Persistence of Genital Human Papillomavirus Infection in a Long-Term Follow-Up Study of Female University Students

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Background. Little is known about the epidemiology of human papillomavirus (HPV) infections that persist for more than a few years.

Methods. Four to 12 years after participation in a longitudinal study of incident HPV infection, a cohort of former university students returned for a follow-up visit that included HPV genotyping of cervical and vulvovaginal swab specimens and collection of colposcopy-directed biopsy specimens.

Results. Of 147 women with HPV infection detected during their undergraduate years, 24 (16.3%) were positive for 1 or more of the same HPV types at follow-up. Overall, 27 (4.8%) of 567 type-specific HPV infections persisted, and DNA sequence analyses of a subset revealed that all were variant specific. Long-term HPV persistence was positively associated with frequent but sporadic detection of the same HPV type early during the course of the infection and with abnormal Pap tests and genital warts; it was negatively associated with marriage and was not associated with the number of intercurrent sex partners.

Conclusions. HPV variant and behavioral risk factor analyses indicated that long-term detection of the same HPV type was more consistent with viral persistence than with reinfection. Although long-term persistence was not common, it was associated with detection of HPV-related pathologies.

Human papillomavirus (HPV) infection is necessary for the development of virtually all invasive cervical cancers, their associated precancerous lesions, and genital warts [1, 2]. Although screening programs have significantly reduced the incidence of cervical cancer, it remains a considerable public health burden [3, 4]. Although hope for the next-generation approach to preventing cervical cancer rests on HPV vaccines, efforts to implement and evaluate a vaccination strategy are dependent on our understanding of the natural history of HPV infection. To date, a great deal of knowledge has been gained from short-term natural history studies, but few studies have addressed the epidemiology of HPV infections that persist for more than a few years. Here, we present evidence of HPV DNA persisting in healthy individuals for upward of 9 years as well as a description of epidemiological factors associated with long-term persistence.

METHODS

Study population and design. The subjects were a subset of 603 University of Washington undergraduates who were enrolled in a longitudinal study of genital HPV infection between 1990 and 1997 when they were 18–20 years of age [5]. Every 4 months throughout the duration of their undergraduate studies (4 years, on average), participants underwent clinical examinations that included collection of genital specimens and colposcopic evaluation with charting of all acetowhite cervical lesions. Between 2001 and 2004, 220 women for whom HPV was detected during the initial study period were contacted by mail, and 156 (71%) enrolled in the long-term follow-up study.

Received 31 January 2008; accepted 10 April 2008; electronically published XX August 2008.

Potential conflicts of interest: none reported.

Presented in part: 23rd International Papillomavirus Conference, 1–7 September 2006, Prague (P-408).

Financial support: National Institutes of Health (grant 2 R01AI 038383). L.K.S. was supported by National Research Service Award CA80416.

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The Journal of Infectious Diseases 2008; 198:xxx

0022-1899/2008/19807-00XX$15.00
DOI: 10.1086/591625

Each long-term follow-up study participant returned for 1 or 2 follow-up visits as close to 10 years after their initial study enrollment as possible (median interval, 10 years; range, 4–12 years). Clinical protocols were approved by the University of Washington's Human Subjects Division. Each long-term follow-up visit began with a face-to-face interview to obtain informed consent, demographic data, and medical/sexual history information. A standardized pelvic examination followed, during which the cervix was examined by colposcopy, a cervical cytologic specimen was obtained, and cervical and vulvovaginal swab specimens were collected for HPV testing. Women with evidence of a high-grade cervical lesion were referred for colposcopy-directed biopsy. On a voluntary basis, other women underwent colposcopy-directed biopsies for pathology and HPV DNA testing of a cervical area(s) with a current or past acetowhite lesion.

