Objective: To examine prospectively the relationship among sun exposure, Betapapillomavirus, and development of actinic keratoses.

Design: Prospective, community-based cohort study.

Setting: Township of Nambour in Southeast Queensland, Australia.

Participants: A total of 291 randomly selected adults aged 36 to 86 years with the presence or absence of Betapapillomavirus DNA in eyebrow hair follicle cells known at baseline in August 1996 and with subsequently documented sun exposure histories.

Main Outcome Measures: Prevalence of actinic keratoses in March 2003 after 7 years of follow-up.

Results: Beyond the known determinants of multiple actinic keratoses, namely, advanced age, male sex, fair skin, and lifetime occupational sun exposure, Betapapillomavirus infection was associated with having more than 10 actinic keratoses (odds ratio, 1.8; 95% confidence interval, 0.7-4.4). However, Betapapillomavirus positivity led to a significant 13-fold increase in the risk of actinic keratoses among those 60 years or older, a nearly 6-fold increase in risk when combined with fair skin color, and a doubling in risk of actinic keratoses when combined with high sun exposure, recent or cumulative, compared with those who had neither Betapapillomavirus infection nor the respective risk factor of interest.

Conclusions: Although the presence of Betapapillomavirus DNA in eyebrow hair follicle cells had only a small independent association with actinic keratoses, Betapapillomavirus infection in combination with key risk factors increased the risk of actinic keratoses, which is consistent with a potentiation by Betapapillomavirus of the effect of established causal factors.

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CTINIC KERATOSES (AKS) are sun-induced skin tumors that are strongly associated with squamous cell carcinomas (SCCs). Prevalence of AKs varies with latitude and skin type. For example, in England the prevalence of AKs is approximately 15% for men and 6% for women, whereas the prevalence in people of the same ancestry in subtropical Australia is approximately 40%. There is increasing evidence from epidemiological and molecular studies that Betapapillomavirus may play a role in the cause of AKs in addition to solar radiation.

Among more than 100 known human papillomavirus (HPV) types, a group of approximately 16 types were classified as epidermodysplasia verruciformis (EV)–HPV types because they were known to infect individuals with EV, who are predisposed to develop warts and skin tumors. Recently, these EV-HPV types and phylogenetically related HPV types were reclassified as the Betapapillomavirus genus. Betapapillomavirus types have been detected not only in SCCs, but also in basal cell carcinomas, and AKs but also in hair follicle cells and healthy skin.

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The association between papillomaviruses and epidermal tumors was established originally in studies of rabbit SCCs in the 1950s. Early human studies of HPV and skin tumor development began in patients with EV, widened to immunosuppressed patients undergoing organ transplantation, who are at high risk of skin cancer, and more recently broadened.
to include healthy populations. All these studies\textsuperscript{6,14,20,21} have provided support for a positive association between \textit{Betapapillomavirus} and skin tumorigenesis. Viral load was found to be highest in AKs rather than SCCs,\textsuperscript{12} indicating that studies of the association between \textit{Betapapillomavirus} and AKs are a legitimate source of data in examining tumorigenesis.

Studies\textsuperscript{7} of this association have relied on various indicators of viral infection, including direct detection of \textit{Betapapillomavirus} DNA with nested polymerase chain reaction primers in skin specimens, detection of \textit{Betapapillomavirus} DNA in hair follicle cells, \textit{Betapapillomavirus} DNA detection from skin swabs (could also indicate surface contamination), and determination of seroreactivity to \textit{Betapapillomavirus} types. The exact role of \textit{Betapapillomavirus} in skin tumor development cannot be determined from any of these laboratory methods in isolation; they need to be combined with epidemiologic studies for further etiological insight. All such studies to date have been cross-sectional, however, so the precise relationship among sun exposure as the chief cause of skin cancers, cutaneous HPV infection, and skin tumors in healthy individuals remains unclear. Indeed, the role of HPV infection remains much more controversial than that of sun exposure, with suggestions that the activity of HPV either decreases,\textsuperscript{22} increases\textsuperscript{5,8} or has no influence\textsuperscript{23,24} on the effect of high UV radiation exposure.

