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HPV-6 Molecular Variants Association With the Development of Genital Warts in Men: The HIM Study

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Background. Human papillomavirus type 6 (HPV-6) and HPV-11 are the etiological agents of approximately 90% of genital warts (GWs). The impact of HPV-6 genetic heterogeneity on persistence and progression to GWs remains undetermined.

Methods. HPV Infection in Men (HIM) Study participants who had HPV-6 genital swabs and/or GWs preceded by a viable normal genital swab were analyzed. Variants characterization was performed by polymerase chain reaction sequencing and samples classified within lineages (A, B) and sublineages (B1, B2, B3, B4, B5). Country- and age-specific analyses were conducted for individual variants; odds ratios and 95% confidence intervals for the risk of GWs according to HPV-6 variants were calculated.

Results. B3 variants were most prevalent. HPV-6 variants distribution differed between countries and case status. HPV-6 B1 variants prevalence was increased in GWs and genital swabs of cases compared to controls. There was difference in B1 and B3 variants detection in GW and the preceding genital swab. We observed significant association of HPV-6 B1 variants detection with GW development.

Conclusions. HPV-6 B1 variants are more prevalent in genital swabs that precede GW development, and confer an increased risk for GW. Further research is warranted to understand the possible involvement of B1 variants in the progression to clinically relevant lesions.

Keywords. HPV-6; variability; genital warts; males; HIM Study; prevalence.

Worldwide, condylomata acuminata (or genital warts [GWs]) are a very common sexually transmitted disease affecting both men and women. Despite their benign nature, GWs are associated with significant morbidity and personal emotional distress [1]. Although about one-third of GWs naturally regress, recurrence frequently occurs and, therefore, repeated treatment represents high medical costs [2]. Low-risk human papillomavirus type 6 (HPV-6) and HPV-11, frequently detected in the anogenital region of both genders, are the etiologic agents of over 90% of GWs [3]. Additionally, HPV-6 and HPV-11 are associated with the development of laryngeal papillomas [4]. These facts warranted the inclusion of both viral types in the quadrivalent HPV vaccine along with high-risk HPV-16 and HPV-18 [5]. Currently, after more than 5 years of high coverage vaccination, a robust reduction in GWs burden is observed among young women in Australia [6].

The prototype HPV-6b clone was initially isolated from a condyloma acuminatum specimen [7] with differences in restriction patterns observed thereafter for HPV-6a and HPV-6vc nonprototypic genomes [8]. Furthermore, a full genome phylogenetic analysis of globally collected HPV-6 isolates revealed the existence of 2 deeply separated variant lineages (named A and B), with the B lineage consisting of 5 sublineages (B1–B5) [9, 10]. Where the HPV-6b-prototype sequence is clustered within lineage A, HPV-6vc and HPV-6a genomes sort into B1 and B3 sublineages, respectively [9].

In the last decade, a growing number of studies have explored the distribution of HPV-6 variants in lesions from different anatomic sites. These reports revealed that, aside from the Asian continent, lineage B variants, mainly from sublineage B1, predominate globally in HPV-6–positive samples from the anogenital region [10–12]. It has also been described that the simultaneous occurrence of 2 or more GWs within the same individual results from infection with a single HPV-6 genomic variant [13]. Interestingly, it has also been shown that in comparison to males, females have higher odds for infection with genetic variants from HPV-6 sublineage B3 than with variants from HPV-6 lineage A [10]. Nevertheless, clinical correlations for HPV-6 variants still deserve extensive analysis [14–16]. An

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important implication arising from this is how HPV-6 genomic heterogeneity impacts on transmission, persistence, and progression to GWs. Unraveling these issues could explain why some HPV-6 infections progress to GWs, while others do not. Here, we address this fundamental question within the multinational HPV Infection in Men (HIM) Study cohort. The aim of our study was to estimate HPV-6 variant prevalence in genital swabs from the healthy skin and in GWs biopsies, and to analyze lineage-specific persistence and infection progression to GWs in otherwise healthy men.

METHODS

Study Population

The HIM Study cohort includes over 4000 men aged 18–70 years, enrolled from July 2005 to June 2009, who were residents of São Paulo, São Paulo, Brazil; Cuernavaca, Morelos, Mexico; and Tampa, Florida. Details of the HIM Study design and procedures are published elsewhere [17]. In 2009, a biopsy and pathology protocol was implemented, allowing for biopsy collection, pathological confirmation, and HPV genotyping of lesions [18]. The study was approved by the ethical review boards of the Institutional Review Boards at the University of South Florida (Tampa, FL), the Ludwig Institute for Cancer Research (São Paulo Branch, Brazil), the Centro de Referência e Treinamento em DST/Aids (São Paulo, Brazil), and the Instituto Nacional de Salud Publica (Cuernavaca, Mexico), and informed consent was obtained from all participants.

