MAJOR ARTICLE

Genomic Polymorphism of Human Papillomavirus Type 52 Predisposes toward Persistent Infection in Sexually Active Women

Joséphine Aho,[1,2] Catherine Hankins,[3,4] Cécile Tremblay,[2,5] Pierre Forest,[1] Karina Pourreaux,[4] Fabrice Rouah,[4] François Coutlée,[1,2,5] and The Canadian Women's HIV Study Group[a]

[1]Laboratoire de Virologie Moléculaire, Centre de Recherche du Centre Hospitalier de l'Université de Montréal, [2]Département de Microbiologie et Immunologie, Université de Montréal, [3]Joint Departments of Epidemiology, Biostatistics and Occupational Health, McGill University, [4]Direction de la Santé Publique de Montréal-Centre, Institut National de la Santé Publique du Québec, [5]Département de Microbiologie et Infectiologie, Centre Hospitalier de l'Université de Montréal, Montréal, Québec, Canada

We investigated the role of human papillomavirus (HPV) type 52 polymorphism in the persistence of HPV infection, which is a predictor for cervical lesions. Cervical samples obtained at 6-month intervals were tested for HPV-52 in 1055 women; 41, 12, and 58 women had persistent, transient, and unclassified HPV-52 infections, respectively. HPV-52 isolates were analyzed by polymerase chain-reaction sequencing of the long control region (LCR), E6, and E7 genes. Although age (odds ratio [OR], 0.90 [95% confidence interval {CI}, 0.81–0.99]), nonprototypic LCR (OR, 9.26 [95% CI, 2.1–41.7]), and E6 variant (OR, 7.04 [95% CI, 1.4–37]) were associated, in univariate analysis, with the persistence of HPV-52 infection, a nonprototypic LCR variant was the only independent predictor of it (OR, 14.1 [95% CI, 1.1–200]). In the latter variants, the loss of a binding site for a repressor of HPV expression was associated with the persistence of HPV infection (OR, 7.25 [95% CI, 1.67–31.25]).

Infection of the uterine cervix with high-risk types of human papillomavirus (HPV) causes the development of squamous intraepithelial lesions (SILs) and invasive cancer [1]. Although the incidence of HPV infection is high in young women, only a minority of infected women will develop persistent infection that may evolve into SIL and progress further to high-grade SIL (HSIL) [2]. Persistent HPV infection with an oncogenic type is considered to be a surrogate marker for cervical SIL and cancer [3]. Viral, environmental, and host factors are thought to play a mitigating role in cervical carcinogenesis and to explain the low rate of high-grade cervical disease, despite the high prevalence of HPV infection [4]. Molecular variants within an HPV type are defined by genomic polymorphisms, compared with the prototype isolate [5]. Whether some variants are more aggressive than others remains a matter of debate, although some studies have shown that HPV-16 nonprototypic variants confer a higher risk for development of high-grade cervical lesions or cervical cancer than does the prototype [6]. The natural history of infection and the role of genomic polymorphism in HPV types other than 16 have not been thoroughly described [7–9]. A better understanding of the association among viral polymorphism, persistent infection, and the risk of cervical cancer could explain, in part, why only a subset of women with HPV infection develop cervical cancer.

HPV-52 is classified as a high-risk genotype, having been identified in SIL and in up to 2% of cancerous lesions of the cervix. The role of HPV-52 polymorphisms in persistent infection and cervical SIL has not been described, although it is one of the most frequent

Received 9 April 2003; accepted 28 November 2003; electronically published 8 June 2004.

Financial support: Canadian Foundation for AIDS Research; Canadian Institutes of Health Research (The Canadian Women's HIV Study); Fonds de la Recherche en Santé du Québec and le Réseau FRSQ-SIDA (support to F.C.).

[a] Study group members are listed after the text.

Reprints or correspondence: François Coutlée, Dépt. de Microbiologie et Infectiologie, Hôpital Notre-Dame du Centre Hospitalier de l'Université de Montréal, 1560 Sherbrooke est, Montréal, Québec H2L 4M1, Canada (francois.coutlee@ssss.gouv.qc.ca).

The Journal of Infectious Diseases 2004;190:46–52

0022-1899/2004/19001-0006$15.00

genotypes in sexually active Canadian women [10]. In the present article, we present results, from an ongoing study of the natural course of HPV infection and cervical disease in a population of women in Canada who are either infected with or at risk for HIV, that demonstrate the association between the persistence of HPV infection and HPV-52 polymorphisms. We have also determined whether the repeated detection of HPV-52 DNA over time represents a persistence of initial infection or repeated transient infection with another variant(s) of HPV-52 [11].

PARTICIPANTS AND METHODS

Participants. Subjects infected with HPV-52 were selected from the Canadian Women's HIV Study. This cross-sectional and cohort study investigates the relationships between HPV/HIV infection and the development of SIL/cervical cancer. The study design and cohort have been described elsewhere, and the latter consists of 1055 women recruited, across Canada during 1993–2000, from outpatient sexually transmitted disease (STD) clinics, family practices, or infectious-disease clinics involved in the management of HIV and STD [10, 12, 13]. Women were eligible to participate if they had tested seropositive for HIV-1 or if they had tested seronegative for HIV but were at risk for STD and agreed to undergo annual serological testing for HIV antibodies [12, 13]. All participants provided written, informed consent. The ethics committee of each participating institution approved the Canadian Women's HIV Study protocol.

