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Cutaneous alpha, beta and gamma human papillomaviruses in relation to squamous cell carcinoma of the skin: a population-based study

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Human papillomavirus (HPV) infection is common worldwide and, in immunodeficient populations, may contribute to the pathogenesis of keratinocyte cancers, particularly squamous cell carcinomas (SCC). However, their role in SCC in the general population is less clear. We conducted a comprehensive analysis to investigate the independent effects of seropositivity for cutaneous alpha, beta and gamma HPV types on risk of SCC, and a meta-analysis of the available literature. In a population-based case-control study from New Hampshire, USA ($n = 1{,}408$), histologically confirmed SCC cases and controls were tested for L1 antibodies to alpha, beta and gamma cutaneous HPV types 2–5, 7–10, 15, 17, 20, 23, 24, 27b, 36, 38, 48–50, 57, 65, 75–77, 88, 92, 95, 96, 101, 103 and 107 using multiplex serology. An increasing risk of SCC with number of beta HPVs to which an individual tested positive was observed even among those seronegative for gamma types (p for trend = 0.016) with an odds ratio of 1.95 (95% confidence interval (CI) = 1.07–3.56) for four or more beta types positive. In a meta-analysis of six case-control studies, increased SCC risks in relation to beta HPV seropositivity were found across studies (meta odds ratio = 1.45, CI = 1.27–1.66). While the prevalence of gamma HPVs assayed was somewhat higher among SCC cases than controls, the association was only weakly evident among those seronegative for beta HPVs. Overall, the association between cutaneous HPVs and skin cancers appears to be specific to SCC and to genus beta HPVs in a general US population.

Keratinocyte cancers (KC) of the skin are the most common type of malignancy in humans.[1] Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the two most frequently occurring forms of KC and, while largely nonfatal, can cause significant morbidity and disfigurement. Immunosuppressed individuals are particularly vulnerable to KC-related mortality, primarily from SCC tumors, which are capable of metastasizing. While cumulative sun exposure is an established environmental risk factor, there remain many questions to be answered regarding the etiology and epidemiology of KCs.

Human papillomavirus (HPV) infection is highly prevalent worldwide and has been hypothesized to contribute to the pathogenesis of KC, in particular SCC.[2–4] HPVs are nonenveloped, double-stranded DNA viruses that infect the skin and mucosa, where they can persist asymptomatically or cause neoplasia. Approximately 90% of the 120 serologically distinct HPV genotypes identified to date fall into either the alpha genus, which tend to infect mucosae, or the beta and gamma genera, which tend to be cutaneous.[5] HPV may be one of the only viruses to exploit the process of keratinocyte differentiation for viral replication.[6] Initial evidence for a role for cutaneous HPVs in the pathogenesis of skin malignancies came from the identification of HPV5 and HPV8 from patients with epidermodysplasia verruciformis (EV), a rare genetic condition characterized by diffuse, wart-like lesions over broad areas of the skin that frequently develop into carcinomas early in life. Cutaneous HPV types have been shown to be prevalent in skin lesions[7–9] and published reports from prospective cohort

Key words: human papillomavirus, squamous cell carcinoma, population-based, case-control, meta-analysis

Abbreviations: HPV: human papillomavirus; SCC: squamous cell carcinoma; KC: keratinocyte cancers; OR: odds ratio; CI: confidence interval; GSEA: gene set enrichment analysis

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What's new?