Laboratory methods. Polymerase chain reaction (PCR) amplification and dot- or line-blot hybridization were used for HPV detection and genotyping. Descriptions of the primers, probes, and methods used in DNA preparation and testing have been published elsewhere [5]. All long-term follow-up study HPV typing was conducted using a line-blot assay that recognizes 37 individual HPV types, as follow: high-risk types, 16, 18, 26, 31, 33, 35, 39, 45, 51–53, 56, 58, 59, 66–68, 73, and 82; low-risk types, 6, 11, 40, 42, 54, 55, 57, 61, 62, 64, 69–72, 81, 83, 84, and CP6108 (Roche Molecular Systems) [6, 7]. Untypeable initial study specimens that tested positive for HPV DNA by a generic probe were also retyped using the Roche assay. Cervical punch biopsy specimens were digested in proteinase K overnight before DNA preparation.

Variant specificity was determined using pairs of samples, 1 from the initial study and 1 from the long-term follow-up study, for 17 of 27 type-specific HPV infections. For the remaining 10 infections, at least 1 of the required samples no longer had sufficient DNA for testing in 2005. PCR amplification of the entire E6 gene was performed using the primers shown in table 1. The amplified sequence was cloned using a TOPO TA Cloning Kit (Invitrogen), and the sequences of 3 separate clones were determined by amplification from purified plasmid using the M13 vector primers. Sequences were analyzed using an ABI PRISM 377 DNA Sequencer (Applied Biosystems) and Sequencher software (version 4.6; Gene Codes). The criteria for determining the predominant variant in each sample was sequence identity in at least 2 of 3 clones across the entire E6 gene. More specifically, nucleotide alterations that were found at least twice in the same sample were counted as variants, and those sequences found only once were counted as potential artifacts introduced by PCR.

Pap smears were read by a cytotechnologist, and all smears with abnormal findings were reviewed by the study pathologist. Pap smear findings were classified according to the Bethesda system [8]. Biopsy tissue was examined by the study pathologist to establish the presence of cervical intraepithelial neoplasia (CIN), adenocarcinoma in situ, or cervical cancer. None of the women developed invasive cervical cancer.

Table 1. Human papillomavirus (HPV) type–specific primers used for polymerase chain reaction amplification of the entire E6 gene.

HPV type, direction	Primer (5'→3')
HPV-16	
Forward	CCGAAACCGGTTAGTATAAAAGC
Reverse	TTCATGCAATGTAGGTGTATCTCC
HPV-31	
Forward	ACACCGTTTTCGGTTACAGTTT
Reverse	TCTTGCAACGTAGGTGTTTCTC
HPV-39	
Forward	TGGATATAAAACGCAGTCACAGTT
Reverse	TCTAATACAATTTCCTGCAAGGTG
HPV-52	
Forward	GTCAGACCGAAACCGGTGTAT
Reverse	GTCAGTTGTTTCAGGTTGCAGAT
HPV-54	
Forward	GCGGTTGTAGAAAACAGTTATTTG
Reverse	TGCATCAGAGTCTTCTAATTGCTC
HPV-66	
Forward	CAGCCTGTTGTGCCTGTAGATA
Reverse	ACCTCTTGCAACGTTGGTACTT
HPV-70	
Forward	AAAAGTTGCTTGCCCATACG
Reverse	GCCGTGGTCCATGCATATT
HPV-81	
Forward	CGACCGGGAAGGATACATATAA
Reverse	CCACCAGCCTAACTAAACACCT

Statistical methods. Long-term persistence was defined as detection of a specific HPV type at 1 or more visits during the initial study followed by detection of the same type at a long-term follow-up visit 3 or more years later. Our study population included 156 women with at least 1 type-specific HPV infection detected during the initial study period and at least 1 adequate long-term follow-up DNA sample. Nine of these women were excluded because all of their infections were first detected <3 years before the long-term follow-up visit and could therefore only provide information on short-term persistence. Altogether, 147 women with a total of 567 HPV infections detected during the initial study were considered to be at risk for detection of a long-term persistent HPV infection and were retained in the analysis.

The dataset used to detect associations between demographic, behavioral, and clinical characteristics and long-term persistence contained 626 observations based on the 567 infections detected in the 147 women during the initial study. Each observation was defined as a type-specific HPV infection detected during the initial study period linked with a particular long-term follow-up visit. By this convention, the 19 women in our study with 2 follow-up visits had each represented independently, be-

cause the designation of an observation as a case or control depended on whether the infection was present at a particular long-term follow-up visit (case) or was absent (control). Thus, the total number of observations a woman contributed to this dataset was the number of different HPV types she had during the initial study period 3 or more years before the long-term follow-up visits multiplied by her number of long-term follow-up visits.

