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HUMAN PAPILLOMAVIRUS TYPES 6 AND 11 SEROPOSITIVITY: RISK FACTORS AND ASSOCIATION WITH ANO-GENITAL WARTS AMONG HOMOSEXUAL MEN

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Abstract

Objectives: Human papillomavirus (HPV) types 6 and 11 are most commonly associated with ano-genital warts. There are few data on the sero-epidemiology of HPV6 and HPV11 among homosexual men.

Methods: Behavioural data and sera for antibodies to HPV6 and HPV11 capsid protein L1 were collected annually for 1427 HIV negative and 245 HIV positive homosexual men. For HIV negative men, a combined variable, HPV6/11, was created (HPV 6 and/or 11) to analyse predictors of seroprevalence and seroincidence.

Results: High rates of HPV6 and HPV11 seroprevalence were found (39.2 - 53.2% of men). For HPV6/11 (HIV negative men only), seroprevalence was associated with higher numbers of sexual partners, longer history of sexual activity and seropositivity for several sexually transmissible infections. Each year, 12.6% of men younger than 25 years seroconverted. Seroincidence (5.9/100PY) was associated with younger age, more recent male sexual partners, receptive anal fingering and anal chlamydia. Seropositivity and seroconversion were strongly associated with past and incident anal warts.

Conclusions: HPV6 and HPV11 seropositivity were common among homosexual men. Among HIV negative men, HPV 6/11 seroprevalence and seroincidence correlated closely with markers of sexual activity. The high numbers of young men seroconverting each year suggests a role for prophylactic vaccination of young gay men.

Summary: Ano-genital warts, caused by human papillomavirus, are very common. In a community cohort of Australian homosexual men, HPV6 and 11 seroprevalence and seroincidence were high and were associated with past and incident anal warts and other markers of sexual activity.

Key words: human papillomavirus, HPV6, HPV11, anal warts, homosexual, MSM

Introduction

Ano-genital warts are an extremely common, highly infectious, sexually transmissible disease manifestation of human papillomavirus (HPV) infection(1). Nearly all ano-genital warts are related to HPV infection with types 6 or 11(2-5) which are primarily transmitted through genital skin-to-skin contact(6). Ano-genital warts are associated with psychological morbidity and substantial health-care costs(7-9). Since 2007, when the quadrivalent HPV (qHPV) vaccine for girls and young women was added to Australia's National Immunization Program, there has been a major reduction in the incidence of ano-genital warts in young women. In addition, there has been evidence of herd immunity among young heterosexual men. However, no decline in genital warts has been seen in homosexual men(10).

Among homosexual men globally, anal HPV6 and HPV11 infection have been reported to be highly prevalent(11-14). Ano-genital warts disproportionately occur in homosexual men, where they are associated with a higher lifetime number of sexual partners(15-17) and positive HIV status(16, 18), among other factors. HPV serology provides an estimate of lifetime cumulative

exposure to or infection with HPV, but there are few data on the seroepidemiology of HPV6 and HPV11 in men and even fewer among homosexual men(12, 19-21).

From 2013, Australia will be the first country in the world to extend its national publicly funded qHPV vaccine program of young girls to boys. By establishing the seroprevalence of qHPV types HPV6 and HPV11 and their correlation with disease, the impact that routine male vaccination program may have on future HPV-associated disease among Australian homosexual men can be measured. To achieve this aim, we explored firstly the prevalence, incidence and risk factors for HPV6 and HPV11 seropositivity, and secondly the association between ano-genital warts and HPV6 and HPV 11 seropositivity in two prospective cohorts of HIV negative and positive homosexual Australian men.

