Papillomavirus Research 5 (2018) 75–79

Contents lists available at ScienceDirect

Papillomavirus Research

journal homepage: www.elsevier.com/locate/pvr

Redetection of human papillomavirus type 16 infections of the cervix in mid-adult life

Aaron Ermel[a], Marcia L. Shew[c], Teresa M. Imburgia[c], Matt Brown[a], Brahim Qadadri[a], Yan Tong[d], Darron R. Brown[a,b,*]

[a] Department of Internal Medicine, Indiana University School of Medicine, USA
[b] Department of Immunology and Microbiology, Indiana University School of Medicine, USA
[c] Department of Pediatrics, Indiana University School of Medicine, USA
[d] Department of Biostatistics, Indiana University School of Medicine, USA

ARTICLE INFO

Keywords:
Human papillomavirus (HPV)
Redetection
Latency
Long control region
Sequencing

ABSTRACT

Purpose: To assess whether HPV 16 originally detected in adolescent women can be redetected in adulthood.
Methods: A convenience sample of 27 adult women with known HPV 16 detection during adolescence was assessed for HPV 16 redetection. A comparison of the long control region (LCR) DNA sequences was performed on some of the original and redetected HPV 16 isolates.
Results: Median age at reenrollment was 27.5 years (interquartile range of 26.7–29.6). Reenrollment occurred six years on average after the original HPV 16 detection. Eleven of 27 women had HPV 16 redetected. Some of these HPV 16 infections had apparently cleared during adolescence. LCR sequencing was successful in paired isolates from 6 women; in 5 of 6 cases the redetected HPV 16 isolates were identical to those detected during adolescence,
Conclusions: HPV 16 may be episodically detected in young women, even over long time periods. HPV 16 redetection with identical LCR sequences suggests low-level persistent infection rather than true clearance, although newly acquired infection with an identical HPV 16 isolate cannot be excluded. However, this study suggests that a new HPV 16-positive test in a clinical setting may not indicate a new infection.

1. Introduction

Despite the high prevalence of HPV infection in women, only a small percentage of these infections lead to cervical dysplasia or cancer. Most become undetectable within 12 months of the initial HPV detection, a phenomenon commonly referred to as clearance [1,2]. However, in studies with longitudinal follow-up periods up to six years, episodic detection of oncogenic HPV (with long time periods of apparent clearance) has been regularly demonstrated in young women [3–6]. Although HPV 16 can be detected in the months or even years immediately prior to the diagnosis of cervical cancer, it is unclear is this represents episodic detection of a previously acquired infection (low-level persistence) or a new HPV 16 infection [8]. HPV, a DNA virus, uses the host cell machinery to replicate. The rate of mutation of HPV mirrors that of the human genome, and is stable over time with some estimating an evolutionary rate of only one magnitude greater than that of their human hosts [9]. Of the nine regions of the HPV 16 genome, the long control region is the most variable of all of the genome regions [10]. The sequence variability in this region may be as high as 5% among HPV 16 isolates and has been used to follow transmission of HPV 16 isolates among cohorts and to understand persistent HPV 16 infections [11,12].

Various models have been proposed to explain detection patterns of HPV after apparent clearance, but the clinical relevance of these patterns of detection are not well understood [13–15]. In certain developed countries, primary cervical cancer screening with HPV DNA followed by type determination will replace cytological screening in upcoming years. Episodically detected high-risk HPV (HR-HPV), including HPV 16, therefore has implications in this new method of screening. In addition, the attributable risks of episodically detected infections vs. incident infections acquired later in life are not known but may differ.

To test the hypothesis that some HPV redetection episodes may be due to reactivation of a previously acquired infection, we reenrolled 30 women from a longitudinal cohort study known as the Young Women's Project (YWP) [16]. During the YWP, these women were tested

* Correspondence to: 635 Barnhill Dr., Van Nuys Medical Sciences Bldg., Suite 224, Indianapolis, IN 46202, USA.
E-mail address: darbrow@iu.edu (D.R. Brown).

https://doi.org/10.1016/j.pvr.2018.01.001
Received 15 August 2017; Received in revised form 20 November 2017; Accepted 12 January 2018
Available online 31 January 2018

Table 1
Long Control Region (LCR) sequencing primers, nucleotide coordinates, and exact sequences.

