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Sunlight Exposure and (Sero)Prevalence of Epidermodysplasia Verruciformis-Associated Human Papillomavirus

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Ultraviolet radiation (UVR) is associated with an increased risk of squamous cell carcinoma (SCC), which is in part due to immunomodulation. In addition, human papilloma virus (HPV), especially the epidermodysplasia verruciformis (EV)-associated types, may be involved. In view of the capacity of UVR to impair host resistance to infections, we investigated the relationship between solar exposure and the prevalence of cutaneous HPV. In a case-control study on skin cancer (320 controls and 156 patients) a lifetime-retrospective questionnaire on sun exposure was administered. The presence of DNA of HPV types 5, 8, 15, 20, 24, and 38 in plucked eyebrow hair and type-specific seroreactivity were assessed and analyzed in relation to estimated exposure. Sunburn episodes in the past, especially at age 13–20 y, appeared to be associated with an enhanced risk of EV–HPV DNA positivity. In contrast, a higher lifetime sun exposure was associated with a lower risk of HPV infection. These results indicate that UVR at erythematogenic doses increases the risk of EV–HPV infection, possibly due to impaired host resistance to HPV and/or a direct effect of UVR on viral replication. The favorable association between lifetime sun exposure and HPV prevalence, however, underscores the enigmatic role of HPV in skin carcinogenesis.

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Human papillomaviruses (HPVs) are associated with both benign and malignant proliferative skin disorders (Boxman *et al*, 2000, 2001; Zur Hausen, 2000; Feltkamp *et al*, 2003; Struijk *et al*, 2003). A quarter of the HPV genotypes that are completely sequenced today belong to the so-called epidermodysplasia verruciformis (EV)-associated HPV types (De Villiers, 2001). These EV–HPV types have originally been found in skin lesions from patients with EV (Jablonska and Orth, 1985). EV patients develop multiple skin lesions on sun-exposed sites that often progress into squamous cell carcinoma (SCC). Recent studies indicate that EV–HPV types are not restricted to EV patients. Skin carcinomas in patients from the general population frequently contain EV–HPV (Pfister and Ter Schegget, 1997; Harwood and Proby, 2002). In immunosuppressed patients, such as renal transplant recipients, high incidences of skin carcinomas and keratotic skin lesions are found on sun-exposed sites in conjunction with a high percentage of EV–HPV DNA positivity, suggesting that these phenomena are associated with an underlying impairment of the immune system and may be causally linked with each other (Bouwes Bavinck *et al*, 1993; Shamanin *et al*, 1994; De Jong-Tieben *et al*, 1995; Berkhout *et al*, 2000).

It is known that exposure to solar ultraviolet radiation (UVR) plays a pivotal role in the genesis of pre-malignant skin lesions and non-melanoma skin cancer. A higher lifetime-cumulative sun exposure is an important risk factor, especially for the induction of SCC (Bouwes Bavinck *et al*, 1993; Kennedy *et al*, 2003). UVR appears to exert its carcinogenic effects by both mutagenic and immunomodulatory mechanisms. In the study by Fisher and Kripke, for example, it was found that pre-exposure of mice to UVR prevented the rejection of transplanted antigenic skin tumor cells (Fisher and Kripke, 1977; Kripke, 1982). UVR exposure resulted in the development of antigen-specific suppressor T cells and, as a consequence, the UVR-induced high susceptibility to these skin tumor cells was probably long lasting. In the human study by Yoshikawa *et al* (1990), it was found that a comparatively high percentage (92%) of skin cancer patients showed a suppressed contact hypersensitivity (CHS) response to 1-chloro-2,4-dinitrobenzene, which was applied epicutaneously, after exposure to UVR. The immunotoxic effects of UVR may have important consequences for the host resistance to a number of viral, bacterial, fungal, and parasitic infections. This was demonstrated in various animal studies (Sleijffers *et al*, 2002b). Immunotoxic effects of UVR, both skin associated and non-

Abbreviations: CHS, contact hypersensitivity; EV, epidermodysplasia verruciformis; HPV, human papillomavirus; OR, odds ratio; SCC, squamous cell carcinoma; UVR, ultraviolet radiation

This work was carried out at Leiden, Leiden University Medical Center, The Netherlands, and Bilthoven, National Institute of Public Health and the Environment (RIVM), The Netherlands.

skin associated, both at the site of irradiation and at a distant (i.e., non-irradiated) skin locus have been demonstrated in humans as well (Hersey *et al*, 1983; Cooper *et al*, 1992; Kelly *et al*, 1998; Duthie *et al*, 1999; Sleijffers *et al*, 2001). But the relevance for the susceptibility to infections in humans remains a matter of debate (Selgrade *et al*, 1997).

