The Journal of Infectious Diseases

MAJOR ARTICLE

Sequential Acquisition of Anal Human Papillomavirus (HPV) Infection Following Genital Infection Among Men Who Have Sex With Women: The HPV Infection in Men (HIM) Study

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Background. The purpose of this study was to assess the risk of sequential acquisition of anal human papillomavirus (HPV) infection following a type-specific genital HPV infection for the 9-valent vaccine HPV types and investigate factors associated with sequential infection among men who have sex with women (MSW).

Methods. Genital and anal specimens were available for 1348 MSW participants, and HPV genotypes were detected using the Roche Linear Array assay. Sequential risk of anal HPV infection was assessed using hazard ratios (HRs) among men with prior genital infection, compared with men with no prior genital infection, in individual HPV type and grouped HPV analyses.

Results. In individual analyses, men with prior HPV 16 genital infections had a significantly higher risk of subsequent anal HPV 16 infections (HR, 4.63; 95% confidence interval [CI], 1.41–15.23). In grouped analyses, a significantly higher risk of sequential type-specific anal HPV infections was observed for any of the 9 types (adjusted HR, 2.80; 95% CI, 1.32–5.99), high-risk types (adjusted HR, 2.65; 95% CI, 1.26, 5.55), and low-risk types (adjusted HR, 5.89; 95% CI, 1.29, 27.01).

Conclusions. MSW with prior genital HPV infections had a higher risk of a subsequent type-specific anal infection. The higher risk was not explained by sexual intercourse with female partners. Autoinoculation is a possible mechanism for the observed association.

Keywords. human papillomavirus (HPV); sequential genital and anal HPV infections; men; HIM study.

Few studies have reported the natural history of anal human papillomavirus (HPV) infections among men [1–4]. In previous studies, anal HPV prevalence was observed to be 47.2% among men who have sex with men (MSM) and 12.2% among men who have sex with women (MSW) [2]. Presence of anal HPV among men who have sex exclusively with women raised questions as to how infection at this anatomic site occurs. One of the proposed mechanisms is autoinoculation, whereby genital HPV infection is transferred by the heterosexual men to the anal epithelium.

Studies evaluating anal infection among women have considered the possibility of sequential cervical and anal infections. In the few studies evaluating concurrent and sequential cervical and anal HPV infections among women [5–10], relative risk estimates as high as 14.2 for anal HPV infection among women with previous high-risk HPV type cervical infections have been reported [6]. Acquisition of anal HPV infections among women was associated with having receptive anal sex [6, 7, 10], which is a less likely explanation for heterosexual men. However, having receptive anal sex did not entirely explain the high rate of sequential cervical to anal HPV infection among women [6, 7, 10]. Thus, autoinoculation or partner-assisted inoculation by transference of previous cervical HPV infection may explain a proportion of the anal HPV infections observed among women.

To date, studies examining sequential genital-anal infections have not been conducted among men. In the current study, we assessed type-specific (genotype-concordant) sequential acquisition of anal HPV infection following a genital HPV infection for 9 vaccine HPV types (HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58) among MSW participants in the HPV Infection in Men (HIM) study cohort. We also investigated factors associated with sequential genital to anal infections.

METHODS

Study Population

The HIM study recruited participants from the United States, Brazil, and Mexico from 2005 to 2009. Study methods and

Received 2 May 2016; accepted 21 July 2016; published online 3 August 2016.

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The Journal of Infectious Diseases® 2016;214:1180–7

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DOI: 10.1093/infdis/jiw334

recruitment of participants have been described in detail previously [11, 12]. In brief, 4123 healthy men from 3 study sites were followed every 6 months for a median follow-up time of 4 years. Eligibility criteria for the participants were (1) age of 18–70 years; (2) residence at one of the study sites; (3) no previous diagnosis of penile or anal cancers; (4) no previous diagnosis of genital warts; (5) no symptoms of a sexually transmitted infection (STI) and no current receipt of STI treatment; (6) no current participation in an HPV vaccine study; (7) no history of human immunodeficiency virus (HIV) infection or AIDS; (8) no history of imprisonment, homelessness, or drug-abuse treatment during the past 6 months; and (9) willingness to comply with 10 scheduled visits every 6 months for 4 years, with no plans to relocate during that time. From these study participants, optional consent was obtained for collection of anal canal exfoliated cell samples. Computer-assisted self-interviewing was used to obtain extensive sexual history and demographic characteristics. Signed informed consent was obtained from all eligible participants, and approval was obtained from the human subjects committees of the University of South Florida (United States), Ludwig Institute for Cancer Research (Brazil), Centro de Referencia e Treinamento em Doencas Sexualmente Transmissíveis e AIDS (Brazil), and Instituto Nacional de Salud Publica de Mexico (Mexico).

External Genital and Anal HPV DNA Samples

Genital cell samples from the coronal sulcus/glans penis, penile shaft, and scrotum were collected with 3 prewetted polyethylene terephthalate–tipped swabs, which were later combined to form a single genital sample [11, 12]. After collection of genital specimens, 360 degrees of the anal epithelium was swabbed between the anal opening and the anal canal dentate line, using a separate swab, among participants who provided consent (81%) for anal specimen collection [1–3]. These swabs were placed in specimen transport medium and stored at −80°C until analyses. Genital and anal HPV DNA was extracted from these samples by the QIAamp DNA Blood Mini Kit (Qiagen). HPV genotyping was performed using the Roche Linear Array assay to detect 37 HPV genotypes, and human β-globin was tested to assess specimen adequacy [13].

