Null Results in Brief

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Lack of Association between Human Papillomavirus Types 6 and 11 Genetic Variants and Cervical Abnormalities: The Ludwig–McGill Cohort Study

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Abstract

Background: Human papillomavirus (HPV) types 6 and 11 are mainly associated with the development of genital warts and recurrent respiratory papillomatosis. We examined intratypic genetic variability of both viral types with the development of cervical cytologic abnormalities in Brazilian women.

Methods: We used PCR sequencing to characterize variants of HPVs 6 and/or 11 in cervical swabs from women in the Ludwig–McGill Cohort Study. We used a binomial generalized estimating equations (GEE) model with logit link to estimate odds ratios (OR) and 95% confidence intervals (CI) for the associations between HPV 6 and 11 variants and cytologic abnormalities.

Results: B1 and B3 HPV6 and A2 HPV11 variants were the most common isolates identified. Compared with HPV6-negative women, the ORs among women harboring HPV6 B1 or B3 variants were 6.3 (95% CI, 2.3–17.0) and 2.3 (95% CI, 0.6–9.7) for atypical cells of undetermined significance (ASCUS)/low squamous intraepithelial lesions (LSIL), respectively, and 1.7 (95% CI, 0.6–5.1) and 1.2 (95% CI, 0.3–4.7) for ASCUS/LSIL/high squamous intraepithelial lesions (HSIL). Respective ORs were 5.0 (95% CI, 1.7–14.6) and 2.8 (95% CI, 1.0–8.1) upon comparing women with HPV11 A2 variants to HPV11-negative women. All associations disappeared when adjusting for coinfections with high-risk HPV types.

Conclusions: Our data do not support an association between low-risk HPVs 6 and 11 genetic variability and cervical abnormalities.

Impact: Risk of cervical cytologic abnormalities is not affected by intratypic polymorphism in HPVs 6 and 11.

Introduction

A fraction of cervical low-grade squamous intraepithelial lesions (LSIL) are caused by low-risk HPVs 6 and 11 (1). Whole-genome variations of 1%–10% and 0.5%–1% define HPV variant lineages and sublineages, respectively (2, 3). Phylogenetic analysis of isolates collected worldwide clusters variants of HPVs 6 and 11 into two lineages termed A and B, additionally divided into sublineages (2, 3). We recently showed that although HPV6 B1 variants are associated with an increased risk for male genital warts (GW) compared with non-B1 variants (4), GW risk was not affected by HPV11 intratypic variability (5). We evaluate the impact of HPVs 6 and 11 variability on the development of cervical cytologic abnormalities among women enrolled in the Ludwig–McGill Cohort Study.

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Materials and Methods

Study population

Details on the design and methods of the Ludwig–McGill Cohort Study of the natural history of HPV infection and precursor lesions of cervical cancer are published (6). Briefly, 2,462 women aged 18–60 years were recruited in Sao Paulo, Brazil from 1993 to 1997, and followed for up to 10 years. Questionnaires were administered and biological specimens were collected. Ethical review boards of the institutions in Brazil and Canada approved the study and informed written consent was obtained from participants.

Characterization of HPV variants

DNA from HPV 6- and HPV 11–positive cervical swabs were submitted to PCR for amplification of a fragment of the *L2* and *E2*/NCR2 regions, respectively (4, 5). Reaction products were sequenced in an ABI PRISM 3130XL GeneticAnalyzer (Applied Biosystems) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). DNA sequences were compared with those of HPVs 6 and 11 prototypes (Genbank X00203 and M14119; refs. 2, 3).

Statistical analysis

We used generalized estimating equations (GEE) with logit link and assuming an exchangeable correlation structure (to account for intraindividual correlations) to study the associations between

Table 1. Frequency of detection of HPV6 and HPV11 variants in the Ludwig–McGill Cohort Study

HPV type	Variant lineage/sublineage	Number of specimens (%)
HPV6	Negative	24,434 (99.55)
	A	7 (0.03)
	B1	39 (0.16)
	B2	1 (0.00)
	B3	38 (0.15)
	B5	1 (0.00)
	Undetermined (no amplification)	25 (0.10)
HPV11	Negative	24,501 (99.82)
	A1	9 (0.04)
	A2	33 (0.13)
	Undetermined (no amplification)	2 (0.01)

HPVs 6/11 variants and cervical abnormalities. The latter included atypical cells of undetermined significance (ASCUS), LSIL, and high-grade squamous intraepithelial lesions (HSIL). We reported results from univariate and bivariate (adjusting for infection with high-risk HPV types) GEE analyses as odds ratios (OR) and 95% confidence intervals (CI). The entire follow-up period (up to 15 visits) was considered. We excluded prevalent lesions. Statistical analysis was performed using Stata version 13 (StataCorp).

