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Anal Human Papillomavirus Genotype Distribution in HIV-Infected Men Who Have Sex with Men by Geographical Origin, Age, and Cytological Status in a Spanish Cohort

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Knowledge of human papillomavirus (HPV) type distribution in populations at risk for anal cancer is needed. Here, we describe the anal HPV genotype distribution in a large Spanish cohort (Cohort of the Spanish HIV Research Network HPV [CoRIS-HPV]) of HIV-positive men who have sex with men (MSM) according to geographical origin, age, and cytological status. A cross-sectional analysis of baseline data from 1,439 HIV-infected MSM (2007 to 2012) was performed. Anal HPV genotyping was performed using the Linear Array HPV genotyping test. Descriptive analyses of subject characteristics, prevalences, and 95% confidence intervals (CI) were performed. The global prevalences of HPV, high-risk HPV (HR-HPV), and low-risk HPV (LR-HPV) types were 95.8%, 83.0%, and 72.7%, respectively. Among the HR-HPV types, HPV16 was the most common, followed by HPV59, -39, -51, -18, and -52. The prevalence of multiple HR-HPV infections was 58.5%. There were no differences in the crude analyses between Spanish and Latin-American MSM for most HPV types, and a peak in prevalence for most HPV types was seen in patients in their late thirties. Globally and by specific HPV groups, men with abnormal anal cytologies had a higher prevalence of infection than those with normal cytologies. This study has the largest number of HIV-positive MSM with HPV genotype data analyzed according to cytological status as far as we know. The information gained from this study can help with the design of anal cancer prevention strategies in HIV-positive patients.

Persistent infections with high-risk types of human papillomavirus (HR-HPV) are responsible for >80% of cases of anal cancer (AC) (1). In the last 20 to 30 years, the incidence of AC has increased significantly, especially in men who have sex with men (MSM), and more so in those also infected with HIV (2–4). Infection with any HPV type is very frequent in HIV-positive MSM, with prevalences of >90%, and HR-HPV infections are present in 48.9% to 94.4% of these patients (4–6). Likewise, infections with multiple HR-HPV types are also very common, being close to 60% (4, 6–8). Differences in the prevalences of HPV, HR-HPV types, and multiple HPV infection have been observed in previous studies mainly because of the molecular genotyping methods used, sample sizes, and the analyzed populations.

Anal squamous intraepithelial lesions (SIL) are precursors of AC and, in a recent review, a high prevalence of anal SIL (up to 57.2%) has been described in patients infected with HIV, similar to the prevalence of 54.7% that was described recently in the Cohort of the Spanish HIV Research Network HPV (CoRIS-HPV) in Spain (4, 9).

As in invasive cervical carcinoma, HPV16 is causally linked to AC, and in European men, it is detected in 87.1% of the cases, while HPV18 is reported in only 6.2% of them (1, 10).

Limited data are available, though, on anal HPV type distribution in HIV-positive subjects and more specifically in MSM. Knowledge of the specific HPV type distribution in populations at high risk for AC and its precursor lesions would be very useful for vaccine design, as well as for establishing the attribution of the different HPV types in the natural history of HPV infection.

The objective of this work was to describe the prevalences and distributions of anal HPV genotypes in HIV-positive MSM in Spain according to their geographical origin, age, and cytological status.

MATERIALS AND METHODS

Subjects and methods. We performed a cross-sectional analysis of baseline samples and data from CoRIS-HPV. CoRIS-HPV is a cohort study within CoRIS, the cohort of the Spanish Network of Excellence on HIV/AIDS Research. Established in January 2004, CoRIS is an open and multicenter cohort of adult patients with confirmed HIV infection who are naive to combined antiretroviral therapy (cART) at study entry. Patients in CoRIS are followed periodically according to routine practices. CoRIS collects baseline and follow-up sociodemographic (age, sex, category of transmission of HIV, educational level, geographical origin), clinical (AIDS and non-AIDS defining conditions), immunological (CD4 T-cell counts), virological (HIV viral load), antiretroviral treatment status, and vital status (including cause of death) data. Ethics approval and signed informed consent were obtained (11). The CoRIS-HPV study has been

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TABLE 1 Sociodemographic characteristics among HIV-infected MSM within CoRIS-HPV with valid HPV DNA and cytological results

Subject characteristic	HIV-positive MSM with:	
	Valid HPV result (n = 1,439)	Valid cytological result (n = 950)
Geographical origin (no. [%])		
Spain	1,003 (69.7)	655 (68.9)
Latin-American country	319 (22.2)	220 (23.2)
Other country	87 (6.0)	58 (6.1)
Unknown	30 (2.1)	17 (1.8)
Age		
Median (IQR) (yr)	33.6 (28.2–40.1)	33.6 (28.0–40.0)
Range (yr)	17.6–77.3	17.6–77.3
≤30 yr (no. [%])	557 (38.7)	375 (39.5)
31–35 yr (no. [%])	312 (21.7)	203 (21.4)
36–40 yr (no. [%])	256 (17.8)	169 (17.8)
≥41 yr (no. [%])	314 (21.8)	203 (21.3)

described elsewhere and was set up in January 2007 to study the epidemiology of HPV coinfection within CoRIS (6, 9). All patients from CoRIS, regardless of ART history, were invited to participate in CoRIS-HPV. The study subjects were informed about the nature of the study and were required to sign an *ad hoc* informed consent form. Specific ethics approval for this study was obtained (reference no. PI-43 of the Instituto de Salud Carlos III [ISCIII] Research Ethics Committee). Besides the aforementioned variables, in CoRIS-HPV, sexual behavior variables were also collected (age of first sexual intercourse, number of lifetime sexual partners, number of sexual partners in the preceding 12 months, and frequency of unprotected intercourse in the preceding 12 months). At entry in CoRIS-HPV, two anal samples were collected for HPV detection and liquid cytological analysis.

HPV DNA detection and genotyping. Anal samples were collected with a cytobrush and placed into Digene specimen transport medium (Qiagen, Hilden, Germany), stored at −20°C, and shipped to the Retroviruses and Papillomavirus Unit of the National Centre for Microbiology in Madrid for testing. DNA was extracted from a 200-μl aliquot of the original anal sample using an automatic DNA extractor (BioRobot M48 Robotic Workstation; Qiagen). For quality control, 10 samples were included in each extraction run, as well as one negative control (PCR-quality water) and one positive control (SiHa cells infected with HPV16). Anal HPV infection and genotyping were determined through the Linear Array HPV genotyping test (Roche Molecular Systems, Inc., Pleasanton, CA), which detects 37 HPV types. Human β-globin gene fragment detection was used as an internal control. The results were considered satisfactory if there were low and high β-globin levels or if at least one HPV type was detected. As the Linear Array HPV52 probe cross-reacts with HPV33, HPV35, and HPV58, in samples in which any of these HPV types were detected, an additional HPV52 infection cannot be excluded. Therefore, in these samples, a specific HPV52 PCR system designed in the E6 gene was performed (12). For the analysis, the HPV types were classified on the basis of their association with cancer using the Muñoz et al. (13) classification. HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, and -59 were considered HR-HPV types, HPV6, -11, -40, -42, -54, -61, -70, -72, and -81, and CP6108 were considered low-risk (LR)-HPV types, HPV26, -53, -66, -68, -73, and -82 were considered probably high-risk (PHR)-HPV types, and HPV55, -62, -64, -67, -69, -71, -83, and -84, and IS39 were considered undetermined risk (UR)-HPV types.

