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Prevalence and type spectrum of human papillomaviruses in healthy skin samples collected in three continents

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In order to investigate whether previous findings of ubiquitous skin papillomavirus infection in Caucasians apply to populations from other parts of the world, skin swab samples from Bangladesh, Japan, Ethiopia and Zambia were analysed in parallel with Swedish samples. The prevalence of HPV DNA in the material from Bangladesh was 68 %, Japan 54 %, Ethiopia 52 %, Zambia 42 % and Sweden 70 %. A great multiplicity of genotypes was demonstrated by the finding of 88 HPV types or putative types in 142 HPV DNA-positive samples in total. Double or multiple genotypes were frequently found in the same sample. The most prevalent HPV type was HPV-5, with an overall prevalence of 6.5 %. This was also the only type that was found in samples from all of the countries in the study. The results presented show that commensal skin HPV infections have a worldwide distribution with a very broad spectrum of genotypes.

INTRODUCTION

Human papillomaviruses (HPVs) are small epitheliotropic DNA viruses that can induce cutaneous and mucosal lesions, and certain high-risk HPV types are the causative agents of anogenital cancers (zur Hausen, 1996). Epidermodysplasia verruciformis (EV) is a rare hereditary skin disease manifesting as multiple warts. A broad variety of HPV types, which have been referred to as EV HPVs, e.g. HPV-5, -8, -9, -12, -14, -15, -17 and -19–25 (Kremsdorf *et al.*, 1984; Orth *et al.*, 1978), has been found in these patients. Some skin HPV types, mainly HPV-5 and HPV-8, have been found in skin cancer lesions, for example in EV patients (Jablonska & Majewski, 1994; Orth *et al.*, 1979) and in immunosuppressed patients (Bens *et al.*, 1998; Berkhout *et al.*, 2000; de Villiers *et al.*, 1997), and have therefore been suggested to be high-risk HPVs for these patients. However, in recent years it has been found that skin HPV types are widely spread among humans, giving rise to subclinical infections generally without causing warts or other lesions (Antonsson *et al.*, 2000; Astori *et al.*, 1998; Boxman *et al.*, 1997).

The GenBank accession numbers of new sequences reported in this paper are AY009879, AY009880, AY009883, AY009884, AY009887, AY040275–AY040280, AY040282–AY040284, AF440445–AF440450 and AF542099–AF542103.

In previous reports, we have shown that normal skin of healthy Swedish adults and children is infected with a great number of different HPV types and putative types, and that colonization of the skin with HPV starts very early in life (Antonsson *et al.*, 2000, 2003). In the present study, we have collected and analysed skin samples from healthy individuals from three continents in order to investigate whether there are any major differences in skin HPV prevalence or genotype distribution between temperate, subtropical and tropical geographical areas or, alternatively, between Caucasian and non-Caucasian population groups.

METHODS

Subjects. Specimens were taken from each of the following areas of five different countries: from healthy skin of 50 individuals from the cities of Dhaka, Bangladesh; Malmö, Sweden; Addis Abeba, Ethiopia; from a rural area in Zambia; and from 48 persons in the city of Kumamoto, Japan. Half of the samples were collected from women and half from men in each country. The age ranges of the individuals were 16–70 years (median 35) for Bangladesh; 15–77 years (median 43.5) for Japan; 16–71 years (median 39) for Sweden; and 18–53 years (median 32) for Zambia and 20–58 years (median 28.5) for Ethiopia. All samples were collected from healthy individuals who, on the whole, were assumed to be representative of the general population of the respective countries.

Samples. Skin swab samples were collected with pre-wetted (0.9% NaCl solution) cotton-tipped swabs (Bio Hospital, Kopparberg, Sweden), which were drawn back and forth five times over the forehead skin within an area of 5 × 10 cm and then suspended in 1 ml 0.9% NaCl solution. The samples from Sweden were kept at 4 °C for a maximum of 24 h before being analysed, while the specimens from Bangladesh, Japan, Zambia and Ethiopia were kept at room temperature for a maximum of 7 days until they reached the laboratory and were analysed.

PCR and HPV type determination. A PCR test with the primer pair FAP59/FAP64 was used for detection of skin HPV DNA, as previously described (Forslund *et al.*, 1999). The protocol was followed as described except for the MgCl₂ concentration, which was modified to 3.5 mM. The PCR products were cloned into the pCR-script SK(+) cloning vector (Stratagene). Between two and four clones per sample were sequenced with both forward and reverse primers (BigDye; Applied Biosystems) and the sequences obtained were compared with available sequences in the GenBank database using the BLAST server (<http://www.ncbi.nlm.nih.gov/blast/>).

