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Human Papillomavirus Genotype Attribution and Estimation of Preventable Fraction of Anal Intraepithelial Neoplasia Cases Among HIV-Infected Men Who Have Sex With Men

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Background. The prevention of human papillomavirus (HPV)–induced anal cancer in high-risk populations such as human immunodeficiency virus (HIV)-infected men who have sex with men (MSM) remains an urgent priority, given rising incidence rates despite widespread antiretroviral therapy use.

Methods. HPV genotypes and anal disease prevalence, by cytology and histopathologic findings, were evaluated among 363 HIV-infected MSM. We modeled fractions of high-grade anal intraepithelial neoplasia (HGAIN) attributable to individual carcinogenic HPV genotypes and estimated the range of the proportion of HGAIN cases potentially preventable by prophylactic HPV vaccines.

Results. HPV16 was the most common genotype overall (26.4% of cases) and among HGAIN cases (55%). Prevalence of multiple (≥2) carcinogenic HPV genotypes increased from 30.9% in cases of AIN grade <1 to 76.3% in cases of AIN grade 3 ($P_{trend} < .001$). The fractions of HGAIN cases attributable to carcinogenic HPV16/18 targeted by currently licensed bivalent and quadrivalent HPV vaccines ranged from 12% to 61.5%, and the fractions attributable to carcinogenic HPV16/18/31/33/45/52/58 targeted by an investigational nonavalent HPV vaccine ranged from 39% to 89.4%.

Conclusions. Our analytical framework allows estimation of HGAIN cases attributable to individual HPV genotypes in the context of multiple concurrent HPV infections, which are very common among HIV-infected MSM. Our results suggest that licensed and investigational HPV prophylactic vaccines have the potential to prevent a substantial proportion of HGAIN cases in this population.

Keywords. Anal cancer; human papillomavirus; human immunodeficiency virus; anal intraepithelial neoplasia; men who have sex with men; genotypes; attribution.

Anal cancer is relatively rare in the general population, yet its incidence is increasing in high-risk groups such as men who have sex with men (MSM), particularly those living with human immunodeficiency virus (HIV)/AIDS [1]. Chronic persistent carcinogenic human papillomavirus (HPV) is associated with anal cancer and its precursor lesions, high-grade anal intraepithelial neoplasia (HGAIN) [2]. However, HPV genotype prevalence among anal intraepithelial neoplasia (AIN) grades in HIV-infected MSM has varied significantly, depending on the characteristics of the population, variability in pathology interpretation, and differences in molecular genotyping assays [3, 4]. The knowledge of individual HPV genotypes within each grade of AIN can allow

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estimation of the impact of prophylactic HPV vaccines in this high-risk population as well as to consider which genotypes should be included in future HPV screening and genotyping assays.

To further our understanding of anal lesions in relation to HPV genotypes in HIV-infected MSM, we analyzed the HPV genotype distribution in anal disease categories based on a rigorous disease end point, using both cytology and histology findings. We used this information to predict the potential efficacy of prophylactic HPV vaccines, under the assumption that vaccination would occur before natural infection and seroconversion.

METHODS

Study Population

We conducted a cross-sectional screening study at an anal-cancer-screening clinic in San Francisco, California, operated by Kaiser Permanente Northern California (KPNC). We enrolled men who were identified as HIV infected through the Kaiser HIV Registry, who were aged ≥18 years, and who had not received a diagnosis of anal cancer prior to enrollment. Written informed consent was obtained from patients, and guidelines of the US Department of Health and Human Services were followed in the conduct of clinical research.

In total, 363 men were enrolled between August 2009 and June 2010. The study was reviewed and approved by the institutional review boards at KPNC and the National Cancer Institute. All participants were asked to complete a self-administered questionnaire for risk factor information. Additional information on HIV status and medication, sexually transmitted diseases, and histopathologic results were abstracted from the KPNC clinical database. For 87 of 271 subjects without HGAIN at the enrollment visit, follow-up information from additional clinic visits up to December 2011 was available and included in the analysis. None of the participants had received the licensed or investigational HPV vaccines.

Cytology, High-Resolution Anoscopy, and Histology

During the clinical examination, 2 cytology specimens were collected by inserting a wetted, flocked nylon swab into the anal canal up to the distal rectal vault and withdrawing with rotation and lateral pressure [5]. Both specimens were transferred to PreservCyt medium (Hologic, Bedford, MA) for anal cytology and HPV DNA and biomarker testing [5]. All participants subsequently underwent a digital anorectal examination, followed by high-resolution anoscopy (HRA). Suspicious lesions visualized during HRA were biopsied and sent for routine histopathologic review by KPNC pathologists.

