

John Cason
Philip Rice
Jennifer M. Best

Department of Virology, Kings College,
London, UK

Transmission of Cervical Cancer-Associated Human Papilloma Viruses from Mother to Child

.....
Key Words

Cancer
Papillomaviruses
Perinatal transmission

.....
Summary

There is now compelling evidence that persistent infection with certain types of human genital papillomaviruses (HPV) may, after many years, lead to cervical cancer. However, HPV have been detected in asymptomatic women, infants and children. Several studies have demonstrated that infants can acquire high-risk HPV infections from their mothers at birth. Thus, the traditional view that cervical-cancer associated HPV infections are primarily sexually transmitted needs to be re-assessed. Accordingly, the role of mother to child transmission of cancer-associated HPVs may need to be investigated further. These facts are pertinent to those developing prophylactic vaccines to prevent high-risk HPV infections and cervical carcinoma.
.....

Introduction

Of the more than 100 human papillomavirus (HPV) types currently identified about 27 cause anogenital infections, with HPV types 6, 11, 16, 18, 31, 33, 35 and 42 being most prevalent [1]. HPV types 16, 18, 31, 33, 51 and 54 are associated with anogenital carcinomas and are thus believed to be of high cancer risk [2–4]. About 95% of cervical cancers contain high-risk HPV DNA, usually HPV-16 [4]. In contrast, HPV-6 or HPV-11 virions occur in genital warts, but are rarely associated with carcinoma, and are classified as low cancer risk. The remaining genital HPVs are of intermediate risk [1]. Evidence linking HPV-16 and HPV-18 with cervical cancer has been the subject of several excellent reviews [3, 4].

Sexual Transmission of High-Risk HPVs

Until recently, it was believed that high-risk genital HPVs caused only genital infections and were transmitted solely by sexual contact. In fact cervical carcinoma exhibits the characteristics of a sexually transmitted disease. Sexual transmission of high-risk HPVs was believed to be dependent upon a reservoir of infected males with sub-clinical HPV infections [5]. Thus initially, there was a very clear story of high-risk HPVs occurring essentially in a small proportion of women, primarily those with cervical neoplasia.

This view was supported by the finding a low prevalence of high-risk HPV infections among women with normal cervical smears. However, this was based upon data collected using insensitive methods to detect viral DNA (e.g. in situ, dot-blot and Southern blot hybridization assays). Even the commercial Hybrid Capture™ test for high-risk HPVs has an analytic sensitivity of only

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 1999 S. Karger AG, Basel
0300–5526/98/0415–0213\$17.50/0

Accessible online at:
<http://BioMedNet.com/karger>

John Cason
Richard Dumbleby Laboratory of Cancer Virology, Department of Virology
Rayne Institute, Guy's, Kings College and St. Thomas' School of Medicine, Kings College
Lambeth Palace Road, London SE1 7EH (UK)
Tel. +44 171 928 9292, ext. 2216/1848, Fax +44 171 922 8394, E-Mail jwcason@aol.com

100,000 HPV genomes/sample. Thus, studies using these techniques will underestimate the true prevalence of infection. More recently, use of sensitive polymerase chain reactions (PCR) has revealed that HPV-16 DNA is more common amongst women with no evidence of cervical lesions [6].

Although there is no doubt that sexual transmission occurs, there remain significant problems with the concept that this mechanism completely explains the transmission of this virus. Several authors have described a low concordance of HPV genital infections between heterosexual partners. Hippelainen et al. [7] showed that of 270 couples investigated for genital HPV DNA by in situ hybridisation, both partners of 66 couples were positive whereas only 15 (23%) of these had identical HPV types in their genital tract. Whilst women with low-risk HPV infections had male partners with identical HPV types in 50 and 37% of cases, respectively, the rate of concordance was much lower amongst those with high-risk HPV-16 or HPV-18 infections (24 and 16%). Another study of heterosexual partners using dot-blot assays found a concordance between partners with HPV-16 infections of 57% and of just 29% for HPV-18 [8]. Unfortunately, interpretation of such studies is frequently confounded by the inclusion of short-term and non-monogamous relationships. In a DNA sequencing study Ho et al. [9] described the occurrence of HPV-16 genomic variants in 8 HPV-16-DNA-positive heterosexual couples. Whilst 4 couples had identical HPV-16 genomic variants, 4 had mismatched variants. The many interpretations of these data were discussed by the authors who formed the opinion that sexual transmission of HPV-16 does occur, but with low infectivity.