Recent work has strengthened the possibility that cutaneous human papillomaviruses (HPVs) may contribute to the development of skin cancers, which worldwide are the most commonly diagnosed malignancies. Here, population-based investigation of alpha, beta, and gamma HPV types in squamous cell carcinoma (SCC) reveals an association between beta HPVs and SCC. Enrichment analyses showed that beta-2 subtypes were specifically associated with elevated risk. These results, together with meta-analysis of previous case-control studies, indicate an increased SCC risk in relation to beta HPV seropositivity across populations.

and case-control studies in immunocompetent adults have begun to provide some support for a role of cutaneous HPV in the etiology of SCC.[10–17] In particular, these prior studies have related cutaneous beta, and more recently gamma, HPV types to increased risks of these malignancies.[11–17]

As part of an expanded population-based case-control study from New Hampshire, USA on whom we previously reported on genus beta human papillomaviruses, we performed a comprehensive serologic analysis of alpha and gamma cutaneous human papillomaviruses and assessed the independent associations of these types along with beta papillomaviruses and squamous cell carcinomas of the skin.[11]

Methods

Study population

Study subjects included those described in our earlier reports.[11,18–20] Briefly, to identify cases we enlisted the collaboration of dermatologists and pathology laboratories throughout New Hampshire and bordering regions.[19] We selected all histologically-confirmed incident invasive cutaneous SCC cases diagnosed from July 1, 1993 through June 30, 1995 in the initial enrollment phase, and from July 1, 1997 through March 31, 2000 in the second enrollment phase. Eligible subjects included New Hampshire residents who, at the time of diagnosis, were aged 25–74 years, spoke English and had a listed telephone number. Individuals with SCC on genital sites were excluded. We identified 1,084 potential participants. Of these, we contacted and confirmed the eligibility on 1036 (96%), of whom 833 (80%) were interviewed.

We chose controls from New Hampshire residents aged 25–74 years who were frequency-matched on age (25–34, 35–44, 45–54, 55–64, 65–69, and 70–74 years) and gender to represent the combined distribution of all KC cases (both SCC and BCC) chosen for the original study.[18,19] We selected controls (roughly a two to one ratio to the SCC cases in the first phase and one to one ratio in the second phase) from lists of New Hampshire residents provided by the New Hampshire Department of Transportation ($<$65 years old) and Center for Medicaid and Medicare Services ($\geq$65 years). As with cases, controls were required to speak English and to have a listed telephone number. For interviewing purposes, controls were randomly assigned reference dates corresponding to the cases' diagnosis dates. Of the 1,527 potential controls, 1,462 (96%) were contacted and confirmed as eligible and 1,066 (73%) of those were interviewed.

Personal interview

All participants provided informed consent in accordance with the Committee for the Protection of Human Subjects at Dartmouth College. Study participants completed a structured personal interview, usually at their homes. To minimize reporting bias, we did not reveal the specific hypotheses of interest to either the interviewer or participant, and did not inform the interviewers of the case-control status of participants. The interview included sociodemographic information (level of education), tobacco use, prolonged use of glucocorticoid drugs (for 1 month or longer) and reasons for use, assessment of pigmentary characteristics and nevi, and questions relating to skin sensitivity to the sun after first exposure in the summer (i.e., tendency to sunburn). To estimate sun exposure, we asked about the amount of time spent outdoors on work days, non-work days and vacations, both in summer and other times of the year, history of sunbathing, number of painful and blistering sunburns, and lifetime residential history using a standardized instrument developed for a case-control study conducted in Australia.[21,22]

Human papillomavirus serology

We collected a venous blood sample (20–30 ml) in heparinized tubes and separated plasma, white blood cells and red blood cells by centrifugation at 3,000 rpm for 20 min at 4°C. Cells were washed twice in saline, aliquoted and stored at −80°C until analysis. Each specimen was labeled and given a unique identifier that did not reveal the subject's case-control status.

We analyzed plasma samples for antibodies to the major capsid protein L1 of HPV types 3, 10, 77 (alpha-2); 2, 27b, 57 (alpha-4); 7 (alpha-8); 4, 65, 95 (gamma-1); 50 (gamma-2); 48 (gamma-3); 88 (gamma-5), 101, 103 (unclassified); and as previously reported, 5, 8, 20, 24, 36 (beta-1); 9, 15, 17, 23, 38, 107 (beta-2); 49, 75, 76 (beta-3); 92 (beta-4); and 96 (beta-5).[11] We based our antibody detection approach on a glutathione S-transferase (GST) capture ELISA method in combination with fluorescent bead technology.[23–25] Full-length viral proteins fused with an N-terminal GST domain were expressed in *E. coli*, coupled to fluorescence-labeled polystyrene beads (Luminex, Austin, TX), and affinity-purified on the beads directly in a one-step procedure. Bead types of different color and carrying different antigens were incubated with human sera. Antibodies bound to beads via the viral antigens were stained by biotinylated anti-human-Ig and