DNA Extraction and HPV Testing

At each biannual visit, 3 different prewetted Dacron swabs were used to sample the external genitalia (coronal sulcus, glans penis, penile shaft) and scrotum of participants; these swabs were further combined to form a single sample [17]. DNA extraction was performed using the Qiagen Media kit (Qiagen, Venlo, The Netherlands), and HPV detection and typing was conducted using the Linear Array HPV Genotyping Test (Roche Molecular Diagnosis, CA) capable of identifying 37 HPV types classified as high risk (HR-HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) or low risk (LR-HPV: 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, IS39, 83, 84, 89).

Additionally, participants underwent clinical examination by a trained clinician at each visit, under 3× light magnification, to detect external genital lesions (EGLs). Each time a lesion was observed, a tissue sample was obtained by shave excision. EGLs were categorized as condyloma, suggestive of condyloma (with several characteristics but not histopathologically defined as condyloma), penile intraepithelial neoplasia, or not HPV-related, according to criteria previously published [19]. Participants could also attend the clinic for an intervening visit whenever a suspicious lesion was present; 66 of the 102 EGLs collected in this study were collected at these intermediate visits. DNA samples from formalin-fixed, paraffin-embedded (FFPE) tissue biopsies were extracted using the QIAamp DNA FFPE Tissue kit (Qiagen), and HPV detection and genotyping was conducted using the INNO-LiPA HPV Genotyping Extra assay (Fujirebio, Göteborg, Sweden), which detects 28 viral types classified as high risk (HR-HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) or low risk (LR-HPV: 6, 11, 26, 40, 43, 44, 53, 54, 66, 69, 70, 71, 73, 74, 82). DNAs were considered adequate if tested positive for β-globin or any HPV type.

HPV-6 Variant Characterization

HPV-6 variants were accessed by polymerase chain reaction (PCR) for amplification of a 617–base pair (bp) fragment within the *L2* gene (nucleotide positions 4430–5047) using AmpliTaq Gold DNA Polymerase (Applied Biosystems, CA). In specimens where no amplification was observed, additional sets of primers, that amplify smaller *L2* fragments, were used to cover the same 617-bp segment. Amplification products were submitted to automated sequencing in an ABI PRISM 3130XL GeneticAnalyzer/HITACHI sequencer (Applied Biosystems) using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). DNA sequences were compared to the HPV-6 prototype (GenBank accession no. X00203) and classified into 2 lineages (A and B) and 5 sublineages (B1, B2, B3, B4, and B5) [9–10].

Statistical Analysis

In the present study, controls met all of the following criteria: (1) had no HPV-6-associated EGLs categorized as condyloma or suggestive of condyloma (named from now on as GWs); (2) HIM participants for whom HPV-6 was detected in at least 1 normal genital skin swab at any study visit between April 2009–December 2013; (3) had at least another normal genital skin swab collected up to 640 days prior to the HPV-6-positive swab (the time period of 640 days [21 months] was selected a priori). Overall, 255 men qualified as controls for this analysis from whom 170 men were randomly selected from the 3 countries (approximately 67% available controls per country): Brazil (78/117), United States (47/71), and Mexico (45/67). Among the controls selected, 15 were excluded because no specimen remained for HPV-6-variant characterization (1 from Brazil; 9 from the United States; 5 from Mexico). For the remaining 155 controls, the most recently collected HPV-6 genital swab specimen underwent molecular variant characterization. Moreover, all HPV-6-positive smears collected in the previous 640 days were also submitted to variant characterization, up to a maximum of 3 specimens.

Cases were men with an incident HPV-6-positive GW, who had at least 1 (and up to 3) viable swabs from normal genital skin collected at a study visit up to 640 days preceding the day when the GW was obtained. The genital swab specimen collected at the visit just prior to the GW excision is referred to as "the most recent swab." A total of 106 men had an HPV-6-positive

GW biopsied during the study, of whom 4 were excluded due to insufficient specimen for variant characterization. Cases were further subdivided into 2 groups: (1) participants harboring HPV-6 in both the GW and in a normal genital skin swab collected prior to lesion development (n = 70); and (2) participants with a HPV-6-positive GW but otherwise negative in all normal genital skin swabs collected up to 640 days prior to EGL development (n = 28).