Is Sexual Transmission the Only Route?

That high-risk HPVs are transmitted exclusively by the sexual route is currently being re-appraised, as high-risk HPVs have been detected in virgins, infants and children (below and table 1). Indeed, it would perhaps be surprising if high-risk HPVs were not transmitted in other ways, particularly from mother-to-child since many other genital infections are transmitted by this route (e.g. herpes simplex, hepatitis B and human immunodeficiency viruses, *Treponema pallidum*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*). Furthermore, there is also evidence for non-sexual parent-to-child transmission of low-risk HPVs [10] and another member of the Papovaviridae, JC polyomavirus.

Table 1. Evidence for the non-sexual transmission of high-risk HPVs

High-risk genital HPVs infect the oral mucosa and are associated with oral carcinomas
Low concordance of HPV types between heterosexual partners and of HPV genomic variants within married couples
Genital HPV DNA was detected in virgins
High-risk genital HPV infections are common amongst infants and children

High-Risk HPV Infections amongst Sexually Inexperienced Populations

Virgins

Whilst some investigators were unable to detect HPV DNA in swabs from vaginal women, or in cervical-vaginal specimens from young girls others have found HPV DNA in vulval swabs from 9 (14.8%) of 61 women who claimed no history of sexual intercourse [11]. Jochmus-Kudielka et al. [12] also investigated vulval swabs from 24 asymptomatic virgins by PCR and in 5 cases (21%) HPV-16 DNA was detected. Similarly, 3 of 15 (20%) women who had never had vaginal intercourse with a man were positive at the vulva, but not at the cervix [13].

The obvious caveat attached to these reports is that the true vaginal state of the women investigated may be questionable. Whilst acquisition of high-risk HPVs may occur in virgins vulval, rather than cervical, HPV infections are most common. Nonetheless, investigations of children [14] and of adult women [15] have indicated that external genital HPV infections are usually accompanied by internal (cervicovaginal) HPV infections.

Children

Whilst HPV-16 is considerably more prevalent at genital sites than either HPV-6 or HPV-11 in the general adult population, few groups have investigated high-risk HPV infections amongst children. This is probably due to the fact that there are only a few reports of clinical lesions in childhood attributable to infection with high-risk HPVs. There are occasional case reports of children with condylomata acuminata which contain HPV-16 [16, 17] or HPV-16 and HPV-18 [18] and one report of a 12-year-old child with a laryngeal carcinoma that contained HPV-18 and HPV-33 [19].

Evidence that high-risk HPV infections may occur among children came initially from the observation that foreskins from 3 of 70 (4.3%) unselected normal new-

borns were HPV DNA-positive, 2 (2.8%) of which contained HPV-16 DNA in dot-blot assays [20]. Further indications that childhood HPV-16 infections occur are provided by seroprevalence studies. Anti-HPV-16 E4 antibodies have been demonstrated in 30–40% of sera from children and adolescents up to 20 years of age, and in another study of 1,707 individuals aged 1–95 years, anti-HPV-16 E4 antibodies were common amongst children and teenagers (20%), but not adults (1.14%). This led the authors to conclude that HPV-16 infections may occur frequently in early life [21]. Antibodies to HPV-16 capsid proteins have also been detected in up to 25% of children when peptides or fusion proteins were used as antigens [22, 23]. In addition, 80 of 200 (40%) sera from children aged between 1 month and 10 years had IgM antibodies to an HPV-16 L2 protein expressed in insect cells via a recombinant baculovirus [24]. In other studies, antibodies reactive with HPV-16 were detected in 6–14% of children [25].

Limited PCR studies have confirmed such serological findings and indicate that up to 19% of children under 6 years have HPV-16 DNA in their buccal cavities [23]. Oral scrapings from 33 of 95 (35%) of children aged 1–11 years were HPV DNA-positive, 16 (17%) were positive for HPV-16/HPV-18 or HPV-6/HPV-11. We are currently performing a large cross-sectional study of the prevalence of buccal infection with HPV-16 amongst 350 London schoolchildren aged 1 through 11 years. To date, of 208 children tested 48% were positive when tested by a highly sensitive PCR (analytic sensitivity \approx 10 HPV-16 copies/sample) [26], whereas only about 15% were positive using the MY09/11 consensus primer PCR (analytic sensitivity \approx 500 HPV-16 copies/sample). Moreover, in 16% of these children we have been able to detect HPV-16 early region mRNA indicating that many of these infections are replicative. Together these data indicate that exposure to and infection with HPV-16 are relatively common in childhood.