Statistical Analysis

HPV types in the 9-valent vaccine (HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58) were assessed for sequential type-specific genital-anal infections. From the HIM study cohort of 4.123 men, 81% of MSW consented for collection of anal canal specimens at baseline and the first follow-up visit [1]. Subjects who did not provide consent were more likely to be of younger age group (18–30 years) and to be less educated (education duration, ≤12 years). Subjects who ever reported having oral or anal sex with a man during the follow-up were excluded from the analysis, which consisted of both MSM and men who have sex with men and women. Anal HPV data from 2 substudies that included baseline and multiple follow-up visit specimens were included in this analysis [1, 2]. The second substudy included same participants of first study with a longer follow-up after visit 2. These subjects were anal HPV DNA positive in previous visits and were susceptible to new anal HPV infection from a different HPV genotype. Participants with both genital and anal HPV DNA samples at baseline and at least 1 follow-up visit were included in this analysis. Subjects with an anal HPV DNA-positive result at baseline were excluded from the genital-anal sequential analyses.

Eligible subjects were assessed for incident anal HPV infection throughout the follow-up period at each 6-month clinical visit. Subjects with an incident anal HPV infection were categorized into 4 groups for evaluation of type-specific sequential genital-to-anal HPV infections (Figure 1). These groups were (1) subjects with genital HPV–positive results at the time of the incident anal HPV–positive visit and genital HPV–negative results in previous visits, (2) subjects with genital HPV–negative results at the time of the incident anal HPV–positive visit and genital HPV–negative results in previous visits, (3) subjects with genital HPV–negative results at the time of the incident anal HPV–positive visit and genital HPV–positive results in previous visits, and (4) subjects with genital HPV–positive results at the time of the incident anal HPV–positive visit and with genital HPV–positive results in previous visits. Subjects in group 1 had concurrent genital and anal infections and were not included in type-specific sequential analyses. Subjects without an incident anal HPV infection were also categorized on the basis of prior genital HPV infection status and constituted the at-risk population for incidence rate calculations. Characteristics of participants who were susceptible to an incident anal HPV infection were compared across prior genital HPV infection status, using χ^2 tests and Monte Carlo estimation of exact P values.

Anal HPV infection incidence rates and 95% confidence intervals (CIs) were calculated among men with a prior genital HPV infection and among men with no prior genital HPV infection. Incidence rate ratios (IRRs) were calculated for each of the 9 HPV types comparing prior genital and no prior genital infection groups. Grouped analyses were also carried out for any of the 9 types, for the low-risk HPV types only (HPV 6 and 11), and for the high-risk HPV types only (HPV 16, 18, 31, 33, 45, 52, and 58). Separate analyses were conducted to assess incidence rates of concurrent genital and anal HPV infections (excluded from the sequential analyses). We also examined whether a prior anal infection is associated with acquisition of a subsequent genital HPV infection by calculating IRRs and 95% CIs.

The risk of sequential detection of anal HPV infections following type-specific genital infections was assessed using a Cox proportional hazards model. Bivariate analyses were carried out in individual HPV type analyses because the sample size was too small to allow for adjustment of multiple covariates.

