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Betapapillomavirus infections : natural history and disease association

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CHAPTER 4

Prevalence and associated factors of betapapillomavirus infections in individuals without cutaneous squamous cell carcinoma

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Prevalence and associated factors of betapapillomavirus infections in individuals without cutaneous squamous cell carcinoma

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Betapapillomavirus (betaPV) infections are often associated with squamous-cell carcinoma (SCC) and the prevalence of betaPV infections in (immunosuppressed) SCC patients is known to be high. The distribution and possible associated factors of betaPV infections in the general population, however, are largely unknown. To address this issue, betaPV infection was studied in 1405 SCC-free immunocompetent ($n=845$) and immunosuppressed ($n=560$) individuals from six countries of different latitudes. A standard study protocol was used to obtain information about age, sex, UV-irradiation and skin type, and from all participants eyebrow hairs were collected for detection and genotyping of 25 established betaPV types using the PM-PCR reverse hybridization assay (RHA) method. The frequency of betaPV-positive participants ranged from 84 to 91% in the immunocompetent population with HPV23 as the most prevalent type, and from 81 to 98% in the immunosuppressed population with HPV23 as the most or the second most prevalent type. The median number of infecting betaPV types ranged from four to six in the immunocompetent and from three to six in the immunosuppressed population. Increasing age in the immunocompetent participants and (duration of) immunosuppression in the immunosuppressed patients were associated with betaPV infection. In both groups, sex, skin phototype, sunburns and sun-exposure were not consistently associated with betaPV infection. This study demonstrates that betaPV infections are also highly prevalent in SCC-free individuals, with similar HPV types prevailing in both immunocompetent and immunosuppressed persons. Age and (duration of) immunosuppression were identified as betaPV infection-associated factors, whereas characteristics related to sun exposure and skin type were not.

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INTRODUCTION

Papillomaviruses (PV) constitute a taxonomic family of non-enveloped double-stranded DNA viruses associated with benign and malignant lesions of cutaneous and mucosal epithelia. So far, more than 100 different human PV (HPV) genotypes have been identified and fully sequenced. Molecular biological and epidemiological data have shown that persistent infection with high-risk mucosal HPV types belonging to the genus *Alphapapillomavirus* causes cervical cancer (Bosch & de Sanjose, 2003; zur Hausen, 2000).

The first evidence that cutaneous HPV types might play a role in the pathogenesis of cutaneous squamous cell carcinoma (SCC) was based on the detection of specific HPV types, predominantly HPV5 and HPV8 and rarely HPV types 14, 17, 20 or 47, in over 90% of SCCs in epidermodysplasia verruciformis (EV) patients (Orth, 2005; Pfister, 2003). EV patients suffer a genetic defect that confers high susceptibility to specific HPV types that induce characteristic, macular skin lesions disseminated over the body that frequently progress to SCC. The HPV types initially found in skin lesions of EV patients, the so-called EV-HPV types, are now classified as belonging to the genus *Betapapillomavirus* (betaPV) (de Villiers *et al.*, 2004). This genus comprises HPV types 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. Sequencing of subgenomic amplicons revealed a large number of putatively novel HPV types belonging to the beta as well as the gamma genus (Forslund, 2007).

Like in EV patients, the risk of developing cutaneous cancer (especially SCC) is markedly increased in organ-transplant patients compared with the normal population (Bouwes Bavinck *et al.*, 2007; Hartevelt *et al.*, 1990). BetaPV DNA has been detected frequently in actinic keratoses and skin cancers of organ-transplant patients, suggesting a role of betaPV in the pathogenesis of skin cancer in these patients (Berkhout *et al.*, 2000; Bouwes Bavinck *et al.*, 2008; De Jong-Tieben *et al.*, 1995; Harwood *et al.*, 2000). Recent epidemiological studies suggest that these betaPV types also play a role in the pathogenesis of cutaneous SCC in the general population. Seroreactivity to betaPV types has been found to be associated with non-melanoma skin cancer (Andersson *et al.*, 2008; Feltkamp *et al.*, 2003; Karagas *et al.*, 2006; Struijk *et al.*, 2006). Also, associations have been found between the detection of betaPV DNA in plucked eyebrow hairs and actinic keratoses (AK) (Boxman *et al.*, 2001; Struijk *et al.*, 2006) and cutaneous SCC (Forslund *et al.*, 2007; Struijk *et al.*, 2003, 2006).

Our recent findings in a small group of 23 healthy study participants (de Koning *et al.*, 2007) indicate that the prevalence of betaPV infections in the general population is also high. However, the prevalence of betaPV (co)infections and betaPV species distribution across larger, SCC-free populations has not been systematically described. In addition, possible determinants of infection with betaPV are poorly understood. Here we sought to investigate these aspects of

betaPV infection in a cross-sectional study of 845 SCC-free immunocompetent participants (ICP) and 560 SCC-free immunosuppressed organ-transplant recipients (OTR) from six countries from different latitudes utilizing a highly sensitive PCR and genotyping method that allows the easy detection of multiple betaPV infections (de Koning *et al.*, 2006).

METHODS

Study participants. Recruitment of SCC-free ICP took place in the scope of an European Commission (EC)-granted multicenter non-melanoma skin cancer case-control study (QLK2-CT-2002-01179) performed in The Netherlands, Italy and Australia (co-funded by the Australian National Health and Medical Research Council) investigating the relationship between betaPV infection, sun exposure and skin cancer. SCC-free OTR were selected from a hospital-based case-control study that was designed to assess possible risk factors for non-melanoma skin cancer and keratotic skin lesions in organ-transplant recipients who had been transplanted at least 2 years previously (Bouwes Bavinck *et al.*, 2007). The study was approved by the local medical ethical committees and the participants had given informed consent.

