Acquisition and Persistence of Human Papillomavirus Infection in Younger Men: A Prospective Follow-up Study among Danish Soldiers

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Abstract

No data is yet available on incidence or persistence of human papillomavirus (HPV) infection in men. We enrolled 374 younger male conscripts (18-29 years) in a prospective study, and they were examined twice with an interval of 6 to 8 months. Data collection included a questionnaire and a sample of cells from the penis for HPV detection using PCR. In addition, the presence of *Chlamydia trachomatis* DNA was assessed in urine samples by means of PCR.The HPV prevalence at the first and second examinations was 33.8% and 31.9%, respectively. The acquisition rate of HPV (overall) during follow-up was 13.8%, and nearly one fourth of the participants were HPV positive at both examinations. Number of sex partners during follow-up was the most important risk factor for acquiring HPV (odds ratio, 17.2; 95% confidence interval, 4.6-64.7, for ≥3 partners versus ≤1 partner). In contrast, acquisition of a new HPV type in initially HPV-positive men was strongly related to having multiple HPV types at enrollment (OR, 4.1; 95% confidence interval, 1.4-12.3). This was also the most important risk factor for HPV persistence together with current smoking and having a high-risk HPV type at enrollment. This is the first study to assess risk factors for acquisition and persistence of HPV. The sexually transmitted nature of the infection is confirmed, and the data point to an important role of having multiple HPV types for persistence. (Cancer Epidemiol Biomarkers Prev 2005;14(6):1528–33)

Introduction

Human papillomavirus (HPV) infection is a common sexually transmitted disease and it has now been established, based on both molecular and epidemiologic evidence, that HPV can cause high-grade cervical intraepithelial neoplasia and invasive cervical cancer. Although genital HPV infections have been extensively studied in different groups of women, the natural history of the infection is still not fully understood, and even less is known about the natural history of HPV in men. Only relatively few studies using a sensitive HPV detection technique as PCR on penile swabs have been conducted (1-7). These studies, all having a cross-sectional design with HPV measurements at only one point in time, generally revealed a similarity in the risk factors for prevalent HPV infection in men and women with number of sex partners as one of the most important determinants. In addition, Svare et al. (4) found different risk factor patterns for oncogenic HPV types and nononcogenic HPV types in males, as has been previously reported for women in some studies (8, 9).

In contrast, the incidence of acquiring an HPV infection and the risk determinants for this have not yet been established. In addition, knowledge about duration, clearance rates, and risk determinants for persistence of the HPV infection is important for our understanding of the natural history. The purpose of the present study was to assess the incidence rate of HPV infection in younger Danish men who were examined twice with an interval of 6 to 8 months. Further, we wanted to examine the risk factors for acquisition of HPV. Finally, it was aim to elucidate factors which are associated with type-specific persistence of HPV.

Materials and Methods

Study Population and Data Collection. All new conscripts starting their mandatory military service at two barracks in the Greater Copenhagen area (Hoevelte and Sjaelsmark) in the autumn 1998 were invited to participate in the study. They were informed about the study orally and in writing, and from August to October 1998, we included 388 men in the study, corresponding to a participation rate of 61%. All participants signed an informed consent and were paid around US$20 for each of two visits for their inconvenience. The study was approved by the scientific ethical committee.

We obtained from all participants penile swabs for HPV testing by means of PCR method. In the present study, analyses were based on cellular material obtained from glans penis and sulcus coronarius using prewetted cotton-tipped (plastic-shafted) swabs. In our hands, this method has yielded the highest amount of cells and the lowest occurrence of β-globin–negative samples, as also reflected in this study (Table 1). The penile swabs were stored in tubes containing 3 mL PBS containing 0.05% methiolate. The tubes were kept at ~4°C until they were sent to the laboratory in Amsterdam at the end of each day. In addition, first voided urine samples were obtained and tested for *Chlamydia trachomatis* DNA using a commercially available PCR assay (Cobas Amplicor, Roche, Basel, Switzerland) as previously described (10).

The soldiers also responded to a structured, self-administered questionnaire, where they provided information on sociodemographic background, contraception, sexual habits, previous sexually transmitted diseases, physical activity, smoking habits, and alcohol consumption.

