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Case–Control Study of Cutaneous Human Papillomavirus Infection in Basal Cell Carcinoma of the Skin

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Abstract

Genus-β human papillomavirus (HPV) DNA has been detected in basal cell carcinoma (BCC) tumors, but most epidemiologic studies have not observed associations between genus-β HPV seropositivity and BCC. A clinic-based case–control study was conducted to investigate cutaneous HPV infection in BCC. BCC cases (*n* = 224) were recruited from a dermatology clinic, and controls (*n* = 300) were patients who were screened negative for skin cancer. Antibodies against cutaneous HPV types in genera α, β, γ, mu, and nu were measured, and tumors from a subset of BCC cases (*n* = 195) were tested for HPV DNA. Overall associations were observed between BCC and seropositivity for HPV types in genus-α (odds ratio (OR) = 1.61; 95% confidence interval (CI) = 1.11–2.35), γ (OR = 1.78; 95% CI = 1.22–2.60), and mu (OR = 1.56; 95% CI = 1.06–2.30). BCC cases with β-HPV DNA in their tumors were more likely to be β-HPV seropositive than controls (OR = 1.76; 95% CI = 1.03–3.01), with type-specific associations observed for HPV8 and HPV23, whereas no association was observed between β-HPV seropositivity and β-HPV DNA–negative BCC. No concordance between seropositivity and tumor

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The work was conducted in Tampa, Florida, USA.

CONFLICT OF INTEREST

The authors state no conflict of interest.

DNA status was observed for HPV types in genera α and γ. In conclusion, the combined serology and tumor DNA results suggest that β HPV types may have a role in BCC. Additional studies of BCC that assess HPV types in multiple genera are needed.

INTRODUCTION

Basal cell carcinoma (BCC) is the most common cancer in the United States (Chinem and Miot, 2011). UVR exposure is the most important environmental risk factor for BCC. Despite public awareness of the harmful effects of UVR exposure and increased use of sunscreen products, the incidence of BCC continues to rise each year.

DNA of cutaneous human papillomavirus (HPV) types has been detected in non-melanoma skin cancer (NMSC), especially in immunosuppressed individuals. Although most studies have focused on HPV DNA detection in squamous cell carcinoma (SCC) (Boxman *et al.*, 2000; Pfister *et al.*, 2003; Struijk *et al.*, 2003; Forslund *et al.*, 2007; McBride *et al.*, 2007; Asgari *et al.*, 2008; Cronin *et al.*, 2008; Madan *et al.*, 2010), genus-β HPV DNA has also been detected in BCC tumors, ranging from 8 to 55% (Shamanin *et al.*, 1996; Harwood *et al.*, 2000; Wieland *et al.*, 2000; Meyer *et al.*, 2003; Forslund *et al.*, 2004; Andersson *et al.*, 2008) in immunocompetent populations. In addition, no single type has been observed to predominate in skin cancers, and cutaneous HPV viral load is low, at less than one copy per cell, requiring highly sensitive techniques to detect viral DNA.

Evidence from the literature supports a potential role for cutaneous HPV seropositivity in NMSC, particularly SCC (Feltkamp *et al.*, 2003; Struijk *et al.*, 2006; Casabonne *et al.*, 2007; Karagas *et al.*, 2010). Studies that have reported on the association between seropositivity to any genus-β HPV type overall and BCC have not observed statistically significant associations (Feltkamp *et al.*, 2003; Andersson *et al.*, 2008; Karagas *et al.*, 2010). However, type-specific associations with BCC have been observed for seropositivity to HPV8 and HPV20 among persons from the Netherlands (Feltkamp *et al.*, 2003).

The aim of the current study was to investigate the association between cutaneous HPV infection and BCC, incorporating multiple biomarkers of infection. To our knowledge, this is a previously unreported case–control study in a US population to investigate the association between BCC and seroreactivity to cutaneous HPV types belonging to five different genera, as well as present serological associations between genus-α, β, or γ HPV types and BCC stratified by the presence of DNA of these HPV types in the tumor tissues.

RESULTS

As presented in Table 1, older age, male sex, ≤12 years of education, light eye and hair color, occupational sunlight exposure, cutaneous sensitivity and tanning ability to sunlight exposure, and history of smoking were associated with BCC. However, none of the risk factors presented in Table 1 were associated with cutaneous HPV serostatus among the controls, as previously described (Iannacone *et al.*, 2012; data not shown).

Genus-mu (odds ratio (OR) = 1.56; 95% confidence interval (CI) = 1.06–2.30; $P = 0.03$) and genus-α (OR = 1.61; 95% CI = 1.11–2.35; $P = 0.01$) HPV seropositivity was significantly associated with BCC, with a greater risk observed among individuals seropositive for ≥2 types in genus-α (OR = 1.75; 95% CI = 1.08–2.85; P_{trend}=0.01; Table 2). However, for both genera, statistical significance was lost after correction for multiple comparisons. A positive association between BCC and genus-γ HPV seropositivity was observed (OR = 1.78; 95% CI = 1.22–2.60; P=0.003), and remained statistically significant after correction for multiple comparisons. No statistically significant associations were observed for genus-β seropositivity or the polyomaviruses JC virus and KI virus (Table 2). Analyses restricted to those aged 40–69 years revealed only minor differences in the associations between genus-specific HPV seropositivity and BCC compared with the total study population (Table 2).

