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Prevalence and stability of antibodies to 37 human papillomavirus types — A population-based longitudinal study

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ABSTRACT

Information about serostability of cutaneous HPV types over time is very limited. We investigated seroprevalence and serostability of 37 different HPV types over 4½ years in an Australian population-based study. Sera and data were analyzed for 390 people who had never been diagnosed with SCC and had blood collected in 1992, 1993 and 1996.

Eighty-six percent of participants were seropositive to at least one of the 37 HPV types at baseline. HPV-4 was the type with the highest seroprevalence (41%), followed by HPV-38 and HPV-8 (both 33%). Over 90% of people retained their baseline serostatus during the 4½ year follow-up. Highest serostability was observed for HPV-88 (99.7% stayed seropositive or seronegative), while HPV-65 was least stable with 17% altering their serostatus during follow-up.

Seroprevalence to cutaneous HPV types are relatively stable over time, and a single measure can be used as a reasonable marker of long-term antibody status.

Introduction

Papillomaviruses are a diverse, epitheliotropic group of viruses recognized as the causative agents of warts, cervical cancer and head and neck cancers. Over 120 different human papillomavirus (HPV) types have been fully characterized and they can be classified into phylogenetic groups which largely parallel with their tissue tropism (Bernard et al., 2010; de Villiers et al., 2004). The mucosal papillomaviruses are found in the alpha genus, and papillomaviruses infecting the skin are found in the beta, gamma, mu and nu genera with a few skin HPV types in the alpha genus.

An etiological role of HPV in skin cancer has been postulated, although not yet proven (2007; Bouvard et al., 2009). The association was first suggested on the basis of studies in patients who suffer from a very rare inherited disorder, epidermodysplasia verruciformis (EV). Patients with EV have mutations in 2 genes that render them susceptible to HPV infection, leading to development of squamous cell carcinomas (SCCs) at a young age (Ramoz et al., 2002). Evidence that HPV may be associated with cutaneous SCC in people without EV came firstly from studies of immunosuppressed organ transplant recipients. These patients have a high prevalence of HPV infection and up to 100-fold increased risk of SCC and a 10-fold increased risk of basal cell carcinoma (BCC) of the skin, resulting in a reversal of the SCC to BCC ratio compared to the general population (Kiviat, 1999). Clinical and histological features of lesions from transplant recipients suggest that SCCs develop from the progression of viral warts through dysplastic lesions to SCC (Barr et al., 1989). Beta HPV types have been identified in up to 80% of SCCs, 90% of actinic keratoses and 60% of BCCs (Berkhout et al., 2000; de Jong-Tieben et al., 2000; Harwood et al., 2000). Studies in immunocompetent patients centered initially on examination of skin lesions, finding that while the prevalence of HPV DNA was lower than in transplant recipients, it was nevertheless substantial, with up to 50% of SCCs in immunocompetent populations showing evidence of HPV infection (Asgari et al., 2008; Forslund et al., 2007).

Despite this suggestive evidence, no high-risk types have emerged as being clearly associated with the development of skin cancer in epidemiological studies. This may be partly because there is a paucity of information about the appropriate marker of infection. Measurements of HPV DNA in eyebrow hairs or skin swabs have been used (Antonsson et al., 2000, 2003; Chen et al., 2008; de Koning et al., 2007), but these samples may not be indicative of infection at the site where the lesion arose. Furthermore, the very high prevalence of HPV DNA in people without skin cancer may indicate that these samples largely measure biologically irrelevant infection (Antonsson et al., 2000, 2003; Chen et al., 2008; de Koning et al., 2007).

More recently HPV antibodies have been used as an indicator of prior HPV infection in case-control and cohort studies (Casabonne

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et al., 2007; Feltkamp et al., 2003; Iannacone et al., 2010; Karagas et al., 2006; Michael et al., 2008; Waterboer et al., 2008, 2009). Antibodies to the mucosal HPV types have been well studied and are an indicator of past infection (Dillner, 1999). However, only 50% of women infected with the mucosal high-risk type HPV-16 develop antibodies to HPV-16, and this can take several months after the infection is established. Once the HPV-16 antibodies are present, low but stable titers are detectable over long periods of time (Dillner, 1999). In contrast, information about serostability over time to cutaneous HPV infections is limited, limiting the interpretation of epidemiological studies.

To add to the knowledge base about HPV antibodies, and thereby facilitate their rational use in research, we aimed to determine the prevalence, determinants and stability of antibody status to 37 HPV types in a population-based longitudinal study of 390 people from Queensland, Australia.