Ascertainment of ICP by study centre. The Dutch study participants were selected from a case-control study investigating several environmental and genetic risk factors for skin cancer (De Hertog *et al.*, 2001; Struijk *et al.*, 2003). Briefly, 278 controls with no history of any type of skin cancer who were age- and sex-matched to a series of cases with SCC were recruited at the ophthalmology outpatient clinic at the Leiden University Medical Center according to criteria that have been described by Struijk *et al.* (2006). The 257 Italian control participants with no skin cancer history were selected from the dermatology out-patients clinics at the Istituto Dermatologico Dell'Immacolata (Rome, Italy). In Australia a total of 310 eligible participants were enlisted; 215 from Brisbane and 95 from Townsville in North Queensland. In Brisbane, 150 controls were randomly recruited from the electoral roll (a register with almost complete population coverage) and 65 from skin cancer-free patients attending primary practice skin cancer clinics for routine self-referred screening. Controls in Townsville were randomly selected from a series of community groups. Due to the common nature of basal-cell carcinoma (BCC) in Australia (Valery *et al.*, 2004), and the poor reliability of self-report, participants with a self-reported past history of BCC were recruited, unless they reported excision of any lesion within the 2 years prior to recruitment. Sixty-seven control participants (22%) reported having had a previous BCC. Their inclusion did not affect the calculated odds ratios for possible associated factors of betaPV (co)infection as determined by sensitivity analyses (data not shown).

Ascertainment of OTR by study centre. Briefly, patients with solid organ transplants without a history of SCC were recruited from the out-patient nephrology and dermatology clinics in the following hospitals: Leiden University Medical Center, Leiden, The Netherlands (111 patients); Barts and the London NHS Trust, London, UK (132 patients); University Clinic Charité, Berlin, Germany (166 patients); Hôpital Edouard Herriot, Lyon, France (54 patients); Ospedali Riuniti di Bergamo, Bergamo and Ospedale Civile Maggiore, Verona, Italy (97 patients). Patients with (Fitzpatrick) skin types V and VI and patients with a history of BCC were not included (Bouwes Bavinck *et al.*, 2007).

Collection of data. Questionnaires and medical charts were used to gather the following information from participants: sex and age, Fitzpatrick-classified skin type, ability to tan, and sun reactivity and sun exposure-related questions. In the ICP from Leiden only the

Table 1. Baseline characteristics of the ICP study populations

Characteristics	The Netherlands <i>n</i> =278	Italy <i>n</i> =257	Australia <i>n</i> =310	<i>P</i> -value
Age in years, <i>n</i> (%)				
<60	93 (33.5)	45 (17.5)	98 (31.6)	<0.001
60–69	110 (39.6)	85 (33.1)	104 (33.5)	
70+	75 (27.0)	127 (49.4)	108 (34.8)	
Sex, <i>n</i> (%)				
Female	155 (55.8)	97 (37.7)	95 (30.6)	<0.001
Male	123 (44.2)	160 (62.3)	215 (69.4)	
Skin phototype*, <i>n</i> (%)				
Dark/olive	145 (52.2)	132 (51.4)	109 (35.3)	<0.001
Medium/fair	133 (47.8)	125 (48.6)	200 (64.7)	
Unknown			1	
Sunburns before 20 years, <i>n</i> (%)				
0 (%)	182 (65.5)	178 (69.3)	74 (23.9)	<0.001
1–4 (%)	78 (28.1)	73 (28.4)	139 (44.8)	
5+ (%)	18 (6.5)	6 (2.3)	97 (31.3)	
Occupational sun exposure, <i>n</i> (%)				
0–3 h	159 (57.2)	150 (58.4)	258 (83.2)	<0.001
4 h and more	119 (42.8)	107 (41.6)	52 (16.8)	
Weekend sun exposure, <i>n</i> (%)				
0–3 h	83 (29.9)	127 (49.4)	231 (74.5)	<0.001
4 h and more	195 (70.1)	130 (50.6)	79 (25.5)	

*In The Netherlands, skin phototype was determined only by Fitzpatrick skin type, whereas for the other populations tanning ability and sun reactivity were also included in the determination of skin phototype (for explanation, see Materials and Methods and Supplementary Fig. S1 in Bouwes Bavinck *et al.*, 2007).

Fitzpatrick skin type was used to determine the skin phototype because tanning ability and sun reactivity were not assessed. As a consequence, in the Dutch ICP the medium and fair skin phototypes were underrepresented compared with those in the Italian and Australian ICP, because in The Netherlands the ICP with Fitzpatrick skin type III and IV were always categorized as dark or olive skin phototypes, whereas in the other two countries individuals with Fitzpatrick skin type III and IV could also be categorized into the medium or fair skin phototypes depending on the answers to the questions about ability to tan and sun reactivity, as previously explained in Supplementary Fig. S1, in Bouwes Bavinck *et al.*, 2007.

Collection of eyebrow hairs. Eight to ten eyebrow hairs were plucked from each participant. Between participants the tweezers were cleaned with a hypochlorite solution or disposable tweezers were used. The samples were kept at -70°C until further processing.

DNA isolation. Purification of the DNA was carried out with the guanidine-thiocyanate-diatom method described by Boom *et al.* (1990) for the samples from the ICP from Leiden or with a QIAamp DNA Mini kit (Qiagen) for all other samples (de Koning *et al.*, 2006). For both DNA isolation methods each tenth sample was a negative isolation control that was processed parallel to the other samples of which 7% was positive with no specific betaPV type standing out.

PCR and hybridization. Isolated DNA for betaPV genotyping of the ICP was available from 277 out of 278 Dutch participants, from all 257 Italian participants and from 295 out of 310 Australian participants. For the OTR populations isolated DNA was available from 111 out of 111 Dutch patients, from 126 out of 132 English patients, from 165 out of 166 German patients, from 53 out of 54 French patients and from 95

out of 97 Italian patients. BetaPV detection and genotyping of all 25 established genotypes (i.e. HPV types 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) was carried out with the skin (beta) HPV prototype research assay (Diassay BV) as described previously (de Koning *et al.*, 2006). Samples from the Dutch, French and Australian participants were tested in a different laboratory than those from the Italian, German and English participants. Previously, interlaboratory reproducibility between both testing sites was extensively tested with bridging panels and agreement was shown to be high (de Koning *et al.*, 2006).

Statistical analyses. All analyses were performed with SPSS version 15 for Windows. Chi-squared tests were used to compare differences in categorical variables between groups, between different countries or other characteristics. Relative risks of cutaneous betaPV infections were estimated using exposure odds ratios from cross-tabulation and logistic regression. Odds ratios were adjusted for age and sex in all study groups and additionally for length of immunosuppression in the OTR groups. Because of missing values for some variables the group sizes may vary. Nine OTR were recruited who had undergone their organ transplant less than 2 years previously (two in the UK, and seven in Germany; range 3–20 months). As exclusion of these OTR made no difference to the results, they were included in the 2–7 years post-transplant category.