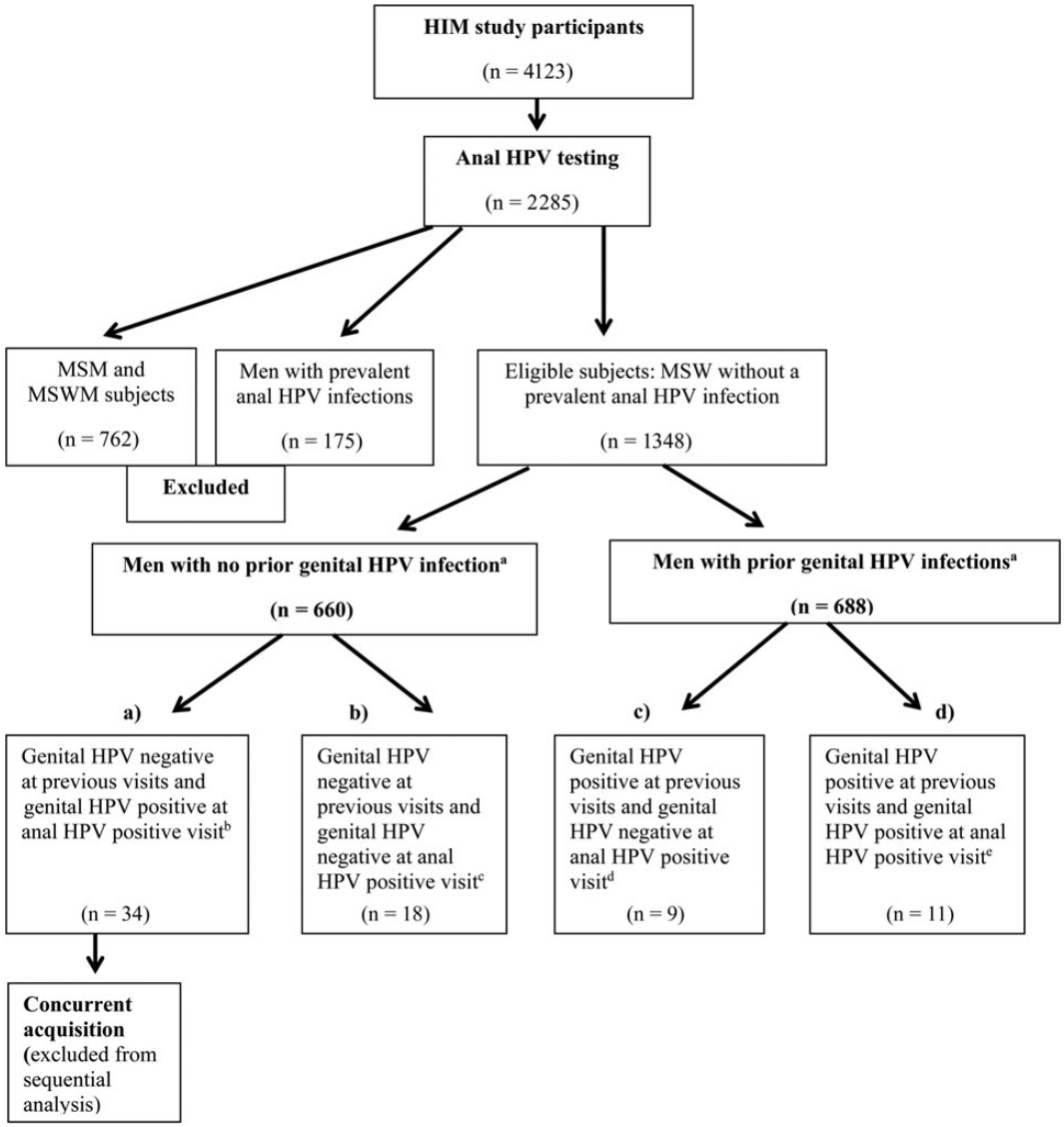

Figure 1. Study population for assessment of sequential genital and anal human papillomavirus (HPV) infections among participants of the HPV Infection in Men (HIM) study. Each genital and anal HPV infection assessed is type specific (genotype concordant). [a]Data are based on the sequential acquisition of anal HPV infections, as indicated by the subboxes below each group. [b]Genital and anal infections occurred at the same visit, so sequential risk could not be assessed. The number of anal HPV–positive men according to each type was as follows: HPV 6, 4; HPV 11, 5; HPV 16, 10; HPV 18, 3; HPV 31, 1; HPV 33, 1; HPV 45, 8; HPV 52, 4; and HPV 58, 2. [c]The number of anal HPV–positive men according to each type was as follows: HPV 6, 3; HPV 11, 0; HPV 16, 5; HPV 18, 4; HPV 31, 0; HPV 33, 1; HPV 45, 3; HPV 52, 2; and HPV 58, 2. [d]The previous genital HPV–positive visit was assessed for the sequential risk of genital/anal infections. The number of anal HPV–positive men according to each type was as follows: HPV 6, 1; HPV 11, 0; HPV 16, 3; HPV 18, 0; HPV 31, 1; HPV 33, 0; HPV 45, 0; HPV 52, 2; and HPV 58, 2. [e]The previous genital HPV–positive visit was used to assess the risk of subsequent anal infections. The number of anal HPV–positive men according to each type was as follows: HPV 6, 2; HPV 11, 1; HPV 16, 3; HPV 18, 0; HPV 31, 0; HPV 33, 0; HPV 45, 0; HPV 52, 3; and HPV 58, 2. Abbreviations: MSM, men who have sex with men; MSW, men who have sex with women; MSWM, men who have sex with women and men.

Demographic variables and time-varying covariates (alcohol use, smoking, and sexual behavior variables) were evaluated in adjusted models. Grouped analyses were performed to increase the statistical power to assess the risk of sequential genital-anal infections. Demographic and sexual behavior variables were evaluated in both backward and forward stepwise models for grouped HPV analyses. Final models included variables using the best-fit approach based on the lowest Akaike information criterion values.

RESULTS

From the original HIM study cohort, anal and genital HPV DNA specimens from baseline and at least 1 follow-up visit were available for 2285 men. Men with prevalent anal HPV infections (n = 175) and those who reported either anal or oral sex with a man at any time through the study follow-up (n = 762) were excluded from the analyses. After these exclusions, 1348 MSW were eligible for inclusion in the current study (Figure 1). A total of 40 incident anal HPV infections were detected during

Table 1. Demographic Characteristics of Men Who Have Sex With Women, According to Prior Genital Infection With Any of the 9 Vaccine-Associated Human Papillomavirus (HPV) Types, Among Subjects Negative for Anal HPV at Baseline in the HPV Infection in Men Study