HPV 16 LCR segment	Nucleotide coordinates spanning the segment	Primer sequence
Segment 1 Forward	7288-7626	5′-TGCTTGTGTAACTATTGTGTC-3′
Segment 1 Reverse		5′-GTTGCACATAGTGCAGTGTAA-3′
Segment 2 Forward	7498-7788	5′-AGTTCTATGTCAGCAACTATGG-3′
Segment 2 Reverse		5′-AACTAGGGTGACATTTAGTTGG-3′
Segment 3 Forward	7681-115	5′-CCTTACATACCGCTGTTAGG – 3′
Segment 3 Reverse		5′-GTCCTGAAACATTGCAGTTCT – 3′

quarterly for HPV using self-collected vaginal swabs and annual cervical sampling. At reenrollment (the current study) data was gathered to assess whether 1) women with prior HPV 16 detection continued to have HPV 16 detected after a decade or longer, and 2) if the original and redetected HPV 16 isolates were identical or nearly identical (suggesting reactivation) or different (suggesting reinfection).

2. Material and methods

2.1. Study population and design

The current study was approved by the Institutional Review Board at Indiana University School of Medicine. Consent for re-contact of women enrolled in a prior study (the YWP) was already in place; however, all participants were consented again at enrollment for this study. We preferentially contacted women who had HPV 16 and or HPV 18 detected during their YWP observation [16–18]. Women in the YWP study (1998 through 2007) were contacted consecutively based on date of study enrollment, beginning with the earliest enrollment, and the first 30 to agree to participate verbally and present for their scheduled appointment constituted the study sample. A convenience sample of 30 women was re-enrolled. For 3 women who were reenrolled, no record of HPV 16 detection was found during their YWP observation, so these 3 women were excluded in this analysis that focused on HPV 16 re-detection.

Two study visits were required. At Visit 1, women were interviewed for intervening sexual histories and behaviors (from last date of YWP observation to current enrollment) as well as lifetime histories of previous and abnormal cervical cytological testing (Pap smear) and treatment for such abnormalities. After the interview, cervical swabs were collected for HPV testing and sequencing as described below, and a Pap smear was obtained. At Visit 2, participants provided a self-obtained vaginal swab for HPV testing. Pap smear and HPV results from Visit 1 were discussed with participants. The median time between Visit 1 and 2 for all participants was 33 days (IQR 22.5–54.5). Arrangements for follow-up with primary providers were made if the Pap smear was abnormal or if the participant's swab was positive for HPV types 16 or 18.

2.2. HPV testing

DNA was extracted from cervical or vaginal swabbed specimens as previously described [19]. The Linear Array HPV Genotyping test (Roche Molecular Diagnostics, Indianapolis, Indiana) (LA-HPV) was used for HPV detection and genotyping. This assay detects 37 HPV types using 5′ biotin-labeled primer pools for polymerase chain reaction (PCR) amplification within the L1 region of the HPV genome. Reactions were amplified in an Eppendorf Mastercycler® proS using the same parameters as previously described [20]. A positive control reaction (sample provided by Roche Molecular Diagnostics) and negative control reaction (no DNA) were performed with each assay. The GH20/PC04 human β-globin target was co-amplified to determine sample adequacy. Determination of specific HPV types was performed using the Roche Linear Assay (Roche Diagnostics, Indianapolis, Indiana) as previously described [19,21]. A semi-quantitative scoring system was used to estimate HPV viral load in samples from adolescent as previously described [22]. The low positive beta-globin band was assigned a value of 2, and the high positive band was assigned a value of 5. The intensity of HPV 16 bands on assay strips (“signal strength”) was compared to the low and high beta-globin bands and scored relative to these bands and given a score of 1–5.