Results

Characteristics of the study population

The mean age at baseline of the 390 participants was 50 years (SD 13), and 219 (56%) were female. Distributions of other characteristics, such as skin color, type of occupation and smoking are presented in Table 1. Eleven (3%) of the 390 participants analyzed were diagnosed at baseline with BCC and 39 (10%) were diagnosed with BCC during the follow-up period (1992–1996). One-hundred-and-fifty (38%) people were diagnosed with actinic keratosis at baseline (Table 1).

HPV seroprevalence

Eighty-six percent (n = 336) of participants were seropositive to at least one of the 37 HPV types tested in 1992 and 1993, and 89% (n = 346) in 1996. Twenty-five (6.4%; 20 women and 5 men) of the participants were seronegative to all HPV types in all three samples during the 4½ year follow-up.

HPV-4 had the highest seroprevalence (41%), followed by HPV-38 (33%), HPV-8 (33%), HPV-65 (29%) and HPV-49 (26%). The lowest seroprevalences were found for HPV-88 (0.5%), HPV-14 (1.3%) and HPV-93 (3.6%; Fig. 1).

Phylogenetically, the overall prevalence was highest for the beta HPV types (65%) and lowest for the two alpha skin HPV types (15%; Table 2).

The MFI values at baseline are shown in Supplementary Table 1. The seroprevalence was relatively insensitive to change in the MFI value used as the cut-off to determine seropositivity (100 MFI and 500 MFI instead of 200 MFI). Changing the cut-off value did not alter the seroprevalence by more than 15% for any HPV type and in most cases not more than 10%. The biggest changes in seroprevalence were seen for HPV-65, (increased from 29% to 44% (100 MFI)) and for HPV-4 (decreased by 14% (500 MFI)). MFI distribution of four selected HPV types are presented in Supplementary Fig. 1.

Table 1
Characteristics of study population at base line.

Characteristic		% (n)
n		390
Age (years), mean (SD)		50 (SD 13)
Age distribution	Up to 40	24 (94)
	41–50	30 (117)
	51–60	22 (86)
	61–70	16 (62)
	71 and older	8 (31)
Eye color	Blue/grey	42 (163)
	Hazel/green	39 (152)
	Light brown	7 (29)
	Dark brown	12 (45)
Hair color	Blonde/Light brown	49 (193)
	Red	8 (31)
	Dark brown/Black	43 (166)
Skin color	Fair	52 (204)
	Medium	38 (149)
	Olive, brown or black	10 (37)
Skin response after exposure to strong sun	Burn only	19 (75)
	Burn, then tan	70 (274)
	Tan only	11 (41)
Type of occupation	Mainly outdoors	17 (66)
	Outdoors and indoors	38 (147)
	Mainly indoors	45 (177)
Smoking	Never smoked	57 (218)
	Current smoker	10 (39)
	Ex-smoker	33 (128)
Basal cell carcinoma	No	97 (379)
	Yes	3 (11)
Actinic keratosis	No	62 (240)
	Yes	38 (150)

Numbers may not sum to total due to missing data.

Characteristics and life-style factors associated with HPV seroprevalence

Men had higher overall seroprevalence than women (19% and 14%, respectively). Men also had higher seroprevalence than women for most individual HPV types (Fig. 1). This difference was significant ($p<0.05$) for the beta types HPV-8, HPV-15, HPV-23, HPV-38, HPV-49 and HPV-76, the gamma types HPV-4 and HPV-95, and the mu types HPV-1 and HPV-63. Seroprevalence was marginally and non-significantly higher in women than in men for the beta type HPV-93, gamma types HPV-50 and HPV-88, and the alpha types HPV-11 and HPV-16.

A significantly higher proportion of men were beta HPV positive (73%) compared with women (58%; $p=0.008$). Seropositivity to at least one of the three HPV types from the mu and nu genera was also more prevalent in men than in women (46% versus 28%, $p=0.0001$). A higher proportion of men (66%) were also seropositive to at least one HPV type from the gamma group compared to women (55%; $p=0.019$). Beta HPV seroprevalence was found to significantly increase with age ($p=0.022$), while seroprevalence to at least one of the HPV types of the mu/nu, and the skin and mucosal alpha HPV groups were decreasing with age ($p=0.018$, 0.017 and 0.0002, respectively). There was no association between age and seroprevalence for the gamma HPV group (Table 2).

People seropositive to the mucosal type HPV-11 were significantly younger than those who were seronegative (mean 44 vs. 51 years; $p<0.001$) which was also the case for HPV-1 (mean age seropositive 47 years and seronegative 51 years; $p=0.033$). There were no differences in prevalence according to age for any of the other HPV types examined.