Sociodemographic and sexual behavior characteristics were compared among cases and controls using the exact Pearson χ^2 test. Fisher exact test was used to compare HPV-6 variant distribution in normal genital skin swabs from controls and cases, and in GWs of cases by age and country. For these analyses, we assessed the most recent specimen from controls and cases. Fisher exact test was also used to study the association between HPV-6 variant detection in GWs and the preceding normal genital skin swabs.

Exact odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for the risk of GW according to HPV-6 variant status in the most recent normal genital swab prior to lesion development. Given that the majority of variants detected on the most recent swabs of controls belonged to sublineage B3, this group was selected as the reference group. All analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC) and attained statistical significance at $\alpha \leq .05$.

RESULTS

We were unable to characterize an HPV-6 variant in specimens from 4 controls and 4 cases due to PCR amplification failure. The majority of the remaining 151 controls and 98 cases were young men (<35 years), white, non-Hispanic, not circumcised, and reported to have never had sex with men. Additionally, most men have never smoked cigarettes and were currently not using any other tobacco products. No significant differences in sociodemographic and sexual behavior characteristics between cases and controls were observed (Table 1).

Table 2 shows the prevalence of the different HPV-6 variants in the most recent swab of normal genital skin obtained from controls, and prior to GW development among cases. Overall, within controls, HPV-6 B3 variants were the most prevalent in all 3 countries, although these variants were significantly higher in Mexico when compared with Brazil and the United States (80.0% Mexico, 60.8% Brazil, 48.7% United States; $P = .006$). Interestingly, the prevalence of HPV-6 B1 variants in the most recent swab of cases was higher compared to controls. HPV-6 B3 variants were the most prevalent variants detected in cases and controls. However, the distribution of variants differed significantly by case status: we observed that cases were more likely to harbor B1 variants and less likely to harbor less common variants (lineage A and sublineages B2 and B5) on normal genital skin when compared to controls ($P = .01$). Among

Table 1. Differences in Sociodemographic and Sexual Behavior Characteristics Among HPV-6-Positive Men Who Did and Did Not Develop GWs During Follow-up in the HIM Study

	Controls (n = 151)		Cases (n = 98)		
Characteristic	n	(%)	n	(%)	*P* Value
Country	…	…	…	…	.06
United States	37	(24.5)	35	(35.7)	
Brazil	74	(49.0)	34	(34.7)	
Mexico	40	(26.5)	29	(29.6)	
Age	…	…	…	…	.69
18–24	30	(19.9)	20	(20.4)	
25–34	56	(37.1)	41	(41.8)	
≥35	65	(43.1)	37	(37.8)	
Race	…	…	…	…	.28
White	77	(51.0)	48	(49.0)	
Black	31	(20.5)	14	(14.3)	
Other	43	(28.5)	36	(36.7)	
Ethnicity	…	…	…	…	1.00
Hispanic	60	(39.7)	39	(39.8)	
Non-Hispanic	91	(60.3)	59	(60.2)	
Education	…	…	…	…	.53
≤12	65	(43.1)	37	(37.8)	
13–15	38	(25.2)	23	(23.5)	
≥16	48	(31.8)	38	(38.8)	
Marital status	…	…	…	…	.82
Single/never married	71	(47.0)	44	(44.9)	
Married/cohabiting	67	(44.4)	47	(48.0)	
Divorced/separated/widowed	13	(8.6)	7	(7.1)	
Circumcision status	…	…	…	…	.08
Not circumcised	95	(66.2)	54	(55.1)	
Circumcised	51	(33.8)	44	(44.9)	
Cigarette smoking status	…	…	…	…	.56
Current	39	(25.8)	27	(27.6)	
Former	24	(15.9)	20	(20.4)	
Never	88	(58.3)	51	(52.0)	
Baseline tobacco use	…	…	…	…	.80
Current	44	(29.1)	30	(30.6)	
Not current	107	(70.9)	68	(69.4)	
Alcohol consumption in last month	…	…	…	…	.25
No alcohol	36	(23.8)	15	(15.3)	
1–4 drinks/occasion	51	(33.8)	31	(31.6)	
≥5 drinks/occasion	61	(40.4)	48	(49.0)	
Missing	3	(2.0)	4	(4.1)	
Lifetime female vaginal sex partners	…	…	…	…	.39
<10	41	(27.2)	29	(29.6)	
10–19	26	(17.2)	22	(22.5)	
20–49	50	(33.1)	27	(27.6)	
≥50	30	(19.9)	20	(20.4)	
Missing	4	(2.7)	0	(0.0)	
Lifetime male anal sex partners	…	…	…	…	.70
0	115	(76.2)	79	(80.6)	
1–9	20	(13.3)	11	(11.2)	
≥10	16	(10.6)	8	(8.2)	
Sexual orientation	…	…	…	…	.55
Never MSM	111	(73.5)	76	(77.6)	
MSM	40	(26.5)	22	(22.5)	

