Human papillomavirus and post-transplant cutaneous squamous-cell carcinoma

Bouwes Bavinck, Jan Nico; Feltkamp, Mariet C. W.; Green, Adele C.; Fiocco, Marta; Euvrard, Sylvie; Harwood, Catherine A.

Published in:
American Journal of Transplantation

DOI:
10.1111/ajt.14537

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

Received: 7 July 2017 | Revised: 20 September 2017 | Accepted: 3 October 2017

DOI: 10.1111/ajt.14537

ORIGINAL ARTICLE

AJT

Human papillomavirus and posttransplantation cutaneous squamous cell carcinoma: A multicenter, prospective cohort study

Jan N. Bouwes Bavinck[1] | Mariet C. W. Feltkamp[2] | Adele C. Green[3] | Marta Fiocco[4,5] | Sylvie Euvrard[6] | Catherine A. Harwood[7] | Charlotte M. Proby[8] | Luigi Naldi[9] | Janouk C. D. Diphoorn[9] | Anna Venturuzzo[9] | Gianpaolo Tessari[10] | Ingo Nindl[11] | Francesca Sampogna[12] | Damiano Abeni[12] | Rachel E. Neale[3] | Jelle J. Goeman[4] | Koen D. Quint[1] | Anne B. Halk[1] | Carmen Sneek[1] | Roel E. Genders[1] | Maurits N. C. de Koning[13] | Wim G. V. Quint[13] | Ulrike Wieland[14] | Sönke Weissenborn[14] | Tim Waterboer[15] | Michael Pawlita[15] | Herbert Pfister[14] | on behalf of the EPI-HPV-UV-CA group

[1]Department of Dermatology, Leiden University Medical Center, Leiden, The Netherlands

[2]Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands

[3]QIMR Berghofer Medical Research Institute, Brisbane, Australia

[4]Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, The Netherlands

[5]Institute of Mathematics, Leiden University, Leiden, The Netherlands

[6]Department of Dermatology, Edouard Herriot Hospital, Hospices Civils de Lyon, Lyon, France

[7]Centre for Cell Biology and Cutaneous Research, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, UK

[8]Division of Cancer Research, University of Dundee, Ninewells Hospital and Medical School, Dundee, UK

[9]Department of Dermatology, Azienda Ospedaliera papa Giovanni XXIII, and GISED Study Center, Bergamo, Italy

[10]Department of Medicine, Section of Dermatology, University of Verona, c/o Ospedale Civile Maggiore, Verona, Italy

[11]Department of Dermatology, University Hospital Charité, Skin Cancer Center Charité, Berlin, Germany

[12]Clinical Epidemiology Unit, IDI-IRCCS FLMM, Rome, Italy

[13]DDL Diagnostic Laboratory, Rijswijk, The Netherlands

[14]Institute of Virology, University of Cologne, Cologne, Germany

[15]German Cancer Research Center (DKFZ), Heidelberg, Germany

Correspondence
Jan Nico Bouwes Bavinck
Email: J.N.Bouwes_Bavinck@lumc.nl

Funding information
EU 5FP Collaborative Research, Grant/Award Number: QLK2-CT-2002-0117; "Progetto Ricerca Corrente" of the Italian Ministry of Health, Rome, Italy

Organ transplant recipients (OTRs) have a 100-fold increased risk of cutaneous squamous cell carcinoma (cSCC). We prospectively evaluated the association between β genus human papillomaviruses (βPV) and keratinocyte carcinoma in OTRs. Two OTR cohorts without cSCC were assembled: cohort 1 was transplanted in 2003-2006 (n = 274) and cohort 2 was transplanted in 1986-2002 (n = 352). Participants were

Abbreviations: CI, confidence interval; BCC, basal cell carcinoma; PV, papillomavirus; cSCC, cutaneous squamous cell carcinoma; HPV, human papillomavirus; HR, hazard ratio; OTR, organ transplant recipient; UVR, ultraviolet radiation.

Members of the EPI-HPV-UV-CA group are listed in the Appendix.

followed until death or cessation of follow-up in 2016. βPV infection was assessed in eyebrow hair by using polymerase chain reaction–based methods. βPV IgG seroresponses were determined with multiplex serology. A competing risk model with delayed entry was used to estimate cumulative incidence of histologically proven cSCC and the effect of βPV by using a multivariable Cox regression model. Results are reported as adjusted hazard ratios (HRs). OTRs with 5 or more different βPV types in eyebrow hair had 1.7 times the risk of cSCC vs OTRs with 0 to 4 different types (HR 1.7, 95% confidence interval 1.1-2.6). A similar risk was seen with high βPV loads (HR 1.8, 95% confidence interval 1.2-2.8). No significant associations were seen between serum antibodies and cSCC or between βPV and basal cell carcinoma. The diversity and load of βPV types in eyebrow hair are associated with cSCC risk in OTRs, providing evidence that βPV is associated with cSCC carcinogenesis and may present a target for future preventive strategies.

KEYWORDS

cancer/malignancy/neoplasia: risk factors, cancer/malignancy/neoplasia: skin - nonmelanoma, clinical research/practice, infection and infectious agents - viral, infection and infectious agents - viral: papillomavirus, organ transplantation in general

1 | INTRODUCTION

Cutaneous squamous cell carcinoma (cSCC) is the most common malignancy in solid organ transplant recipients (OTRs), followed by basal cell carcinoma (BCC).[1,2] The incidence of cSCC and BCC increases with duration of immunosuppressive therapy.[1] Important risk factors are male sex, increasing age, fair skin type, sun exposure, and smoking.[3] cSCCs are usually preceded by multiple viral warts from human papillomavirus (HPV) infection and by actinic keratoses, which also often contain HPV.[3]

The virome component of the human microbiome is known to play an important role in disease,[4,5] and HPV is particularly prevalent in the normal skin virome.[6,7] HPVs are double-stranded DNA viruses that are classified into 5 genera (α, β, γ, μ, and ν).[8,9] αHPVs (eg, mucosal HPV types 16 and 18) are responsible for the development of cervical carcinoma and other mucosal SCCs, including anogenital and oropharyngeal carcinomas.[10] The role of skin βPVs in cSCC carcinogenesis is controversial,[9,11,12] but evidence for etiologic involvement is accumulating.[13-17] βPV cause latent, persistent skin infections[18] and were first discovered in cSCCs from patients with epidermodysplasia verruciformis, a rare genetic skin disease characterized by increased susceptibility to βPV with a high risk of cSCC on sun-exposed skin.[9]