The PCR products obtained spanned the HPV L1 gene from nt 6044 to 6480 (numbering relative to HPV 20). Since full-length L1 sequences were not obtained, the new HPV isolates detected in this study were designated putative HPV types. The guidelines from the Papillomavirus Nomenclature Committee 1995 (14th International Papillomavirus Conference, Quebec City, Quebec, Canada) were followed in defining a new putative HPV type (de Villiers, 2001).

All samples were also analysed for human DNA by PCR, targeting the L1 repetitive sequence (Deragon *et al.*, 1990).

Statistical analysis. The chi-square test was used to compare the prevalence of HPV DNA and human DNA among the different countries.

Nucleotide sequence accession numbers. Sequences of FA46, FA48–49, FA52, FA56–60, FA62–64, FA73–78 and FA93–97 have been submitted to GenBank with the following accession numbers; FA46, AY009880; FA48, AY009879; FA49, AY009884; FA52, AY009883; FA56, AY040275; FA57, AY040276; FA58, AY040277; FA59, AY040278; FA60.1, AY040279; FA60.2, AY040280; FA62, AY040282; FA63, AY040283; FA64, AY040284; FA73, AF440445; FA74, AF440446; FA75, AF440447; FA76, AF44048; FA78, AF440450; FA93, AF542099; FA94, AF542100; FA95, AF542101; FA96, AF542102; FA97, AF542103.

RESULTS

Bangladesh

Thirty-four (68%), 17 men and 17 women, of the 50 individuals from Bangladesh were HPV DNA positive. Thirteen of the samples tested negative for human DNA, out of which 10 were HPV DNA positive. The HPV type determination revealed four new putative types (FA46, FA48, FA49 and FA52) together with 29 HPV types or previously reported putative types (Table 1). One sample from a male contained a subtype of FA20 (92% sequence homology). It was not possible to determine the HPV type of two samples collected from males, despite several attempts.

Japan

HPV DNA was detected in 26 (54%) of the 48 Japanese samples, collected from 14 men and 12 women. Thirty-one

of the samples tested negative for human DNA and 20 of these were HPV DNA positive. The HPV DNA-positive specimens were HPV type determined and 20 previously described HPV types or putative types plus eight new putative HPV types were found (Table 1).

Sweden

Thirty-five (70%) of the 50 Swedish samples were HPV DNA-positive, 20 from men and 15 from women. Five of the samples tested negative for human DNA and two of these were HPV DNA positive. HPV type determination of the positive specimens revealed 34 previously described HPV types or putative types and five new putative types, FA73–76 and FA78 (Table 1).

Zambia

Twenty-one (42%), 14 men and 7 women, of the 50 Zambian individuals were HPV DNA positive. Twenty-nine of the specimens tested negative for human DNA by PCR, out of which 16 were HPV DNA positive. HPV type determination revealed two new putative types (FA93 and FA94) together with 11 previously described skin HPV types or putative types (Table 1). Two subtypes, one of FA27 (92% sequence homology) and another of HPV-20 (91% sequence homology) were found in samples from two males.

Ethiopia

Twenty-six (52%) of the 50 Ethiopian samples were HPV DNA positive, 11 from men and 15 from women. Thirty-one of the samples tested negative for human DNA and 17 of these were HPV DNA positive. The HPV types were determined in the HPV DNA-positive specimens and 25 previously described HPV types or putative types plus three new putative HPV types (FA95–FA97) were found (Table 1). Two putative subtypes were detected, one of HPV-37 (92% sequence homology) and another of FA25 (92% sequence homology). Three samples could not be HPV type determined; one was collected from a male and two from females.

Summary

The HPV DNA prevalence found in the Zambian samples was significantly lower than that of the samples collected in Sweden ($P < 0.01$) and in Bangladesh ($P < 0.05$).

All of the putative HPV types that have not been reported previously, together with the types most closely related to them, are presented in Table 2.

Altogether, 142 samples proved positive for HPV DNA and 88 different HPV types or putative types were found, 22 of which have not been described before. Thirty-three different HPV types or putative types were seen in the samples from Bangladesh, 28 in the Japanese samples, 40 in the Swedish, 13 in the Zambian and 28 in the Ethiopian samples. Fifty of the 88 HPV types or putative types detected in this study

Table 1. HPV types and putative types found in samples collected in Bangladesh, Japan, Sweden, Zambia and Ethiopia

The total number of individuals with each specific HPV type is given together with human L1 DNA-positive and human L1 DNA-negative results (respectively) in parentheses.