In view of the capacity of UVR to promote tumor genesis and impair host resistance to infections, and the high prevalence of HPV in non-melanoma skin cancer, it is likely that both UVR and HPV contribute to cutaneous malignancy. HPV may interfere with UVR-induced apoptosis, as has been described for the EV–HPV type 5 and appeared to be mediated by a p53-independent abrogation of the pro-apoptotic functions of Bak proteins (Jackson *et al*, 2000). Furthermore, UVR may increase the risk of HPV infection itself. One possible mechanism may be the direct effect of UVR on HPV replication, as has been described for HIV in human skin (Breuer-McHam *et al*, 2001). UVR responsiveness has been described for HPV77, and appears to be mediated by a p53-dependent stimulation of viral promoter activity (Purdie *et al*, 1999). In addition, enhanced viral replication in the epidermis may follow UVR-induced local or systemic immunosuppression, as has been described for herpes simplex virus (Taylor *et al*, 1994; Norval and El-Ghorr, 1998).

The aim of the study was to investigate the relationship between exposure to sunlight on the one hand and the presence of DNA of six EV–HPV types (HPV5, 8, 15, 20, 24, 38) in plucked eyebrow hair and the presence of antibodies against these HPV types on the other. This was done in the framework of an epidemiological study on risk factors for skin cancer. We hypothesized that a higher lifetime-cumulative sun exposure and sunburn episodes in the past are associated with a higher prevalence of EV–HPV DNA and/or a higher EV–HPV seroprevalence. This was examined for healthy controls and patients with SCC separately. The presence of SCC may confound the relationship between UVR exposure and HPV, as it is already known that SCC is associated with both a higher lifetime sun exposure and a higher prevalence of HPV.

A positive (i.e., unfavorable) association between sun exposure and prevalence of HPV, irrespective of the presence of SCC, would indicate that UVR exposure might have adverse effects on susceptibility to cutaneous HPV infections in humans. Furthermore, such an association between sun exposure and HPV infection would indicate that UVR might promote skin carcinogenesis through an infectious route, in addition to the known mutagenic and immunomodulatory pathways.

Results

Population characteristics (Table I) Characteristics of the study population are shown for controls and SCC cases separately in Table I. The mean age of controls was 59.4 y, which was statistically significantly lower than the mean age of 65.9 y in SCC patients. The percentage of males and participants with skin type I or II ("fair skin") was statistically significantly lower in controls than in SCC patients. Lifetime sun exposure was categorized according to the tertiles of the exposure distribution in the study population as a whole. The majority of control subjects fell in the low and intermediate exposure tertiles, whereas the majority of SCC patients fell in the intermediate and high tertiles. The reporting of painful sunburns in different age periods was more frequent in patients with SCC, especially at young ages (2–12 y). The majority of participants were infected with at least one of the EV–HPV types 5, 8, 15, 20, 24, or 38. Among participants with positivity for at least one EV–HPV DNA type, infection with multiple EV–HPV types was observed more frequently in SCC patients than in healthy controls (60.4% *vs* 44.8%, $p < 0.05$). With the exception of EV–HPV type 38, the prevalence of EV–HPV DNA positivity for these types in controls was significantly lower than the prevalence of positivity for the same type in SCC patients. The prevalence of anti-HPV antibodies against at least one of the EV–HPV types was low. The prevalence of seropositivity was higher in patients with SCC than in control participants. Statistical significance was reached for HPV type 8, 24, and 38 seropositivity.

