Evidence for the Association of Human Papillomavirus Infection and Cutaneous Squamous Cell Carcinoma in Immunocompetent Individuals

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Objective: The aim of our study was to evaluate human papillomavirus (HPV) infection as a risk factor for cutaneous squamous cell carcinoma (SCC) in immunocompetent individuals.

Design: Hospital-based case-control study.

Setting: Referral center for dermatologic diseases for central and southern Italy.

Participants: Consecutive patients with histologically confirmed cutaneous SCC (n=46) and control subjects (n=84) chosen by frequency matching (age and sex) among patients admitted with unrelated diseases.

Main Outcome Measures: Infection with epidermodysplasia verruciformis–related HPV types, blindly assessed by serologic testing (viruslike particle enzyme-linked immunosorbent assay). Information was obtained on known potentially confounding risk factors (family history, history and signs of sun exposure, and pigmentary traits) and on history of HPV-related lesions and diseases, assessed by interview and examination by a dermatologist.

Results: Positive serologic findings for HPV type 8 were associated with SCC (odds ratio, 3.2; 95% confidence interval, 1.3-7.9) independently of other risk factors, whereas positive serologic findings for HPV type 15 were negatively associated with SCC (odds ratio, 0.4; 95% confidence interval, 0.2-0.9). Other variables significantly associated with the tumor were family history of skin cancer, professional or recreational sun exposure, light eye color, high number of solar keratoses and seborrheic keratoses on the body surface, and residency in radon-emitting buildings.

Conclusions: Positive serologic findings for HPV type 8 are associated with SCC occurrence in immunocompetent individuals. Viral infection could act as a cofactor in the tumor development, along with genetic predisposition, solar radiation, and other environmental exposures. If confirmed, these findings could open new perspectives for treatment and prevention of SCC.

Arch Dermatol. 2003;139:890-894

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†Dr Fuchs died July 10, 2002.
tion so far on the molecular mechanisms involved in the HPV-dependent skin carcinogenesis. As antibodies to HPV capsid antigens are reliable markers of past or present HPV infection, and seroepidemiologic methods have been successfully used in studies that linked HPV infections to both anogenital and cutaneous lesions, we designed this seroepidemiologic study with the aim to evaluate EV-HPV infection as a risk factor for the development of cutaneous SCC in immunocompetent individuals.

METHODS

SUBJECTS AND STUDY DESIGN

This case-control study was conducted in Rome at the Istituto Dermopatico dell’Immacolata, a referral dermatological center for central and southern Italy. The institutional ethical committee approved the study protocol before the study began.

Cases were subjects newly diagnosed as having histologically confirmed cutaneous invasive SCC in one surgical ward from October 1, 1999, to July 31, 2000, who gave their written informed consent to participate in the study. Control subjects were selected among patients with unrelated dermatologic conditions (mostly dermatitis, leg ulcers, alopecia, bullous diseases, and bacterial infections) admitted to 2 medical wards and were frequency matched to the cases by age and sex. Exclusion criteria for cases and controls were the presence of HPV-related skin lesions (warts and condylomas); history of SCC of the mucosa (cervix and head and neck); history or signs and symptoms of immunosuppression (including iatrogenic); and history of skin diseases for which UV radiation therapy was indicated, such as psoriasis or vitiligo, or sun avoidance was recommended, such as connective tissue diseases. Personal history of nonmelanoma skin cancer was an additional exclusion criterion for the controls.

A serum sample was obtained from each study subject, divided into aliquots, and stored at −20°C at Istituto Dermopatico dell’Immacolata until shipped in dry ice by air courier to Cologne, Germany, for testing.

With the use of a standardized questionnaire, detailed information was collected on demographic factors and lifestyle (smoking, sexual habits, and attendance sport facilities such as swimming pools); history of professional and recreational sun exposure; history of sunburns; history of HPV-related skin lesions (warts, condylomas); and personal and family history of any cancer.

A complete skin examination was performed by a dermatologist to assess pigmentary characteristics (ie, eye color), the presence of sunlight-induced skin lesions (ie, solar keratoses, solar lentigines, and solar elastosis), and the number of seborrheic keratoses.

LABORATORY METHODS

The serologic response to the major capsid protein L1 of EV-HPV type 8 (EV-HPV-8), -15, -36, and -23 was tested by enzyme-linked immunosorbent assay with the use of viruslike particles (VLPs). Generation of HPV-8, -15, -36, and -23 specific VLPs has been described previously.22,24 In case of HPV-8–, HPV-15–, and HPV-36–specific VLPs was identical to that described by Wieland and coworkers.24

For each serum sample, the optical density (OD) value of an antigen-free well was subtracted from the mean value of duplicate wells coated with VLPs. A positive serum sample with an OD of 1.0 was included on each plate.