2.3. Sequencing of the LCR region

The long control region (LCR) is the most variable region within the HPV genome with as much as 5% variability within variants, and has been used to characterize HPV isolates of various types [23–25]. When a participant tested positive for HPV 16 at one of the two study visits, a paired sampled was identified for comparison of LCR sequence. The paired sample consisted of the last HPV 16-positive swab during their YWP observation and an HPV 16-positive swab identified at re-enrollment. If amplification from the last positive swab during their YWP was unsuccessful, the next closest collected HPV 16-positive swab from that participant's longitudinal testing was used.

For sequencing, three overlapping primer pairs were developed such that the entire LCR was amplified (Table 1). The overlapping primer design was used to reduce error per sequencing reaction and increase sensitivity of the amplification [26]. The HPV 16 sequence used to determine the nucleotide number is located in GenBank under the accession number K02718.1. The percent homology between HPV 16 isolates was calculated by dividing the number of nucleotide non-identities by the total number of nucleotides sequenced, multiplied by 100. If sequencing for the identified pairs of HPV was more than 98% homologous, the pair was considered identical. As all of the participants were from a geographically limited area, determining the intertype variants may not have provided sufficient sequence variation to differentiate two isolates within the same type. de Villiers, et al. has noted a 2 to 5% variabilityin the LCR among HPV isolates of the same type [9]. Therefore, we selected this highly variable region for sequencing, and a cut-off of $\leq$2% difference in this region was used to differentiate HPV 16 isolates.

Five microliters of extracted DNA from each sample were used for amplification of the LCR region. PCR reactions were carried out using 5 μL of each primer (0.5 μM final concentration), 25 μL of Roche FastStart™ Master Mix (Roche Diagnostics), 10 μL of water on an Eppendorf Mastercycler® pro S for a final reaction volume of 50 μL. Reaction parameters were as follows: 94 °C for 9 min, 94 °C for 30 s, 56 °C for 30 s, 72 °C for 45 s all for 40 cycles followed by 72 °C for 7 min for the final extension step.

Amplification products were visualized on an agarose gel and the products corresponding to the expected size were excised and purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Purified LCR segments were sequenced using BIgDye terminator version 3.1 on an ABI 3730 XL instrument by ACGT, Inc. (Wheeling, IL). The sequences of the overlapping segments from the same sample were aligned using Basic Local Alignment Search Tool (BLAST) available on the NCBI website to create the full LCR sequence for that sample. The complete LCR sequence from one sample collected during the original YWP observation was then aligned with a sample from the re-enrollment period using BLAST.

2.4. Statistical analyses

Descriptive analysis (mean, standard deviation, median, interquartile range [IQR]) of the study population, HPV 16 detection, and Pap smears were conducted and reported. Two terms were used in the YWP study to describe HPV events for analysis and epidemiology of HPV infections [3]. First, if an HPV 16 infection had not been detected for the last two observation points in the YWP (at the end of the study observation), the infection was said to have "cleared". If an HPV infection was detected, then followed a period of non-detection of greater than or equal to six months (2 or more consecutive quarterly visits), then redetected, the term "episodic detection" was applied. The analysis of HR-HPV types was restricted to the 13 types considered Group 1 carcinogens (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66) [27].

3. Results

3.1. Characteristics of participants

Twenty-seven women were re-enrolled with HPV 16 detected during their YWP observation period. The median age at YWP enrollment was (interquartile range) 15.9 years (IQR 14.9–16.7), and the median age at the time of re-enrollment into the current study was 27.5 years (IQR 26.7–29.6). The median time between participant's final YWP visit and re-enrollment was 5.9 years (IQR 5.5–6.4). The majority of women identified themselves as African-American or Black (N = 24, or 88%). The comparison of the cohort characteristics from the YWP (N = 147) and the re-enrolled cohort are shown in Table 2. There were no significant differences found between the larger YWP cohort and the subset of 27 women who were re-enrolled.

Table 2
Comparison of the cohort characteristics between the YWP and those participants who were re-enrolled.

