Multicenter Study of the Association between Betapapillomavirus Infection and Cutaneous Squamous Cell Carcinoma

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Abstract

Human papillomaviruses (betaPV) from the beta genus cannot be classified according to their oncogenicity due to a paucity of information. This study evaluates the association between betaPV infection and cutaneous squamous cell carcinoma in conjunction with measures of UV exposure and susceptibility. We performed case–control studies in the Netherlands, Italy, and Australia, countries with profoundly different UV exposures. The presence of 25 betaPV types in eyebrow hair follicles was determined using a highly sensitive HPV DNA genotyping assay, and antibodies for the 15 most prevalent betaPV types in a total of 689 squamous cell carcinoma cases and 845 controls were detected using multiplex serology. Multivariate logistic regression models were used for case–control comparisons and interaction analyses. BetaPV DNA was detected in eyebrow hairs of more than 90% of all participants. The presence of betaPV DNA was associated with an increased risk of squamous cell carcinoma in the Netherlands (OR = 2.8; 95% CI 1.3–5.8) and Italy (OR = 1.7; 95% CI 0.79–3.6), but not in Australia (OR = 0.91; 95% CI 0.53–1.6). Seropositivity for betaPV in controls ranged between 52% and 67%. A positive antibody response against 4 or more betaPV types was associated with squamous cell carcinoma in Australia (OR = 2.2; 95% CI 1.4–3.3), the Netherlands (OR = 2.0; 95% CI 1.2–3.4) and fair-skinned Italians (OR = 1.6, 95% CI 0.94–2.7). The association between UV susceptibility and squamous cell carcinoma was stronger in betaPV-seropositive people. These combined data support the hypothesis that betaPV may play a role in the development of cutaneous squamous cell carcinoma.

Cancer Res; 70(23); 9777–86. ©2010 AACR.

Introduction

Cutaneous squamous cell carcinoma is the second most common skin cancer after basal-cell carcinoma (1). Lifetime risks for cutaneous squamous cell carcinoma are estimated to be 7% to 11% in the United States and are even higher in Australia (2). Increasing age, male sex, fair complexion, and decreased immunity are host-related risk factors for squamous cell carcinoma, and exposure to sunlight is the dominant environmental risk factor (3).

Human papillomaviruses (HPV) can be classified on the basis of their tropism into either mucosal or cutaneous types...
(4). HPV16 and related mucosal types are common "carcinogenic" viruses and play a crucial role in the development of cervical and anogenital carcinomas (5, 6). Links between cutaneous HPV and skin cancer were first suspected in the rare genetic disease, epidermodysplasia verruciformis (EV). Patients with EV have diminished cell-mediated immunity, high susceptibility to infection with betapapillomavirus (betaPV) types (formerly called EV-associated HPV types) and develop keratotic skin lesions with a high rate of progression to squamous cell carcinoma (7, 8). Further evidence for a role of betaPV in cutaneous squamous cell carcinoma came from the observation that keratotic skin lesions in immunosuppressed patients following organ transplant are strongly associated with squamous cell carcinoma (9).

BetaPV infection can be determined by identifying viral DNA in human tissues. BetaPV DNA has been detected in a high proportion of cutaneous squamous cell carcinomas and their precursors, actinic keratoses, in organ-transplant recipients (10, 11) and immunocompetent people (12–15). Hair bulbs are thought to be the most likely reservoir of betaPV, and eyebrow hairs harbor persistent betaPV (16–18). The prevalence of betaPV infection in people without squamous cell carcinoma is now known to exceed 80% (19). Several studies have shown significant associations between the presence of betaPV DNA and squamous cell carcinoma or actinic keratoses (12, 18, 20, 21). The presence of betaPV evokes a serologic response (22) in a proportion of people infected (23). Little is known about the relation between betaPV infection and seroreactivity (24), but it is conceivable that detectable betaPV antibodies are indicators of an infection that is present at a higher viral load, perhaps due to local proliferation and inflammation of the skin (25). Several studies have shown associations between serologic responses to betaPV and squamous cell carcinoma or actinic keratoses (26–28). However, these studies have used variable approaches to antibody measures, have been underpowered, or have not included measures of betaPV DNA alongside the antibody measurement.