From the first specimen container, a slide was prepared for routine liquid cytology (Thin Prep) Papanicolaou staining. Cytology results were reported analogous to the revised 2001 Bethesda classification for cervical cytology [6], using the categories negative for intraepithelial lesions or malignancy, atypical squamous cells of undetermined significance, atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion, low-grade squamous intraepithelial lesions, and high-grade squamous intraepithelial lesions (HSIL). The study pathologists made a further distinction of HSIL, categorizing it as HSIL-AIN grade 2 (AIN2) or HSIL-AIN grade 3 (AIN3). We observed good agreement ($\kappa = 0.54$) between 2 independent expert cytology reviews [7]. In this analysis, we report the primary cytology result from KPNC pathologists. Histology results were reported as negative, condyloma, and AIN grades 1–3.

HPV DNA Testing

All HPV DNA tests were conducted on the second sample by Roche, using the Linear Array HPV Genotyping Test (LA-HPV; Roche Molecular Systems, Pleasanton, CA) [8]. The pool of consensus L1 PGMY09/11 primers used in this assay is designed to amplify HPV-DNA from 37 genotypes. These include genotypes classified by the International Agency for Research on Cancer (IARC) as group 1 or 2A carcinogens (ie, "carcinogenic genotypes") [9]: HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68; group 2B carcinogens (ie, "possibly carcinogenic genotypes"): HPV types 26, 53, 66, 67, 70, 73, and 82; and group 3 carcinogens or unclassified genotypes (ie, "noncarcinogenic genotypes/genotypes of unknown carcinogenicity"): HPV types 6, 11, 40, 42, 54, 55, 61, 62, 64, 69, 71, 72, 81, 83, 84, CP6108 (HPV89), and IS39 (HPV82v). The probe for HPV52 in LA-HPV is a mixed probe that cross-reacts with HPV33, HPV35, and HPV58 hence, HPV52 estimates presented here only include those without concurrent presence of the latter three types. Amplification of the β-globin gene was used as a control for cellular adequacy and sufficient DNA.

Classification of Disease Grades

To improve the accuracy of the estimation of the patient's anal disease status, a combination of results of HRA-guided biopsy, results of anal cytology, and the follow-up data were used to improve the classification of disease status, as has been done in previous analyses in context of cervical neoplasia [10, 11]. Four distinct disease categories were defined (Supplementary Table 1): (1) AIN grade <1 (<AIN1), including men with a nondysplastic biopsy finding or without a biopsy and with cytology findings less severe than HSIL; (2) AIN grade 1 (AIN1), including men with AIN1 histology findings and cytology findings less severe than HSIL; (3) AIN2, including men with AIN2 histology findings or with lower grade, normal, or no histology findings and HSIL-AIN2; and (4) AIN3, including men with AIN3 histology findings or with less severe, normal, or no histology findings but with HSIL-AIN3 cytology. HGAIN comprised of the disease categories AIN2 and AIN3, as defined above.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics 19 and Intercooled Stata, version 11.2. All statistical tests were 2-sided and considered to be significant at a P value of $< .05$. We calculated the prevalence of HPV genotypes (any/carcinogenic type), stratified by AIN categories and by single versus multiple genotypes. The Mantel-Haenszel χ^2 test for trend was used to analyze trends with respect to increasing severity of AIN and increasing age categories. We explored trends in genotype composition and number of anal sex partners (lifetime and in the past 6 months), by age categories.

We evaluated fractions of anal disease categories attributable to individual carcinogenic HPV genotypes. We assumed that individual HPV genotype infections were biologically independent (ie, that there is no synergy between HPV types) and that HPV is a necessary cause of anal neoplastic disease. For each disease category, the maximum estimate of the fraction caused by an individual genotype was calculated by assigning the frequency of that genotype in that category, under the assumption of a causal association with every case in which that genotype is present. The minimum estimate was calculated by assigning the frequency of an individual HPV genotype when it was present as a single carcinogenic infection in that disease category. Two attribution models were used to derive estimates that ranged between the maximum and minimum proportions of disease caused by individual HPV genotypes (similar to approaches previously described in the cervix literature by Insinga et al [12] and Wentzensen et al [11]). A "proportional attribution" estimate was calculated, in which a case is proportionally attributed according to the frequency of that type at the respective disease category. A "hierarchical attribution" estimate was calculated, in which individual cases were assigned by the frequency of the most frequent overall type in that respective disease category. In the proportional attribution model, a fraction of each case is attributed to every multiple genotype present in the lesion, whereas in the hierarchical attribution model, a case is completely attributed to the most frequent type. Thus, the hierarchical attribution tends to favor the more frequent types, while the proportional attribution is more likely to attribute some cases to less common types, including types with little carcinogenic potential. For example, consider a case of AIN3 with the presence of 3 carcinogenic types: HPV16, HPV18 and HPV59. The frequencies ("any type infections") of these individual types in AIN3 are 61%, 15%, and 10% (sum, 86%). By using the proportional attribution, the case would be attributed to the 3 types in proportion to their prevalence in AIN3 ($61/86 = 70.9\%$ for HPV16, $15/86 = 17.4\%$ for HPV18, and $10/86 = 11.7\%$ for HPV59). Since HPV16 is the most common carcinogenic genotype in AIN3, by using of the hierarchical attribution, the case would be completely attributed to HPV16.