Characteristic	YWP, mean (SD) N = 147	Reenrolled, mean (SD) N = 27	p-value
Age at enrollment (years)	15.41 (1.04)	15.27 (1.08)	0.52[a]
Age at first sex (years)	14.4 (1.7)	14.2 (1.4)	0.72[a]
Duration of follow-up (years)	5.79 (1.5)	6.05 (1.8)	0.51[c]
Number of partners[d]			
Enrollment	2.92 (3.50)	3.33 (4.57)	0.10[c]
Last Visit	10.6 (6.8)	12.6 (6.3)	0.13[c]
	YWP, N (%) N = 147	Reenrolled N, (%) N = 27	
Race			
African American	139 (94.6)	26 (96.3)	0.10[b]
White	8 (5.4)	1 (3.7)	
Sexual Experience			
At enrollment	125 (85.0)	25 (92.6)	0.38[b]
By the last visit	146 (99.3)	27 (100)	0.10[b]
History of STI**			
At enrollment	26 (17.7)	4 (14.8)	0.10[b]
By the last visit	125 (85.0)	25 (92.6)	0.38[b]

YWP women (n = 147) includes 146 women from HPV project & 1 woman from original YWP project.
Reenrolled women (n = 27) includes 26 women from HPV project & 1 woman from original YWP project.
Time point of acquisition of data is dependent upon the characteristic mentioned.
†STI = cumulative number of sexually transmitted infections other than human papillomavirus at enrollment and at the last visit. These include Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis.
[a] *t*-test.
[b] Fisher's Exam test.
[c] Wilcoxon sum rank test.
[d] Mean number of unique cumulative lifetime partners within 2 months of enrollment and at the end of the study.

Table 3
HPV 16 detection based on collection method for each visit in the reenrollment study by study subject.

Participant number	Cervical swab HPV 16 Visit 1 (Score)*	Self-collected HPV 16 Visit 2 (Score)*	HPV 16 Redetection from YWP	Status of HPV 16 detection at the end of YWP†
1	1	–	‡	Censored
2	–	2	‡	Censored
3	–	1	‡	Censored
4	–	1	‡	Censored
5	1	–	‡	Censored
6	2	–	‡	Cleared
7	1	–	‡	Cleared
8	5	–	‡	Cleared
9	1	–	‡	Cleared
10	1	–	‡	Cleared
11	3	–	‡	Cleared
12	–	–		Censored
13	–	–		Censored
14	–	–		Censored
15	–	–		Censored
16	–	–		Censored
17	–	–		Cleared
18	–	–		Cleared
19	–	–		Cleared
20	–	–		Cleared
21	–	–		Cleared
22	–	–		Cleared
23	–	–		Cleared
24	–	–		Cleared
25	–	–		Cleared
26	–	–		Cleared
27	–	–		Cleared

3.2. HPV 16 infection and redetection

HPV 16 was detected during the YWP for an average of 2.58 years (SD = 2.46) for this group of 27 women. Eleven of 27 (40.7%) had HPV 16 detected again in the reenrollment study. For these 11 women, the mean time between the last detected HPV 16 in the YWP until redetection was 8.5 years (SD = 3.3) with a median (interquartile range) of 7.1 (5.6–11.2). The median time between visit 1 and visit 2 was 33.0 days (IQR 22.5–54.5).

Examining the patterns of HPV 16 detection during the YWP observation period, 6 of the 11 women with HPV 16 redetections had "cleared" their infections (minimally having the last two observation points negative for the HPV type) while 5 met the study definition for ongoing infection or were "right censored" (HPV 16 detected in one or both of the last two points of observation in the YWP). For those women who had "cleared" their HPV 16 infection, the mean time from last HPV 16 detection was 10.7 years versus 5.7 years for those participants whose HPV 16 infection was "right censored" (t = 4.45, p = .005). The status of HPV 16 detection for all 27 women with HPV 16 infection during and at the last observation period in the YWP is shown in Table 3. Among the eleven women with redetection of HPV 16, HPV was detected only in the cervical swab in 8 women, and in the vaginal swab in 3 women. None of the 11 women had HPV 16 detected in both cervical and vaginal swabs (Table 3).