βPVs are ubiquitous, with more than 90% of people carrying these viruses as part of their normal skin virome.[7,19,20] Hair follicles are the likely reservoir.[18] βPV-infected cells may have impaired DNA repair and decreased sensitivity to apoptosis induced by ultraviolet radiation (UVR), facilitating escape from normal cellular defense mechanisms.[9,13] A substantial body of epidemiologic data show an association between HPV infection and cSCC,[12,20-24] in particular, in OTRs.[25,26] In most studies, HPV infection has been determined by detection of serologic responses to a large series of βPV and sometimes γPV types[12,21–23,25–31] and less frequently by detecting genomic DNA from 1 or multiple HPV types in eyebrow hair or skin scrapings.[20,21,25,32-34]

Case-control studies in both OTR and immunocompetent populations have shown that the presence of βPV DNA or antibodies was associated with a 1.5- to 3-fold increased risk of cSCC.[12,20–26,29,31,32] It was not possible, however, to determine whether the βPV infection increases risk of cSCC development or whether cSCC formation promotes active proliferation of βPV. To our best knowledge, there is only 1 published cohort study in OTRs that investigated the influence of βPV infection on the development of cSCC.[26] In a single-center study in 445 patients, OTRs who were βPV seropositive around the time of transplantation had an almost 3-fold increased risk of developing cSCC during the 22-year follow-up period.[26] There are no prospective studies in which the associations between cSCC and both the number and DNA load of βPV types have been examined.

In this study, we present data from 2 prospective multicenter cohort studies confirming that the presence of human skin βPV infection is associated with cSCC development in OTRs.

2 | METHODS

2.1 | Participants and study design

As part of a European Union–funded fifth framework program (5FP) collaborative research grant (QLK2-CT-2002-0117), the EPI-HPV-UV-CA study group collected data for a prospective[35] and a case-control study between 2003 and 2006.[3,25] A description of the inclusion and exclusion criteria is provided in the supplementary material (Figures S1A-S1C). Cohort 1 was assembled from 441 OTRs

who participated in the prospective study.[35] Of these, 167 were excluded due to lack of sufficient follow-up data, leaving a final cohort of 274 OTRs. Cohort 2 was established from 915 OTRs who participated in the case-control study.[3,25] Exclusion of OTRs with lack of sufficient follow-up data (n = 242), an cSCC diagnosed before recruitment (n = 210), or transplanted before 1986 (n = 111) gave a total of 352 OTRs. Both cohorts were under prospective observation until 2016.

Between 2003 and 2006, relevant demographic data and information about skin cancer risk factors were collected.[3,25,35] Plucked eyebrow hair was collected; DNA was extracted for detection and genotyping of 25 βPV types using the skin βPV prototype research assay (Labo Bio-medical Products BV, Rijswijk, The Netherlands)[36] and for viral load determination using quantitative polymerase chain reaction.[32] Because more than 95% of OTRs had 1 or more βPV types in their eyebrow hair,[25] we dichotomized the number of βPV as 0 to 4 and as 5 or more, as previously published.[25] High viral load was defined as 1 βPV copy per 1 to 19 cells and low load as 1 copy per 20 or more cells. Serum was collected and tested for 16 βPV, 6 γPV, 4 αPV, 2 μPV, and 1 νPV IgG antibodies with a multiplex serology technique based on glutathione s-transferase capture ELISA in combination with fluorescent bead technology.[37,38] All 3 techniques used have been previously described in more detail.[25,32,35-38]

In cohort 1, eyebrow hair and serum were collected at 1, 3, 6, 9, 12, and 18 months posttransplantation. The 12-month posttransplantation time point was selected for the final analyses, because this time point best represented the βPV DNA measurement, as separate analyses have shown (Figures S2A and S2B). No participants had developed cSCC before this time point. In cohort 2, DNA and serum were collected at the time of recruitment, a median of 10 (range 2-19) years after transplantation. This was assumed to be representative of βPV DNA status in the years before sampling as βPV infection is characterized by a chronic persistent course with βPV DNA content in eyebrow hair being stable over time.[18]

All OTRs were routinely seen 1 to 4 times per year in specialized centers dedicated to surveillance of OTRs with skin lesions. Clinical information was collected from the time of enrolment between 2003 and 2006 until the last follow-up in 2016 and included the first histologically confirmed cSCC and BCC and the date of the last follow-up or death. We used medical charts, local pathology records, and local oncology databases to collect this information. Both cohort studies adhered to the Declaration of Helsinki principles, and the local medical ethical committees of the hospitals in the participating countries had approved the study design.

2.2 | Statistical analysis

Cohorts 1 and 2 were analyzed separately and in combination. To estimate the independent effect of risk factors on the occurrence of cSCC or BCC, a Cox model was used. Second and subsequent cSCCs or BCCs were not considered, because these data were not collected. The starting date for analysis was the day of transplantation; the end dates for those not diagnosed with cSCC or BCC were the end of follow-up or death. Proportionality of the hazard was assessed by plotting Schoenfeld residuals for relevant covariates and by introducing interactions of relevant covariates with time in the Cox model. A value of $P < .05$ indicated a violation of the proportionality assumption.

A competing risk model with death as competing event and delayed entry (also referred to as left truncation) was used to estimate the cumulative incidence of first cSCC or BCC since transplantation.[39,40] Competing risk models take into account situations where more than 1 cause of failure is possible. In our study, the 2 competing events were cSCC or BCC and death, as patients might have died beforethe occurrence of cSCC or BCC. Delayed entry was used to adjust for the fact that in cohort 2 the HPV DNA testing and serology were not performed at the time of transplantation and that we had taken the 12-month time point in cohort 1.

We had previously defined a new variable based on concordance of βPV DNA and serology. We classified OTR according to whether they were both antibody and DNA positive for the same βPV type as follows: antibody negative, regardless of DNA status; antibody positive but with no types for which DNA was also found; and antibody positive with at least 1 type concordant for DNA.[25]

The final analyses were adjusted for sex, age, skin type, and study center (study center being an important composite variable representing differences in patient populations, immunosuppression regimens, and sun exposure between the 3 clinical sites). We also investigated the other clinical variables, and none changed the estimates of interest. These were, therefore, not included in the analysis. Analyses of cohort 2 or of the combined cohorts showed that adjustment for type of immunosuppression, type of organ transplanted, average sun exposure, number of sunburns, smoking, or alcohol consumption did not substantially change the hazard ratios (HRs) (data not shown). We did not adjust for keratotic skin lesions and common viral warts, as these potentially lie on the causal pathway between HPV infection and cSCC or BCC and their inclusion in the model may lead to overadjustment, biasing the result toward the null.[41]

The analyses concerning the competing risk model were performed in R environment (http://www.R-project.org) with the mstate library.[42] All other analyses were performed with SPSS Statistics for Windows version 23.0 (IBM Corp., Armonk, NY).