Perinatal Transmission of High-Risk HPVs

Pregnancy

Whether there is an increase in the prevalence of genital HPV infections during pregnancy is controversial, although several studies have reported an increase of such infections [27, 28]. Rando et al. [28] using Southern blotting reported that 52.5% of patients were HPV DNA-positive in the third trimester of pregnancy, compared with only 17.5% postpartum. Schneider et al. [27] reported

that 28% of pregnant women and 12.5% of nonpregnant controls were HPV positive, with HPV-16 being the dominant genotype amongst the former group. This apparent increase in detectable HPV infections may be the result of two factors which may permit pre-existing low-copy-number, latent, and de novo infections, to be detected. These are the hormonal changes which may encourage increased transcription mediated by the glucocorticoid response elements in the non-coding region of HPV-16 and the transient immunosuppression which accompanies pregnancy.

Detection of High-Risk HPV DNA in Mothers and Their Infants

The concept of HPV transmission from mother-to-infant at birth was first proposed by Sedlacek et al. [29] after showing that HPV DNA could be detected by Southern blotting in nasopharyngeal aspirates from 11 of 23 (48%) infants born to HPV DNA-positive mothers. However, although HPV DNA in maternal cervical cells was genotyped DNA from infant samples was not. Smith et al. [30] reported HPV DNA in oropharyngeal cells from two of 72 (2.8%) infants delivered to HPV positive mothers, but these authors used the ViraPap/ViraType assay which is neither as sensitive, nor as specific as Southern blotting.

One report of 3-year-old children and their HIV-infected mothers in Zaire revealed that whilst 10 of 81 (12.3%) children were positive for high-risk HPV DNA this did not correlate with HPV infection in the mothers [31]. This ambiguity may again be explained by use of the ViraPap/ViraType™ kit or alternatively that transient maternal HPV infections among mothers may have regressed during the 3 years since delivery.

More convincing evidence of perinatal transmission of high-risk HPVs comes from other studies [32] in which HPV-16 or HPV-18 DNA was demonstrated by PCR on swabs from the external genitalia and/or in buccal cavities of 50% of 24-hour-old infants delivered to HPV-16- or HPV-18-infected mothers. Whilst at 24 h HPV-16 DNA-positive infants could just be smeared with infected maternal cells, high-risk HPV DNA persisted in at least half of the infants until they were 6 weeks of age, suggesting that replicative infections had been established. At 6 months of age, over half of the children remained positive and 13 HPV-16 DNA-positive children followed to 2 years of age remained infected [33, 34].

Fredericks et al. [35] were also able to demonstrate high-risk HPV DNA by PCR analysis of samples from the genital tracts of mothers and in the buccal cavities of their

Table 2. Potential routes for the transmission of high-risk HPVs to infants

Probable
Mother to infant during parturition
Ascending infection via ruptured amniotic membranes
Horizontal spread from relatives/friends
Shared bathing with parents
Sexual abuse
Possible
Infected fomites/clothing
Improbable
Breast milk
HPV genome in gametes
Blood

infants when swabs were taken 6 weeks after parturition. HPV DNA was detected in cervical epithelial cells of 11 of 30 (37%) women and in buccal cells from 8 of 11 (73%) infants born to HPV-positive women. Concordant HPV-18 infections were detected in 6 mothers and their infants. Whilst these data suggest HPV-18 may be transmitted at birth from mother to child it remains possible that some children acquired infection from another source in the intervening period between delivery and testing.

Source and Routes of Infant Infections with HPV

It seems probable that passage of the neonate through an infected birth canal might explain the source and route of high-risk HPV DNA detected in infants (tables 1, 2). Indeed, estimation of viral load in the genital tracts of HPV-16 DNA-positive pregnant women revealed that women who transmitted infection to their infants had significantly greater quantities of HPV-16 DNA than those who did not [36]. Furthermore, amongst 13 HPV-16 DNA-positive children, we have also shown that mothers are usually the source of infant infections by using DNA sequencing to detect the occurrence of concordant HPV-16 variants in maternal and infant samples [34]. Other evidence for a maternal source of infant infections comes from a report of 2 mothers with dual HPV-16/HPV-18 infections who both produced infants who were similarly co-infected [33]. In addition to passage through an infected birth canal, transplacental HPV-16/HPV-18 infections have been documented. HPV DNA has been detected in 75% (12 of 16) of amniotic fluids from cervical HPV DNA-positive pregnant women, implying that ascending infections may occur [37].