We found a significant association between hair color and gamma HPV positivity ($p=0.009$), with blonde and red-haired people having lower prevalence than those with dark hair. Fair-skinned people had higher gamma, skin and mucosal alpha HPV seropositivity than those with darker skin (all three p values <0.05). Significantly higher seropositivity to the mu and nu HPV types were found for people that reported burning only as a response to strong sun exposure compared with people that tanned only ($p<0.0001$). Conversely, seroprevalence to the mucosal alpha types was higher in those who tanned compared with those who burnt easily ($p=0.013$). Smokers had a significantly higher seroprevalence for beta and both skin and mucosal alpha HPV types than non-smokers ($p=0.001$, 0.012 and 0.012, respectively; Table 2).

HPV-75 antibodies were less prevalent in people that had been diagnosed with BCC ($p=0.039$) but there was no association between BCC and antibodies to any other type or specific genus. Actinic keratosis was associated with seropositivity to at least one beta or gamma HPV type ($p=0.040$ and $p=0.012$, respectively).

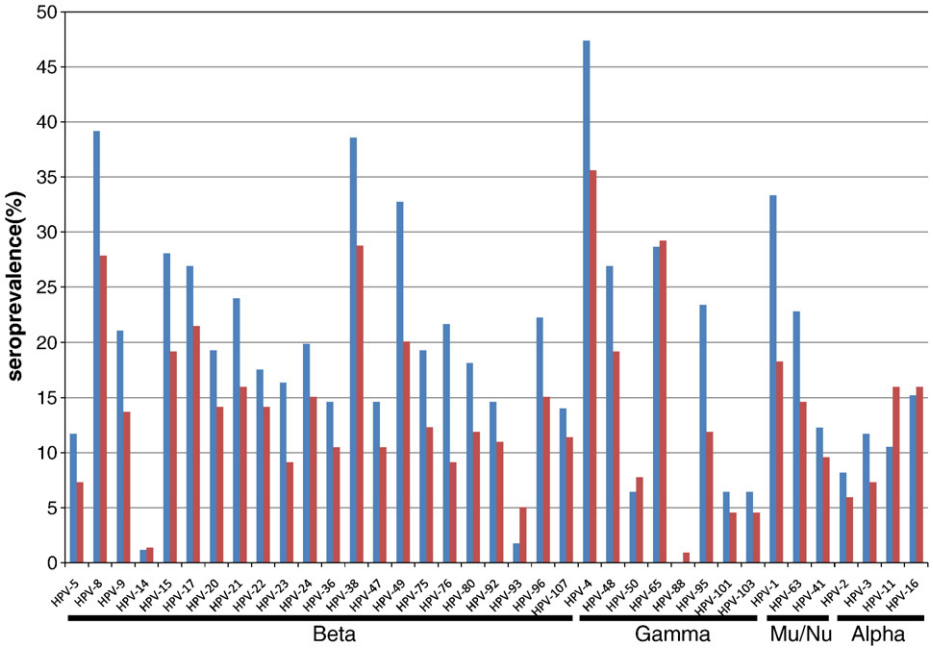

Fig. 1. Seroprevalence for men and women for all 37 HPV antibodies analyzed at baseline (1992). Blue bar — male seroprevalence, Red bar — female seroprevalence.

Stability of HPV antibodies over time

We analyzed changes in HPV antibodies over time by examining changes in serostatus (positive versus negative) and also in MFI with no cut-off specified. In each case we stratified the analysis according to baseline serostatus.

Over 90% of people that were seronegative at baseline stayed seronegative during the 4½ year follow-up and people that changed status were more likely to seroconvert than to fluctuate (Supplementary Table 2). The highest serostability in people seronegative at baseline was seen for HPV-88 (99.7% remained seropositive or seronegative), while HPV-65 was least stable with 15% of those negative at baseline altering their serostatus (seroconversion or fluctuation) during follow-up. Seroprevalence to the beta HPV types increased significantly from year 1992 to 1996 ($p_{trend} = 0.031$; Table 2).

In participants seropositive at baseline there was greater variability in serostability across types. For example, of the 36 people positive for HPV-5, 20 either seroreverted or had fluctuating status over the 4.5 years, while for HPV-3 32 of the 36 people who were positive at baseline remained positive. Across all HPV types, on average 80% of people seropositive at baseline remained seropositive (Supplementary Table 2).

We investigated the extent to which fluctuation was due to MFI values being close to the cut-off in the HPV types where there were at least 15% of people fluctuating. For HPV-5 and HPV-23, 15 people had fluctuating serostatus, and for each type 67% (10/15) had at least one of the three MFI values within 50 MFI units of the cut-off. Twelve out of the 15 people (80%) with fluctuating HPV-22 serostatus, 11 out of the 17 for HPV-80 (65%) and 15 out of 16 for HPV-63 (94%) also had at least one of the MFI values within 50 MFI units of the cut-off.