The primary aim of this study was to investigate whether \textit{Betapapillomavirus} influences the subsequent development of AKs independently or in combination with sun exposure. We have therefore performed a prospective study to assess whether \textit{Betapapillomavirus} infection of hair follicle cells was associated with subsequent development of AKs. If it was associated, we aimed to assess in more detail the relation of \textit{Betapapillomavirus} to AKs, taking into account other known risk factors.

\section*{METHODS}

The participants comprised a subset of the ongoing Nambour Skin Cancer Study, which has been described in detail previously\textsuperscript{5,23} (\textbf{Figure}). Briefly, participants aged 20 to 69 years were randomly selected from residents of Nambour, a subtropical Australian township (latitude 26°S), in 1986 and invited to complete a skin cancer prevalence survey in 1986.\textsuperscript{4} In 1992 they participated in a 5-year field trial of skin cancer prevention, which entailed full-body skin examinations by specialist dermatologists in February 1992, August 1994, and August 1996, including site-specific counts of all AKs.\textsuperscript{26} Participants gave written informed consent before taking part in skin examinations and collection of biological samples. Ethical approval for all aspects of the study was obtained through Bancroft Centre Ethics Committee, Queensland Institute of Medical Research.

\section*{BASELINE DATA}

An unselected half of the 1250 trial participants who underwent skin examinations were invited to take part in a study of HPV and skin cancer in August 1996.\textsuperscript{5} Eyebrow hair samples were collected from 507 participants, and samples were analyzed for the presence of \textit{Betapapillomavirus} types 5, 8, 9, 12, 14, 15, 17, 19 through 25, 36 through 38, and 47 using a nested polymerase chain reaction to detect \textit{Betapapillomavirus} DNA.\textsuperscript{3} A participant was considered to be positive for HPV if DNA from any of these viruses was detected and negative otherwise. The \textit{Betapapillomavirus} detection was performed according to methods described in detail by Boxman et al\textsuperscript{13} and Berkhout et al.\textsuperscript{27} The polymerase chain reaction primers used were designed to detect all EV-HPV types (now \textit{Betapapillomavirus} types) known in 1995 at low viral DNA copy numbers.\textsuperscript{25} In the current study population, no negative controls were positive for EV-HPV DNA.\textsuperscript{3}

Information about skin color (fair, medium, or olive), skin response to short-term sun exposure (always burn, never tan; burn, then tan; or tan only), and lifetime sun exposure was obtained from questionnaires administered during the Nambour Skin Cancer Study.\textsuperscript{26,30} Sun exposure history included the proportion of time spent outdoors or in the sun each day during childhood and teenage years, lifetime occupational sun exposure, and daily sun exposure in a typical week (hardly ever [up to 1 hour], less than 50% of the time [more than 1 hour and up to 4 hours], or more than 50% of the time [more than 4 hours]) for each day of the week (separate average daily values were used for weekdays and weekend days). Baseline counts of AKs on the face, forearms, and hands by survey dermatologists in August 1996 were also used in the present analysis.

\section*{FOLLOW-UP}

Of the 507 participants with known \textit{Betapapillomavirus} infection status in August 1996, those who had consented to long-term follow-up were invited to take part in a follow-up study in March 2003. A single dermatologically trained clinician with several years of experience (P.M.) counted all AKs
RESULTS

Of the original 507 participants in the baseline Betapapillomavirus study in 1996, 359 participants were eligible for participation in the present follow-up study. The 148 participants not eligible for this study included 96 participants who had withdrawn completely from the Nambour Skin Cancer Study, 40 participants who consented only to tracking of their skin cancers through medical records, and 12 who had died. Approximately 60% of those who gave no consent for follow-up were men (mean age, 65 years). In most cases no reason was given, but of those with a reason, illness and geographical distance were the most common ones cited. Of the eligible participants, 10 refused to participate, 13 were unable to attend on the data collection dates, 4 had died since the last date of confirmation of their participation status, and 41 no longer lived in the region. Thus, 291 (81.1%) of the 359 eligible participants were successfully examined in 2003.