3 | RESULTS

The median and maximum follow-up times since transplantation were 9.5 and 12.4 years in cohort 1 and 18.7 and 29.9 years in cohort 2, respectively. The median and maximum follow-up times since DNA and serum sampling did not differ between the cohorts and were 9.8 and 13.4 years, respectively. At the end of follow-up, 161 (26%) of 626 OTRs had died: 52 (19%) of 274 OTRs in cohort 1 and 109 (31%) of 352 OTRs in cohort 2.

The clinical profile of transplanted patients differed between cohorts 1 and 2 (Table 1), but there were no significant differences in virologic results and association with cSCC. OTRs in cohort 1 were more often female, had a darker skin type, were less often smokers, and had lower alcohol consumption. Almost all received mycophenolate

TABLE 1 Baseline characteristics of the 626 organ transplant recipients

	Cohort 1	Cohort 2	Combined cohort
No. of patients	274	352	626
Years of first transplantation	2003-2006	1986-2002	1986-2006
Study center, n (%)			
Leiden (The Netherlands)	101 (36.9)	91 (25.9)	192 (30.7)
Lyon (France)	99 (36.1)	64 (18.2)	163 (26.0)
London (UK)	32 (11.7)	123 (34.9)	155 (24.8)
Bergamo (Italy)	42 (15.3)	74 (21.0)	116 (18.5)
			***P* < .001**
Sex, n (%)			
Women	98 (35.8)	96 (27.3)	194 (31.0)
Men	176 (64.2)	256 (72.7)	432 (69.0)
			***P* = .023**
Age at transplantation, y			
Median	56.4	50.3	52.4
IQR (25%-75%)	47.9-62.6	38.4-57.3	42.0-58.9
			***P* = .003**
Age at physical examination, y			
Median	56.4	59.8	58.2
IQR (25%-75%)	48.0-62.6	52.7-67.1	51.3-65.7
			***P* < .001**
Skin phototype, n (%)			
Dark/olive	157 (57.5)	162 (46.0)	319 (51.1)
Medium	91 (33.3)	128 (36.4)	219 (35.0)
Fair	25 (9.2)	62 (17.6)	87 (13.9)
Missing values	(1)		***P* = .002**
Type of organ, n (%)			
Kidney	220 (80.3)	288 (81.9)	508 (81.2)
Kidney and pancreas	34 (12.4)	29 (8.2)	63 (10.0)
Heart	20 (7.3)	35 (9.9)	55 (8.8)
			P = .140
Immunosuppressive therapy, n (%)			
Aza in any combination	14 (5.1)	148 (42.0)	162 (25.9)
MMF in any combination	251 (92.0)	108 (30.7)	359 (57.5)
CyA or Tac without Aza or MMF	8 (2.9)	96 (27.3)	104 (16.6)
Missing values	(1)		*P* < .001
Average daily sun exposure, n (%)			
1-3 h	175 (63.9)	208 (59.1)	383 (61.2)
≥4 h	99 (36.1)	144 (40.9)	243 (38.8)
			P = .224
Sunburns before the age of 20 y, n (%)			
0	110 (40.4)	174 (49.4)	284 (45.5)
1-4	108 (39.7)	123 (34.9)	231 (37.0)
≥5	54 (19.9)	55 (15.6)	109 (17.5)
Missing values	(2)		*P* = .073

(Continues)

TABLE 1 (Continued)

	Cohort 1	Cohort 2	Combined cohort
Smoking, n (%)			
No	139 (50.8)	133 (38.1)	272 (43.7)
1-19 pack-y	76 (27.7)	136 (39.0)	212 (34.0)
≥20 pack-y	59 (21.5)	80 (22.9)	139 (22.3)
Missing values		(3)	***P* = .003**
Alcohol consumption, n (%)			
No	112 (57.1)	98 (27.8)	210 (38.3)
1-19 g/d	64 (32.7)	188 (53.4)	252 (56.0)
≥20 g/d	20 (10.2)	66 (18.8)	86 (15.7)
Missing values	(78)		***P* < .001**
Keratotic skin lesions, n (%)			
0	205 (74.8)	111 (31.6)	316 (50.6)
1-49	65 (23.7)	208 (59.3)	273 (43.6)
≥50	4 (1.5)	32 (9.1)	36 (5.8)
Missing values		(1)	***P* < .001**
Common viral warts, n (%)			
0	234 (85.7)	203 (57.8)	437 (70.0)
1-49	33 (12.1)	103 (29.3)	136 (21.8)
≥50	6 (2.2)	45 (12.8)	51 (8.2)
Missing values	(1)	(1)	***P* < .001**
No. of βPV types in hair			
Median	4.5	4.0	4.0
IQR (25%-75%)	2.3-6.8	2.0-8.0	2.0-7.0
			P = .643
No. of βPV types in hair			
0-4	142 (51.8)	174 (49.4)	316 (50.5)
≥5	132 (48.2)	178 (50.6)	310 (49.5)
			P = .552
βPV load (based on 8 βPV types)			
Low load (1 copy per ≥20 cells)	195 (71.2)	252 (71.6)	447 (71.4)
High load (1 copy per <20 cells)	79 (28.8)	100 (28.4)	179 (28.6)
			P = .907
HPV seropositivity			
αPV	165 (60.2)	188 (53.4)	353 (56.4)
βPV	137 (50.0)	176 (50.0)	313 (50.0)
γPV	133 (48.5)	165 (46.9)	298 (47.6)
μPV	91 (33.2)	100 (28.4)	192 (30.5)
νPV	31 (11.3)	31 (8.8)	62 (9.9)
			P > .05

The *P*-values refer to the differences between cohorts 1 and 2 and are calculated with a χ^2 test (ordinal data) or ANOVA (continuous data). Significant *P*-values are indicated in bold.

Aza, azathioprine; CyA, cyclosporin A; MMF, mycophenolate mofetil; Tac, tacrolimus.

mofetil in combination with tacrolimus, rather than azathioprine, which was the main immunosuppressive drug in cohort 2. The numbers of keratotic lesions and common viral warts were higher in cohort 2, consistent with their longer duration of immunosuppression (Table 1).