3.3. Pap smears

Pap smear results among 27 women included 23 normal smears and 4 with low-grade changes (2 ASCUS and 2 LGSIL) (Table 4). Twenty-two of these 27 women reported a history of previously abnormal Pap tests. Three women had documented histories of treatment for cytological abnormalities including one woman who underwent cold knife conization.

Table 4
Pap smear and HPV results from Visit 1.

Pap smear result	HPV 16 Positive	Any HR-HPV Positive	HR-HPV Negative
Normal pap (N = 23)	11	12	11
ASCUS (N = 2)	0	1	1
LGSIL (N = 2)	0	1	1

3.4. Analysis of LCR sequencing of HPV 16 isolates

Of the eleven HPV 16 isolates that were redetected during the re-enrollment study, six were successfully amplified in all three segments for both the original and redetected isolates. The mean number of nucleotides sequenced for these 6 isolates was 747 base pairs with a range of 744–752. The median number of non-identities identified between HPV 16 isolates from the original observational period and the re-enrollment period was 9 1–15 nucleotides), and all non-identities were single nucleotide changes. The number of nucleotides sequenced, number of non-identities, and percent similarity between the HPV 16 isolates from the first observation period and the reenrollment period are shown in Table 5. The mean percentage of non-identities was 1.1% (range 0.1–2.0%), and the percentage of non-identities was less than 2.0% in isolates from all but one participant. There were no deletions or insertions found within the LCR segments between HPV 16 isolates from the original observational period and the re-recruitment period. For the woman whose HPV 16 isolates were 2% different, the band intensity corresponding to this result from the YWP specimen was 5 + and for the reenrollment specimen was 3 +, indicating a high viral load or a large number of HPV 16-infected cells in the specimen.

For 5 of 11 women with HPV 16 redetection, either the original isolate from the YWP or the HPV 16 isolate from the reenrollment study could not be amplified sufficiently (i.e., one of the three segments failed multiple attempts at amplification) for sequencing and therefore could not be included in the comparison.

4. Discussion

Many women are infected with HPV 16 and other oncogenic types in the first years after sexual debut. In most cases, type-specific HPV DNA can be detected by PCR for a few months after the initial detection, then the infection “clears”, meaning that the type-specific HPV DNA is no longer detected in one or two subsequent specimens. Whether the HPV has been truly eradicated has not been established. However, it is possible that a few infected cells remain in basal layers of epithelium in women with cleared infections, but the specimen contains only a few actual copies of HPV 16, below the level of detection of the PCR assay.

In the current study, 27 closely followed women with HPV 16 infections detected during adolescence were reenrolled nearly a decade after the initial detection. Redetection of HPV 16 occurred in 11 (40.7%) of these women. Were these new HPV 16 infections or a re-detection of a low-level persistent infection? This is a difficult if not impossible question to answer, but a nearly identical LCR sequence of earlier and later isolates occurred in most cases in which careful sequencing was performed. Such an identical sequence of the highly variable LCR in isolates taken over longer time periods suggests that either 1) the HPV 16 detected at the later time is actually the same as the original one, and never actually cleared, or 2) the woman has been re-infected with an identical HPV 16 isolate. Based on current beliefs that type-specific HPV re-infections are uncommon, it is most likely that these apparently “cleared” HPV 16 infections were actually infections that persisted at low levels, as was previously suggested by shorter studies of this same cohort of women [28]. Those that did appear to “clear” their infection (as defined by the study under the first observation period) did have a longer interval between the last detection and redetection from those that were censored in our study that was statistically significant. However, the purpose of the study was to investigate the redetection of HPV 16 and to demonstrate that the same isolate could persist for many years. We do not have information as to what occurred during the intervening period and HPV 16 could have been present at low-levels during this time.

Table 5
Nucleotide sequencing of the LCR segment in redetected HPV 16 paired isolates.

Participant Number	Number of nucleotides sequenced	Number of non-identities	Percent similarity[a]
1	744	13	98.3
2	745	1	99.9
5	747	5	99.3
7	748	13	98.3
10	752	3	99.6
11	744	15	98.0

[a] Calculated percent similarity: (# of nucleotides that are non-identities / total # of nucleotides) multiplied by 100.