EV–HPV DNA positivity (Table II) A higher age and a history of SCC were significantly and independently associated with a higher prevalence of EV–HPV DNA when evaluated for the study population as a whole (Table II). Male gender appeared to be borderline significantly associated with a higher EV–HPV prevalence ($p = 0.098$). In addition, lifetime sun exposure was negatively associated with EV–HPV prevalence. Exposure in the highest 33.3% category was significantly associated with a lower prevalence (OR = 0.41, 95% CI: 0.23–0.74). Lower exposure categories were associated with a higher prevalence, which means that an inverse relationship between lifetime sun exposure and HPV prevalence was found. This inverse association was not substantially modified after excluding the history of SCC from the model or when the analyses were performed for controls and SCC patients separately. A statistically significant inverse association between HPV prevalence and lifetime sun exposure was found among controls (Table II). Among patients with SCC, an inverse association was suggested with borderline statistical significance (overall test: $p = 0.092$) (Table II).

Contrary to the inverse association between lifetime sun exposure and the presence of EV–HPV DNA, painful sunburns appeared to be associated with a higher prevalence of EV–HPV DNA (Table II). The reporting of painful sunburns at age 13–19 y appeared to be significantly associated with a higher HPV prevalence when evaluated for the study population as a whole (OR = 1.54, $p = 0.047$). This association could also be established when restricting the analysis to control persons (OR = 1.74, $p = 0.04$), but was less and non-significant among participants with SCC (OR = 1.18, $p = 0.68$). Adding the reported painful sunburns by age period to the model did not substantially modify the found associations between lifetime-cumulative exposure to sunlight and EV–HPV DNA prevalence. Reporting of sunburn during the age period 40–59 y was non-significantly associated with a higher risk of EV–HPV DNA positivity (OR = 1.55, 95% CI: 0.76–3.19). As time at risk during this age period depended on the age at the time of study entry, and, as a consequence, a fair comparison is

Table I. Characteristics of study population according to the presence of SCC

	Controls N = 320	Patients with history of SCC N = 156
Age		
Mean (SD)	59.4 (10.7)	65.9 (8.4)***
5%–95% percentiles	41.2–74.7	48.4–76.2
Gender (N and %)		
Female	184 (57.5)	56 (35.9)***
Male	136 (42.5)	100 (64.1)
Skin type (N and %)		
III/ IV	173 (54.1)	53 (34.0)***
I/II	147 (45.9)	103 (66.0)
EV–HPV DNA positivity (N and %)		
Any type (5, 8, 15, 20, 24, or 38)	174 (54.4)	111 (71.2)**
Type 5	38 (11.9)	37 (23.7)**
Type 8	32 (10.0)	27 (17.4)*
Type 15	26 (8.1)	26 (16.8)**
Type 20	73 (22.8)	51 (32.7)*
Type 24	49 (15.3)	39 (25.0)*
Type 38	81 (25.3)	50 (32.3)
EV–HPV seropositivity (N and %)		
Any type (5, 8, 15, 20, 24, or 38)	39 (12.5)	30 (19.9)*
Type 5	1 (0.3)	2 (1.3)
Type 8	1 (0.3)	5 (3.2)*
Type 15	7 (2.2)	6 (3.9)
Type 20	7 (2.2)	7 (4.5)
Type 24	22 (6.9)	20 (12.8)*
Type 38	7 (2.2)	9 (5.8)*
Lifetime sun exposure (N and %)		
<26,642 h	124 (38.8)	31 (19.9)***,[a]
26,642–37,734 h	122 (35.0)	49 (31.4)
>37,734 h	84 (26.2)	76 (48.7)
Painful sunburns at different age periods (N and %)		
Age 2–5 y	10 (3.1)	11 (7.3)*
6–12 y	54 (14.1)	39 (25.3)**
13–19 y	94 (29.4)	52 (33.6)
20–39 y	97 (30.3)	55 (35.5)

***$p<0.0001$; **$p<0.01$; *$P<0.05$.
[a]One overall test procedure with χ^2 test for categorized data (df = 2).
SCC, squamous cell carcinoma; SD, standard deviation; EV, epidermodysplasia verruciformis; HPV, human papilloma virus.

hampered, findings for the reported sunburns during the age period 40–59 y were not included in Table II.

The multivariate analyses for the study group as a whole were performed for the different types of EV–HPV separately. A (borderline) significant association with the presence of SCC was found for HPV-5 (OR = 1.99, p = 0.02), HPV-15 (OR = 2.08, p = 0.03), and HPV-20 (OR = 1.58, p = 0.055). A significant association between the reporting of sunburn at age 13–19 y and HPV–DNA positivity was found for HPV-5 (OR = 1.88, p = 0.02) and HPV-15 (OR = 1.81, p = 0.06). No significant associations between sunburn at age 13–19 y and the other HPV types (HPV-8, HPV-20, HPV-24, HPV-38) could be established. With respect to the association with lifetime sun exposure, no single type stood out. Inverse associations with HPV-5, HPV-8, HPV-15, HPV-20, and HPV-38 were suggested, but did not reach the level of statistical significance (data not shown).