With a few exceptions, there was little difference in serostability between men and women. For HPV-49, women were more likely to be stably seropositive or seronegative than men ($p = 0.008$). In women, 93% ($N = 41$) of those HPV-49 seropositive at baseline remained seropositive compared with 75% ($N = 42$) of men, and 97% of seronegative women remained seronegative compared with 88% of men ($p = 0.005$). Women's serum antibody status was also significantly more stable than men's for HPV-23, HPV-65 and HPV-95, with men more likely to serorevert for HPV-23 and more likely to seroconvert for the latter two. No associations between serostability and any other characteristics (age, skin type, diagnosis of BCC or AK) were found. People diagnosed with BCC or AK at baseline or with BCC between 1992 and 1996 were not more likely to seroconvert than people who were not newly diagnosed with skin lesions.

We used linear regression to investigate changes in MFI over the three time points for selected HPV types stratified by serostatus at baseline and adjusted for sex and age (Table 3). People who were seronegative for HPV-8, HPV-38, HPV-4, HPV-1 and HPV-16 at baseline had significantly increasing MFI over time ($p<0.05$ for all five HPV types; Table 3). The group of people that was HPV-38 seropositive at baseline also had significantly increasing MFI over time ($p = 0.025$), but there were no significant trends for the people that were seropositive to the other HPV types investigated. In most cases there was a decrease in MFI in 1993 but a return to baseline levels in 1996. Despite changes in MFI, changing the cut-off used to determine positivity did not substantially alter the proportion of people who remained serostable for most types.

Discussion

Previous studies of HPV antibodies have mostly focused on the high-risk HPV types from the alpha genus, and cutaneous HPVs have been studied less extensively. To our knowledge, this is the first longitudinal serology study investigating the stability of antibodies to different cutaneous HPV types. We investigated seroprevalence and antibody stability over 4½ years for 37 different HPV types in 390 people. At baseline, 86% of participants were seropositive to at least one of the HPV types analyzed, which is similar to recent reports from the United Kingdom and Florida (86–91%) (Casabonne et al., 2009; Iannacone et al., 2010), but higher than has been reported in European studies (20–60%) (Casabonne et al., 2007; Michael et al., 2008). However, comparisons of overall seropositivity are arguably inappropriate due to variability in the types and number of different HPVs tested, along with variation resulting from different testing techniques.

We found HPV-4 to have the highest seroprevalence (41%). High HPV-4 seroprevalence has been reported in previous studies from Queensland (35%) (Waterboer et al., 2009), Florida (46%) (Iannacone et al., 2010) and the United Kingdom (31%) (Casabonne et al., 2007).

Table 2
Associations with HPV seropositivity, grouped by phylogenetic genera and adjusted for age and sex. Significant P values in bold.