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Table 1. HPV testing results (PCR) from the first and second examinations of young Danish male conscripts

First examination	Second examination				
	Negative	Positive	Not participating	β-Globin negative	Total
Negative	144	23	33	23	223
Positive	23	60	25	6	114
β-Globin negative	17	3	9	8	37
Total	184	86	67	37	374

About 6 months following the initial visit, the conscripts were invited for a new examination, the mean interval between the two examinations being 6.6 months (range, 5.4-7.8 months). A total of 321 conscripts (~83%) were examined in the follow-up phase of the study. At this second examination, they had a new penile swab taken and urine samples collected, and they also responded to another questionnaire focusing on the time period between the two visits.

We excluded from the analyses men who at the second examination reported never to have had sexual intercourse (n = 14), leaving 374 men for analysis. In addition, we excluded men with penile swabs testing β-globin negative, which applied to 37 samples from the first phase and 37 samples from the second phase. This left 337 men with an HPV result from the first phase, 270 men with a result from the second phase, and 250 men contributing with HPV results from both examinations (Table 1).

Human Papillomavirus DNA Analysis (PCR). The penile swab was collected as described above and stored in 3 mL PBS containing 0.05% methiolate. The samples were sent to the laboratory (Amsterdam, the Netherlands) on the day of collection. After vortexing vigorously, the suspensions were centrifuged for 10 minutes at 3,000 × g. The cell pellets were resuspended in 100 μL of 10 mmol/L Tris-HCl (pH 7.4) and frozen at −80°C.

For PCR, 10 μL pretreated crude cell suspensions of the penile scrapes were boiled for 10 minutes at 100°C, cooled on ice, and centrifuged for 1 minute at 3,000 × g before addition of the PCR mixture. To analyze the quality of the DNA, the penile swabs were first subjected to a 209 bp amplifying β-globin PCR using the primers $BGPCO_3$ and $BGPCO_5$. The detection of a broad spectrum of mucosotropic HPV types was done by a general primer GP5+/6+-mediated PCR-enzyme immunoassay as previously described in detail (11), except that another PCR processor and program was used. Briefly, 10 μL of the crude penile cell suspensions were added to the PCR mixture, which consists of 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 3.5 mmol/L $MgCl_2$, 1 unit of thermostable DNA polymerase (Amplitaq, Perkin-Elmer, Norwalk, CT), 200 μmol of each deoxynucleotide triphosphate, and 25 pmol of each primer (GP5+, Bio-GP6+). The mixture was incubated for 5 minutes at 94°C for DNA denaturation, followed by 40 cycles of amplification using a PCR processor (PTC-225, Biozym, Landgraaf, the Netherlands). The GP-PCR products were analyzed by enzyme immunoassay using two cocktail oligoprobes to identify high-risk HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and low-risk HPVs (6, 11, 26, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 70, 71, 72, 73, 81, 82, 83, 84, and 89). The low-risk oligoprobe contains HPV 73 and 82, but, as these have been epidemiologically classified as high-risk HPV types (12), they were included in the HPV high-risk group in this study. HPV types 26, 34, and 53, also contained in the low-risk probe, have been epidemiologically classified as probably high-risk types (12). In the present analysis, these types stayed in the low-risk group because the oncogenic risk seems less clear (ref. 12; only one man in the present study had HPV 34, and none had HPV 26 or HPV 53). Subsequently, high-risk and low-risk positive samples were subjected to individual typing using enzyme immunoassay and HPV type–specific oligoprobes. Finally, to detect HPV types (named HPV X) which were not identified by high-risk/low-risk enzyme immunoassays, GP-PCR products were subjected to Southern blot analysis under low stringent conditions with a cocktail probe consisting of GP-PCR products that were specific for HPV 6, 11, 16, 18, 31, and 33 (13).

Statistical Analysis. In this follow-up study, we estimated the HPV acquisition rate in men who were HPV negative at the start of the study. The acquisition rate of a specific HPV type was assessed in men who at enrollment were either HPV negative or positive for another HPV type (i.e., the incidence rate of a specific HPV type was calculated as the number of men infected with a certain type at the second examination divided by the number of men who were negative for that HPV type at the first examination). We analyzed the risk factors for acquisition of an HPV infection both among initially HPV-negative men and among men who were HPV positive at enrollment but acquired a new type during follow-up.