The issue of low-level persistence, or latency of HPV has been a controversial issue. In the current study, most of the redetections of HPV 16 were found in the first visit where the sampling area included the cervix as opposed to the second self-obtained swab. As shown in Table 2, these redetections were associated with quantitatively low viral loads (compared to those found in the initial HPV detection), having a band intensity of 1 or 2, based on our semi-quantitative 1 through 5 scoring system as previously published [22]. These lower viral loads may suggest local immune control by the host or viral integration. Interestingly, the HPV 16 redetection with the strongest band intensity (Participant 11) was also the isolate that differed the most from the HPV 16 detected during the YWP study (that is, the highest percentage of non-identities). This relatively high band intensity of HPV 16 associated with non-identity may be a clue to true reinfection with a different HPV 16 variant.

Animal models have suggested that papillomaviruses indeed can exist in a latent state [29]. In a cottontail rabbit papillomavirus (CRPV) model, persistence of viral DNA was seen in experimental inoculation of the skin of the rabbit after the resolution of the papilloma that formed in response to the initial inoculation. DNA at these sites was associated with a lower viral copy number than was in the papilloma. However, these areas were virologically competent, as the latent sites were capable of forming papillomas with or without skin irritation [30]. Canine oral papillomavirus (COPV) reactivation has also been shown when immunosuppression is induced [31].

Few studies have investigated the persistence of HR-HPV types with such long intervening periods of non-observation as in ourstudy, and even fewer have utilized sequencing of HPV isolates in follow-up studies. Sycuro et al., [7] reenrolled a cohort of university-aged women who had followed longitudinally for HPV infections. In their follow-up, 16.3% of these women had the same HR-HPV type redetected. Sequencing of the E6 gene revealed that some HPV 16 redetection events were due to the same variant [7]. They also noted that redetection of the same HPV type was positively associated with sporadic detection of that type during their first observation period, a finding that further supports the notion that long-term persistent infections (with the same HPV isolate) account for a significant proportion of HPV DNA detection later in life.

Human studies of HPV latency are limited by infrequent sampling, reporting biases, and short periods of observation; thus the proportion of new HPV detection resulting from recent sexual acquisition versus reactivation of a prior infection is not known [13]. In spite of these limitations, Kocjan et al., using partial and full-length genome sequencing, showed that recurrence of laryngeal papillomas caused by HPV 6 or HPV 11 were likely due to the persistence of a single HPV

variant obtained in the original isolate and subsequent specimens, 1–22 years later [32]. Recently, van der Weele, et al., successfully utilized a Sanger-based method of whole genome sequencing to analyze persistent variants of HPV 16. In this study, the majority of the isolates were found to be identical during periods of repeated detection, with only one reinfection having been detected. However, there were no intervening periods of non-detection in this study [33]. Studies of women during prolonged periods of sexual inactivity have detected new HPV type-specific infections suggesting the possibility of reactivation [34,35]. These studies represent the mounting evidence in support of an HPV latent state.

There are several limitations to this study. First, only 27 women were included in the analysis. These women were part of a larger cohort (147 women) who were followed intensively for up to 8 years. Second, not every HPV 16 isolate could be sequenced, possibly due to either a very low abundance of DNA or due to degradation of DNA. Our sequencing results are suggestive of persistent isolates of HPV 16 in most participants whose isolates could be sequenced, but this was limited to a small, yet highly variable, region of the HPV genome. The application of techniques such as next generation sequencing may be useful in future studies to further clarify the true pathophysiology of episodic HPV detection [36]. The use of next generation sequencing techniques is relatively new in its application to the study of HPV persistence and different techniques may provide slightly different results. The lack of consensus as to what threshold of non-identities is considered to be a different isolate, especially in the setting of an infection that has potentially persisted for nearly ten years and in a potentially altered cell that may have reduced DNA proofreading capabilities, further hampers the determination of an HPV latent state. Mutations in the human genome are thought to accumulate in persistently infected cells in HPV related cancers, and this may also be true in the HPV genome. As more women are followed with serial HPV DNA tests, more opportunities to answer this question will arise.