Unlike high-risk mucosal HPV, which can immortalize and transform cells and eventually cause cervical cancer (4), cutaneous betaPVs appear not to integrate into the cellular DNA and are thought to act by potentiating the harmful effects of UV radiation, for example by impairing DNA repair and apoptosis following UV-induced damage (29–33). Given the hypothesized biological action of betaPV, it is important to conduct studies in which interaction between UV and HPV can be assessed.

Highlighting the need for additional research in this area, the International Agency for Research on Cancer has recently found that it is not possible to classify HPV4 from the beta genus according to their oncogenicity, due to a paucity of evidence (34). The purpose of this study was therefore to provide additional, detailed evidence regarding a possible association between HPV infection and the development of cutaneous squamous cell carcinoma using state-of-the-art methodology for both betaPV DNA detection and betaPV serology in 3 large-scale epidemiologic studies in the context of varying skin phototypes and ambient UV levels.

Materials and Methods

Study design

Recruitment of participants for the case–control studies took place between 1998 and 1999 in the Netherlands (Leiden, 52°N), and between 2003 and 2005 in Italy (Rome, 42°N) and Australia (Brisbane, 27°S and Townsville, 19°S; refs. 19, 35). Individual studies were approved by local human research ethics committees and all participants gave their written informed consent.

Participants

The studies from the Netherlands and Italy were hospital based, with cases being recruited from hospital dermatology departments. The controls were selected from the out-patient department of ophthalmology in the Netherlands and the outpatient department of dermatology in Italy. In Australia, cases were selected from primary care skin cancer clinics and controls from the same clinics (21%), a population register (48%), or from community groups (31%; 35). All cases had a histologically confirmed diagnosis of primary squamous cell carcinoma, excluding in situ carcinoma (Bowen disease). In Italy and Australia, cases were newly diagnosed; in the Netherlands, the squamous cell carcinomas may have been diagnosed at any time previously. The response proportions in the cases were 83% in Australia and 80% in Italy; we did not keep track of the response proportions for cases in the Netherlands. Controls from all settings had no history of squamous cell carcinoma and were matched to the cases by age (within 5 years) and sex in Italy and Australia. The Dutch study had preceded the studies in Italy and Australia and had included cases with basal-cell carcinoma and malignant melanoma, as well as squamous cell carcinoma (26). Controls were matched in the Netherlands to all skin cancer cases irrespective of the type of skin cancer. In the current study only cases with squamous cell carcinoma were included, but we included all controls. As a result, the matching for age and sex was lost for the Dutch study since melanoma cases were younger and more often women. The response proportions in the controls were 60% in Australia and 81% in Italy, and were not monitored in the Netherlands.

Questionnaire

The questionnaire from the Dutch study was used and some additional questions were added to the questionnaires used in Italy and Australia. The questionnaires were used to gather the following information from participants in each country: sex and age; cigarette and alcohol consumption; skin type (36), ability to tan and sensitivity to sunburn (Italy and Australia only); and amount of spring and summertime weekday and weekend sun exposure during the longest held occupation.

Physical examination

All participants underwent full skin examinations and suspected skin cancers were biopsied for histologic diagnosis.

BetaPV detection and genotyping

BetaPV infection was assessed by the detection of betaPV DNA in eyebrow hairs. (37). Eight to ten eyebrow hairs were...
plucked from each participant and stored at −70°C until processing. All DNA analyses were performed blinded with respect to the case or control status of study participants. DNA purification was carried out with the guanidine-thiocyanate-diatom method (38) or with the QIAamp DNA Mini Kit (Qiagen GmbH) as described (39). In all samples, detection and betaPV genotyping were carried out with the skin (beta) HPV prototype research assay (Diassay BV) as described previously (19, 39).