We also estimated the maximum and minimum estimates and the proportional and hierarchical attribution fractions of anal disease categories potentially preventable by prophylactic bivalent and quadrivalent HPV vaccines currently approved by the Food and Drug Administration that target carcinogenic types HPV16/18 and by an investigational nonavalent vaccine (currently being evaluated in clinical trials) that targets carcinogenic types HPV16/18/31/33/45/52/58. For this analysis, we assumed full protection of the vaccine against acquisition of the specific HPV genotypes included in the vaccines, but we did not assume cross-protection. Another underlying caveat of our analysis was that the HIV-infected MSM population would have been vaccinated before being exposed (natural infection) to the carcinogenic HPV genotypes that are contained in the licensed and investigational HPV vaccines.

RESULTS

Participant Characteristics

The median age of the participants was 53 years (range, 26–79 years). The median age at first anal sex was 20 years (range, 7–66 years), 40% of the participants reported ≥40 anal sex partners during their life, and 15% reported ≥3 partners in the past 6 months. The $CD4^+$ T-cell count at the time of study enrollment was ≤350 cells/μL in 18.3%, and the HIV load was <75 copies/mL in 90%. A vast majority (93.8%) were taking antiretroviral drugs at the time of enrollment.

Prevalence of HPV Genotypes, by categories of anal neoplastic disease

HPV genotyping results were available for 342 of 363 participants (94.2%). Of these 342, 139 (40.6%) had <AIN1, 99 (29%) had AIN1, 45 (13.2%) had AIN2, and 59 (17.2%) had AIN3; thus, the HGAIN (AIN2 plus AIN3) prevalence was 30.4% (95% confidence interval [CI], 25.8%–35.5%; Supplementary Table 1).

The prevalence of any HPV genotype was 94.4% (95% CI, 91.4%–96.6%), and the prevalence of any carcinogenic HPV genotype was 75.4% (95% CI, 70.5%–79.9%; Table 1). HPV16 was the most common individual genotype, present in 96 participants (28.1%). There were 9 genotypes classified as non/unknown or possibly carcinogenic (ie, HPV6, HPV53, HPV42, HPV84, HPV61, HPV62, HPV70, HPVCP6108, and HPV55) that were more frequently detected than the next most common carcinogenic type (HPV31). Among carcinogenic HPV types, HPV16, HPV18, HPV33, HPV35, HPV39, HPV51, and HPV56 showed a trend of increasing prevalence from <AIN1 to AIN3 that was statistically significant ($P < .05$).

Single carcinogenic type infections were present in just over a quarter of all participants (96 of 342 [28.1%]) and were progressively infrequent with increasing AIN severity, with such infections detected in 33.3% of AIN1 cases but only 18.6% of

Table 1. Prevalence and Distribution of Human Papillomavirus (HPV) Genotypes, by Anal Intraepithelial Neoplasia (AIN) Grade