Materials and methods

Study populations

Two prospective community based cohort studies of homosexual men were conducted in Sydney, Australia. The Health in Men (HIM) study involved HIV negative homosexually active men. The methodology for the HIM study has been published previously (22). Men who were 18 years or older, tested HIV negative, had sexual contact with at least one man in the past 5 years, lived in Sydney or had regular contact with gay events and venues in Sydney were eligible to enrol. The study recruited 1427 participants from June 2001 to December 2004 and concluded interviews in June 2007. The Positive Health (pH) study recruited HIV positive men from 1998

to 2006, who reported sex with at least one man during the previous five years or who identified as homosexual, using similar community based methods as the HIM study (23). The HIM and pH studies and the serological research within the two studies received ethics approval from the University of New South Wales Human Research Ethics committee.

Data collection

Participants underwent annual structured face-to-face interviews on a wide range of topics, including sexual relationships and practices. Quantitative sexual behaviour data were collected. At approximately 6 months between annual face-to-face interviews, information on recent sexual relationships and practices and injecting drug use was collected from HIM participants via a short-version telephone interview. In the HIM study, serological testing for HIV and storage of serum samples were undertaken annually. In the pH study, annual serological testing was only performed from 2005 to 2007.

Serum sample collection, shipment and testing

The laboratory methodology has been previously published(24). Briefly, serological analysis was performed at the German Cancer Research Center (DFKZ), Heidelberg, Germany. The samples were analysed for antibodies to the capsid (L1) proteins of the mucosal alpha HPV types 6, 11, 16, 18, 31, 33, 35, 45, 52 and 58 as previously described(25, 26). All samples were analysed for HPV antibodies by glutathione S-transferase (GST)-L1 serology(27, 28) in combination with fluorescent bead technology(25, 29). Cut-points to determine antibody positivity were selected by visual inspection of the distribution of median fluorescence intensity (MFI) values among all study participants(30, 31) and seropositivity was defined as having an MFI value of 400 or more

for all HPV types, except HPV 6 and HPV 35 where more stringent cut-offs of 800 and 600 MFI were used. Seroconversion was defined by two conditions: 1) at least 2-fold increase of the previous serum MFI-value, and 2) MFI-value above the cut-off. The current manuscript describes only the results of the HPV6 and HPV11 L1 data.

Statistical analysis

Firstly, seroprevalences and seroincidence were calculated separately for HPV6 and for HPV11. Seroincidence per 100 person-years (PY) for HPV6 and for HPV11 were calculated in those initially HPV6 and HPV11 seronegative respectively.. Due to the likely cross-reactivity between HPV6 and HPV11 antibody responses(32), a composite variable, “HPV6/11”, was created. A positive anti-HPV6/11 result was defined as positive prevalent or incident serology for either HPV6 and/or HPV11. Predictors of seroprevalent HPV6/11 were analysed by logistic regression. Sociodemographic, biological and behavioural characteristics and their relation to HPV6/11 seroprevalence were examined. These included: age, occupation, education, circumcision status, level of gay community involvement, lifetime number of male sexual partners, lifetime number of female sexual partners, duration since first sexual intercourse, duration since first anal sexual intercourse, cigarette smoking, marijuana use, past history of syphilis, serological evidence of past infection with hepatitis A, hepatitis B, hepatitis C, HSV-1 and HSV-2. Association with a history of anal warts and/or history of genital warts was analysed separately. In the pH study, very limited data were collected on lifetime sexual risk behavioural factors and no data were collected on ano-genital warts. Thus, risk factors for HPV6/11 seropositivity and association with warts were only examined in HIV negative HIM participants.

Predictors of seroincident HPV6/11 among participants seronegative to both at baseline were analysed by Cox regression for HIV negative participants only. Similar to the analysis of seroprevalence, sociodemographic, biological and behavioural characteristics and their relation to HPV6/11 seroincidence were examined. These included: age, occupation, education, circumcision status, level of gay community involvement, number of male sexual partners in the past 6 months, cigarette smoking, marijuana use, recent report (in the past six months) of unprotected anal intercourse (UAI) by partner type (regular or casual), UAI by anal intercourse position (insertive or receptive), UAI by partner's HIV status (positive, negative or unknown), receptive oral sex with casual partners, insertive oral sex with casual partners, receptive rimming, receptive fingering and receptive fisting, reported urethral gonorrhoea and chlamydia, pharyngeal gonorrhoea, anal gonorrhoea and chlamydia, past syphilis infection, incident hepatitis A, incident hepatitis B, incident hepatitis C, incident HSV-1, incident HSV-2. Prevalent and incident anal warts as well as prevalent and incident genital warts were analysed separately.

