use of highly discriminatory beta-HPV genotyping assays that hair follicles are frequently, if not always, infected with multiple beta-HPV genotypes (10, 11). However, since hair follicle specimens usually used in prevalence studies consist of multiple individual hairs pooled together in a single sample, the distribution of beta-HPV genotypes in single hairs and the contribution of an individual hair to the beta-HPV profile of pooled hairs remain largely unknown.

In the present study, we systematically explored and compared beta-HPV genotype profiles of individual and pooled eyebrow hair specimens obtained from 21 immunocompetent individuals from Slovenia. The detection and genotyping of beta-HPVs was done using a reverse-line blot-based HPV genotyping assay able to identify 25 different beta-HPV genotypes in a single specimen.

Material and methods

Eyebrow hairs were plucked from 21 immunocompetent male and female patients (age range 22-54 years; mean age 35.8 years) treated for anogenital warts. Altogether, 21 pools of hairs (consisting of 3-8 hairs) and 64 single hair specimens were collected (Table 1). A pair of sterile tweezers and disposable gloves were used for each individual. Only eyebrow hairs containing hair follicles were used. Samples were stored at -80°C until further processing.

HPV DNA from eyebrow specimens was extracted using a High Pure PCR Template Preparation Kit (Roche diagnostics GmbH, Mannheim, Germany), following the protocol for nucleic acid purification from mammalian tissue. DNA was eluted in a final volume of 50 μl and stored at -20°C until use.

The quality of each DNA sample was verified by real-time PCR amplification of a 268-bp fragment of the human beta-globin gene using PC04 and GH20 primers, as described previously (12). Detection and genotyping of beta-HPVs was performed using an RHA skin (beta) HPV assay (Diassay BV, Rijswijk, The Netherlands), which enables simultaneous identification of 25 different beta-HPV genotypes, including HPV-5, -8, -9, -12, -14, -15, -17, -19, -20, -21, -22, -23, -24, -25, -36, -37, -38, -47, -49, -75, -76, -80, -92, -93, and -96. The assay was performed according to the manufacturer's instructions; for PCR amplification, 5 μl of each sample was used per 25 μl reaction.

Results

A 286-bp fragment of the beta-globin gene was successfully amplified from 84/85 (98.8%) samples included in the study. A sample containing a single eyebrow hair with negative internal control amplification was excluded from further study (individual no. 16, Table 1). Overall, beta-HPV DNA was detected in 82/84 (97.6%) samples. Interestingly, both beta-HPV negative samples were obtained from the same individual (individual no. 16, Table 1). Specimens positive for the universal beta-HPV probe only (a positive signal indicates the presence of an unspecified (unknown) beta-HPV(s)), were excluded from genotype prevalence analysis.

An overview of our results is presented in Table 1 and the prevalence of individual beta-HPV genotypes in Figure 1. As shown in Table 1, beta-HPV DNA was detected in all 21 hair pool specimens: the great majority of hair pools (19/21; 90.5%) contained multiple beta-HPVs, one pool contained a single beta-HPV (patient no. 1, Table 1) and one hair pool was positive only for unknown beta-HPV(s) (patient no. 16, Table 1). HPV-23 and HPV-24 were the most prevalent genotypes, followed by HPV-9 (Figure 1). The mean number of identified beta-HPV genotypes per hair pool was 5.2 (ranging from 1 to 12).

In individual hairs, beta-HPV DNA was detected in 61/63 (96.8%) samples (Table 1). As shown in Table 1, the great majority of individual hairs (43/63; 68.3%) contained multiple beta-HPVs, eight hairs contained a single beta-HPV and 10 individual hairs were positive for unknown beta-HPV(s). The mean number of identified beta-HPV genotypes per individual hair was 4 (ranging from 1 to 12). The most prevalent genotype in individual hairs was HPV-23, followed by HPV-36 and HPV-38. The mean number of detected beta-HPV genotypes per patient was 6.9 (ranging from 2 to 18) when all beta-HPV profiles from different hairs obtained from individual patient were taken together.

Overall, HPV-23 was the most prevalent genotype, followed by HPV-24 and HPV-38; these three genotypes together accounted for 23.3% of all identified beta-HPVs.

A comparison of beta-HPV genotype distribution in pooled hair specimens and in at least one individual hair within a single patient revealed that 5/20 patients had a complete match between the number and profile of identified genotypes (patients no. 10, 14, 15, 19 and 20; Table 1), 2/20 patients had the same/similar

number of HPV genotypes but different genotype profile (patients no. 8 and 18; Table 1), 9/20 patients had more HPV genotypes identified in pools than in the majority of individual hairs (patients no. 2, 3, 6, 9, 11, 12, 13, 17 and 21; Table 1) and 4/20 patients had at least one individual hair with more HPV genotypes identified than in the corresponding pool (patients no. 1, 4, 5 and 7; Table 1).