	Overall (n = 342)		<AIN1 (n = 139)		AIN1 (n = 99)		AIN2 (n = 45)		AIN3 (n = 59)		
Variable	No.	%	No.	%	No.	%	No.	%	No.	%	P[b]
No HPV type	19	5.6	15	10.8	3	3.0	0	0.0	1	1.7	<.01
Any HPV type	323	94.4	124	89.2	96	97.0	45	100.0	58	98.3	<.01
Single HPV type	47	13.7	32	23.0	10	10.1	2	4.4	3	5.1	.44
Multiple (≥2) HPV types	276	80.7	92	66.2	86	86.9	43	95.6	55	93.2	<.01
Any carcinogenic[a] HPV type											
Absent	84	24.6	58	41.7	21	21.2	2	4.4	3	5.1	<.01
Present	258	75.4	81	58.3	78	78.8	43	95.6	56	94.9	<.01
Single type	96	28.1	38	27.3	33	33.3	14	31.1	11	18.6	<.01
Multiple (≥2) types	162	47.4	43	30.9	45	45.5	29	64.4	45	76.3	<.01
Individual HPV types											
Carcinogenic[a]											
HPV16	96	28.1	13	9.4	26	26.3	21	46.7	36	61.0	<.01
HPV18	36	10.5	9	6.5	9	9.1	9	20.0	9	15.3	.01
HPV31	51	14.9	15	10.8	13	13.1	14	31.1	9	15.3	.07
HPV33	37	10.8	8	5.8	8	8.1	6	13.3	15	25.4	<.01
HPV35	39	11.4	11	7.9	12	12.1	4	8.9	12	20.3	.03
HPV39	42	12.3	12	8.6	10	10.1	8	17.8	12	20.3	.01
HPV45	41	12.0	15	10.8	11	11.1	3	6.7	12	20.3	.17
HPV51	44	12.9	14	10.1	10	10.1	4	8.9	16	27.1	.01
HPV52	41	12.0	10	7.2	18	18.2	6	13.3	7	11.9	.28
HPV56	37	10.8	12	8.6	8	8.1	7	15.6	10	16.9	.05
HPV58	46	13.5	14	10.1	14	14.1	6	13.3	12	20.3	.07
HPV59	36	10.5	9	6.5	13	13.1	8	17.8	6	10.2	.18
HPV68	26	7.6	8	5.8	7	7.1	5	11.1	6	10.2	.19
Possibly carcinogenic[c]											
HPV26	4	1.2	2	1.4	0	0.0	0	0.0	2	3.4	.44
HPV53	66	19.3	23	16.5	22	22.2	10	22.2	11	18.6	.59
HPV66	35	10.2	8	5.8	5	5.1	8	17.8	14	23.7	<.01
HPV67	8	2.3	0	0.0	2	2.0	2	4.4	4	6.8	<.01
HPV70	54	15.8	21	15.1	12	12.1	8	17.8	13	22.0	.22
HPV73	32	9.4	6	4.3	10	10.1	8	17.8	8	13.6	.01
HPV82	9	2.6	2	1.4	2	2.0	1	2.2	4	6.8	.05
Noncarcinogenic/unknown carcinogenicity[d]											
HPV6	68	19.9	7	5.0	34	34.3	10	22.2	17	28.8	<.01
HPV11	26	7.6	1	0.7	15	15.2	5	11.1	5	8.5	.03
HPV40	9	2.6	3	2.2	5	5.1	1	2.2	0	0.0	.42
HPV42	63	18.4	14	10.1	26	26.3	9	20.0	14	23.7	.02
HPV54	49	14.3	19	13.7	11	11.1	11	24.4	8	13.6	.53
HPV55	53	15.5	15	10.8	18	18.2	8	17.8	12	20.3	.07
HPV61	57	16.7	20	14.4	19	19.2	7	15.6	11	18.6	.51
HPV62	57	16.7	20	14.4	16	16.2	10	22.2	11	18.6	.30
HPV64	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	…
HPV69	12	3.5	3	2.2	1	1.0	1	2.2	7	11.9	<.01
HPV71	6	1.8	2	1.4	1	1.0	2	4.4	1	1.7	.56
HPV72	37	10.8	10	7.2	12	12.1	5	11.1	10	16.9	.05
HPV81	37	10.8	13	9.4	14	14.1	6	13.3	4	6.8	.80
HPV83	21	6.1	4	2.9	11	11.1	3	6.7	3	5.1	.47
HPV84	63	18.4	21	15.1	25	25.3	9	20.0	8	13.6	.96
HPVIS39	16	4.7	2	1.4	9	9.1	1	2.2	4	6.8	.03

Table 1 continued.

Variable	Overall (n = 342)		<AIN1 (n = 139)		AIN1 (n = 99)		AIN2 (n = 45)		AIN3 (n = 59)		P^b
	No.	%	No.	%	No.	%	No.	%	No.	%	
HPVCP6108	54	15.8	16	11.5	19	19.2	8	17.8	11	18.6	.35
HPV vaccine types[e]											
Bivalent	117	34.2	21	15.1	32	32.3	27	60.0	37	62.7	<.01
Quadrivalent	163	47.7	29	20.9	59	59.6	33	73.3	42	71.2	<.01
Nonavalent	245	71.6	69	49.6	81	81.8	42	93.3	53	89.8	<.01

See Methods for definitions of AIN grades.

Abbreviation: IARC, International Agency for Research on Cancer.

[a] IARC group 1/group 2A carcinogens.

[b] Calculated by the χ^2 test of trend in proportions across the <AIN1, AIN1, AIN2, and AIN3 categories.

[c] IARC group 2B carcinogens.

[d] IARC group 3 carcinogens HPV types not currently classified as carcinogenic.

[e] For comparison of bivalent(HPV16/18), quadrivalent (HPV16/18/6/11), and nonavalent (HPV16/18/6/11/31/33/45/52/58) vaccine types, only cases in which any of the component vaccine types were present were considered (ie, this was not an additive comparison of the individual component vaccine types as presented in the preceding rows).

AIN3 cases (P for trend, < .01). Multiple (≥2) HPV genotypes (in 276 of 342 participants [81%]) and multiple carcinogenic HPV genotypes (in 162 of 342 [47%]) were very common. Up to 14 concurrent HPV genotypes and up to 7 concurrent carcinogenic HPV genotypes were detected in individual specimens. The proportion of cases with multiple carcinogenic HPV types increased with increasing disease severity, ranging from 45.5% of AIN1 cases to 76.3% of AIN3 cases (Table 1).

Increased severity of anal disease was also associated with the proportion of participants reporting ≥40 anal sex partners in their lifetime (P < .04; data not shown). The prevalence of HGAIN was higher among men aged 46–65 years (approximately 33%), compared with men aged ≤45 years and men aged ≥66 years, although these differences were not statistically significant (P > .05; Figure 1). The relative proportion of multiple carcinogenic HPV genotypes decreased with increasing age categories (P < .001; Figure 1). Increasing age correlated with a decline in the number of participants reporting ≥10 anal sex partners in their lifetime and ≥3 anal sex partners in the past 6 months (P < .001; Figure 1).