AGE, SEX, AND SKIN PHENOTYPE

Participants were divided into 2 age categories (36-59 years and ≥60 years) based on the categories used previously and collapsed to allow for the cohort's aging. The only significant difference between the characteristics of the 507 participants whose HPV status was measured in 1996 and those who were followed up in 2003 was that the 291 participants seen in 2003 were almost 4 years younger on average (P = .002) than the 216 who were not included (Table 1). Despite this preponderance of younger participants in 2003, the average age of those examined was 5 years older than the average age of the sample in 1996 (7 years earlier). The proportions of Betapapillomavirus DNA–positive participants were identical in those followed up and those not followed up (Table 1).

There were almost equal proportions of men and women (Table 1). Approximately half the participants reported fair skin color, and only 9.0% described their skin color as olive. More than two thirds reported that their skin burned, then tanned, after short-term sun exposure (Table 1). Almost half had an indoor occupation, and only 19.9% worked mostly outdoors (Table 1).

RISK FACTORS FOR AKs

When examining independent risk factors for AKs, AK counts were grouped into 3 categories as previously. A total of 179 participants (112 with Betapapillomavirus and 67 without) had no AKs, 71 (29 with Betapapillomavirus and 42 without) had 1 to 10 AKs, and 41 (14 with Betapapillomavirus and 27 without) had more than 10 AKs. Interviews with the primary physicians of the 24 participants who showed a decrease of more than 4 AKs between 1996 and 2003 revealed that 4 of these participants had more than 10 AKs treated in the 6 months before the 2003 skin examination, which led to their reclassification into the highest category of AK.

Age was the strongest predictor of AK: age older than 60 years increased the odds of having 1 to 10 AKs by 9 times (OR, 9.0; 95% confidence interval [CI], 4.6-17.4) and increased the odds of having more than 10 AKs by almost 26 times (OR, 25.6; 95% CI, 9.7-67.7) compared with those with no AKs. There was a positive relationship with male sex and fair skin.

Most people in the study reported being in the sun less than 50% of the time in childhood and as a teenager, and no relationship was found between early-life sun exposure and prevalent AK. High cumulative sun ex-
posure from occupation or leisure increased the risk of prevalent AK, but the associations were not statistically significant after adjustment for age and sex (P=.15 for high occupational exposure and P=.15 for moderate leisure exposure). No effect of smoking on prevalent AK was found.

There was no independent association of AK prevalence in 2003 with Betapapillomavirus infection in 1996 (OR, 1.4; 95% CI, 0.7-2.7; for 1-10 AKs), although there was a nonsignificant association (P=.17) between Betapapillomavirus and the presence of more than 10 AKs (OR, 1.8; 95% CI, 0.7-4.4). A further logistic regression analysis was performed, including only those 149 participants who had no AKs at baseline in 1996. The odds of developing AKs in the Betapapillomavirus-positive group were similar to those observed overall, but the precision of estimated odds of effect was much lower. When change in individual AK counts was examined, an overall decrease in AK counts occurred in this population between 1996 and 2003, but the decrease was 11.0% greater in those without detectable Betapapillomavirus DNA in eyebrow hair follicles compared with those with detectable Betapapillomavirus DNA in 1996.

**JOINT EFFECT OF BETAPAPILLOMAVIRUS INFECTION AND OTHER RISK FACTORS FOR AKs**

To maximize the statistical power of the analysis of the joint effects of Betapapillomavirus infection and other risk factors, the presence or absence of AKs was used rather than several categories of AK counts. A stratified analysis according to Betapapillomavirus positivity or negativity and the main risk factors for AK, namely, age, sex, skin color, and propensity to sunburn, revealed substantially higher odds of AKs if another risk factor was combined with Betapapillomavirus positivity (Table 2). The subset of participants with advanced age and Betapapillomavirus positivity had double the odds of AKs compared with their Betapapillomavirus-negative counterparts. In men with Betapapillomavirus infection, the odds of having AKs were almost 3. In the fair-skinned subgroup with Betapapillomavirus infection, the odds of AKs were even higher at almost 6 (Table 2). Similarly, Betapapillomavirus infection and high occupational sun exposure doubled the odds of having AKs in 2003 (Table 3), whereas weekday sun exposure in 2002 in combination with Betapapillomavirus positivity doubled the odds of AKs in 2003 (OR, 2.2; 95% CI, 1.0-5.0).