Sex-stratified analyses demonstrated a statistically significant greater association between BCC and seropositivity to the single type in genus-mu among women (OR = 2.46; 95% CI = 1.40–4.30) compared with men (OR = 1.00; 95% CI = 0.58–1.72; $P_{interaction}$=0.02; Table 3) within the total study population, as well among individuals aged 40–69 years (P=0.007; data not shown). No significant differences were observed by sex between BCC and seropositivity to HPV type(s) in genera α, β, γ, and nu (Table 3).

Among BCC cases, the greatest type-specific seroprevalence was observed for HPV1, and it was statistically significantly associated with BCC (OR = 1.56; 95% CI = 1.06–2.30; Table 4). In genus-α, compared with controls, seropositivity to HPV types 3 (OR = 1.70; 95% CI = 1.03–2.82) and 27 (OR = 2.18; 95% CI = 1.28–3.71) were significantly associated with BCC; however, none of these associations remained statistically significant after correcting for multiple comparisons. No associations were observed between BCC and genera β, γ, or nu seropositivity (Table 4).

Of the 238 BCC tumor tissues (98.3%) that tested positive for the β-globin gene, 120 (50.4%) tested positive for DNA to at least one genus-β HPV type, including 43 tumors (18.1%) with a single-type infection and 77 (32.4%) with multiple-type infections (Table 5). Individuals with genus-β HPV DNA–positive tumors were significantly older (mean age (SD) = 65.1 (12.2) compared with those with genus-β HPV DNA–negative tumors (mean age (SD) = 60.1 (11.7); $P_{wilcoxon}$=0.001). No additional factors listed in Table 1 were statistically significantly associated with genus-β HPV DNA status in the tumor (data not shown). In contrast to the high DNA prevalence observed for genus-β HPV types, the prevalence for HPV type(s) in genera α, γ, and mu was much lower. Twenty (8.4%) BCC tumors tested DNA positive to at least one genus-γ HPV type, seven (2.9%) tumors tested positive for genus-α HPV DNA, although none were DNA positive for HPV2 or HPV3, and only one (0.4%) BCC tumor tested HPV DNA positive for the single type in genus-mu (Table 5). In addition, within genus, only single-type infections were present in the tumors for α and γ HPV types, and a single tumor was DNA positive for HPV27 in genus-α and HPV50 in genus-γ.

Roughly half of the BCC cases (49.7%) were HPV DNA positive, corresponding to at least one type in genus-β. When comparing seropositivity between controls and BCC cases stratified by HPV DNA status, a statistically significantly greater seroprevalence of genus-β

antibodies was observed among BCC cases with HPV DNA–positive tumors compared with controls (OR = 1.76; 95% CI=1.03–3.01), whereas no association was observed between genus-β seropositivity and BCC cases negative for HPV DNA (OR = 1.06; 95% CI = 0.65–1.72; Table 6). Of the 97 BCC cases with genus-β HPV DNA–positive tumors, 36.1% tested seropositive for the same HPV type detected in the tumor tissue (data not shown). Among cases with HPV8 and HPV23 DNA–positive tumors, statistically significant associations were observed between BCC and seropositivity for HPV types 8 (OR = 2.77; 95% CI = 1.11–6.94) and 23 (OR = 15.8; 95% CI = 1.60–155.0; Table 6). No differences in seroprevalence were observed between HPV DNA–negative BCC cases and controls for any single HPV type in genus-β (Table 6).

Among the seven BCC cases with genus-α HPV DNA–positive tumors, none was seropositive for the same genus-α HPV type. In addition, compared with controls, the seroprevalence for genus-α HPV types was greater among cases with genus-α HPV DNA–positive (OR = 2.53; 95% CI = 0.55–11.72) or DNA–negative tumor tissues (OR = 1.64; 95% CI = 1.11–2.44; Table 6). Only 17 (8.7%) BCC cases had genus-γ HPV DNA–positive tumors (Table 6), of which one tested seropositive for the same type (HPV50). Using the controls as a common reference group, no association was observed between genus-γ seropositivity and BCC cases with tumors that were HPV DNA positive (OR = 1.40; 95% CI = 0.49–4.00), whereas a statistically significant positive association was observed between genus-γ HPV seropositivity and BCC cases with tumors that were negative for γ HPV DNA (OR = 1.97; 95% CI = 1.31–2.97; Table 6).

DISCUSSION

To our knowledge, this is the first study to report seroprevalence for cutaneous HPV types from five different genera and to include both serological and DNA-based markers of infection for cutaneous HPV types in relation to BCC. Similar associations with SCC have been previously reported (Iannacone *et al.*, 2012).

The current study did not observe an association between overall genus-β seropositivity and BCC, which is consistent with previous studies (Feltkamp *et al.*, 2003; Andersson *et al.*, 2008; Karagas *et al.*, 2010). However, stratified analyses revealed that BCC cases with genus-β HPV DNA–positive tumors were significantly more likely to have genus-β HPV antibodies compared with controls. In addition, among BCC cases DNA positive for HPV types 8 and 23, a higher seroprevalence for the same type was observed in the cases compared with the controls. These results may indicate that HPV infection is involved in some but not all skin cancers, and as a result the association between genus-β HPV seropositivity and BCC would be obscured when HPV DNA status of the tumors is not taken into account.