We also identified risk determinants for persistent HPV infection, comparing men with persistent HPV infection to those with a transient infection. In this study, an HPV infection was defined as persistent if at least one HPV type detected at enrollment was also detected at follow-up. As we were interested in examining the importance of certain characteristics of the HPV infection (high/low risk types, single/multiple infections) for the risk of acquisition of a new type and persistence, respectively, we excluded men with HPV X infection at enrollment from these analyses. In the risk factor analyses, we used multivariate logistic regression analysis to assess the association between acquisition/persistence and various risk factors, and simultaneously adjusted for potentially confounding factors, the measure of association being odds ratios (OR) with 95% confidence intervals (CI). Initially, we assessed the same variables for all outcomes.

The variables were selected on the basis of the available literature about HPV infection in men and women. In the subsequent analyses of the different outcomes, a variable was retained in the statistical model if it was significantly associated (on a 10% level) with the outcome or if it affected the estimate of other variables.

Results

The 374 men who participated in our study were young with 92% between ages of 19 and 22 years (mean age, 20.3 years; range, 18-29). At enrollment, 45% of the men had ever smoked and 37% were current smokers. The median number of drinks per week was 15 (range, 0-80), and 26.3% of the men reported an alcohol consumption of ≥21 drinks per week. The median time since first intercourse was 4 years (range, 0-15 years). Approximately one third reported to have had ≤3 partners, 40% reported 4 to 5 partners, and 25% had 10 or more sexual partners. Finally, the majority (64%) had been sexually active (intercourse) in the last month before enrollment in the study (data not shown). The distribution of these variables was nearly identical when analysis was restricted to the 337 men with HPV results from the first examination or to the 250 men with HPV results from both examinations (data not shown).

At the initial examination, 114 of 337 men (33.8%) were positive for HPV DNA and a similar HPV prevalence rate was observed at the second examination (86 of 270, 31.9%; Table 1). Among the 250 men who contributed with HPV results from both examinations, 144 men (57.6%) were HPV negative at both occasions, 46 (18.4%) were HPV positive at either enrollment or follow-up, and 60 men (24.0%) were HPV positive at both examinations.

Acquisition of Human Papillomavirus. The acquisition rate of HPV infection during follow-up in men who were HPV negative at baseline was 13.8% (23 of 167; 95% CI, 8.6-19.0). Among the men who were HPV positive at the first examination (n = 83), 10 had HPV X and were excluded from the acquisition analyses. Among the remainders, 39.7% (29 of 73) acquired a new HPV type in the time period between the two examinations. Only 4.3% (95% CI, >0 to 12.7) of the initially HPV-negative men acquired more than one HPV type, whereas 27.6% (95% CI, 11.3-43.9) of the HPV-positive men acquired multiple new types (P = 0.06; Table 2). In contrast to this, there were no significant differences in variables known to be related to the risk of HPV infection such as age, number of sexual partners, and number of years since first intercourse (Table 2).

The type-specific incidences during the 6 to 8 months of follow-up for HPV types with an incidence of >0.5% are shown in Fig. 1. The incidence was highest (1.6-2.6%) for HPV types 16, 42, 11, 6, 51, 31, 56, and 83. The proportion of men ever positive for the different types of HPV at some time during the 6- to 8-month study period (i.e., positive at enrollment and/or at the follow-up examination) ranged from 0.8% (HPV 52) to 10% (HPV 16; data not shown).

The risk factors for acquisition of HPV between the two examinations among initially HPV-negative men are presented in Table 3. Number of sex partners between enrollment and the second examination was a significant risk factor for HPV acquisition; men with ≥3 sex partners have an adjusted OR of 17.2 (95% CI, 4.6-64.7) when compared with men with ≤1 partner. The risk was also related to age (OR, 2.6; 95% CI, 1.0-7.1, for age ≥21 versus ≤20 years). Condom use reduced the risk of acquiring HPV [i.e., compared with men who had not used condoms between the two examinations, those who used it (always/occasionally combined) had a decreased risk (OR, 0.2; 95% CI, 0.1-0.8)]. Three of the initially HPV-negative men acquired HPV infection in spite of reporting no intercourse during the follow-up (two acquired HPV 11 and one acquired HPV 18). Neither smoking nor education exerted any significant effect on the risk. Only one case and two noncases had *C. trachomatis* DNA in the urine at the first examination, yielding a nonsignificant OR of 1.6. No association was observed with self-reported sexually transmitted disease (*Chlamydia*, genital warts; data not shown).