### Table 2. Joint Effect of Betapapillomavirus and Age, Sex, and Skin Phenotype

<table>
<thead>
<tr>
<th>Characteristic and Betapapillomavirus Status</th>
<th>AKs in 2003, No. (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group, y &amp; Moderate or high occupational exposure in 2002 in combination with Betapapillomavirus infection</td>
<td>None (n=178)</td>
<td>≥1 (n=113)</td>
</tr>
<tr>
<td>Negative (n=112)</td>
<td>92 (82.1)</td>
<td>20 (17.9)</td>
</tr>
<tr>
<td>Positive (n=56)</td>
<td>45 (80.4)</td>
<td>11 (19.6)</td>
</tr>
<tr>
<td>Negative (n=43)</td>
<td>19 (44.2)</td>
<td>24 (55.8)</td>
</tr>
<tr>
<td>Positive (n=60)</td>
<td>22 (27.5)</td>
<td>59 (72.5)</td>
</tr>
<tr>
<td>Sex &amp; High occupational sun exposure</td>
<td>Female (n=79)</td>
<td>57 (72.2)</td>
</tr>
<tr>
<td>Positive (n=72)</td>
<td>41 (56.9)</td>
<td>31 (43.1)</td>
</tr>
<tr>
<td>Male (n=76)</td>
<td>54 (71.1)</td>
<td>22 (28.9)</td>
</tr>
<tr>
<td>Positive (n=64)</td>
<td>26 (40.6)</td>
<td>38 (59.4)</td>
</tr>
<tr>
<td>Skin color &amp; Presence of more than 10 AKs</td>
<td>Olive/medium (n=83)</td>
<td>52 (62.6)</td>
</tr>
<tr>
<td>Negative (n=69)</td>
<td>29 (42.0)</td>
<td>40 (58.0)</td>
</tr>
<tr>
<td>Positive (n=67)</td>
<td>38 (56.7)</td>
<td>29 (43.3)</td>
</tr>
<tr>
<td>Fair &amp; High occupational sun exposure</td>
<td>Negative (n=72)</td>
<td>59 (81.9)</td>
</tr>
<tr>
<td>Positive (n=67)</td>
<td>38 (56.7)</td>
<td>29 (43.3)</td>
</tr>
<tr>
<td>Tendency to sunburn &amp; Always burn</td>
<td>Negative (n=79)</td>
<td>57 (72.2)</td>
</tr>
<tr>
<td>Positive (n=72)</td>
<td>41 (56.9)</td>
<td>31 (43.1)</td>
</tr>
<tr>
<td>Tan or burn, then tan</td>
<td>Negative (n=29)</td>
<td>19 (65.5)</td>
</tr>
<tr>
<td>Positive (n=24)</td>
<td>10 (41.7)</td>
<td>14 (58.3)</td>
</tr>
<tr>
<td>Always burn &amp; High occupational sun exposure</td>
<td>Negative (n=126)</td>
<td>92 (73.0)</td>
</tr>
<tr>
<td>Positive (n=112)</td>
<td>57 (50.9)</td>
<td>55 (49.1)</td>
</tr>
</tbody>
</table>

Abbreviations: AKs, actinic keratoses; CI, confidence interval; OR, odds ratio.

*Adjusted for age and sex.