Characteristic	Entire Population, No. (%) (n = 1348)	No Prior Genital Infection, No. (%)[a] (n = 660)	Prior Genital Infection, No. (%)[b] (n = 688)	*P* Value[c]
Country of residence				
United States	510 (37.8)	265 (52.0)	245 (48.0)	<.0001
Brazil	325 (24.1)	115 (35.4)	210 (64.6)	
Mexico	513 (38.1)	280 (54.6)	233 (45.4)	
Age, y				
18–30	641 (47.6)	313 (48.8)	328 (51.2)	.8082
31–44	501 (37.2)	242 (48.3)	259 (51.7)	
45–73	206 (15.3)	105 (51.0)	101 (49.0)	
Marital status				
Single or never married	543 (40.4)	256 (47.1)	287 (52.9)	.0710
Married/cohabitating	670 (49.9)	347 (51.8)	323 (48.2)	
Divorced/separated/ widowed	130 (9.7)	55 (42.3)	75 (57.7)	
Race				
White	589 (44.1)	273 (46.3)	316 (53.7)	<.0001
African Americans	160 (12.0)	61 (38.1)	99 (61.9)	
Asian/Pacific Islander	33 (2.5)	26 (78.8)	7 (21.2)	
Mixed race/other[d]	555 (41.5)	296 (53.3)	259 (46.7)	
Education duration, y				
≤12	578 (43.0)	285 (49.3)	293 (50.7)	.9708
13–15	363 (27.0)	178 (49.0)	185 (51.0)	
≥16	404 (30.0)	196 (48.5)	208 (51.5)	
Alcohol use, drinks/d, no.				
0	276 (20.9)	144 (52.2)	132 (47.8)	.0010
<0.5	484 (36.6)	258 (53.3)	226 (46.7)	
0.5–2	347 (26.2)	165 (47.6)	182 (52.4)	
>2	216 (16.3)	83 (38.4)	133 (61.6)	
Smoking status				
Never	776 (57.6)	376 (48.5)	400 (51.5)	.3432
Former	275 (20.4)	145 (52.7)	130 (47.3)	
Current	296 (22.0)	139 (47.0)	157 (53.0)	
Circumcision				
No	771 (57.2)	377 (48.9)	394 (51.1)	1.0000
Yes	577 (42.8)	283 (49.0)	294 (51.0)	
Lifetime female sex partners, no.				
0–1	114 (8.5)	79 (69.3)	35 (30.7)	<.0001
2–9	662 (49.1)	358 (54.1)	304 (45.9)	
10–49	484 (35.9)	183 (37.8)	301 (62.2)	
≥50	88 (6.5)	40 (45.5)	48 (54.5)	
New female sex partners in previous 6 mo, no.				
None	843 (65.0)	440 (52.2)	403 (47.8)	.0011
1	340 (26.2)	146 (42.9)	194 (57.1)	
≥2	114 (8.8)	44 (38.6)	70 (61.4)	

Vaccine HPV types are as follows: HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58.

[a] Includes participants who are susceptible to acquire incident anal HPV infection and do not have prior type-specific genital HPV infection.

[b] Includes participants who are susceptible to acquire incident anal infection and have a prior type specific genital HPV infection.

[c] *P* values for categorical variables were calculated using the χ^2 test with Monte Carlo estimation of *P* values; $\alpha = 0.05$.

[d] Includes mixed race, American Indian, Alaska native, Native Hawaiian, and other races.

the follow-up period. As a result of the limited follow-up time and selection strategy for collection anal HPV samples discussed earlier, most men only had data from 2 consecutive visits, and therefore 85% of the incident infections were detected during the first follow-up visit. The median follow-up time in this subcohort was 6.8 months (mean, 8.4 months; interquartile range, 6.5–7.4 months). For group 3, the median times to infection in the genital region to that in the anal region were 13.5 months (for HPV 6), 14.3 months (for HPV 16), 18.8 months (for HPV 31), 15.3 months (for HPV 52), and 19.5 months (for

Table 2. Incidence Rate of Type-Specific Anal Human Papillomavirus (HPV) Infection, by Prior Genital HPV Infection Status, Among Men Who Have Sex With Women in the HPV Infection in Men Study

	No Prior Genital HPV Infection				Prior Genital HPV Infection				
HPV Type	Subjects at Risk, No.	Incident Infections, No.	Person-Months	Incidence per 1000 Person-Months (95% CI)	Subjects at Risk, No.	Incident Infections, No.	Person-Months	Incidence per 1000 Person-Months (95% CI)	Incidence Rate Ratio (95% CI)[a]
Any[b]	660	12	5704.0	2.10 (.12, 3.70)	688	25[c]	5817.8	4.29 (2.90, 6.36)	2.04 (.99, 4.46)
Low risk[d]	1086	3	9542.3	0.31 (.10, .98)	262	4	2288.8	1.75 (.65, 4.66)	5.56 (.94, 37.95)
High risk[e]	772	11	6695.1	1.64 (.91, 2.97)	576	21[c]	4895.0	4.30 (2.80, 6.58)	2.61 (1.20, 6.00)
HPV 6	1104	3	9554.1	0.31 (.10, .97)	224	3	1967.3	1.52 (.49, 4.73)	4.86 (.65, 36.26)
HPV 11	1299	0	11441.5	0.00 (NE)	47	1	437.7	2.28 (.32,16.22)	NE
HPV 16	1036	5	9034.6	0.55 (.23, 1.33)	286	6	2415.7	2.48 (1.11, 5.53)	4.49 (1.14, 18.59)
HPV 18	1249	4	10902.3	0.37 (.14, .98)	95	0	856.2	0.00 (NE)	NE
HPV 31	1259	0	11164.6	0.00 (NE)	84	1	707.2	1.41 (.20, 1.00)	NE
HPV 33	1325	1	11691.3	0.09 (.01, .61)	21	0	180.6	0.00 (NE)	NE
HPV 45	1261	3	11102	0.27 (.09, .84)	81	0	682.1	0.00 (NE)	NE
HPV 52	1169	2	10142.3	0.20 (.24, 1.17)	176	5	1640.3	3.05 (1.10, 6.33)	15.46 (2.53, 162.33)
HPV 58	1241	2	10875.8	0.18 (.08, 0.76)	104	4	950.1	4.21 (1.40, 9.95)	22.89 (3.28, 253.09)

Prior genital HPV infections were with the same HPV genotypes. No MSW reported having anal or oral sex with men during the 4 year follow-up time.

