Prospective seroepidemiological study of role of human papillomavirus in non-cervical anogenital cancers

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Abstract

Objective: To evaluate the association between infection with the major oncogenic types of human papillomavirus and the risk of developing non-cervical anogenital cancers in a cohort followed up prospectively.

Design: Data from two large serum banks to which about 700 000 people had donated serum samples were followed up for a mean of 8 years. People who developed non-cervical anogenital cancers during follow up were identified by registry linkage with the nationwide cancer registries in Finland and Norway. Within this cohort a nested case-control study was conducted based on the serological diagnosis of infection with human papillomavirus types 16, 18, and 33.

Subjects: 81 cases and 240 controls matched for sex, age, and storage time of serum samples.

Main outcome measures: Odds ratios of developing non-cervical anogenital cancers in presence of IgG antibodies to specific micro-organisms.

Results: Subjects seropositive for human papillomavirus type 16 had an increased risk of developing non-cervical anogenital cancers (odds ratio 3.1 (95% confidence interval 1.4 to 6.9)). Subjects seropositive for type 33 also had an increased risk (odds ratio 2.8 (1.0 to 8.3)) but not significantly after adjustment for infection with type 16. Seropositivity for human papillomavirus type 16 was associated with an increased risk of developing vulvar and vaginal cancers (odds ratio 4.5 (1.1 to 22)) and a strongly increased risk of developing preinvasive vulvar and vaginal lesions (odds ratio ∞ (3.8 to ∞)). Seropositivity for human papillomavirus type 18 increased the risk of developing preinvasive lesions (odds ratio 12 (1.2 to 590)). High, but non-significant odds ratios for types 16 and 33 were seen for penile cancers.

Conclusions: This study provides prospective seroepidemiological evidence that infection with human papillomavirus type 16 confers an increased risk of developing non-cervical genital cancers, particularly vulvar and vaginal cancers.

Introduction

Experimental and epidemiological data have implicated human papillomavirus as a cause of cervical cancer.^{1,2} Human papillomavirus DNA has been detected in more than 90% of invasive cancers.³ Human papillomavirus type 16 is the dominant subtype, found in about 50% of tumours, and type 18 is detected in nearly 20%.⁴ Cervical intraepithelial neoplasia has also been attributed to infection with human papillomavirus.⁵

In addition to cervical cancer, the genomes of the oncogenic human papillomavirus types 16 and 18 have been found in a substantial proportion of anal, penile, vaginal, and vulvar neoplasms.¹ Patients with preinvasive and invasive cervical cancers are at increased risk of developing secondary cancers at specific sites—notably, vulvar and vaginal cancers and anal cancer—suggesting the existence of shared risk factors.^{6,7}

Human papillomavirus serology using capsids of human papillomavirus types 16, 18, and 33 has been extensively validated as a type restricted marker of sexually transmitted human papillomavirus infections.⁸⁻¹⁰ Use of serology based on type 16 capsids has provided prospective epidemiological evidence of a link to cervical cancer.¹¹

Serum banks were established in the early 1980s in Finland and in the early 1970s in Norway, and national cancer registries have been in operation since the early 1950s in both countries. We used these data sources to conduct a prospective seroepidemiological evaluation of the risk of developing non-cervical anogenital cancers among subjects seropositive for three major oncogenic types of human papillomavirus.

Subjects and methods

Serum banks

The *Finnish maternity cohort* contains blood specimens from 390 000 women. Samples have been collected since 1983 by the National Public Health Institute in Oulu, Finland. By 1993 a total of 710 000 serum samples had been stored. The samples are collected at maternity clinics from almost all (about 98%) pregnant women in Finland during early pregnancy (first trimester) to screen for congenital infections. The samples are stored at -20°C .

The *Janus project* was started in 1973 and contains about 500 000 serum samples from about 300 000 donors.¹² The samples have been collected from people who participated in county health examinations, mostly for cardiovascular diseases, and from blood donors. The participants in the health examinations were recruited from several counties in Norway. The blood donors were from the Red Cross Blood Donor Centre in Oslo. In 1997 these donors were aged 24-86, whereas most of the other donors in Norway were aged 52-72. The samples are stored at -25°C .

