

ORIGINAL ARTICLE

Risk factors for genital HPV DNA in men resemble those found in women: a study of male attendees at a Danish STD clinic

E I Svare, S K Kjaer, A M Worm, A Østerlind, C J L M Meijer, A J C van den Brule

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See end of article for authors' affiliations

Correspondence to: Edith Ingerslev Svare, Danish Cancer Society, Institute of Cancer Epidemiology, Strandboulevarden 49, Box 839, DK-2100 Copenhagen Ø, Denmark; svare@cancer.dk

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Objectives: Genital infection with certain types of human papillomavirus (HPV) is the most important risk factor for cervical cancer. The male sexual partner is supposed to be the vector of the infection. However, the knowledge of risk factors for genital HPV DNA in men is limited. The objective of this paper is to study the risk factors for HPV infection in men and to compare them with those found in women, including the study of whether there are different risk profiles for oncogenic and non-oncogenic HPV types.

Methods: From a sexually transmitted diseases (STD) clinic in Denmark, 216 men were consecutively included. A personal interview was done and material for genital HPV DNA detection was obtained with swabs. HPV DNA was detected by polymerase chain reaction (PCR). Odds ratios (OR) for HPV as well as for oncogenic and non-oncogenic types separately were computed with a 95% confidence interval (CI) by means of unconditional multiple logistic regression.

Results: The most important predictors of any HPV were lifetime number of sex partners (OR = 4.3; 95% CI 1.4 to 13.1 for 25–39 v 1–9 partners), young age, and being uncircumcised. The most important risk factor for oncogenic HPV types was lifetime number of partners, whereas number of partners in the past year and ever having genital warts were risk factors for the non-oncogenic HPV types. Young age predicted risk of both oncogenic and non-oncogenic HPV types.

Conclusions: Most risk factors for HPV DNA detection in men resemble those found in women. As in women, the risk factor profile for the oncogenic HPV types was different from that of the non-oncogenic HPV types.

Genital infection with certain human papillomavirus (HPV) types, especially HPV 16, has consistently been reported as the most important risk factor for cervical neoplasia¹ and the male sexual partner has been implicated as the vector of the virus.² Variables such as age, proximity to first intercourse, and number of male sexual partners (during lifetime and more recently) have consistently been found to predict risk of genital HPV detection in women.^{3–7} Furthermore, in women, there seems to be different profiles of risk factors for acquiring oncogenic HPV types and non-oncogenic HPV types.^{8–11} In men, however, only few studies have focused on risk factors for genital HPV DNA detection and they have shown association to variables related to sexual behavior such as multiple sexual partners, previous sexually transmitted diseases (STDs), and non-use of condoms.^{12–13} Studies of risk factors in men for genital infection with oncogenic HPV types and non-oncogenic HPV types as separate groups have not been done. To improve the understanding of the natural history of genital HPV infection it is important to know if there is gender related differences in the determinants of the infection. Therefore, the objective of this study was to determine the risk factors for HPV infection in men and to compare them with those previously described in women. Moreover, it was the goal to compare the risk factor pattern for oncogenic and non-oncogenic HPV types in men.

METHODS

Study population and questionnaire

On randomly chosen days during March to December 1993, men attending a STD clinic in Copenhagen, Denmark, were consecutively invited to participate in the study. After written informed consent, 216 men were included. Information about

social and demographic factors, smoking, genital hygiene, circumcision, sexual habits, contraceptive use, and history of STDs was obtained by means of a personal interview using a structured questionnaire. Except for HIV, we did not obtain information about the concurrent presence of other STDs. If any clinical samples were taken, the participants were informed about these results but not about the HPV result, as this test was taken for research purposes only.

Biological material

Material for HPV detection was collected by firmly scraping with two pre-wetted cotton tipped plastic shafted swabs over the external genital area; one over glans and sulcus coronarius of the penis, and another over the shaft of the penis, the scrotum, and the perianal region. Immediately afterwards, both swabs were placed in the same plastic tube containing 3 ml phosphate buffered saline (PBS) with 0.005% thiomersal and kept at 4°C until forwarded by mail for analysis. After centrifugation for 10 minutes at 2755 *g*, the pellet was resuspended in 100 µl 10mM TrisHCl, pH 8.3.

