CHAPTER 2

TRANSMISSION OF BETAPAPILLOMA VIRUSES BETWEEN DOMESTIC PARTNERS IN AN AUSTRALIAN COMMUNITY

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**Abstract**

Betapapillomaviruses may be associated with the development of cutaneous squamous cell carcinoma but little is known about their transmission. One suggestion is that they are transmitted through close skin contact.

To test this hypothesis we assessed whether co-habiting opposite-sex couples were more or less likely to share betaPV types than each member of the couple and an age-matched, opposite-sex control. Betapapillomavirus was measured in eyebrow hairs of 57 couples and 114 age- and sex-matched controls. We compared the proportion of partners who shared at least one betaPV type with the proportion of control partnerships sharing a betaPV type. We further subdivided those who shared at least one type into those who shared only one and those who shared more than one. We tested the significance of differences in these proportions using Chi-squared tests. A case-wise concordance index was used to calculate the overall concordance of the partners and the control pairings.

At least one betaPV type was shared by 39% of the co-habiting couples and 26% of the control pairs (p=0.10). When restricted to all people with at least one virus infection (26 couples) 74% of the partners and 46% of the control pairs shared at least one type (p=0.02). The case-wise concordance index for partners was 0.28 (95% CI 0.21-0.35) and for the matched control pairs 0.16 (95% CI 0.12-0.20) (p<0.001).

Our results support the hypothesis that skin-to-skin contact is the primary means of betapapillomavirus transmission.
**Introduction**

Human papillomaviruses of the beta-genus (betaPV) are cutanotropic viruses that are associated with cutaneous squamous cell carcinoma (SCC) (1). So far 31 different betaPV types have been fully sequenced and more than 100 types partially sequenced (2;3). Epidemiological studies have shown that all currently identified betaPV types are frequently found in hair bulbs of eyebrows and body hairs, normal skin swabs and biopsies from healthy controls and transplant recipients, as well as in tumour tissue from patients with SCC (4-6). Usually multiple infections are detected within a sample (7).

Little is known about the transmission of betaPV. In healthy people no clinical signs of initial infection are observed. We have found only 5 previous studies addressing transmission of betaPV, several of which are very small (8-12). The data about transmission between parents and children is ambiguous: one study involving 38 infants showed parents and babies as young as 4 weeks of age to share betaPV types (8) and another study showed that transmission between parents and children occurs frequently (13;14). However in a third study transmission between parents and children was observed rarely (15). In this cohort transmission between couples was also infrequently seen (15). A cohort of 23 participants showed that the 5 students sharing a household were not likely to obtain each other’s betaPV, but instead kept their own infection profile (10). Despite different outcomes, all of these studies concluded that betaPV transmission probably takes place during close (skin-to-skin) contact.

To test this hypothesis we assessed whether co-habiting married or *de facto* opposite-sex couples (herein called ‘partners’) were more or less likely to share betaPV types than each member of the couple and an age-matched, opposite-sex control.

**Material and methods**

**Study population and sample collection**

Participants were an unselected subset of the study population of the Nambour Skin Cancer Study which has been described in detail previously (16;17). Briefly, in 1986, 2095 of 3000 randomly selected residents of Nambour, a subtropical township in Australia (latitude 26°S), who were aged 20-69 years, participated in a skin cancer prevalence survey. From 1992 to 1996, 1621 of these took part in a trial of sunscreen application and beta-carotene supplementation for the prevention of skin cancer. In 1996, 507 randomly selected members of the
cohort participated in a sub-study aiming to understand the association between HPV and skin cancer (18), and 10 eyebrow hairs were plucked from each participant and processed as described below. Participants’ relationships with one another in 1996 were recorded. For the analysis described here we selected all 57 male-female co-habiting couples. For each of these 114 people, we randomly selected an opposite-sex control from the remaining 393 participants, matched to the age of his/her partner. For example, a 60-year-old man and his 50-year-old wife were matched to a 50-year-old woman and a 60-year-old man respectively.

**DNA isolation, PCR and hybridization**

DNA from eyebrow hairs was isolated according to a method described previously (19). BetaPV detection and genotyping were performed using a reversed hybridization assay as described previously (20). All amplimers generated with the broad spectrum PCR were analysed with a reverse hybridization assay (RHA) that permitted specific detection and identification of 25 established betaPV genotypes (i.e., 5, 8, 9, 12, 14, 15, 17, 19-25, 36-38, 47, 49, 75, 76, 80, 92, 93 and 96). The RHA was performed according to the manufacturer’s instructions (skin (beta) HPV prototype research assay; Diassay BV, Rijswijk, The Netherlands).

**Statistical analyses**

We compared the proportion of partners who shared at least one betaPV type with the proportion of control partnerships sharing a betaPV type. We further subdivided those who shared at least one type into those who shared only one and those who shared more than one. We tested the significance of differences in these proportions using Chi-squared tests. These analyses were performed for all participants and for those in whom we identified at least one betaPV. In addition, case-wise concordance was calculated, which is defined as the conditional probability that one member of the matched pair is positive to a specific betaPV type given that the other member is positive. It was estimated as the ratio of the number of concordant pairs to the total of concordant and average discordant pairs. The standard error and 95% confidence interval were estimated according to methods documented by Huang and Tai (21). The totals for concordant and discordant pairs have been pooled across the 25 individual betaPV types. One estimate was calculated for partner pairs and a second for the pairs formed by matched control couples. Statistical analyses were performed in SAS 9.1.
Results

The mean age of the men was 55 years (SD 11) and of the women 51 (SD 11). Seventy-four percent of the male partners were betaPV-positive (median number of types: 2, range 1-12), compared with 86% of the male controls (p=0.07) (median number of types: 2, range 1-15), 70% of the female partners (median number of types: 2, range 1-11) and 74% of the female controls (p=0.65) (median number of types: 1, range 1-11).