streptavidin-R-phycoerythrin, analyzed in a Luminex analyzer that identified the antigen by bead color and quantified via the median R-phycoerythrin fluorescence intensity (MFI) of at least 100 beads of the same internal color. Standard cut points for a positive MFI reaction were derived from a survey of the German general population.[26] A reanalysis of our earlier data with these cut-offs made no substantial difference to the results (data not shown).[20]

The study sera were analyzed once on three consecutive days. As a quality control, a subset of 166 study sera supplemented with 21 sera with known reactivity was tested daily.[27] Pearson correlation coefficients (R^2) for individual antigens ranged from 0.634 to 0.967 (median 0.849) for day 2 versus 1 and from 0.544 to 0.980 (median 0.903) for day 3 versus 1. As additional quality control, a standard serum with known reactivity pattern to a subset of the antigens was analyzed on each 96-well plate, and loading of the different beads with antigens was monitored by a monoclonal antibody against a peptide fused to the C-terminus of all expressed antigens.[24]

Systematic review and meta-analysis

We reviewed all previous case-control and prospective studies of HPV serology and histologically confirmed SCC of the skin published through September 2012. A Medline search included the terms: human papillomavirus, skin cancer, nonmelanoma, keratinocyte, cutaneous and squamous cell carcinoma. We crosschecked and supplemented our review by examining reference lists and tables from literature reviews and recent IARC reports.[28–31] Our meta-analysis included only studies of SCC of the skin (excluding anogenital sites) among immunocompetent adults and we calculated a meta-odds ratio for SCC in relation to one or more positive serological measurements for beta HPV L1 antibodies.

Statistical analysis

Primary analysis. We examined alpha, beta, and gamma type-specific prevalence of HPV antibody positivity according to skin cancer risk factors. Statistical analyses were conducted with SAS 9.2 (SAS Institute, Cary, NC). We computed the odds ratios (OR) and 95% confidence intervals (CI) of SCC and BCC associated with seropositivity to alpha or gamma HPV types (overall, and by individual types). Based on earlier work, we examined seropositivity to multiple types for both alpha and gamma types and computed a P for trend based both on these categories and on a continuous variable of the number of types positive.[20,26,32] We further examined the independent effects of gamma and beta seropositivity (*i.e.,* each of the gamma types and the number of gamma types positive in the absence of beta types and vice versa) and computed OR and 95% CI of SCC for each combination. In each of these analyses, we used unconditional logistic regression, taking into account multiple confounding factors.[33] These covariates included age, level of education, smoking status, skin sensitivity to the sun and lifetime number of painful sunburns.

We classified cases according to their status as of the date of their first skin cancer diagnosed during the study period, or for controls, as of their reference date. This subject classification plan results in relative risk estimates of incidence density ratios.[34] As a sensitivity analysis, we performed analyses restricted to subjects who have had no previous skin cancers to assess whether the risk estimates differ from those obtained for all subjects. Additionally, we conducted analyses of SCC excluding individuals who had a concomitant BCC ($n = 58$; 6.7% of cases). All risk estimates were ultimately adjusted for or stratified by age, gender and sun sensitivity, and cigarette smoking. No other factors appreciably influenced the results.

Secondary analyses. We assessed the potential modifying effects of ultraviolet light exposure by examining ORs stratified by skin reaction to the sun and lifetime number of painful sunburns. As HPVs have been related to skin cancers in immunosuppressed populations, we also considered whether associations might be stronger among those with prolonged oral glucocorticoid use for reasons other than organ transplantation.[11,35] In these stratified analyses we classified individuals as users if they reported glucocorticoid use for one month or longer and excluded individuals who reported having an organ transplant.