We also looked at the risk factors for acquisition of a new HPV type in men who were HPV positive at enrollment (Table 4). The most important risk determinant for HPV acquisition in this group was having multiple HPV types at enrollment, which increased the risk (OR, 4.1; 95% CI, 1.4-12.3). In addition, presence of *Chlamydia* DNA (urine) at the first examination also seemed to increase the risk of acquiring a new HPV type during follow-up (OR, 5.2; 95% CI, 1.0-27.1). The overall pattern of the other risk factors was similar to that in the group of initially HPV-negative men, with number of partners between the two visits as the most important factor followed by age, although they did not reach statistical significance. Also among the initially HPV-positive men, three acquired HPV (new type) during follow-up in spite of reporting not having had sexual intercourse during this period (one acquired HPV 6, one acquired HPV 73, and one acquired HPV 84 + HPV 39).

Table 2. Characteristics of men acquiring HPV infection

	HPV-negative men acquiring HPV infection (n = 23)	HPV-positive men acquiring a new HPV type (n = 29)
Age (mean)	20.6	20.5
No. of sex partners between the two examinations (mean)	2.3	1.9
Years since first sexual intercourse (mean)	4.5	4.9
Proportion acquiring multiple types	4.3% (0-12.7)	27.6% (11.3-43.9)

Human Papillomavirus Persistence. Among the 73 men testing positive for a known HPV type at enrollment, 42 had at least one identical type detected at follow-up. The risk for persistent HPV infection was most strongly related to infection with multiple HPV types at enrollment (OR, 4.2; 95% CI, 1.3-12.7; Table 5), and infection with a high-risk HPV type increased the risk, although after adjustment, the association was not statistically significant (OR, 2.2; 95% CI, 0.9-7.4). Similarly, current smoking at enrollment was related to an increased risk of persistence; however, this association did not reach statistical significance after adjustment for confounding factors (OR, 2.5; 95% CI, 0.9-7.1). In contrast, current asymptomatic presence of *Chlamydia* DNA in the urine seemed to be related to a decreased risk of HPV persistence, although nonsignificantly (OR, 0.3; 95% CI, 0.1-1.6). Age was not strongly associated with persistence of HPV.

Acquisition and Persistence in Relation to Human Papillomavirus Type at Enrollment. Table 6 shows HPV status at follow-up for men who at enrollment were positive for HPV 16, other high-risk HPV types, or only low-risk HPV types. Among the 19 men who had HPV 16 (+/− other high-risk or low-risk HPV types) at the first examination, 15% became HPV negative, 26% acquired a new type, and 63% had HPV 16 persistence.

In the group of men who did not have HPV 16 but other high-risk types (+/− low-risk HPV types), 26% were HPV negative at follow-up, 44% acquired a new type, and 56% had type-specific persistence. For the corresponding numbers of men who only harbored low-risk HPV types, 30% tested HPV negative at the second examination, 45% had a new HPV type, and 35% still had the same HPV type after the 6- to 8-month follow-up.

Discussion

The risk of incident HPV infection during a 6- to 8-month period was nearly 14% in young Danish male conscripts, pointing to a high level of exposure to HPV among younger men in the military. The determinants for acquisition of HPV infection among HPV-negative men were factors associated with sexual behavior during follow-up, notably number of sex partners, and also condom use between the two visits, supporting the sexual transmission of the infection. In contrast to women, where practically no HPV has been found in cervical swabs from virgins (14, 15) and where acquisition of HPV largely is dependent on sexual intercourse (15), HPV in the present study was detected in 4 of 9 men (21%) who at enrollment reported that they had never had sexual intercourse. In addition, we found that among men reporting no sexual intercourse in the time period between the first and the second examinations, several acquired HPV, notably low-risk types. This may be explained by HPV being transmitted to men also by means of sexual activity not involving intercourse. As HPV is often present in the vulva as well as in the cervix, a noncoital sexual transmission of HPV from woman to man is not an unlikely phenomenon. However, we were not able to confirm this in our study, as we did not obtain information on sexual activity other than sexual intercourse.