**BetaPV serology**

Sera were stored at −20°C and shipped on dry ice to the German Cancer Research Center (DKFZ) in Heidelberg, where all serum analyses were performed in the same laboratory at the same time, blinded with respect to disease status. The presence of antibodies to the major capsid protein L1 of 15 betaPV types (i.e., HPV5, 8, 9, 15, 17, 20, 23, 24, 36, 38, 49, 75, 76, 92, and 93) was analyzed in a multiplex analyzer (Luminex) as previously described (22, 40). Concerning quality control measures, the gluthathione–casein-coupled bead sets were loaded with their respective antigens in 1 batch. Correct antigen loading was verified by using 28 reference sera with known HPV antibody patterns from 2 earlier studies. Study sera were analyzed once on 3 consecutive assay days. Every day, binding of gluthathione S-transferase (GST)–L1–tag fusion proteins to gluthathione–casein-coated beads was quantified by anti-tag monoclonal antibody. Anti-tag median fluorescence intensity (MFI) values for the first day varied less than 2-fold (range 7773–14904 MFI) and the inter-antigen coefficient of variation (CV) was 17.2%, indicating similar full-length L1 fusion protein density for the different HPV types. Antigen-specific ratios of anti-tag MFI for the second (mean ± SD, 0.99 ± 0.19) and third (0.99 ± 0.11) day revealed stable antigen binding to the beads throughout the 3 assay days. A quality control (QC) panel of 94 sera was included each day, resulting in 3 QC datasets to determine interday variation. Pearson correlation coefficients (R²) of raw MFI values for the individual antigens ranged from 0.742 to 0.999 (median 0.976) for day 2 versus day 1, and from 0.796 to 0.999 (median 0.971) for day 3 versus day 1. The raw data from days 2 and 3 for each antigen were divided by the slopes of the regression lines of the QC data pairs of days 1 and 2 or days 1 and 3, respectively, to correct for interday variation. The reference sera used for correct antigen loading were also pooled and included as standard on each plate; this was seropositive for all antigens except HPV-93. The interplate CV of the plate standard for the various antigens across all assay days ranged from 4.9% to 26.8%, with a median of 13.7%, indicating low plate-to-plate variation.

**Statistical analysis**

With respect to betaPV infection of hair follicles, participants were categorized according to whether or not DNA for any of 25 viruses was detected and according to the number of detected betaPV types. Similarly, participants were categorized depending on whether or not antibodies to any of 15 viruses were detected. When analyses were performed according to individual virus type, no one type emerged as being specifically associated with squamous cell carcinoma risk and there was a very high degree of colinearity, so these analyses are not displayed.

Fitzpatrick skin type and responses to the questions about tanning ability and sun reactivity were combined to create a measure of UV susceptibility, referred to as fair, medium, and dark/olive skin phototype as described previously (9). The amount of self-reported sun exposure occurring on weekdays and on weekend days were combined into a new variable called average daily sun exposure (35). For a combined country analysis, this was also multiplied by a country-specific erythemal UV index to enable analysis of UV exposure across all countries combined (35).

We used logistic regression models to calculate odds ratios as estimates of relative risk, adjusted for sex, age, and other potentially confounding factors. Possible interaction was calculated by including the product of the 2 potentially interacting factors into the logistic model. Analyses were stratified by country unless stated otherwise. Statistical analysis was performed with SPSS software (version 16.0, SPSS).

**Results**

**Participants and associations with known risk factors for squamous cell carcinoma**

A total of 689 cases and 845 controls were recruited across the 3 study sites: 155 and 278 from the Netherlands; 228 and 257 from Italy, and 306 and 310 from Australia. Fair skin, painful sunburns before the age of 20 years, and average daily sun exposure were associated with an increased risk of squamous cell carcinoma (Table 1).

**BetaPV infection and seropositivity are high in the 3 countries**

The prevalence of betaPV infection and seropositivity was generally high with the following proportions positive in control participants: 84% and 52% in the Dutch, 91% and 67% in the Italian, and 90% and 54% in the Australian populations, respectively (Table 2).

The prevalences of betaPV infection and antibodies for all betaPV types are presented for all 3 countries and for cases and controls separately in Fig. 1. DNA of HPV23 was most frequently detected in all 3 countries, whereas a serological response to HPV8 was the most frequent. In the Netherlands, DNA in eyebrow hairs and serum antibodies were detected more frequently in cases than in controls for almost all HPV types. In Australia, this was true for antibodies, but the prevalence of DNA was the same in cases and controls. Italy appeared to have an intermediate position.