Analyses of the frequency and regularity of HPV detection early during the course of a given infection were performed with a dataset containing 1 observation per type-specific infection detected in the 24 women who had at least 1 long-term persistent infection. Infections that were incident at the last initial study visit were excluded from analyses of consecutive detection. For analyses pertaining to sporadic detection of the same HPV type early during the course of a given infection, infections that were detected only once during the initial study were excluded, as were infections first detected at the last or second-to-last initial study visits.

Generalized estimating equations with robust variance estimates for P values and 95% confidence intervals were used to account for intrasubject correlation. Associations were deemed statistically significant if $P \leq .05$. All statistical analyses were performed using Stata software (version 8.0) [9].

RESULTS

Long-term HPV persistence. The 147 women who participated in our long-term follow-up study completed an average of 8.4 clinic visits (beginning with the first HPV positive visit) and tested positive for an average of 3.9 type-specific HPV infections (SD, 3.0; range, 1–15) during the initial study period, which usually ended when they graduated from the university. Although 73 (49.7%) of the 147 women were HPV positive at 1 or both long-term follow-up visits, only 24 (16.3%) had the same HPV type(s) detected as in the initial study. Of the 19 women who returned for 2 long-term follow-up visits, 5 had a persistent HPV type detected at 1 long-term follow-up visit, and only 1 was positive for a persistent HPV type (type 6) at both visits. At their first HPV-positive visit, the point in time at which they began to be at risk for long-term HPV persistence, women who did and did not develop a long-term persistent HPV infection had similar demographic, behavioral, and clinical characteristics ($P > .05$ for all characteristics analyzed, by χ^2 statistics and Student's t test with unequal variances) (table 2).

Of 567 type-specific HPV infections detected during the initial study period among the 147 women enrolled in the long-term follow-up study (table 3), 353 (62.3%) were classified as high-risk HPV types and 214 (37.7%) as low-risk types. Twenty-seven (4.8%) of the 567 type-specific HPV infections were detected again during the long-term follow-up period. The frequency of long-term persistence for the high-risk (5.4%) and the low-risk (3.7%) HPV type groups was similar.

Table 2. Demographic and clinical characteristics of women who were at risk for long-term persistent human papillomavirus (HPV) infection and who participated in the long term follow-up study.

Characteristic	Women with short-term or transient infection (n = 123)	Women with ⩾1 long-term persistent infection (n = 24)
Age, mean ± SD, years	20.5 ± 1.3	20.5 ± 1.3
Lifetime no. of male sex partners, mean ± SD	3.8 ± 3.7	3.6 ± 2.6
Race		
White	95 (77.2)	22 (91.7)
Other	28 (22.8)	2 (8.3)
Smoking status		
Never	80 (65.0)	19 (79.2)
Prior	15 (12.2)	0 (0)
Current	14 (11.4)	3 (12.5)
Prior and current	14 (11.4)	2 (8.3)
Currently using hormonal contraceptives		
No	67 (54.5)	12 (50.0)
Yes	56 (45.5)	12 (50.0)
Pap smear result		
Normal	95 (77.9)	19 (79.1)
ASC-US	10 (8.2)	1 (4.2)
LSIL	17 (13.9)	4 (16.7)
HPV risk type(s) detected		
Low	30 (24.4)	6 (25.0)
High	69 (56.1)	14 (58.3)
Both	24 (19.5)	4 (16.7)

NOTE. Data are no. (%) of women, unless otherwise indicated. Data shown were collected on the date each woman first became HPV positive and was thus at risk for HPV persistence. ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion.

Variant specificity. Sequence analysis of the E6 gene was performed on 17 pairs of samples consisting of 1 initial study sample and 1 long-term study sample. A total of 10 different HPV types were analyzed, and sequencing of the initial study samples revealed that 5 types (HPV-53, -54, -62, -70, and -84) were each represented by a single variant, that 4 types (HPV-31, -39, -52, and -66) were each represented by 2 unique variants, and that 1 type (HPV-16) was represented by 3 unique variants. Two women had the same HPV-16 variant. A comparison of the initial study samples and the long-term follow-up study samples revealed 100% E6 sequence identity, indicating the presence of a single variant for all 17 infections.