Abbreviations: CI, confidence interval; NE, not estimable.

[a] Data were calculated using a Poisson model with log link function.

[b] Includes infections due to any of the 9 vaccine-type HPVs (6, 11, 16, 18, 31, 33, 45 52, and 58).

[c] Combining different HPV types for analyses resulted in higher number of incident anal infections among men with prior genital infection with any of the 9 types and the high-risk HPV types.

[d] Includes infections due to HPV 6 and 11.

[e] Includes infections due to HPV 16, 18, 31, 33, 45, 52, and 58.

HPV 58). For group 4, the median times to infection in the genital region to that in the anal region were 8.7 months (for HPV 6), 6.1 months (for HPV 11), 8.9 months (for HPV 16), 6.9 months (for HPV 52), and 10.6 months (for HPV 58). Thus, the interval was shorter for sequential genital-to-anal infection for group 4 as compared to group 3.

Overall, 660 men had no prior genital HPV infections, and 688 men had a prior genital HPV infection with any of the 9 HPV types (Table 1). These 2 groups differed significantly by country, with men from Brazil constituting the highest proportion (64.6%) of men with a prior genital HPV infection. The 2 groups also differed by race, with black individuals (61.9%) constituting the highest proportion of men with a prior genital infection. In addition, men with prior genital HPV infection reported higher (>2 drinks/day) alcohol use ($P = .001$), greater number of lifetime female sex partners (10–49 partners; $P < .001$), and greater number of new female sex partners in the previous 6 months (≥2 partners; $P = .001$).

In individual HPV type analyses, men with HPV 16 genital infections had a >4- fold increased risk of acquiring a subsequent anal HPV 16 infection (IRR, 4.49; 95% CI, 1.14–18.59) as compared to men with no prior genital HPV 16 infection (Table 2). Similarly, the risk of acquisition of anal HPV was significantly higher for HPV 52 (IRR, 15.46; 95% CI, 2.53–162.33) and HPV 58 (IRR, 22.89; 95% CI, 3.28–253.09). In grouped analyses, men with prior high-risk genital HPV infections had a >2-fold increased risk of detection of a subsequent anal HPV infection as compared to men with no prior genital infections (IRR, 2.61; 95% CI, 1.20–6.00; Table 2). Overall, men with prior genital HPV infections had higher incidence rates of subsequent anal HPV infections. In contrast, prior anal infections were not associated with risk of subsequent genital infections (IRR, 1.18 [95% CI, .64–2.01] for high-risk type HPVs and 0.72 [95% CI, .09–2.65] for HPV 16; data not shown). Incident rates for concurrent genital and anal HPV infection were low for HPV 18 (0.18; 95% CI, .05–.73) and HPV 45 (0.18; 95% CI, .4–.72); the incidence rate could not be assessed for other HPV types.

In Cox proportional hazard analyses, statistically significant HRs for anal HPV infection after a genital infection were observed for HPV 16 (HR, 4.63; 95% CI, 1.41–15.23), HPV 52 (HR, 15.78; 95% CI, 3.04–81.92) and HPV 58 (HR, 19.60; 95% CI, 3.56–107.79; Table 3). These HRs remained statistically significant even after adjustment for demographic and time-varying covariates one at a time (Table 3). Similar to the IRR analyses, a higher but nonsignificant HR was observed for HPV 6 (4.6; 95% CI, .93–22.84) for an anal HPV infection after a genital infection. In grouped HPV analyses, significantly higher aHRs were observed for infection with any of the 9 HPV types (2.8; 95% CI, 1.32–5.99), low-risk HPV types (5.89; 95% CI, 1.29–27.01), and high-risk HPV types (2.65; 95% CI, 1.26–5.55), among men with prior genital infections with these types, compared with men who did not have prior genital infections with these types. Infection with any of the 9 HPV types, low-risk HPV types, and high-risk HPV types were also evaluated

Table 3. Risk of Sequential Acquisition of Anal Human Papillomavirus (HPV) Infection Following a Genital HPV Infection Among Men Who Have Sex With Women in the HPV Infection in Men Study