Table 2 shows the cause-specific HRs for prognostic factors associated with cSCC in the 2 cohorts combined. Results generated from individual cohort analyses are comparable (Tables S1 and S2). Male sex, increasing age, fair skin type, and immunosuppression with azathioprine were the strongest risk factors for cSCC. Because the

TABLE 2 Possible risk factors for the development of cutaneous squamous cell carcinoma in 626 organ transplant recipients (combined cohort)

	N (%)		Cause-specific univariate HR	Adjusted HR[a]
	No SCC	SCC		
No. of patients	536	90		
Study center, n (%)				
Leiden (The Netherlands)	172 (32.1)	20 (22.3)	1	1
Lyon (France)	144 (26.9)	19 (21.1)	1.4 (0.72-2.6)	0.93 (0.49-1.8)
London (UK)	126 (23.5)	29 (32.2)	1.8 (0.99-3.2)	1.6 (0.88-2.9)
Bergamo (Italy)	94 (17.5)	22 (24.4)	1.5 (0.83-2.8)	1.7 (0.90-3.1)
Sex, n (%)				
Women	176 (32.8)	18 (20.0)	***1***	***1***
Men	360 (67.2)	72 (80.0)	***1.7 (1.01-2.7)***	***2.0 (1.2-3.5)***
Age at transplantation per 10 y				
Median	51.9	54.8		
IQR (25%-75%)	41.9-58.8	46.8-61.8	***1.8 (1.4-2.1)***	***2.1 (1.7-2.6)***
Age at physical examination per 10 y				
Median	57.8	64.5		
IQR (25%-75%)	51.2-65.43	58.1-67.6	***1.9 (1.5-2.3)***	***2.1 (1.7-2.7)***
Skin phototype, n (%)				
Dark/olive	287 (53.6)	32 (35.6)	1	***1***
Medium	184 (34.4)	35 (38.9)	1.6 (0.99-2.6)	***1.9 (1.2-3.2)***
Fair	64 (12.0)	23 (25.5)	***3.3 (1.9-5.6)***	***3.9 (2.2-7.0)***
Missing values	(1)			
Type of organ, n (%)				
Kidney	434 (81.0)	74 (82.2)	1	
Kidney and pancreas	59 (11.0)	4 (4.4)	0.45 (0.16-1.2)	0.81 (0.28-2.4)
Heart	43 (8.0)	12 (13.4)	1.8 (0.97-3.3)	1.1 (0.53-2.2)
Immunosuppressive therapy, n (%)				
Aza in any combination	108 (20.2)	54 (60.0)	***1***	***1***
MMF in any combination	334 (62.4)	25 (27.8)	***0.22 (0.13-0.38)***	***0.28 (0.15-0.50)***
CyA or Tac without Aza or MMF	93 (17.4)	11 (12.2)	***0.33 (0.17-0.64)***	***0.25 (0.13-0.50)***
Missing values	(1)			
Average daily sun exposure, n (%)				
1-3 h	335 (62.5)	48 (53.3)	1	1
≥4 h	201 (37.5)	42 (46.7)	1.4 (0.90-2.1)	1.1 (0.71-1.7)
Sunburns before the age of 20 y, n (%)				
0	243 (45.4)	41 (46.1)	1	1
1-4	196 (36.7)	35 (39.3)	1.1 (0.68-1.7)	0.91 (0.56-1. 5)
≥5	96 (17.9)	13 (14.6)	0.92 (0.49-1.7)	0.66 (0.33-1.3)
Missing values	(1)	(1)		
Smoking, n (%)				
No	240 (44.9)	32 (36.0)	1	1
1-19 pack-y	182 (34.1)	30 (33.7)	1.1 (0.69-1.9)	0.94 (0.56-1.6)
≥20 pack-y	112 (21.0)	27 (30.3)	***1.9 (1.1-3.2)***	1.1 (0.65-1.9)
Missing values	(2)	(1)		

(Continues)

TABLE 2 (Continued)

	N (%)		Cause-specific univariate HR	Adjusted HR[a]
	No SCC	SCC		
Alcohol consumption, n (%)				
No	190 (41.0)	20 (23.5)	1	1
1-19 g/d	207 (44.7)	45 (52.9)	1.6 (0.97-2.8)	1.4 (0.83-2.5)
≥20 g/d	66 (14.3)	20 (23.6)	***2.5 (1.3-4.6)***	1.8 (0.93-3.5)
Missing values	(73)	(5)		
Keratotic skin lesions, n (%)				
0	299 (55.9)	17 (18.9)	***1***	***1***
1-49	213 (39.8)	60 (66.7)	***4.1 (2.3-7.1)***	***3.1 (1.7-5. 5)***
≥50	23 (4.3)	13 (14.4)	***8.0 (3.8-16.6)***	***4.7 (2.0-10.9)***
Missing values	(1)			
Common viral warts, n (%)				
0	387 (72.5)	50 (55.6)	1	1
1-49	113 (21.1)	23 (25.6)	1.3 (0.80-2.2)	1.3 (0.77-2.2)
≥50	34 (6.4)	17 (18.8)	***2.3 (1.3-4.1)***	***2.5 (1.3-5.0)***
Missing values	(2)			

All hazard ratios (HRs) are calculated using delayed entry and adjusting for competitive risk of death. Significant HRs are indicated in bold.
Aza, azathioprine; CyA, cyclosporin A; MMF, mycophenolate mofetil; SCC, squamous cell carcinoma; Tac, tacrolimus.
[a]The adjusted HRs are calculated with the factor of interest and sex, age at physical examination, skin type, and study center included in the model.

majority (>80%) of both cohorts were kidney transplant recipients, we had insufficient statistical power to assess differences in risk of cSCC according to type of organ transplant received. As we have reported earlier,[3] the number of keratotic skin lesions and common viral warts was strongly associated with the development of cSCC, with an adjusted HR of 4.7 (95% confidence interval [CI] 2.0-10.9) for OTRs with 50 or more lesions compared with those OTRs with fewer lesions. After adjustment, sun exposure, painful sunburns, and smoking were not associated with cSCC in the combined cohort (Table 2).

The number of βPV types in eyebrow hair, βPV DNA load, and serologic responses to HPV did not significantly differ between the 2 cohorts (Table 1). Figure 1 shows a higher overall cumulative incidence of cSCC in OTRs infected with multiple (≥5) HPV types measured 12 months posttransplantation in cohort 1 and after a median of 10 years posttransplantation in cohort 2 compared with OTR with fewer βPV types or noninfected OTRs (Figure 1A). The cumulative incidence of cSCC for the separate cohorts is provided in Figures S3A and S3B. Table 3 reports the association between presence of βPV DNA in eyebrow hair and subsequent development of cSCC. The combined adjusted HR was 1.7 (95% CI 1.1-2.6). More detailed information is provided in the Tables S3-S5.

High βPV load was also associated with a significantly higher cumulative incidence of cSCC compared with a low load or absent βPV types (Figure 1B and Table 3). In the combined cohort, the adjusted HR was 1.8 (95% CI 1.2-2.8). The βPV load risk factor in cohort 1 was probably driving the HRs in the combined analyses.