Characteristic		Any β-HPV positive (%)[a]	OR (95% CI)	P value (P_{trend})	Any γ-HPV positive (%)[b]	OR (95% CI)	P value (P_{trend})	Any μ/ν HPV positive (%)[c]	OR (95% CI)	P value (P_{trend})	Skin α-HPV positive (%)[d]	OR (95% CI)	P value (P_{trend})	Mucosal α-HPV positive (%)[e]	OR (95% CI)	P value (P_{trend})
Total % (n)		64.9	–	–	59.9	–	–	35.9	–	–	14.8	–	–	25.7	–	-
Age distribution:																
	up to 40	62.5	1.00	0.601	56.0	1.00	0.151	43.3	1.00	0.511	16.7	1.00	0.107	33.3	1.00	0.143
	40–50	61.5	0.96 (0.57–1.62)	**(0.022**)	59.4	1.14 (0.69–1.90)	(0.939)	32.8	0.66 (0.39–1.10)	**(0.018)**	18.5	1.16 (0.59–2.30)	**(0.017)**	25.4	0.67 (0.39–1.13)	**(0.0002**)
	50–60	66.8	1.17 (0.66–2.08)		68.2	1.61 (0.94–2.76)		36.1	0.72 (0.40–1.30)		9.3	0.50 (0.21–1.19)		25.2	0.68 (0.38–1.22)	
	60–70	67.2	1.22 (0.66–2.25)		62.4	1.28 (0.71–2.32)		32.3	0.64 (0.34–1.19)		16.1	0.95 (0.43–2.10)		20.4	0.51 (0.27–0.99)	
	70 and older	75.3	1.78 (0.74–4.25)		45.2	0.63 (0.29–1.34)		32.3	0.62 (0.28–1.41)		7.5	0.40 (0.10–1.55)		16.1	0.38 (0.15–0.96)	
Sex:	Female	58.3	1.00	**0.0008**	55.1	1.00	**0.019**	28.2	1.00	**0.0001**	12.5	1.00	0.083	28.8	1.00	0.085
	Male	73.4	1.97 (1.32–2.90)		66.0	1.56 (1.08–2.27)		45.8	2.19 (1.47–3.25)		17.7	1.60 (0.94–2.71)		21.8	0.69 (0.46–1.05)	
Eye color:	Dark brown	71.9	1.00	0.056	57.0	1.00	0.285	34.1	1.00	0.285	21.7	1.00	0.201	26.8	1.00	0.102
	Light brown	63.2	0.60 (0.24–1.53)		64.0	1.36 (0.58–3.28)		33.3	0.98 (0.65–2.25)		14.9	0.67 (0.31–1.48)		33.3	1.49 (0.62–3.55)	
	Hazel/green	64.0	0.62 (0.32–1.21)		65.3	1.38 (0.75–2.47)		34.0	1.00 (0.53–1.48)		11.2	0.45 (0.20–1.04)		28.7	1.16 (0.58–2.35)	
	Blue/grey	63.9	0.58 (0.30–1.15)		55.2	0.94 (0.52–1.66)		38.9	1.21 (0.65–2.25)		16.2	0.67 (0.31–1.48)		21.3	0.81 (0.40–1.63)	
Hair color:																
	Dark brown/ Black	68.4	1.00	0.113	64.0	1.00	**0.009**	33.7	1.00	0.065	17.3	1.00	0.115	27.7	1.00	0.706
	Blonde/Light brown	62.7	0.80 (0.53–1.21)		58.6	0.83 (0.56–1.23)		38.0	1.36 (0. 90–2.06)		13.1	0.77 (0.44–1.33)		22.4	0.74 (0.48–1.14)	
	Red	60.2	0.79 (0.40–1.60)		46.2	0.53 (0.25–1.11)		34.4	1.22 (0.56–2.63)		11.8	0.70 (0.23–2.09)		35.5	1.31 (0.66–2.57)	
Skin color:	Dark	73.0	1.00	0.103	51.3	1.00	**0.044**	25.2	1.00	0.739	12.6	1.00	**0.022**	37.8	1.00	**0.012**
	Medium	66.1	0.77 (0.36–1.66)		59.6	1.43 (0.75–2.72)		41.4	2.19 (1.02–4.71)		12.1	0.97 (0.34–2.76)		24.2	0.49 (0.24–1.00)	
	Fair	62.6	0.68 (0.32–1.43)		61.6	1.61 (0.85–3.03)		33.8	1.65 (0.76–3.48)		17.2	1.50 (0.56–4.07)		24.7	0.48 (0.24–0.94)	
Skin response to strong sun exposure:	Tan only	54.5	1.00	0.070	60.2	1.00	0.325	24.4	1.00	<**0.0001**	10.6	1.00	0.676	30.1	1.00	**0.013**
	Burn, then tan	67.3	2.13 (1.11–4.12)		58.8	0.90 (0.48–1.70)		35.5	1.75 (0.80–3.84)		16.4	1.52 (0.54–4.27)		25.9	0.65 (0.32–1.29)	
	Burn only	62.0	1.92 (0.91–4.06)		63.6	1.14 (0.54–2.43)		43.6	2.88 (1.17–7.02)		11.1	1.04 (0.32–3.40)		22.7	0.50 (0.23–1.11)	
Type of occupation:																
	Mainly indoors	62.5	1.00	0.518	60.6	1.00	0.123	35.0	1.00	0.685	13.7	1.00	0.574	28.1	1.00	0.996
	Out–and indoors	65.7	1.09 (0.70–1.71)		57.6	0.78 (0. 51–1.20)		32.6	0.99 (0.55–1.80)		14.3	1.07 (0.58–1.97)		24.0	0.99 (0.61–1.60)	
	Mainly outdoors	69.9	1.10 (0.59–2.06)		62.9	1.10 (0.70–1.62)		45.5	1.24 (0.78–1.98)		18.7	1.19 (0.57–2.50)		23.2	0.98 (0.52–1.91)	
Smoking:	Never	58.9	1.00	**0.001**	59.0	1.00	0.270	30.4	1.00	0.141	11.5	1.00	**0.012**	23.7	1.00	**0.012**
	Current	74.5	1.91 (0.88–4.16)		69.6	1.40 (0.71–2.75)		49.0	1.88 (0.92–3.85)		20.6	1.89 (0.76–4.65)		31.4	1.57 (0.72–3.40)	
	Ex-smoker	72.0	1.56 (1.00–2.42)		58.9	0.84 (0.55–1.27)		41.2	1.37 (0.88–2.14)		18.5	1.60 (0.88–2.93)		27.6	1.45 (0.92–2.28)	
Basal cell carcinoma:	No	64.7	1.00	0.828	59.2	1.00	0.777	35.7	1.00	0.798	14.8	1.00	0.868	25.6	1.00	0.616
	Yes	65.7	1.06 (0.64–1.73)		62.8	1.08 (0.67–1.75)		36.7	1.07 (0.63–1.82)		15.0	1.06 (0.54–2.08)		26.6	1.15 (0.67–2.00)	
Actinic keratosis	No	63.8	1.00	0.291	58.3	1.00	0.092	35.7	1.00	0.862	14.9	1.00	0.803	26.2	1.00	0.798
	Yes	72.1	1.37 (0.75–2.53)		69.8	1.62 (0.90–2.88)		37.1	1.05 (0.59–1.89)		13.8	0.90 (0.40–2.03)		22.6	0.91 (0.48–1.76)	
Year	1992	62.5	1.00		60.3	1.00		34.6	1.00		14.1	1.00		24.4	1.00	
	1993	63.1	1.01 (0.88–1.17)	**0.0002**	56.9	0.87 (0.75–1.00)	**0.004**	35.4	1.04 (0.90–1.19)	0.065	15.1	1.09 (0.91–1.31)	0.597	24.6	1.01 (0.86–1.20)	0.072
	1996	69.1	1.35 (1.15–1.57)	**(0.031**)	62.5	1.10 (0.93–1.31)	(0.304)	37.7	1.15 (1.02–1.30)	(0.341)	15.1	1.09 (0.91–1.31)	(0.744)	28.2	1.22 (1.02–1.47)	(0.173)