Cancer registries

Both the Finnish and the Norwegian cancer registries are country wide and population based. Since 1953 they have received notifications from hospitals, pathology and haematology laboratories, and doctors. They provide information about site, histological type, and stage of disease at the time of diagnosis. The

registration of solid cancers is regarded as practically complete.¹³ Coding of primary site is based on a modified version of ICD-7 (international classification of diseases, seventh revision).

Identification of cases and controls

The data files of the serum banks and cancer registries were linked on the basis of personal identification number to identify non-cervical anogenital cancers. If there were several serum samples available for each case the first sample was chosen. Three controls were selected from the cohorts for each case. The controls were individually matched for sex, age at serum sampling (within 2 years), storage time (within 2 months), and for county (in Norway alone). If three controls could not be found the matching criteria on age at serum sampling and storage time were widened successively—for example, age at serum sampling 3 years, storage time 3 months and age at serum sampling 4 years, storage time 4 months, and so on.

In Finland 19 cases and 57 controls were identified initially, all of them in women under 50. A serum sample was not available for one control. In Norway 62 cases and 186 controls were identified initially. Serum samples were not available for two controls. Consequently, 81 cases (34 men and 47 women) and 240 controls were left for analysis—19 cases and 56 controls from Finland and 62 cases and 184 controls from Norway. The matching criteria were widened for two of the Finnish and eight of the Norwegian controls.

Ten preinvasive vulvar and vaginal lesions, one anal cancer, and eight vulvar and vaginal cancers comprised the Finnish cases; the remaining cases were from Norway (table 1). Most of the cases were vulvar and vaginal cancers (25), followed by anal cancer (20), and penile and scrotal cancers (16). Cases with invasive cancer were aged 28–71, with a median age of 53. The median age at diagnosis ranged from 45 for vulvar and vaginal cancers to 60 for urethral cancers. The median age at diagnosis was 35 years for preinvasive vulvar and vaginal lesions.

Median time between withdrawal of serum and diagnosis was 8.7 years (range 1 month to 19 years) for the invasive cancers and 4.7 years (range 1 month to 8.8 years) for the preinvasive lesions.

Laboratory methods

Seropositivity to human papillomavirus capsids was determined by an established and validated enzyme linked immunosorbent assay (ELISA) using baculovirus expressed capsids comprising both the L1 and L2 proteins, with disrupted capsids of bovine papillomavirus as control.¹⁴ Seropositivity was assessed using two cut off points, both having been used in previous studies.^{14–16} A cut off point of 0.100 absorbance units was considered to be the lowest reasonable point. For human papillomavirus type 16 analysis was also performed with a cut off point of 0.239, which relative to internal standards was the same as the one giving optimal discrimination of cases and controls in a previous study of cervical cancer.¹⁷ Cut off points that are optimal for infection with human papillomavirus types 18 and 33 have not been established, so we used an arbitrary cut off point of 0.200. The specificity of the serological assays was high because no antibodies could be found in a panel of serum samples obtained

Table 1 Cases in Finland and Norway included in study with median age at diagnosis

Cancer	ICD-7 code	Median age at diagnosis (years)	Finland (n=19)	Norway (n=62)	Total (n=81)
Anus	154.1	54	1	19	20
Vulva, vagina	176	45	8	17	25
Penis, scrotum	179	53	0	16	16
Urethra	181.1	60	0	5	5
Skin (perianal)	191.4	59	0	5	5
Preinvasive lesion	NA	35	10	0	10

NA=not applicable.

from virginal women.¹⁶ Estimates of sensitivity for detection of human papillomavirus type 16 infection have been found to be about 50%.^{9 16 18 19}

IgG antibodies to *Chlamydia trachomatis* were determined by a microimmunofluorescence method using the serovars B, E, D/C, H, I, J/G, F, and K.²⁰ A combination of the results obtained for the different serovars with a preassigned cut off point of $\geq 1:16$ was used to distinguish between seropositivity and seronegativity.

All laboratory analyses were performed on masked samples.

Statistical analyses

Odds ratios and their 95% confidence intervals were derived from conditional exact logistic regression models²¹ using the program package LogXact.²²

Results

Overall, 35% of the cases of cancer and 21% of the controls were seropositive for human papillomavirus types 16, 18, or 33. There was an increased risk of developing non-cervical anogenital cancers among subjects seropositive for type 16 (odds ratio 3.1 (95% confidence interval 1.4 to 6.9)) and also among subjects seropositive for type 33 (odds ratio 2.8 (1.0 to 8.3)) (table 2). The odds ratio of human papillomavirus type 16 adjusted for type 33 was 2.8 (1.2 to 6.2), and the odds ratio of type 33 adjusted for type 16 was 2.2 (0.7 to 6.7). Infection with *Chlamydia trachomatis* was not associated with the development of non-cervical anogenital cancers.