EV–HPV seropositivity (Table III) In 14.2% of the participants with EV–HPV DNA positivity for any of the above-mentioned types, EV–HPV seropositivity could be established. This percentage was not different from the percentage EV–HPV seropositivity in participants who were EV–HPV DNA negative for these types (15.8%, p = 0.64). When these correlations were calculated for the different EV–HPV types separately, significant correlations between EV–HPV DNA positivity and EV–HPV seropositivity could not be established either.

Fair skin type (I/II) was independently correlated with a higher EV–HPV seroprevalence (OR = 2.10, p = 0.01). This correlation was found especially among patients with SCC (OR = 3.45, p = 0.02). In patients with SCC, higher age and male gender were also borderline significantly associated with a higher EV–HPV seroprevalence (Table III). No significant association between lifetime sun exposure and EV–HPV seroprevalence could be established, either in the study group as a whole or in controls and participants with a history of SCC separately (Table III). No association was found when a history of SCC was excluded from the model for the study group as a whole. Furthermore, no significant associations between the reported sunburns by age period and EV–HPV seropositivity could be established. A borderline significantly higher EV–HPV seropositivity was found in participants with SCC who reported one or more painful sunburns during the age period 20–39 y (OR = 2.31, p = 0.056). Because of the small numbers, the analyses on EV–HPV seropositivity were not performed for the different EV–HPV types separately.

Discussion

In view of the known relationship between lifetime sun exposure and sunburns in the past and the occurrence of non-melanoma skin cancer, especially SCC, on the one hand, and the involvement of HPV in non-melanoma skin cancer on the other, we investigated whether parallel associations between solar exposure and current HPV infection (DNA positivity) or past HPV infection (presence of anti-HPV antibodies) might be established. Painful sunburns in the past, especially at age 13–19 y, appeared to be associated with an enhanced prevalence of EV–HPV DNA. This finding agrees with our hypothesis of a higher susceptibility to viral skin infections following exposure to solar UVR. Unexpectedly, a statistically significant inverse

Table II. Association between lifetime sun exposure, episodes of painful sunburn, and the prevalence of EV–HPV DNA (type 5, 8, 15, 20, 24, or 38)

	Controls and SCC patients (n = 476)			Controls only (n = 320)			SCC patients (n = 156)		
	OR	95% CI	p-Value	OR	95% CI	p-Value	OR	95% CI	p-Value
Multivariate model									
Age (1 y increase)	**1.03**	**1.01–1.05**	0.009	**1.04**	**1.01–1.06**	0.01	1.01	0.96–1.06	0.63
Gender (ref. = female)	1.40	0.94–2.08	0.098	1.50	0.94–2.40	0.092	1.16	0.54–2.49	0.70
Skin type I/II (ref. = III/IV)	0.97	0.66–1.42	0.86	1.04	0.66–1.64	0.87	0.89	0.41–1.91	0.76
Presence of SCC (ref. = no)	**1.96**	**1.25–3.07**	0.003	–	–		–	–	
Lifetime sun exposure									
> 37,734 h	**0.41**	**0.23–0.74**	0.013[a]	**0.45**	**0.22–0.92**	0.057[a]	0.42	0.14–1.27	0.092[a]
26,642–37,734 h	0.63	0.37–1.06		**0.54**	**0.30–0.98**		1.00	0.31–3.26	
< 26,642 h (= ref.)	1.00	–		1.00	–		1.00	–	
Painful sunburns at different age periods									
2–5 y (ref. = no)	1.63	0.60–4.44	0.34	1.96	0.48–7.98	0.35	1.31	0.31–5.48	0.71
6–12 y (ref. = no)	1.08	0.64–1.82	0.78	1.68	0.83–3.38	0.15	0.54	0.24–1.22	0.14
13–19 y (ref. = no)	**1.54**	**1.01–2.37**	0.047	**1.74**	**1.04–2.91**	0.04	1.18	0.54–2.60	0.68
20–39 y (ref. = no)	1.13	0.75–1.71	0.57	1.04	0.63–1.72	0.88	1.39	0.65–2.98	0.40