[a] β-HPV types analyzed: HPV-5, -8, -9, -14, -15, -17, -20, -21, -22, -23, -24, -36, -38, -47, -49, -75, -76, -80, -92, -93, -96 and -107.
[b] γ-HPV types: HPV-4, -48, -50, -65, -88, -95, -101 and -103.
[c] μ/ν HPV types: HPV-1, -63 and -41.
[d] Skin α-HPV types: HPV-2 and -3.
[e] Mucosal α-HPV types: HPV-11 and -16.

Table 3
Association between MFI values and the year of serum collection, expressed as the ratio of the geometric means with 1992 used as the reference category. Results are stratified by serostatus at baseline and adjusted for sex and age. Significant P values are in bold.

	1992			1993			1996			
	Mean	Median	Ratio[a] (REF)	Mean	Median	Ratio[a] (95% CI)	Mean	Median	Ratio[a] (95% CI)	P_{trend}
HPV-8										
Seropositive	1577	811	1.00	1446	810	0.93 (0.84–1.02)	1580	687	1.06 (0.96–1.17)	0.100
Seronegative	48	32	1.00	43	26	0.89 (0.78–1.02)	50	33	1.16 (1.01–1.33)	**0.006**
HPV-38										
Seropositive	1785	669	1.00	1556	718	0.98 (0.88–1.09)	1709	709	1.09 (0.99–1.19)	**0.025**
Seronegative	49	32	1.00	48	29	0.84 (0.74–0.95)	46	33	1.01 (0.88–1.15)	**0.004**
HPV-4										
Seropositive	1099	730	1.00	1048	940	0.91 (0.85–0.97)	1067	720	0.96 (0.90–1.03)	0.674
Seronegative	44	22	1.00	47	29	0.96 (0.86–1.08)	48	27	1.15 (1.02–1.29)	**0.006**
HPV-1										
Seropositive	956	558	1.00	877	590	0.91 (0.84–0.98)	861	511	0.93 (0.84–1.04)	0.381
Seronegative	30	9	1.00	28	8	0.97 (0.89–1.05)	29	10	1.08 (0.99–1.18)	**0.036**
HPV-16										
Seropositive	918	418	1.00	917	508	1.02 (0.86–1.21)	1067	477	1.17 (0.97–1. 04)	0.085
Seronegative	46	30	1.00	48	33	0.94 (0.86–1.03)	52	39	1.26 (1.17–1.36)	<**0.0001**

[a] Ratio of the geometric mean generated from the linear regression model, using the log of the MFI value as the outcome variable.

This is not surprising as HPV-4 is the most prevalent HPV type in common cutaneous warts which are highly prevalent in the general population, especially in children and adolescents (Chen et al., 1993; Kilkenny et al., 1998). The second most seroprevalent type and the most common beta type in our study population was HPV-8. This is consistent with other reports (Andersson et al., 2008; Karagas et al., 2006; Michael et al., 2008), and the seroprevalence is almost identical to that reported for a different Queensland population (Waterboer et al., 2009). Furthermore, other type-specific seroprevalences shown here are similar to those previously reported in Caucasians and show that seroprevalence to skin HPV types have a similar worldwide distribution.