Individual serum samples were scored as antibody positive or negative by using a cutoff OD value based on the distribution of OD values of 291 healthy blood donors, aged 18 to 65 years. After individual values exceeding the mean +3 SD were excluded, the remaining readings were averaged again and the cutoff point was chosen at the 90th percentile of the OD value distribution observed in the healthy donors, to maximize specificity.

STATISTICAL ANALYSIS

A power calculation was performed before the study began, and was based on the hypothesis that 20% of the controls and 50% of the cases would have been seropositive for HPV-8. Considering a power of 0.80 and α = .05, a sample composed of 35 cases and 70 controls should have been enrolled.

All statistical analyses were performed with the statistical packages SPSS for Windows, release 8.0.27 and Stata for Windows, release 4.0.28 Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for known risk factors (family history of skin cancer, light eye color, sunlight-related skin lesions), occupational and recreational sun exposure, history of sunburns, lifestyle-related habits (smoking and housing conditions, serologic findings for EV-HPV, and history of HPV-related diseases).

Unconditional logistic regression models were built to allow for multiple adjusting of confounding and thus to identify variables independently associated with SCC.

In all models, age was considered as a continuous variable. For categorical variables, point estimates were first computed for each category. Adjacent categories for which the calculated ORs were very similar were collapsed in a single category whenever possible, to increase robustness of the estimates. Variables that were measured on a continuous scale were categorized by dichotomizing or dividing in tertiles the distributions of exposed controls, and, where appropriate, adjacent categories were eventually collapsed, as described.

RESULTS

At the end of the study, questionnaires, clinical information, and corresponding serum samples were available for 46 cases and 84 controls. Of 51 clinically eligible case subjects, 2 refused to participate in the study, and in 3 cases the clinical diagnosis was not confirmed by histologic examination. For the enrolled cases, the location of SCC was the lip (n=13), other regions of the head (n=27), or other body areas (n=6). Of 137 eligible controls, 52 refused to participate in the study and 1 was erroneously not requested to donate serum samples.

The demographic characteristics of the study subjects are summarized in Table 1. As expected, cases were mainly elderly men (mean age, 72 years), and no differences in age and sex were observed between cases and controls, indicating that frequency matching on these 2 variables was successful. No differences were observed between cases and controls for place of residency.

The known risk factors for SCC (family history of skin cancer, sun exposure, and light eye color) and sun-
induced skin lesions, such as solar keratoses, were strongly associated with disease in our study population (Table 2). The risk of developing SCC was increased also for people with 10 or more seborrheic keratoses on body surfaces and for people who lived in radon-emitting tuff buildings.

Of the 4 EV-HPVs for which antibodies were sought in the serum samples of the study subjects (Table 3), HPV-8 and HPV-36 (members of subgroup A of EV-HPV) were associated with SCC, while HPV-15 and HPV-23 (subgroup B) were not, although the small number of patients positive for HPV-36 and HPV-23 did not allow any inference (Figure). Positive serologic findings for HPV-8 were also associated with male sex (OR, 3.3; 95% CI, 1.5-7.5).

In multivariate analysis, positive serologic findings for HPV-8 were confirmed to be associated with SCC, independently of age and sex, other known risk factors (sun exposure [as assessed by profession, history of exposure, and sunburns] and pigmented traits [as assessed by eye color]), and other variables of interest such as number of seborrheic warts and radon exposure.

Positive serologic findings for HPV-15 were shown to be negatively associated with SCC, and this was confirmed in multivariate analysis.

The main finding of our study in immunocompetent individuals was that subjects with positive serologic findings for HPV-8 had a 3-fold risk of cutaneous SCC (OR, 3.2; 95% CI, 1.3-7.9), independently of other risk factors. This association is in accordance with previous findings of HPV-specific DNA sequences in tumoral tissue and in eyebrow hairs of immunocompetent individuals with SCC. Two previous seroepidemiologic studies performed with the same method used in this study showed a high prevalence of antibodies against HPV-8 in patients with SCC (10 positive results in 14 patients compared with about 7% in the general German population) and an association of seropositivity for HPV-8 with the development of actinic keratoses.