Fig. 2 shows the cumulative number of betaPV infection and seroresponse detections for all betaPV types together per 100 participants. Italians harbored the highest cumulative number of betaPV infections, followed by Australian and Dutch participants. The difference between cases and controls was highest in the Netherlands and lowest in Australia. In contrast to the viral DNA measurements, there was a difference in the number of serologic responses between cases and controls in Australia. This difference was also

www.aacrjournals.org Cancer Res; 70(23) December 1, 2010 9779

Published OnlineFirst November 23, 2010; DOI: 10.1158/0008-5472.CAN-10-0352

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### Table 1. Baseline characteristics of the populations and associations with squamous cell carcinoma

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Netherlands</th>
<th>Italy</th>
<th>Australia</th>
<th>All countries combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>SCC</td>
<td>OR (^a)</td>
<td>Controls</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>155 (56)</td>
<td>55 (36)</td>
<td>b</td>
<td>97 (38)</td>
</tr>
<tr>
<td>Male</td>
<td>123 (44)</td>
<td>100 (64)</td>
<td></td>
<td>160 (62)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>64.4</td>
<td>67.6</td>
<td>b</td>
<td>69.6</td>
</tr>
<tr>
<td>25%–75%</td>
<td>57.3–70.3</td>
<td>61.2–71.9</td>
<td>b</td>
<td>63.4–76.0</td>
</tr>
<tr>
<td><strong>Skin phototyped(^c)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark/Olive</td>
<td>145 (52)</td>
<td>53 (34)</td>
<td>1</td>
<td>132 (51)</td>
</tr>
<tr>
<td>Medium</td>
<td>120 (43)</td>
<td>77 (50)</td>
<td>2.0 (1.3–3.1)</td>
<td>115 (45)</td>
</tr>
<tr>
<td>Fair</td>
<td>13 (5)</td>
<td>25 (16)</td>
<td>5.5 (2.6–11.8)</td>
<td>10 (4)</td>
</tr>
<tr>
<td><strong>Sunburns before the age of 20 years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>182 (66)</td>
<td>82 (53)</td>
<td>1</td>
<td>178 (69)</td>
</tr>
<tr>
<td>1–4</td>
<td>78 (28)</td>
<td>48 (31)</td>
<td>1.4 (0.86–2.2)</td>
<td>73 (28)</td>
</tr>
<tr>
<td>5 or more</td>
<td>18 (6)</td>
<td>24 (16)</td>
<td>3.5 (1.7–7.1)</td>
<td>6 (3)</td>
</tr>
<tr>
<td><strong>Average daily sun exposure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–3 h</td>
<td>166 (60)</td>
<td>82 (53)</td>
<td>1</td>
<td>157 (61)</td>
</tr>
<tr>
<td>4 or more hours</td>
<td>73 (47)</td>
<td>1.1 (0.74–1.7)</td>
<td>100 (39)</td>
<td>106 (47)</td>
</tr>
<tr>
<td><strong>Average daily sun exposure(^d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower tertile</td>
<td>e</td>
<td>e</td>
<td>e</td>
<td>e</td>
</tr>
<tr>
<td>Middle tertile</td>
<td>e</td>
<td>e</td>
<td>e</td>
<td>e</td>
</tr>
<tr>
<td>Upper tertile</td>
<td>384 (33)</td>
<td>289 (42)</td>
<td>1.2 (0.86–1.7)</td>
<td>275 (33)</td>
</tr>
</tbody>
</table>

\(^a\)The odds ratios are adjusted for sex and age and in the last column also for the 3 countries.

\(^b\)These odds ratios were not calculated because cases and controls were matched for sex and age.

\(^c\)In the Netherlands, Fitzpatrick-classified skin type was used to define individuals with dark/olive (skin type III or IV) and medium (skin type II) or fair (skin type I) skin phototype.

\(^d\)Average daily sun exposure multiplied by country specific UV index.