Although condom use decreased the risk of acquisition, even among men who reported to use condom at every intercourse, acquisition of HPV could not be avoided. However, Bleeker et al. (16) found that regression of flat penile lesions in male partners of women with cervical intraepithelial neoplasia was HPV dependent and accelerated

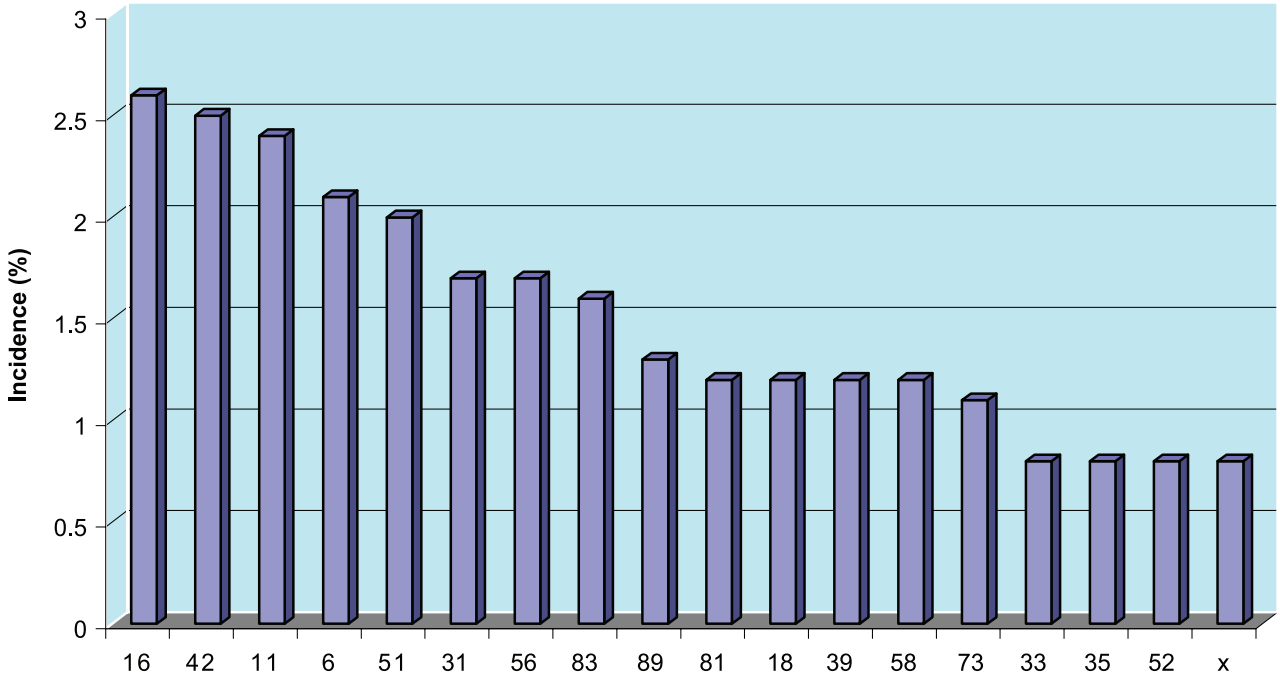

Figure 1. Incidence rates (6-8 months) of specific HPV types in younger Danish men (number of men infected with a certain HPV type at the second examination divided by the number of men who were negative for that type at the first examination). HPV types not mentioned if the incidence was <0.5%.

by condom use, suggesting that this effect is probably the result of blocking viral transmission between several partners. The potential protective effect of condom is still controversial in both men and women, and our results are in line with some (1, 17), but not all (5), previous findings. It now seems like a generally accepted concept that condom use may imply a lesser protection against HPV infection than against several other sexually transmitted diseases like *Chlamydia*, HIV, and gonorrhea (5). The fact that condoms are often not used during the entire intercourse, taken together with the multifocal nature of the HPV infection in women, makes the limited protective effect of condom use in relation to HPV acquisition in men understandable.

Table 3. Risk factors for acquisition of HPV among young Danish male conscripts who were HPV negative at enrollment

Variable	No. of cases/noncases	OR	Adj. OR*	95% CI
No. of sex partners between the two examinations				
0-1	10/113	1.0	1.0	
2	3/16	2.1	2.9	0.6-13.2
≥3	10/14	8.1†	17.2	4.6-64.7
Age				
≤20	13/105	1.0	1.0	
≥21	10/39	2.1	2.6	1.0-7.1
Condom use between at the two examinations				
Never	11/48	1.0	1.0	
No intercourse between the two examinations	3/20	0.5	1.2	0.3-5.4
Yes (always/occasionally)	9/76	0.7	0.2	0.1-0.8
Chlamydia DNA (urine) at the first examination				
No	22/142	1.0	1.0	
Yes	1/2	3.2	1.6	0.2-23.8
Current smoking				
No	17/102	1.0	1.0	
Yes	6/42	0.9	0.6	0.2-2.0
Schooling (y)				
≤10	8/38	1.5	1.1	0.3-3.4
≥11	15/106	1.0	1.0	

*Adjusted for age, condom use, and number of sex partners between the two examinations.
† 95% CI excludes 1.0.