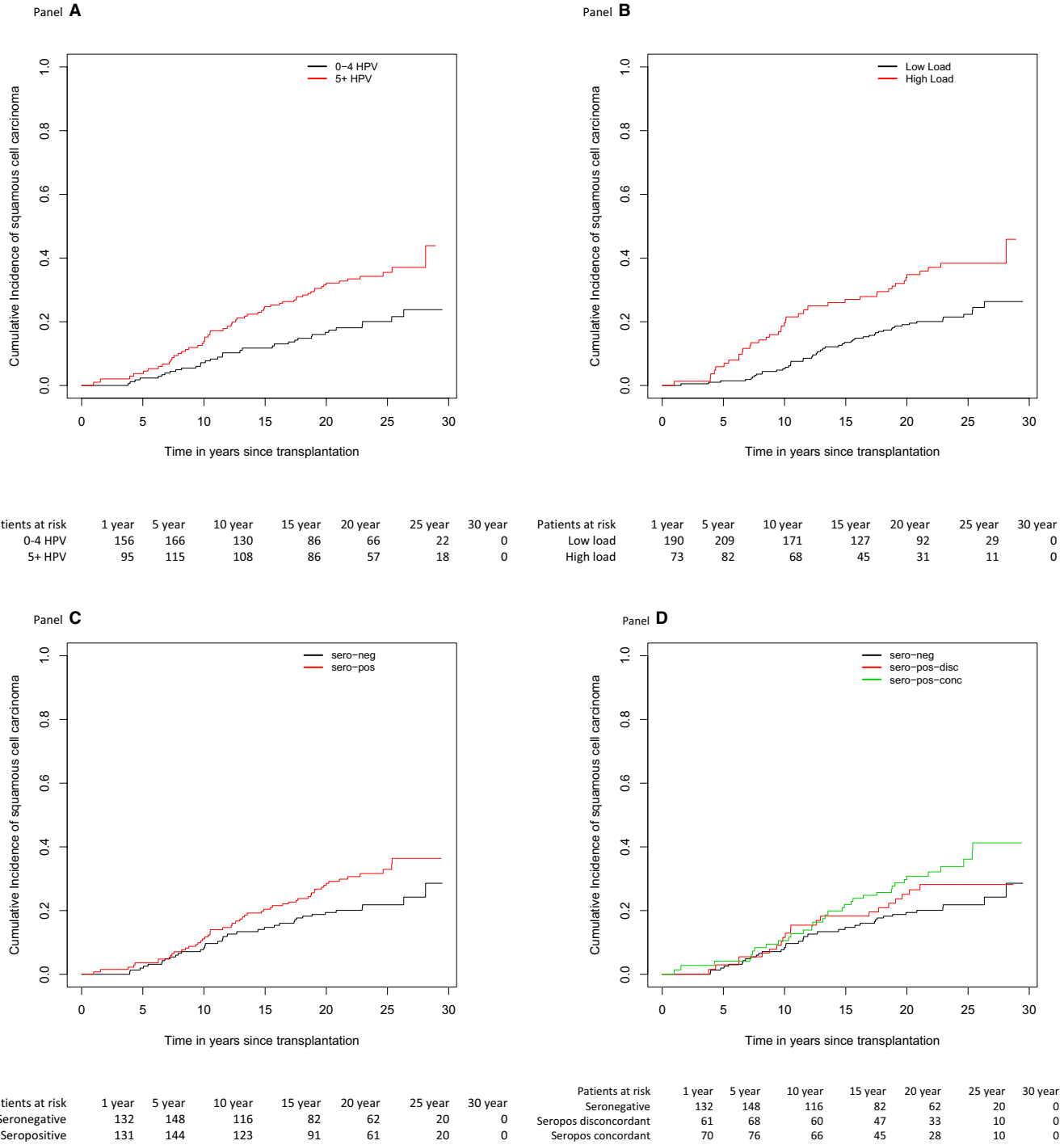
HPV seropositivity at the time of sampling was not associated with cSCC risk (Figures 1C and 1D and Table 3). The association was stronger but still not significant for concordant serologic responses (Figure 1D and Table 3). We observed a weak association between γPV seropositivity and cSCC (Table 3) but no association with αPV, μPV, and νPV seropositivity (Tables S3-S5). We examined possible heterogeneity of βPV effects between cohorts 1 and 2, but this was not the case in the univariate and adjusted analyses for any of the variables in Table 3. We were unable to assess the influence of type of organ transplanted because our study populations were mostly kidney transplant recipients. There was no evidence of violation of the proportional hazard assumption.

Data on individual HPV types suggested that βPV of species 1, specifically HPV types 5, 8, 20, 21, and 36, are those most likely to be involved in cutaneous squamous carcinogenesis, with possible roles for HPV76 (species 3) and HPV92 (species 4) (Table S6). The increased risk of skin cancer was specific for cSCC; there were no statistically significant associations between HPV infection and the development of BCC (Table S7).

4 | DISCUSSION

In this observational hospital-based study of 2 OTR cohorts with a follow-up period after HPV sampling of longer than 10 years, we found that the diversity and load of infecting βPV types were associated with an approximately doubled risk of cSCC. Our results were robust after adjustment for multiple potential confounders. This level of increased risk is equivalent to that associated with other well-established risk factors such as skin phototype[3] and points to an important role for the skin virome and specifically HPV in the pathogenesis of cSCC.

HPV constitute an important part of the normal human skin virome.[6,7,19,20] There is strong evidence from laboratory studies to suggest



Patients at risk	1 year	5 year	10 year	15 year	20 year	25 year	30 year
0-4 HPV	156	166	130	86	66	22	0
5+ HPV	95	115	108	86	57	18	0

Patients at risk	1 year	5 year	10 year	15 year	20 year	25 year	30 year
Low load	190	209	171	127	92	29	0
High load	73	82	68	45	31	11	0

Patients at risk	1 year	5 year	10 year	15 year	20 year	25 year	30 year
Seronegative	132	148	116	82	62	20	0
Seropositive	131	144	123	91	61	20	0

Patients at risk	1 year	5 year	10 year	15 year	20 year	25 year	30 year
Seronegative	132	148	116	82	62	20	0
Seropos disconcordant	61	68	60	47	33	10	0
Seropos concordant	70	76	66	45	28	10	0

FIGURE 1 Cumulative incidence of cutaneous squamous cell carcinoma in organ-transplant recipients with 5 and more HPV types in eyebrow hair measured 12 mo post-transplant in cohort 1 and after a median of 10 years post-transplant in cohort 2 (red line) compared with transplant recipients with 0-4 HPV types (black line) (Panel A); with a high βPV load in plucked eyebrow hair (red line) compared with transplant recipients with a low load or absence of these 8 specific βPV types (black line) (Panel B); with a positive serologic response to βPV (red line) compared with transplant recipients without a positive response (black line) (Panel C); with a positive serologic response to βPV in combination with the same βPV type(s) in the eyebrow hair (green line), and with a positive serologic response to βPV in combination with different βPV types in the eyebrow hair (red line) compared with transplant recipients without a positive response (black line) (Panel D). The curves are adjusted for competitive risk of death and for delayed inclusion of the patients. Due to the delayed inclusion, the numbers of patients at risk initially increase with time before they decrease