Polymerase chain reaction

HPV DNA was detected by polymerase chain reaction (PCR) using general primers (GP) 5/6 which allows the detection of at least 27 mucosotropic HPV types.^{14–15} Type specific (TS) PCR

Abbreviations: GP, general primers; HPV, human papillomavirus; PBS, phosphate buffered saline; PCR, polymerase chain reaction; STD, sexually transmitted disease; TS-PCR, type specific polymerase chain reaction

Table 1 Determinants for HPV positivity (any type)

	No (% positive)	Crude OR	Adjusted OR (95% CI)
Age			
40+	25 (40)	1.0	1.0
30–39	66 (38)	0.9	1.1 (0.4 to 2.9)
25–29	63 (52)	1.7	1.7 (0.6 to 4.9)
18–24	44 (48)	1.4	1.8 (0.6 to 5.9)
Lifetime number of sex partners			
1–9	44 (25)	1.0	1.0
10–24	55 (55)	3.6*	4.1 (1.5 to 11.1)
25–39	38 (55)	3.7*	4.3 (1.4 to 13.1)
40+	61 (44)	2.4*	2.0 (0.6 to 6.7)
Sex partners in the past year			
≤2	79 (34)	1.0	1.0
3–4	46 (50)	1.9	2.5 (1.1 to 5.8)
5–9	46 (48)	1.8	1.3 (0.5 to 3.3)
10+	27 (63)	3.3*	3.6 (1.1 to 12.0)
Genital warts			
Never	142 (42)	1.0	1.0
Ever	51 (53)	1.5	1.1 (0.5 to 2.3)
Circumcision			
No	174 (49)	1.0	1.0
Yes	24 (17)	0.2*	0.2 (0.06 to 0.6)

*95% CI excludes 1.0; Adjusted OR, odds ratio adjusted for the other variables in the table.

for HPV 6, 11, 16, 18, 31, and 33 was performed using combinations of HPV 6, 16, 33 and HPV 11, 18, 31 specific primers. Samples positive for GP-PCR but negative for TS-PCR were characterised as unclassified HPV, HPV X. All samples tested positive for the β globin gene. A cut point for HPV positivity on 100 pg was chosen and 18 samples with a weaker signal were excluded leaving 198 samples for analysis.

Statistical analysis

Odds ratios (OR) were computed with 95% confidence interval (CI) by means of unconditional multiple logistic regression to assess the association between potential risk factors and presence of HPV DNA while simultaneously adjusting for potential confounding variables. OR were computed for any HPV type as well as for oncogenic and non-oncogenic types separately. In all three analyses, the same control group was used—that is, the HPV negative (and β globin positive) men. HPV 16, 18, 31, and 33 were grouped together in the oncogenic group, whereas HPV 6 and HPV 11 formed the non-oncogenic group. The uncharacterised HPV types (HPV X) were only included in the overall analysis of risk factors for HPV (any type). All analyses were done using the SAS software package.¹⁶

RESULTS

Fifty four per cent of the participants were younger than 30 years, and respectively 33% and 13% were 30–39 years and 40 years or older. Most of the men (58%) reported 20 or more partners during lifetime whereas 16 men (8%) had ≤ 4 lifetime partners. Forty per cent, 23%, and 37% of the men reported respectively ≤ 2 , 3–4 partners, and ≥ 5 partners during the past year. A total of 24 men (12%) were circumcised, and among the 46 study subjects who had sex with men, 35 were bisexual. Using condoms at every intercourse, only occasionally, or never was reported by, respectively, 38%, 54%, and 8%. Ever having had genital warts or *Chlamydia trachomatis* was reported by, respectively, 51 (25%) and 49 men (26%), three men were HIV positive, and 50% of the men were current smokers.