HPV type/putative type	Bangladesh	Japan	Sweden	Zambia	Ethiopia
HPV-5	4 (3+1)	2 (0+2)	3 (3+0)	4 (0+4)	3 (1+2)
HPV-8			1 (1+0)	4 (1+3)	1 (0+1)
HPV-9		1 (1+0)			
HPV-12		1 (0+1)	3 (3+0)		1 (1+0)
HPV-14D			1 (1+0)	1 (1+0)	
HPV-17			2 (2+0)		
HPV-20	1 (1+0)	3 (0+3)	2 (2+0)		2 (0+2)
HPV-21	1 (1+0)	1 (1+0)			
HPV-22			1 (0+1)		
HPV-23	2 (1+1)	3 (0+3)		6 (1+5)	
HPV-24				1 (0+1)	2 (0+2)
HPV-36			1 (1+0)		
HPV-37			1 (0+1)		1 (0+1)
HPV-38			2 (2+0)		1 (0+1)
HPV-47	1 (1+0)	1 (1+0)	2 (2+0)		1 (0+1)
HPV-49		2 (0+2)		1 (0+1)	
HPV-50	3 (3+0)	1 (1+0)			
HPV-65	1 (1+0)				
HPV-75					2 (0+2)
HPV-76	1 (1+0)	1 (0+1)	2 (2+0)		
HPV-80	1 (0+1)				
HPVvs20-4			1 (1+0)		
HPVvs42-1					1 (0+1)
HPVvs73-1	1 (1+0)	1 (0+1)			
HPVvs92-1	1 (0+1)				1 (0+1)
HPVvs102-4		1 (1+0)			
RTRX7					1 (1+0)
FA1		1 (1+0)			1 (1+0)
FA2		1 (1+0)			
FA5	1 (0+1)		2 (2+0)		
FA8	1 (1+0)	2 (0+2)			1 (0+1)
FA12			2 (2+0)		
FA13	1 (1+0)				
FA14			1 (1+0)		
FA15			1 (1+0)		
FA16		2 (2+0)	2 (2+0)	1 (1+0)	
FA18			1 (1+0)		
FA20	1 (0+1)		1 (1+0)		1 (0+1)
FA22			1 (1+0)	1 (0+1)	
FA23		4 (1+3)	2 (2+0)	1 (0+1)	
FA25	1 (1+0)	7 (2+5)	1 (1+0)		1 (0+1)
FA26			1 (1+0)		
FA27	2 (0+2)			1 (0+1)	
FA28	1 (1+0)				
FA32			2 (2+0)		
FA34			1 (1+0)		
FA36		2 (1+1)			
FA37	1 (1+0)				
FA39	2 (1+1)				1 (0+1)
FA40	1 (0+1)				
FA42	1 (1+0)				
FA44	1 (1+0)		1 (1+0)		

Table 1. (*cont.*)

HPV type/putative type	Bangladesh	Japan	Sweden	Zambia	Ethiopia
FA45	1 (1+0)				1 (1+0)
FA46	1 (1+0)				
FA47	1 (1+0)				
FA48	1 (1+0)				
FA49	1 (1+0)				
FA50	1 (1+0)		2 (2+0)		
FA51	3 (2+1)				1 (1+0)
FA52	1 (0+1)				1 (1+0)
FA54	1 (1+0)				
FA55	1 (1+0)		1 (1+0)		
FA56		1 (0+1)			
FA57		1 (0+1)			1 (0+1)
FA58		1 (0+1)			
FA59		1 (0+1)			
FA60		1 (0+1)	1 (1+0)	1 (0+1)	
FA61		1 (0+1)			
FA62		1 (0+1)	1 (1+0)		
FA63		1 (0+1)	1 (1+0)		
FA64		1 (0+1)			
FA69			1 (0+1)		
FA71			1 (1+0)		
FA73			1 (1+0)		
FA74			1 (1+0)		
FA75			1 (1+0)		1 (0+1)
FA76			1 (1+0)		
FA78			1 (1+0)		
FA85					1 (1+0)
FA88					1 (0+1)
FA93				2 (1+1)	
FA94				1 (0+1)	
FA95					1 (0+1)
FA96					1 (0+1)
FA97					1 (0+1)
FAIMVS11·3					1 (1+0)
HPV DNA prevalence	68 %	54 %	70 %	42 %	52 %
Total no. of HPV types/putative types	33	28	39	13	28
HPV types/putative types found uniquely in the country	12	9	18	2	9
No. of HPV types/putative types found per no. of HPV DNA-positive individuals	33/34 (0·97)	28/26 (1·08)	39/35 (1·11)	13/21 (0·56)	28/26 (1·08)
Number of HPV types/putative types found per no. of individuals tested	33/50 (0·66)	28/48 (0·58)	39/50 (0·78)	13/50 (0·26)	28/50 (0·56)