Gene set enrichment analysis. We also applied the methodology of gene set enrichment analysis (GSEA), first used for gene expression data, to identify important gene classes.[36] We used this approach to determine whether SCC was associated with specific subtypes within the three HPV genera. In this analysis, we used the MFI values as continuous variables with positive values "log+1" transformed and negative values corrected to 0. The 40 HPV variables were grouped based on genera and subtype, resulting in 4 alpha, 4 beta, 2 gamma and 2 miscellaneous groups. GSEA produces an enrichment score (ES) to represent the degree to which a group of HPV types is overrepresented among the highest (positively related) and lowest (inversely related) ranked HPV types that differentiate cases from controls. The significance of the ES is evaluated via a permutation test.

Results

We obtained a plasma sample for HPV serology on 663 (80%) of the 833 interviewed SCC cases and 805 (76%) of the 1,066 interviewed controls (excluding 7 SCC and 7 controls with insufficient or unprocessed samples). No appreciable case-control differences were noted in the characteristics of individuals on whom we did not obtain serology data (data not shown).

Men had a slightly higher proportion of seropositivity to beta and gamma HPVs than women, which was only statistically significant for gamma types (43.6% in men, 34.9% in women; $p = 0.01$; Table 1). Individuals with sun sensitive skin types were less likely to be seropositive for the alpha types than those without a sensitive skin type ($p = 0.03$; Table

Table 1. Distribution of HPV seropositivity among control subjects by age, education, smoking status, and sunlight-related factors[1]

Variable	Total *N*	Genus α Positive *N* (%)	Genus β Positive *N* (%)	Genus γ Positive *N* (%)
Overall	805	278 (34.5)	369 (45.8)	324 (40.3)
Gender				
Men	493	175 (35.5)	237 (48.1)	215 (43.6)
Women	312	103 (33.0)	132 (42.3)	109 (34.9)
Age (years)				
25–34	5	2 (40.0)	1 (20.0)	0 (0.0)
35–49	118	47 (39.8)	53 (44.9)	50 (42.4)
50–54	59	19 (32.2)	28 (47.5)	33 (55.9)
55–59	96	32 (33.3)	41 (42.7)	36 (37.5)
60–64	111	45 (40.5)	49 (44.1)	44 (39.6)
65–69	223	77 (34.5)	111 (49.8)	83 (37.2)
70–74	193	56 (29.0)	86 (44.6)	78 (40.4)
Education				
High school or technical school	376	126 (33.5)	170 (45.2)	154 (41.0)
College	262	88 (33.6)	124 (47.3)	95 (36.3)
Graduate or professional school	167	64 (38.3)	75 (44.9)	75 (44.9)
Smoking status				
Never	276	96 (34.8)	125 (45.3)	115 (41.7)
Former	399	139 (34.8)	186 (46.6)	165 (41.4)
Current	130	43 (33.1)	58 (44.6)	44 (33.9)
Skin Sensitivity[2]				
Severe sunburn with blistering	43	10 (23.3)	19 (44.2)	17 (39.5)
Painful sunburn followed by peeling	198	62 (31.3)	84 (42.4)	77 (38.9)
Mild sunburn with some tanning	408	138 (33.8)	189 (46.3)	162 (39.7)
Tan without sunburn	154	67 (43.5)	77 (50.0)	67 (43.5)
Lifetime number of painful sunburns				
None	256	98 (38.3)	114 (44.5)	109 (42.6)
1 to 2	231	79 (34.2)	104 (45.0)	83 (35.9)
3 or more	309	99 (32.0)	147 (47.6)	127 (41.1)
Glucocorticoid use				
No	737	252 (34.2)	336 (45.6)	299 (40.6)
Yes	43	18 (41.9)	18 (41.9)	13 (30.2)

[1]Numbers may not sum to the overall total due to missing data. Two subjects were missing skin sun sensitivity, nine were missing number of sunburns and twenty-five were missing glucocorticoid use. They were excluded from analyses.
[2]Sun sensitivity was defined as the reaction to 1 hr of sun exposure the first time in the summer.

