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Low-risk human papillomavirus type 6 DNA load and integration in cervical samples from women with squamous intraepithelial lesions

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ABSTRACT

Background: The association between human papillomavirus (HPV) viral load and high-grade squamous intraepithelial lesion (HSIL) of the uterine cervix has been demonstrated for high-risk HPV-16 but has not been investigated for low-risk HPV types.
Objective: To determine the association between the presence of low-grade SIL (LSIL) and viral load of low-risk HPV type 6.
Study design: 107 HPV-6-positive cytobrush samples collected from 90 women (67 without SIL, 11 with LSIL, 5 with HSIL, 6 with SIL of unknown grade and 1 with a smear with LSIL and a normal colposcopy) were analyzed for their content of HPV-6 DNA.
Methods: HPV-6 E6 and E2 DNA loads were measured in the cytobrush samples with two real-time PCR assays. HPV-6 integration was confirmed with restriction-site PCR.
Results: HPV-6 DNA in cervical samples ranged from 1.8×10^2 to 4.25×10^8 copies per μg of cellular DNA. HPV-6 E6 DNA loads were higher in women with LSIL, with or without co-infection with high-risk HPV types, than in women without SIL ($p = 0.03$). HPV-6 loads greater than 8.76×10^6 copies per μg DNA, corresponding to the mean HPV-6 load measured in women with LSIL, were more frequently detected in these women with LSIL than in women without LSIL, controlling for age (odds ratio 13.5, 95% confidence interval 1.1–172.4). Although 10 samples generated HPV-6 E6/E2 ratios above 2, there was no evidence of integration of HPV-6 by restriction-site PCR.
Conclusion: Samples from women with LSIL contained higher HPV-6 loads than women without SIL. HPV-6 DNA was not integrated into cellular DNA in these samples.

Human papillomavirus (HPV) causes squamous intraepithelial lesions (SIL) of the uterine cervix as defined cytologically, or equivalently, cervical intraepithelial neoplasia (CIN) as defined by histology. High-risk HPV types cause low-grade CIN (CIN-1) and high-grade CIN (CIN-2,3), while low-risk types cause CIN-1 and condylomata acuminata. About 1.5 million women living in the United States are diagnosed each year with LSIL.[1] Up to 94% of genital warts and 8% of CIN-1 are caused by HPV-6.[2–4] Most studies of HPV viral load have focused on high-risk HPV types associated with CIN-2,3 and cancer.[5] Less is known on the HPV viral load in CIN induced by low-risk HPV types.

Investigation of the amount of HPV DNA present in various CIN grades associated with low-risk HPV types or during persistent HPV infection would indicate if HPV replication is associated with CIN. This information has never been reported for HPV-6, a prevalent low-risk genotype .[2–4] To address this issue, we investigated the association between HPV-6 DNA load and CIN grade in women participating in several epidemiological studies. We also assessed if HPV-6 DNA was integrated into the genome of cervical cells from these women.

1. Materials and methods

1.1. Population studied and clinical specimens

We studied 114 cervical samples collected from 97 women whose cervical specimens tested positive for HPV-6 DNA by consensus L1 PCR in previous studies. As shown in Fig. 1, 52 women

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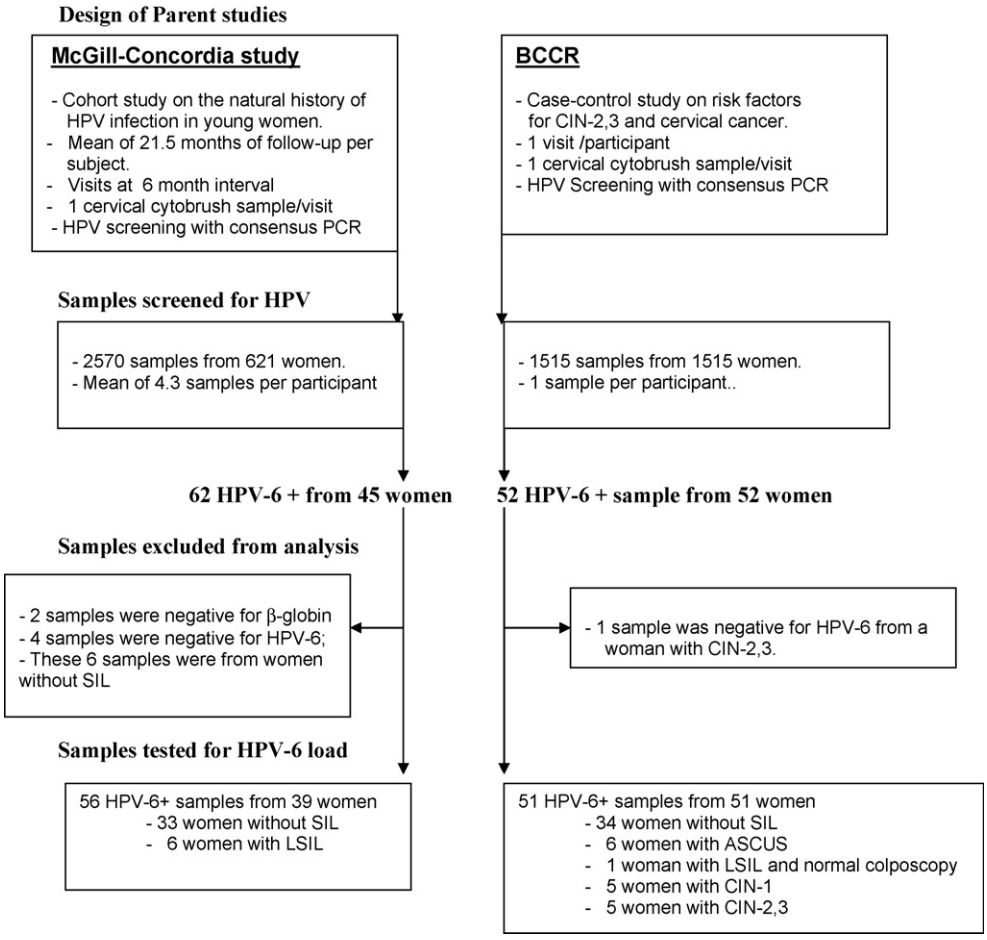