[a]One overall test procedure with Wald χ^2 (df = 2).
EV, epidermodysplasia verruciformis; HPV, human papilloma virus; SCC, squamous cell carcinoma; OR, odds ratio; CI, confidence interval.
Note: **Bold** indicates statistical difference ($p < 0.05$) as established either by 95%-C1 or by overall p value. Non Bold: non-significance ($p > 0.10$) or borderline significance (p value in range of 0.05–0.10).

association between lifetime sun exposure and EV–HPV DNA positivity was found in this analysis. No significant and consistent association between sunlight exposure and EV–HPV seropositivity could be established.

In this study, sunlight exposure and number of sunburns were assessed on the basis of self-reported information, which has probably introduced inaccuracy. Skin cancer patients may remember their exposure histories differently from healthy controls. But we analyzed the relationships between exposure and HPV infection for SCC cases and controls separately. In addition, consciousness of being HPV infected or not is unlikely, which makes that "information bias" is probably not relevant to our results. For this study, it may be argued that the inaccuracy of remembered sun exposure histories may have introduced a non-systematic error, which mostly results in attenuation of the observed associations.

Various human studies indicate that acute erythemagenic doses of UVR may adversely influence immune responses that follow the first contact with an antigen, either at the site of irradiation or at a distant non-irradiated skin site (Cooper *et al*, 1992; Duthie *et al*, 1999; Kelly *et al*, 2000; Termorshuizen *et al*, 2002). In addition, induction of antigen-specific tolerance following erythemagenic doses of UVR was demonstrated in humans (Cooper *et al*, 1992). In view of the found association between the immunosuppressive/-tolerogenic and erythemagenic effects of UVR in humans, we may argue that sunburn experienced shortly prior to or at the time of first infection with an HPV type may impair the host resistance to that virus (and probably also other viral infections) and result in a higher HPV prevalence in adulthood.

UVR may alter certain immune functions in humans at sub-erythemal doses as well (Gilmour *et al*, 1993; Kelly *et al*, 2000). The possible subtle effect of UVR at sub-erythemal doses on host resistance to infections in humans, however, may be overruled by many other factors associated with outdoor behavior. As a consequence, this effect may be difficult to establish in retrospective epidemiological studies, especially in those of a restricted nature in terms of size, exposure assessment, and adjustments for confounding variables (Termorshuizen *et al*, 2002).

Although epidemiological and experimental studies overwhelmingly implicate HPV as an important causal agent in cervical cancer, the causal link between HPV and skin carcinogenesis remains a matter of debate (Harwood and Proby, 2002). We cannot exclude the possibility that HPV is just "hitching a ride" (De Gruijl, 2002), and has no carcinogenic properties in itself. We found a high prevalence of EV–HPV DNA in healthy controls (54%), which indicates that HPV is ubiquitous. In the study of Antonsson *et al* (2000), HPV DNA was detected in at least one of the samples from five different body sites from 64 of 80 healthy humans (80%). Most of the HPV types detected in this study belonged to the EV-associated HPVs. In the same study (Antonsson *et al*, 2000), HPV prevalence was found to be higher on the forehead compared with other (UVR non-exposed) body sites. This suggests a role for UVR-induced local immunosuppression. Our data did not allow an analysis of HPV prevalence by different skin sites. Still, the

Table III. Association between lifetime sun exposure, episodes of painful sunburn, and the serorecognition of EV–HPV (type 5, 8, 15, 20, 24, or 38)