Other routes of infant infection with high-risk HPVs may also exist (table 2). Some viruses (e.g. HIV) can be transmitted via breast milk, but it seems unlikely that HPVs are present in breast milk since there is no viraemic phase and HPVs are not associated with breast lesions. Certain retroviruses can be transmitted vertically by infecting the gametes and there is one report of HPV DNA sequences in purified human sperm cells [38]; this again seems unlikely given the high degree of tissue specificity of genital HPVs for keratinocytes.

Discussion

There is now convincing evidence that high-risk HPVs may be transmitted from mother to infant, although it is unknown whether such infections play a role in the aetiology of cervical neoplasia in later life. The main areas of controversy on this issue are the incidence of mother-to-child infection and whether persistent replicative infections are established. High-risk HPV infections may be common in asymptomatic women, however only a minority of such infections are believed to result in malignancy and then probably only when co-carcinogens are present. Thus at present, there is no rationale either for testing neonates for high-risk HPV DNA or for offering Caesarean section to pregnant women with such infections. Furthermore, based upon the evidence presented, detection of infant infection with genital HPVs should not be considered as evidence for sexual abuse.

What is currently required is the determination of age-related prevalences of high-risk HPV infections according to age amongst unselected children. In particular studies of genital infections are virtually impossible to perform since virus replication could be restricted to the transformation zone which would be unethical to sample in young virginal females. However, high-risk HPV DNA on infants can be detected at external genital and at buccal sites with sensitive type-specific PCRs [39].

Serological evidence suggests that infant infections with high-risk HPVs may persist and utilisation of improved immunoassays employing assembled HPV-16 virus-like particles [40, 41] would make epidemiological studies of childhood infections considerably easier. However, the authors of one preliminary study (using HPV-16-virus-like particles as antigens) were unable to detect antibodies in 94 16-year-old virginal female students [42].

HPV infection acquired before the infant's immune system is mature may lead to immunological tolerance. This could permit persistence of such infections and/or a

reduced ability to clear subsequent high-risk HPV re-infections in later life. Thus, acquisition of high-risk HPV infections at birth has significant implications for the design of, and timing of the delivery of vaccines against genital HPV.

Finally, the potential impact of vertical transmission on the epidemiology of high-risk HPV infections may have been underestimated since (1) between 5 and 40% of the young female population have asymptomatic HPV-16 infections [43, 44], (2) this may increase to about 52% during pregnancy [27, 28] and (3) HPV-16/HPV-18 ap-

pear to be transmitted vertically in 50–73% of children born to infected mothers [33, 35]. Thus, between 26 and 38% of children may be infected with high-risk HPVs at birth or in early infancy.

Acknowledgements

We are grateful for continued financial support from the Wellcome Trust, the Special Trustees of St. Thomas' Hospital, Smiths Charities and the Richard Dimbleby Cancer Fund.