HPV Type	Crude HR (95% CI)	Adjusted HR (95% CI)	Adjusted[a] HR (95% CI)	Adjusted[b] HR (95% CI)	Adjusted[c] HR (95% CI)	Adjusted[d] HR (95% CI)
Any[e]	2.19 (1.10, 4.39)	2.81 (1.32, 5.99)[f]	2.28 (1.10, 4.72)	2.53 (1.21, 5.29)	5.14 (1.40, 18.82)	5.14 (1.40, 18.82)
Low risk[g]	5.41 (1.21, 24.16)	5.89 (1.29, 27.01)[h]	5.16 (1.15, 23.17)	7.80 (1.41, 43.16)	NE	NE
High risk[i]	2.89 (1.38, 6.02)	2.65 (1.26, 5.55)[j]	3.13 (1.43, 6.83)	3.34 (1.53, 7.28)	6.95 (1.91, 25.27)	6.95 (1.91, 25.27)
6	4.61 (.93, 22.84)	NE	4.66 (.92, 23.56)	6.19 (1.03, 37.25)	NE	5.65 (1.13, 28.24)
11	NE	NE	NE	NE	NE	NE
16	4.63 (1.41, 15.23)	NE	4.46 (1.34, 14.85)	6.44 (1.91, 21.66)	6.50 (1.06, 39.69)	5.22 (1.55, 17.57)
18	NE	NE	NE	NE	NE	NE
31	NE	NE	NE	NE	NE	NE
33	NE	NE	NE	NE	NE	NE
45	NE	NE	NE	NE	NE	NE
52	15.78 (3.04, 81.92)	NE	23.35 (2.57, 211.94)	14.52 (2.77, 76.03)	17.95 (1.33, 242.47)	NE
58	19.60 (3.56, 107.79)	NE	23.50 (4.19, 131.84)	23.56 (3.72, 149.07)	40.54 (4.51, 364.28)	35.70 (5.11, 249.28)

HRs were calculated by Cox proportional hazard model. The reference group consists of men without a prior genital HPV infection of the same genotype.

Abbreviations: CI, confidence interval; HR, hazard ratio; NE, not estimable.

[a] Adjusted for alcohol use as a time-varying covariate.

[b] Adjusted for lifetime female sex partners as a time-varying covariate.

[c] Adjusted for number of new female partners in past 6–12 months as a time-varying covariate.

[d] Adjusted for frequency of sexual intercourse with female partners as a time-varying covariate.

[e] Includes infections due to any of the 9 vaccine-type HPVs (6, 11, 16, 18, 31, 33, 45, 52, and 58).

[f] Adjusted for marital status and frequency of sexual intercourse with female partners as a time-varying covariate, based on a best fit model.

[g] Includes infections due to HPV 6 and 11.

[h] Adjusted for country, based on a best fit model.

[i] Includes HPV 16, 18, 31, 33, 45, 52, and 58.

[j] Adjusted for marital status, based on a best fit model.

by adjusting one variable at a time, with HRs remaining significant after adjustment for demographic and time-varying covariates (Table 3).

DISCUSSION

This is the first study to report sequential type-specific genital-to-anal HPV infections among MSW for the HPV types included in the 9-valent vaccine (HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58). Overall, having a prior genital HPV infection increased the risk of detection of subsequent anal HPV of the same type, and this association was unaffected by adjusting for sexual behavior. Men with a prior genital HPV 16 infection had >4-fold increased risk of anal HPV 16 infection than men with no prior genital HPV 16 infections. A significantly higher risk of sequential anal infection after a genital infection was observed in combined analyses for high-risk HPV types, low-risk HPV types, and any of the 9 HPV types targeted by vaccine.

In previous publications, anal HPV infections were detected among MSW with a prevalence of 12.2% for any HPV type and 2.2% for HPV 16 [2], and HPV was detected at a higher prevalence in the perianal region (21.3%) as compared to the anal canal (16.6%) [14]. It is not clear how MSW acquire these infections. Our study provides the first indication of autoinoculation of genital infections as one of the possible mechanisms. Among women, only 2 studies have assessed sequential acquisition of type-specific anal HPV infection from a prior cervical infection. A study in the Hawaii HPV cohort found a high risk for sequential cervical-to-anal HPV infection with an aHR of 17.33 (95% CI, 10.22–29.39) for any type and an aHR of 13.25 (95% CI, 3.43–51.22) for HPV types 16 and 18 [5]. Another study from the same cohort also found a high risk for sequential acquisition of anal infections after cervical infection, with a relative risk (RR) of 20.5 (95% CI, 16.3–25.7) for any HPV type and a RR of 14.2 (95% CI, 9.86–20.5) for high-risk HPV types [6]. Other studies have also indicated associations between anal and cervical HPV infections, although they did not conduct sequential analyses. A high concurrence rate of anal and cervical HPV infections with an adjusted odds ratio (aOR) of 3.3 (95% CI, 2.5–4.4) was observed in one study [7]. In a study among women in American Samoa, the authors' found that women with anal HPV infections were more likely to have a history of prior cervical infections (aOR, 3.32; 95% CI, 1.10–10.00) [8]. Another study found that anal HPV infections were associated with cervical HPV infection with a RR of 1.3 (95% CI, 1.1–1.4) [9]. A concordance rate of 20% for anal HPV infections among women with preexisting cervical HPV infections was observed in another study [10].