Attribution of HPV Genotypes to Disease Categories

Tables 2–4 and Supplementary Tables 2 and 3 present the maximum and minimum attribution estimates of anal disease categories attributable to individual carcinogenic HPV genotypes. The maximum estimate is based on the inclusion of any case in which the genotype is detected (equivalent to the type prevalence in Table 1), whereas the minimum estimate is restricted to the frequency in cases with single carcinogenic type infections. The tables also include estimates by the hierarchical attribution and proportional attribution models for individual carcinogenic HPV genotypes in the respective disease categories.

HPV16 was the most frequent genotype and the most frequent carcinogenic genotype in both single and multiple infections in HGAIN, as well as individually in AIN3 and AIN2 (Tables 2–4). The proportions of attribution fractions by the hierarchical and proportional schemes attributable to HPV16 in each high-grade AIN category were higher than that for any other carcinogenic genotype. The proportion of AIN2 or worse cases attributable to HPV16 was 55% in cases with any HPV (maximum estimate) and by the hierarchical attribution model estimate, 38% by the proportional attribution model estimate, and 12% when considering single carcinogenic infections only (minimum estimate). The proportion of AIN3 cases attributable to HPV16 ranged from 61% to 9%, whereas for AIN2 these fractions ranged from 47% to 16%.

The relative prevalence of other non-HPV16 carcinogenic types varied between individual disease categories according to the method of estimation of attribution fractions. Of note, HPV31, which was the second most common carcinogenic HPV type overall (14%), was ranked tenth most common out of 13 carcinogenic types in AIN3 lesions but was the second most common in AIN2 lesions. HPV18 also had a pattern similar to that of HPV31, while HPV51 and HPV33 had higher attribution fractions for AIN3 lesions (≥25%) but much lower fractions for AIN2 lesions (≤13%).

While HPV16 was the most common carcinogenic HPV genotype in all AIN categories (AIN1, AIN2, and AIN3), it was less common than the others in men with <AIN1 (Supplementary Tables 2 and 3). No single HPV genotype dominated the <AIN1 group in lieu of HPV16. Consequently, the range of proportional attribution fractions between individual types was much narrower (from 2% for HPV68 to 7% for HPV31), compared with the corresponding range in high-grade lesions

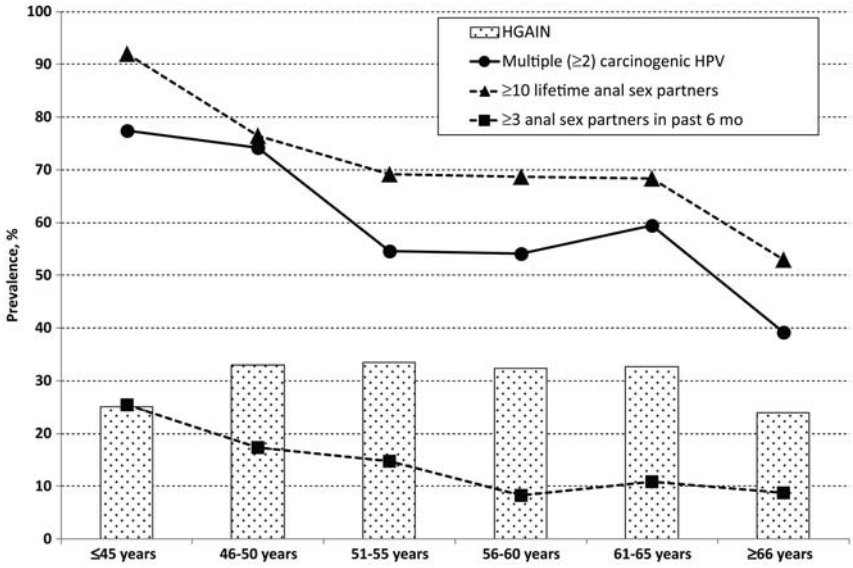


Figure 1. Composite line and bar graphs showing age-category-specific prevalence levels of anal human papillomavirus (HPV) related clinical and behavioral parameters among human immunodeficiency virus–infected men who have sex with men. The line graphs show proportions of (1) multiple carcinogenic HPV genotypes, (2) ≥10 lifetime anal sex partners, and (3) ≥3 recent (ie, within the past 6 months) anal sex partners. The bar graphs depict the high-grade anal intraepithelial neoplasia (HGAIN) disease prevalence. See Methods for a definition of HGAIN. Abbreviation: HGAIN, high-grade AIN.