Seropositivity for human papillomavirus type 16 was associated with an increased risk of developing vulvar and vaginal cancers (odds ratio 4.5 (1.1 to 22)) (table 3). The odds ratio for type 16 adjusted for type 33 was 4.0 (0.9 to 19). At the preassigned cut off point (0.239) five of the 18 women who developed vulvar

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Table 2 Risk of developing invasive non-cervical anogenital cancers according to presence of IgG antibodies to different micro-organisms

Micro-organism	Percentage positive		Odds ratio (95% CI)
	Cases (n=71)	Controls (n=210)	
Human papillomavirus:			
Type 16:			
Cut off point 0.239	24	9	3.1 (1.4 to 6.9)
Cut off point 0.100	30	12	3.2 (1.5 to 6.9)
Type 18*	13	11	1.2 (0.4 to 3.0)
Type 33*	14	6	2.8 (1.0 to 8.3)
Types 16, 18, or 33	35	21	2.1 (1.1 to 3.9)
<i>Chlamydia trachomatis</i>	20	19	1.0 (0.5 to 2.1)

*Preassigned cut off point 0.100. Calculations with cut off point of 0.200 yielded similar results.

Table 3 Risk of developing cancers and preinvasive lesions of vulva and vagina according to presence of IgG antibodies to different micro-organisms

Micro-organism	Vulvar and vaginal cancers			Preinvasive vulvar and vaginal lesions*		
	Percentage positive			Percentage positive		
	Cases (n=25)	Controls (n=73)	Odds ratio (95% CI)	Cases (n=10)	Controls (n=30)	Odds ratio (95% CI)
Human papillomavirus:						
Type 16:						
Cut off point 0.239	24	5	4.5 (1.1 to 22)	70	3	∞ (3.8 to ∞)
Cut off point 0.100	36	10	5.5 (1.5 to 25)	70	17	7.8 (1.4 to 80)
Type 18†	20	15	1.5 (0.3 to 7.5)	40	3	12 (1.2 to 590)
Type 33†	16	5	3.3 (0.5 to 23)	30	7	4.5 (0.5 to 54)
Types 16, 18, or 33	36	19	2.5 (0.8 to 8.4)	70	13	15 (1.9 to 710)
<i>Chlamydia trachomatis</i>	20	26	0.7 (0.2 to 2.3)	30	10	∞ (0.5 to ∞)

*Nine of carcinoma in situ and one of dysplasia gravis.

†Preassigned cut off point 0.100. Calculations with cut off point of 0.200 yielded similar results.

Table 4 Risk of developing anal and penile and scrotal cancers according to presence of IgG antibodies to different micro-organisms

Micro-organism	Anal cancer			Penile and scrotal* cancers		
	Percentage positive			Percentage positive		
	Cases (n=20)	Controls (n=59)	Odds ratio (95% CI)	Cases (n=16)	Controls (n=48)	Odds ratio (95% CI)
Human papillomavirus:						
Type 16:						
Cut off point 0.239	25	19	1.5 (0.3 to 5.6)	19	4	7.2 (0.5 to 390)
Cut off point 0.100	25	24	1.1 (0.3 to 4.1)	25	4	10 (1.0 to 510)
Type 18†	15	10	1.5 (0.2 to 9.6)	6	8	0.7 (0.0 to 7.6)
Type 33†	10	12	0.8 (0.1 to 6.2)	19	4	7.2 (0.5 to 390)
Types 16, 18, or 33	35	32	1.1 (0.3 to 3.5)	38	13	4.4 (0.9 to 28)
<i>Chlamydia trachomatis</i>	15	19	0.8 (0.1 to 3.6)	31	17	2.3 (0.5 to 10)

*Only case of scrotal cancer was seropositive for human papillomavirus type 18.

†Preassigned cut off point 0.100. Calculations with cut off point of 0.200 yielded similar results.

cancers were seropositive for type 16, giving an odds ratio of 5.0 (1.0 to 32).