RESULTS

Baseline characteristics

The baseline characteristics, stratified by country, of the 845 ICP and the 560 OTR are shown in Tables 1 and 2,

Table 2. Baseline characteristics of the OTR study populations

Characteristics	The Netherlands <i>n</i> =111	UK <i>n</i> =132	Germany <i>n</i> =166	France <i>n</i> =54	Italy <i>n</i> =97	<i>P</i> -value
Age in years, <i>n</i> (%)						
<50	45 (40.5)	54 (41.5)	81 (48.8)	8 (14.8)	33 (34.0)	<0.001
50–59	42 (37.8)	47 (36.2)	37 (22.3)	14 (25.9)	34 (35.1)	
60+	24 (21.6)	29 (22.3)	48 (28.9)	32 (59.3)	30 (30.9)	
Unknown		2				
Sex, <i>n</i> (%)						
Female	36 (32.4)	49 (37.1)	61 (36.7)	11 (20.4)	19 (19.6)	0.009
Male	75 (67.6)	83 (62.9)	105 (63.3)	43 (79.6)	78 (80.4)	
Years after transplantation, <i>n</i> (%)						
2–7	27 (24.3)	41 (31.5)	88 (53.0)	17 (31.5)	29 (29.9)	<0.001
8–12	24 (21.6)	38 (29.2)	22 (13.3)	15 (27.8)	32 (33.0)	
13–17	22 (19.8)	30 (23.1)	30 (18.1)	11 (20.4)	15 (15.5)	
18–22	19 (17.1)	11 (8.5)	16 (9.6)	7 (13.0)	13 (13.4)	
23+	19 (17.1)	10 (7.7)	10 (6.0)	4 (7.4)	8 (8.2)	
Unknown		2				
Skin phototype, <i>n</i> (%)						
Dark/olive	48 (43.2)	51 (38.6)	26 (15.7)	34 (63.0)	63 (64.9)	<0.001
Medium/fair	63 (56.8)	81 (61.4)	140 (84.3)	20 (37.0)	34 (35.1)	
Sunburns before 20 years, <i>n</i> (%)						
0	48 (43.2)	79 (59.8)	120 (72.7)	23 (42.6)	65 (67.0)	<0.001
1–4	42 (37.8)	41 (31.1)	42 (25.5)	17 (31.5)	24 (24.7)	
5+	21 (18.9)	12 (9.1)	3 (1.8)	14 (25.9)	8 (8.2)	
Unknown			1			
Occupational sun exposure, <i>n</i> (%)						
0–3 h	67 (60.4)	89 (67.4)	157 (94.6)	26 (48.1)	49 (50.5)	<0.001
4 h and more	44 (39.6)	43 (32.6)	9 (5.4)	28 (51.9)	48 (49.5)	
Weekend sun exposure, <i>n</i> (%)						
0–3 h	45 (40.5)	56 (42.4)	135 (81.3)	21 (38.9)	31 (32.0)	<0.001
4 h and more	66 (59.5)	76 (57.6)	31 (18.7)	33 (61.1)	66 (68.0)	

respectively. The distribution across all variables tested was significantly different between each study centre across the two populations. For instance the Dutch ICP (median age of 64 years) were significantly younger than those from Australia (median age of 66 years) and Italy (median age of 69 years), and the percentage of males ranged from 44% in The Netherlands to 69% in Australia. Among the OTR the median age was 52 years in The Netherlands and the UK, 50 years in Germany, 62 years in France and 56 years in Italy, and in each study centre there was a variable male preponderance (63–80%). Sunburns before 20 years of age were particularly frequent in Australia (ICP) and France (OTR), whereas occupational and weekend sun exposure was low in Australia (ICP) and also in Germany (OTR). Among the OTR, time after transplantation was not correlated with age (data not shown).

BetaPV prevalence and type distribution

The overall percentage of betaPV-infected ICP was 84% in the Netherlands and 91% in Italy and Australia. For the OTR this percentage was 81% in Germany, 88% in the

UK, 95% in Italy, 96% in France and 98% in the Netherlands. The prevalences per betaPV type in the ICP and OTR are shown in Fig. 1a and b, respectively. Sixty-six to 83% of the ICP and 72–87% of the OTR populations were infected with betaPV species 1 (HPV5, 8, 12, 14, 19, 20, 21, 24, 25, 36, 47 and 93). Species 2 betaPV types (HPV9, 15, 17, 22, 23, 37, 38 and 80) were found in 71–78% of the ICP and in 65–91% of the OTR populations. Species 3 betaPV types (HPV49, 75 and 76) were present in 18–34% of the ICP and in 15–37% of the OTR from the different countries.

HPV23 was the most prevalent betaPV type in the ICP study centres (Fig. 1a). HPV24 and HPV36 were also highly prevalent in each ICP centre, with more than 25% of the participants infected with either one of these types. In the OTR study groups, HPV23 was either the most prevalent betaPV type (The Netherlands, Germany and France) or the second most prevalent betaPV type (UK and Italy) (Fig. 1b). In the UK and Italy, HPV38 and HPV24 were the most prevalent types, respectively. HPV36 was also highly prevalent in the OTR participants, with more than 23% of infected participants.