Abbreviations: GWs, genital warts; HIM Study, HPV Infection in Men Study; HPV, human papillomavirus; MSM, men having sex with men.

Table 2. Distribution of HPV-6 Variants Detected in the Most Recent Swab of Normal Genital Skin Prior to Wart Detection, by Case Status, Country, and Age Group

	Sublineage B1		Sublineage B3		Lineage A Sublineages B2, B5		Total		
	n	%	n	%	n	%	n	%	*P* Value
Controls (n = 151)	…	…	…	…	…	…	…	…	…
Country	…	…	…	…	…	…	…	…	.006
Brazil	21	28.4	45	60.8	8	10.8	74	49.0	…
Mexico	3	7.5	32	80.0	5	12.5	40	26.5	…
US	16	43.2	18	48.7	3	8.1	37	24.5	…
Age Group	…	…	…	…	…	…	…	…	.09
18–24	12	40.0	17	56.7	1	3.3	30	19.9	…
25–34	10	17.9	41	73.2	5	8.9	56	37.1	…
≥35	18	27.7	37	56.9	10	15.4	65	43.1	…
Total	40	26.5	95	62.9	16[a]	10.6	151	100.0	…
Cases (n = 70)[b]	…	…	…	…	…	…	…	…	…
Country	…	…	…	…	…	…	…	…	.007
Brazil	13	61.9	8	38.1	0	0.0	21	30.0	…
Mexico	4	17.4	17	73.9	2	8.7	23	32.9	…
US	14	53.9	12	46.2	0	0.0	26	37.1	…
Age group	…	…	…	…	…	…	…	…	.19
18–24	11	68.8	5	31.3	0	0.0	16	22.9	…
25–34	9	34.6	16	61.5	1	3.9	26	37.1	…
≥35	11	39.3	16	57.1	1	3.6	28	10.0	…
Total	31	44.3	37	52.9	2[c]	2.9	70	100.0	…

Abbreviations: BZ, Brazil; EGL, external genital lesion; HPV, human papillomavirus; MX, Mexico; US, United States.

[a]Among controls, lineage A, and sublineages B2 and B5 were detected in 5, 7, and 4 swabs, respectively.

[b]Twenty-eight cases lacked an HPV-6-positive normal genital skin swabs within 640 days prior of EGL detection (US = 9, MX = 6, BZ = 13).

[c]Among cases, lineage A and sublineage B2 variants were detected in 1 case each.

cases, the distribution of HPV-6 variants across countries was significantly different, with HPV-6 B1 variants most commonly detected in Brazil and the United States, and HPV-6 B3 variants more frequently found in Mexico ($P = .007$). For both cases and controls, the distribution of HPV-6 variants among genital swabs was independent of age (Table 2).

We further analyzed the prevalence of HPV-6 variants among all 98 GW biopsy samples included in this study. The distribution of HPV-6 variants in these lesions closely resembled that of the lesion-preceding genital swabs. Although HPV-6 B3 variants were more prevalent in men from Mexico compared with men from Brazil and the United States, this difference did not reach statistical significance in the biopsies ($P = .21$) (Table 3). However, we observed significant association between age and HPV-6 variants detected on GWs: men aged 18–24 years predominantly presented HPV-6 B1–positive lesions (70.0%), whereas HPV-6 B3 variants were more frequently detected in GWs of older men (>34 years; 54.1%) ($P = .02$).