	Controls and SCC patients (n = 464)			Controls only (n = 313)			SCC patients (n = 151)		
	OR	95% CI	p-Value	OR	95% CI	p-Value	OR	95% CI	p-Value
Multivariate model									
Age (1 y increase)	1.02	0.99–1.05	0.27	1.00	0.96–1.04	0.91	1.06	0.99–1.12	0.08
Gender (ref. = female)	1.58	0.91–2.76	0.11	1.14	0.56–2.32	0.71	2.61	0.95–7.14	0.06
Skin type I/II (ref. = III/IV)	**2.10**	**1.18–3.65**	0.01	1.68	0.84–3.36	0.14	**3.45**	**1.18–10.0**	0.02
Presence of SCC (ref. = no)	1.25	0.70–2.22	0.45	–	–		–	–	
Lifetime sun exposure									
> 37,734 h	0.95	0.44–2.06	0.99[a]	1.45	0.50–4.16	0.77[a]	0.65	0.20–2.14	0.70[a]
26,642–37,734 h	0.95	0.46–1.96		1.10	0.44–2.73		0.91	0.26–3.24	
< 26,642 h (= ref.)	1.00	–		1.00	–		1.00	–	
Painful sunburns at different age periods									
2–5 y (ref. = no)	0.92	0.25–3.36	0.90	0.69	0.10–5.76	0.73	1.21	0.21–6.92	0.83
6–12 y (ref. = no)	1.41	0.75–2.65	0.29	1.18	0.46–3.01	0.73	2.06	0.81–5.24	0.13
13–19 y (ref. = no)	0.68	0.37–1.23	0.20	0.63	0.28–1.41	0.26	0.75	0.30–1.89	0.54
20–39 y (ref. = no)	1.29	0.75–2.23	0.36	0.80	0.37–1.73	0.58	2.31	0.98–5.44	0.056

[a]One overall test procedure with Wald χ^2 (df = 2).
EV, epidermodysplasia verruciformis; HPV, human papilloma virus; SCC, squamous cell carcinoma; OR, odds ratio; CI, confidence interval.
Note: **Bold** indicates statistical difference ($p < 0.05$) as established either by 95%-C1 or by overall p value. Non Bold: non-significance ($p > 0.10$) or borderline significance (p value in range of 0.05–0.10).

inverse association between lifetime-cumulative solar exposure and the prevalence of skin EV–HPV DNA throws doubt on the hypothetical causal role of these viruses in UVR-associated skin carcinogenesis.

The concomitantly positive (i.e., unfavorable) association with sunburn, however, indicates that the relationship between sunlight and human health is not one-dimensional and that exposure to sunlight may be associated with both favorable and adverse health consequences. Opposite associations between sun exposure and sunburn on the one hand and health outcome on the other have been described for cutaneous melanoma in adults (Kaskel *et al*, 2001; Kennedy *et al*, 2003) and respiratory tract symptoms in young children (Termorshuizen *et al*, 2002). Whether the favorable association between lifetime sun exposure and HPV infection is related to melanogenesis and increased thickness of the epidermis, a diminished exposure to microorganisms, or to the reported anti-proliferative and immunological effects of vitamin D remains to be established (Manolagas *et al*, 1985; De Gruijl, 1997; Kaskel *et al*, 2001). The idea that "light" or UVR may have a beneficial effect on immunity and infections in humans has a long history (Finsen, 1901; Barenberg *et al*, 1926; De Gruijl, 1997); however, further mechanistic studies, preferentially in human volunteers, are required to establish evidence for a possible favorable influence of sunlight, probably UVR over a certain range of low doses, on immunity and related infections.

The finding of a positive association between sunburn and HPV–DNA positivity may also be due to keratinocyte proliferation and associated activation of the HPV life cycle. In the study of Favre *et al* (2000), it was found that second-degree burns were associated with a short-term and probably transient generation of anti-HPV5 antibodies. Extensive re-epithelialization may account for the higher susceptibility to HPV infection or to a higher expression of HPV after a latently established infection, which may enhance the ability of HPV to induce humoral immune responses (Favre *et al*, 1998).

We could not establish significant associations between HPV seropositivity and sunlight exposure or sunburn. This may be explained by the fact that both estimates of UVR exposure relate to exposure in the past and antibodies to HPV develop transitionally, or probably often do not develop at all after HPV infection.

The discrepancy between virological and serological data indicates that current HPV infection and the presence of anti-HPV antibodies show no important association. In addition, suppressive effects of UVR on humoral immunity, both Th-1 and Th-2 associated, have been described, which further complicates interpretation of the results (Garssen *et al*, 1999; Sleijffers *et al*, 2002a). Erythemal doses of UVR may promote local HPV skin infection and, as a consequence, enhance the ability of HPV to induce humoral immune responses. On the other hand, UVR may concomitantly suppress serological responses. These possible opposite effects on serology associated with UVR exposure are probably hard to disentangle, especially in the observational design of this study.