SCC, squamous cell carcinoma.
**Table 2. Association between markers of betapapillomavirus infection and squamous cell carcinoma**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Netherlands</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls&lt;sup&gt;a&lt;/sup&gt;</td>
<td>SCC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>OR&lt;sup&gt;b&lt;/sup&gt; (95% CI)</td>
<td>Controls&lt;sup&gt;a&lt;/sup&gt;</td>
<td>SCC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>OR&lt;sup&gt;b&lt;/sup&gt; (95% CI)</td>
<td>Controls&lt;sup&gt;a&lt;/sup&gt;</td>
<td>SCC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>OR&lt;sup&gt;b&lt;/sup&gt; (95% CI)</td>
</tr>
<tr>
<td>Presence of betaPV DNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (reference)</td>
<td>44 (16)</td>
<td>10 (7)</td>
<td>1</td>
<td>22 (9)</td>
<td>11 (5)</td>
<td>1</td>
<td>28 (10)</td>
<td>30 (10)</td>
<td>1</td>
</tr>
<tr>
<td>Positive</td>
<td>233 (84)</td>
<td>144 (93)</td>
<td>2.8 (1.3–5.8)</td>
<td>235 (91)</td>
<td>215 (95)</td>
<td>1.7 (0.79–3.6)</td>
<td>267 (90)</td>
<td>265 (90)</td>
<td>0.91 (0.53–1.6)</td>
</tr>
<tr>
<td>0 types (reference)</td>
<td>44 (16)</td>
<td>10 (7)</td>
<td>1</td>
<td>22 (9)</td>
<td>11 (5)</td>
<td>1</td>
<td>28 (10)</td>
<td>30 (10)</td>
<td>1</td>
</tr>
<tr>
<td>1–4 types</td>
<td>131 (47)</td>
<td>78 (50)</td>
<td>2.8 (1.3–6.1)</td>
<td>93 (36)</td>
<td>77 (34)</td>
<td>1.6 (0.72–3.5)</td>
<td>138 (47)</td>
<td>128 (43)</td>
<td>0.87 (0.49–1.5)</td>
</tr>
<tr>
<td>5 or more types</td>
<td>102 (37)</td>
<td>66 (43)</td>
<td>2.7 (1.3–5.9)</td>
<td>142 (55)</td>
<td>138 (61)</td>
<td>1.7 (0.80–3.7)</td>
<td>129 (43)</td>
<td>137 (47)</td>
<td>0.96 (0.54–1.7)</td>
</tr>
<tr>
<td>P-trend</td>
<td>0.022</td>
<td></td>
<td>0.378</td>
<td></td>
<td>0.794</td>
<td></td>
<td></td>
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<tr>
<td>Serologic response to betaPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Negative (reference)</td>
<td>132 (48)</td>
<td>60 (39)</td>
<td>1</td>
<td>84 (33)</td>
<td>62 (27)</td>
<td>1</td>
<td>128 (46)</td>
<td>86 (32)</td>
<td>1</td>
</tr>
<tr>
<td>Positive</td>
<td>143 (52)</td>
<td>93 (61)</td>
<td>1.4 (0.93–2.1)</td>
<td>172 (67)</td>
<td>164 (73)</td>
<td>1.2 (0.81–1.8)</td>
<td>148 (54)</td>
<td>180 (68)</td>
<td>1.8 (1.3–2.6)</td>
</tr>
<tr>
<td>0 Types (reference)</td>
<td>132 (48)</td>
<td>60 (39)</td>
<td>1</td>
<td>84 (33)</td>
<td>62 (27)</td>
<td>1</td>
<td>128 (46)</td>
<td>86 (32)</td>
<td>1</td>
</tr>
<tr>
<td>1–3 Types</td>
<td>94 (34)</td>
<td>45 (29)</td>
<td>1.1 (0.67–1.7)</td>
<td>102 (40)</td>
<td>87 (39)</td>
<td>1.1 (0.69–1.7)</td>
<td>81 (29)</td>
<td>81 (31)</td>
<td>1.5 (0.97–2.2)</td>
</tr>
<tr>
<td>4 or more types</td>
<td>49 (18)</td>
<td>48 (32)</td>
<td>2.0 (1.2–3.4)</td>
<td>70 (27)</td>
<td>77 (34)</td>
<td>1.4 (0.88–2.2)</td>
<td>67 (25)</td>
<td>99 (37)</td>
<td>2.2 (1.4–3.3)</td>
</tr>
<tr>
<td>P-trend</td>
<td>0.013</td>
<td></td>
<td>0.152</td>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>The number of controls and cases do not add up to the same number, because of some missing values.

<sup>b</sup>The odds ratios are adjusted for sex and age and in the last column also for average daily sun exposure and the three different countries.

SCC, squamous cell carcinoma.
observed in the Netherlands and to some extent in Italy, albeit to a lesser extent.