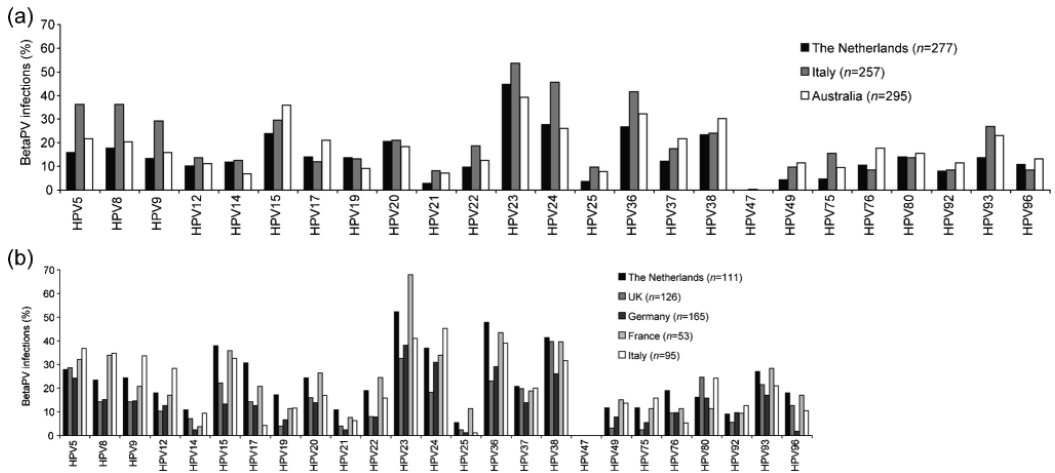


Fig. 1. BetaPV type distribution in the ICP (a) and the OTR (b) study populations.

BetaPV coinfections

The percentages of ICP who were infected with two or more betaPV types were 69 % in The Netherlands, 81 % in Italy and 76 % in Australia. The median number of betaPV types infecting a single participant was 4 in the Dutch and Australian populations (range 1–12 and 1–14, respectively), and 6 in the Italian population (range 1–16) (Fig. 2a). The percentage of OTR infected with two or more types was 96 % in The Netherlands, 75 % in the UK, 70 % in Germany, 89 % in France and 84 % in Italy. In these groups the median number of infecting types was 5 in The Netherlands (range 1–12), 3 in the UK (range 1–10), 4 in Germany (range 1–13), 6 in France (range 1–12) and 5 in Italy (range 1–11) (Fig. 2b).

Assessment of associated factors for betaPV infection

Potentially associated factors for betaPV infection were determined in two ways. First we compared the betaPV-positive with the betaPV-negative participants (1+ vs 0, Table 3). In the ICP, increasing age appeared to be an associated factor for betaPV infection in Italy and Australia, although this was of marginal statistical significance. Sex, skin phototype, sunburns before the age of 20, UV exposure during the week or weekend and years after transplantation where applicable were not consistently associated with betaPV infection in any study group (Tables 3 and 4). In the OTR, these calculations were only performed for the UK and Germany as the number of betaPV-negative participants was too low in the other study centres (Table 4).

As betaPV coinfections were frequently found in all study centres, we next determined the potentially associated factors for multiple betaPV infection. For this purpose betaPV-infected participants infected with 5 or more types were compared with those infected with fewer types (5+ vs 1–4, Tables 3 and 4). In the ICP, age again turned out to be an associated factor. In Australia multiple betaPV infections were strongly and significantly associated with older

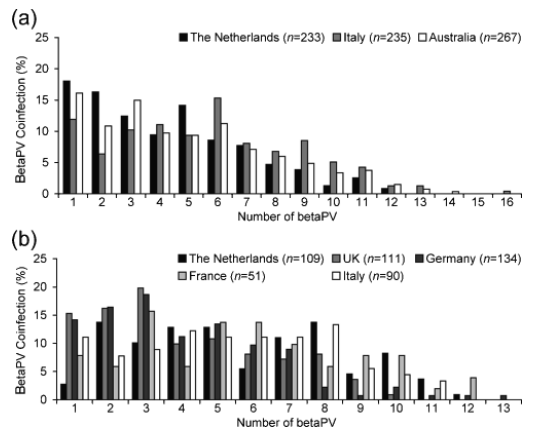


Fig. 2. BetaPV coinfections in the ICP (a) and OTR (b) study populations. The bars show the presence of betaPV coinfection as a percentage of the total number of infected participants in the populations.

Table 3. Assessment of possible associated factors for betaPV (co)infection in the ICP

CI, Confidence interval.

Characteristics	The Netherlands					Italy				
	No. detected betaPV types		Odds ratio (95% CI)*			No. detected betaPV types		Odds ratio (95% CI)*		
	0 n=44	1-4 n=131	5+ n=102	1+ vs 0 types	5+ vs 1-4 types	0 n=22	1-4 n=93	5+ n=142	1+ vs 0 types	5+ vs 1-4 types
Age in years, n (%)										
<60	14 (15.1)	51 (54.8)	28 (30.1)	1.0	1.0	7 (15.6)	16 (35.6)	22 (48.9)	1.0	1.0
60-69	16 (14.7)	48 (44.0)	45 (41.3)	1.1 (0.48-2.3)	1.6 (0.88-3.0)	7 (8.2)	36 (42.4)	42 (49.4)	2.0 (0.67-6.1)	0.83 (0.38-1.8)
70+	14 (18.7)	32 (42.7)	29 (38.7)	0.80 (0.35-1.8)	1.5 (0.76-3.0)	8 (6.3)	41 (32.3)	78 (61.4)	2.7 (0.92-8.1)	1.3 (0.62-2.8)
Sex, n (%)										
Female	23 (14.8)	81 (52.3)	51 (32.9)	1.0	1.0	9 (9.3)	39 (40.2)	49 (50.5)	1.0	1.0
Male	21 (17.2)	50 (41.0)	51 (41.8)	0.81 (0.42-1.6)	1.5 (0.91-2.6)	13 (8.1)	54 (33.8)	93 (58.1)	1.1 (0.44-2.7)	1.3 (0.75-2.2)
Skin phototype, n (%)										
Dark/olive	22 (15.3)	67 (46.5)	55 (38.2)	1.0	1.0	12 (9.1)	50 (37.9)	70 (53.0)	1.0	1.0
Medium/fair	22 (16.5)	64 (48.1)	47 (35.3)	0.92 (0.48-1.8)	0.99 (0.58-1.7)	10 (8.0)	43 (34.4)	72 (57.6)	1.2 (0.51-3.0)	1.3 (0.76-2.2)
Unknown										
Sunburns before 20 years, n (%)										
0 (%)	25 (13.7)	84 (46.2)	73 (40.1)	1.0	1.0	17 (9.6)	64 (36.0)	97 (54.5)	1.0	1.0
1-4 (%)	14 (18.2)	38 (49.4)	25 (32.5)	0.72 (0.35-1.5)	0.27 (0.39-1.3)	5 (6.8)	26 (35.6)	42 (57.5)	1.7 (0.56-4.9)	1.1 (0.59-2.0)
5+ (%)	5 (27.8)	9 (50.0)	4 (22.2)	0.44 (0.14-1.4)	0.50 (0.14-1.7)	0 (0.0)	3 (50.0)	3 (50.0)	No power	0.69 (0.13-3.6)
Occupational sun exposure, n (%)										
0-3 h	20 (12.7)	73 (46.2)	65 (41.1)	1.0	1.0	13 (8.7)	58 (38.7)	79 (52.7)	1.0	1.0
4 h and more	24 (20.2)	58 (48.7)	37 (31.1)	0.54 (0.28-1.0)	0.65 (0.37-1.1)	9 (8.4)	35 (32.7)	63 (58.9)	1.1 (0.43-2.9)	1.4 (0.76-2.4)
Weekend sun exposure, n (%)										
0-3 h	12 (14.6)	40 (48.8)	30 (36.6)	1.0	1.0	10 (7.9)	49 (38.6)	68 (53.5)	1.0	1.0
4 h and more	32 (16.4)	91 (46.7)	72 (36.9)	0.97 (0.45-2.0)	1.1 (0.60-2.0)	12 (9.2)	44 (33.8)	74 (56.9)	0.95 (0.36-2.6)	1.3 (0.73-2.4)