Table 3 also shows a highly increased risk of developing preinvasive vulvar and vaginal lesions among subjects seropositive for human papillomavirus type 16 (odds ratio ∞ (3.8 to ∞)) and also among subjects seropositive for type 18 (odds ratio 12 (1.2 to 590)). High but non-significant increases in odds ratio for type 33 and *Chlamydia trachomatis* were also found.

High but non-significant increases in odds ratio for human papillomavirus types 16 and 33 were found for penile and scrotal cancers (table 4). The odds ratio for type 16 adjusted for type 33 was 3.9 (0.3 to 230), and the odds ratio for type 33 adjusted for type 16 was 4.4 (0.2 to 270). The development of anal cancer was not significantly associated with seropositivity for types 16, 18, or 33, although a comparatively high proportion of the cases were seropositive for type 16.

A high but non-significant increase in odds ratio was found for seropositivity for human papillomavirus type 16 and the development of perianal skin cancer (odds ratio 9.0 (0.7 to 470)). None of the five cases of urethral cancer (all in men) were seropositive for any of the human papillomavirus types.

No substantial difference in odds ratios was found for the development of non-cervical anogenital cancers and seropositivity for one or several types of human papillomavirus with lag time (time between serum sampling and diagnosis) below or above 5 years (data not shown). Analyses that excluded controls who

differed from cases in age at serum sampling by more than 2 years or in storage time by more than 2 months did not affect the results.

Discussion

To our knowledge, this is the first study providing prospective epidemiological evidence of an association between human papillomavirus and non-cervical anogenital cancers. We also evaluated the risk associated with seropositivity to *Chlamydia trachomatis* but did not find a significant association.

Vulvar and vaginal lesions

We found increased risks of cancers and preinvasive lesions of the vulva and vagina in subjects seropositive for human papillomavirus type 16. We found an increased risk of preinvasive lesions with seropositivity for human papillomavirus type 18.

Vulvar neoplasms are related to sexual factors and a history of genital warts.²³ Human papillomavirus DNA has been found in a substantial proportion of cases of vulvar intraepithelial neoplasia (about 90%).¹ The prevalence of human papillomavirus DNA in vulvar cancer is, however, considerably lower than it is in cervical cancer, around 30%.¹⁻²⁶ Although human papillomavirus type 16 is the most prevalent subtype in both cancers, the positivity rate is much lower in vulvar than in cervical carcinoma.¹⁻²⁶ However, human papillomavirus DNA is found more often in younger patients with vulvar cancer.²⁵ Vulvar cancer may have two different causes, only one of which may be associated with infection with human papillomavirus.²⁷ Besides human papillomavirus type 16, factors such as mutation of the p53 tumour suppressor gene or squamous cell hyperplasia may play a part in vulvar carcinogenesis, particularly in older women.²⁶

The number of sexual partners and a history of genital warts are also risk factors for vaginal cancer.²⁸⁻²⁹ In addition, human papillomavirus DNA, mostly types 16 and 18, has been found in up to 76% of these cancers.²⁸

Malignant tumours of the vulva and vagina account for about 6% of all cancers of the female genital tract. These tumours primarily occur in older women, but a trend towards occurrence in younger women has been reported.³⁰ The median age of cases was 45 in our study, partly because of the young age of women in the Finnish maternity cohort.

Other non-cervical anogenital cancers

Anal epidermoid carcinoma is a comparatively rare tumour, but its incidence has increased over the past 30 years.³¹⁻³² The incidence peaks between 60 and 70 years of age and is generally two to three times higher among women than men. The median age of the cases of anal cancer in this study (eight men and 12 women) was 54.

Registry studies have shown an epidemiological association between anal and cervical cancer.⁷ Human papillomavirus DNA has been found in anal squamous intraepithelial lesions and in invasive anal cancers.¹ About 60% of the anal cancers contain human papillomavirus DNA, mostly types 16 and 18. A serological association of type 16 with incident anal cancer has been shown.¹⁴ In that study 55% of the

Key messages

- Human papillomavirus has emerged as a leading infectious cause of human cancer, notably cervical and other anogenital cancers, but prospective epidemiological evidence of causality is lacking
- This study used six million person years of follow up to investigate the relation between seropositivity for human papillomavirus and the development of non-cervical anogenital cancers
- Infection with human papillomavirus type 16 increases the risk of developing non-cervical genital cancers, particularly vulvar and vaginal cancers
- Infection with human papillomavirus type 16 should be considered in future intervention strategies for cervical and other genital cancers

patients with anal cancer were seropositive for human papillomavirus type 16 compared with 4% of the controls.¹⁴ In our study equal proportions of cases and controls were seropositive for type 16. The reason for the high proportion of seropositive controls is unclear but may relate to overmatching or to small numbers.