If HPV can exist in a latent state, then low-level persistence and episodic detection may be lifelong phenomena. This is important for two reasons: First, low-level persistence of HPV 16 would obviously mean that the virus did not actually clear, and could eventually lead to a dysplastic lesion in a woman's future, even if the infection is present at times at levels below the detection limit of standard PCR assays. Second, when a “new” infection is detected in a screening test of a mature woman, this may in fact represent episodic detection of a low-level persistent infection acquired many years before. As stated earlier, understanding the trajectory of low-level persistent infection versus infections that are newly acquired in mid-adult life becomes even more imperative clinically.

In conclusion, these data support the low-level persistence and episodic detection of HPV 16 even after many years after initial detection. The clinical implications of these findings support the need of early vaccination among adolescents. As traditional cytology screening is replaced by primary HPV DNA testing, a better understanding of HPV epidemiology is imperative when assessing a positive HPV test with negative cytology or in women with a lack of recent sexual activity.

AcknowledgementsFunding

Center of HPV Research (Indiana University Purdue University at Indianapolis Signature Center Initiatives and Indiana University School of Medicine). The funding body had no role in the collection, review or publication of the data presented in this article.

Conflict of Interest

Authors A. Ermel, M. Shew, T. Imburgia, B. Qadadri and M. Brown have no conflicts to report. Dr. Brown has received funding, honoraria, and royalties from Merck and Company, Inc.