Discussion

To the best of our knowledge, this is the first study to investigate the distribution of beta-HPV genotypes in single hair specimens and the first to have estimated the contribution of an individual hair to the beta-HPV profile in a pool made of multiple hairs.

Beta-HPV DNA was detected in eyebrow hair specimens of all 21 patients included in the study. This is similar to the results of previous studies that have investigated the prevalence of beta-HPVs in immunocompetent populations, indicating that beta-HPVs are widely (ubiquitously) present in humans (6, 10, 11). All 25 beta-HPVs that were targeted by the genotyping assay used in the present study were detected, suggesting a variety of beta-HPV genotypes is circulating in our country. However, the total spectrum of detected beta-HPV genotypes was very probably underestimated in the present study, since at least 36 novel putative beta-HPVs exist that were not covered by our genotyping method (13). HPV-23 was the most prevalent beta-HPV genotype identified in both single and pooled hair specimens (Figure 1).

The great majority of eyebrow hair specimens contained multiple beta-HPVs; however, the number of genotypes per specimen was generally greater in pooled hair specimens. We detected a much higher rate of multiple beta-HPV infections in our study than was observed in a recently published study, which showed that 69% of immunocompetent individuals and immunocompromised patients are infected with two or more beta-HPV genotypes (10). The most reasonable explanation for this discrepancy would be that the DNA used in our PCR amplification mixture was about twice as concentrated as that used in the other study (14).

Previous studies that have investigated the presence of beta-HPVs in hair samples from different parts of the body, including scrotal, pubic and eyebrow areas, have used pooled hair samples consisting of multiple hairs. In this study, we showed that HPV DNA can be successfully extracted and further analyzed for the presence of beta-HPV even from a single hair. However, Table 1 clearly indicates that only five patients (no. 10, 14, 15, 19 and 20) had identical beta-HPV genotype profiles in pooled hairs and in at least one of the investigated single hairs. Nine patients had more beta-HPVs detected in pools, while four patients had a wider beta-HPVs spectrum detected in at least one single hair. Our results suggest that beta-HPVs are unevenly distributed over the eyebrows and even pools made of several hairs do not necessarily provide information on the whole spectrum of HPV genotypes present in eyebrows. However, it must also be kept in mind that PCR-amplification of several viral targets in one reaction can lead to selective and preferable amplification of particular beta-HPVs that amplify more efficiently and/or are present in higher amounts, consequently producing conflicting results.

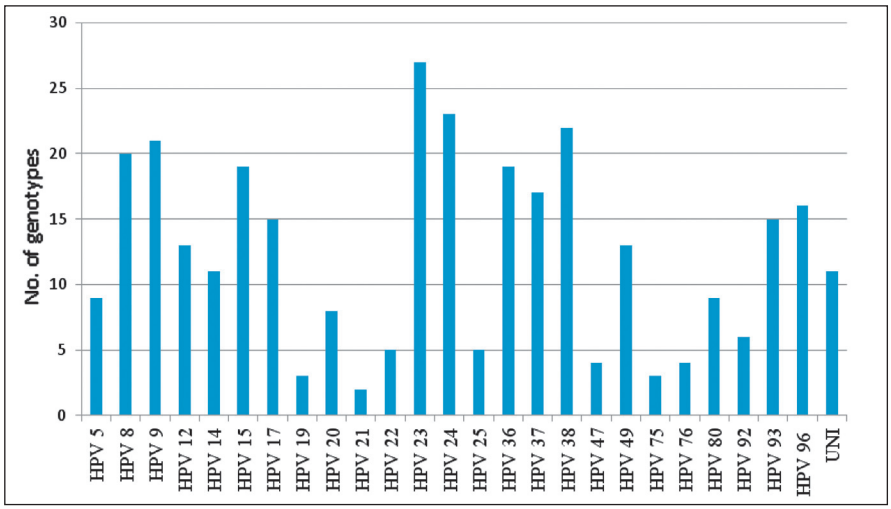

Figure 1. Number of detected individual beta-HPV genotypes.

Table 1: Distribution of beta-HPV genotypes in individual eyebrow hair samples and pools of eyebrow hair samples. Samples positive for universal beta-HPV genotype are indicated by the letter U and by X in relation to the number of identified beta-HPV genotypes. Italicized beta-HPV genotypes are shared between different samples from the same patient. NT: negative internal control amplification.