Penile cancer is a rare tumour in developed countries, comprising less than 1% of all cancers in men. Geographical clustering of penile and cervical cancer suggests a common cause.³³ In addition, the wives of men with penile cancer have increased rates of cervical cancer.³⁴ The role of human papillomavirus infection in the development of penile cancer is unclear. High grade penile squamous intraepithelial lesions are regularly positive for human papillomavirus DNA, predominantly type 16.³⁵ The percentage of invasive penile squamous cell carcinomas that are positive for human papillomavirus (mostly type 16) is typically much lower. We found high, but non-significant increases in odds ratios of human papillomavirus types 16 and 33 for penile and scrotal cancers. However, at the higher cut off point we found an increase of borderline significance in odds ratio for type 16.

In summary, this prospective, seroepidemiological study showed that the presence of serum antibodies to human papillomavirus type 16 confers an increased risk of developing non-cervical genital cancers, particularly vulvar and vaginal cancers.

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Conflict of interest: None.

- 1 International Agency for Research on Cancer. *Human papillomaviruses*. Lyons: IARC, 1995. (IARC monographs on the evaluation of carcinogenic risks to humans, vol 64.)
- 2 Cannistra SA, Niloff JM. Cancer of the uterine cervix. *N Engl J Med* 1996;334:1030-8.
- 3 Bosch FX, Manos MM, Muñoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International Biological Study on Cervical Cancer (IBSCC) Study Group. *J Natl Cancer Inst* 1995;87:796-802.
- 4 Zur Hausen H, de Villiers EM. Human papillomaviruses. *Annu Rev Microbiol* 1994;48:427-47.
- 5 Schiffman MH, Bauer HM, Hoover RN, Glass AG, Cadell DM, Rush BB, et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. *J Natl Cancer Inst* 1993;85:958-64.