1). We did not observe any clear trends in antibody prevalence by age, level of education, smoking status, number of painful sunburns or prolonged glucocorticoid use (Table 1).

Similar to our previously published observations that demonstrated an association between SCC and beta seropositivity,[11] we observed an association between SCC and gamma seropositivity, with an overall OR of 1.41 (CI = 1.13–1.75) for positivity to at least one gamma type (Supporting Information Table S2). We also found an increasing trend in risk of SCC with increasing number of gamma types positive (OR per gamma type positive = 1.23 (CI = 1.10–1.38); p for trend <0.001, Supporting Information Table S2). Elevated odds ratios for SCC were detected for each of the individual gamma HPV types (Supporting Information Table S2). In contrast, no relation was observed with any alpha types for SCC or for BCC (Supporting Information Table S1).

Overall, 31% of SCC cases and 24% of controls ($n = 203$ and 193 individuals, respectively) were positive for both beta and gamma types. To examine the independent effects of beta and gamma type on SCC risk, we performed stratified analyses of individuals seropositive for either beta or gamma types while seronegative for the other. There remained a

Table 2. Independent odds ratios (95% confidence intervals) of squamous cell carcinoma of the skin in relation to number of gamma and beta HPVs to which individuals tested positive

	Control subjects (%)	SCC case patients (%)	Adjusted OR (95% CI)[1]
γ HPV serology results (β seronegative)			
No. of positive γ types[2]			
0	305 (70.0)	203 (65.3)	1.00 (referent)
1	99 (22.7)	80 (25.7)	1.33 (0.93-1.92)
2+	32 (7.3)	28 (9.0)	1.23 (0.70-2.16)
			p for trend = 0.18
β HPV serology results (γ seronegative)			
No. of positive β types			
0	305 (63.4)	203 (57.7)	1.00 (referent)
1	101 (21.0)	62 (17.6)	0.83 (0.56-1.21)
2–3	50 (10.4)	58 (16.5)	1.57 (1.01–2.45)
4+	25 (5.2)	29 (8.2)	1.95 (1.07-3.56)
			p for trend = 0.016

[1]Adjusted by age, sex, level of education, cigarette smoking status, skin sensitivity as measured by skin reaction after 1 hr of sun exposure the first time in the summer and the number of lifetime painful sunburns.
[2]Too few subjects were seropositive for four or more gamma types (in absence of beta) to calculate in a similar manner as for beta types.

weak association with gamma types among individuals seronegative for beta types, (OR = 1.31, CI = 0.95–1.82) but lack of trend by number of types to which an individual tested positive (Table 2). In contrast, the trend in odds ratios for number of beta HPVs persisted even among those seronegative for gamma types (p for trend = 0.016), with an odds ratio of 1.95 (CI = 1.07–3.56) for four or more beta types positive (Table 2). In further analyses of gamma HPV seropositivity (restricted to beta HPV seronegatives), we did not find any differences in SCC risk when stratified by sun-related factors (*i.e.,* lifetime painful sunburns or skin sensitivity) or by glucocorticoid use (Supporting Information Table S3).

In a comparison of SCC cases and controls using gene set enrichment analysis, we found beta-2 types had a high enrichment score (ES = 0.8; p-value = 0.025) (Supporting Information Fig. S1). After False Discovery Rate (FDR) adjustment, the estimated q-value was 0.062, with six HPV types (HPV 9, 15, 17, 23, 38, 107) within the beta-2 subgroup being positively associated with an elevated risk of SCC. These beta-2 types ranked at 1st, 3rd, 6th, 8th, 9th and 24th among all 40 HPV types.