### Table 3. Association of the Presence or Absence of AKs in 2003 With Betapapillomavirus Status and Occupational Sun Exposure and Sun Exposure in 2002

<table>
<thead>
<tr>
<th>Characteristic and Betapapillomavirus Status</th>
<th>AKs in 2003, No. (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative sun exposure &amp; Low cumulative sun exposure</td>
<td>None (n=178)</td>
<td>≥1 (n=113)</td>
</tr>
<tr>
<td>Negative (n=74)</td>
<td>56 (75.7)</td>
<td>18 (24.3)</td>
</tr>
<tr>
<td>Positive (n=52)</td>
<td>34 (65.4)</td>
<td>18 (34.6)</td>
</tr>
<tr>
<td>High cumulative sun exposure</td>
<td>Negative (n=81)</td>
<td>55 (67.9)</td>
</tr>
<tr>
<td>Positive (n=84)</td>
<td>33 (39.3)</td>
<td>51 (60.7)</td>
</tr>
<tr>
<td>Weekday sun exposure in 2002 &amp; Low weekday sun exposure in 2002</td>
<td>None (n=177)</td>
<td>≥1 (n=110)</td>
</tr>
<tr>
<td>Negative (n=85)</td>
<td>62 (72.9)</td>
<td>23 (27.1)</td>
</tr>
<tr>
<td>Positive (n=59)</td>
<td>39 (66.1)</td>
<td>20 (33.9)</td>
</tr>
<tr>
<td>High weekday sun exposure</td>
<td>Negative (n=70)</td>
<td>49 (70.0)</td>
</tr>
<tr>
<td>Positive (n=73)</td>
<td>27 (37.0)</td>
<td>46 (63.0)</td>
</tr>
</tbody>
</table>

Abbreviations: AKs, actinic keratoses; CI, confidence interval; OR, odds ratio.

*Adjusted for age and sex.

### COMMENT

This study is a novel prospective study of the association of Betapapillomavirus and the development of AKs. Previous studies have relied on tissue samples obtained at the same time as AK counts in an attempt to investigate the longitudinal association of Betapapillomavirus and AK (and other skin tumors).

Advanced age, male sex, and fair skin that burned easily were significant predictors of moderate or high num-
bers of AKs. Lifelong outdoor occupation increased the odds of having prevalent AKs but not significantly \((P = .15)\). These results confirm known risk factors for AKs.\(^1,3,5,38,39\) We sought to investigate if Beta
apillomavirus DNA detected in eyebrow hair follicle cells had any independent influence on the subsequent development of AKs, and if so, whether there might be a synergistic relationship with high sun exposure. When considered on its own, the presence of Beta
apillomavirus DNA in eyebrow hair follicle cells appeared to increase the odds of developing AK, although this was not statistically significant \((P = .24)\). Beta
apillomavirus positivity increased the ORs for the presence of AKs for each confirmed risk factor.

Because AKs do not follow the course of typical skin tumors (ie, they frequently spontaneously regress),\(^3\) prevalence was considered the most appropriate measure to use in this study. We showed that against the background of an overall decrease in AK counts in this population (due to natural regression,\(^3\) lower rate of accumulation, treatment, or a combination of these factors), people with EV-HPV DNA detected in eyebrow hair follicles in 1996 had an 11.0% higher persistence rate of AKs compared with those without EV-HPV infection.

Evidence from several large studies\(^1,4,20,21\) that compared skin tumors with healthy skin in healthy people suggests a positive association between presence of Beta
apillomavirus and development of SCCs and AKs. Studies of Beta
apillomavirus DNA in plucked eyebrow hair follicle cells have also supported a role for Beta
apillomavirus in the development of skin tumors, most convincingly in European studies\(^20,37\) with corroborations from research using serological methods.\(^23,36,40\) Studies of SCCs and AKs have been reviewed together because of the similar pathogenesis of both lesions.

The combination of high sun exposure and Beta
apillomavirus infection was found to predispose to skin tumor development in previous studies,\(^20,21\) but findings have been contradictory, especially when different sample types are studied. Studies of tumor samples and healthy skin samples support the relationship. In a small study\(^9\) of Australian immunocompetent and immunosuppressed patients with skin cancer, a tendency was noted for HPV DNA to be present in more tumors from sun-exposed sites than from nonexposed sites. Further evidence supporting a joint effect between UV radiation and HPV in tumorigenesis was found in a British study\(^21\) in which the association between Beta
apillomavirus DNA and skin cancer on sun exposed sites was stronger than on nonexposed sites.