A standardized questionnaire was administered at enrollment in the study and at 6-month intervals thereafter [12]. For all HIV-seropositive women, CD4 cell counts, Pap smears obtained by use of a cytobrush and Ayre spatula, vaginal tampon specimens, and cervicovaginal lavages were obtained and processed as described elsewhere [10], at enrollment and at 6-month intervals. For HIV-seronegative women, vaginal tampons were obtained at 6-month intervals, and cervicovaginal lavages and Pap smears were collected at 1-year intervals. Cell suspensions from genital specimens were processed as described elsewhere [10].

Molecular HPV-52–variant analysis. An aliquot of 5 μL from each processed sample was amplified for β-globin DNA, by use of PC04-GH20 [10]. β-Globin–negative samples were extracted with phenol-chloroform and were precipitated with ethanol; β-globin–positive samples (lysate or extracted DNA) were tested for the detection and typing of HPV DNA, by use of MY09-MY11-HMB01 consensus L1 polymerase chain reaction (PCR) and type-specific probes [10, 14]. HPV-52 isolates from cervicovaginal lavages were further characterized by PCR sequencing of the 3′ end of the long control repeat (LCR) (primers 5′-TTGCACCCACATGAGTAACA-3′and 5′-AGTGCACACCTGGTGAGTAA-3′), the complete E6 gene (primers 5′-GAACACAGTGTAGCTAACGCACG-3′ and 5′-GCATGACGTTACACTTGGGTCA-3′), and the complete E7 gene (primers 5′-ATATTATGGGTCGTTGGACA-3′ and 5′-TTCAAACCAGCCTGTACATC-3′), according to protocols described elsewhere [15]. In brief, amplification reactions for each HPV region were performed with 5 μL of processed sample in a 100-μL reaction volume containing 10 mmol Tris-HCl/L (pH 8.3), 50 mmol KCl/L, 2.5 U of Expand High Fidelity PCR enzyme (Boehringer Mannheim), 0.5 μmol each primer/L, and 0.25 mmol each dCTP, dTTP, dGTP, and dATP/L. The $MgCl_2$ concentration was adjusted to 2.5, 2.0, and 1.0 mmol/L for the LCR primers, the E6 primers, and the E7 primers, respectively. Temperature cycling was different between the 3 targets, as described elsewhere [15]. When amplicons produced by these protocols generated faint bands on gel electrophoresis, 10 U of Ampli*Taq* Gold DNA polymerase (Roche Molecular Systems) was used instead of the Expand mixture [11, 15]. Depending on the number of bands visible on the gel, PCR-amplified HPV-52 DNA fragments were purified with the QIAquick gel extraction kit (Qiagen). Direct double-stranded PCR sequencing was performed by the fluorescent cycle-sequencing method (BigDye terminator ready-reaction kit; Perkin-Elmer), on an ABI Prism 3100 Genetic Analyzer system. Any variation from the prototype sequence was confirmed by a second PCR sequencing.

Statistical analysis. Women who did not have detectable HPV-52 DNA at enrollment but who tested positive at a subsequent visit were considered to have incident infection. Women were considered to have persistent HPV-52 infection if, over a $\geq$9-month period, they had consecutive genital specimens containing the same HPV-52 variant [2, 16]. Both definitions used for transient HPV-52 infection required that women be infected for <9 months and that they have HPV-52–negative specimens after having had HPV-52–positive specimens. The first definition of transient infections included only women with incident infections, thus excluding those who were infected at baseline with HPV-52 and who therefore could represent either true transient infection or the end of a persistent infection; in the second definition, all women observed to have HPV-52 infections for <9 months were considered to have transient infection.

Because the prevalence of each molecular variant of HPV-52 was often low, the association between the persistence of HPV infection and HPV-52 variance could not be evaluated individually for each variant. The isolates were thus classified as belonging to one or the other of 2 broad categories: prototype strains and nonprototype strains. Isolates with a DNA sequence different from that of the prototype strain were classified as nonprototype variants. Univariate analysis was first performed to identify factors significantly associated with the persistence of HPV infection, by use of either Fisher's exact test or Pearson's χ^2 test, for proportions, and by use of Student's t test and the Kruskal-Wallis rank test, for continuous variables. Double-sided $P < .05$ was considered to be significant. The magnitude of the association between persistent HPV-52 infections and risk factors was assessed by calculation of the odds

ratios (ORs) and 95% confidence intervals (CIs). Unconditional multiple logistic-regression analysis was performed to obtain maximum-likelihood estimates of the OR for HPV-52 polymorphisms while controlling for the confounding effects of age, HIV status, and CD4 cell count.