Risk factors for long-term HPV persistence. The number of exposures reported or detected during the intercurrent interval from the first detection of a given infection to the long-term

Table 3. Frequency of type-specific human papillomavirus (HPV) infections and long-term persistent HPV detection.

Risk category, HPV type	Type-specific HPV infections in the initial study, no. (n = 567)	Long-term persistent HPV infections, no. (%) (n = 27)
Low risk		
6	35	1 (2.9)
11	8	0 (0)
40	10	0 (0)
42	19	0 (0)
54	24	2 (8.3)
55	5	0 (0)
61	8	0 (0)
62	20	1 (5.0)
64	3	0 (0)
70	5	1 (20.0)
72	2	0 (0)
81	7	1 (14.3)
83	7	0 (0)
84	31	2 (6.5)
CP6108	30	0 (0)
Total	214	8 (3.7)
High risk		
16	59	5 (8.5)
18	23	1 (4.4)
26	3	0 (0)
31	20	2 (10.0)
33	12	0 (0)
35	4	0 (0)
39	31	2 (6.5)
45	9	0 (0)
51	30	0 (0)
52	26	4 (15.4)
53	26	1 (3.9)
56	35	0 (0)
58	12	0 (0)
59	20	0 (0)
66	24	4 (16.7)
67	5	0 (0)
68	3	0 (0)
73	7	0 (0)
82	4	0 (0)
Total	353	19 (5.4)

follow-up visit were investigated as potential predictors of long-term persistent HPV infection. A potential confounder of these associations was the length of the intercurrent time interval, yet we found that this interval, which ranged from 3 to 12 years, did not differ significantly between women with long-term persistent HPV infection (cases) and women with short-term or transient HPV infections (controls) (table 4). The multivariate model, which included all factors associated with long-term persistence in univariate regression analyses as well as the number of initial study visits subsequent to the first detection, indicated that frequent detection of a given HPV type shortly after the initial detection increased the odds that the type would be detected years later, whereas being married decreased the odds (table 4). Other factors that were evaluated but showed no relationship with long-term persistence included age, race/ethnicity, cumulative lifetime number of sex partners, total number of infecting HPV types, simultaneous detection of multiple HPV types, whether an infection was prevalent at the first initial study visit, and the presence of another sexually transmitted infection (data not shown).

Early patterns of HPV DNA detection. An analysis of the frequency and regularity of HPV DNA detection was performed on all 140 type-specific infections detected in the 24 women with long-term persistent HPV infection (figure 1). This particular subset was used to eliminate confounding due to variability in the timing of clinic visits. The percentage of high-risk HPV types was similar in both the subset and the full dataset. Although nearly half of the 140 HPV infections were detected only once during the initial study period, long-term persistent infections were more likely to be detected at 3 or more visits during the initial study, compared with infections that appeared to clear before a long-term follow-up visit (40.7% vs. 18.6%; $P = .014$). This difference was not based on the number of consecutive positive visits but rather on the number of sporadically positive visits, because long-term persistent infections were twice as likely as transient or short-term infections to have 1 or more periods of intercurrent negativity during the initial study period (50.0% vs. 22.2%; $P = .039$).

Clinical findings at long-term follow-up visits. We investigated associations between long-term persistent HPV infections and abnormal clinical findings at a given long-term follow-up visit. Because it was possible that clinical abnormalities were associated with more recently acquired HPV infections, we split the control observations into 2 separate groups: (1) observations from women who had HPV DNA detected in the initial study but who did not have any HPV DNA detected at a long-term follow-up visit and (2) observations from women who tested negative at the long-term follow-up visit for the HPV type(s) detected in the initial study but who tested positive for a new HPV type(s). Genital warts and Pap smear abnormalities were positively associated with detection of a long-term persistent infection (table 5). Among women who underwent cervical biopsy at a long-term follow-up visit, CIN was positively associated with persistence in both comparisons, but the association was statistically significant only for the comparison with the HPV-negative controls.