Multivariate models were developed using a backwards selection method. A significance threshold of a p of 0.10 or smaller in the univariate models was used to identify variables to be included in the final multivariate models. Odds ratios (ORs) and hazard ratios (HRs) with corresponding 95% confidence intervals (CIs) were calculated for seroprevalent and seroincident HPV6/11 risk factors respectively.

Data analyses were performed using Stata 10.0 (Stata Corporation, College Station, TX, USA).

Results

A total of 1427 initially HIV-negative men were enrolled in the HIM study, and a total of 5959 serological samples were available for analysis. The median age at enrolment was 35 years (range 18–75 years) and men were followed for a mean of 3.5 years, with a total follow-up time of 4538 PY. In the pH study, a total of 245 men had sera collected at least once, with 394 serological samples stored. The median age at enrolment was a decade older than in the HIM study (45 years, range 22–71 years).

Almost half (46.9%, 95% CI 44.2–49.6%) of the HIV negative participants were HPV6 seropositive and over a third (39.2%, 95% CI 36.5–41.8%) were HPV11 seropositive at baseline. Similar results for HPV6 seroprevalence were seen for the HIV positive participants (53.2%, 95% CI 46.1–60.2%), whereas HPV11 seroprevalence was higher (48.3%, 95% CI 41.2–55.4%) among HIV positive participants. In contrast, HPV seroconversion occurred much more frequently among HIV negative men. HPV6 seroconversion was almost twice as common among HIV negative (4.3/100PY, 95% CI 3.5–5.3/100PY) than among HIV positive men (2.4/100PY, 95% CI 0.8–7.3/100PY) and HPV11 seroconversion was almost three times as common among HIV negative (4.3/100PY, 95% CI 3.5–5.2/100PY) than among HIV positive men (1.5/100PY, 95% CI 0.4–5.9/100PY). For the combined variable HPV6/11, Over half the HIV negative participants (55.3%, 95% CI 52.6–58.0%) and almost two thirds of the HIV positive participants (55.3%, 95% CI 52.6–58.0%) were HPV6/11 seropositive at baseline. Overall HPV6/11 seroconversion was 5.9/100PY (95% CI 4.9–7.2/100PY) and 4.0/100PY (95% CI 1.50–10.6) for HIV negative and HIV positive men respectively. These results should be interpreted with

caution, as numbers were small in the pH study leading to wide confidence intervals, and HIV positive men were on average a decade older than HIV negative men.

Predictors of HPV6/11 seroprevalence among HIV negative men

In univariate analysis among HIV negative men, older age (p for trend <0.001), greater involvement in the gay community (p for trend =0.007), higher numbers of lifetime male (p for trend <0.001) and female partners (p=0.024), longer duration since first sexual intercourse (p for trend <0.001) and first anal intercourse (p for trend <0.001), marijuana use (p for trend =0.002) and serological evidence of a number of STIs were all significantly associated with seroprevalent HPV6/11 (Table 2). HPV6/11 seropositivity was not associated with the other factors examined, including circumcision or cigarette smoking. In multivariate analyses, seroprevalent HPV6/11 was associated with a higher number of lifetime male sexual partners (p for trend <0.001), longer duration since first anal intercourse (p for trend =0.02) and serological evidence of past infection with hepatitis B virus (HBV) (OR=1.59, 95%CI 1.17-2.16) and HSV type 2 (OR=1.62, 95%CI 1.10-2.37 (Table 2).