References

[1] G.Y.F. Ho, et al., Natural history of cervicovaginal papillomavirus infection in young women, N. Engl. J. Med. 338 (1998) 423–428.
[2] A.-B. Moscicki, et al., Updating the natural history of human papillomavirus and anogenital cancers, Vaccine 30 (Supplement 5(0)) (2012) F24–F33.
[3] M.L. Shew, et al., Episodic detection of human papillomavirus within a longitudinal cohort of young women, J. Med. Virol. 87 (12) (2015) 2122–2129.
[4] R.P. Insinga, et al., Incidence, duration, and reappearance of type-specific cervical human papillomavirus infections in young women, Cancer Epidemiol. Biomark. Prev. 19 (6) (2010) 1585–1594.
[5] A.C. Rodriguez, et al., Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection, J. Natl. Cancer Inst. 102 (5) (2010) 315–324.
[6] R.L. Winer, et al., Early natural history of incident, type-specific human papillomavirus infections in newly sexually active young women, Cancer Epidemiol. Biomark. Prev. 20 (4) (2011) 699–707.
[7] L.K. Sycuro, et al., Persistence of Genital Human Papillomavirus Infection in a Long-Term Follow-Up Study of Female University, J. Infect. Dis. 198 (7) (2008) 971–987.
[8] M.R.E. McCredie, et al., Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study, Lancet Oncol. 9 (5) (2008) 425–434.
[9] K. Van Doorslaer, Evolution of the papillomaviridae, Virology 445 (1) (2013) 11–20.
[10] E.-M. de Villiers, et al., Classification of papillomaviruses, Virology 324 (1) (2004) 17–27.
[11] E. Tanzi, et al., Human papillomavirus genotypes and phylogenetic analysis of HPV-16 variants in HIV-1 infected subjects in Italy, Vaccine 27 (Supplement 1) (2009) A17–A23.
[12] Z. Sun, et al., Genetic variations of E6 and long control region of human papillomavirus type 16 from patients with cervical lesion in Liaoning, China, BMC Cancer 13 (2013) (459-459).
[13] P.E. Gravitt, The known unknowns of HPV natural history, J. Clin. Investig. 121 (12) (2011) 4593.
[14] C.E. Depuydt, et al., Serial type-specific human papillomavirus (HPV) load measurement allows differentiation between regressing cervical lesions and serial virion productive transient infections, Cancer Med. 4 (8) (2015) 1294–1302.
[15] J. Doorbar, Latent papillomavirus infections and their regulation, Curr. Opin. Virol. 3 (4) (2013) 416–421.
[16] W. Tu, et al., Time from first intercourse to first sexually transmitted infection diagnosis Among adolescent women, Arch. Pediatr. Adolesc. Med. 163 (12) (2009) 1106–1111.
[17] B.P. Katz, J.D. Fortenberry, J. Harezlak, Sexual behavior of adolescent women at high risk for sexually transmitted infections, Sex. Transm. Dis. 28 (2001) 247–251.
[18] B.E. Batteiger, et al., Repeated Chlamydia trachomatis genital infections in adolescent women, J. Infect. Dis. 201 (1) (2010) 42–51.
[19] D.R. Brown, et al., A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women, J. Infect. Dis. 191 (2) (2005) 182–192.
[20] K.H. Fife, et al., Prevalence and persistence of cervical human papillomavirus infection in HIV-positive women initiating highly active antiretroviral therapy, J. Acquir. Immune Defic. Syndr. 51 (3) (2009) 274–282.
[21] M.L. Shew, et al., Association of condom use, sexual behaviors, and sexually transmitted infections with the duration of genital human papillomavirus infection among adolescent women, Arch. Pediatr. Adolesc. Med. 160 (2006) 151–156.
[22] Y. Tong, et al., Association of HPV types 6, 11, 16, and 18 DNA detection and serological response in unvaccinated adolescent women, J. Med. Virol. 85 (10) (2013) 1786–1793.
[23] L. Ho, et al., The genetic drift of human papillomavirus type 16 is a means of reconstructing prehistoric viral spread and the movement of ancient human populations, J. Virol. 67 (11) (1993) 6413–6423.
[24] Y.-J. Chang, et al., Intratypic variants of human papillomavirus type 16 and risk of cervical Neoplasia in Taiwan, J. Med. Virol. 85 (9) (2013) 1567–1576.
[25] C. Pientong, et al., Association of human papillomavirus type 16 long control region mutation and cervical cancer, Virol. J. 10 (2013) (30-30).
[26] A.A. Chen, et al., Human papillomavirus 18 genetic variation and cervical cancer risk worldwide, J. Virol. 89 (20) (2015) 10680–10687.
[27] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, Human papillomaviruses, IARC Monogr Eval Carcinog Risks Hum 90 (2007) 1567–1576.
[28] B. Weaver, et al., Low-level persistence of human papillomavirus 16 DNA in a cohort of closely followed adolescent women, J. Med. Virol. 83 (8) (2011) 1362–1369.
[29] G.A. Maglennon, J. Doorbar, The biology of papillomavirus latency, Open Virol. J. 6 (Suppl 2) (2012) 190–197.
[30] R. Selvakumar, et al., Regression of papillomas induced by cottontail rabbit papillomavirus is associated with infiltration of CD8+ cells and persistence of viral DNA after regression, J. Virol. 71 (7) (1997) 5540–5548.
[31] G.A. Maglennon, P.B. McIntosh, J. Doorbar, Immunosuppression facilitates the reactivation of latent papillomavirus infections, J. Virol. 88 (1) (2014) 710–716.
[32] B.J. Kocjan, et al., Identical human papillomavirus (HPV) genomic variants persist in recurrent respiratory papillomatosis for up to 22 years, J. Infect. Dis. 207 (4) (2013) 583–587.
[33] P. van der Weele, C.J. Meijer, A.J. King, Whole-genone sequencing and variant analysis of HPV16 infections, J. Virol. (2017) 00844-17 (p. JVI).
[34] H.D. Strickler, et al., Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women, J. Natl. Cancer Inst. 97 (8) (2005) 577–586.
[35] R.N. Theiler, et al., High-risk human papillomavirus reactivation in human immunodeficiency virus-infected women risk factors for cervical viral shedding, Obstet. Gynecol. 115 (6) (2010) 1150–1158.
[36] M. Cullen, et al., Deep sequencing of HPV16 genomes: a new high-throughput tool for exploring the carcinogenicity and natural history of HPV16 infection, Papillomavirus Res. 1 (2015) 3–11.