HPV DNA was found in 89 men (45%). HPV 16 was the most prevalent type, accounting for 15% of the HPV positives. The other oncogenic types—that is, HPV 18, HPV 31, and HPV 33 contributed with respectively, 8%, 4%, and 3%, and the non-oncogenic types HPV 6 and HPV 11 with respectively, 14% and 8%. HPV of unclassified type (HPV X) was found in 49 men (55%). Six of the HPV positive men (7%) had multiple infections.

Risk factors for HPV positivity (any type)

Risk of HPV increased with a greater number lifetime number of sexual partners. Compared to men with 1–9 partners, men with 10–24 partners had an OR = 4.1 (95% CI 1.5 to 11.1) and men with 25–39 partners had an OR = 4.3 (95% CI 1.4 to 13.1) (table 1). However, for 40 or more partners the risk estimate declined (OR = 2.0 (95% CI 0.6 to 6.7)). Similarly, an increasing number of partners in the past year was associated with an increased risk of HPV being 3.6 times higher for ≥ 10 partners than for ≤ 2 partners (95% CI 1.1 to 12.0). Risk of HPV was significantly lower in circumcised men compared to uncircumcised men and this association could not be explained taking the other variables into account (OR = 0.2 (95% CI 0.06 to 0.6)). Frequency of genital washing, however, was not associated with HPV and there was no clear influence of current condom use (data not shown). Other variables unrelated to risk of HPV DNA detection included age at first intercourse, contact with prostitutes, sexual preference, marital status (including years living with the current partner), years at school, and smoking habits (data not shown).

Risk factors for oncogenic HPV types

Age was the most important determinant for oncogenic HPV types with OR = 4.7 (95% CI 1.0 to 23.4) for men aged 18–24 years compared to those aged ≥ 35 years (table 2). Risk of oncogenic HPV types also increased with number of lifetime sexual partners and was 3.0 times (95% CI 1.0 to 9.6) more common in men with 20–39 partners than in men with fewer partners. However, risk of the oncogenic HPV types was not related to the number of partners in the past year. Oncogenic HPV types were less frequently found in circumcised men than in non-circumcised men, but the association did not reach statistical significance after adjustment (OR = 0.4; 95% CI 0.08 to 1.7). Finally, risk of these HPV types was not related to ever having had genital warts.

Risk factors for non-oncogenic HPV types

As with the oncogenic types, risk of the non-oncogenic HPV types increased with young age, being 6.3 times (95% CI 1.1 to 39.4) more common in the youngest men aged 18–24 years compared with those aged ≥ 35 years. Regarding the variables related to sexual behaviour, number of recent partners was the most important risk factor. Men who reported five or more different sexual partners in the past year were 4.1 times more likely to have non-oncogenic HPV types compared with the

Table 2 Determinants for positivity of oncogenic and non-oncogenic HPV types

	Oncogenic HPV types			Non-oncogenic HPV types		
	No (% positive)	Crude OR	OR ¹ (95% CI)	No (% positive)	Crude OR	OR ² (95% CI)
Age						
35+	36 (8)	1.0	1.0	35 (6)	1.0	1.0
25–34	68 (22)	3.1	3.0 (0.8 to 11.7)	63 (16)	3.1	2.5 (0.5 to 13.7)
18–24	30 (23)	3.3	4.7 (1.0 to 23.4)	29 (21)	4.3	6.3 (1.1 to 39.4)
Lifetime number of sex partners						
1–19	60 (13)	1.0	1.0	56 (7)	1.0	1.0
20–39	32 (28)	2.5	3.0 (1.0 to 9.6)	29 (21)	3.4	2.0 (0.3 to 12.0)
40+	42 (19)	1.5	2.6 (0.8 to 9.0)	42 (19)	3.1	2.3 (0.4 to 14.8)
Number of partners in the past year						
≤4	92 (18)	1.0	1.0	81 (7)	1.0	1.0
5+	42 (19)	1.0	0.6 (0.2 to 1.8)	46 (26)	4.4*	4.1 (1.3 to 13.3)
Genital warts						
Never	100 (18)	1.0	1.0	89 (8)	1.0	1.0
Ever	31 (23)	1.3	1.0 (0.4 to 3.0)	34 (29)	4.9*	5.9 (1.8 to 18.9)
Circumcision						
No	112 (21)	1.0	1.0	105 (15)	1.0	1.0
Yes	22 (9)	0.4	0.4 (0.08 to 1.7)	22 (9)	0.6	0.8 (0.1 to 4.1)