HPV DNA in basal/suprabasal cell layers. Our study was designed to evaluate whether evidence of long-term persistent HPV infection was more likely to be detected in the basal/suprabasal epithelial layers than in exfoliated cells collected by swab-

Table 4. Potential predictors of long-term persistent human papillomavirus (HPV) infection.

Predictor	Controls (short-term or transient HPV) ($n = 598$)	Cases (long-term persistent HPV) ($n = 28$[a])	Univariate analyses, crude OR (95% CI[b])	Multivariate analyses, adjusted OR[c] (95% CI[b])
Intercurrent time,[d] mean ± SD, years	7.1 ± 2.0	6.9 ± 2.1	1.0 (0.8–1.2)	
Visits at which HPV type detected,[e] mean ± SD, no.	1.9 ± 1.2	2.7 ± 2.1	1.4 (1.2–1.8)	1.5 (1.1–2.0)
New high-risk types detected,[f] mean ± SD, no.	2.4 ± 1.8	2.3 ± 1.7	1.0 (0.8–1.2)	
New low-risk types detected,[f] mean ± SD, no.	1.8 ± 1.7	1.3 ± 1.3	0.8 (0.6–1.02)	
New male sex partners acquired,[f] mean ± SD, no.	4.8 ± 6.8	4.8 ± 6.6	1.0 (0.9–1.1)	
HPV risk type				
Low risk	229 (38.3)	9 (32.1)	1.0 (reference)	
High risk	369 (61.7)	19 (67.9)	1.3 (0.6–3.1)	
HPV species				
Other	459 (76.8)	17 (60.7)	1.0 (reference)	1.0 (reference)
A9	139 (23.2)	11 (39.3)	2.1 (1.03–4.4)	1.5 (0.6–3.4)
Anatomical location of HPV[g]				
Vulvovaginal only	206 (34.4)	12 (42.9)	1.0 (reference)	
Cervix only	83 (13.9)	3 (10.7)	0.6 (0.2–2.2)	
Both	309 (51.7)	13 (46.4)	0.7 (0.3–1.7)	
Ever used hormonal contraceptives[f]				
No	66 (11.0)	4 (14.3)	1.0 (reference)	
Yes	532 (89.0)	24 (85.7)	0.7 (0.3–2.3)	
Intercurrent smoking				
No	291 (48.7)	13 (46.4)	1.0 (reference)	
Yes	307 (51.3)	15 (53.6)	1.1 (0.5–2.3)	
Ever married				
No	357 (59.8)	23 (82.1)	1.0 (reference)	1.0 (reference)
Yes	240 (40.2)	5 (17.9)	0.3 (0.1–0.8)	0.4 (0.2–0.97)
Intercurrent parity				
0	465 (77.8)	25 (89.3)	1.0 (reference)	
⩾1	133 (22.2)	3 (10.7)	0.4 (0.2–1.2)	
Intercurrent genital warts				
No	501 (83.8)	23 (82.1)	1.0 (reference)	
Yes	97 (16.2)	5 (17.9)	1.1 (0.3–3.8)	
Intercurrent acetowhitening[h]				
No	135 (22.6)	6 (21.4)	1.0 (reference)	
Yes	463 (77.4)	22 (78.6)	1.1 (0.4–2.6)	
Worst intercurrent Pap smear result				
Normal	162 (27.1)	10 (35.7)	1.0 (reference)	
ASC-US	108 (18.1)	4 (14.3)	0.6 (0.2–2.0)	
SIL	328 (54.8)	14 (50.0)	0.7 (0.3–1.7)	
Intercurrent cervical biopsy				
No	325 (54.4)	14 (50.0)	1.0 (reference)	
Yes	273 (45.6)	14 (50.0)	1.2 (0.5–2.6)	
Intercurrent cervical treatment				
No	480 (80.3)	23 (82.1)	1.0 (reference)	
Yes	118 (19.7)	5 (17.9)	0.9 (0.4–2.1)	

NOTE. Data are no. (%) of women, unless otherwise indicated. Data shown are drawn from analyses using a dataset with 626 total observations, each representing a type-specific HPV infection linked to a follow-up visit (see Methods), and are cumulative through the intercurrent period of each infection, including the visit at which the HPV type was first detected but not including the follow-up visit. ASC-US, atypical squamous cells of undetermined significance; SIL, squamous intraepithelial lesion.