*Odds ratios were adjusted for age and sex when applicable.

age. In the OTR, in some centres betaPV multiplicity tended to increase with age (e.g. in The Netherlands) but significance was not reached. The time after transplantation, adjusted for age and sex, was associated with multiple betaPV infection in The Netherlands, Germany and Italy, but not in the UK and France. None of the other studied factors were associated with multiple betaPV infection.

Finally, we investigated the effect of immunosuppression on betaPV prevalence by comparing the combined ICP and OTR study centres. Immunosuppression was significantly associated with betaPV infection as well as multiple betaPV infection, with age and sex adjusted odds ratios of 1.6 (95% confidence interval 1.1-2.5) and 1.5 (95% confidence interval 1.1-1.9), respectively. Stratification for geographical region (i.e. Northern and Southern Europe) did not reveal any relevant differences (data not shown).

DISCUSSION

This is the first study of substantial size that investigated betaPV prevalence and possible risk factors for infection in

SCC-free immunocompetent and immunosuppressed individuals with a highly reproducible technique suitable to detect and identify 25 established betaPV types. The observed prevalence of cutaneous betaPV infection in all study populations was very high with positivity rates ranging from 81 to 98%, depending on the study centre. Furthermore, we observed that the number of betaPV types present in betaPV-positive participants was high, with a median number of three to six types and a maximum of 16 types. The overall betaPV detection frequency in these populations is probably still an underestimation, because recently 36 putative new betaPV types were described (Forslund, 2007) that were not included in our test.

An important finding of this study was that the most common HPV types were similar for all study centres, both ICP and OTR, with HPV23 in general being the most prevalent type. This finding is strengthened by the reported analytical sensitivity of the assay that does not vary more than tenfold between the different betaPV types (de Koning *et al.*, 2006) as differential sensitivity for specific HPV types of detection and genotyping methods might influence

Table 3. cont.

Characteristics	Australia					Pooled analyses				
	No. detected betaPV types		Odds ratio (95% CI)*			No. detected betaPV types			Odds ratio (95% CI)†	
	0 n=28	1-4 n=138	5+ n=129	1+ vs 0 types	5+ vs 1-4 types	0 n=94	1-4 n=362	5+ n=373	1+ vs 0 types	5+ vs 1-4 types
Age in years, n (%)										
<60	13 (13.7)	57 (60.0)	25 (26.3)	1.0	1.0	34 (14.6)	124 (53.2)	75 (32.2)	1.0	1.0
60-69	9 (9.3)	43 (44.3)	45 (46.4)	1.6 (0.63-3.9)	2.4 (1.3-4.5)	32 (11.0)	127 (43.6)	132 (45.4)	1.4 (0.82-2.3)	1.6 (1.1-2.4)
70+	6 (5.8)	38 (36.9)	59 (57.3)	2.6 (0.95-7.2)	3.5 (1.9-6.5)	28 (9.2)	111 (36.4)	166 (54.4)	1.6 (0.91-2.7)	2.1 (1.4-3.1)
Sex, n (%)										
Female	7 (8.1)	47 (54.7)	32 (37.2)	1.0	1.0	39 (11.5)	167 (49.4)	132 (39.1)	1.0	1.0
Male	21 (10.0)	91 (43.5)	97 (46.4)	0.74 (0.30-1.8)	1.5 (0.86-2.6)	55 (11.2)	195 (39.7)	241 (49.1)	0.84 (0.53-1.3)	1.4 (1.0-1.9)
Skin phototype, n (%)										
Dark/olive	12 (11.8)	48 (47.1)	42 (41.2)	1.0	1.0	46 (12.2)	165 (43.7)	167 (44.2)	1.0	1.0
Medium/fair	16 (8.3)	90 (46.9)	86 (44.8)	1.7 (0.73-3.8)	1.4 (0.80-2.4)	48 (10.7)	197 (43.8)	205 (45.6)	1.2 (0.54-1.4)	1.2 (0.89-1.6)
Unknown			1					1		
Sunburns before 20 years, n (%)										
0 (%)	4 (5.8)	29 (42.0)	36 (52.2)	1.0	1.0	46 (10.7)	177 (41.3)	206 (48.0)	1.0	1.0
1-4 (%)	21 (15.8)	58 (43.6)	54 (40.6)	0.39 (0.12-1.2)	1.0 (0.52-1.9)	40 (14.1)	122 (43.1)	121 (42.8)	0.72 (0.44-1.2)	0.88 (0.62-1.2)
5+ (%)	3 (3.2)	51 (54.8)	39 (41.9)	2.3 (0.49-11.1)	0.86 (0.44-1.7)	8 (6.8)	63 (53.8)	46 (39.3)	1.6 (0.70-3.8)	0.71 (0.43-1.2)
Occupational sun exposure, n (%)										
0-3 h	24 (9.7)	111 (44.9)	112 (45.3)	1.0	1.0	57 (10.3)	242 (43.6)	256 (46.1)	1.0	1.0
4 h and more	4 (8.3)	27 (56.3)	17 (35.4)	1.1 (0.34-3.3)	0.45 (0.22-0.90)	37 (13.5)	120 (43.8)	117 (42.7)	0.76 (0.47-1.2)	0.78 (0.60-1.1)
Weekend sun exposure, n (%)										
0-3 h	20 (9.2)	104 (47.7)	94 (43.1)	1.0	1.0	42 (9.8)	193 (45.2)	192 (45.0)	1.0	1.0
4 h and more	8 (10.4)	34 (44.2)	35 (45.5)	0.86 (0.35-2.1)	0.95 (0.53-1.7)	52 (12.9)	169 (42.0)	181 (45.0)	0.97 (0.60-1.6)	1.2 (0.84-1.6)