Of those infected (controls and cases combined), 87% were infected with 2 or more betaPV types, 53% with 5 or more types, and 10% with 10 or more types with a maximum of 17 different betaPV types in 1 eyebrow hair sample (1 patient). Simultaneous detection of antibodies to multiple betaPV was also frequent, with 71% of the betaPV-seropositive participants having antibodies against 2 or more types, 38% against 5 or more types, 16% against 10 or more types, and 0.3% against all 15 betaPV types. The number of simultaneously detected antibodies was associated with the number of betaPV types present in eyebrow hairs \((P < 0.001)\). Antibodies against HPV5, 8, 15, 20, 24, 38, 49, and 92 were statistically significantly associated with the presence of the same type in eyebrow hairs \((P < 0.05)\), whereas this association was not apparent for HPV 9, 17, 23, 36, 75, 76, and 93.

**BetaPV infection and seropositivity are associated with squamous cell carcinoma**

The prevalence of betaPV infection was significantly higher in cases than controls in the Netherlands (93% vs. 84%, observed in the Netherlands and to some extent in Italy, albeit to a lesser extent.

Of those infected (controls and cases combined), 87% were infected with 2 or more betaPV types, 53% with 5 or more types, and 10% with 10 or more types with a maximum of 17 different betaPV types in 1 eyebrow hair sample (1 patient). Simultaneous detection of antibodies to multiple betaPV was also frequent, with 71% of the betaPV-seropositive participants having antibodies against 2 or more types, 38% against 5 or more types, 16% against 10 or more types, and 0.3% against all 15 betaPV types. The number of simultaneously detected antibodies was associated with the number of betaPV types present in eyebrow hairs \((P < 0.001)\). Antibodies against HPV5, 8, 15, 20, 24, 38, 49, and 92 were statistically significantly associated with the presence of the same type in eyebrow hairs \((P < 0.05)\), whereas this association was not apparent for HPV 9, 17, 23, 36, 75, 76, and 93.

**BetaPV infection and seropositivity are associated with squamous cell carcinoma**

The prevalence of betaPV infection was significantly higher in cases than controls in the Netherlands (93% vs. 84%,
non-significantly higher in Italy (95% vs. 91%, \( P = 0.108 \)), and was not different in Australia (90% in both, \( P = 0.782 \); Table 2). In the Netherlands, the presence of betaPV infection was associated with an almost 3-fold increased risk of squamous cell carcinoma compared with no infection, after adjustment for sex and age (OR 2.8; 95% CI 1.3–5.8), but there was no significant association in Italy and Australia. There was also no evidence that risk increased with increasing number of betaPV types (Table 2).

A significant association between betaPV seropositivity and squamous cell carcinoma was found in Australia [(OR 1.8, 95% CI 1.3–2.6) (Table 2)]. Smaller and non-significant positive associations were found in the Netherlands (OR 1.4, 95% CI 0.93–2.1) and Italy (OR 1.2, 95% CI 0.81–1.8) overall, but they were stronger among people with fair or medium skin phototype (the Netherlands, OR 2.3, 95% CI 1.3–4.1; and Italy, OR 1.6, 95% CI 0.94–2.7).

**Discussion**

This international, multicenter epidemiologic study conducted at different latitudes and using the same highly sensitive and reproducible laboratory techniques in all centers supports the hypothesis that betaPV is associated with squamous cell carcinoma, regardless of latitude, and therefore of ambient UV radiation. There were significant associations between 1 of the 2 markers of betaPV infection tested and squamous cell carcinoma in the Netherlands and in Australia and an indication of a trend in Italy, but the strength of these associations differed across the 3 countries. In Australia an association with a positive antibody response to betaPV, but not with betaPV DNA in eyebrow hairs, was apparent whereas in the Netherlands the association with betaPV infection, as measured by the presence of betaPV DNA in eyebrow hairs, was strongest.

The reason for the lack of a consistent association between the presence of betaPV DNA or antibodies and squamous cell carcinoma across the 3 countries is unclear. It may be that differences in UV radiation of the susceptible skin cells alter any causal relation between betaPV and squamous cell carcinoma. Alternatively, this inconsistency might indicate that betaPV is not causally related to squamous cell carcinoma, and that the variability is due to methodologic differences or to other population differences. These issues are explored further below.