Individual	Sex	Age (yr)	No. hairs	Beta PV types	No. types
1	M	38	5	80	1
			1	U	X
			1	U	X
			1	8+15+36	3
2	F	47	4	*36*+49+75+*96*	4
			1	*96*	1
			1	*36*	1
			1	U	X
			1	U	X
3	F	54	5	*38*+*49*+75+*96*	4
			1	*49*+*96*	2
			1	*38*+*96*	2
			1	*49*+*96*	2
4	M	22	3	*9*+*15*+24+47	4
			1	23+93	2
			1	*9*+12+14+*15*+38+96	6
			1	U	X
5	F	22	5	9+14+*20*+23+80+93+*96*	7
			1	5+12+15+*20*+47+49+92+*96*	8
			1	U	X
			1	U	X
6	M	38	4	*9*+19+*23*+24+*38*	5
			1	*23*	1
			1	*23*	1
			1	*9*+*38*	2
7	F	23	4	37+*80*	2
			1	12+15+47+*80*	4
			1	36+*80*	2
			1	U	X
8	M	26	8	*9*+*15*+17+*21*+*23*+*24*+25+37+47+*49*+*76*+*93*	12
			1	*8*+*9*+*12*+*15*+*19*+*23*+*24*+*36*+*49*+*76*+*92*+*93*	12
			1	*8*+*9*+*12*+*15*+*19*+*23*+*24*+*36*+*49*+*76*+*93*+*96*	12
			1	*9*+*12*+*15*+*21*+*23*+*24*+*36*+*49*+*76*+*93*+*96*	11
9	M	53	5	12+*15*+*24*	3
			1	17+*24*	2
			1	*15*+*36*	2
			1	*36*	1
10	M	45	6	*23*+*38*+*49*	3
			1	*23*+*38*+*49*	3
			1	*23*+*38*+*49*	3
			1	*23*+*38*	2

11	M	24	4	8+*15*+23	3
			1	14+36	2
			1	U	X
			1	*15*+80	2
12	M	35	5	5+*17*+*22*+24+37	5
			1	*17*+36	2
			1	*22*	1
			1	12	1
13	F	31	5	*8+9+12+15+20+23+24+37+38*+49+*80*	11
			1	*12+15+20+23+37*	5
			1	*8+9+15+20+23+24+37+38+80*+93	10
			1	*8+9+15+20+23+24+37+38+80*	9
14	M	24	7	*8+15+38*	3
			1	*8+15+38*	3
			1	*8+38*	2
			1	*8+38*	2
15	F	38	3	*8+23*	2
			1	*23*	1
			1	*8+23*	2
			1	*8+23*	2
16	M	28	4	U	X
			1	NEG	0
			1	NEG	0
			1	NT	/
17	M	47	3	*5*+8+9+*24*+*37*+75	6
			1	*5+20+24+37*	4
			1	*20+24+37*	3
			1	*5+37*	2
18	F	41	3	*14+17+22+24+36+96*	6
			1	*14+17+24+36+96*	5
			1	9+*14+17+22+24+36*	6
			1	*14+17+22+24+36*	5
19	M	46	6	*5+8+9+17+23+24+25+36+37+38+92+93*	12
			1	*5+8+17+23+24+25+36+37+38+92+93*	11
			1	*5+8+9+17+23+24+25+36+37+38+92+93*	12
			1	*5+8+9+17+23+24+25+36+37+38+92+93*	12
20	F	35	5	*9+14+37+38+93+96*	6
			1	*9+14+37+38+93+96*	6
			1	*9+14+93+96*	4
			1	*9+14+93*	3
21	M	35	3	8+*12*+*17*+23	4
			1	U	X
			1	*12+17*	2
			1	*12+17*	2

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AUTHORS' ADDRESSES

Kristina Fujs Komloš, BSc, Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1105 Ljubljana, Slovenia

Boštjan J. Kocjan, PhD, same address

Anja Šterbenc, same address

Mateja M. Jelen, BSc, same address

Assoc. Prof. Katja Seme, MD, PhD, same address

Assist. Prof. Pavle Košorok, MD, PhD, Medical centre IATROS, Parmova 51b, Ljubljana, Slovenia

Prof. Mario Poljak, MD, PhD, Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1105 Ljubljana, Slovenia. E-mail: mario.poljak@mf.uni-lj.si. Phone: +386 1 543 74 53