Table 2. Attribution of Carcinogenic Human Papillomavirus (HPV) Types to 104 High-Grade Anal Intraepithelial Neoplasia (HGAIN) Cases

	Any Type Infection[a]		Hierarchical Attribution[b]		Proportional Attribution[c]		Single Carcinogenic Type Infection[d]	
HPV Type	No.	%	No.	%	No.	%	No.	%
HPV16	57	55	57	55	39.04	38	12	12
HPV31	23	22	13	13	8.86	9	2	2
HPV33	21	20	8	8	8.28	8	3	3
HPV39	20	19	6	6	5.87	6	1	1
HPV51	20	19	5	5	6.19	6	1	1
HPV18	18	17	2	2	4.53	4	0	0
HPV58	18	17	2	2	4.78	5	1	1
HPV56	17	16	0	0	3.97	4	0	0
HPV35	16	15	2	2	4.69	5	2	2
HPV45	15	14	1	1	3.54	3	1	1
HPV59	14	14	1	1	2.71	3	0	0
HPV52	13	13	2	2	4.69	5	2	2
HPV68	11	11	0	0	1.84	2	0	0
Overall	…	…	99	95	99	95	25	24

HGAIN comprised of a total of 45 cases of AIN2, and 59 cases of AIN3. See Methods for definitions of AIN grades.

[a] Frequency of HPV types, with inclusion of all cases in which an HPV type is detected (maximum attribution).

[b] The most frequent type (according to its frequency in the respective disease category) is attributed to the case.

[c] Each type is proportionally attributed to a case according to its frequency in the respective disease category.

[d] Frequency of HPV types, with inclusion of cases with single carcinogenic infections, irrespective of additional noncarcinogenic type infections.

Table 3. Attribution of Carcinogenic Human Papillomavirus (HPV) Types to 59 Anal Intraepithelial Neoplasia Grade 3 (AIN3) cases

	Any Type Infection[a]		Hierarchical Attribution[b]		Proportional Attribution[c]		Single Carcinogenic Type Infection[d]	
HPV Type	No.	%	No.	%	No.	%	No.	%
HPV16	36	61	36	61	23.01	39	5	9
HPV51	16	27	9	15	5.91	10	1	2
HPV33	15	25	5	9	5.98	10	2	3
HPV35	12	20	2	3	4.1	7	2	3
HPV39	12	20	1	2	2.9	5	0	0
HPV45	12	20	1	2	2.6	4	0	0
HPV58	12	20	0	0	2.54	4	0	0
HPV56	10	17	1	2	2.22	4	0	0
HPV18	9	15	0	0	1.55	3	0	0
HPV31	9	15	0	0	1.55	3	0	0
HPV52	7	12	1	2	2.15	4	1	2
HPV59	6	10	0	0	0.68	1	0	0
HPV68	6	10	0	0	0.81	1	0	0
Overall	…	…	56	95	56	95	11	19

See Methods for definition of AIN3.

[a] Frequency of HPV types, with inclusion of all cases in which an HPV type is detected (maximum attribution).

[b] The most frequent type (according to its frequency in the respective disease category) is attributed to the case.

[c] Each type is proportionally attributed to a case according to its frequency in the respective disease category.

[d] Frequency of HPV types, with inclusion of cases with single carcinogenic infections, irrespective of additional noncarcinogenic type infections.

Table 4. Attribution of Carcinogenic Human Papillomavirus (HPV) Types to 45 Anal Intraepithelial Neoplasia Grade 2 (AIN2) Cases

	Any Type Infection[a]		Hierarchical Attribution[b]		Proportional Attribution[c]		Single Carcinogenic Type Infection[d]	
HPV Type	No.	%	No.	%	No.	No.	%	No.
HPV16	21	47	21	47	15.49	34	7	16
HPV31	14	31	10	22	7.66	17	2	4
HPV18	9	20	3	7	3.01	7	0	0
HPV39	8	18	3	7	2.89	6	1	2
HPV59	8	18	1	2	2.21	5	0	0
HPV56	7	16	0	0	1.73	4	0	0
HPV33	6	13	2	4	2.3	5	1	2
HPV52	6	13	1	2	2.28	5	1	2
HPV58	6	13	1	2	2.26	5	1	2
HPV68	5	11	0	0	0.83	2	0	0
HPV35	4	9	0	0	0.51	1	0	0
HPV51	4	9	0	0	0.62	1	0	0
HPV45	3	7	1	2	1.19	3	1	2
Overall	…	…	43	96	43	96	14	31

See Methods for definition of AIN2.

[a] Frequency of HPV types, with inclusion of all cases in which an HPV type is detected (maximum attribution).

[b] The most frequent type (according to its frequency in the respective disease category) is attributed to the case.

[c] Each type is proportionally attributed to a case according to its frequency in the respective disease category.

[d] Frequency of HPV types, with inclusion of cases with single carcinogenic infections, irrespective of additional noncarcinogenic type infections.

(eg, in AIN3, the fractions ranged from 1% for HPV68 to 39% for HPV16; Table 2).