- 6 Bjørge T, Hennig EM, Skare GB, Søreide O, Thoresen SØ. Second primary cancers in patients with carcinoma in situ of the uterine cervix. The Norwegian experience 1970-1992. *Int J Cancer* 1995;62:29-33.
- 7 Melbye M, Sprøgel P. Aetiological parallel between anal cancer and cervical cancer. *Lancet* 1991;338:657-9.
- 8 Dillner J. Serology of human papillomavirus. *Cancer J* 1995;8:264-9.
- 9 Dillner J, Kallings I, Brühmer C, Sikström B, Schiller JT, Sapp M, et al. Seropositivities to human papillomavirus types 16, 18 and 33 capsids and to Chlamydia trachomatis are markers of sexual behaviour. *J Infect Dis* 1996;173:1394-8.
- 10 Cheng G, Icenogle JP, Kirnbauer R, Hubbert NL, St Louis ME, Han C, et al. Divergent human papillomavirus type 16 variants are serologically cross-reactive. *J Infect Dis* 1995;172:1584-7.
- 11 Lehtinen M, Dillner J, Knekt P, Loustariainen T, Aromaa A, Kirnbauer R, et al. Serologically diagnosed infection with human papillomavirus type 16 and risk for subsequent development of cervical cancer: nested case-control study. *BMJ* 1996;312:537-9.
- 12 Jellum E, Andersen A, Lund-Larsen P, Theodorsen L, Ørjasæter H. Experiences of the Janus Serum Bank in Norway. *Environ Health Perspect* 1995;103 (suppl 3):85-8.
- 13 Teppo L, Pukkala E, Lehtonen M. Data quality and quality control of a population-based cancer registry. Experience in Finland. *Acta Oncol* 1994;33:365-9.
- 14 Heino P, Eklund C, Fredriksson-Shanazarian V, Goldman S, Schiller JT, Dillner J. Association of serum immunoglobulin G antibodies against human papillomavirus type 16 capsids with anal epidermoid carcinoma. *J Natl Cancer Inst* 1995;87:437-40.
- 15 Andersson-Ellström A, Dillner J, Hagmar B, Schiller J, Forssman L. No serological evidence for non-sexual spread of HPV16. *Lancet* 1994;344:1435.
- 16 Andersson-Ellström A, Dillner J, Hagmar B, Schiller J, Sapp M, Forssman L, et al. Comparison of development of serum antibodies to HPV 16 and HPV 33 and acquisition of cervical HPV DNA among sexually experienced and virginal young girls. A longitudinal cohort study. *Sex Trans Dis* 1996;23:234-8.
- 17 Dillner J, Wiklund F, Lenner P, Eklund C, Fredriksson-Shanazarian V, Schiller JT, et al. Antibodies against linear and conformational epitopes of human papillomavirus type 16 that independently associate with incident cervical cancer. *Int J Cancer* 1995;60:377-82.
- 18 Kirnbauer 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;86:494-9.
- 19 Chua KL, Wiklund F, Lenner P, Ingström T, Hallmans G, Sapp M, et al. A prospective cohort study on the risk to develop cervical intraepithelial neoplasia among healthy subjects with serum antibodies to HPV, in comparison with presence of HPV DNA in Papanicolaou smears. *Int J Cancer* 1996;68:54-9.
- 20 Wang SP, Grayston JT. Immunologic relationship between genital TRIC, lymphogranuloma venereum, and related organism in a new microtiter indirect immunofluorescence test. *Am J Ophthalmol* 1970;70:367-74.
- 21 Mehta CR, Patel NR. Exact logistic regression: theory and examples. *Stat Med* 1995;14:2143-60.
- 22 Cytel Software Corporation. *LogXact-Turbo version 1.1*. Cambridge, MA: Cytel Software Corporation, 1993.
- 23 Brinton LA, Nasca PC, Mallin K, Baptiste MS, Wilbanks GD, Richart RM. Case-control study of cancer of the vulva. *Obstet Gynecol* 1990;75:859-66.
- 24 Ansink AC, Krul MR, De Weger RA, Kleyn JA, Pijpers H, Van Tinteren H, et al. Human papillomavirus, lichen sclerosus, and squamous cell carcinoma of the vulva: detection and prognostic significance. *Gynecol Oncol* 1994;52:180-4.
- 25 Hørding U, Junge J, Dagaard S, Lundvall F, Poulsen H, Bock JE. Vulvar squamous cell carcinoma and papillomaviruses: indications for two different etiologies. *Gynecol Oncol* 1994;52:241-6.
- 26 Milde-Langosch K, Albrecht K, Joram S, Schlechte H, Giessing M, Löning T. Presence and persistence of HPV infection and p53 mutation in cancer of the cervix uteri and the vulva. *Int J Cancer* 1995;63:639-45.
- 27 Trimble CL, Hildesheim A, Brinton LA, Shah KV, Kurman RJ. Heterogeneous etiology of squamous carcinoma of the vulva. *Obstet Gynecol* 1996;87:59-64.
- 28 Daling JR, Sherman KJ. Relationship between human papillomavirus infection and tumours of anogenital sites other than the cervix. *IARC Sci Publ* 1992;119:223-41.
- 29 Brinton LA, Nasca PC, Mallin K, Schairer C, Rosenthal J, Rothenberg R, et al. Case-control study of *in situ* and invasive carcinoma of the vagina. *Gynecol Oncol* 1990;38:49-54.
- 30 Messing MJ, Gallup DG. Carcinoma of the vulva in young women. *Obstet Gynecol* 1995;86:51-4.
- 31 Frisch M, Melbye M, Møller H. Trends in incidence of anal cancer in Denmark. *BMJ* 1993;306:419-22.
- 32 Goldman S, Glimelius B, Nilsson B, Pählman L. Incidence of anal epidermoid carcinoma in Sweden 1970-1984. *Acta Chir Scand* 1989;155:191-7.
- 33 Li J, Li FP, Blot WJ, Miller RW, Fraumeni JF Jr. Correlation between cancers of the uterine cervix and penis in China. *J Natl Cancer Inst* 1982;69:1063-5.
- 34 Graham S, Priore R, Graham M, Browne R, Burnett W, West D. Genital cancer in wives of penile cancer patients. *Cancer* 1979;44:1870-4.
- 35 Aynaud O, Ionesco M, Barrasso R. Penile intraepithelial neoplasia. Specific clinical features correlate with histologic and virologic findings. *Cancer* 1994;74:1762-7.

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