We found that subsequent infection with other HPV types among men who were HPV positive at enrollment was common as nearly one third acquired a new HPV type during the 6- to 8-month follow-up. Having multiple HPV types at enrollment was the most important risk factor for subsequent acquisition of a new HPV type. Whether this finding can be explained by some yet unknown biological mechanism or whether this is due to chance remains to be seen. However, it is noteworthy that these HPV-positive men acquiring a new type were more likely to acquire multiple HPV types than HPV-negative men acquiring HPV during follow-up in spite of the two groups being nearly identical in terms of age, number of partners between the two examinations, and years since first intercourse. It has been hypothesized that the acquisition of one HPV type facilitates the acquisition of another type by some yet unknown biological mechanism (18). Another explanation may be that some individuals have an increased susceptibility, and may thus be more prone to HPV infection(s). Our observation of a high rate of acquisition of multiple HPV types in men is in line with findings in women (18, 19). Due to the small numbers of men within each category of specific HPV types, we were not able to assess whether men with a certain HPV type were less likely (compared with those not having this HPV type) to be infected with another HPV type.

The present study is to our knowledge the first to report on risk determinants for persistence of genital HPV infection in men. The major risk factors were related to the HPV infection itself, as having multiple HPV infections at the start of the study was the most important variable. The results are in agreement with those reported in women as Ho et al. found

Table 4. Risk factors for acquisition of a new HPV type among young Danish male conscripts who were HPV positive at enrollment

Variable	No. of cases/noncases	OR	Adj. OR*	95% CI
Multiple HPV types at the first examination				
No	11/29	1.0	1.0	
Yes	18/15	3.2†	4.1	1.4-12.3
Chlamydia DNA (urine) at the first examination				
No	23/41	1.0	1.0	
Yes	6/3	3.6	5.2	1.0-27.1
No. of sex partners between the two examinations				
0-1	12/29	1.0	1.0	
≥2	17/15	2.7†	2.3	0.8-6.5
Age				
≤20	17/32	1.0	1.0	
≥21	12/12	1.9	1.8	0.6-3.5
Condom use between the two examinations				
Never	12/19	1.0	1.0	
No intercourse between the two examinations	3/5	1.0	1.2	0.2-8.2
Yes (always/occasionally)	13/18	1.1	0.8	0.3-3.2
Current smoking				
No	12/21	1.0	1.0	
Yes	17/23	1.2	0.8	0.3-2.2
Schooling (y)				
≤10	14/20	1.0	1.0	
≥11	14/24	1.2	0.9	0.3-2.7

*Adjusted for number of sex partners between the two examinations, multiple HPV types at enrollment, and presence of Chlamydia DNA (urine) at enrollment.
†95% CI excludes 1.0.

that having multiple HPV types detected at the previous visit was strongly related to risk of HPV persistence in women. Our results could indicate that men who have multiple HPV types may possess certain characteristics, like an impaired immune response, implying that within the time frame of this study

Table 5. Persistence of HPV among young Danish male conscripts

Variable	No. of cases/noncases	OR	Adj. OR*	95% CI
Multiple HPV types at the first examination				
No	16/24	1.0	1.0	
Yes	26/7	5.6†	4.2	1.3-12.7
High-risk HPV type at the first examination				
No	7/13	1.0	1.0	
Yes	35/18	3.6†	2.2	0.9-7.4
Current smoking				
No	17/20	1.0	1.0	
Yes	25/15	2.7†	2.5	0.9-7.1
Chlamydia DNA (urine) at the first examination				
No	39/25	1.0	1.0	
Yes	3/6	0.3	0.3	0.1-1.6
Age				
≤20	27/22	1.0	1.0	
≥21	15/9	1.4	1.2	0.4-3.8
No. of sex partners between the two examinations				
0-1	21/20	1.0	1.0	
≥2	21/11	1.8	1.5	0.5-4.4

*Adjusted for multiple HPV types at enrollment, high-risk HPV type at enrollment, and current smoking at enrollment.
†95% CI excludes 1.0.