TABLE 3 Risk of cutaneous squamous cell carcinoma in organ transplant recipients with human papillomavirus infection according to the presence of βPV DNA and/or serologic response to βPV

No. of squamous cell carcinoma cases/total population[a]	Cohort 1 14/274	Cohort 2 76/352		Both cohorts combined 90/626	
Risk factors	Cause-specific univariate HR[b]	Cause-specific univariate HR	Adjusted HR[c]	Cause-specific univariate HR	Adjusted HR[c]
βPV DNA (based on 25 βPV types)					
0-4 types	1	***1***	1	***1***	***1***
≥5	2.6 (0.82-8.3)	***1.8 (1.1-2.8)***	1.6 (0.998-2.7)	***1.9 (1.2-2.9)***	***1.7 (1.1-2.6)***
βPV load (based on 8 βPV types)					
Low load (1 copy per ≥20 cells)	***1***	***1***	1	***1***	***1***
High load (1 copy per <20 cells)	***4.6 (1.5-13.8)***	***1.8 (1.1-2.8)***	1.5 (0.92-2.4)	***2.1 (1.4-3.1)***	***1.8 (1.2-2.8)***
βPV serology (based on 16 βPV types)					
Seronegative	1	***1***	1	1	1
Seropositive	1.0 (0.36-2.9)	***1.6 (1.02-2.6)***	1.5 (0.90-2.4)	1.5 (0.97-2.3)	1.4 (0.90-2.1)
Combination βPV DNA and serology					
Seronegative irrespective DNA	1	1	1	1	1
Seropositive without concordant DNA	0.98 (0.25-3.9)	1.2 (0.69-2.2)	1.2 (0.66-2.2)	1.2 (0.69-2.0)	1.2 (0.71-2.1)
Seropositive with concordant DNA	1.1 (0.31-3.6)	***2.0 (1.2-3.3)***	***1.7 (1.002-3.0)***	***1.8 (1.1-2.9)***	1.5 (0.93-2.5)
γPV serology (based on 6 γPV types)					
Seronegative	1	1	***1***	1	1
Seropositive	1.1 (0.38-3.1)	1.5 (0.93-2.3)	***1.7 (1.1-2.8)***	1.4 (0.91-2.1)	***1.6 (1.01-2.4)***

All hazard ratios (HRs) are calculated using delayed entry and adjusting for competitive risk of death. Significant HRs are indicated in bold.
[a]The detailed numbers of squamous cell carcinoma per cohort and per exposure are provided in the supplementary material (Tables S3-S5).
[b]Adjusted HRs are not available for cohort 1 because of low number of events.
[c]The HRs are adjusted for sex, age at physical examination, skin type, and study center.

that HPV plays a role in cSCC carcinogenesis, principally through synergy with UVR.[13-15] In normal cells, UVR upregulates cellular defense processes leading to *p53* activation, cell cycle arrest, apoptosis, or DNA repair. Several βPV types (eg, HPV5, HPV8, HPV38, and HPV49) deregulate these crucial cellular regulatory pathways by targeting multiple transcription factors and/or transcriptional regulators, resulting in infected cells that are highly susceptible to chromosomal instability and malignant transformation by UVR.[9,15,43] Synergy between βPV and UVR has also been documented in animal models. In transgenic mice expressing the HPV8 early region or *E6* gene alone, a single dose of UVR rapidly promoted papillomas and cSCC formation,[16] and in HPV38 *E6/E7* transgenic mice, UVR also induced the development of actinic keratoses and cSCC.[17] Certain βPV types also appear to have enhanced replication in the context of host immunosuppression, likely accompanied by increased oncogene expression and oncogenic activity.[44]

Previous epidemiologic studies investigating the association between βPV and cutaneous squamous carcinogenesis have generated inconsistent findings.[9,11,12] One of the challenges is the diversity of recognized HPV types within the normal skin virome.[33,34] More than 200 HPV types have been identified, and the number of putative new types is increasing.[33,34,45] We tested for only a subset of HPV types. Our polymerase chain reaction–based βPV DNA detection technique was state-of-the-art when we initiated this study and remains a reliable and reproducible method. It is possible, however, that the true association between βPV infection and cSCC is even stronger, as we may have failed to detect additional relevant HPV infections. We also observed that seropositivity to γPV was associated with subsequent cSCC, supporting recent findings that γPV type HPV197 is commonly present in skin tumors[34] and that its E6 and E7 proteins interact with a set of cellular proteins similar to those encoded by genital HPVs linked to human carcinogenesis.[45]

Another limitation of this study was the lack of follow-up data in Berlin and Verona. While this decreased the power of the study, we believe that it is unlikely to have introduced bias because all patients in these 2 centers were excluded, independent of their medical history. Although cohort 1 was ideally designed for analysis of the involvement of HPV in cSCC development, we were hampered by the low number of events during the first 10 years after transplantation. Therefore, cohorts 1 and 2 were combined, which in the crude associations between the presence, number, and load of βPV DNA with the later development of cSCC appeared similar. There were also differences between the 2 cohorts. In recent years, older patients were more frequently transplanted, the immunosuppressive regimen changed, and a larger proportion of patients with a darker skin phototype were transplanted, especially in Leiden and London.

Other potential limitations of this study are missing data in the clinical characteristics included in the multivariable analyses. Of

particular concern is the 28% missing data for alcohol consumption, but we found no evidence that adjusting for alcohol in those with complete information made any difference to the estimates of association between HPV and cSCC. We have no good explanation why we did not find a statistically significant association with βPV serology. We have shown a 40% increased risk of cSCC overall in seropositive OTRs but had to exclude a substantial number of patients because of missing data, decreasing the power of our study, which may be a possible explanation of our failure to show a statistically significant association between βPV serology and cSCC.

This cohort study provides evidence that infection with βPV is associated with the development of cSCC. βPV vaccines are now in development, and our data provide a rationale for routine pretransplantation screening of the skin virome for βPV content and βPV vaccination of high-risk individuals as a possible future strategy for reducing the burden of posttransplantation cSCC.

ACKNOWLEDGMENTS

The first part of the study was funded by an EU 5FP collaborative research grant (QLK2-CT-2002-0117). D.A., F.S., and the other group members of IDI-IRCCS FLMM were also supported, in part, by the "Progetto Ricerca Corrente" of the Italian Ministry of Health, Rome, Italy.