In conclusion, the presented epidemiological data indicate that at sub-erythemal UVR doses no adverse effects on HPV infection can be detected. The higher occurrence of HPV

DNA found in association with sunburn is in accordance with other recent human studies that indicate adverse effects of UVR on immunity, infections, and skin tumor genesis, at least at doses that cause erythema. As no unequivocal relationships between solar exposure in the past and HPV infection could be established, the involvement of HPV in UVR-associated skin cancer remains enigmatic.

Materials and Methods

Study population This study was embedded in the "Leiden Skin Cancer Study" (LSS) that was initiated at the Leiden University Medical Center (LUMC) in 1997 as a case–control study on the causes of skin cancer in the Dutch population. The medical ethical committee approved the protocol, and all participants gave informed consent. The design of this study was described earlier (De Hertog *et al*, 2001). In short, 580 newly diagnosed cases from 1985 till 1997 with histologically confirmed skin cancer were studied; 161 persons out of these cases were diagnosed with SCC. Controls ($N = 386$) were recruited at the ophthalmology outpatient clinic. Controls were excluded when they had an intra-ocular melanoma or skin cancer in their history. Furthermore, both cases and controls were excluded when they were transplant recipients or suffered from hereditary skin disorders with an increased risk of skin cancer. Dark skinned persons (Fitzpatrick skin type classification $\geqslant$V) were also excluded, since they very rarely develop skin cancers. For this study, we selected persons for whom the (sero)prevalence of EV–HPV types 5, 8, 15, 20, 24, 38 in serum and in plucked hair was assessed, respectively (Feltkamp *et al*, 2003; Struijk *et al*, 2003). Three hundred and twenty controls and 156 patients with SCC were eligible for this analysis.

Data collection Participants were sent an invitation for a visit at the Dermatology outpatient clinic. Along with this invitation, a so-called Residence Work Calendar was sent. In this form, every change in residence or working environment during the lifetime had to be marked, and this was done to facilitate the assessment of sun exposure in the past during the interview at the outpatient clinic. The visit at the Dermatology outpatient clinic consisted of a standardized interview and a physical examination. Information on the propensity to burn rather than tan (skin type) and type and duration of sun exposure was collected during the interview using a standardized questionnaire (which can be obtained from Dr Bouwes Bavinck). Hours spent outdoors were recorded for working and non-working days between 09:00 and 16:00 hours in the months May–September. The whole year was taken into account when people had lived near the equator. Sunburns were asked for by different age periods that corresponded with infancy (2–5 y), primary school (6–12 y), secondary school (13–19 y), and adulthood (20–39 y).

Assessment of EV–HPV DNA in plucked hair DNA was isolated from 8–10 plucked eyebrow hairs collected per patient, as described (Boxman *et al*, 2000). Within each preparation, EV–HPV DNA was detected by specific PCR for EV–HPV types 5, 8, 15, 20, 24, and 38 with primers located in the viral E7 gene using amplitaq Gold DNA polymerase (Applied Biosystems, Branchburg, NJ), as recently described (Struijk *et al*, 2003). The DNA quality of each sample was ascertained by PCR amplification of the household gene Myb (Boxman *et al*, 1997).

Assessment of anti-EV–HPV antibodies The detection of EV–HPV-reactive IgG antibodies in patient sera was performed by ELISA, as described (Feltkamp *et al*, 2003). For this purpose, L1 virus-like particles of EV–HPV types 5, 8, 15, 20, 24, and 38 were expressed, purified, and coated to Polysorp microtiter plates (Nunc). Patient sera were tested in a 1:100 dilution, and optical densities were measured and corrected, as described. Calculations of the EV–HPV type specific ELISA cut-off values above which a serum is called seropositive were based on the mean reactivity plus $3 \times$ SD of a group of 100 randomly selected anonymous LUMC employees, excluding outliers.

Statistical analysis Univariate analyses were performed using the non-parametric one-way Wilcoxon test for continuous data and the χ^2 test for categorized data. The presence of EV–HPV DNA and anti-EV–HPV antibodies in relation to sunlight exposure was also analyzed in multivariate logistic regression models, in which adjustments were made for age, gender, skin type, and the presence of SCC. All calculations were performed with SAS statistical software, version 8.0 for Windows. The statistical contribution of a categorized variable with more than two levels was evaluated by individual odds ratios (ORs) and associated 95% confidence intervals (CIs) and by one overall-test procedure (Wald χ^2 test).

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