Anal liquid cytology. Anal liquid cytology samples were collected by clinicians using an endocervical brush device (Cytobrush; Hologic, Inc., Bedford, MA). The brush was rinsed in ThinPrep PreservCyt solution (Hologic, Inc.) to resuspend the cells. Cytological slides were obtained using a ThinPrep 2000 processor (Hologic, Inc.). Samples with poor cellularity were considered inadequate for the cytological analysis. The cytological results were reported following the Bethesda 2001 classification, also accepted for anal cytology as negative for an intraepithelial lesion, atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (L-SIL), or high-grade squamous intraepithelial lesion (H-SIL) (14).

Definition of variables. The outcome variable for the current analyses was type-specific HPV prevalence. Separate analyses for specific HPV type groups were performed according to any HPV type, HR-HPV types, LR-HPV types, PHR-HPV types, UR-HPV types, HR-HPV types belonging to alpha-7 species (HPV18, -39, -45, and -59), HR-HPV types belonging to alpha-9 species (HPV16, -31, -33, -35, -52, and -58), HPV types included in the quadrivalent vaccine (HPV6, -11, -16, and -18), and the most frequent HR-HPV types detected in cases of AC (HPV16, -18, -31, and -33), as described by De Vuyst et al. (1). The independent sociodemographic variables analyzed were geographical origin (categorized as Spanish, Latin-American, or other) and age (categorized as ≤30 years, 31 to 35 years, 36 to 40 years, or ≥41 years). The anal cytology results were categorized as negative or abnormal, which included ASC-US, L-SIL, and H-SIL.

Statistical analysis. We analyzed all the subjects with baseline samples for HPV who were included in CoRIS-HPV until June 2012. Descriptive analyses of the subject characteristics were performed. Frequency distributions are presented for qualitative variables, and medians and interquartile ranges (IQRs) are presented for quantitative variables with asymmetrical distributions. We used the chi-square test for the comparison of qualitative variables and nonparametric tests for the comparison of medians. Prevalence and 95% confidence intervals (CI) were calculated. The analyses were conducted using Stata 12 (Stata Corp, LP, College Station, TX).

RESULTS

Overall, 1,477 patients were included in the analyses, of which 1,439 had a valid HPV DNA result (38 samples with inadequate cellularity were excluded). Of these 1,439 subjects, 1,037 also had a cytology performed, the results of 87 of which were considered inadequate because of poor cellularity, leaving a final sample size of 950 subjects with a valid cytological result. The majority (69.7%) of the patients were Spaniards, 45% were naive for antiretroviral therapy, the median age was 33.6 years (IQR, 28.2 to 40.1), and there were no differences by geographical origin or age between those with a valid cytological result and those without (Table 1).

Prevalence of HPV genotypes. The global prevalences for any HPV, HR-HPV, LR-HPV, PHR-HPV, and UR-HPV types were 95.8% (95% CI, 94.6 to 96.7), 83.0% (95% CI, 80.9 to 84.9), 72.7% (95% CI, 70.3 to 75.0), 57.5% (95% CI, 54.9 to 60.0), and 51.1% (95% CI, 48.5 to 53.7), respectively (Fig. 1). The most common HR-HPV types were HPV16 (34.7%), HPV59 (20.5%), HPV39 (18.8%), HPV51 (18.6%), HPV18 (18.1%), and HPV52 (18.1%). HPV52 prevalence obtained using an additional and HPV52-specific PCR in samples infected with HPV33, -35, and -58 was twice (18.1% versus 9.2%) that obtained using only the Linear Array HPV genotyping test. HPV6 and CP6108 were the most frequently detected LR-HPV types (21.6% and 20.0%, respectively). HPV53 and HPV66 were the most prevalent types found from the PHR-HPV group (25.8% and 18.3%, respectively), and HPV84 and HPV62 were the most prevalent types found in the UR-HPV group (21.8% and 14.9%, respectively).

HPV types belonging to the alpha-7 and alpha-9 species were detected in 54.6% (95% CI, 52.0 to 57.2) and 63.9% (95% CI, 61.3 to 66.4) of the patients, respectively. The HR-HPV types most frequently detected in cases of AC (HPV16, -18, -31, and -33) were

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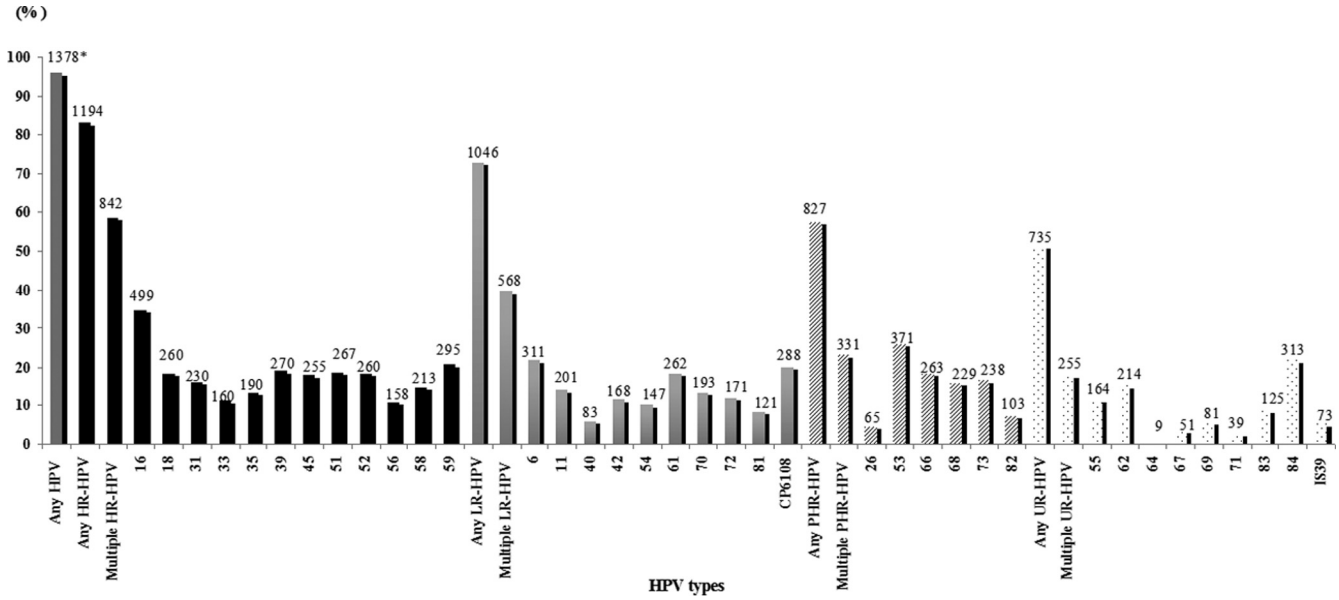

FIG 1 Prevalences of HPV types in 1,439 HIV-infected MSM within CoRIS-HPV. The numbers above the bars indicate the number of patients in each category.

present in 57.1% (95% CI, 54.5 to 59.7) of the anal samples, and 881 patients (61.2%) (95% CI, 58.7 to 63.8) harbored one of the HPV types HPV6, -11, -16, or -18.