At least one betaPV type was shared by 39% of the co-habiting couples (Table). For the control pairs this was 26% (p=0.10). Fourteen percent of partners versus 11% of control pairs shared more than one type (p=0.25). When we repeated the analyses for all people with at least one virus infection (26 couples) 74% of the partners and 46% of the control pairs shared at least one type (p=0.02), and 32% versus 19% shared more than 1 type (p=0.08) (Table). The case-wise concordance index for partners was 0.28 (95% CI 0.21-0.35) and for the matched control pairs 0.16 (95% CI 0.12-0.20) (p<0.001).

Table: Number of betaPV types shared by partners and by partners and their controls.

<table>
<thead>
<tr>
<th>No. of shared types</th>
<th>Partners, n=57</th>
<th>Control-pairs, n=114</th>
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<tr>
<td></td>
<td>N (%)</td>
<td></td>
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<tr>
<td>Including all</td>
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<td></td>
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<tr>
<td>participants</td>
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<td></td>
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<tr>
<td>0</td>
<td>35 (61)</td>
<td>84 (74)</td>
</tr>
<tr>
<td>1+</td>
<td>22 (39)</td>
<td>30 (26)</td>
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<tr>
<td>Chi-square</td>
<td>2.71 (p=0.10)</td>
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<tr>
<td>0</td>
<td>35 (61)</td>
<td>84 (74)</td>
</tr>
<tr>
<td>1</td>
<td>14 (25)</td>
<td>18 (16)</td>
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<tr>
<td>&gt;1</td>
<td>8 (14)</td>
<td>12 (11)</td>
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<tr>
<td>Chi-square</td>
<td>2.79 (p=0.25)</td>
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</table>

<table>
<thead>
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<th>No. of shared types</th>
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<th>Control-pairs, n=52</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td></td>
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<tr>
<td>Including only betaPV positive participants</td>
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<tr>
<td>0</td>
<td>7 (26)</td>
<td>28 (54)</td>
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<tr>
<td>1+</td>
<td>19 (74)</td>
<td>24 (46)</td>
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<td>7 (26)</td>
<td>28 (54)</td>
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<tr>
<td>1</td>
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<td>14 (27)</td>
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<td>&gt;1</td>
<td>8 (32)</td>
<td>10 (19)</td>
</tr>
<tr>
<td>Chi-square</td>
<td>5.08 (p=0.08)</td>
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Discussion

In this study we found that participants more often shared at least one betaPV type with their opposite-sex domestic partner than with random controls of the same age and sex as their partner. This difference was significant when the analysis was restricted to people who had at least one betaPV infection. Partners also were likely to share more than one type than control pairs, although due to small numbers significant differences could not be observed. We found a highly significant difference in the concordance index. We assessed skin type, sun exposure and skin cancer rate as possible confounders and found no differences between the male and female partners and male and female controls. The borderline significant difference in betaPV prevalence between the male partners and male controls is most likely to be due to random sampling error and is not likely to have caused differences. The higher number of viruses seen in male controls than in male partners suggests that they would have an increased chance of sharing types with the female partner, so if anything, this variability may have led to an underestimation of the difference in shared types found.

The most likely explanation for our findings is the frequent close contact likely to occur between partners, which was also postulated to be the main cause of HPV transmission in babies (8). A study among tenants in a student share house showed that transmission was rare (10), suggesting that living in close proximity may not be sufficient for betaPV transmission and skin-to-skin contact may be required. A recent study about betaPV transmission in families with an overall HPV prevalence of 42% found that the frequency of shared types was higher among couples than among randomly selected individuals, but the frequency of sharing at least one type was only 21% and in all cases only one type was shared (15). We found a much higher proportion of couples with at least one shared type (39%), and 14% of these shared more than one type, possibly due to the higher overall prevalence of betaPV in our sample. The higher prevalence might be due to the fact that we used a different PCR and typing method than those used by Gottschling and colleagues (15). Furthermore, the mean age of our participants was higher (over 50 compared with 42 years), and age is independently associated with betaPV acquisition or detection (7). Weissenborn and colleagues studied the betaPV-spectrum in 10 families with up to 3 generations sampled over a period of time and found comparable results to ours with respect to partner transmission, despite using skin swabs rather than eyebrow hairs as the sample for viral DNA detection (22). Their longitudinal measures showed that persistent infections in one person of a family were shared by their family members in 30-50% of the cases.
Our data are cross-sectional and we therefore cannot address the issue of whether or not persistent betaPV types are shared between couples.

In conclusion, these cross-sectional data demonstrate that co-habiting partners of the opposite-sex share a greater number of betaPV types than with randomly selected matched members of the population. This finding supports the hypothesis that close contact is the primary means of betaPV transmission, probably through skin-to-skin contact. Larger, longitudinal studies are needed to confirm this finding and to give more insight into the sustainability of the shared infections.

**Acknowledgements**

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References