**Figure 2.** Cumulative numbers of 25 betaPV type-specific infections and seroresponses to 15 different betaPV types in controls and cases with squamous cell carcinoma in the Netherlands, Italy, and Australia ranked according to the frequency of occurrence of the individual betaPV types in all 3 countries. The numbers are expressed as number of participants with the specific infection per 100 participants tested and sum to more than 100 because many participants had multiple betaPV types in their eyebrow hairs or were seropositive to multiple betaPV types.
## Table 3. Association between skin phototype, sunburns before the age of 20 years, average daily sun exposure, and squamous cell carcinoma stratified by betapapillomavirus serostatus, categorized according to country

<table>
<thead>
<tr>
<th>Country</th>
<th>Risk factor</th>
<th>BetaPV seronegative</th>
<th>BetaPV seropositive</th>
<th>Test for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Controls n (%)</td>
<td>SCC n (%)</td>
<td>OR&lt;sup&gt;a&lt;/sup&gt; (95% CI)</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Skin phototype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dark/Olive</td>
<td>66 (50)</td>
<td>30 (50)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Medium/Fair</td>
<td>66 (50)</td>
<td>30 (50)</td>
<td>1.1 (0.57–2.0)</td>
</tr>
<tr>
<td>Italy</td>
<td>Skin phototype</td>
<td>39 (46)</td>
<td>25 (40)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Dark/Olive</td>
<td>45 (54)</td>
<td>37 (60)</td>
<td>1.3 (0.67–2.7)</td>
</tr>
<tr>
<td>Australia</td>
<td>Skin phototype</td>
<td>44 (35)</td>
<td>22 (26)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Dark/Olive</td>
<td>83 (65)</td>
<td>64 (74)</td>
<td>1.8 (0.96–3.4)</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Sunburns before the age of 20</td>
<td>82 (62)</td>
<td>37 (52)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1–4</td>
<td>40 (30)</td>
<td>16 (26)</td>
<td>0.89 (0.43–1.8)</td>
</tr>
<tr>
<td></td>
<td>5 or more</td>
<td>10 (8)</td>
<td>7 (12)</td>
<td>1.8 (0.59–5.6)</td>
</tr>
<tr>
<td>Italy</td>
<td>Sunburns before the age of 20</td>
<td>59 (70)</td>
<td>30 (48)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1–4</td>
<td>22 (26)</td>
<td>32 (52)</td>
<td>2.8 (1.4–5.8)</td>
</tr>
<tr>
<td></td>
<td>5 or more</td>
<td>3 (4)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td>Sunburns before the age of 20</td>
<td>31 (24)</td>
<td>17 (20)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1–4</td>
<td>63 (49)</td>
<td>42 (49)</td>
<td>1.4 (0.69–3.0)</td>
</tr>
<tr>
<td></td>
<td>5 or more</td>
<td>34 (27)</td>
<td>27 (31)</td>
<td>1.7 (0.76–3.7)</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Average daily sun exposure</td>
<td>74 (56)</td>
<td>32 (53)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1–3 h</td>
<td>58 (44)</td>
<td>28 (47)</td>
<td>1.0 (0.53–1.9)</td>
</tr>
<tr>
<td>Italy</td>
<td>Average daily sun exposure</td>
<td>56 (67)</td>
<td>30 (48)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1–3 h</td>
<td>28 (33)</td>
<td>32 (52)</td>
<td>2.1 (1.0–4.3)</td>
</tr>
<tr>
<td>Australia</td>
<td>Average daily sun exposure</td>
<td>113 (88)</td>
<td>63 (73)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1–3 h</td>
<td>15 (12)</td>
<td>23 (27)</td>
<td>2.6 (1.2–5.5)</td>
</tr>
</tbody>
</table>

<sup>a</sup>The odds ratios are adjusted for sex and age.
BetaPV DNA in eyebrow hairs has been used as an indicator of skin infection at the site of the lesion, and 2 studies have suggested that the former is indeed a reasonable proxy despite some degree of misclassification (37, 41). However, the highly sensitive laboratory test used may result in ascertainment of infections that are present at very low copy number or are only transient. In people exposed to very high levels of ambient UV, such as in Queensland, infection of this nature may not further increase the risk of squamous cell carcinoma. Antibodies might be a more precise measure of persistent infection that may be sufficiently pathogenic to potentiate the effect of UV, even in an environment with high levels of ambient solar UV. Alternatively, the presence of betaPV antibodies may indicate proliferation of betaPV infected cells in actinic keratoses, which occur more frequently in cases, or the development of the squamous cell carcinoma may lead to presentation of the betaPV antigen to the immune system, which could explain the observed association.