References

- de Villiers E-M: Laboratory techniques in the investigation of human papillomavirus infection. *Genitourin Med* 1992;68:50–54.
- Syrjanen KJ: Epidemiology of human papillomavirus infections and their associations with genital squamous cell cancer. *APMIS* 1989;97: 957–970.
- zur Hausen H: Papillomavirus in anogenital cancer as a model to understand the role of viruses in human cancer. *Cancer Res* 1989;49: 4677–4681.
- zur Hausen H: Molecular pathogenesis of cancer of the cervix and its causation by specific human papillomavirus types. *Curr Top Microbiol Immunol* 1994;186:131–156.
- Campion MJ, Singer A, Clarkson PK, McCance DJ: Increased risk of cervical neoplasia in consorts of men with penile condylomata acuminata. *Lancet* 1985;i:943–946.
- Agrastos T, Bontis J, Lambropoulos F: Epidemiology of human papillomavirus infection in Greek asymptomatic women. *Eur J Cancer Prev* 1995;4:159–167.
- Hippeläinen MI, Yliskoski M, Syrjanen S, Saastamoinen J, Hippeläinen M, Saarikoski S, Syrjanen K: Low concordance of genital human papillomavirus lesions and viral types in HPV infected women and their sexual partners. *Sex Transm Dis* 1994;21:76–82.
- Monsonogo J, Zerat L, Catalan F, Coscas Y: Genital human papillomavirus infections: Correlation of cytological, colposcopic and histological features with viral types in women and their male partners. *Int J STD AIDS* 1993;4: 13–20.
- Ho L, Tay S-K, Chan S-Y, Bernard HU: Sequence variants of human papillomavirus type 16 from couples suggest sexual transmission with low infectivity and polyclonality in genital neoplasia. *J Infect Dis* 1993;168:803–809.
- Gissmann L, Wolnik L, Ikenberg H, Koldovsky U, Schurch HG, zur Hausen H: Human papillomavirus types 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers. *Proc Nat Acad Sci USA* 1983; 80:560–563.
- Pao CC, Tsai PL, Chang YL: Possible non-sexual transmission of genital human papillomavirus infections in young women. *Eur J Clin Microbiol Infect Dis* 1993;12:221–223.
- Jochmus-Kudielka I, Schneider A, Braun R, Kimmig R, Koldovsky U, Schneeweis KE, Seedorf K, Gissmann L: Antibodies against the human papillomavirus type 16 early proteins in human sera: Correlation of anti-E7 reactivity with cervical cancer. *J Natl Cancer Inst* 1989;81:1698–1704.
- Ley C, Bauer H, Schiffman M, Chambers JC, Tashiro CJ, Reingold A, Manos MM: Determinants of genital HPV infection in young women. *J Natl Cancer Inst* 1991;83:997–1003.
- Gutman LT, St Claire KK, Everett VD: Cervico-vaginal and intraanal human papillomavirus infection of young girls with external genital warts. *J Infect Dis* 1994;170:339–344.
- Horn JE, McQuillan GM, Shah KV: Genital human papillomavirus infections in patients attending an inner city STD clinic. *Sex Transm Dis* 1991;18:183–187.
- Rock B, Naghasfar Z, Barnett Z: Genital tract papillomavirus infections in children. *Arch Dermatol* 1986;122:1129–1132.
- Matsumura N, Kumasaka K, Maki H: Giant condyloma acuminatum in a baby boy. *J Dermatol* 1992;19:432–435.
- Gibson PE, Gardner SD, Best SJ: Human papillomavirus types in anogenital warts of children. *J Med Virol* 1990;30:142–145.
- Simon M, Khan T, Schneider A, Pirsig W: Laryngeal carcinoma in a 12-year-old child: Association with human papillomavirus types 18 and 33. *Arch Otolaryngol Head Neck Surg* 1994;120:277–282.
- Roman A, Fife K: Human papillomavirus DNA associated with foreskins of normal newborns. *J Infect Dis* 1986;153:855–860.
- Muller M, Viscidi RP, Ulken V: Antibodies to the E4, E6 and E7 proteins of human papillomavirus type-16 in patients with HPV associated diseases and in the normal population. *J Invest Dermatol* 1995;104:138–141.
- Cason J, Kambo PK, Best JM, McCance DJ: Detection of antibodies to a linear epitope on the major capsid protein of human papillomavirus type 16 in sera from patients with cervical intraepithelial neoplasia and children. *Int J Cancer* 1992;50:349–355.
- Jenison SA, Yu X-P, Valentine JM, Koutsky LA, Christiansen AE, Beckmann AM, Gallo way DA: Evidence of prevalent genital type human papillomavirus infections in adults and children. *J Infect Dis* 1990;162:60–69.
- Cason J, Kambo PK, Shergill B, et al: Detection of class-specific antibodies to baculovirus derived human papillomavirus type 16 capsid proteins; in Stanley MA (ed): *Immunology of Human Papillomaviruses*. New York, Plenum Press, 1994, pp 155–160.
- Marais D, Rose RC, Williamson A-L: Age distribution of antibodies to human papillomaviruses in children, women with cervical intraepithelial neoplasia and blood donors from South Africa. *J Med Virol* 1997;51:126–131.
- Cavuslu S, Mant C, Starkey WG, Bible JM, Biswas C, Kell B, Rice P, Best JM, Cason J: Analytic sensitivities of hybrid capture, consensus and type-specific PCRs for the detection of human papillomavirus type 16 DNA. *J Virol Methods* 1996;49:319–324.
- Schneider A, Hotz M, Gissmann L: Increased prevalence of human papillomaviruses in the lower genital tract of pregnant women. *Int J Cancer* 1987;40:198–201.
- Rando RF, Lindheim S, Hasty L, Sedlacek TV, Woodland M, Eder C: Increased frequency of detection of human papillomavirus DNA in exfoliated cervical cells during pregnancy. *Am J Obstet Gynecol* 1989;161:50–59.
- Sedlacek TV, Lindheim S, Eder C: Mechanism for human papillomavirus transmission at birth. *Am J Obstet Gynecol* 1989;161:55–59.
- Smith EM, Johnson SR, Cripe TP, Pignatari S, Turek L: Perinatal transmission of human papillomavirus and subsequent development of respiratory tract papillomatosis. *Ann Otol Laryngol* 1991;100:479–483.