Table 3. Distribution of HPV-6 Variants Detected in GW Biopsies, by Country and Age Group

	Sublineage B1		Sublineage B3		Lineage A Sublineages B2, B5[a]		Total		
	n	%	n	%	n	%	n	%	*P* Value
Country	…	…	…	…	…	…	…	…	.21
Brazil	18	52.9	15	44.1	1	2.9	34	34.7	…
Mexico	8	27.6	20	69.0	1	3.5	29	29.6	…
US	17	48.6	16	45.7	2	5.7	35	35.7	…
Age group	…	…	…	…	…	…	…	…	.02
18–24	14	70.0	6	30.0	0	0.0	20	20.4	…
25–34	16	39.0	25	61.0	0	0.0	41	41.8	…
≥35	13	35.1	20	54.1	4	10.8	37	37.8	…
Total	43	43.9	51	52.0	4	4.1	98	100.0	…

Abbreviations: GW, genital wart; HPV, human papillomavirus.

[a]Lineage A, and sublineages B2 and B5 were detected in 1, 2 and 1 EGLs, respectively.

Table 4. Cases With the Same HPV-6 Variant Detected in the EGL and in Normal Genital Skin Swab Collected Prior to GW Development[a]

	Previous Genital Swab With the Same Variant		
HPV-6 Variant in EGL Biopsy	n	%	*P* Value
Sublineage B1	25/27	92.6	…
Sublineage B3	34/39	87.2	…
Lineage B/sublineages B2, B5, A	1/4	25.0	…
Overall	60/70	85.7	.01

Abbreviations: EGL, external genital lesion; GW, genital wart; HPV, human papillomavirus.
[a]HPV-6 variant detected at any visit (maximum of 4 visits) prior to wart development.

Among genital swabs and GWs biopsies, HPV-6 variants from lineage A or sublineages B2 and B5 were rarely detected. Additionally, no variants from the B4 sublineage were identified. Interestingly, GWs harboring these less frequently detected variants were only detected in men ≥35 years old.

We next sought to investigate HPV-6 infection progression to wart development among cases that had an HPV-6-positive normal genital skin prior to GW development. Among men who developed an HPV-6 B1–positive GW, 92.6% had a previous swab positive for the same variant. Similarly, 87.2% of men who developed an HPV-6 B3–positive GW had a previous swab positive for the same variant (Table 4). Taken together, our results indicate that men with an HPV-6 B1–positive GW were more likely to have an infection with the same variant detected in a normal genital skin swab collected prior to lesion development compared to those with HPV-6 B3 variants or with less common variants (lineage A and sublineages B2 and B5) ($P = .01$).

Finally, Table 5 shows the risk for GW development by HPV-6 status in the most recent genital swab of cases. We observed a significant association of HPV-6 B1 variants infection with GW development (OR = 1.98, 95% CI = 1.04, 3.80; $P = .04$) compared to HPV-6 B3 variants.

DISCUSSION

In the present study, we examined the prevalence and natural history of HPV-6 variants during a follow-up period spanning about 2 years within a multicentric cohort of men from the United States, Brazil, and Mexico. Molecular variants of HPV-6 were characterized based on the sequence analysis of a 617-bp fragment of the *L2* gene. This fragment is contained within the 960-bp segment shown by Jelen and colleagues [10] to be representative of whole-genome-based phylogenetic clustering, and which contains sufficient single-nucleotide polymorphism patterns to discriminate between major HPV-6 lineages and sublineages. The present study revealed the predominance of HPV-6 B1 and B3 variants with some differences in their distribution by geographic region and case status; specifically, we detected an increase in the frequency of B1 variants in cases compared to controls. We further observed that among cases, most genital HPV-6 swabs preceding GW development contained the same variant present in the lesion. Most notably, we showed for the first time that infections with HPV-6 B1 variants are associated with an increased risk of GW development compared to the most commonly detected HPV-6 B3 variants. To the best of our knowledge, this study is unique in investigating prospectively the impact of HPV-6 intratypic variability in the development of GWs in men.

Table 5. Unadjusted OR (and respective 95% CI) for the Risk of GW by HPV-6 Variant Status in the Most Recent Genital Swab Collected Prior to Lesion Development[a]

	Cases (n = 70)	Controls (n = 151)	Unadjusted OR (95% CI)	*P* Value
Sublineage B3	37	95	1 (Ref.)	
Sublineage B1	31	40	1.98 (1.04–3.80)	.04
Lineage A Sublineages B2, B5,	2	16	0.32 (0.03–1.48)	.20

Abbreviations: CI, confidence interval; GW, genital wart; HPV, human papillomavirus; OR, odds ratio.
[a]HPV-6 sublineage B3 was selected as the reference (Ref.) group.