Predictors of HPV6/11 seroincidence among HIV negative men

Among the 569 HIV negative men who were seronegative to both HPV6 and HPV11 at baseline and completed at least one follow-up visit, overall HPV6/11 seroincidence was 5.9/100PY (95% CI 4.9-7.2/100PY), 108 men seroconverted to HPV6 and/or HPV11, with 44 men seroconverting

to HPV6 and HPV11, 33 men to HPV6 only and 31 men to HPV11 only. HPV6/11 seroincidence declined with age from 12.6/100 PY (95% CI 7.6- 20.9/100 PY) in those aged less than 25 to 4.3/100 PY (95% CI 1.9- 9.6/100 PY) in those aged 55 or older. This decrease occurred rapidly after 25 years of age, with seroincidence falling to 6.0/100 PY in those aged between 25 and 35 years. In univariate analyses, behavioural predictors of HPV6/11 seroconversion included reporting in the past six months higher numbers of male sexual partners (p for trend=0.004), unprotected anal intercourse (UAI) with both casual and regular partners ($p=0.028$) and with partners of unknown or positive HIV status (p for trend =0.032). Some receptive non-intercourse anal sexual practices were also strongly associated with seroincident HPV6/11, including receptive rimming (HR=1.59, 95% CI 1.04-2.42) and receptive fingering (HR=1.81, 95% CI 1.15-2.86) in the past six months. Several recent bacterial STIs were significantly associated with HPV6/11 seroconversion in the univariate model, though there was no association with any viral STIs (Table 3). In multivariate analyses, seroincident HPV6/11 remained significantly associated with younger age (p for trend =0.034), higher numbers of recent male sexual partners in the past 6 months (p for trend =0.031), receptive anal fingering (HR=1.81, 95% CI 1.11-2.98) in the past six months and anal chlamydia (HR=3.04, 95% CI 1.44-6.42) (Table 3).

Association between HPV6/11 seropositivity and ano-genital warts among HIV negative men

At baseline, of those men with available HPV serology results and information on warts, 934 (75.3%) reported no history of ano-genital warts, 196 (15.8%) reported a history of anal warts only, 61 reported genital warts only (4.92%), and 50 reported both (4.03%). HPV6/11 seroprevalence was 52.1% among those with no history of ano-genital warts. In comparison,

seroprevalence was significantly raised in those with anal (75.5%, $p < 0.001$) and both anal and genital warts (78.0%, $p < 0.001$), but was not raised in those with genital warts only (52.5%, $p = 0.96$).

HPV6/11 seroconversion during the study was strongly associated with a baseline history of anal warts (HR 4.02, 95%CI 2.33-6.96, $p < 0.001$) and of genital warts (HR 3.01, 95%CI 1.22-7.40, $p = 0.016$). Overall, there were 89 cases of anal warts and 52 cases of genital warts reported during the study period. Reporting anal warts (HR 5.64, 95%CI 3.14-10.12, $p < 0.001$) and genital warts (HR 3.59, 95%CI 1.32-9.79, $p = 0.012$) during follow up were also highly predictive of HPV6/11 seroconversion. Of those men with no history of anal warts at baseline, 59 reported incident anal warts during the study period. Of the 53 of these men who had HPV serology at baseline and at least once during follow-up, 39.6% (21) were HPV6/11 seropositive at baseline. Eighteen (56.3%) of the remaining 32 men seroconverted during the study; 4 (22.2%) seroconverted up to three years prior to developing incident anal warts, 11 (61.1%) seroconverted in the same interview round as they reported incident anal warts and three (16.7%) seroconverted between one and three years later. Of those men with no history of genital warts at baseline, 38 reported incident genital warts during the study period. Of the 34 men who had HPV serology at baseline and at least once during follow-up, 50% (17) were HPV6/11 seropositive at baseline. Nine (52.9%) of the remaining 17 men seroconverted during the study; 4 (44.4%) seroconverted up to three years prior to developing incident genital warts, 4 (44.4%) seroconverted in the same interview round as they reported incident genital warts and one (11.1%) seroconverted between one and three years later.