were found in only one country (Table 1). Only one HPV type, HPV-5, was detected in samples from all five countries and HPV-5 was found as the single type or together with one or more other HPV types or putative types in 16 of the 248 samples analysed (6·5 %). HPV-47 and FA25 were also common and were detected in samples from all countries except Zambia.

Fifty-three (39 %) of the 137 HPV type-determined samples contained two or more HPV types or putative types: eight

samples from Bangladesh, 14 from Japan, 18 from Sweden, four from Zambia and nine from Ethiopia.

DISCUSSION

The present study shows that the previously reported high prevalence and type diversity of skin papillomavirus in healthy skin of Caucasians (Antonsson *et al.*, 2000, 2003) also applies to people from other parts of the world.

Table 2. Twenty-two new putative HPV types and their most closely related HPV type or putative type

Putative HPV type	Country of origin of skin sample	Size of fragment (bp)	Most closely related HPV type/putative type	Nucleotide sequence homology (%)
FA46	Bangladesh	443	FA15	78
FA48	Bangladesh	440	FA35	82
FA49	Bangladesh	440	FA66	73
FA52	Bangladesh	440	FA22	90
FA56	Japan	440	FA7	75
FA57	Japan	434	FA11	77
FA58	Japan	434	HPV-50	83
FA59	Japan	449	FA65	66
FA60	Japan	446	FA40	77
FA62	Japan	449	FA24	69
FA63	Japan	434	FA81	78
FA64	Japan	443	FA1	85
FA73	Sweden	437	HPV-14D	67
FA74	Sweden	440	FA22	85
FA75	Sweden	437	FA85	79
FA76	Sweden	440	FA4	78
FA78	Sweden	437	FA10	80
FA93	Zambia	431	HPV-47	89
FA94	Zambia	218	HPV-5	80
FA95	Ethiopia	443	HPV-60	66
FA96	Ethiopia	443	HPV-21	65
FA97	Ethiopia	434	FA63	81

The quality of the cellular human DNA as determined by PCR amplification of the human L1 repetitive DNA sequence varied significantly ($P < 0.001$) between the samples collected from the different countries, ranging from five negative Swedish samples to 29 in Zambia and 31 in Ethiopia and Japan. The Zambian, Ethiopian and Japanese samples had been handled without a cold chain, which might have caused the observed decrease in the number of samples positive in the human DNA test. However, even in samples that were negative in the human DNA PCR test, we were able to detect HPV DNA at as high a prevalence as in the human DNA-positive samples, indicating that the viral DNA contained in the virions was protected against DNA degradation, contrary to the human cellular DNA in the samples.

Altogether, 88 different HPV types or putative types were found and of these 38 were detected in at least two countries. However, the majority of the HPV types and putative types were seen in one of the countries only. Furthermore, of the 137 HPV type-determined specimens, 53 (39%) contained two or more HPV types or putative types each.

It is worth noting that five different subtypes of previously described HPV types or putative types were found in specimens from Bangladesh, Zambia and Ethiopia. In our previous studies spanning some 1000 HPV type determinations by DNA sequencing, we have only been confronted with putative HPV subtypes twice (Antonsson *et al.*, 2000), which involved 90–98% sequence homology with a

previously described HPV type. However, the two putative types that were found previously had a sequence homology close to 98%, while the sequence homology of the subtypes detected in this study was between 91 and 92%.

The findings in this and previous studies together emphasize the ubiquity and impressive multiplicity of genotypes amongst the skin papillomaviruses, both in humans (Antonsson *et al.*, 2000, 2003) and in animals (Antonsson & Hansson, 2002). Adapted to their hosts presumably over hundreds of millions of years, these viruses are the first viral skin commensals to be described.

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