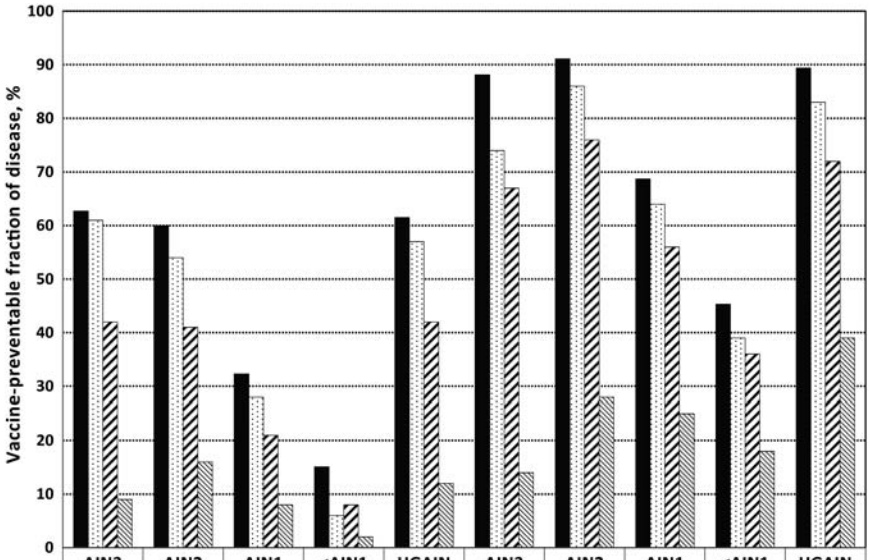
While HPV16 was the most common carcinogenic HPV type present singly in AIN2 and AIN3 lesions, HPV18, HPV56, HPV59, and HPV68 were never present as single carcinogenic HPV infections (ie, they were always present concurrently with other carcinogenic HPV types; Tables 3 and 4). When type attribution was evaluated across all HPV types (regardless of carcinogenicity potential), HPV16 was still the most frequent type and had higher attribution fractions for AIN3, AIN2, and HGAIN lesions, compared with other HPV genotypes (data not shown).

Attribution of HGAIN to Vaccine-Preventable HPV Genotypes

The ranges of HGAIN fractions attributable to carcinogenic HPV genotypes included in prophylactic HPV vaccines were wide (Figure 2). For the currently licensed bivalent and quadrivalent HPV vaccines that target the carcinogenic types HPV16 and HPV18, the estimates ranged from 9% to 62.7% for AIN3 lesions, from 16% to 60% for AIN2 lesions, and from 12% to 61.5% for HGAIN lesions (Figure 2). Similarly, the range of fractions attributable to the carcinogenic HPV types (HPV 16/18/31/33/45/52/58) targeted by an investigational nonavalent vaccine ranged from 14% to 88.1% for AIN3 lesions, from 28% to 91.1% for AIN2 lesions, and from 39% to 89.4% for HGAIN lesions. The estimates by the hierarchical and proportional attribution schemes were intermediate between the low-end estimates (which considered only the sum of percentages of the vaccine genotypes in cases with single carcinogenic infections) and the high-end estimates (which considered the sum of percentages of the vaccine genotypes in all cases, regardless of concurrency).

DISCUSSION

The significant diversity and high prevalence of multiple carcinogenic HPV infections complicates a fuller understanding of the role and risk of individual HPV genotypes in anal carcinogenesis among HIV-infected MSM, for whom the need for anal cancer prevention is the highest [13, 14]. The clarification of the role of these individual genotypes can allow estimation of the efficacy of prophylactic vaccines, as well as inform the spectrum of HPV genotypes for future HPV-based screening



Any type	62.7%	60.0%	32.3%	15.1%	61.5%	88.1%	91.1%	68.7%	45.3%	89.4%
Hierarchical attribution	61.0%	54.0%	28.0%	6.0%	57.0%	74.0%	86.0%	64.0%	39.0%	83.0%
Proportional attribution	42.0%	41.0%	21.0%	8.0%	42.0%	67.0%	76.0%	56.0%	36.0%	72.0%
Single carcinogenic infection	9.0%	16.0%	8.0%	2.0%	12.0%	14.0%	28.0%	25.0%	18.0%	39.0%

Figure 2. Bar graphs showing the range of human papillomavirus (HPV) vaccine–preventable fractions of various anal disease categories among human immunodeficiency virus–infected men who have sex with men. The proportions depicted include the maximum estimates (any type) and the minimum estimates (single carcinogenic infection), along with the hierarchical and proportional attribution model estimates. These ranges are separately depicted for the carcinogenic genotypes included in the currently licensed (bivalent and quadrivalent) and investigational (nonavalent) HPV prophylactic vaccines. See Methods for definitions of anal intraepithelial neoplasia (AIN) grades. Abbreviation: HGAIN, high-grade AIN.

assays [15]. Because HPV vaccination will be expanded among men [16], especially among higher-risk populations, the resulting changes in anal genotypes composition will influence associations between HPV genotypes and disease ascertainment [17–19].

In this cross-sectional study, we have used a novel framework, previously validated in studies of cervical lesions [11, 12], for addressing the methodological challenge of attributing a causative role for individual HPV genotypes due to the concurrent presence of multiple HPV infections. To our knowledge, this is the first report that attempts to address this issue in the context of anal HPV genotypes and uses this to estimate the fraction of AIN lesions potentially preventable by prophylactic HPV vaccines. HPV16, which has been shown to cause an even greater proportion of anal cancers than cervical cancers [20–22], is seen as the dominant genotype in HGAIN disease categories, whereas the other HPV genotypes vary substantially in their prevalence and attribution estimates in our study.