In the current study, BCC was associated with genus-α seropositivity, with type-specific associations observed for HPV3 and HPV27. The single published study reporting results for BCC and seroreactivity to cutaneous HPV types outside of genus-β (Andersson *et al.*, 2008) reported a null association with genus-α seropositivity. Differences across study findings may be due to differences in the HPV types and number of types investigated. Only

seven BCC tumors were DNA positive for genus-α HPV types; however, no tumors were positive for HPV3 and only two were positive for HPV27. Regardless of HPV DNA status in the tumors, seroprevalence was greater among BCC cases compared with controls. These findings may indicate that seroresponse for genus-α HPV types is not related to the presence of DNA for these types in the tumor. In addition, if in fact genus-α HPV types are involved in BCC development, the low detection of these HPV types in the tumor tissues may indicate that HPV has a role in the earlier stages of skin cancer tumorigenesis. A similar phenomenon may hold true for the single HPV type in genus-mu, where 38% of BCC cases were observed to be seropositive while DNA for HPV1 was detected in a single tumor tissue only.

A statistically significant association was observed between genus-γ HPV seropositivity and BCC; however, only 8.4% of BCC tumors were DNA positive for genus-γ HPV types. Furthermore, when stratified by the presence or absence of DNA in the tumor tissue, an association with genus-β HPV seropositivity was observed among BCC cases with HPV DNA–negative tumors but not among BCC cases with DNA-positive tumors. This trend is contrary to that observed for genus-β HPV seropositivity in relation to DNA status in the tumor. No other studies have presented information on genus-γ HPV seroreactivity or detection of DNA in tumor tissue among BCC cases. Furthermore, there is no information available on the functionality of genus-γ HPV types. Thus, other than chance, the explanation for the observed association between genus-γ seropositivity and γ-HPV DNA–negative BCC is unknown.

The associations with BCC observed in the present analysis differ from those previously reported for SCC from the same underlying clinic population (Iannacone *et al.*, 2012), with seroprevalence being significantly elevated for genus-β HPV types in SCC but for genus-γ types in BCC. In addition, in genus-α, HPV10 seropositivity was associated with SCC, whereas HPV types 3 and 27 were associated with BCC. The observed differences in risk of cutaneous HPV infection by NMSC type within the same study population support the hypothesis that these malignancies have distinct etiologies. Recent evidence *in vitro* demonstrated functional differences in the E6 and E7 oncoproteins encoded for by different genus-β HPV types, as they relate to the life span and immortalization of primary foreskin keratinocytes (Cornet *et al.*, 2012). For example, Cornet and colleagues demonstrated the ability of HPV38 and HPV49 E6 to inactivate p53, although the mechanisms for each HPV type may not be the same. These functional differences may translate to their differential involvement in BCC versus SCC tumorigenesis by targeting signaling proteins implicated in NMSC, such as TP53, PTCH1, and telomerase (Madan *et al.*, 2010). The functional activity of oncoproteins encoded for by HPV types in genera α and γ have not yet been characterized.

The clinic-based study design may introduce selection bias if referral patterns differ between cases and controls. However, in the current study, the cases and a portion of the controls were selected from University of South Florida (USF) clinics that serve the same underlying community. BCC cases were significantly older than controls and tended to be male, but all analyses adjusted for these factors. In addition, the age-adjusted analysis restricted to a narrower age range revealed findings similar to those obtained from the overall study

population, indicating that the observed associations are not due to confounding by age. Men were significantly more likely than women to be seropositive for genus-γ HPV types. However, the only statistically significant interaction observed was between sex and genus-mu seropositivity in relationship with BCC. Similar to the current study, a single study from the United States (Karagas *et al.*, 2010) reported a nonstatistically significant higher seroprevalence for any HPV type in genus-β among men compared with women. However, the study did not discuss potential interaction effects between sex and genus-β seropositivity in relation to BCC (Karagas *et al.*, 2010).

Several strengths of the current case–control study should also be noted. This is the first study to examine associations between BCC and seroreactivity for cutaneous HPV types outside of genus-α and -β utilizing the same multiplex serologic methods used in recent previously published studies (Casabonne *et al.*, 2007; Karagas *et al.*, 2010), allowing for direct comparisons across studies with regard to genus-α and -β seroreactivity. A major strength of our study was the unique opportunity to investigate the specificity of serologic response and the presence of DNA for the same HPV type in the BCC tumor tissues. Only one study in the published literature has presented similar results among a Swedish/Austrian population; however, the concordance between seropositivity and DNA status was presented for NMSC overall and not by skin cancer type (Andersson *et al.*, 2008). Furthermore, the type-specific multiplex genotyping assay used for genera β and γ HPV DNA detection afforded many strengths including high sensitivity, allowing for the detection of low amounts of viral genomes and high specificity for the identification of specific HPV types in both single and multiple infections (Gheit *et al.*, 2007). Another strength of the current study includes the full-body skin screening exams undergone by all control subjects to identify previously undetected cases of skin cancer.

In conclusion, the current study provided evidence suggesting a potential association between cutaneous HPV infection and BCC. However, cutaneous HPVs are ubiquitous in healthy, immunocompetent populations, and the mechanisms by which HPV may have a role in NMSC carcinogenesis are not yet well understood. To rule out the possibility of reverse causality, additional studies are necessary to address the functional role of, and the natural history of, cutaneous HPV infections as they relate to NMSC development.

MATERIALS AND METHODS

Study design and population

To investigate the association between cutaneous HPV infection and BCC, a clinic-based case–control study was conducted in Tampa, FL. The study design and population have been previously described in detail (Rollison *et al.*, 2012). Briefly, histologically confirmed BCC cases were recruited from the USF Dermatology clinic (n = 236). Control subjects were recruited from the affiliated USF Family Medicine and Moffitt Lifetime Cancer Screening and Prevention clinics and could not have a history of any type of cancer, including skin cancer (n = 281). To exclude prevalent cases of undetected skin cancer, all potential control participants underwent a full-body skin cancer screening at the time of study enrollment. If a patient had a suspicious lesion detected during the skin screening that was later determined to be benign on the basis of pathology review, the patient was included as a control (n = 77).