[a] Included in these 28 observations of long-term persistent HPV infections are 2 observations of an HPV-6 infection that were contributed by 1 woman who had HPV-6 detected at both long-term follow-up visits.

[b] Calculated using robust variances.

[c] Adjusted for all other variables in the column and for the no. of initial study visits subsequent to first detection of the HPV type.

[d] Time from first detection to follow-up visit.

[e] During the initial study.

[f] During the intercurrent period.

[g] Includes all intercurrent detections.

[h] Detected on colposcopy.

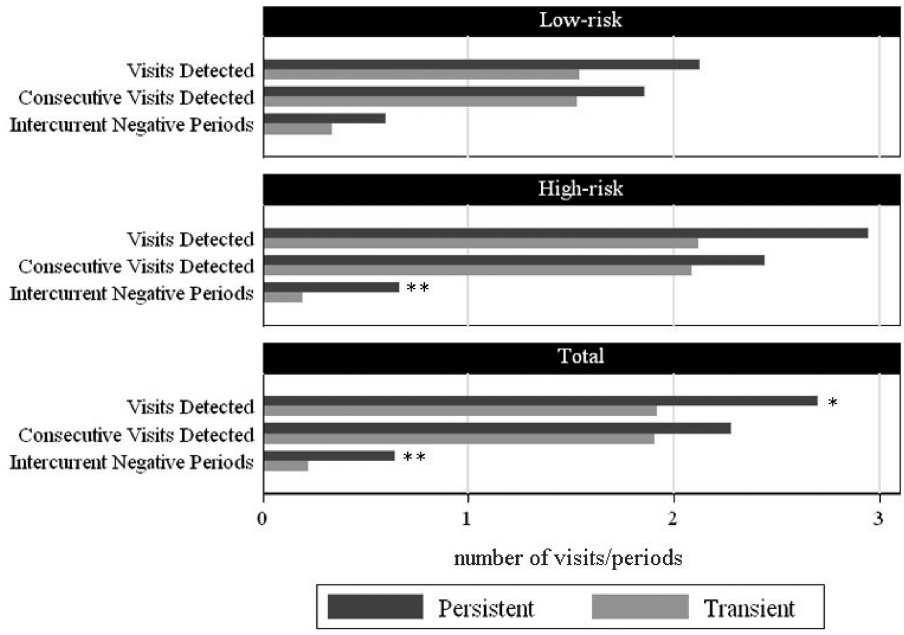

Figure 1. Presentations of the frequency and regularity of type-specific human papillomavirus (HPV) DNA detection in 24 women with 1 or more long-term persistent HPV infections, of whom 23 had at least 1 transient or short-term HPV infection as well. Horizontal bars show the mean no. of detection visits or intervals between detection visits during the initial study period. Black bars represent long-term persistent infections, and gray bars represent transient or short-term HPV infections. The total no. of infections in the subset of 24 women was 140 (47 low risk and 93 high risk). Because of the exclusion criteria detailed in Methods, the no. of infections considered for the consecutive visits detected and intercurrent negative period analyses was reduced to 125 (39 low risk and 86 high risk) and 68 (17 low risk and 51 high risk), respectively. A single asterisk indicates that univariate regression analysis of the persistent vs. transient means (with robust variances) approached significance ($P < .10$); a double asterisk indicates significance ($P < .05$).

bing the surface. Sixty-five women who were at risk for long-term persistence provided cervical punch biopsy specimens from the site of a past lesion. Fourteen of these women had long-term persistent infection detected in a swab specimen, yet only 4 of them (28.6%) had their persistent HPV type(s) detected in their biopsy specimen. Furthermore, no additional long-term persistent HPV infections were detected exclusively by HPV DNA testing of biopsy specimens, even after microdissection and specific analysis of the basal/suprabasal cell layers.