In this study, risk of sequential acquisition of anal HPV infections remained high even after adjusting for sexual behavior and other demographic characteristics. Our analyses included exclusively MSW subjects and excluded any subjects who reported either oral or anal sex with men throughout the

follow-up period. As the number of female sex partners and frequency of sexual intercourse with women did not explain the presence of anal HPV infections, a likely explanation of these infections is autoinoculation through prior genital infections. Autoinoculation from genital infections may occur through contact of the hand, toilet paper, undergarments, towel, or other objects [15]. Apart from autoinoculation, partner-assisted inoculation of HPV infection from the genital region to the anal site is also a likely explanation. Digital contact through female sex partners or sex toys may lead to partner-assisted inoculation; however, we did not have information available to assess transmission outside penetrative sex. Based on the absence of other explanatory factors and the high risk of sequential infections observed in these analyses, we hypothesize that autoinoculation or partner-assisted inoculations are the likely explanations of the observed association. In current study, high risk of sequential genital to anal HPV infection indicates potential auto-inoculation of HPV 16 infections. In addition, suggestions of a similar phenomenon for HPV 52 and 58 were observed, although the CIs were wide for these types. As such, autoinoculation is a likely mechanism for HPV 16, as well as other HPV types. It is also possible that HPV 16 has higher infectivity than other HPV types, and therefore it may be more readily transmitted to other anatomical sites, including the anal canal. More research is needed to fully elucidate the factors leading to anal HPV detection among MSW.

To the best of our knowledge, no other study has assessed the risk of type-specific sequential genital-anal infections among heterosexual men. Strengths of the current study are the relatively large sample size, the multinational nature of the cohort, and the standard HPV DNA genotyping methods [11, 12]. Despite these strengths, there are limitations that need to be considered when interpreting study results. We did not have information regarding HPV infections prior to the start of the study; therefore, participants may have had genital HPV infections prior to enrollment that subsequently cleared. This may have led to misclassification of participants with respect to their prior genital infection status. It is also possible that participants acquired anal HPV infections prior to the study's start. These infections may have been latent and later detected and classified as incident anal infections during follow-up. Similar to genital HPV infections, it is possible that anal HPV infections may be retained in a latent state in basal epithelial stem cell pool [16–20]. However, reactivation of a latent infection is less likely in immunocompetent HIM study participants, and the detection of a very low load of a latent virus is unlikely with current HPV DNA detection methods [17–19]. Future studies are needed to assess the role of latent HPV infections, particularly in immunocompetent individuals.

Although 2285 subjects provided anal specimens at baseline, fewer specimens were available after 24 months. Data used in current analysis were obtained from previously completed anal HPV studies [11, 12]. Those studies had limited funding that led to restriction of analyses to the first 2 years of study duration. Subjects were also lost to follow-up or excluded if they reported having anal/oral sex with men during follow-up visits. Therefore, we performed grouped analyses to increase the power to assess sequential genital-anal infection for any of the 9 vaccine types, high-risk types, and low-risk HPV types. As discussed previously, subjects for the second substudy (inclusive of the 12-, 18-, and 24-month visits) were more likely to have anal infections, which can potentially lead to overestimation of incidence rates. These subjects represented only 15% of the total incident anal HPV infections. We performed a sensitivity analysis by excluding participants in the second substudy and observed similar results for grouped analysis and individual type analysis (HPV 16 and 52). Sample size was insufficient to perform individual type analysis for HPV 6 and HPV 58. Partner-assisted transmission, autoinoculation, or transmission from inanimate objects shortly after sexual intercourse may explain simultaneous (concurrent) genital and anal infections. HPV infections prior to the study start can also help explain concurrent genital and anal infections at the same visit. As indicated previously, this information was not available to assess the origin of concurrent (simultaneous) HPV infections.

Eligible participants may not have reported having male sex partners, owing to social desirability. Use of computer-assisted techniques for recording sexual behavior history should have minimized the effect of such bias. Misclassification of genital and anal HPV infection status is also possible, especially for infections with low viral load and long periods between initial infection and assessment of HPV DNA. Given the robust methods used for HPV DNA detection in our study, the effects of such misclassification should be minimal [21–23]. Furthermore, subjects included in the analyses did not differ from excluded subjects with regard to sexual behavior variables. As indicated previously [14], with a higher prevalence of HPV infection at the perianal site, it may have been easier to assess sequential genital-to-perianal HPV infection for other HPV types. Unfortunately, samples for only the anal canal region were collected for the current study. As a result, it was not possible to compare intraanal infections with perianal infections in this study.

In conclusion, men with prior genital HPV infections are at higher risk of acquiring anal infections with the same type, with a >4-fold increased risk for HPV 16. Autoinoculation or partner-assisted inoculation of genital HPV infection, along with other unknown factors, may explain the presence of anal HPV among MSW. Future studies are needed to assess the role of autoinoculation and explore other factors associated with high rates of anal HPV infection following a genital infection among MSW. These findings also suggest that the prevention of genital HPV infection through vaccination and other methods may help curtail anal HPV infection among heterosexual men.

Notes

Acknowledgments. We thank the Human Papillomavirus Infection in Men (HIM) study teams and participants in the United States (Moffitt Cancer Center, Tampa), Brazil (Centro de Referência e Treinamento em DST/AIDS, Fundação Faculdade de Medicina Instituto do Câncer do Estado de São Paulo, Ludwig Institute for Cancer Research, São Paulo), and Mexico (Instituto Mexicano del Seguro Social, Instituto Nacional de Salud Pública, Cuernavaca).

Financial support. This work was supported by the National Cancer Institute (grant CA R01CA098803 to A. R. G.) and the National Institute of Allergy and Infectious Diseases (grant 5R21AI101417-3 to A. G. N.), National Institutes of Health; and the Merck Investigator-Initiated Studies Program (grant IISP33707 to A. G. N.).