If a patient's screen-detected lesion was histological-confirmed BCC, then that patient was included as a case (n = 8). All eligible study participants were aged 18–80 years.

Participants completed a self-administered questionnaire, including information on demographic (e.g., age, sex, race, education, and US residency), lifestyle (e.g., history of smoking and alcohol consumption), and skin cancer (e.g., eye, hair, and untanned skin color, occupational sunlight exposure, history of blistering sunburn, cutaneous sensitivity, and tanning ability to sunlight exposure) risk factors. Analyses were restricted to those individuals who reported being White, with the exception of two non-White BCC cases and two non-White controls retained to match to the non-White cases. Blood samples were obtained from 226 (92.2%) BCC cases and 340 (95.0%) controls. The final sample size for the analysis of cutaneous HPV seroreactivity was 224 BCC cases and 300 controls. For BCC patients undergoing surgical excision, a 3-mm punch of the residual BCC tumor was obtained and flash-frozen in liquid nitrogen (n = 242). Analyses were restricted to tumor specimens that tested positive for β-globin (98.3%), corresponding to 238 BCC tumors from 230 individual patients. Information on both HPV seroreactivity and DNA status of the tumor was available for 195 BCC cases. All study procedures adhere to the Declaration of Helsinki Principles. All study participants provided written informed consent, and the institutional review board at USF approved all study procedures.

HPV antibody measurement

Sera were tested for antibodies to the major capsid protein L1 of cutaneous HPV type(s) within genera α (2, 3, 7, 10, 27, 57, and 77), β (5, 8, 9, 15, 17, 20, 23, 24, 36, 38, 49, 75, 76, 92, 96, and 107), γ (4, 48, 50, 65, 88, 95, 101, and 103), mu (1), and nu (41). Sera were also tested for antibodies to the VP1 capsid protein of two human polyomaviruses, JC virus and KI virus, to test the specificity of associations observed between cutaneous HPV and BCC. The antibody detection method used was based on glutathione-*S*-transferase capture ELISA (Sehr *et al.*, 2001, 2002) in combination with fluorescent bead technology (Luminex) (Waterboer *et al.*, 2005, 2006), as described previously. Individual cutoff values to define HPV type–specific seropositivity were applied as described previously to allow for the direct comparison of cutaneous HPV seroprevalence across studies that used the same assay (Casabonne *et al.*, 2007; Michael *et al.*, 2008).

DNA extraction

DNA extraction from fresh-frozen BCC tumor tissues was conducted with the QIAGEN BioRobot EZ1 with the EZ1 DNA Tissue Kit according to the manufacturer's instructions (QIAGEN, Hilden, Germany). Briefly, frozen tissues were incubated in proteinase K and a buffer G2 (QIAGEN) at 56 °C until the tissue was completely lysed. To monitor the possible occurrence of cross-contamination between the different specimens during DNA extraction, tubes containing only buffer were also included.

HPV DNA typing

HPV typing was performed by a type-specific multiplex genotyping assay (Schmitt *et al.*, 2006; Gheit *et al.*, 2007; Ruer *et al.*, 2009; Schmitt *et al.*, 2010). For specificity evaluation, cloned HPV genomes and human specimens were used. PCR products were obtained even

when only 10 copies of the viral genome for each HPV type were used as a template, demonstrating high sensitivity for type-specific detection. The assay detects HPV DNA of 25 genus-β 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), 16 genus-γ types (4, 65, 95, 60, 48, 50, 88, 101, 103, 108, 109, 112, 116, 119, 121, and 123), 5 genus-α types (2, 3, 10, 27, and 57), and the single type in genus-mu (1). Two primers for the amplification of β-globin were added to provide a positive control for the quality of the template DNA (Saiki *et al.*, 1988).

Statistical analysis

Logistic regression (LR) was used to estimate crude ORs and 95% CIs between BCC and demographic, lifestyle, and phenotypic characteristics. Seroprevalence for individual HPV types was estimated as the proportion of patients who tested positive for antibodies to a single HPV type. Subsequently, genus-specific HPV seroprevalence was estimated as the proportion of patients who tested positive for antibodies to at least one HPV type in a given genus. Using LR, OR and 95% CI were estimated for the associations between BCC and genus-specific and type-specific cutaneous HPV seropositivity. LR was also used to assess the association between BCC and the number of HPV types in a given genus for which an individual tested seropositive. To test for trends in risk for BCC associated with seropositivity to an increasing number of cutaneous HPV types within a given genus, an ordinal variable was included in the model. Bonferroni correction of *P*-values was used to account for multiple comparisons, reducing the statistical significance level for genus-specific HPV seropositivity to $P<0.01$ and type-specific HPV seropositivity to $P<0.002$ (Iannacone *et al.*, 2012).

BCC cases that provided tumor tissues were classified as either positive or negative for the presence of HPV DNA for type(s) in genera α, β, and γ. The eight BCC cases that provided two tumor tissues were categorized as genus-specific HPV DNA positive if at least one of the two specimens tested positive for any HPV type belonging to genera α, β, or γ. To calculate OR and 95% CI for the association between seropositivity to any genus-α, β, or γ HPV type and BCC, LR was used, stratifying by the presence and absence of DNA in the tumor tissue. The analysis was restricted to HPV types included in both antibody and PCR assays for genus-α (2, 3, 10, 27, 57), genus-β (5, 8, 20, 24, 36, 9, 15, 17, 23, 38, 75, 76, 92, 96), and genus-γ (4, 48, 50, 65, 88, 95, 101, 103).