Table 1. Demographic Characteristics of 46 Case Patients With Cutaneous Squamous Cell Carcinoma and 84 Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Cases, No. (%)</th>
<th>Controls, No. (%)</th>
<th>χ²</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33-63</td>
<td>12 (26.1)</td>
<td>27 (32.1)</td>
<td></td>
<td>.67</td>
</tr>
<tr>
<td>64-74</td>
<td>16 (34.8)</td>
<td>30 (35.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75-94</td>
<td>19 (39.1)</td>
<td>27 (32.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>14 (30.4)</td>
<td>33 (39.3)</td>
<td></td>
<td>.32</td>
</tr>
<tr>
<td>Male</td>
<td>32 (69.6)</td>
<td>51 (60.7)</td>
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<td></td>
</tr>
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</table>

Table 2. Odds Ratios of Squamous Cell Carcinoma by Known and Suspected Risk Factors

<table>
<thead>
<tr>
<th>Professional/recreational lifetime sun exposure</th>
<th>Cases, No. (%)</th>
<th>Controls, No. (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>8 (18.2)</td>
<td>33 (41.3)</td>
<td>1.0</td>
</tr>
<tr>
<td>High</td>
<td>36 (81.8)</td>
<td>47 (58.8)</td>
<td>3.2 (1.3-7.7)</td>
</tr>
<tr>
<td>Work</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoor</td>
<td>31 (70.5)</td>
<td>72 (91.1)</td>
<td>1.0</td>
</tr>
<tr>
<td>Outdoor</td>
<td>13 (29.5)</td>
<td>7 (8.9)</td>
<td>4.3 (1.6-11.9)</td>
</tr>
<tr>
<td>Lifetime sunburns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never, sometimes</td>
<td>28 (62.2)</td>
<td>72 (88.9)</td>
<td>1.0</td>
</tr>
<tr>
<td>Often</td>
<td>17 (37.8)</td>
<td>9 (11.1)</td>
<td>4.9 (1.9-12.2)</td>
</tr>
<tr>
<td>Actinic keratoses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>28 (65.1)</td>
<td>80 (98.8)</td>
<td>1.0</td>
</tr>
<tr>
<td>≥10</td>
<td>15 (34.9)</td>
<td>1 (1.2)</td>
<td>42.8 (5.4-339.2)</td>
</tr>
<tr>
<td>Seborrheic keratoses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>31 (70.5)</td>
<td>76 (93.8)</td>
<td>1.0</td>
</tr>
<tr>
<td>≥10</td>
<td>13 (29.5)</td>
<td>5 (6.2)</td>
<td>6.4 (2.1-19.4)</td>
</tr>
<tr>
<td>Eye color</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark</td>
<td>9 (19.6)</td>
<td>43 (53.1)</td>
<td>1.0</td>
</tr>
<tr>
<td>Green, hazel</td>
<td>15 (32.6)</td>
<td>24 (29.6)</td>
<td>1.0</td>
</tr>
<tr>
<td>Blue, gray</td>
<td>22 (47.8)</td>
<td>14 (17.3)</td>
<td>2.8 (1.2-5.8)</td>
</tr>
<tr>
<td>House building material</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other materials</td>
<td>23 (52.3)</td>
<td>63 (77.8)</td>
<td>1.0</td>
</tr>
<tr>
<td>Toff (radon emitting)</td>
<td>21 (47.7)</td>
<td>18 (22.2)</td>
<td>3.2 (1.4-7.0)</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>15 (33.3)</td>
<td>28 (34.6)</td>
<td>1.0</td>
</tr>
<tr>
<td>Ever</td>
<td>30 (66.7)</td>
<td>53 (65.4)</td>
<td>1.1 (0.5-2.3)</td>
</tr>
<tr>
<td>History of warts or condylomas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>35 (76.1)</td>
<td>67 (82.7)</td>
<td>1.0</td>
</tr>
<tr>
<td>Yes</td>
<td>11 (23.9)</td>
<td>14 (17.3)</td>
<td>1.5 (0.6-3.7)</td>
</tr>
<tr>
<td>Family history of nonmelanoma skin cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>35 (77.8)</td>
<td>80 (100.0)</td>
<td>1.0</td>
</tr>
<tr>
<td>Yes</td>
<td>10 (22.2)</td>
<td>0 (0.0)</td>
<td>47.6 (2.7-835.2)</td>
</tr>
</tbody>
</table>

Table 3. Odds Ratios of Cutaneous Squamous Cell Carcinoma by Serologic Testing for Human Papillomaviruses