Discussion

In two community based cohorts of Australian homosexual men HPV6 and HPV11 seropositivity was very common among both HIV negative and HIV positive men, with around half of the men seropositive at enrolment. More than 10% of HIV negative men younger than 25 years seroconverted to the composite variable, HPV6/11 each year. Among HIV negative men, those with a history of anal warts were around five times more likely to be HPV6/11 seropositive. There was a very strong relationship between seroincident HPV6/11 and past and incident anal warts, though a weaker association was apparent with past genital warts. Among HIV negative men, both HPV6/11 seroprevalence and seroincidence correlated closely with markers of sexual activity, including non-intercourse receptive anal sexual practices.

The HPV6 and HPV11 seroprevalence found in this research is comparable to, though slightly higher than, findings from the few other published studies in homosexual men, though the prevalence figures in the cited studies have been determined with different assay systems and individual cut-off definitions and must therefore be viewed with caution. In a sub-study of the HPV in Men study, a multi-national longitudinal study of HPV infection in men, HPV6 and HPV11 seroprevalence were 34.4% and 31.3% respectively among 64 men who have sex with men (MSM). Combined HPV6/11 seroprevalence was 51.6% (95% CI: 38.7-64.2)(12). A 41% HPV6 seroprevalence among 101 homosexual HIV negative men and a 32% HPV6 seroprevalence among 154 homosexual HIV positive men was reported in a serosurvey

conducted in Seattle, USA(33). In a clinic-based Austrian study including 59 HIV positive homosexual men, 21 (35.6%) were HPV6/11 seropositive(21). In a nationally representative US cross-sectional national survey, HPV11 seroprevalence among MSM (27.7%) was lower than our findings (34). Not surprisingly, HPV6/11 seroprevalence was also much lower in a study of 602 young HIV negative MSM with less than five lifetime sexual partners, where HPV6, HPV11 and HPV6/11 seropositivity at enrolment were 14.0%, 6.4% and 18.2%, respectively(19).

The only other Australian study to describe HPV seroprevalence among men found an overall HPV6 seroprevalence of 9.1% and HPV11 seroprevalence of 5.2% in the male general population aged older than 15 years(35). Similar findings have been reported in a number of US studies involving heterosexual men (12, 34, 36) providing consistent evidence that heterosexual men have lower HPV6/11 seroprevalence than homosexual men. This disparity between heterosexual and homosexual men may be due to differences in the number of sexual partners and/or to decreased antibody production in response to infection of the squamous epithelium of the penis from vaginal intercourse compared with infection of the mucosal surfaces of the anal area as well as the squamous epithelium of the peri-anal area from receptive anal intercourse (12, 37, 38).

Age was strongly related to HPV6/11 seroincidence and seroprevalence in this study, with over one in 10 men younger than 25 seroconverting each year. This high seroincidence among young HIV negative men suggests that quadrivalent HPV vaccination of young men may be effective in preventing HPV-related disease. This key finding provides support for a substantial impact of vaccination programs among young men, especially those targeted at young MSM. Conversely,

older men were much more likely to be seropositive at enrolment. This is consistent with the only other study reporting the association with age and HPV6/11 seroprevalence among MSM, where HPV 6 seroprevalence was associated with being over 35 years of age (OR, 2.2; 95% CI: 1.0-4.8, $p=0.06$)(33). In other studies where findings included both heterosexual and homosexual men, HPV 6 and 11 seroprevalence increased with age, peaking between 35 and 44-49 years and then declined in older age(12, 34).

This continued high HPV6/11 seroprevalence into older age among homosexual men may represent more persistent HPV infection in the anus than at other sites. As a substantial proportion of homosexual men continue to be sexually active into their later years(39), it also may reflect frequent re-infection or re-exposure. Other risk factors associated with seroprevalent HPV6/11, such as higher numbers of lifetime male sexual partners and duration since first sexual intercourse are consistent with the findings from other studies(12, 34). The association between serological evidence of past infection with HBV and HSV2 and seroprevalent HPV6/11 may reflect the sexual behavior of participants, with positive serology for HBV and HSV2 a marker for higher levels of sexual activity. Our findings of an association between HPV6/11 seroconversion and receptive anal practices such as fingering but not with genital exposures, further strengthen the theory that anal mucosal exposure leads to greater induction of a systemic antibody response(12, 37). As with seroprevalence, the association of HPV6/11 seroconversion with anal chlamydia may be a marker of higher levels of sexual activity. This is supported by the fact that reporting higher numbers of male sexual partners in the past six months remained significantly associated with HPV6/11 seroconversion in the multivariate model.