Genus-β type-specific concordance was calculated among the BCC cases as the proportion that tested seropositive for a given HPV type and had DNA in their tumor tissue corresponding to the same HPV type. Using LR, additional stratified analyses were then conducted to compare genus-β type-specific HPV seropositivity between controls and BCC cases that had DNA in their tumors corresponding to the same HPV type for which antibodies were detected. LR was also used to assess the associations between genus-β type-specific seropositivity and BCC among cases with HPV DNA–negative tumor tissues. Similar analyses were conducted for genus-α and genus-γ HPV types and BCC.

Skin cancer risk factors were investigated as potential confounders of the associations between genus-specific HPV seroreactivity and BCC. With the exception of age and sex, none of the factors investigated altered the ORs and corresponding 95% CIs for serological

associations by more than 10%. To examine residual confounding resulting from the case–control differences in age and sex, analyses were conducted stratified by sex and restricted to a narrower age range of 40–69 years. The SAS statistical software package (version 9.2; SAS Institute, Cary, NC) was used to conduct all study analyses.

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Abbreviations

BCC	basal cell carcinoma
CI	confidence interval
HPV	human papillomavirus
LR	logistic regression
NMSC	non-melanoma skin cancer
OR	odds ratio
SCC	squamous cell carcinoma
TS-MPG	type-specific multiplex genotyping
USF	University of South Florida

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Table 1

Crude associations between demographic, skin cancer, and lifestyle risk factors and BCC

	Control (*n*=300)	BCC (*n*=224)	
Variable	***n* (%)**	***n* (%)**	**OR (95% CI)**
Age in years (mean, SD)	55.4 (11.7)	62.5 (11.9)	1.05 (1.04–1.76)
Age			
18–39	28 (9.3)	8 (3.6)	1.00 (Reference)
40–49	54 (18)	28 (12.5)	1.82 (0.73–4.50)
50–59	104 (34.7)	46 (20.5)	1.55 (0.66–3.66)
60–69	85 (28.3)	67 (29.9)	2.76 (1.18–6.45)
70–80	29 (9.7)	75 (33.5)	9.05 (3.70–22.15)
Sex			
Female	186 (62)	89 (39.7)	1.00 (Reference)
Male	114 (38)	135 (60.3)	2.48 (1.74–3.53)
Education			
> 12 years	267 (90.2)	157 (77.3)	1.00 (Reference)
≤ 12 years	29 (9.8)	46 (22.7)	2.70 (1.63 -4.47)
Eye color			
Dark brown	81 (27.3)	33 (16)	1.00 (Reference)
Blue	85 (28.6)	85 (41.3)	2.45 (1.48–4.06)
Green	48 (16.2)	21 (10.2)	1.07 (0.56–2.06)
Hazel	48 (16.2)	46 (22.3)	2.35 (1.33–4.17)
Light brown	35 (11.8)	21 (10.2)	1.47 (0.75–2.89)
Hair color			
Black/brown	234 (78.3)	144 (69.9)	1.00 (Reference)
Blonde/red	65 (21.7)	62 (30.1)	1.55 (1.03–2.32)
Job in sun for ≥ 3 months			
No	216 (72.7)	114 (55.1)	1.00 (Reference)
Yes	81 (27.3)	93 (44.9)	2.18 (1.50–3.16)

Variable	Control (*n*=300) *n* (%)	BCC (*n*=224) *n* (%)	BCC (*n*=224) OR (95% CI)
History of blistering sunburn			
No	95 (32)	54 (26.3)	1.00 (Reference)
Yes	202 (68)	151 (73.7)	1.32 (0.89–1.95)
Skin reaction to season's first sun exposure			
Tan or no change in skin color	44 (14.8)	22 (10.8)	1.00 (Reference)
Mild sunburn turns to a tan	136 (45.6)	61 (30.1)	2.03 (1.15–3.60)
Sunburn with or without blisters	118 (39.6)	120 (59.1)	0.9 (0.50–1.63)
Skin reaction to repeated sun exposure			
It tans easily	174 (59)	99 (49.3)	1.00 (Reference)
It can tan if you work at it	99 (33.6)	87 (43.3)	1.55 (1.06–2.26)
It is unable to tan	22 (7.5)	15 (7.5)	1.20 (0.59–2.42)
Alcohol consumption			
No drinks in past year	38 (12.8)	36 (17.6)	1.00 (Reference)
≥ 1 drink in the past year	260 (87.3)	169 (82.4)	0.69 (0.42–1.13)
Ever smoked 100 cigarettes			
No	150 (50.2)	78 (38.2)	1.00 (Reference)
Yes	149 (49.8)	126 (61.8)	1.63 (1.13–2.34)

Abbreviations: BCC, basal cell carcinoma; CI, confidence interval; OR, odds ratio.