It has been suggested that the key steps and role of carcinogenic HPV genotypes in anal carcinogenesis mirror those in cervical carcinogenesis [23]. While there is significant overlap in the types causing high-grade lesions of the anal canal and the cervix, there is not complete concordance. Indeed, HPV16 is likely overrepresented in anal lesions as compared to cervical lesions. A relative preponderance of non-HPV16 cervical HPV genotypes has been reported for immunocompromised HIV-infected women [21, 22]. In contrast, we observed a higher prevalence of HPV16 in HIV-infected MSM who were relatively immunocompromised ($CD4^+$ T-cell count <350/μL), compared with HIV-infected MSM who were not immunocompromised ($CD4^+$ T-cell count ≥350/μL); ($P = .04$; data not shown), although this was unadjusted for other factors. Larger studies are needed to further explore these findings and understand whether attribution fractions differ by levels of immunosuppression.

Similar to patterns described in cervical infection [11], we observed that the proportion of MSM with multiple carcinogenic infections declined with advancing age (Figure 1); in contrast to cervical disease [11], increasing grades of anal disease were associated with increasing multiplicity of carcinogenic HPV infections. The etiologic significance of these multiple carcinogenic HPV infections in HGAIN remains to be established, although they likely represent several transient carcinogenic HPV infections populating the anal transformation zone, along with a single causal carcinogenic HPV genotype. We observed that the age-specific prevalence of multiple carcinogenic HPV infections correlated with higher lifetime and recent (ie, within the past 6 months) numbers of sex partners, which are proxies for sexual behaviors and multiple exposures (Figure 1). It also remains to be investigated whether immunosuppression and treatment status may be independently associated with increased detection of multiple carcinogenic HPV genotypes in high-grade lesions.

In the absence of functional data, tissue-based genotyping evidence (such as laser-capture microdissection [24]), or evidence from longitudinal studies, the framework that we have used in this study allows presentation of a range of attribution fractions that can be assigned to individual HPV genotypes. We did not have data to weigh genotypes in the proportional attribution model by their carcinogenic risk, other than their overall frequency in that disease category. Hence, our analysis was restricted to attribution to carcinogenic HPV genotypes in which other/noncarcinogenic types did not dilute the proportional attribution fractions. When these other/noncarcinogenic types were considered to make attribution decisions, the fraction of HGAIN lesions attributable to HPV16 was lower (23%; data not shown), compared with the fraction when restricted to lesions attributable to carcinogenic HPV genotypes only (38%). Ideally, the frequency of types in single infections should constitute the underlying assumptions of hierarchy/proportions (as previously described by Insinga et al [12]). However, the very infrequent single genotype infections in our study did not permit us to meaningfully assign these frequencies.

The ranges of the attribution fractions between the minimum estimates (ie, cases with single carcinogenic HPV) and maximum estimates (ie, cases with any HPV) are wide. Given the high rate of multiple infections, the minimum estimate likely represents an underestimation and the maximum estimate an overestimation of the attribution fractions of individual HPV genotypes. The ranges also differ significantly between types. By using biologically and clinical relevant assumptions, we have therefore modeled the proportional and hierarchical attribution fractions that provide the closest approximations of the preventable fraction. These estimates can inform assumptions of HPV vaccine efficacy for cost-effectiveness and decision-analysis models [25].

Some key strengths of our study include the use of a highly sensitive HPV assay and the detailed characterization of anal disease end points, using diagnostic categories that were based on both cytology and histology results to maximize disease ascertainment. Since this study was focused on the evaluation of anal precancers, we are unable to determine the HPV genotype composition of anal cancers, an area with a substantial need for additional research. While the prevalences of carcinogenic HPV (75.4% [95% CI, 70.5%–79.9%]) and HGAIN (30.4% [95% CI, 25.8–35.5]), as estimated in our study, are in line with those reported from a recent global meta-analysis of similar studies in other HIV-positive MSM (73.5% [95% CI, 63.9–83.0] and 29.1% [95% CI, 22.8–35.4], respectively) [3], the geographic differences in attribution fractions in anal precancers and cancers will be important to clarify in future studies.

The prevention of anal cancer in high-risk populations such as HIV-infected MSM remains an urgent priority, given the

rising incidence rates, despite widespread antiretroviral therapy use [1]. The analytical framework presented in this study can be applied to larger and pooled efforts to improve estimations of the causative role of individual genotypes and expand our understanding of the natural history of and prevention approaches for anal neoplastic disease.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. P. E. C. is compensated for serving on a Merck data and safety monitoring board for HPV vaccines. T. M. D. is compensated for serving on the boards of Arbor Vita and Onco Health and has stock options in Onco Health. Roche Molecular Systems provided HPV genotyping assays and tests without charge. S. B., M. S., and S. D. T. are employees of Roche Molecular Systems. All other authors report no potential conflicts.

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