Ano-genital warts were common in this cohort of HIV negative men, with anal warts almost twice as common as genital warts. The self-reported incidence rates of genital and anal warts were 0.94 and 1.92 per 100 person years, respectively (15). Our major finding of a very strong association between HPV6/11 seroprevalence and seroincidence and both past and incident anal warts (and less strongly with past and incident genital warts) reinforces findings from other studies of this association among homosexual men(21, 33). Only three quarters of men with a history of anal warts and one half of men with a history of genital warts at baseline had positive HPV6/11 serology, indicating that serological testing underestimates the cumulative incidence of HPV infection. This disconnect between clinical history and serological status may represent a delay in the development of an immune response or indeed the fact that many men may never develop a humoral immune response to HPV6/11(37). It could also indicate that the seropositivity cut-off points chosen to ensure high type-specificity may have been set too high and seropositivity and seroconversions may be underestimated.

The HIM and pH studies have the strength of being large-scale prospective cohort studies. The samples were primarily community based, and we believe that they can be considered as broadly representative of homosexual men in Sydney. We were able to correlate HPV6/11 seroprevalence and seroincidence with prevalent and incident anal and genital warts. Both are areas with limited published data available.

There were several limitations in this research. Multiplex serology was utilised, a serological method which uses an L1 capsomer fluorescent bead system, rather than the more widely used

systems based on virus-like particles (VLPs) either in ELISA or bead format. Though this methodology has been used in numerous other studies, the other publications with HPV 6 & 11 antibody data referred to here, used the VLP-based antibody methodology.. These differences in serological methods and variations between cut-off definitions for positivity between laboratories may limit direct comparisons with other studies. It is highly likely that there is some cross-reactivity between HPV6 and HPV11 antibody responses with the Luminex system(32). To account for this, a composite variable was created and seroprevalence, seroincidence and risk factors were assessed using this new variable. HPV seroconversion can occur up to 18 months after HPV DNA detection (29), and thus it is likely that some seroincident cases represented infection prior to study entry. Even though HPV serology is believed to be type-specific, it is only a moderately sensitive measure of infection. Therefore, with estimated seroconversion rates of 60% in women and even lower estimated rates in men(40) , not all those infected with HPV will develop antibodies(4, 41). The true HPV infection rate is potentially underestimated, which may lead to misclassification bias(20, 41). Exposure misclassification may also occur due to waning of antibodies over time(42) and due to varying immune response based on exposure via mucosal rather than squamous epithelium, as mentioned previously and also to viral load differences depending on the site of infection.

These results clearly demonstrate that HPV6/11 seropositivity is common among both HIV negative and HIV positive homosexual men and is strongly associated with past and present anogenital warts. HPV6/11 seroprevalence and seroincidence correlated well with markers of sexual activity and with receptive non-intercourse anal sexual practices. With more than one in 10 men younger than 25 seroconverting each year, prophylactic vaccination of young homosexual men

could potentially lead to a dramatic decrease in the burden of this costly and distressing HPV-related disease.