As in previous studies (Feltkamp et al., 2003; Karagas et al., 2006; Waterboer et al., 2008, 2009), we found that men generally have higher HPV seroprevalence than women, particularly for beta, gamma, mu and nu HPV types. The reason for this difference is unclear, but similar findings for HPV DNA in plucked eye brow hairs have also been reported (Struijk et al., 2003) suggesting that the difference may be related to propensity for infection rather than to whether or not antibodies are produced.

Seroprevalence to HPV-1, one of the HPV types causing common warts, was significantly higher in people younger than 50 years than in older people. Comparable findings have been reported in a population-based German study (Michael et al., 2008). The low-risk alpha HPV type, HPV-11, was also more commonly found in younger participants. It has been shown that antibodies to high-risk mucosal HPV types are more stable over time than antibodies to low-risk types which seem to decay 1 to 2 years after the infection has been cleared (Carter et al., 2000). Genital HPV infections are more prevalent in younger people and loss of seropositivity over time could explain why seroprevalence to HPV-11 decreases with higher age.

We found that fair-skinned people had higher seroprevalence for gamma and skin alpha HPV types than those with darker skin. This is consistent with a recent study from Florida (Iannacone et al., 2010). The reason for this is unknown, but it is possible that fair-skinned people have had a greater number of sunburns which may increase the likelihood of seroconversion (Favre et al., 2000).

Current smokers had significantly higher seroprevalence to HPV types from the alpha genus compared to non-smokers. However without information about potential confounders, such as the number of sexual partners, we cannot assess whether or not this association is causal. Smokers also had significantly higher prevalence of beta HPV types. Some previous studies have found smoking to be associated with beta HPV seropositivity (Andersson et al., 2008; Iannacone et al., 2010), while others have not found any association (Casabonne et al., 2009) but this inconsistency may be due to analysis of different numbers and types of beta HPV.

We did not find any associations between the presence of antibodies from any HPV genera and a diagnosis of actinic keratosis at baseline. Two previous studies have found higher HPV-8 seropositivity in patients with actinic keratosis (Bouwes Bavinck et al., 2000; Struijk et al., 2006) while another did not find any association (Andersson et al., 2008). Actinic keratosis is one of the known precursors to cutaneous SCC, and it has been estimated that approximately 10% of actinic keratoses progress to SCC (Boukamp, 2005). HPV seroprevalence in relation to cutaneous SCCs has been studied in more detail and while most studies find higher seroprevalence in SCC patients than in controls, others have not identified any associations between HPV seropositivity and SCC (Andersson et al., 2008; Casabonne et al., 2009, 2007; Feltkamp et al., 2003; Karagas et al., 2006). Additional work is needed to clarify the role of HPV in the etiology of actinic keratosis and cutaneous SCCs.

There is limited information about HPV antibody stability over time, and the studies available have studied the most common alpha HPV types in women which have mucosal tropism (Carter et al., 2000; Syrjanen et al., 2009; Wang et al., 2004). We found cutaneous HPV antibodies to be relatively stable over 4½ years. The HPV types where we observed larger changes were those with low seroprevalence, leading to statistical instability. For most HPV types the majority of people retained their seropositive or seronegative status over time. The most common change was seroreversion (loss of seropositivity) while seroconversion and fluctuation of antibody levels occurred less frequently. Antibody levels for the mucosal high-risk HPV types that are the causative agents of cervical cancer are usually stable over time with little or no variation (af Geijersstam et al., 1998; Carter et al., 1996; Shah et al., 1997). In contrast, antibodies to low-risk HPV types seems to decay within two years after the infection has been cleared (Carter et al., 2000). In this study we did observe a slightly higher proportion of people seroreverting for HPV-11 (low-risk) than for HPV-16 (high risk). However, our population was considerably older than those studied previously and consisted of both women and men.

We recognize that the cut-off value used in this study is arbitrary. However this is a common feature of most cutaneous HPV serology studies, as long as there are no reference sera to use as standards. We observed variations in MFI values over time, even in those whose antibody status based on a categorization of the MFI did not change. In general, the MFI was higher in 1996 than in 1992, although in most cases there was a small decrease in MFI in 1993. This may be due to physiological variation, but sample storage, other methodological issues or longer follow-up time could be responsible for the variability

we observed. Despite the variation, the actual seroprevalence and serostability were relatively robust to changes in MFI values.

It has been suggested that diagnosis of skin lesions might lead to inflammatory changes that either increase HPV proliferation or lead to presentation of HPV to the immune system leading to an antibody response (Favre et al., 2000). However we found no evidence that a diagnosis of AK or BCC increased the rate of seroconversion.