AUTHOR CONTRIBUTIONS

Conception and design: Jan Nico Bouwes Bavinck, Mariet C. W. Feltkamp, Adele C. Green, Sylvie Euvrard, Catherine A. Harwood, Charlotte M. Proby, Luigi Naldi, Ingo Nindl, Francesca Sampogna, Damiano Abeni, Rachel E Neale, Maurits N.C. de Koning, Wim G.V. Quint, Ulrike Wieland, Tim Waterboer, Michael Pawlita, and Herbert Pfister. Collection of data: Jan Nico Bouwes Bavinck, Sylvie Euvrard, Catherine A. Harwood, Charlotte M. Proby, Luigi Naldi, Janouk C.D. Diphoorn, Anna Venturuzzo, Gianpaolo Tessari, Anne Berthe Halk, and Carmen Sneek. Data analyses and interpretation: Marta Fiocco, Jelle J. Goeman, Jan Nico Bouwes Bavinck, Mariet C.W. Feltkamp, Adele C. Green, Sylvie Euvrard, Catherine A. Harwood, Charlotte M. Proby, Luigi Naldi, Ingo Nindl, Francesca Sampogna, Damiano Abeni, Rachel E Neale, Maurits N.C. de Koning, Wim G.V. Quint, Ulrike Wieland, Tim Waterboer, Michael Pawlita, and Herbert Pfister. Manuscript writing: all authors. Final approval of manuscript: all authors. Accountable for all aspects of the work: all authors.

DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

REFERENCES

1. Euvrard S, Kanitakis J, Claudy A. Skin cancers after organ transplantation. *N Engl J Med*. 2003;348:1681-1691.
2. Hartevelt MM, Bouwes Bavinck JN, Kootte AM, Vermeer BJ, Vandenbroucke JP. Incidence of skin cancer after renal transplantation in The Netherlands. *Transplantation*. 1990;49:506-509.
3. Bouwes Bavinck JN, Euvrard S, Naldi L, et al. Keratotic skin lesions and other risk factors are associated with skin cancer in organ-transplant recipients: a case-control study in The Netherlands, United Kingdom, Germany, France, and Italy. *J Invest Dermatol*. 2007;127:1647-1656.
4. Kong HH, Segre JA. The molecular revolution in cutaneous biology: investigating the skin microbiome. *J Invest Dermatol*. 2017;137:e119-e122.
5. Oh J, Byrd AL, Deming C, et al. Biogeography and individuality shape function in the human skin metagenome. *Nature*. 2014;514:59-64.
6. Foulongne V, Sauvage V, Hebert C, et al. Human skin microbiota: high diversity of DNA viruses identified on the human skin by high throughput sequencing. *PLoS ONE*. 2012;7:e38499.
7. Ma Y, Madupu R, Karaoz U, et al. Human papillomavirus community in healthy persons, defined by metagenomics analysis of human microbiome project shotgun sequencing data sets. *J Virol*. 2014;88:4786-4797.
8. Doorbar J, Egawa N, Griffin H, Kranjec C, Murakami I. Human papillomavirus molecular biology and disease association. *Rev Med Virol*. 2015;25(Suppl 1):2-23.
9. Howley PM, Pfister HJ. Beta genus papillomaviruses and skin cancer. *Virology*. 2015;479-480:290-296.
10. Uijterwaal MH, Polman NJ, van Kemenade FJ, et al. Five-year cervical (pre)cancer risk of women screened by HPV and cytology testing. *Cancer Prev Res (Phila)*. 2015;8:502-508.
11. Accardi R, Gheit T. Cutaneous HPV and skin cancer. *Presse Med*. 2014;43:e435-e443.
12. Chahoud J, Semaan A, Chen Y, et al. Association between beta-genus human papillomavirus and cutaneous squamous cell carcinoma in immunocompetent individuals—a meta-analysis. *JAMA Dermatol*. 2015;152:1354.
13. Bouwes Bavinck JN, Feltkamp MC. Milk of human kindness?–HAMLET, human papillomavirus, and warts. *N Engl J Med*. 2004;350:2639-2642.
14. Connolly K, Manders P, Earls P, Epstein RJ. Papillomavirus-associated squamous skin cancers following transplant immunosuppression: one Notch closer to control. *Cancer Treat Rev*. 2014;40:205-214.
15. Quint KD, Genders RE, de Koning MN, et al. Human Beta-papillomavirus infection and keratinocyte carcinomas. *J Pathol*. 2015;235:342-354.
16. Marcuzzi GP, Hufbauer M, Kasper HU, Weissenborn SJ, Smola S, Pfister H. Spontaneous tumour development in human papillomavirus type 8 E6 transgenic mice and rapid induction by UV-light exposure and wounding. *J Gen Virol*. 2009;90:2855-2864.
17. Viarisio D, Mueller-Decker K, Kloz U, et al. E6 and E7 from beta HPV38 cooperate with ultraviolet light in the development of actinic keratosis-like lesions and squamous cell carcinoma in mice. *PLoS Pathog*. 2011;7:e1002125.
18. de Koning MN, Struijk L, Bouwes Bavinck JN, et al. Betapapillomaviruses frequently persist in the skin of healthy individuals. *J Gen Virol*. 2007;88:1489-1495.
19. de Koning MN, Weissenborn SJ, Abeni D, et al. Prevalence and associated factors of betapapillomavirus infections in individuals without cutaneous squamous cell carcinoma. *J Gen Virol*. 2009;90:1611-1621.
20. Iannacone MR, Gheit T, Pfister H, et al. Case-control study of genus-beta human papillomaviruses in plucked eyebrow hairs and cutaneous squamous cell carcinoma. *Int J Cancer*. 2014;134:2231-2244.
21. Bouwes Bavinck JN, Neale RE, Abeni D, et al. Multicenter study of the association between betapapillomavirus infection and cutaneous squamous cell carcinoma. *Cancer Res*. 2010;70:9777-9786.
22. Karagas MR, Waterboer T, Li Z, et al. Genus beta human papillomaviruses and incidence of basal cell and squamous cell carcinomas of skin: population based case-control study. *BMJ*. 2010;341:c2986.
23. Andersson K, Michael KM, Luostarinen T, et al. Prospective study of human papillomavirus seropositivity and risk of nonmelanoma skin cancer. *Am J Epidemiol*. 2012;175:685-695.