If the hypothesis that betaPV acts by potentiating the effects of UV radiation is correct, an interaction between indicators of UV exposure or skin susceptibility should be present. Our finding that the presence of antibodies to betaPV significantly modified the association between squamous cell carcinoma and skin phototype variously in all countries (and nonsignificantly with painful sunburns in 2 countries) is in agreement with this hypothesis and with findings recently described in a fair-skinned population living in New Hampshire, United States (43°N; 27°). The lack of effect modification in Italy in relation to sunburns may be due to the small proportion of participants with sun-sensitive skin type. The lack of interaction between the presence of antibodies to betaPV and measures of UV exposure is likely to be the result of the known poor recall of past sun exposure and the instrument used to assess exposure.

There was no single betaPV type that was preferentially associated with the development of squamous cell carcinoma. This finding is in stark contrast to the predominance of HPV16 in the pathogenesis of cervical carcinoma. The almost ubiquitous presence of betaPV infection in eyebrow hairs, together with very low amounts of betaPV DNA in the skin (42), raises questions about appropriate markers of pathogenic infection and how betaPV could contribute to the development of cutaneous squamous cell carcinoma (43–45). In contrast to HPV16 and HPV18, the viral load in squamous cell carcinoma seldom reaches the level of 1 viral copy per cell on average (42), indicating that betaPV are not involved in maintenance of the transformed state of the tumor cells. As the betaPV prevalence and loads in actinic keratoses have been reported to exceed those found in squamous cell carcinoma (42), betaPV may play a role earlier in carcinogenesis, possibly through a hit-and-run mechanism and/or by facilitating or enhancing carcinogenic processes that are initiated by sunlight or other environmental factors. This is in keeping with functional studies showing that betaPV abrogate UV-induced apoptosis, thus cooperating with the mutagenic properties of UV radiation (32, 33, 43, 44).

This is the largest and one of the most comprehensive studies of betaPV and cutaneous squamous cell carcinoma conducted to date. We have previously published results from the Dutch part of this study in which betaPV infection was ascertained using the different laboratory tests that were available at the time (20, 26). The current study enables a comparison of the association between betaPV and squamous cell carcinoma across 3 different countries with different UV exposure using the same state-of-the-art laboratory techniques. However, some site-specific requirements did result in small methodological differences that may contribute to the lack of consistency across centers. As well, measurement of sun exposure history across all studies was not optimal.

In conclusion, although sun exposure and sun sensitivity are the dominant risk factors for squamous cell carcinoma, our data provide additional evidence that betaPV infection might play an aetiopathological role. However, the lack of consistency across centers highlights the ongoing uncertainty in this area and the need for renewed efforts involving collaboration between epidemiologists and laboratory scientists to understand the mechanisms by which cutaneous PV potentially could contribute to skin carcinogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank the persons who participated in this study.

Grant Support

These studies were funded by an EC grant (QLK2-CT-2002-01179), with the Australian data collection funded by the National Health and Medical Research Council (Australia). MCVF was supported by a Clinical Fellowship from the Netherlands Organization for Health Research and Development (grant 907-00-150). REN is funded by a NHMRC (Australia) Career Development Award. TW was supported by the Peter und Traudl Engelhorn-Stiftung zur Förderung der Biotechnologie und Genteknik. We thank J. Lindeman and Labo Bio-Medical Products B.V. (Rijswijk, the Netherlands) for providing the RHA strips.

Received 02/02/2010; revised 09/23/2010; accepted 09/24/2010; published OnlineFirst 11/23/2010.

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www.aacrgournals.org Cancer Res; 70(23) December 1, 2010 9785

Published OnlineFirst November 23, 2010; DOI: 10.1158/0008-5472.CAN-10-0352
Bouwes Bavinck et al.


Multicenter Study of the Association between Betapapillomavirus Infection and Cutaneous Squamous Cell Carcinoma


Cancer Res 2010;70:9777-9786. Published OnlineFirst November 23, 2010.

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