†Odds ratios adjusted for study centre and age and sex when applicable.

observed HPV type prevalence. The differences in species distribution appeared to be mainly driven by the number of betaPV types present within the species, as betaPV species 1 contains 12 betaPV types and species 2 contains 8. Species 3, 4 and 5 contain only 3, 1 and 1 type(s), respectively. Among the five most prevalent types in each study centre, no species stood out (data not shown). Overall it seems that betaPV infections in Caucasians (Fitzpatrick skin types \geq V were excluded from the study) are ubiquitously present, with universal type distribution regardless of geographical region.

The most marked difference in prevalence of betaPV (co)infections was observed between countries and may be due to the selection of study participants in the different centres. In addition, it is not inconceivable that this difference in betaPV prevalence at least partly has a technical reason, as there were indications that the average number of hairs that were plucked per patient somewhat varied per study centre. Also the DNA isolation method may have influenced HPV genotyping (Dunn *et al.*, 2007), as DNA from the Dutch ICP samples was purified differently than that from the other samples. As the amount of human genomic DNA present in the samples seems to influence the outcome of betaPV testing to a

certain degree (S. Weissenborn and H. Pfister, manuscript in preparation), this may have contributed to the differences in betaPV prevalence among study centres as well.

Age was the only factor which was identified as an associated factor for (multiple) betaPV infection in the ICP. Although most individual comparisons did not show a statistically significant association, the trend for almost all comparisons was in the same direction, showing that the frequency of betaPV infection increased with age, confirming earlier data (Boxman *et al.*, 2001; Struijk *et al.*, 2003). In this study we also showed that the number of betaPV types increased with age. In contrast to the ICP, we did not find a consistent association between age and betaPV infection in the five OTR study groups. It has been suggested that with increasing age the immune system deteriorates (Pawelec & Larbi, 2008), an effect called immunosenescence. This deterioration of the immune system might therefore be the underlying mechanism explaining the increasing prevalence of betaPV infection with age. Alternatively, contraction of *de novo* betaPV infections with increasing age might cause the observed association. The fact that age did not appear to be associated with infection in the OTR may be explained

Table 4. Assessment of possible associated factors for betaPV (co)infection in the OTR populations

CI, Confidence interval; NA, not analysed.