Potential conflicts of interest. A. G. N. received funding for related research from 2009–2011 from Merck through a grant to Moffitt Cancer Center. A. R. G. and L. L. V. are members of the Merck Advisory Board. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Nyitray AG, Carvalho da Silva RJ, Baggio ML, et al. Six-month incidence, persistence, and factors associated with persistence of anal human papillomavirus in men: the HPV in men study. J Infect Dis **2011**; 204:1711–22.
2. Nyitray AG, Carvalho da Silva RJ, Baggio ML, et al. Age-specific prevalence of and risk factors for anal human papillomavirus (HPV) among men who have sex with women and men who have sex with men: the HPV in men (HIM) study. J Infect Dis **2011**; 203:49–57.
3. Nyitray AG, Smith D, Villa L, et al. Prevalence of and risk factors for anal human papillomavirus infection in men who have sex with women: a cross-national study. J Infect Dis **2010**; 201:1498–508.
4. Nyitray AG, Carvalho da Silva RJ, Chang M, et al. Incidence, duration, persistence, and factors associated with high-risk anal HPV persistence among HIV-negative men having sex with men: a multi-national study. Clin Infect Dis **2016**; 62:1367–74.
5. Goodman MT, McDuffie K, Hernandez BY, et al. The influence of multiple human papillomavirus types on the risk of genotype-concordant incident infections of the anus and cervix: the Hawaii HPV cohort study. J Infect Dis **2011**; 203:335–40.
6. Goodman MT, Shvetsov YB, McDuffie K, et al. Sequential acquisition of human papillomavirus (HPV) infection of the anus and cervix: the Hawaii HPV Cohort Study. J Infect Dis **2010**; 201:1331–9.
7. Hernandez BY, McDuffie K, Zhu X, et al. Anal human papillomavirus infection in women and its relationship with cervical infection. Cancer Epidemiol Biomarkers Prev **2005**; 14(11 Pt 1):2550–6.
8. Hernandez BY, Ka'opua LS, Scanlan L, et al. Cervical and anal human papillomavirus infection in adult women in American Samoa. Asia Pac J Public Health **2013**; 25:19–31.
9. Palefsky JM, Holly EA, Ralston ML, Da Costa M, Greenblatt RM. Prevalence and risk factors for anal human papillomavirus infection in human immunodeficiency virus (HIV)-positive and high-risk HIV-negative women. J Infect Dis **2001**; 183:383–91.
10. Guler T, Uygur D, Uncu M, et al. Coexisting anal human papilloma virus infection in heterosexual women with cervical HPV infection. Arch Gynecol Obstet **2013**; 288:667–72.
11. Giuliano AR, Lee JH, Fulp W, et al. Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. Lancet **2011**; 377:932–40.
12. Giuliano AR, Lazcano-Ponce E, Villa LL, et al. The human papillomavirus infection in men study: human papillomavirus prevalence and type distribution among men residing in Brazil, Mexico, and the United States. Cancer Epidemiol Biomarkers Prev **2008**; 17:2036–43.
13. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens–Part B: biological agents. Lancet Oncol **2009**; 10:321–2.
14. Nyitray A, Nielson CM, Harris RB, et al. Prevalence of and risk factors for anal human papillomavirus infection in heterosexual men. J Infect Dis **2008**; 197:1676–84.
15. Liu Z, Rashid T, Nyitray AG. Penises not required: a systematic review of the potential for human papillomavirus horizontal transmission that is non-sexual or does not include penile penetration. Sex Health **2016**; 13:10–21.
16. Gravitt PE. Evidence and impact of human papillomavirus latency. Open Virol J **2012**; 6:198–203.
17. Lu B, Viscidi R, Wu Y, et al. Prevalent serum antibody is not a marker of immune protection against acquisition of oncogenic HPV16 in men. Cancer Res **2012**; 72:676–85.
18. Rositch AF, Burke AE, Viscidi RP, Silver MI, Chang K, Gravitt PE. Contributions of recent and past sexual partnerships on incident human papillomavirus detection: acquisition and reactivation in older women. Cancer Res **2012**; 72:6183–90.
19. Strickler HD, Burk RD, Fazzari M, et al. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. J Natl Cancer Inst **2005**; 97:577–86.
20. Theiler RN, Farr SL, Karon JM, et al. High-risk human papillomavirus reactivation in human immunodeficiency virus-infected women: risk factors for cervical viral shedding. Obstet Gynecol **2010**; 115:1150–8.
21. Coutlée F, Rouleau D, Petignat P, et al. Enhanced detection and typing of human papillomavirus (HPV) DNA in anogenital samples with PGMY primers and the Linear array HPV genotyping test. J Clin Microbiol **2006**; 44:1998–2006.
22. Stevens MP, Rudland E, Garland SM, Tabrizi SN. Assessment of MagNA pure LC extraction system for detection of human papillomavirus (HPV) DNA in PreservCyt samples by the Roche AMPLICOR and linear array HPV tests. J Clin Microbiol **2006**; 44:2428–33.
23. Gravitt PE, Peyton CL, Apple RJ, Wheeler CM. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. J Clin Microbiol **1998**; 36:3020–7.