Table 2

Associations between genus-specific HPV seroreactivity and BCC cases and controls

	Control (*n*=300)	BCC (*n*=224)		BCC (*n*=141)
	***n* (%)**	***n* (%)**	**OR (95% CI)[1]**	**OR (95% CI)[2]**
Alpha				
Seronegative	194 (64.7)	121 (54.0)	1.00 (Reference)	1.00 (Reference)
Seropositive	106 (35.3)	103 (46.0)	1.61 (1.11–2.35)	1.68 (1.09–2.59)
1 type	59 (19.7)	52 (23.2)	1.50 (0.94–2.38)	1.55 (0.91–2.65)
≥ 2 types	92 (30.7)	80 (35.7)	1.59 (1.03–2.45)	1.38 (0.84–2.29)
			p_{trend}=0.01	p_{trend}=0.02
Beta				
Seronegative	119 (39.7)	72 (32.1)	1.00 (Reference)	1.00 (Reference)
Seropositive	181 (60.3)	152 (67.9)	1.32 (0.90–1.93)	1.07 (0.69–1.66)
1 type	47 (15.7)	42 (18.8)	1.35 (0.79–2.32)	0.93 (0.70–1.73)
2-3 types	51 (17.0)	40 (17.8)	1.30 (0.76–2.21)	1.30 (0.70–2.42)
4-8 types	36 (12.0)	36 (15.2)	1.75 (0.97–3.15)	1.01 (0.51–2.01)
> 8 types	47 (15.7)	36 (16.1)	1.02 (0.59–1.78)	1.08 (0.57–2.02)
			p_{trend}=0.43	p_{trend}=0.72
Gamma				
Seronegative	145 (48.3)	73 (32.6)	1.00 (Reference)	1.00 (Reference)
Seropositive	155 (51.7)	151 (67.4)	1.78 (1.22–2.60)[3]	1.58 (1.02–2.44)
1 type	63 (21.0)	71 (31.7)	2.06 (1.29–3.27)[3]	1.86 (1.09–3.17)
≥2 types	47 (15.7)	51 (22.8)	1.75 (1.08–2.85)	1.84 (1.04–3.24)
			p_{trend}=0.03	p_{trend}=0.17
Mu				
Seronegative	204 (68.0)	138 (61.6)	1.00 (Reference)	1.00 (Reference)
Seropositive	96 (32.0)	86 (38.4)	1.56 (1.06–2.30)	1.38 (0.89–2.13)

	Control (*n*=300)	BCC (*n*=224)		BCC (*n*=141)
	***n* (%)**	***n* (%)**	**OR (95% CI)[1]**	**OR (95% CI)[2]**
Nu				
Seronegative	266 (88.7)	198 (88.4)	1.00 (Reference)	1.00 (Reference)
Seropositive	34 (11.3)	26 (11.6)	1.00 (0.56–1.77)	1.25 (0.64–2.42)
Control antigens				
JCV				
Seronegative	108 (36.0)	62 (27.7)	1.00 (Reference)	1.00 (Reference)
Seropositive	192 (64.0)	162 (72.3)	1.32 (0.89–1.96)	1.34 (0.84–2.11)
KIV				
Seronegative	24 (8.0)	14 (6.3)	1.00 (Reference)	1.00 (Reference)
Seropositive	276 (92.0)	210 (93.8)	0.88 (0.42–1.83)	0.81 (0.35–1.88)

Abbreviations: BCC, basal cell carcinoma; CI, confidence interval; HPV, human papillomavirus; JCV, JC virus; KIV, KI virus; OR, odds ratio.

[1] OR and 95% CI adjusted for age and sex.

[2] OR and 95% CI adjusted for age and sex, restricted to participants aged 40–69 years. The age-restricted analysis included 141 BCC cases and 243 controls.

[3] Statistical significance retained after correction for multiple comparisons (β-coefficient $P<0.01$).

Table 3

Associations between (a) genus-specific HPV seroreactivity and BCC stratified by sex; (b) genus-specific seropositivity and BCC stratified by gender, restricted to population aged 40–69 years

	Male (*n*=249)		Female (*n*=275)		
HPV genus	***n* (%)**	**OR (95% CI)[1]**	***n* (%)**	**OR (95% CI)[1]**	***P*-value[2]**
(a)					
Alpha					
Seronegative	145 (58.2)	1.00 (Reference)	170 (61.8)	1.00 (Reference)	
Seropositive	104 (41.8)	1.92 (1.13-3.27)	105 (38.2)	1.35 (0.79-2.29)	0.36
Beta					
Seronegative	83 (33.3)	1.00 (Reference)	108 (39.3)	1.00 (Reference)	
Seropositive	166 (66.7)	1.24 (0.72-2.13)	167 (60.7)	1.39 (0.81-2.39)	0.76
Gamma					
Seronegative	87 (34.9)	1.00 (Reference)	131 (47.6)	1.00 (Reference)	
Seropositive	162 (65.1)	1.64 (0.95-2.81)	144 (52.4)	1.93 (1.13-3.28)	0.67
Mu					
Seronegative	162 (65.1)	1.00 (Reference)	180 (65.5)	1.00 (Reference)	
Seropositive	87 (34.9)	1.00 (0.58-1.72)	95 (34.5)	2.46 (1.40-4.30)	0.02
Nu					
Seronegative	216 (86.8)	1.00 (Reference)	248 (90.2)	1.00 (Reference)	
Seropositive	33 (13.2)	1.00 (0.47-2.12)	27 (9.8)	0.99 (0.40-2.42)	0.99
(b)					
	Male (age 40–69, *n*=177)		**Female (age 40–69, *n*=207)**		
HPV Genus	**OR (95% CI)[1]**		**OR (95% CI)[1]**		
Alpha	2.05 (1.11-3.79)		1.37 (0.73-2.55)		
Beta	1.13 (0.61-2.09)		1.01 (0.55-1.88)		
Gamma	1.43 (0.77-2.66)		1.74 (0.94-3.22)		
Mu	0.83 (0.45-1.52)		2.37 (1.27-4.43)		

	Male (*n*=249)		Female (*n*=275)		
HPV genus	***n* (%)**	**OR (95% CI)[1]**	***n* (%)**	**OR (95% CI)[1]**	***P*-value[2]**
Nu		1.30 (0.54-3.12)		1.19 (0.43-3.31)	

Abbreviations: BCC, basal cell carcinoma; CI, confidence interval; HPV, human papillomavirus; OR, odds ratio.