Multiple HR-, HR/PHR-, and HR/LR-HPV infections were detected in 58.5% (95% CI, 55.9 to 61.1), 51.1% (95% CI, 48.5 to 53.8), and 63.6% (95% CI, 61.0 to 66.1) of all samples, respectively. The median number of any HPV type was 5 (IQR, 3 to 7), and for HR-HPV types, the median number was 2 (IQR, 1 to 3). In 932 MSM (64.8%; 95% CI, 62.2 to 67.2), we detected more than four HPV types, and in 292 MSM (20.3%; 95% CI, 18.2 to 22.5), we detected more than four HR-HPV types.

HPV type distribution by geographical origin. Differences in specific HPV type distribution by geographical origin were analyzed (Fig. 2), comparing patients from Spain (n = 1,003) and patients from Latin-American countries (n = 319). Specific HPV type prevalences were similar in both Spaniards and Latin-Amer-

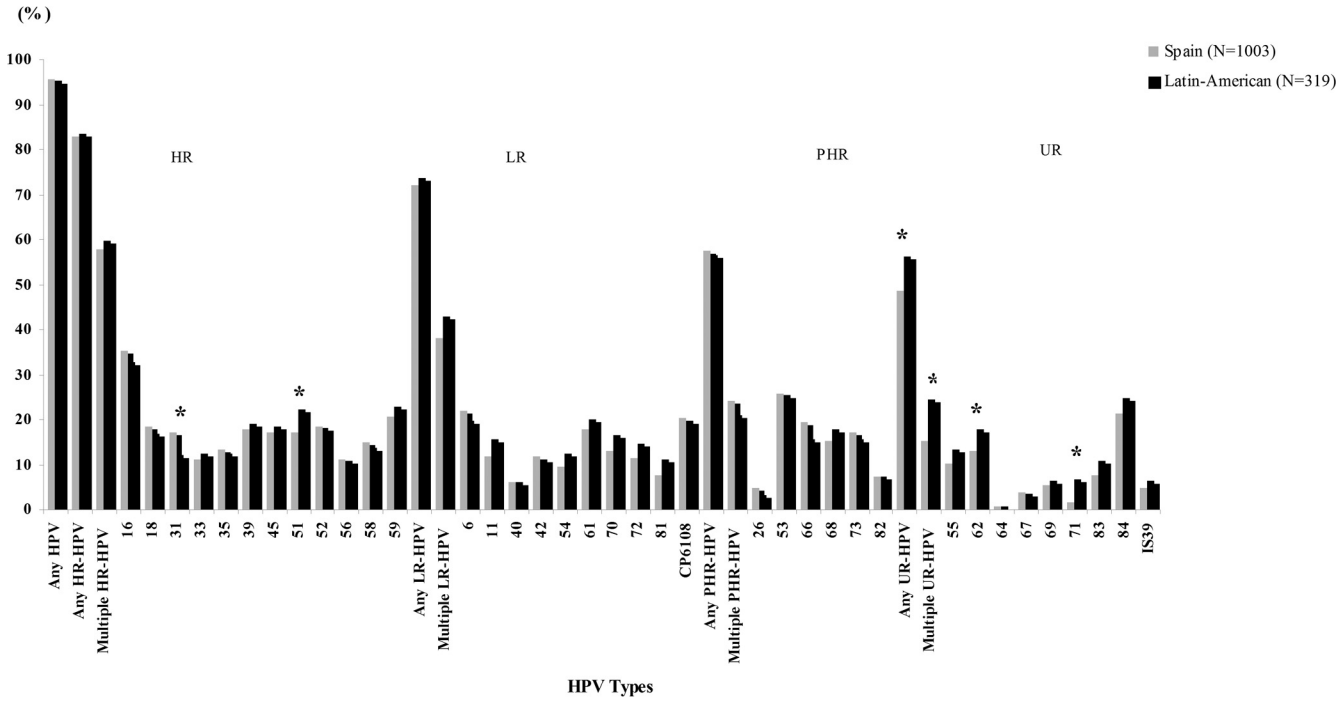

FIG 2 Prevalences of HPV types in HIV-infected MSM within CoRIS-HPV by geographical origin. *, $P < 0.05$.

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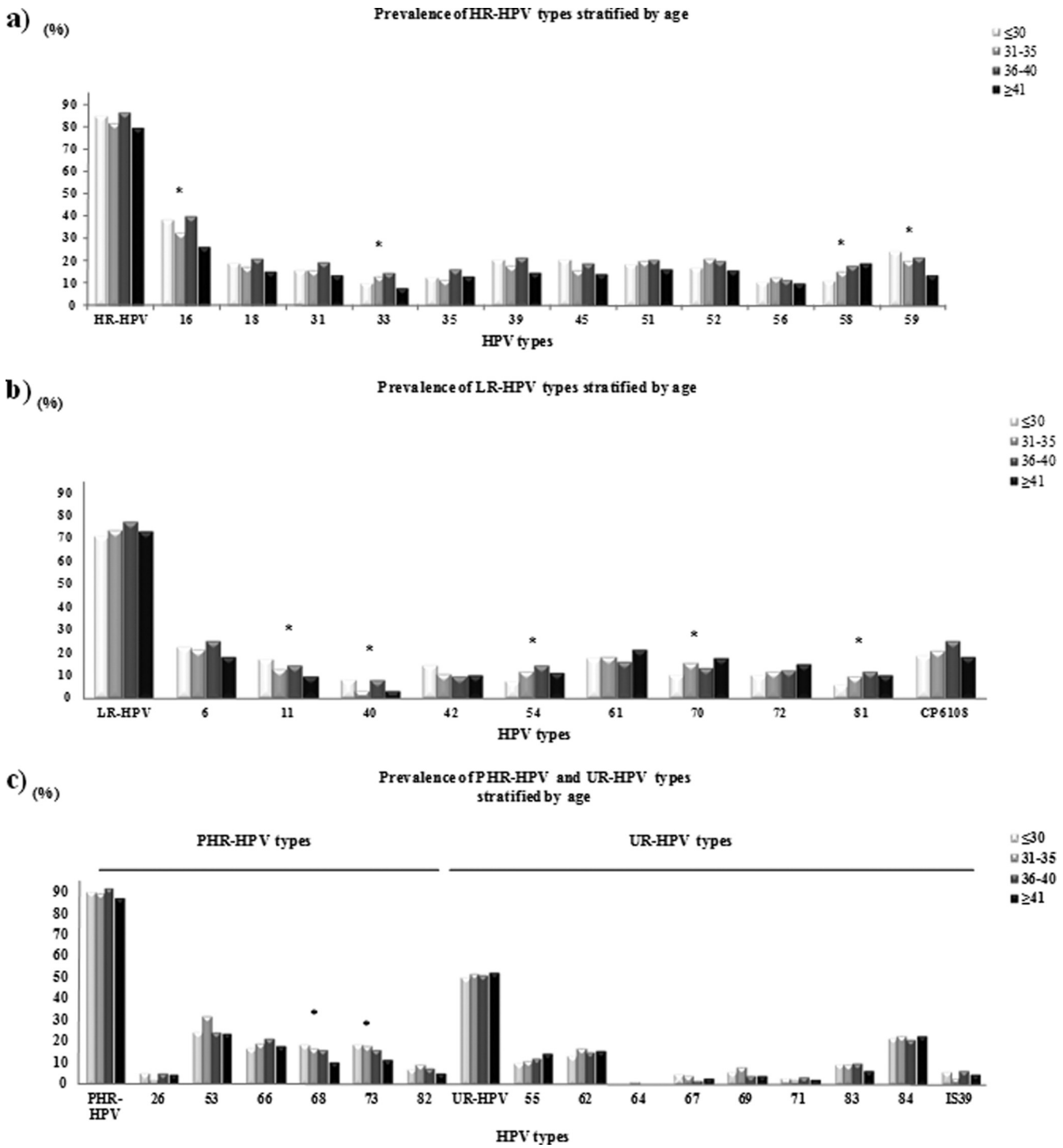