A total of six case-control studies met our inclusion criteria for the meta-analysis (Fig. 1).[11,14,15,32,37,38] We were able to calculate a meta-odds ratio for SCC in relation to one or more positive serological measurements for beta HPV L1 antibodies, as this was measured in the majority of studies. Of these, all reported an elevated risk of SCC associated with seropositivity to at least one beta HPV, four of which were statistically significant. The meta-analytic odds ratio of SCC was 1.45 (CI = 1.27–1.66), without evidence of heterogeneity (χ^2 for heterogeneity, $Q = 6.89$, $p = 0.23$).

Three prospective studies evaluated cutaneous HPV and SCC occurrence with evidence of associations with beta HPV serology and SCC risk for certain subgroups (*e.g.*, beta -2 HPVs of those <50 years at diagnosis) or in relation to subsequent SCCs in those with a prior history of these malignancies.[13,16,17] Prior studies also have measured risk of SCC associated with seropositivity to other types, including alpha and gamma, with inconsistent findings; we were unable to compute a summary odds ratio for these other types because too few studies reported their data in a comparable way.

Discussion

Principal findings of the study

Findings from our large, population based case-control study provide further evidence to support an association between genus beta HPV seropositivity and SCC. We tested a wide range of human papillomaviruses using a multiplex serologic assay. The excess risk of SCC associated with beta HPVs was exclusive of seropositivity to gamma HPVs and there was no association with cutaneous alpha HPVs. In a gene enrichment analysis, we found that beta-2 was most highly associated with SCC of the various HPV subtypes.

Previous studies have raised the possibility of a relation between cutaneous gamma types and SCC risk.[12,16] We observed associations in our data with several gamma types, but they appeared to be dependent on beta HPV seropositivity (*i.e.*, with a only a weak association among those seronegative for beta types). When we restricted to individuals seronegative for gamma types, there remained an increasing trend in risk of SCC with the number of beta types detected, with an approximate 12% increase in risk of SCC per beta type detected. Dose-response relationships with beta types have also been shown in other studies, including a large, multi-center case-control study ($n = 1534$) that observed an increasing SCC risk with increasing numbers of beta HPV types present in sera (≥4 beta types positive OR = 1.8, CI = 1.4–2.4; p <0.0001 for trend), an association that was consistent across study centers.[14] As in our study, another US study detected an increasing risk with the accumulation of beta types ($p = 0.04$ for trend)—a trend they did not observe for gamma or alpha types.[15] Altogether, these results portray a consistent trend of increasing risk of SCC with seropositivity to an increasing number of beta type HPVs.

Evidence that beta HPVs play a role in the malignant transformation of EV lesions gave the first indication of the oncogenic potential of this phylogenetic subgroup, a role supported by the relation between beta HPV infection and keratinocytic skin tumors in immunosuppressed populations.[28] Organ-transplant recipients are exceptionally susceptible to SCCs: with up to a 250-fold higher risk of SCC than the general population.[28,39,40] Long-term users of glucocorticoids, an immunosuppressed population analogous to transplant