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Conflict of interest:

IMP, FJ, DJT, GP and MP have no conflicts of interest. TW is currently employed by Boehringer Ingelheim. BD has received research funding from CSL Biotherapies Ltd, and has been on the speakers' bureau for Merck and Sanofi-PasteurMSD. CKF has received honoraria and research funding from CSL Biotherapies. CKF owns shares in CSL Biotherapies the manufacturer for Gardasil and has received honoraria from Merck. SMG has received advisory board fees and grant support from CSL and GlaxoSmithKline and lecture fees from Merck, GSK and Sanofi Pasteur. SMG has received funding through her institution to conduct HPV vaccine studies for MSD and GSK, is a member of the Merck Global Advisory Board and the Merck Scientific Advisory Committee for HPV. AEG has received consulting fees and conference sponsorship from Merck, the manufacturer of the Gardasil quadrivalent HPV vaccine, and research funding, consulting fees and conference sponsorship from CSL Biotherapies, the Australian distributor of the Gardasil vaccine.

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Table 1. Seroprevalence and seroincidence of HPV6, HPV11 and HPV6/11 in HIV positive and HIV negative men

	Seroprevalence				Seroincidence		
	N	%	95% CI	N	Incidence per 100PY	95% CI	per 100PY
HIV negative men							
HPV6	625	46.9	44.2 - 49.6	91	4.3	3.5 – 5.3	
HPV11	522	39.2	36.5 – 41.8	90	4.3	3.5 – 5.2	
HPV6/11	737	55.3	52.6 – 58.0	108	5.9	4.9 – 7.2	
HIV positive men							
HPV6	108	53.2	46.1 - 60.2	3	2.4	0.80 – 7.3	
HPV11	98	48.3	41.2 – 55.4	2	1.5	0.40 – 5.9	
HPV6/11	125	61.6	54.5 – 68.3	4	4.0	1.50 – 10.6	

Table 2. Univariate and multivariate risk factors for seroprevalent HPV6/11 in a cohort of HIV negative men

	Univariate		OR	95% CI	p value	Multivariate		
	n ¹	%				Adjusted OR	95% CI	p value
Age								
<25	54	38.6	1	---	p ² <0.001			
25-34	256	50.4	1.62	1.10-2.37				
35-44	280	61.5	2.55	1.73-3.76				
45-54	119	68.0	3.38	2.12-5.39				
>54	28	50.9	1.65	0.88-3.10				
Gay community involvement								
Not at all	29	53.7	1	---	p ² =0.007			
Not very	202	50.1	0.87	0.49-1.53				
Somewhat	360	56.3	1.11	0.63-1.94				
Very	146	61.9	1.40	0.77-2.54				
Number of lifetime male partners								
0-10	39	36.5	1	---	p ² <0.001	1	---	p ² <0.001
11-50	113	40.2	1.17	0.74-1.86		0.86	0.52-1.40	
51-200	198	57.0	2.31	1.48-3.61		1.43	0.87-2.33	
201-500	156	65.3	3.23	2.04-5.27		1.80	1.05-3.07	
>500	232	64.6	3.19	2.03-4.99		1.57	0.93-2.65	
Number of lifetime female partners								
0	290	51.6	1	---	p ² =0.024			
1	103	53.9	1.10	0.79-1.53				
2-5	238	60.7	1.45	1.12-1.88				
>5	106	56.4	1.21	0.87-1.69				
Duration since first sex (yrs)								
0-9	135	42.2	1	---	p ² <0.001			
10-17	179	53.1	1.55	1.14-2.11				
18-25	201	62.2	2.26	1.65-3.10				
>26	222	62.9	2.32	1.70-3.17				
Duration since first anal sex (yrs)								
0-6	129	40.3	1	---	p ³ <0.001	1	---	p ² =0.02
7-11	162	54.4	1.76	1.28-2.43		1.40	0.99-1.99	
12-20	221	61.4	2.35	1.73-3.20		1.59	1.12-2.26	
>21	224	65.9	2.86	2.08-3.92		1.56	1.06-2.32	
Marijuana use								
Never	303	51.7	1	---	p ³ =0.002			
Once or twice	196	55.5	1.17	0.89-1.52				
Once a month	84	54.9	1.34	0.80-1.63				
>Once a month	154	64.2	1.67	1.23-2.28				
HSV-1 serology								
Negative	157	48.5	1	---	p ³ =0.003			
Positive	569	58.0	1.47	1.14-1.89				
HSV-2 serology								
Negative	517	51.5	1	---	p ³ <0.001	1	---	p ³ =0.003
Positive	209	69.4	2.14	1.63-2.82		1.59	1.17-2.16	
Hepatitis A								