<table>
<thead>
<tr>
<th>Serologic test</th>
<th>Cases, No. (%)</th>
<th>Controls, No. (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative HPV-8</td>
<td>20 (43.5)</td>
<td>57 (67.9)</td>
<td>1.0</td>
</tr>
<tr>
<td>Positive HPV-8</td>
<td>26 (56.5)</td>
<td>27 (32.1)</td>
<td>2.7 (1.3-5.8)</td>
</tr>
<tr>
<td>Negative HPV-15</td>
<td>30 (65.2)</td>
<td>44 (52.4)</td>
<td>1.0</td>
</tr>
<tr>
<td>Positive HPV-15</td>
<td>16 (34.8)</td>
<td>40 (47.6)</td>
<td>0.6 (0.3-1.2)</td>
</tr>
<tr>
<td>Negative HPV-23</td>
<td>39 (84.8)</td>
<td>74 (88.1)</td>
<td>1.0</td>
</tr>
<tr>
<td>Positive HPV-23</td>
<td>7 (15.2)</td>
<td>10 (11.9)</td>
<td>1.3 (0.5-3.8)</td>
</tr>
<tr>
<td>Negative HPV-36</td>
<td>37 (80.4)</td>
<td>77 (91.7)</td>
<td>1.0</td>
</tr>
<tr>
<td>Positive HPV-36</td>
<td>9 (19.6)</td>
<td>7 (8.3)</td>
<td>2.7 (0.9-7.7)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HPV, human papillomavirus; OR, odds ratio.
*Totals may vary because of missing values.
†Odds ratios are adjusted for age, sex, history of lifetime professional or recreational sun exposure, and eye color.
founded by differences in lifestyle. Instead, it seems plausible, suggests that the HPV-associated risk is not con-

troversial, but not with the other EV-HPV types we tested (even if they probably share similar modes of transmis-

sion), HPV-36, but not with the other EV-HPV types we tested.

risk was associated only with HPV-8 and possibly with

Our finding that an excess of solar keratoses was not significant (OR, 1.63; 95% CI, 0.57-4.69)

have been extensively discussed in previous articles by our group and others. As the VLP–enzyme-linked immuno-
sorbent assay is able to detect present as well as past infec-
tions, its validity does not depend on a precise sampling of the tissue, and no obvious cross-reactivity between different HPV types is known, we believed that this method could be particularly useful for the preliminary assessment of cancer risk. Because the viral genome is maintained and expressed in the lesions in most cervical SCC and EV skin cancers, further studies assessing the presence of viral DNA and RNA also in cutaneous SCC in immunocompetent individuals would be needed to prove that HPV-8 may be causally involved in skin malignancy. However, we do not know at present whether expression of the viral genome needs to be maintained or is necessary only in the early steps of malignant transformation in cutaneous carcinogenesis.

Moreover, epidermal proliferation limited to small cutaneous areas, such as nonmelanoma skin cancer, was not found sufficient to evoke anti–HPV-5 antibody formation as a marker of process linked to epidermal proliferation, as in conditions implying extensive reepithelialization. Similarly, it seems difficult to consider HPV-8 antibody formation simply as a marker of process in cutaneous carcinogenesis, especially in immunocompetent individuals.

The presence of 10 or more solar keratoses was strongly associated with the presence of the tumor (OR, 42.8; 95% CI, 5.4-339.2), and all 8 HPV-8–positive subjects with 10 or more solar keratoses had SCC. This result was not surprising, confirming that solar keratoses are to be considered an SCC “in situ,” undergoing a long precancerous phase before progressing to invasive carcinoma. However, association between HPV-8 positive serologic findings and presence of 10 or more solar keratoses was not significant (OR, 1.63; 95% CI, 0.57-4.69).

The strong association of seborrheic keratoses with SCC was an unexpected finding. We recorded this variable to verify a reported association with HPV infection and sun exposure. In fact, the presence and number of
terplay with other factors to which miners may be under-
derground miners exposed to high radon concentrations,
have not shown excess mortality from skin cancers in un-
might be a chance finding, since important cohort studies
environmental exposures.

of “high-risk” cutaneous HPV strains are associated with
ther studies are needed to determine whether a number
lar keratoses, family history of skin cancer, and presence
seborrhoeic warts (seborrheic keratoses was not associated with seropositiv-
ors. On the basis of these findings, a high number of
were the association between SCC and living in tuff build-
ably be regarded as a risk marker for SCC occurrence.
This study was presented in part as a poster at the World
Corresponding author and reprints: Cinzia Masini, MD,
leeds, UK. for Molecular Medicine, Cologne University, Cologne.

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