24. Farzan SF, Waterboer T, Gui J, et al. Cutaneous alpha, beta and gamma human papillomaviruses in relation to squamous cell carcinoma of the skin: a population-based study. *Int J Cancer*. 2013;133:1713-1720.
25. Proby CM, Harwood CA, Neale RE, et al. A case-control study of beta-papillomavirus infection and cutaneous squamous cell carcinoma in organ transplant recipients. *Am J Transplant*. 2011;11:1498-1508.
26. Genders RE, Mazlom H, Michel A, et al. The presence of betapapillomavirus antibodies around transplantation predicts the development of keratinocyte carcinoma in organ transplant recipients: a cohort study. *J Invest Dermatol*. 2015;135:1275-1282.
27. Paradisi A, Waterboer T, Sampogna F, et al. Seropositivity for human papillomavirus and incidence of subsequent squamous cell and basal cell carcinomas of the skin in patients with a previous nonmelanoma skin cancer. *Br J Dermatol*. 2011;165:782-791.
28. Plasmeijer EI, Pandeya N, O'Rourke P, et al. The Association between cutaneous squamous cell carcinoma and betapapillomavirus seropositivity: a cohort study. *Cancer Epidemiol Biomarkers Prev*. 2011;20:1171-1177.
29. Iannacone MR, Gheit T, Waterboer T, et al. Case-control study of cutaneous human papillomaviruses in squamous cell carcinoma of the skin. *Cancer Epidemiol Biomarkers Prev*. 2012;21:1303-1313.
30. Iannacone MR, Wang W, Stockwell HG, et al. Sunlight exposure and cutaneous human papillomavirus seroreactivity in basal cell and squamous cell carcinomas of the skin. *J Infect Dis*. 2012;206:399-406.
31. Faust H, Andersson K, Luostarinen T, Gislefoss RE, Dillner J. Cutaneous human papillomaviruses and squamous cell carcinoma of the skin: nested case-control study. *Cancer Epidemiol Biomarkers Prev*. 2016;25:721-724.
32. Neale RE, Weissenborn S, Abeni D, et al. Human papillomavirus load in eyebrow hair follicles and risk of cutaneous squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev*. 2013;22:719-727.
33. Bzhalava D, Muhr LS, Lagheden C, et al. Deep sequencing extends the diversity of human papillomaviruses in human skin. *Sci Rep*. 2014;4:5807.
34. Arroyo Muhr LS, Hultin E, Bzhalava D, et al. Human papillomavirus type 197 is commonly present in skin tumors. *Int J Cancer*. 2015;136:2546-2555.
35. Antonsson A, Waterboer T, Bouwes Bavinck JN, et al. Longitudinal study of seroprevalence and serostability of 34 human papillomavirus types in European organ transplant recipients. *Virology*. 2013;436:91-99.
36. de Koning M, Quint W, Struijk L, et al. Evaluation of a novel highly sensitive, broad-spectrum PCR-reverse hybridization assay for detection and identification of beta-papillomavirus DNA. *J Clin Microbiol*. 2006;44:1792-1800.
37. Michael KM, Waterboer T, Sehr P, et al. Seroprevalence of 34 human papillomavirus types in the German general population. *PLoS Pathog*. 2008;4:e1000091.
38. Waterboer T, Sehr P, Michael KM, et al. Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion proteins. *Clin Chem*. 2005;51:1845-1853.
39. Keurentjes JC, Fiocco M, Schreurs BW, Pijls BG, Nouta KA, Nelissen RG. Revision surgery is overestimated in hip replacement. *Bone Joint Res*. 2012;1:258-262.
40. Putter H, Fiocco M, Geskus RB. Tutorial in biostatistics: competing risks and multi-state models. *Stat Med*. 2007;26:2389-2430.
41. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. *Epidemiology*. 2009;20:488-495.
42. de Wreede LC, Fiocco M, Putter H. The mstate package for estimation and prediction in non- and semi-parametric multi-state and competing risks models. *Comput Methods Programs Biomed*. 2010;99:261-274.
43. Arron ST, Jennings L, Nindl I, et al. Viral oncogenesis and its role in nonmelanoma skin cancer. *Br J Dermatol*. 2011;164:1201-1213.
44. Weissenborn S, Neale RE, Waterboer T, et al. Beta-papillomavirus DNA loads in hair follicles of immunocompetent people and organ transplant recipients. *Med Microbiol Immunol*. 2012;201:117-125.
45. Grace M, Munger K. Proteomic analysis of the gamma human papillomavirus type 197 E6 and E7 associated cellular proteins. *Virology*. 2017;500:71-81.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Bouwes Bavinck JN, Feltkamp MCW, Green AC, et al. Human papillomavirus and posttransplantation cutaneous squamous cell carcinoma: A multicenter, prospective cohort study. *Am J Transplant*. 2017;00:1–11. https://doi.org/10.1111/ajt.14537

APPENDIX

Department of Dermatology, Leiden University Medical Center, Leiden, The Netherlands: J. N. Bouwes Bavinck, P. van der Zwan-Kralt, Y. G. L. de Graaf, L. E. Vos, E. J. Uphoff-Meijerink, A. B. Halk, C. Sneek, R. Willemze; Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands: M. C. W. Feltkamp, L. Struijk, P. Wanningen, E. van der Meijden, E. I. Plasmeijer; Department of Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands: R. Wolterbeek; Department of Dermatology, Hospices Civils de Lyon, Lyon, France: S. Euvrard, A. C. M. A. Ocampo, J. Kanitakis; Department of Dermatology, University Hospital Charité, Skin Cancer Center Charité, Berlin, Germany: I. Nindl, E. Stockfleth, T. Forschner; Department of Dermatology, Azienda Ospedaliera papa Giovanni XXIII, and GISED Study Center Bergamo, Italy: L. Naldi, A. Pizzagalli, F. Sassi, E. Gotti, R. Fiocchi, J. C. D. Diphoorn; Department of Biomedical and Surgical Sciences, Section of Dermatology, University of Verona, c/o Ospedale Civile Maggiore, Verona, Italy: G. Tessari; Centre for Cutaneous Research, Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK: C. A. Harwood, C. M. Proby, J. Breuer, L. Mitchell, K. Purdie, S. R. Lambert, H. Ran; Institute of Virology, University of Cologne, Cologne, Germany: H. Pfister, U. Wieland, S. Weissenborn; German Cancer Research Center (DKFZ), Heidelberg, Germany: M. Pawlita, T. Waterboer, P. Sehr, K. M. Michael; DDL Diagnostic Laboratory, Rijswijk, The Netherlands: W. G. V. Quint, M. N. C. de Koning*, J. ter Schegget*, B. Kleter, L. J. van Doorn. *Also employed by Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands; Clinical Epidemiology Unit, IDI-IRCCS, Rome, Italy: D. Abeni, F. Sampogna, S. Simoni, G. P. Petasecca Donati, C. Masini; Queensland Institute of Medical Research, Brisbane, Australia: A. C. Green, R. E. Neale, C. Olsen, P. O'Rourke; James Cook University; S. Harrison, P. Buttner.