FIG 3 Prevalences of HR-HPV (a), LR-HPV (b), and PHR-HPV and UR-HPV (c) types in 1,439 HIV-infected MSM within CoRIS-HPV by age. *, $P < 0.05$.

icans; the only statistically significant differences were observed for HPV31, which was more common in Spaniards (17.0% versus 12.2%, $P = 0.040$), and for HPV51, -62, and -71, which were more frequently detected in Latin-American patients ($P = 0.045$, 0.025, and <0.001, respectively).

HPV type distribution by age. The distribution of HPV types across four age categories is described in Fig. 3. Overall, HR-HPV infection was not associated with age, but a statistically significant association with age was observed for HR-HPV16, -33, -58, and -59. HPV58 prevalence increased with age ($P = 0.004$). A slight peak in prevalence for the 36- to 40-year age category was observed for HPV16, -18, -31, -33, -35, -39, and -59.

For LR-HPV types, statistically significant associations with age were observed for HPV11, -40, -54, -70, and -81, and this effect

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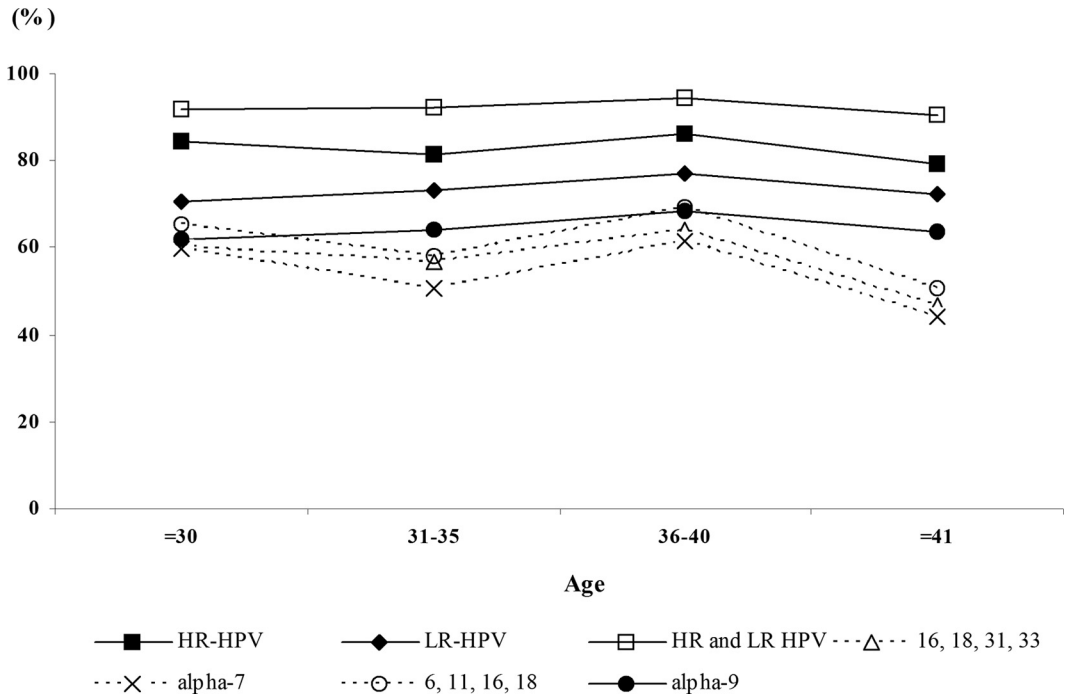

FIG 4 Prevalences of HPV type groups by age in 1,439 HIV-infected MSM within CoRIS-HPV. Statistically significant differences are indicated by dotted lines; HPV16, -18, -31, and -33 are the most frequent types found in AC cases, and HPV6, -11, -16, and -18 are the HPV types included in the approved quadrivalent vaccine.

was not observed when any LR-HPV infection was considered. A slight peak in the prevalence for the 36- to 40-year age category was observed for HPV6, -11, -40, -54, and -81, and for CP6108.

For PHR- and UR-HPV types, a statistically significant effect of age was observed only for HPV68 and -73. Age-specific curves for HPV types according to various classifications are shown in Fig. 4. Only infections with any of the HR-HPV types most detected in AC cases, any of the HPV types included in the quadrivalent approval vaccine, and HR-HPV types belonging to the alpha-7 species had a statistically significantly association with age. We also analyzed HPV prevalence data in the <30-year-old age group (data not shown), and no differences were observed.

HPV type distribution by cytological status. Of the 950 subjects with valid cytology results, 441 (46.4%) had negative anal cytologies and 509 (53.6%) had abnormal anal cytologies, which included 103 cases of ASC-US (10.8%), 362 cases of L-SIL (38.1%), and 44 cases of H-SIL (4.6%). No cases of squamous cell carcinoma were diagnosed.

HPV type-specific prevalence by cytological status is described in Table 2. Patients with abnormal cytology results presented with a higher prevalence of any HPV, HR-HPV, LR-HPV, and PHR-HPV types than those with negative cytology results ($P < 0.05$, for all categories). Likewise, individual HR-HPV type prevalence in patients with an abnormal cytology result was significantly higher for all types, except for HPV33, -58, and -59. HPV16 was the most frequently detected HR-HPV type in both negative and abnormal cytologies (27.9% and 44.2%, respectively). The next most frequent HR-HPV types were HPV59, -52, -58, and -39 in MSM with negative cytologies and HPV51, -39, and -52 in patients with abnormal cytologies.