Characteristics	The Netherlands					UK					Germany				
	No. detected betaPV types		Odds ratio (95% CI)*			No. detected betaPV types		Odds ratio (95% CI)*			No. detected betaPV types		Odds ratio (95% CI)†		
	0 n=2	1-4 n=43	5+ n=66	1+ vs 0 types	5+ vs 1-4 types	0 n=15	1-4 n=68	5+ n=43	1+ vs 0 types	5+ vs 1-4 types	0 n=31	1-4 n=81	5+ n=53	1+ vs 0 types	5+ vs 1-4 types
Age in years, n (%)															
<50	0	23	22	NA	1.0	6	31	16	1.0	1.0	14	43	24	1.0	1.0
		(51.1)	(48.9)			(11.3)	(58.5)	(30.2)			(17.3)	(53.1)	(29.6)		
50-59	2	13	27	NA	1.8	6	22	16	0.73	1.6	6	18	13	1.1	1.3
		(4.8)	(31.0)	(64.3)	(0.73-4.6)	(13.6)	(50.0)	(36.4)	(0.21-2.5)	(0.64-4.0)	(16.2)	(48.6)	(35.1)	(0.37-3.1)	(0.53-3.1)
60+	0	7	17	NA	2.7	3	15	11	1.1	1.6	11	20	16	0.71	1.5
		(29.2)	(70.8)		(0.66-8.1)	(10.3)	(51.7)	(37.9)	(0.23-4.9)	(0.59-4.5)	(23.4)	(42.6)	(34.0)	(0.29-1.7)	(0.66-3.5)
Sex, n (%)															
Female	1	16	19	NA	1.0	2	32	14	1.0	1.0	10	27	24	1.0	1.0
		(2.8)	(44.4)	(52.8)			(4.2)	(66.7)	(29.2)			(16.4)	(44.3)	(39.3)	
Male	1	27	47	NA	1.5	13	36	29	0.21	2.0	21	54	29	0.78	0.60
		(1.3)	(36.0)	(62.7)	(0.65-3.7)	(16.7)	(46.2)	(37.2)	(0.05-0.99)	(0.87-4.5)	(20.2)	(51.9)	(27.9)	(0.34-1.8)	(0.29-1.2)
Years after transplantation, n (%)															
2-7	1	14	12	NA	1.0	2	20	17	1.0	1.0	17	45	26	1.0	1.0
		(3.7)	(51.9)	(44.4)			(5.1)	(51.3)	(43.6)			(19.3)	(51.1)	(29.5)	
8-12	0	10	14	NA	1.6	5	20	12	0.33	0.73	7	9	5	0.45	0.94
		(41.7)	(58.3)		(0.51-5.4)	(13.5)	(54.1)	(32.4)	(0.06-1.9)	(0.27-2.0)	(33.3)	(42.9)	(23.8)	(0.15-1.3)	(0.27-3.2)
13-17	1	8	13	NA	1.3	6	17	6	0.16	0.44	5	16	9	1.2	1.1
		(4.5)	(36.4)	(59.1)	(0.39-4.6)	(20.7)	(58.6)	(20.7)	(0.03-0.9)	(0.14-1.4)	(16.7)	(53.3)	(30.0)	(0.41-3.7)	(0.41-2.8)
18-22	0	7	12	NA	1.8	1	6	4	0.57	0.69	1	9	6	3.7	1.2
		(36.8)	(63.2)		(0.50-6.2)	(9.1)	(54.5)	(36.4)	(0.05-7.1)	(0.16-2.9)	(6.3)	(56.3)	(37.5)	(0.46-30.4)	(0.38-3.8)
23+	0	4	15	NA	4.2	1	5	4	0.38	1.1	1	2	7	2.0	5.6
		(21.1)	(78.9)		(1.1-16.6)	(10.0)	(50.0)	(40.0)	(0.03-4.9)	(0.24-4.9)	(10.0)	(20.0)	(70.0)	(0.23-17.0)	(1.1-29.2)
Skin phototype, n (%)															
Dark/olive	1	22	25	NA	1.0	6	24	20	1.0	1.0	9	11	6	1.0	1.0
		(2.1)	(45.8)	(52.1)			(12.0)	(48.0)	(40.0)			(34.6)	(42.3)	(23.1)	
Medium/fair	1	21	41	NA	2.0	9	44	23	1.1	0.62	22	70	47	2.8	1.1
		(1.6)	(33.3)	(65.1)	(0.86-4.7)	(11.8)	(57.9)	(30.3)	(0.34-3.3)	(0.28-1.4)	(15.8)	(50.4)	(33.8)	(1.1-7.1)	(0.37-3.4)
Sunburns before 20 years, n (%)															
0	1	14	33	NA	1.0	8	40	27	1.0	1.0	23	61	35	1.0	1.0
		(2.1)	(29.2)	(68.8)			(10.7)	(53.3)	(36.0)			(19.3)	(51.3)	(29.4)	
1-4	1	20	21	NA	0.44	6	24	9	0.66	0.57	8	17	17	1.0	1.8
		(2.4)	(47.6)	(50.0)	(0.17-1.1)	(15.4)	(61.5)	(23.1)	(0.19-2.3)	(0.22-1.5)	(19.0)	(40.5)	(40.5)	(0.41-2.5)	(0.79-4.0)
5+	0	9	12	NA	0.68	1	4	7	1.6	2.5	0	3	0	NA	NA
		(42.9)	(57.1)		(0.22-2.1)	(8.3)	(33.3)	(58.3)	(0.17-15.6)	(0.61-10.0)	(100.0)	1			
Unknown															
Occupational sun exposure, n (%)															
0-3 h	2	26	39	NA	1.0	11	48	27	1.0	1.0	28	78	50	1.0	1.0
		(3.0)	(38.8)	(58.2)			(12.8)	(55.8)	(31.4)			(17.9)	(50.0)	(32.1)	
4 h and more	0	17	27		0.95	4	20	16	1.7	1.1	3	3	3	0.48	1.9
		(38.6)	(61.4)		(0.41-2.2)	(10.0)	(50.0)	(40.0)	(0.46-5.9)	(0.44-2.6)	(33.3)	(33.3)	(33.3)	(0.11-2.1)	(0.35-10.7)
Weekend sun exposure, n (%)															
0-3 h	1	16	28	NA	1.0	4	33	17	1.0	1.0	22	67	45	1.0	1.0
		(2.2)	(35.6)	(62.2)			(7.4)	(61.1)	(31.5)			(16.4)	(50.0)	(33.6)	
4 h and more	1	27	38	NA	0.85	11	35	26	0.50	1.6	9	14	8	0.46	0.81
		(1.5)	(40.9)	(57.6)	(0.36-2.0)	(15.3)	(48.6)	(36.1)	(0.15-1.7)	(0.72-3.7)	(29.0)	(45.2)	(25.8)	(0.18-1.2)	(0.31-2.2)

*Odds ratios were adjusted for age, sex and years after transplantation when applicable.

Table 4. cont.