[1] Adjusted for age.

[2] *P*-value for β-coefficient corresponding to the genus-specific HPV×sex interaction term in logistic regression model.

Table 4

Association between type-specific HPV seropositivity and BCC

	Controls (*n*=300)	BCC (*n*=224)	
HPV genus	***n* (%)**	***n* (%)**	**OR (95% CI)[1]**
Any HPV type	256 (85.3)	203 (90.6)	1.72 (0.96–3.05)
α			
2	29 (9.7)	29 (12.9)	1.55 (0.87–2.77)
3	36 (12)	43 (19.2)	1.70 (1.03–2.82)
7	22 (7.3)	14 (6.3)	0.87 (0.42–1.82)
10	17 (5.7)	22 (9.8)	1.70 (0.84–3.44)
27	33 (11)	42 (18.8)	2.18 (1.28–3.71)
57	22 (7.3)	21 (9.4)	1.39 (0.71–2.72)
77	47 (15.7)	40 (17.9)	1.18 (0.72–1.92)
β			
Any β_1	100 (33.3)	94 (42)	1.30 (0.89–1.90)
5	38 (12.7)	30 (13.4)	1.01 (0.59–1.75)
8	69 (23)	69 (30.8)	1.32 (0.87–2.00)
20	63 (21)	54 (24.1)	1.11 (0.72–1.73)
24	37 (12.3)	42 (18.8)	1.37 (0.82–2.28)
36	52 (17.3)	40 (17.9)	0.90 (0.55–1.46)
Any β_2	143 (47.7)	114 (50.9)	1.14 (0.79–1.64)
9	75 (25)	57 (25.4)	0.88 (0.58–1.35)
15	76 (25.3)	67 (29.9)	1.18 (0.78–1.77)
17	77 (25.7)	59 (26.3)	1.06 (0.70–1.61)
23	61 (20.3)	52 (23.2)	1.28 (0.82–1.99)
38	74 (24.7)	58 (25.9)	1.05 (0.69–1.59)
107	48 (16)	33 (14.7)	0.83 (0.50–1.38)
Any β_3	101 (33.7)	76 (33.9)	0.96 (0.65–1.42)

	Controls (*n*=300)	BCC (*n*=224)	
HPV genus	***n* (%)**	***n* (%)**	**OR (95% CI)[1]**
49	89 (29.7)	67 (29.9)	0.96 (0.65–1.44)
75	46 (15.3)	35 (15.6)	1.01 (0.61–1.68)
76	54 (18)	45 (20.1)	1.07 (0.67–1.70)
β_4			
(92)	50 (16.7)	30 (13.4)	0.78 (0.46–1.31)
β_5			
$(96)_5$	45 (15)	33 (14.7)	0.86 (0.52–1.44)
γ			
4	103 (34.3)	84 (37.5)	1.11 (0.76–1.62)
48	64 (21.3)	56 (25)	1.23 (0.80–1.91)
50	47 (15.7)	34 (15.2)	1.01 (0.61–1.67)
65	78 (26)	62 (27.7)	0.98 (0.65–1.48)
88	20 (6.7)	13 (5.8)	1.05 (0.49–2.24)
95	67 (22.3)	55 (24.6)	1.00 (0.65–1.54)
101	33 (11)	24 (10.7)	0.97 (0.54–1.74)
103	13 (4.3)	15 (6.7)	1.37 (0.61–3.05)
Mu (1)	96 (32)	86 (38.4)	1.56 (1.06–2.30)
Nu (41)	34 (11.3)	26 (11.6)	1.00 (0.56–1.77)

Abbreviations: BCC, basal cell carcinoma; CI, confidence interval; HPV, human papillomavirus; OR, odds ratio.

[1]ORs and 95% CIs adjusted for age and sex.

Table 5

Type-specific HPV DNA prevalence among 238 BCC tumor tissues

	HPV DNA+
HPV genus/type	***n* (%)**
Any β type	120 (50.4)
β Species	
β[1]	
5	31 (13.0)
8	31 (13.0)
12	9 (3.8)
14	7 (2.9)
19	3 (1.3)
20	2 (0.8)
21	0 (0.0)
24	35 (14.7)
25	3 (1.3)
36	21 (8.8)
47	10 (4.2)
93	9 (3.8)
β[2]	
9	10 (4.2)
15	6 (2.5)
17	9 (3.8)
22	17 (7.1)
23	7 (2.9)
37	17 (7.1)
38	32 (13.5)
80	3 (1.3)
β[3]	

	HPV DNA+
HPV genus/type	***n* (%)**
49	0 (0.0)
75	4 (1.7)
76	18 (7.6)
β[4]	
92	6 (2.5)
β[5]	
96	7 (2.9)
Number of β HPV infections	
1 Type	43 (18.1)
2 Types	31 (13.0)
3 Types	22 (9.2)
4 Types	9 (3.8)
≥5 Types	15 (6.3)
Any α type [1]	7 (3.0)
2	0 (0.0)
3	0 (0.0)
10	4 (1.7)
27	2 (0.8)
57	1 (0.4)
IAny γ type	20 (8.4)
4	1 (0.4)
50	11 (4.6)
60	1 (0.4)
65	3 (1.3)
88	1 (0.4)
103	1 (0.4)
121	2 (0.8)

	HPV DNA+
HPV genus/type	***n* (%)**
Mu[1] (1)	1 (0.4)

Abbreviations: BCC, basal cell carcinoma; HPV, human papillomavirus.