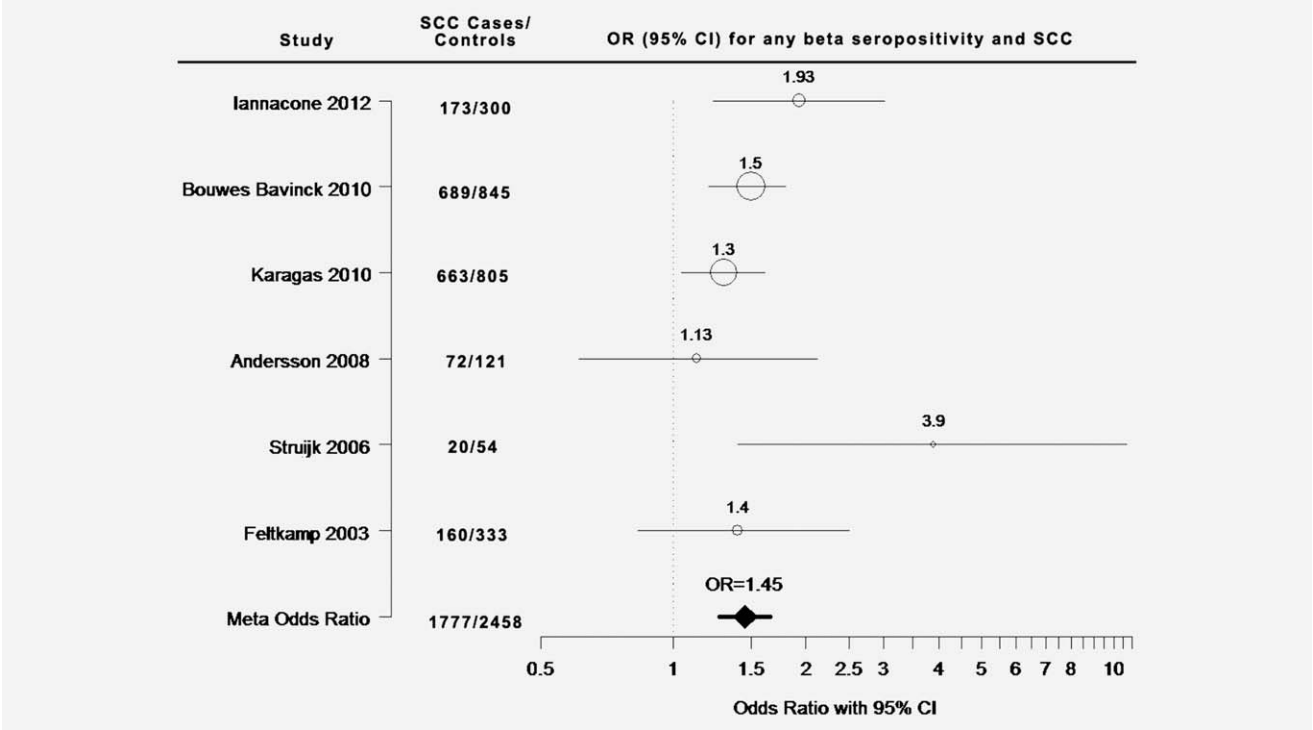

Figure 1. Forest plot of odds ratios and confidence intervals for case-control studies of SCC in relation to seropositivity for any beta HPV type. Horizontal lines represent the 95% confidence interval. Circles represent individual odds ratios and the area of the circle is proportional to the relative size of the studies. The meta odds ratio (diamond) and confidence interval was calculated from all studies and represents a weighted average of odds.

recipients (albeit to a lesser extent), provided proof of principle in our previous work as we observed both an increased risk of SCC associated with glucocorticoid use (OR = 2.31, CI = 1.27–4.18) as well as a greater magnitude of SCC risk in relation to beta seropositivity among glucocorticoid users than nonusers.[11,35] It is noteworthy that in the present study glucocorticoid use did not modify risk of SCC in relation to gamma HPV seropositivity when restricted to beta HPV seronegative individuals, further supporting the notion that risk of SCC appears to be specific to beta HPV types.

Indeed, many studies have reported that phylogenetically related beta type HPVs are associated with enhanced SCC risk, as summarized by our meta-analysis.[8,9,11,12,14,15,20,32] Several reports from prospective cohort and case-control studies on the relation between HPV and SCC risk in immunocompetent adults have been published since the 2009 International Agency for Research on Cancer (IARC) monograph update on papillomaviruses.[11,13–17,31] Assessment of previous literature, reviewed along with more recent studies, including ours, indicates relatively consistent findings with respect to cutaneous beta HPVs and SCC. In addition, beta-2 types have begun to emerge as a possible "high-risk" subtype. Consistent with our results, a prospective study from Sweden and Norway by Andersson *et al.* found that risk of SCC increased with baseline seropositivity to any beta-2 type, as well as with persistent beta-2 seropositivity.[13] Waterboer *et al.* also observed significant associations between SCC diagnoses and beta-2 seropositivity (OR = 3.3, CI = 1.2–8.7) in their clinic-based case-control study from Italy.[12] They further identified associations specifically to beta-2 types HPV15, HPV17, and HPV38, which also ranked highly in our enrichment set analysis.[12]