Oncogenic HPV types: 16, 18, 31, and 33. Non-oncogenic HPV types: 6 and 11.

*95% CI excludes 1.0.

OR¹, odds ratio adjusted for age, lifetime number of sex partners, and circumcision; OR², odds ratio adjusted for age, number of sex partners during the past year, and ever having had genital warts.

men who reported fewer partners (table 2). The association with lifetime number of partners was weaker and not statistically significant. Men who had ever had genital warts were 5.9 times (95% CI 1.8 to 18.9) more likely to have non-oncogenic HPV types. No association was found with circumcision.

DISCUSSION

This study shows that the determinants for genital HPV infection in men resemble those previously found in women. Lifetime number of sexual partners was an important predictor of HPV infection in men. In studies of women with a high sexual activity, risk of HPV has been found to level off after a certain number of partners.^{5,7} We find the same pattern for the risk estimates in men. Likewise, the number of recent sexual partners—that is, in the past year—predicts risk of HPV in men with a high sexual activity as it has been found in women.^{5,7}

Decreasing risk of HPV with age has been found in several female populations.^{3–5} We have previously reported that the age specific HPV prevalence declines in the same way among men participating in this study as it does in female attendees from the same clinic.³ It has been suggested that at least part of the explanation for the HPV age pattern in women is an acquired immunity that is strengthened over time due to repeated exposure.⁵ As shown in table 3, men with many recent partners (≥ 10 in the past year) had a lesser risk of HPV if they had been sexually active for many years (≥ 10 years) than if they had been sexually active for a shorter period. A similar relationship between proximity to first intercourse (exposure time) and number of partners in the past year (recent exposure) has previously been described in women.^{5,7} Thus, our data support that some acquired immunity to genital HPV infection also occurs in men.

Some studies,¹⁷ but not all,¹⁸ have pointed to a lower risk of cervical cancer in women whose sexual partner is circumcised. In this study, the risk of HPV DNA in circumcised men was only one fifth of the risk in uncircumcised men, and this could not be explained by other variables such as number of partners and age. Thus, our results may suggest that the female partners of circumcised men are less exposed to cervical cancer because these men are less likely to be infected with HPV.

Different risk determinant profiles for oncogenic HPV types and non-oncogenic HPV types have been found in women.^{8–11} Lifetime number of partners was more strongly related to risk of the oncogenic HPV types than to the risk of the non-oncogenic HPV types. Moreover, other variables related to lifetime exposure were associated with the risk of oncogenic types but not with risk of non-oncogenic types—for example, age^{8,11} and years of sexual activity.^{8,9} A possible explanation could be that the infections with oncogenic types last longer than the infections with the non-oncogenic types. Correspondingly, we find different patterns of risk factors for oncogenic and non-oncogenic HPV types in men. The risk of oncogenic HPV types was related to lifetime number of sexual partners whereas risk of non-oncogenic types was only related to recent sexual behaviour (number of sex partners in the past year). It seems, though, that the hypothesis of oncogenic HPV types causing infection of a longer duration and the non-oncogenic HPV types causing infections of a more transient nature also applies to HPV infection in men. In this study, however, young age seemed to be equally important for the risk of having oncogenic and non-oncogenic HPV types. Whereas ever having genital warts (caused by the non-oncogenic types HPV 6 and 11) was the most important risk factor for current presence of HPV 6 and 11, this variable was not related to risk of the oncogenic HPV types.