[1] DNA prevalence estimation based on a sample size of 236 BCC tumor tissues.

Table 6

Association between genus-specific HPV seroposivity and BCC, stratified by HPV DNA status of the same HPV type in the tumor

			BCC DNA status			
			Positive		Negative	
HPV type	Antibody status	Controls *n* (%)	*n* (%)	OR (95% CI)[1]	*n* (%)	OR (95% CI)[1]
Genus-β						
N		300	97		98	
Any β	Negative	121 (40.3)	26 (26.8)	1.00 (Reference)	31 (37.8)	1.00 (Reference)
	Positive	179 (59.7)	71 (73.2)	1.76 (1.03–3.01)	61 (62.2)	1.06 (0.65–1.72)
β[1]						
5	Negative	262 (87.3)	21 (84)	1.00 (Reference)	86 (87.8)	1.00 (Reference)
	Positive	38 (12.7)	4 (16)	1.17 (0.36–3.87)	12 (12.2)	0.91 (0.45–1.86)
8	Negative	231 (77)	14 (53.8)	1.00 (Reference)	73 (74.5)	1.00 (Reference)
	Positive	69 (23)	12 (46.2)	2.77 (1.11–6.94)	25 (25.5)	1.02 (0.59–1.77)
20	Negative	237 (79)	0 (0)	1.00 (Reference)	74 (75.5)	1.00 (Reference)
	Positive	63 (21)	2 (100)	NA (NA)	24 (24.5)	1.13 (0.65–1.96)
24	Negative	263 (87.7)	22 (73.3)	1.00 (Reference)	82 (83.7)	1.00 (Reference)
	Positive	37 (12.3)	8 (26.7)	1.80 (0.69–4.70)	16 (16.3)	1.15 (0.59–2.22)
36	Negative	248 (82.7)	14 (70)	1.00 (Reference)	81 (82.7)	1.00 (Reference)
	Positive	52 (17.3)	6 (30)	1.12 (0.37–3.39)	17 (17.3)	0.85 (0.46–1.59)
β[2]						
9	Negative	225 (75)	6 (66.7)	1.00 (Reference)	74 (75.5)	1.00 (Reference)
	Positive	75 (25)	3 (33.3)	1.02 (0.22–4.63)	24 (24.5)	0.93 (0.54–1.60)
15	Negative	224 (74.7)	3 (60)	1.00 (Reference)	72 (73.5)	1.00 (Reference)
	Positive	76 (25.3)	2 (40)	1.48 (0.23–9.48)	26 (26.5)	1.00 (0.59–1.71)
17	Negative	223 (74.3)	4 (57.1)	1.00 (Reference)	78 (79.6)	1.00 (Reference)
	Positive	77 (25.7)	3 (42.9)	2.40 (0.49–11.70)	20 (20.4)	0.81 (0.46–1.43)
23	Negative	239 (79.7)	1 (20)	1.00 (Reference)	81 (82.7)	1.00 (Reference)

			BCC DNA status			
			Positive		Negative	
HPV type	Antibody status	Controls *n* (%)	*n* (%)	OR (95% CI)[1]	*n* (%)	OR (95% CI)[1]
	Positive	61 (20.3)	4 (80)	15.80 (1.60–155.00)	17 (17.3)	0.85 (0.46–1.56)
38	Negative	226 (75.3)	21 (75)	1.00 (Reference)	78 (79.6)	1.00 (Reference)
	Positive	74 (24.7)	7 (25)	0.97 (0.37–2.56)	20 (20.4)	0.80 (0.45–1.43)
β[3]						
75	Negative	254 (84.7)	3 (75)	1.00 (Reference)	87 (88.8)	1.00 (Reference)
	Positive	46 (15.3)	1 (25)	2.14 (0.19–24.40)	11 (11.2)	0.67 (0.32–1.37)
β[4]						
92	Negative	250 (83.3)	5 (100)	1.00 (Reference)	86 (87.8)	1.00 (Reference)
	Positive	50 (16.7)	0 (0)	0.00 (NA)	12 (12.2)	0.64 (0.32–1.28)
β[5]						
96	Negative	255 (85)	6 (85.7)	1.00 (Reference)	82 (83.7)	1.00 (Reference)
	Positive	45 (15)	1 (14.3)	0.70 (0.08–6.42)	16 (16.3)	0.96 (0.50–1.82)
Genus-α[2]						
N		300	7		186	
	Negative	194 (64.7)	3 (42.9)	1.00 (Reference)	98 (52.7)	1.00 (Reference)
	Positive	106 (35.3)	4 (57.1)	2.53 (0.55–11.72)	88 (47.3)	1.64 (1.11–2.44)
Genus-*γ*						
N		300	17		178	
	Negative	145 (48.3)	6 (35.3)	1.00 (Reference)	55 (30.9)	1.00 (Reference)
	Positive	155 (51.7)	11 (64.7)	1.40 (0.49–4.00)	123 (69.1)	1.97 (1.31–2.97)

Abbreviations: BCC, basal cell carcinoma; CI, confidence interval; HPV, human papillomavirus; NA, not applicable; OR, odds ratio.

[1] ORs and 95% CIs adjusted for age and sex.

[2] OR and 95% CI estimations based on a sample size of 193 BCC cases and 300 controls.