Questions remain regarding the underlying carcinogenic mechanism of beta HPVs. Lack of actively transcribed HPV mRNA in skin tumors is cited as incompatible with a role for beta HPVs in tumorigenesis.[41] However, there is evidence for beta HPVs hijacking control of cell cycle progression, DNA repair, and immune surveillance, promoting both viral infection and carcinogenesis. Over a decade ago, Storey *et al.* reported beta HPV E6 protein protected keratinocytes from Bak-induced apoptosis.[42] Although beta HPV E6 and E7 proteins may not completely inactivate the p53 and Rb proteins, as found with high-risk HPV types (*e.g.*, HPV16/18), beta E6 and E7 can deregulate these pathways and immortalize primary keratinocytes, as well as sensitize mice to UV-induced skin lesions resembling actinic keratoses and SCC.[43–45] E6 also may disrupt cell adhesion and growth factor signaling pathways, and induce secretion of anti-apoptotic factors to inhibit UV-induced apoptosis.[46,47] Additional work suggests beta HPV may act transiently, evidenced by loss of HPV protein expression with cancer progression, contributing to the initiating events of carcinogenesis, and allowing the HPV infection to persist by suppressing the local immune response.[48,49] While further work is needed to fully

understand these mechanisms, an early role in carcinogenesis may be one explanation for the limited number of studies that have been able to relate beta HPV seropositivity to beta HPV DNA presence in SCC tumors.[14,15]

Nearly all individuals are exposed to cutaneous HPV, thus it is possible that certain subsets of the population may be more susceptible to HPV-induced skin tumors. A GWAS recently identified a common MHCII variant associated with HPV8 seropositivity, suggesting that in some individuals HPV may more easily evade the immune system, enabling proliferation of infected pre-cancerous cells.[50] The possibility of genetically susceptible subgroups to HPV-induced skin cancer also was suggested in our earlier study of *EVER2/TMC8* (rs7208422) polymorphisms.[51]

Strengths and limitations of this study

We performed a comprehensive assessment of cutaneous HPV infection in relation to keratinocyte skin cancers. The strength of our study lies in the large number of histologically confirmed cases of invasive cutaneous SCC identified through a population-based surveillance network of dermatologists, dermopathologists, and pathologists. This population-based design is representative of the general population and less susceptible to selection bias than hospital and clinic-based studies. However, we cannot rule out the possibility that nonparticipation introduced selection bias or residual confounding might exist. Further, our study has the potential for lack of generalizability due to the fact that it is located at a higher latitude relative to other at-risk populations. Our study is based on serology, which is an indirect measure of infection. Using a case-control design, we collected blood for serological testing after cancer diagnosis. Therefore, we also must consider the idea that induction of HPV seroresponses may have occurred during the course of tumor development and thus represent recent rather than past infection. However, this possibility is unlikely, as prospective studies have provided evidence to support an association between HPV seropositivity at baseline and later skin carcinoma development.[13,16,17]

One limitation to our meta-analysis is that two of the studies that were included were done prior to the availability of multiplex serologic assays and used an ELISA-based HPV detection method.[32,38] It is possible that the different methodology may influence serology results, although no clear differences are apparent and both of these studies showed elevated SCC risk in relation to beta seropositivity.

Conclusions and Implications

Additional prospective and mechanistic studies are needed to elucidate the natural history and role of beta HPV infection in carcinogenesis. A recent meta-analysis of infection-related cancers estimated that ~2 million (16%) new cancer cases diagnosed worldwide in 2008 were attributable to infections, including HPV.[52] As keratinocyte skin cancers continue to be the most commonly diagnosed cancers and a growing problem worldwide, defining the role of HPV infection in these malignancies could have wide reaching economic and public health impacts.[53]

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APPENDIX

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