Prevalence values of LR-HPV types HPV6, -11, -40, -42, and -72 and CP6108 were also significantly higher in MSM with abnormal cytologies; HPV6 was the most frequently detected LR-HPV type in patients with L-SIL and H-SIL and HPV CP6108 was the second most common LR-HPV type in all cytological categories. PHR-HPV types HPV66, -68, and -73 were significantly more prevalent in MSM with abnormal cytologies ($P < 0.05$ for all categories).

MSM with abnormal cytology results had a higher prevalence of HPV types belonging to the alpha-7 and alpha-9 species groups than MSM with negative cytology results (62.9% and 74.7% versus 50.6% and 56.0%, respectively). In 67% of the MSM with an abnormal cytology, at least one of the HPV types most frequently detected in cases of AC (HPV16, -18, -31, and -33) were identified, and 74.5% harbored any of the four HPV types included in the quadrivalent vaccine (HPV16, -18, -6, and -11).

Patients with abnormal cytology results had a higher prevalence of multiple HPV infections than those with negative cytology results ($P < 0.001$) and were infected more frequently with more than four of any HPV or HR-HPV types.

As shown in Fig. 5, the prevalence of each HR-HPV type present as part of multiple infections was significantly higher in patients with cytological abnormalities, except for HPV33, -58, and -59, in which the observed differences were not significant.

We also analyzed anal HPV infections in patients with abnormal cytologies stratified by age and by geographical origin. No statistically significant differences were observed by geographical origin (data not shown) or age in the four categories for prevalence values of any HPV, HR-HPV, LR-HPV, PHR-HPV, UR-HPV, and HPV types belonging to the alpha-9 species. MSM aged >40 years presented with a significantly lower prevalence of HPV

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TABLE 2 Prevalence of anal HPV infection by cytological status in 950 HIV-infected MSM within CoRIS-HPV

HPV infection status/type	No. (%) of patients with negative cytology results	No. (%) of patients with abnormal cytology result of:				P^a
		ASC-US	L-SIL	H-SIL	Total	
Negative for HPV	26 (6.0)	2 (2.0)	3 (0.8)	1 (2.3)	6 (1.2)	
Any HPV	415 (94.1)	101 (98.1)	359 (99.2)	43 (97.7)	503 (99.0)	**<0.001**
HR-HPV						
Any HR-HPV	336 (76.2)	95 (92.2)	327 (90.3)	43 (97.7)	465 (91.4)	**<0.001**
Multiple HR-HPV types	229 (52.0)	75 (72.8)	251 (69.3)	39 (88.6)	365 (71.7)	**<0.001**
HPV16	123 (27.9)	49 (47.6)	150 (41.4)	26 (59.1)	225 (44.2)	**<0.001**
HPV18	67 (15.2)	24 (23.3)	79 (21.8)	7 (15.9)	110 (21.6)	**0.011**
HPV31	61 (13.8)	25 (24.3)	67 (18.5)	12 (27.3)	104 (20.4)	**0.007**
HPV33	38 (8.6)	13 (12.6)	41 (11.3)	8 (18.2)	62 (12.2)	0.074
HPV35	32 (7.3)	22 (21.4)	66 (18.2)	11 (25.0)	99 (19.4)	**<0.001**
HPV39	71 (16.1)	24 (23.3)	81 (22.4)	10 (22.7)	115 (22.6)	**0.012**
HPV45	64 (14.5)	16 (15.5)	80 (22.1)	10 (22.7)	106 (20.8)	**0.011**
HPV51	61 (13.8)	20 (19.4)	88 (24.3)	11 (25.0)	119 (23.4)	**<0.001**
HPV52	73 (16.6)	23 (22.3)	78 (21.5)	13 (29.5)	114 (22.4)	**0.024**
HPV56	37 (8.4)	14 (13.6)	51 (14.1)	7 (15.9)	72 (14.1)	**0.005**
HPV58	71 (16.1)	23 (22.3)	52 (14.4)	9 (20.5)	84 (16.5)	0.867
HPV59	90 (20.4)	25 (24.3)	76 (21.0)	9 (20.5)	110 (21.6)	0.650
LR-HPV						
Any LR-HPV	304 (68.9)	82 (79.6)	288 (79.6)	36 (81.8)	406 (79.8)	**<0.001**
Multiple LR-HPV types	142 (32.2)	49 (47.6)	174 (48.1)	19 (43.2)	242 (47.6)	**<0.001**
HPV6	60 (13.6)	22 (21.4)	110 (30.4)	15 (34.1)	147 (28.9)	**<0.001**
HPV11	46 (10.4)	12 (11.7)	77 (21.3)	5 (11.4)	94 (18.5)	**<0.001**
HPV40	17 (3.9)	13 (12.6)	27 (7.5)	2 (4.5)	42 (8.3)	**0.005**
HPV42	38 (8.6)	15 (14.6)	51 (14.1)	7 (15.9)	73 (14.3)	**0.006**
HPV54	50 (11.3)	7 (6.8)	35 (9.7)	4 (9.1)	46 (9.0)	0.241
HPV61	93 (21.1)	25 (24.3)	66 (18.2)	7 (15.9)	98 (19.3)	0.481
HPV70	56 (12.7)	18 (17.5)	53 (14.6)	5 (11.4)	76 (14.9)	0.321
HPV72	43 (9.8)	16 (15.5)	53 (14.6)	3 (6.8)	72 (14.1)	**0.038**
HPV81	42 (9.5)	10 (9.7)	28 (7.7)	6 (13.6)	44 (8.6)	0.638
CP6108	73 (16.6)	24 (23.3)	92 (25.4)	9 (20.5)	125 (24.6)	**0.002**
PHR-HPV						
Any PHR-HPV	254 (57.6)	64 (62.1)	233 (64.4)	28 (63.6)	325 (63.8)	**0.049**
Multiple PHR-HPV	83 (18.8)	31 (30.1)	113 (31.2)	16 (36.3)	160 (31.4)	**<0.001**
HPV26	15 (3.4)	6 (5.8)	16 (4.4)	4 (9.1)	26 (5.1)	0.197
HPV53	120 (27.2)	31 (30.1)	97 (26.8)	13 (29.5)	141 (27.7)	0.866
HPV66	69 (15.6)	21 (20.4)	85 (23.5)	14 (31.8)	120 (23.6)	**0.002**
HPV68	61 (13.8)	18 (17.5)	81 (22.4)	6 (13.6)	105 (20.6)	**0.006**
HPV73	62 (14.1)	19 (18.4)	81 (22.4)	9 (20.5)	109 (21.4)	**0.003**
HPV82	31 (7.0)	9 (8.7)	35 (9.7)	7 (15.9)	51 (10.0)	0.102
UR-HPV						
Any UR-HPV	229 (51.9)	49 (47.6)	190 (52.5)	26 (59)	265 (52.1)	0.967
Multiple UR-HPV	69 (15.6)	20 (19.4)	72 (19.9)	13 (29.5)	105 (20.7)	**0.048**
HPV55	52 (11.8)	11 (10.7)	39 (10.8)	7 (15.9)	57 (11.2)	0.775
HPV62	71 (16.1)	10 (9.7)	62 (17.1)	9 (20.5)	81 (15.9)	0.938
HPV64	3 (0.7)	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.2)	0.251
HPV67	12 (2.7)	4 (3.9)	17 (4.7)	1 (2.3)	22 (4.3)	0.185
HPV69	18 (4.1)	7 (6.8)	25 (6.9)	6 (13.6)	38 (7.5)	**0.027**
HPV71	9 (2.0)	4 (3.9)	14 (3.9)	1 (2.3)	19 (3.7)	0.124
HPV83	31 (7.0)	11 (10.7)	32 (8.8)	5 (11.4)	48 (9.4)	0.182
HPV84	101 (22.9)	17 (61.5)	80 (22.1)	11 (25.0)	108 (21.2)	0.532
IS39	19 (4.3)	12 (11.7)	18 (5.0)	4 (9.1)	34 (6.7)	0.112
HR- and LR-HPV	394 (89.3)	99 (96.1)	352 (97.2)	43 (97.7)	494 (97.1)	**<0.001**
Alpha-7 species	223 (50.6)	64 (62.1)	230 (63.5)	26 (59.1)	320 (62.9)	**<0.001**