Fig. 1. Consort diagram of the two parent studies from which HPV-6-positive samples were obtained. BCCR is for Biomarkers of Cervical Cancer Risk" (BCCR) case–control study. CIN is for cervical intraepithelial neoplasia. SIL is for squamous intraepithelial lesion. ASCUS is for atypical squamous cell of unknown significance. The age of HPV-6-positive participants ranged from 16 to 72 years (mean: 23, median: 21).

provided 52 HPV-6-positive samples while participating in the "Biomarkers of Cervical Cancer Risk" (BCCR) case–control study,[6] and 45 women provided 62 HPV-6-positive samples while participating in the McGill-Concordia cohort study of HPV persistence.[7] Samples were collected at 6-month intervals for women recruited in the McGill-Concordia cohort. We could thus compare HPV-6 viral loads obtained in samples from women with transient and persistent (>1 HPV-6-positive sample obtained at different visits) HPV-6 infections. Six HPV-6-positive women from the BCCR had HSIL smears confirmed to be CIN-2,3 on biopsies. Eleven women had low-grade cervical disease, including 6 women from the cohort study with repeated LSIL smears and 5 women from BCCR with LSIL smears confirmed as CIN-1. A total of 73 women did not have SIL, including 34 women who had no previous abnormal Pap smears and a normal smear at recruitment, and 39 women from the cohort study with $\geq$2 normal smears obtained at 6-month intervals. Seven women had one smear with ASCUS or LSIL, but were not further investigated by colposcopy. Informed consent was obtained from all study participants, and Ethics committees from each participating institution approved procedures and consent forms. Cervical cells were collected with a cytobrush and processed with QIAamp columns (QIAGEN Inc., CA, USA)[7] or Master pure.[6] During the parent studies, HPV DNA was amplified using MY09-MY11-HMB01 or PGMY primers, and typed with type-specific probes in the Line blot assay (Roche Molecular systems, CA).[6,7]

1.2. HPV-6 and β-globin real-time PCR assays

HPV-6-positive samples were screened before quantitation of HPV-6 DNA for the presence of PCR inhibitors by amplification of an internal control for HPV-6 DNA.[8] Amplification of the internal control did not show any inhibition in these samples as measured by a signal corresponding to at least 80% of the expected signal.[8] HPV-6 DNA and β-globin DNA were quantitated with real-time PCR assays targeting E6 and E2, as described previously.[8] Two microliters of each processed sample was tested in duplicate in each HPV-6 E6 and E2 assays, and for β-globin DNA.[8] Cycle thresholds were compared to those of serial ten-fold dilutions of an HPV-6 plasmid in DNA extracted from 10^4 human fibroblasts. Titration curves of human DNA were obtained by serial dilutions of a stock of human genomic DNA (Roche Molecular Biochemicals). HPV-6 load was expressed as the number of HPV-6 E6 DNA copies per μg of human DNA.

1.3. Restricted-site PCR

Since HPV integration often results in the disruption of the E2 gene in high-risk genotypes, detection of a greater quantity of HPV-6 E6 compared to HPV-6 E2 suggests the presence of integrated HPV DNA. The presence of integrated HPV-6 DNA was suspected for specimens with ratios of HPV-6 E6 and E2 copies (HPV-6 E6/E2) at or above 2, as previously discussed for types 16 and 33.[8] HPV-6 integration was confirmed by restriction-site PCR (RS-PCR), a