Several studies have suggested that high-risk HPV-16 and HPV-18 coevolved with the human species and that the distribution of variants of both viral types varies geographically [20–22]. However, with regard to variants of low-risk HPV types, only slight geographical correlations have been reported, which strongly suggest that HPV-6 and HPV-11 variants are detected with similar frequencies around the world [10, 15, 16, 23]. In the present study, HPV-6 B3 variants predominated in all 3 countries among genital swabs of controls, although these variants were significantly less frequent in men from Brazil and the United States compared to men from Mexico. Interestingly, the prevalence of HPV-6 B1 variants was higher in GW biopsies of cases and in normal genital skin swabs from cases compared to controls from all 3 countries. Moreover, among men from Brazil and the United States who developed a GW, HPV-6 B1 variants were more prevalent than HPV-6 B3. Our results corroborate previous data reporting that worldwide (including samples from Brazil and the United States), the majority of HPV-6 variants in GWs and laryngeal papillomas cluster into the B1 sublineage, with the B3 sublineage being the second most frequently detected [10, 11, 12, 16, 24]. The low prevalence, or even absence, of HPV-6 A, B2, or B4 variants in GWs in the United States and Brazil has also been observed by others [10]. It is of note that most studies conducted to date were restricted to lesion specimens with very scarce data, focusing on preceding normal skin genital swabs.

To date, the HIM Study is the most extensive analysis of the natural history of HPV infection in healthy and asymptomatic men aged 18 years and older and mostly men who have sex with women. Overall, genital HPV prevalence among HIM participants at baseline was 65.2% of which about one-third are

low-risk viral types [17, 25]; HPV-6 was the third most common viral type in genital smears of these men and was identified in 9.4%, 4.1%, and 6.3% of men from Brazil, Mexico, and the United States, respectively. It was further observed that after 24 months of follow-up, 27% of genital HPV-6 infections progressed to an HPV-6-positive condyloma (median time of 7.8 months) [26]. Although younger men (<30 years) enrolled in the HIM Study cohort were more likely to develop any EGL [26], among HPV-6-positive men included in the present analysis, we did not detect any significant association between age and the development of GWs.

We observed that the great majority of HPV-6 variants detected in GWs were previously detected in normal genital skin swabs collected up to 640 days prior to lesion development, suggesting that persistence (even if short-term persistence) of HPV-6 infection precedes lesion development in most cases, even if significant differences were observed among variants. Among cases, 28 men included in this study did not have an HPV-6-positive swab prior to GW development; this could be due to the fact that in the HIM protocol design, we tested for genital HPV infection every 6 months, and it is likely that some infections progressed to GWs during the short time frame between study visits. Additionally, it is possible that latent and/or persistent infection remained subclinical, likely at low-copy number and thus undetectable. This hypothesis is reinforced by the observed recurrence of laryngeal papillomas and genital warts as a consequence of long-term persistence of unique HPV genomic variant rather than of repeated reinfections with novel HPV isolates [13, 27].

It is well established, at least for populations with a multiethnic composition, that HPV-16 Asian-American variants are associated with an increased risk of clinically relevant anogenital lesions development [28–30]. We report here for the first time that HPV-6 B1 variants are associated with 2-fold increased risk of GW development when compared to B3 variants in this cohort of men from 3 different countries. At the same time, in other reports, the association of clinical correlates to specific HPV-6 variants was not observed [15, 16, 31].

Functionally, for high-risk HPV-16, it has been extensively described in literature that Asian-American variants have the increased ability of inducing differentiation-resistant colonies of human foreskin keratinocytes [32, 33] and in cooperative transformation involving other signaling pathways [34, 35]. Conversely, there are no data available regarding whether the E6 and E7 proteins from different HPV-6 variants differ in their biological and biochemical properties. We recently demonstrated, using luciferase reporter assays, that the HPV-6 B1 reference variant is 10 times more transcriptionally active than the HPV-6 B3 reference genome [31]. The more efficient E1/E2 expression could confer an elevated viral replication potential to HPV-6 B1 variants; thus, one may assume that an infection with a more replicative viral variant could implicate more efficient proliferation and higher frequency of GW development.

In summary, we present for the first time the impact of HPV-6 nucleotide variability on GW development in the male genitalia. Our results provide the background for the need of future efforts on elucidating the influence of intraviral heterogeneity on the clinical outcome of HPV-6 infection and the mechanisms by which B1 variants endow a higher risk of progression to clinically relevant lesions.

Notes

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