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TABLE 2 Continued

HPV infection status/type	No. (%) of patients with negative cytology results	No. (%) of patients with abnormal cytology result of:				P^a
		ASC-US	L-SIL	H-SIL	Total	
Alpha-9 species	247 (56.0)	77 (74.7)	263 (72.6)	40 (91.0)	380 (74.7)	**<0.001**
HPV16, -18, -31, -33	216 (49.0)	72 (70.0)	232 (64.1)	37 (84.1)	341 (67.0)	**<0.001**
HPV6, -11, -16, -18	227 (51.5)	75 (72.8)	269 (74.3)	35 (79.5)	379 (74.5)	**<0.001**
No. of infecting HPV types						
1	32 (7.3)	4 (0.9)	15 (4.1)	0 (0)	19 (3.7)	**0.016**
2	64 (14.5)	9 (2.0)	30 (8.3)	1 (2.3)	40 (7.9)	**0.011**
3	62 (14.0)	5 (1.1)	37 (10.2)	8 (18.2)	50 (9.8)	**0.043**
≥4	257 (58.3)	83 (18.8)	277 (76.5)	34 (77.3)	394 (77.4)	**<0.001**
No. of infecting HR-HPV types						
1	103 (23.4)	20 (19.4)	66 (18.2)	3 (6.8)	89 (17.5)	**0.024**
2	102 (23.1)	24 (23.3)	83 (23.0)	14 (31.8)	121 (23.8)	0.815
3	75 (17.0)	18 (17.5)	64 (17.7)	8 (18.2)	90 (17.7)	0.784
≥4	67 (15.2)	35 (34.0)	117 (32.3)	18 (41.0)	170 (33.4)	**<0.001**

[a] The *P* values were calculated between categories with normal and abnormal cytological results. Statistically significant values are shown in bold type.

types belonging to the alpha-7 species and to any of the HPV types included in the available quadrivalent vaccine (Fig. 6).

DISCUSSION

This study found the prevalences of any HPV, HR-HPV, and LR-HPV types to be 96%, 83%, and 73%, respectively, in HIV-positive MSM. Among HR-HPV types, HPV16 was the most common, followed by HPV59, -39, -51, -18, and -52. As for LR-HPV types, HPV6 and CP6108 were the most frequently detected. The prevalence of multiple HPV infections was also very high. There were no major differences in crude analyses between the Spanish and Latin-American MSM for most HPV types, and a peak in prevalence for most HPV types was seen in HIV-positive MSM in their late thirties. Globally and by specific HPV groups, men with abnormal anal cytologies had a higher prevalence of any HPV type than those with no cytological abnormalities. As far as we know, this study has the largest number of HIV-positive MSM (1,439) with available HPV genotype data among published studies.

Our results are consistent with those of previous publications regarding HIV-infected MSM, although we acknowledge that differences in overall and/or specific HPV prevalences between studies are due not only to the characteristics of the populations and the sample size but also to the molecular methods used for HPV detection and genotyping. In this sense, it is important to have in mind the different HPV types targeted and the specific HPV type sensitivities and specificities of the molecular methods employed (15). Salit et al. (16), in 401 HIV-positive MSM in Canada, Conley et al. (17), in 379 MSM from four U.S. cities, and Sahasrabuddhe et al. (8), in 363 MSM from San Francisco, obtained prevalence values for any HPV type of 93%, 88.7%, and 94.4%, respectively, and HR-HPV prevalences of 88%, 88.7%, and 75.4%, respectively (8, 16, 17). Consistent with previous reports conducted in Europe,

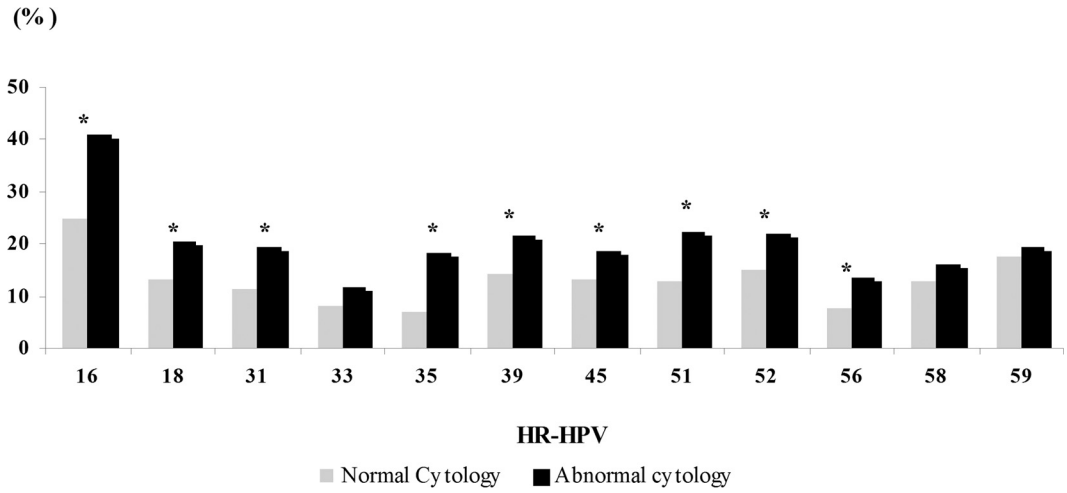

FIG 5 Prevalence of multiple infections of HR-HPV types in 950 HIV-infected MSM with valid cytological results within CoRIS-HPV. *, $P < 0.05$.