Table 3 Combined effect of years since first intercourse and recent number of partners on risk of HPV (any type)

	No (% positive)	Crude OR	Adjusted OR (95% CI)
"Many years" since 1st intercourse/"few" partners last year	32 (28)	1.0	1.0
"Many years" since 1st intercourse/"many" partners last year	4 (25)	0.9	0.7 (0.06 to 8.4)
"Few years" since 1st intercourse/"few" partners last year	139 (45)	2.1	5.2 (1.1 to 25.5)
"Few years" since 1st intercourse/"many" partners last year	23 (70)	5.8*	14.3 (2.3 to 87.3)

*95% CI excludes 1.0; Adjusted OR, odds ratio adjusted for age and lifetime number of partners; Years since first intercourse, few ≤ 9 and many ≥ 10 ; Number of partners last year, few ≤ 9 and many ≥ 10 .

Detection of genital HPV is technically more complicated in men than in women because cells are more difficult to harvest from skin than from moist mucosal surfaces. However, a demand for a high sensitivity of the DNA detection method is fulfilled by the use of the PCR technique.^{14–19} We have chosen to collect cells from all over the external genital surface—that is, the area that might come into contact with the female genital area during intercourse. It is possible that the sampling method used in this study has facilitated sampling of skin types of HPV as well as mucosotropic genital HPV types. Since other HPV types than the mucosotropic are not amplified as well by the GP5/6-PCR, this may explain the high prevalence of HPV X. The HPV X group probably represents a mixture of oncogenic and non-oncogenic types. Supporting this hypothesis, we found the determinants of HPV X to be a mixture of the risk factors described above for the oncogenic and non-oncogenic type (data not shown) and this probably explains some of the variation in the ORs for any HPV compared to the ORs for the specific HPV types—for example, in relation to age and to circumcision status. HPV X was not associated with age and this dominates the analysis of any HPV leaving the estimates insignificant whereas excluding HPV X reveals a strong association to both the oncogenic and the non-oncogenic HPV types. HPV X was strongly related to circumcision status as none of the circumcised men had HPV X so a significant association was found with risk of any HPV but not with risk of the specific HPV types. Since these analyses were done, newer PCR techniques have been developed which type a broader spectrum of HPV types. Thus applying newer PCR techniques (as the 5+/6+ PCR) would probably reduce the number of HPV X but not induce any marked changes in the determinant analysis for overall HPV.

In conclusion, the most important risk factors for HPV detection in men resemble those reported in women—that is, number of sex partners and age. Moreover, the profiles of risk factors for oncogenic and non-oncogenic HPV types correspond well with those found in women. Finally, the lower HPV DNA detection rate observed in circumcised men is consistent with the suggested lower risk of cervical cancer in female partners of circumcised men.

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ETHICS

The study was approved by the Ethical Committee of Copenhagen and Frederiksberg Municipality, Denmark, Journal KF V92–343.

COMPETING INTEREST

There is no competing interests as no organisation may gain or lose financially from the results of this study.

CONTRIBUTION OF AUTHORS

The study was initiated by Dr S Krüger Kjaer. Drs EI Svare and S Krüger Kjaer were responsible for the central coordinating of the study, for training the staff at the STD clinic, for collecting and registration of the data, for the statistical analysis and for the reporting of

the results including writing this paper. Drs AM Worm and A Østerlind were responsible for recruiting participants and for the collecting of data at the STD clinic. Drs CJLM Meijer and AJV van den Brule were responsible for the PCR analysis.

Authors' affiliations

E I Svare, S K Kjaer, Danish Cancer Society, Institute of Cancer Epidemiology, Copenhagen, Denmark
A M Worm, A Østerlind, Copenhagen Outpatient Venereal Clinic, Rudolph Berghs Hospital, Copenhagen, Denmark
C J L M Meijer, A J C van den Brule, Department of Pathology, University Hospital Vrije Universiteit, Amsterdam, The Netherlands

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