Results

During the entire study period HPVs 6 and 11 were detected at least once in 79 (111 smears) and 39 women (44 smears), respectively. Eleven HPV6-positive women and two HPV11-positive women were diagnosed with ASCUS, 6 and 3, respectively, developed LSIL during follow-up, and none developed HSIL.

Because of PCR or sequencing failure, we were unable to characterize the variants in 25 HPV6 and 2 HPV11-positive smears. B1 and B3 were the most commonly found HPV6 variants, with similar frequencies of detection (Table 1). A2 was the most common HPV11 variant. On age- and race-adjusted analyses, women harboring HPV6 B1 variants had a significantly higher risk for ASCUS/LSIL (OR = 6.3; 95%CI, 2.3–17.0) and ASCUS/LSIL/HSIL (OR = 5.4; 95%CI, 2.0–14.8) compared with HPV6-negative women, although the excess risks were not statistically distinguishable from those associated with HPV6 B3 variants (Table 2). Women with A2 HPV11 variants were at higher risk for ASCUS/LSIL (OR = 5.0; 95%CI, 1.7–14.6) and ASCUS/LSIL/HSIL (OR = 4.0; 95%CI, 1.0–8.1). However, when adjusting the analyses for coinfections with high-risk HPV types, the magnitude of the associations decreased substantially and lost statistical significance.

Discussion

We examined the frequency and clinical correlates of HPVs 6 and 11 variants infection in women throughout a follow-up period up to 10 years. Although on unadjusted analyses there seemed to be excess risks for HPV6 and HPV11 positivity and a slight between-variant heterogeneity in risk, these associations nearly disappeared when we controlled for the presence of HR HPV types, which are established causal agents in cervical carcinogenesis. In fact, we previously reported that within this population cytologic abnormalities were more commonly observed among women infected with oncogenic HPVs in comparison with

Table 2. Association between variants of HPV6 and HPV11 and cytologic abnormalities in the Ludwig–McGill Cohort Study

		ORs (95% CIs) according to cytologic outcome[a]			
		ASCUS+LSIL		ASCUS+LSIL+HSIL	
HPV type	Variants	Simple model[b]	Adjusted model[c]	Simple model[b]	Adjusted model[c]
HPV6	Negative	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
	HPV6 B1	6.3 (2.3–17.0)	2.1 (0.7–6.0)	5.4 (2.0–14.8)	1.7 (0.6–5.1)
	HPV6 B3	2.3 (0.6–9.7)	1.4 (0.4–5.5)	2.0 (0.5–8.2)	1.2 (0.3–4.7)
	HPV6 others[d]	12.4 (5.6–27.4)	12.2 (5.0–29.6)	10.5 (4.8–23.2)	10.8 (4.4–26.5)
HPV11	Negative	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
	HPV11 A2	5.0 (1.7–14.6)	3.2 (1.1–9.4)	4.0 (1.3–12.0)	2.8 (1.0–8.1)
	HPV11 others[e]	Undetermined	Undetermined	Undetermined	Undetermined

[a]Outcome refers to the highest cytologic grade attained during follow-up.
[b]Adjusted for age and race.
[c]Adjusted for age, race, and high-risk HPV coinfections.
[d]Includes the lineages/sublineages A, B2, and B5 in addition to samples that failed DNA amplification or sequencing.
[e]Includes the sublineages A1, in addition to samples that failed DNA amplification or sequencing. No A1 HPV11-positive women developed cervical cytologic abnormalities during follow-up.

those infected with nononcogenic types (1), and also among women harboring multiple infections (7).

The small number of women who developed cervical lesion in this cohort constitutes an important limitation of this study, in addition to the relatively high proportion of samples that were not evaluable for HPV6 and HPV11 variants, which made our study underpowered to detect small to moderate heterogeneity in risk between variants. The latter problem likely results from the lower sensibility of PCR sequencing for variant analysis in comparison with PCR hybridization employed for HPV identification and typing (6). Further analysis of possible clinical correlates to specific HPVs 6 and 11 variants is thus warranted.

Disclosure of Potential Conflicts of Interest

E.L. Franco is a consultant/advisory board member for Roche and has provided expert testimony for Elsevier. L.L Villa has received speakers bureau honoraria from Merck Sharp & Dohme. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The funders of the study had no involvement in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the article.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Sichero, S. Ferreira, E.L. Franco, L.L. Villa
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Writing, review, and/or revision of the manuscript: L. Sichero, M. El-Zein, S. Ferreira, E.L. Franco, L.L. Villa
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