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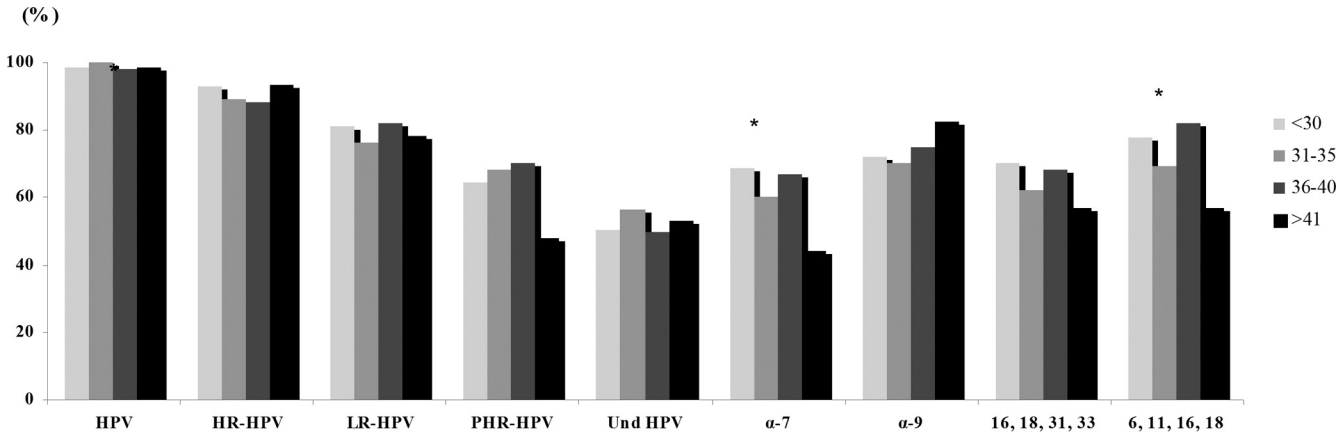

FIG 6 Prevalence of anal HPV infections in HIV-infected MSM with abnormal cytological results stratified by age. *, $P < 0.05$.

the United States, Australia, and Canada, in the present study, the prevalences of multiple HR-HPV infections and infections with more than four HPV types or more than four HR-HPV types are high (1, 6, 8, 9, 16–19). HPV16, as expected, was the most common HR-HPV type detected, with a prevalence of 34.7%.

The association between geographical origin and the specific HPV type prevalence was statistically significant only for HR-HPV types HPV31 (most prevalent in Spaniards) and HPV51 (most common in Latin-Americans). It is highly plausible that Latin-American MSM have acquired their HPV infections in Spain as a result of sexual mixing with Spanish MSM, probably due to the higher prevalence of partnership concurrency (20). But, this cannot be concluded because no sexual behavior data were analyzed in the present study.

In this study, the peaks in prevalence for most HPV types in the MSM aged 36 to 40 years is consistent with a previous study from our group. In reference 6, we described a strong nonlinear association between the number of HR-HPV types with age, with a peak in the mid-thirties, and a positive association between the number of HR-HPV types and the number of recent sexual partners (6). Similar results were obtained by Parisi et al. in Italy (21). In the present study, we found a peak in the prevalence not only for the most common HR-HPV infections but also for LR-HPV infections. Further analyses with a longitudinal design are under way to explore if this peak in prevalence is attributable to sexual behavior and/or to HPV persistence.

We have also found a high prevalence of abnormal anal cytologies (just over half), which is also consistent with previously reported data in HIV-infected MSM in Europe, the United States, and Canada, as well the prevalences of L-SIL and H-SIL that we have found of 38.1% and 4.6%, respectively (4, 9, 17, 18, 22, 23). Indeed, in a recent publication from our group, González et al. (9) described a high prevalence of anal SIL and a 7-fold-increased risk of anal SIL in MSM with five or more detectable HR-HPV types. Other groups have described that HPV multiplicity (including HR and LR types), HR-HPV infections, and an increased number of HPV types are associated with cytological abnormalities (9, 17, 18, 24). In our study, patients with abnormal cytology results consistently showed a higher prevalence of any HPV, HR-HPV, LR-HPV, HR- and LR-HPV combined, and multiple HPV type infections. Likewise, the increased number of HPV or HR-HPV types was associated with abnormal cytological results. HPV16 was the most common type detected, and its prevalence was significantly higher in H-SIL than in L-SIL, similar to that reported by Hoots et al. (25). This pattern was also observed for genotypes HPV31, -33, -35, and -58. It is remarkable that the prevalence of HPV68, considered in some epidemiological classifications to be an HR-HPV type, was higher in patients with abnormal cytologies (26). In our study, high prevalences of LR-HPV types HPV6, -11, -40, -42, and -72, as well as CP6108, were also observed in those with abnormal cytologies, but further analyses are necessary in order to establish whether this observation is only a consequence of the presence of multiple HR and LR HPV infections in these patients or which other factors are associated (e.g., a history of condyloma). A limitation of our study is that we do not have high-resolution anoscopy (HRA)-guided biopsy confirmation for the anal SIL. We are aware that liquid cytology is far from perfect, but it may be used as a screening tool in a high-risk population, such as HIV-infected MSM (27). In Spain, no anal screening program has been established yet and few centers have implemented HRA.

To conclude, in this large study of 1,439 HIV-positive MSM, we report the quasiuniversal presence of any HPV type in the anal canal and a high disease burden determined by anal liquid cytology. Follow-up of this cohort will provide insight into the natural history of HPV infection in terms of the persistence and clearance of the different types. Due to the high prevalence of multiple HPV infections in this population, the proportion attributable to each specific genotype to anal SIL requires more exhaustive analysis. The roles of the CD4 cell count and ART have been partially explored in a previous study in our cohort (6) together with other covariates, but a detailed study on their relationship with the prevalence of HPV types, more specifically with HPV type-specific prevalence, is being carried out currently. From a clinical and public health perspective, we echo other voices in calling for the need to establish evidence-based data on anal screening programs and the lack of impact of vaccinating these men after their HIV diagnoses unless evidence is published otherwise.