DISCUSSION

Although viral DNA from a single HPV variant was capable of persisting in the female genital tract for several years, long-term persistence was uncommon. Only 16% of women infected with 1 or more HPV types as undergraduates had persistent infection detected 3 to 12 years later, and overall only 5% of type-specific HPV infections persisted. Most studies of HPV persistence have defined persistence as the detection of the same HPV type at 2–3 consecutive visits, each 2–24 months apart [10–18]. Previous attempts to identify HPV persistence beyond 3–4 years include a study that failed to detect HPV DNA in cervical swab samples collected from 99 women 7–11 years later [19] and a 7-year study of postmenopausal women that did not present persistence findings at the level of HPV type specificity [20]. More recently, a cohort of >7000 women in Guanacaste, Costa Rica, were tested for HPV annually over 5–7 years [21, 22]. With persistence being defined as detection of the same HPV type at the first and last study visits, ~5–30% of type-specific infections were persistent, with the frequency increasing with increasing age at enrollment.

The lowest age at enrollment in the Guanacaste cohort (<25 years) corresponds to that of our initial study cohort (18–20 years). Within this age range, the frequency of persistence was ~6%–7% for the Guanacaste cohort and ~5% for our cohort, with similar persistence estimates for high- and low-risk HPV types. These findings indicate that, in contrast to what has been frequently reported in the short-term HPV persistence literature [10, 15, 23–27], classifying a given HPV type as high risk or low risk may not be a good indicator of long-term persistence.

Our findings diverged further from the paradigm of short-term HPV persistence when we analyzed how frequently and regularly viruses that persisted long term were detected. In the literature on short-term persistence, 2 or more consecutive HPV positive tests typically constitute persistence, but the meaning of an intercurrent negative test is inconsistently defined. Several investigators have considered a persistent infection to be cleared if 2 consecutive positive test results are followed by 1 negative test result [15–17], others have required 2 consecutive negative test results [23], and another has suggested that 3 or more would

Table 5. Clinical findings at the long-term follow-up visits: comparisons between those with long-term persistent human papillomavirus (HPV) infection and those with (1) HPV detected only during the initial study or (2) HPV detected during the initial study and a new HPV type(s) detected at a long-term follow-up visit.

Finding	Controls (short-term or transient HPV) (n = 403)	Cases (long-term persistent HPV) (n = 28)[a]	Crude OR (95% CI)[b]	Controls (short-term or transient HPV and detection of new HPV type) (n = 195)	Cases (long-term persistent HPV) (n = 28)[a]	Crude OR (95% CI)[b]
Genital warts						
No	402 (99.7)	25 (89.3)	1.0 (reference)	188 (96.4)	25 (89.3)	1.0 (reference)
Yes	1 (0.3)	3 (10.7)	48.2 (6.8–340.0)	7 (3.6)	3 (10.7)	3.2 (1.4–7.7)
Colposcopic acetowhitening						
No	204 (50.9)	14 (50.0)	1.0 (reference)	98 (50.3)	14 (50.0)	1.0 (reference)
Yes	197 (49.1)	14 (50.0)	1.0 (0.5–2.4)	97 (49.7)	14 (50.0)	1.0 (0.4–2.5)
Pap smear result						
Normal	361 (94.0)	22 (78.6)	1.0 (reference)	187 (95.9)	22 (78.6)	1.0 (reference)
Abnormal[c]	23 (6.0)	6 (21.4)	4.3 (1.4–13.5)	8 (4.1)	6 (21.4)	6.4 (1.9–22.0)
CIN on cervical biopsy[d]						
No	234 (99.2)	9 (69.2)	1.0 (reference)	71 (81.6)	9 (69.2)	1.0 (reference)
Yes	2 (0.9)	4 (30.8)	52.0 (7.3–369.7)	16 (18.4)	4 (30.8)	2.0 (0.7–6.0)

NOTE. Data are no. (%) of women, unless otherwise indicated. Data shown are from analyses of a dataset with 626 total observations, each representing a type-specific HPV infection linked to a long-term follow-up visit (see Methods), enabling the analysis of clinical findings associated with long-term persistent type-specific HPV infection. Analyses were conducted using 2 separate comparison groups: (1) observations from women with a type-specific HPV infection(s) detected during the initial study period and no infections detected at the relevant long-term follow-up visit (short-term or transient HPV) and (2) observations from women with a type-specific HPV infection(s) detected during the initial study period and a new type-specific HPV infection(s) detected at a long-term follow-up visit (short-term or transient HPV and detection of new HPV type). CI, confidence interval; CIN, cervical intraepithelial neoplasia; OR, odds ratio.