We conducted many different analyses, and it is likely that some of the associations we found with HPV seropositivity arose due to chance. Our participants were all over 26 years limiting the generalisability of these findings to younger people, and we do not know if the stability we observed here would persist over a longer follow-up time. However, the participants in the Nambour Skin Study have been shown to be similar to the population from which they were drawn (Green et al., 1999), and we did not find any demographic or behavioral differences between the 390 people included in this study and the remainder of the original cohort (n = 1231) who did not have three serum samples collected and analyzed.

To conclude, we found that the majority of the Australian adults investigated here were seropositive to at least one cutaneous HPV type. Furthermore, seroprevalence to the cutaneous HPV types appears to be largely very stable over the time period investigated. This would suggest that measuring antibodies at a single time point is appropriate in studies aiming to understand disease associations.

Materials and methods

Study population

This study was a part of the Nambour Skin Cancer Study which has previously been described in detail (Green et al., 1999). Briefly, in 1992 1621 randomly selected members of the Nambour Township in southeast Queensland, Australia were enrolled in a trial of sunscreen and beta-carotene for the prevention of skin cancer. All participants completed questionnaires in which information about skin phenotype and sun exposure was obtained. Serum samples were collected from 693 randomly selected participants in 1992, 549 in 1993, and 1211 in 1996. In this study, we analyzed data and sera for 390 people who had blood collected in 1992, 1993 and 1996 and who had never been diagnosed with cutaneous SCC. The subgroup of 390 people had similar characteristics to the remainder of the Nambour cohort (mean age 50 years (range 26 to 76 years) and 55% female).

This study was approved by the Ethics Committee at the Queensland Institute for Medical Research and all participants gave written informed consent.

HPV serology analysis

Serum samples were stored at −80 °C and shipped on dry ice to the German Cancer Research Center (DFKZ), Heidelberg, Germany. The samples were analyzed for antibodies to the capsid protein (L1) of the following 37 HPV types; beta types HPV-5, -8, -9, -14, -15, -17, -20, -21, -22, -23, -24, -36, -38, -47, -49, -75, -76, -80, -92, -93, -96 and -107; gamma types HPV-4, -48, -50, -65, -88, -95, -101 and -103; mu types HPV-1 and -63, the nu type HPV-41, the cutaneous alpha types HPV-2 and -3, and the mucosal alpha types HPV-11 and -16 (Bernard et al., 2010). The antibody detection method was based on glutathione *S*-transferase (GST) capture ELISA (Sehr et al., 2001) in combination with fluorescent bead technology (Waterboer et al., 2005). Briefly, full-length viral proteins were expressed in bacteria in fusion with an N-terminal GST-domain. Glutathione cross-linked to casein was coupled to fluorescence-labeled polystyrene beads (SeroMap, Luminex, Austin, TX) and GST-fusion proteins were affinity-purified on the beads. Bead types of different color and each carrying a different antigen were mixed and incubated with human sera at 1:100 dilution. Antibody bound to the beads via the viral antigens was stained by biotinylated anti-human-Ig and streptavidin-R-phycoerythrin. A Luminex analyser (xMAP, Luminex) was used to identify the internal color of the individual beads and to quantify their reporter fluorescence (expressed as median fluorescence intensity (MFI) of at least 100 beads per set per serum).

Statistical analysis

All analysis was carried out using SAS 9.1. Cut-points to determine antibody positivity were selected by visual inspection of the distribution of MFI values among all study participants (Carter et al., 2009), and seropositivity was defined as having an MFI value of 200 or more (Michael et al., 2008). Factors associated with positivity were analyzed using logistic regression with generalized estimating equations (GEE) to take account of serostatus at all three time points.

In order to analyze HPV antibody stability over time we classified participants as being seropositive at all time points (stably seropositive), seronegative at all time points (stably seronegative), seroconverting (change from seronegative to seropositive over time), seroreverting (change from seropositive to seronegative) and fluctuating between seropositive and seronegative over the three time points (fluctuating). Chi-squared tests were used to analyze associations between variables such as age, sex, skin type, sun exposure, BCC, actinic keratosis (AK) and antibody prevalence and stability.

In addition, for five HPV types selected to represent the most common type in each genus, we examined changes in MFI over time using linear regression with GEE to account for intra-person correlation. Because MFI was not normally distributed, we used the log of the MFI value as the outcome variable in each analysis, and expressed the results as the ratio in the geometric means using 1992 as the reference point. These analyses were stratified by serostatus at baseline and adjusted for age and sex.

Supplementary data to this article can be found online at doi:10.1016/j.virol.2010.07.046.

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