There was no such concurrence between 2 studies that relied on Beta
apillomavirus detection in hair follicle cells. A recent study\(^22\) conducted in the Netherlands found that high lifetime sun exposure decreased the risk of Beta
apillomavirus DNA positivity when plucked eyebrow hair follicle cells were examined. However, in a community-based study\(^5\) conducted in Nambour, high occupational sun exposure was significantly associated with higher risk of Beta
apillomavirus DNA detection in hair follicles.

Serological investigations have yielded conflicting results. Feltkamp et al\(^2\) found no association between HPV seropositivity and sun exposure. Two more recent studies\(^41,42\) of SCCs have provided further evidence of a positive combined influence of sun exposure and HPV infection in tumor development. Karagas et al\(^41\) found that the highest risk of SCCs was found in those with antibodies to several HPV types and with a tendency to sunburn. Hall et al\(^42\) also found the risk of SCCs highest for the Beta
apillomavirus-seropositive group, and a joint effect was observed for each separate classic risk factor (such as older age, male sex, fair skin, tendency to sunburn, and high sun exposure).

Laboratory evidence is conflicting regarding the possible role of Beta
apillomavirus infection and UV radiation in the development of skin tumors. In support of a biological interaction, the promoter activity of HPV-77 (a cutaneous HPV type) has been directly stimulated by UV radiation,\(^43\) but other cutaneous HPV types (such as 1, 2, 3, 5, 20, 23, 27, 38, and 41) all reacted differently to UV radiation treatment in vitro.\(^44\)

In considering potential weaknesses of this study, it is likely that participants had developed different attitudes and behaviors with regard to sun exposure and possibly skin cancer treatment than the general Australian population because of their involvement in the Nambour Skin Cancer Study since 1986. The active participants who were followed up in 2003 are likely to represent an especially sun-aware, motivated subgroup with greater sun-avoidant behavior than other community members. Although this may contribute to the lower prevalence of AKs than in the general public, it is unlikely to have influenced the relationships in this study. Our measurement of the prevalence of AKs was dependent on clinical recognition and has been shown to have high validity in our previous studies,\(^32\) although no histopathological confirmation was available in this particular study.

The Beta
apillomavirus DNA detection method used in 1996 was highly sensitive and was not likely to have biased results greatly,\(^27,45\) although with newer methods, other Beta
apillomavirus types are being discovered, and thus there may have been a systematic underestimation of the prevalence of infection in this study. Although it is possible that Beta
apillomavirus status changed during the observation period, it has been shown elsewhere that cutaneous HPV infection persists.\(^46\) None of the current common investigative methods alone are able to provide direct evidence to determine the role of Beta
apillomavirus in skin tumor development, however. Currently, detection of the presence of Beta
apillomavirus in tumors and comparison of the prevalence between individuals with and without tumors are the most frequent and probably the most accurate investigative approaches. Better evidence of an independent association between Beta
apillomavirus infection and skin cancer is likely to come from further prospective studies using several markers of infection, such as tissue DNA detection and seroreactivity.

To establish a causal role for Beta
apillomavirus in skin cancer, molecular techniques that examine possible
mechanisms of viral carcinogenesis may yield the vital clue. A recent study into the mechanisms by which Beta-papillomavirus might induce tumors found that infected keratinocytes express viral genes. In conclusion, the results of this prospective study show a weaker association between the presence of Betapapillomavirus in hair follicles and the increased prevalence of AKs than that established in previous cross-sectional analyses of viral infection of eyebrow hair follicle cells. However, the findings presented herein add to early evidence that suggests that Betapapillomavirus enhances the effects of increasing age, sun-sensitive phenotypes, and high-dose UV radiation to further increase the risk of developing AKs.

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Author Contributions: Ms McBride had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: McBride, Neale, and Green. Acquisition of data: McBride and Green. Analysis and interpretation of data: McBride, Neale, Pandeya, and Green. Drafting of the manuscript: McBride and Green. Critical revision of the manuscript for important intellectual content: Neale, Pandeya, and Green. Statistical analysis: Neale and Pandeya. Obtained funding: Green. Study supervision: Neale and Green.

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