[a] Included in these 28 observations of long-term persistent HPV infections are 2 observations of an HPV-6 infection that were contributed by 1 woman who had HPV-6 detected at both long-term follow-up visits.

[b] Calculated using robust variances.

[c] Abnormal incorporates both atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesions. High-grade lesions were not observed.

[d] Restricted to women with cervical biopsy specimens.

be ideal [26]. In a previous analysis, we considered intercurrent negative visits to be false-negative results [5], because the same HPV-16 variant remained in each woman who again tested positive for HPV-16 after an intercurrent negative sample [28]. In the present study, we extended this observation by including sequencing data for 10 HPV types representing 17 different infections and again found the same variant detected in all pairs of samples. Because these data provided evidence for persistence rather than reinfection, it was not surprising to find no association between persistent infection and report of new male sex partners during the intercurrent period. We also found that, although persistent infections were detected at more visits on average than transient infections, detection visits were not necessarily consecutive. Moreover, nearly half of the persistent infections were detected at only 1 initial study visit. In light of our findings, we propose that HPV persistence during the first several years of infection might be characterized by frequent periods of detection interspersed with periods of negativity. Presumably, the persistent virus remains present during these periods of negativity, with levels of shed virus being below the threshold of detection.

In an effort to maximize the sensitivity of persistent HPV detection, we investigated whether HPV DNA could be frequently detected in biopsy tissue samples that contained cells of the basal/suprabasal layers. Because all persistent infections detected in biopsy specimens were concurrently detected in swab specimens, our HPV DNA detection methods might not have been sensitive enough to detect persisting low-copy episomal DNA. Additionally, a single punch biopsy specimen might have a low probability of being properly localized to capture foci of infected basal/suprabasal cells from a former lesion. To more directly isolate the basal/suprabasal cell layers and ensure their complete digestion, we applied laser-capture microdissection to some of the paraffin-embedded biopsy specimens, but our detection rate was not improved (data not shown). We conclude that swab sample testing remains the best method available for detecting persistent HPV DNA because of its lower degree of invasiveness and as-yet-unsurpassed sensitivity.

Our efforts to identify demographic and clinical factors predictive of long-term HPV persistence yielded some surprising results that might provide insight into the poorly understood interplay between persistent virus and host. One unexpected

finding was the protective effect of marriage. Biologically, this association could result from regular boosting of the immune response due to frequent HPV transmission between male and female partners during barrier-free vaginal intercourse. As has been shown for HPV-16 infections [29], regular exposure to L1 antigen could result in prolonged periods of high antibody titer, which could in turn reduce levels of virus, resulting in less-frequent HPV DNA detection. Analysis of clinical findings at follow-up indicated that detection of a long-term persistent infection was a stronger marker of benign genital warts, abnormal Pap test results, and CIN (not statistically significant) than was detection of a new HPV type. A previous study similarly found that detection of type-specific infections 5–6 years after baseline correlated with invasive cervical cancers, which were likely proceeded by undetected precancerous lesions [30].

Our study has important limitations. Although a high number of type-specific HPV infections were followed, statistical power was low because long-term persistence was rare. Having only 1 or 2 follow-up visits was also a constraint that likely resulted in underestimation of the true frequency of long-term persistence. Thus, although the results of this study generated new hypotheses about the natural history of long-term HPV infection, additional studies with larger sample sizes and more frequent sampling are needed to fully characterize long-term HPV persistence.

In summary, HPV variant and behavioral risk factor analyses indicated that long-term detection of the same HPV type was more consistent with viral persistence than with reinfection. Although long-term persistence was not common, it was associated with sporadic rather than consistent viral DNA detection early during the course of infection and with the development of HPV-related lesions.

Acknowledgment

We thank Janice Morihara for her diligent performance of the laser-capture microdissection analyses.

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