Negative	194	48.1	1	---	p ³ =0.001			
Positive	531	58.3	1.51	1.19-1.91				
Hepatitis B								
Negative	166	44.7	1	---	p ³ <0.001	1	---	p ³ =0.014
Past infection	175	69.2	2.77	1.98-3.88		1.62	1.10-2.37	
History of anal warts								
No	519	52.2	1	---	p ³ <0.001			
Yes	188	75.5	4.94	1.13-21.62				

1) n= number of participants who are HPV16 seropositive; 2) p for trend; 3) p for heterogeneity

Table 3. Univariate and multivariate risk factors for seroincident HPV6/11 in a cohort of HIV negative men

	Univariate		Multivariate					
	PY	n ¹	HR	95% CI	p value	HR	95% CI	p value
Age								
<25	119	15	1	---	p ² =0.021	1	---	p ² =0.034
25-34	604	36	0.45	0.25-0.83		0.46	0.24-0.87	
35-44	673	37	0.42	0.23-0.77		0.39	0.21-0.74	
45-54	282	14	0.37	0.18-0.77		0.45	0.21-0.96	
>54	139	6	0.32	0.12-0.82		0.30	0.11-0.86	
Number of male partners in past 6 months								
0-1	463	18	1	---	p ² =0.004	1	---	p ² =0.031
2-5	423	21	1.24	0.66-2.32		1.11	0.59-2.10	
6-10	298	20	1.69	0.90-3.20		1.51	0.79-2.89	
11-50	461	31	1.64	0.92-2.93		1.42	0.78-2.58	
>50	81	11	3.62	1.70-7.68		3.12	1.44-6.74	
Unprotected anal intercourse (UAI) in past 6 months by partner type								
No UAI	736	35	1	---	p ² =0.028			
UAI with regular	640	38	1.26	0.80-2.00				
UAI with casual	270	21	1.59	0.93-2.74				
UAI with both	168	14	1.81	0.97-3.38				
Unprotected anal intercourse (UAI) in past 6 months by partner HIV status								
No UAI	736	35	1	---	p ² =0.032			
UAI with negative	658	42	1.33	0.85-2.08				
UAI with unknown	371	25	1.46	0.87-2.44				
UAI with positive	49	6	2.60	1.09-6.21				
Receptive rimming in past 6 months								
No	693	30	1	---	p ³ =0.032			
Yes	1125	78	1.59	1.04-2.42				
Receptive fingering in past 6 months								
No	634	24	1	---	p ³ =0.010	1	---	p ³ =0.019
Yes	1184	84	1.81	1.15-2.86		1.81	1.11-2.98	
Anal gonorrhoea								
Negative	1467	83	1	---	p ³ =0.025			
Positive	33	5	2.84	1.14-7.08				
Anal chlamydia								
Negative	1443	80	1	---	p ³ =0<0.001	1	---	p ³ =0.004
Positive	51	9	3.53	1.77-7.06		3.04	1.44-6.42	
Urethral Chlamydia								

Negative	1498	86	1	---	p ³ =0.045
Positive	3	1	7.58	1.04-55.17	
History of anal warts					
No	1750	93	1	---	p ³ <0.001
Yes	63	15	4.02	1.21-4.47	
Incident anal warts					
No	1774	95	1	---	p ³ <0.001
Yes	40	13	5.64	3.14-10.12	
History of genital warts					
No	1784	103	1	---	p ³ =0.016
Yes	30	5	3.01	1.22-7.40	
Incident genital warts					
No	1792	104	1	---	p ³ =0.012
Yes	21	4	3.59	1.32-9.79	

1) n= number of participants who are HPV16 seropositive; 2) p for trend; 3) p for heterogeneity