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REFERENCES

1. **De Vuyst H, Clifford G, Li N, Franceschi S.** 2009. HPV infection in Europe. Eur. J. Cancer **45:**2632–2639.
2. **Daling JR, Madeleine MM, Johnson LG, Schwartz SM, Shera KA, Wurscher MA, Carter JJ, Porter PL, Galloway DA, McDougall JK.** 2004. Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. Cancer **101:**270–280.
3. **D'Souza G, Wiley DJ, Li X, Chmiel JS, Margolick JB, Cranston RD, Jacobson LP.** 2008. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. J. Acquir. Immune Defic. Syndr. **48:**491–499.
4. **Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM, Hillman RJ, Petoumenos K, Roberts J, Tabrizi SN, Templeton DJ, Grulich AE.** 2012. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. Lancet Oncol. **13:**487–500.
5. **de Pokomandy A, Rouleau D, Ghattas G, Vézina S, Coté P, Macleod J, Allaire G, Franco EL, Coutlée F, HIPVIRG Study Group.** 2009. Prevalence, clearance, and incidence of anal human papillomavirus infection in HIV-infected men: the HIPVIRG cohort study. J. Infect. Dis. **199:**965–973.
6. **del Amo J, González C, Geskus RB, Torres M, Del Romero J, Vinciana P, Masiá M, Blanco JR, Hernández-Novoa B, Ortiz M, CoRIS-HPV Study Group.** 2013. What drives the number of high-risk human papillomavirus types in the anal canal in HIV-positive men who have sex with men? J. Infect. Dis. **207:**1235–1241.
7. **Piketty C, Darragh TM, Da Costa M, Bruneval P, Heard I, Kazatchkine MD, Palefsky JM.** 2003. High prevalence of anal human papillomavirus infection and anal cancer precursors among HIV-infected persons in the absence of anal intercourse. Ann. Intern. Med. **138:**453–459.
8. **Sahasrabuddhe VV, Castle PE, Follansbee S, Borgonovo S, Tokugawa D, Schwartz LM, Lorey TS, LaMere BJ, Gage JC, Fetterman B, Boyle S, Sadorra M, Tang SD, Darragh TM, Wentzensen N.** 2013. Human papillomavirus genotype attribution and estimation of preventable fraction of anal intraepithelial neoplasia cases among HIV-infected men who have sex with men. J. Infect. Dis. **207:**392–401.
9. **González C, Torres M, Benito A, Del Romero J, Rodríguez C, Fontillón M, Trastoy M, Viciana P, Del Amo J, Ortiz M, Hernández-Novoa B, CoRIS-HPV Study Group.** 2013. Anal squamous intraepithelial lesions are frequent among young HIV-infected men who have sex with men followed up at the Spanish AIDS Research Network Cohort (CoRIS-HPV). Int. J. Cancer **133:**1164–1172.
10. **Hartwig S, Syrjänen S, Dominiak-Felden G, Brotons M, Castellsagué X.** 2012. Estimation of the epidemiological burden of human papillomavirus-related cancers and non-malignant diseases in men in Europe: a review. BMC Cancer. **12:**30. doi:10.1186/1471-2407-12-30.
11. **Sobrino-Vegas P, Gutiérrez F, Berenguer J, Labarga P, García F, Alejos-Ferreras B, Muñoz MA, Moreno S, del Amo J, CoRIS.** 2011. The Cohort of the Spanish HIV Research Network (CoRIS) and its associated biobank; organizational issues, main findings and losses to follow-up. Enferm. Infecc. Microbiol. Clin. **29:**645–653. (Article in Spanish.)
12. **Aho J, Hankins C, Tremblay C, Forest P, Pourreaux K, Rouah F, Coutlée F, Canadian Women's HIV Study Group.** 2004. Genomic polymorphism of human papillomavirus type 52 predisposes toward persistent infection in sexually active women. J. Infect. Dis. **190:**46–52.
13. **Muñoz N, Castellsagué X, de González AB, Gissmann L.** 2006. Chapter 1: HPV in the etiology of human cancer. Vaccine **24**(Suppl 3)**:**S3/1–10.
14. **Nayar R, Solomon D.** 2004. Second edition of 'The Bethesda System for reporting cervical cytology' - atlas, website, and Bethesda interobserver reproducibility project. Cytojournal **1:**4. doi:10.1186/1742-6413-1-4.
15. **Torres M, Fraile L, Echevarria J, Hernandez Novoa B, Ortiz M.** 2012. Human papillomavirus (HPV) genotyping: automation and application in routine laboratory testing. Open Virol. J. **6:**144–150.
16. **Salit IE, Lytwyn A, Raboud J, Sano M, Chong S, Diong C, Chapman W, Mahony JB, Tinmouth J.** 2010. The role of cytology (Pap tests) and human papillomavirus testing in anal cancer screening. AIDS **24:**1307–1313.
17. **Conley L, Bush T, Darragh TM, Palefsky JM, Unger ER, Patel P, Kojic EM, Cu-Uvin S, Martin H, Overton ET, Hammer J, Henry K, Vellozzi C, Wood K, Brooks JT, Study to Understand the Natural History of HIV and AIDS in the Era of Effective Therapy (SUN Study) Investigators.** 2010. Factors associated with prevalent abnormal anal cytology in a large cohort of HIV-infected adults in the United States. J. Infect. Dis. **202:**1567–1576.
18. **Damay A, Fabre J, Costes V, Didelot JM, Didelot MN, Boulle N, Segondy M.** 2010. Human papillomavirus (HPV) prevalence and type distribution, and HPV-associated cytological abnormalities in anal specimens from men infected with HIV who have sex with men. J. Med. Virol. **82:**592–596.
19. **Sirera G, Videla S, Piñol M, Cañadas MP, Llatjos M, Ballesteros AL, García-Cuyás F, Castellá E, Guerola R, Tural C, Rey-Joly C, Clotet B.** 2006. High prevalence of human papillomavirus infection in the anus, penis and mouth in HIV-positive men. AIDS **20:**1201–1204.
20. **Glick SN, Morris M, Foxman B, Aral SO, Manhart LE, Holmes KK, Golden MR.** 2012. A comparison of sexual behavior patterns among men who have sex with men and heterosexual men and women. J. Acquir. Immune Defic. Syndr. **60:**83–90.
21. **Parisi SG, Cruciani M, Scaggiante R, Boldrin C, Andreis S, Dal Bello F, Pagni S, Barelli A, Sattin A, Mengoli C, Palù G.** 2011. Anal and oral human papillomavirus (HPV) infection in HIV-infected subjects in northern Italy: a longitudinal cohort study among men who have sex with men. BMC Infect. Dis. **11:**150. doi:10.1186/1471-2334-11-150.
22. **Berry JM, Palefsky JM, Jay N, Cheng SC, Darragh TM, Chin-Hong PV.** 2009. Performance characteristics of anal cytology and human papillomavirus testing in patients with high-resolution anoscopy-guided biopsy of high-grade anal intraepithelial neoplasia. Dis. Colon Rectum **52:**239–247.
23. **Gohy L, Gorska I, Rouleau D, Ghattas G, Pokomandy Ad, Vézina S, Coté P, Macleod J, Allaire G, Hadjeres R, Kornegay JR, Franco E, HIPVIRG Study Group, Coutlée F.** 2008. Genotyping of human papillomavirus DNA in anal biopsies and anal swabs collected from HIV-seropositive men with anal dysplasia. J. Acquir. Immune Defic. Syndr. **49:**32–39.
24. **Palefsky JM.** 1998. Human papillomavirus infection and anogenital neoplasia in human immunodeficiency virus-positive men and women. J. Natl. Cancer Inst. Monogr. **1998:**15–20.
25. **Hoots BE, Palefsky JM, Pimenta JM, Smith JS.** 2009. Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. Int. J. Cancer **124:**2375–2383.
26. **Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ, International Agency for Research on Cancer Multicenter Cervical Cancer Study Group.** 2003. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N. Engl. J. Med. **348:**518–527.
27. **Darragh TM, Winkler B.** 2011. Anal cancer and cervical cancer screening: key differences. Cancer Cytopathol. **119:**5–19.

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