Characteristics	France					Italy					Pooled analyses				
	No. detected betaPV types			Odds ratio (95% CI)*		No. detected betaPV types			Odds ratio (95% CI)*		No. detected betaPV types			Odds ratio (95% CI)†	
	0 n=2	1-4 n=18	5+ n=33	1+ vs 0 types	5+ vs 1-4 types	0 n=5	1-4 n=36	5+ n=54	1+ vs 0 types	5+ vs 1-4 types	0 n=55	1-4 n=246	5+ n=249	1+ vs 0 types	5+ vs 1-4 types
Age in years, n (%)															
<50	1 (12.5)	2 (25.0)	5 (62.5)	NA	1.0	1 (3.0)	11 (33.3)	21 (63.6)	NA	1.0	22 (10.0)	110 (50.0)	88 (40.0)	1.0	1.0
50-59	0 (28.6)	4 (71.4)	10	NA	0.86 (0.10-7.0)	3 (8.8)	16 (47.1)	15 (44.1)	NA	0.48 (0.17-1.4)	17 (9.9)	73 (42.7)	81 (47.4)	0.75 (0.37-1.5)	1.3 (0.81-2.0)
60+	1 (3.2)	12 (38.7)	18 (58.1)	NA	0.53 (0.8-3.5)	1 (3.6)	9 (32.1)	18 (64.3)	NA	1.6 (0.49-5.3)	16 (10.1)	63 (39.6)	80 (50.3)	0.82 (0.40-1.7)	1.5 (0.94-2.3)
Sex, n (%)															
Female	0 (40.0)	4 (60.0)	6	NA	1.0	0 (42.1)	8 (57.9)	11	NA	1.0	13 (7.5)	87 (50.0)	74 (42.5)	1.0	1.0
Male	2 (4.7)	14 (32.6)	27 (62.8)	NA	0.98 (0.22-4.4)	5 (6.6)	28 (36.8)	43 (56.6)	NA	1.3 (0.45-3.9)	42 (11.2)	159 (42.3)	175 (46.5)	0.54 (0.27-1.1)	1.2 (0.80-1.8)
Years after transplantation, n (%)															
2-7	1 (5.9)	3 (17.6)	13 (76.5)	NA	1.0	3 (10.3)	15 (51.7)	11 (37.9)	NA	1.0	24 (12.0)	97 (48.5)	79 (39.5)	1.0	1.0
8-12	1 (6.7)	6 (40.0)	8 (53.3)	NA	0.34 (0.06-1.9)	2 (6.5)	9 (29.0)	20 (64.5)	NA	3.9 (1.2-13.3)	15 (11.7)	54 (42.2)	59 (46.1)	0.71 (0.34-1.5)	1.2 (0.70-1.9)
13-17	0 (50.0)	5 (50.0)	5	NA	0.17 (0.03-1.2)	0 (42.9)	6 (57.1)	8	NA	2.3 (0.57-9.5)	12 (11.4)	52 (49.5)	41 (39.0)	0.87 (0.41-1.9)	0.92 (0.54-1.5)
18-22	0 (42.9)	3 (57.1)	4	NA	0.18 (0.02-1.6)	0 (23.1)	3 (76.9)	10	NA	6.2 (1.2-31.4)	2 (3.0)	28 (42.4)	36 (54.5)	3.2 (0.71-14)	1.4 (0.77-2.6)
23+	0 (25.0)	1 (75.0)	3	NA	0.54 (0.03-8.7)	0 (37.5)	3 (62.5)	5	NA	3.0 (0.54-16.6)	2 (3.9)	15 (29.4)	34 (66.7)	2.0 (0.45-9.3)	2.5 (1.2-4.9)
Skin phototype, n (%)															
Dark/olive	2 (6.1)	12 (36.4)	19 (57.6)	NA	1.0	5 (8.2)	23 (37.7)	33 (54.1)	NA	1.0	23 (10.6)	92 (42.2)	103 (47.2)	1.0	1.0
Medium/fair	0 (30.0)	6 (70.0)	14	NA	1.3 (0.36-4.8)	0 (38.2)	13 (61.8)	21	NA	1.2 (0.48-3.0)	32 (9.6)	154 (46.4)	146 (44.0)	2.1 (1.1-4.0)	1.2 (0.78-1.8)
Sunburns before 20 years, n (%)															
0	1 (4.5)	6 (27.3)	15 (68.2)	NA	1.0	4 (6.3)	22 (34.9)	37 (58.7)	NA	1.0	37 (11.3)	143 (43.7)	147 (45.0)	1.0	1.0
1-4	1 (5.9)	9 (52.9)	7 (41.2)	NA	0.21 (0.05-0.96)	1 (4.2)	10 (41.7)	13 (54.2)	NA	0.84 (0.29-2.4)	17 (10.4)	80 (48.8)	67 (40.9)	0.97 (0.51-1.8)	0.77 (0.50-1.2)
5+	0 (21.4)	3 (78.6)	11	NA	1.1 (0.20-6.6)	0 (50.0)	4 (50.0)	4	NA	0.62 (0.13-2.9)	1 (1.7)	23 (39.7)	34 (58.6)	4.7 (0.60-36)	1.2 (0.61-2.2)
Unknown															
Occupational sun exposure, n (%)															
0-3 h	1 (4.0)	10 (40.0)	14 (56.0)	NA	1.0	1 (2.1)	19 (39.6)	28 (58.3)	NA	1.0	43 (11.3)	181 (47.4)	158 (41.4)	1.0	1.0
4 h and more	1 (3.6)	8 (28.6)	19 (67.9)	NA	1.6 (0.48-5.2)	4 (8.5)	17 (36.2)	26 (55.3)	NA	1.4 (0.51-3.6)	12 (7.1)	65 (38.7)	91 (54.2)	0.96 (0.44-2.1)	1.2 (0.80-1.9)
Weekend sun exposure, n (%)															
0-3 h	1 (5.0)	7 (35.0)	12 (60.0)	NA	1.0	1 (3.3)	12 (40.0)	17 (56.7)	NA	1.0	29 (10.2)	135 (47.7)	119 (42.0)	1.0	1.0
4 h and more	1 (3.0)	11 (33.3)	21 (63.6)	NA	0.92 (0.27-3.2)	4 (6.2)	24 (36.9)	37 (56.9)	NA	1.3 (0.49-3.4)	26 (9.7)	111 (41.6)	130 (48.7)	0.55 (0.28-1.1)	1.1 (0.71-1.6)

†Odds ratios adjusted for study centre and age, sex and years after transplantation when applicable.

by the iatrogenic immunosuppression that overshadows the effect of reduced immunity conferred by increasing age. Although this study was not designed as a case-control study comparing ICP with OTR, and our dataset contained significant inter-centre differences, preliminary calculations indeed seem to assign immunosuppression as a risk factor for betaPV (co)infection. After adjustment for age and sex, the odds of betaPV infection in OTR was 60% higher than in the immunocompetent population and the odds of multiple betaPV infection (5+ vs 1–4 types) was 50% higher.

Previously, an association between male sex and betaPV infection has been shown in ICP (Struijk *et al.*, 2003). We found no significant differences in the prevalence of betaPV, although the ICP men were more likely than the women to have more than five viruses present. This association was also found in three of the five OTR centres. In a previous study of betaPV and actinic keratoses, different associations were observed according to sex (Boxman *et al.*, 2001). This, along with our knowledge that males generally have thicker hair bulbs than women, which in turn might influence betaPV detection (unpublished observation), led us to conduct analyses stratified by sex. No consistent differences between males and females were found for the investigated associated factors (data not shown). Sun exposure, skin type and sunburns were not found to be associated with betaPV infection in this study, suggesting that a possible effect of UV-radiation on betaPV replication and/or immunity is probably limited.

This study, as well as others (Antonsson *et al.*, 2000, 2003; Boxman *et al.*, 1997; de Koning *et al.*, 2007; Hazard *et al.*, 2007), highlights the extraordinarily high prevalence of betaPV infection in humans. Generally, ubiquitous viruses maintain a harmonious relationship with their host, but this does not imply that betaPV infections cannot play a role in the pathogenesis of cutaneous SCC, particularly as several studies have shown that infections with betaPV are associated with the development of cutaneous SCC in immunosuppressed OTR, in EV patients and also in the general population. For instance, betaPV infections may play a role as an auxiliary factor to sunlight in the pathogenesis of cutaneous SCC (Pfister, 2003).

In conclusion, this study increases the knowledge of natural betaPV infection by describing the prevalence and type distribution in immunocompetent and immunosuppressed persons without (a history of) cutaneous SCC and thereby aids future investigations into betaPV-related skin carcinogenesis.

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