- 31 St Louis ME, Icenogle JP, Manzila T: Genital types of papillomavirus in children of women with HIV infection in Kinshasa, Zaire. *Int J Cancer* 1993;54: 181–184.
- 32 Pakarian FB, Kaye J, Cason JB, Jewers RJJ, Raju KS, Best JM: Cancer-associated human papillomaviruses: Perinatal transmission, and persistence. *Br J Obstet Gynaecol* 1994;101: 514–517.
- 33 Cason J, Kaye JN, Jewers RJ, Kambo PK, Bible J, Kell B, Shergill B, Pakarian FB, Raju KS, Best JM: Perinatal infection and persistence of human papillomavirus types 16 and 18 in infants. *J Med Virol* 1995;47:209–218.
- 34 Kaye JN, Starkey WG, Kell B, Biswas C, Raju KS, Best JM, Cason J: Human papillomavirus type-16 (HPV-16) in infants: Use of DNA sequence analyses to establish the source of infection. *J Gen Virol* 1996;77:1139–1143.
- 35 Fredericks BD, Balkin A, Daniel HW, Schonrock J, Ward B, Frazer IH: Transmission of human papillomavirus from mother to child. *Aust NZ J Obstet Gynaecol* 1993;33:30–32.
- 36 Kaye JN, Pakarian F, Cason J, Jewers RJ, Kell B, Bible J, Raju KS, Best JM: Viral load as a determinant for the transmission of human papillomavirus type 16 from mother to child. *J Med Virol* 1994;44:415–421.
- 37 Armbruster-Moraes E, Ioshimoto LM, Leao E, Zugaib M: Presence of human papillomavirus DNA in amniotic fluids of pregnant women with cervical lesions. *Gynecol Oncol* 1994;52: 152–158.
- 38 Chan PJ, Su BC, Kalugdan T, Seraj IM, Tredway DR, King A: Human papillomavirus gene sequences in washed human sperm deoxyribonucleic acid. *Fertil Steril* 1994;61:982–985.
- 39 Cavuslu S, Starkey WG, Kaye JN, Biswas C, Mant C, Kell B, Rice P, Best JM, Cason J: Detection of human papillomavirus type-16 (HPV-16) DNA utilising microtitre-plate based amplification reactions and a solid-phase enzyme-immunoassay detection system. *J Virol Methods* 1996;58:59–69.
- 40 Kimbauer R, Hubbert NL, Wheeler CM, Becker TM, Lowy DR, Schiller JT: A virus-like particle enzyme-linked immunosorbent assay detects serum antibodies in a majority of women infected with human papillomavirus type 16. *J Natl Cancer Inst* 1994;86:494–499.
- 41 Galoway DA: Papillomavirus capsids: A new approach to identify serological markers of HPV infection. *J Natl Cancer Inst* 1994;86: 474–475.
- 42 Andersson-Ellstrom A, Dillner J, Hagmer B, Schiller J, Forssman J: No serological evidence for non-sexual spread of HPV-16. *Lancet* 1994; ii:1435.
- 43 Hildesheim A, Schiffman MH, Gravitt PE: Persistence of type-specific human papillomavirus infection among cytologically normal women. *J Infect Dis* 1994;169:235–240.
- 44 Bauer HM, Ting X, Greer CF, Chambers JC, Tashiro CJ, Chimera J: Genital human papillomavirus infection in female university students as determined by a PCR based method. *JAMA* 1991;265:472–477.