Table 6. Acquisition and persistence of HPV according to type of HPV infection at enrollment

HPV status at enrollment	HPV status at follow-up*
HPV 16† (total: n = 19)	3 HPV negative (15%) 5 new type of HPV (26%) 12 HPV 16 persistence (63%)
Non-16 high-risk HPV type† (total: n = 34)	9 HPV negative (26%) 15 new type of HPV (44%) 19 HPV type–specific persistence (56%)
Only low-risk HPV types (total: n = 20)	6 HPV negative (30%) 9 new type of HPV (45%) 7 HPV type–specific persistence (35%)

*Numbers add up to more than total, and percentages add up to >100 as some men had both persistence of a certain HPV type and acquired a new HPV type.
† +/− other high-risk and or low-risk HPV types.

they were less likely to clear the HPV infection. The association between having multiple types at enrollment and HPV persistence (i.e., at least one identical type at follow-up) could not be merely explained by the phenomenon that the more types present, the greater the likelihood that at least one type is still present at the second examination, as among the men with multiple HPV types at enrollment not only one type persisted, but rather several types tended to persist (data not shown).

Our results are also in accordance with the finding of studies on women that the oncogenic HPV types have a higher risk of persistence than the nononcogenic types (19-21). Although based on limited numbers, our data seem to suggest a slightly higher persistence rate for HPV 16 compared with the other oncogenic types, which again had a higher persistence rate than the nononcogenic types—a pattern very similar to observations in women (22).

In this study, smoking was not related to acquisition of HPV. This is in line with most previous studies which failed to find an association between smoking and detection of HPV infection in men (3-5). In contrast, smoking did only seem to associate with the risk of persistence. This may well be in line with the previously suggested immunosuppressive effect of smoking which could reinforce persistence. It is also in agreement with a recent study reporting that female smokers maintained an HPV infection significantly longer than non-smokers (23). However, our results are somewhat in contrast to studies of determinants of HPV persistence in women as Ho et al. (21) found a decreasing risk of HPV persistence lasting 6 months or more with increasing number of cigarettes smoked per day; Hildesheim et al. (20) also reported on a two times decreased risk for HPV type–specific positivity among ever smokers, the median interval between the two examinations being around 15 months (9-30 months). Thus, the potential effect and mechanism of smoking in relation to persistence of HPV is still unknown in both women and men.

Although based on a rather limited number of infected persons, *C. trachomatis* infection, as determined by the detection of *Chlamydia* DNA in the urine at enrollment in the study, seemed to be a risk factor for acquiring HPV, notably the acquisition of a new HPV type in already HPV-positive men. The association was present also after adjustment for the other risk factors including number of partners between enrollment visit and follow-up visit. In contrast, presence of *Chlamydia* infection at the start of the study seemed to decrease the risk of HPV persistence. Whether this is due to chance because of small numbers in the study or is a reflection of a true biological association cannot be disentangled in our study. It could be speculated that the *Chlamydia* infection may cause some immunologic reaction that somehow assists in the

clearance of HPV. Some support for a biological mechanism comes from a recent study reporting results which could indicate a protective effect of *C. trachomatis* against the carcinogenic effect of HPV 16 (24). This effect could be hypothetically mediated through a decreased risk of HPV persistence as our results may point to. However, larger studies are needed to assess the relationship between *Chlamydia* infection and the natural history of HPV infection. It is important to consider the limitations of this study. Due to the fact that the GP5+/6+ PCR method is using one single primer set, the detection of multiple types might be a little underestimated due to competition in case of high copy numbers of a particular HPV type are present together with low copy numbers of another HPV type. The age range in our study population was narrow as only men of ages 18 to 29 years were enrolled, with the majority being 19 to 22 years old. As our study only included two examinations, we were not able to examine the duration of the HPV infection and our definition of persistence was based on prevalent HPV infection. This, taken together with the relatively short interval between the two examinations, may have led to an overestimation of persistence. Larger studies including a broader age range, applying HPV detection at several time points, are needed to better define acquisition, persistence, and clearance of HPV infection.

In spite of these limitations, this study is important as it is the first to report on the frequency and determinants of acquisition and persistence of HPV infection in men. Both acquisition of HPV in men who were HPV negative at baseline and subsequent infection with other HPV types among men who were HPV positive at enrollment were common. The most important risk factor for acquisition of HPV (in HPV-negative men) was number of sex partners during follow-up, whereas the key risk factor for HPV persistence and acquisition of a new HPV type in HPV-positive men was having multiple HPV types at enrollment, corroborating previous findings in women.

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