RESULTS

Of the 1055 participants (732 HIV seropositive and 323 HIV seronegative) evaluated, at their first 3 study visits, for HPV infection and cervical SIL, 366 HPV-infected women (207 HIV seropositive and 159 HIV seronegative) were monitored prospectively for HPV persistence and cervical SIL. Overall, 114 (10.8%) of the 1055 women had ⩾1 genital specimen containing HPV-52 DNA and were selected for the study. PCR sequencing of HPV-52 could not completed for 3 of these women, leaving 111 women for the study of HPV-52 variance. The prevalence of HPV infection in The Canadian Women's HIV Study reaches 67%, as has been reported elsewhere [12], and nearly 29% of participants are infected with >1 HPV type. In that cohort, 8.5% of HIV-seropositive women had abnormal cytological smear results; determinants of HPV infection in HIV-seropositive women included a low CD4 cell count, younger age, inconsistent condom use, and race [12].

For the 111 women studied for HPV-52 variance, the median follow-up time after the first HPV-52 positive specimen was obtained was 23.5 months (mean, 27.5 months [range, 0–89 months]), for a total follow-up of 244.4 women-years. Of the 787 genital samples provided by these 111 women, 15 (1.9%) were inadequate for PCR analysis, but this had no effect on the classification of infections as either transient or persistent (data not shown). The median age of the HPV-52–infected women was 32.0 years (mean, 31.9 years [range, 19–52 years]). The mean and median CD4 cell counts closest to those of the first HPV-52–positive sample were measured in 84 HIV-seropositive women and reached 322×10^6 and 303×10^6 cells/L, respectively (range, 12–933×10^6 cells/L). In 13 (14.9%) of the 87 HIV-infected women, AIDS was diagnosed. We identified 28 different HPV-52 molecular variants, including the prototype based on the genetic polymorphism of the LCR, as described elsewhere [15]. The prototype strain was encountered most frequently (43 [38.7%] of 111 isolates); 17 E6 variants were defined by 17 variation sites, and 8 E7 variants were defined by 18 variation sites, as detailed elsewhere [15]. All women were infected with only 1 HPV-52 variant per positive specimen. Because, for several variants, the number of women infected was small, each isolate was classified, for the purpose of our analyses, as being either a prototype strain or a nonprototype strain [15].

A total of 41 women were considered to have persistent infection; that is, all had consecutive specimens containing HPV-52 DNA over a period ⩾9 months. To refine the definition of persistence, PCR sequencing of the LCR was applied to HPV-52 isolates detected in 2 samples from each woman that were collected ⩾9 months apart. The same variants were identified in all pairs of specimens, demonstrating true persistence. In these 41 women with persistent HPV-52 infection, the median number of HPV-52–positive visits was 3 (3.8 ± 1.6 visits [range, 2–7 visits]), and the mean period of HPV-52 positivity was 26.5 months (median, 22.5 [range, 9–62 months]). A total of 12 women had transient HPV-52 infection according to the first definition for transient infections (see Participants and Methods). Thus, the nature of HPV-52 infection could not be determined in 58 (52.3%) of the 111 HPV-52–infected women: 24 of these 58 women were infected at enrollment and for <9 months subsequently, preventing discrimination between transient infection or the end of a persistent infection; 21 provided only 1 specimen; 12 were infected with HPV-52 at their last visit only; and 1 had HPV-52 infection detected only in vaginal tampon specimens (i.e., HPV-52 infection was not detected in cervicovaginal lavages).

In the first set of analyses, 41 women with persistent HPV-52 infection were compared with 12 women with incident transient HPV-52 infections, to assess risk factors for persistence. As shown in table 1, younger age (OR, 0.90 [95% CI, 0.81–0.99]) and infection with either a nonprototype LCR (OR, 0.11 [95% CI, 0.02–0.48]) or a nonprototype E6 (OR, 0.14 [95% CI, 0.03–0.73]) variant were significantly associated with the persistence of HPV-52 infection, according to the results of univariate analysis; however, when considered as a continuous variable, age was marginally associated with the persistence of HPV-52 infection. When participants were classified into age categories, age was no longer associated with the persistence of HPV, according to the results of univariate analysis (data not shown; $P = .37$). No other factors—including HIV status, level of immune deficiency, and race—were significantly associated with the persistence of HPV-52 infection. The lifetime number of sexual partners, whether considered as a continuous (table 1; $P = .54$) or as a categorical (data not shown; $P = .22$) variable, was not associated with the persistence of HPV-52 infection. Because our cohort was enrolled in 1993, only 4 HIV-seropositive women were receiving highly active antiretroviral therapy (HAART) while they were infected with HPV-52. Only 1 (25%) of these 4 women receiving HAART had persistent infection, compared with 34 (81%) of 42 women not receiving HAART (OR, 0.078 [95% CI, 0.007–0.857]; $P = .037$).

When the variables of age, HIV status, CD4 cell count, and E6 and LCR variants were entered into multivariate analysis, infection with a nonprototype LCR variant was the only independent predictor for the persistence of HPV-52 infections (table 2). The most frequent variation in nonprototypic isolates was at nucleotide position 7624 (where a T was substituted for a C or a G) found in 57 (84%) of 68 nonprototypic isolates [15], which resulted in the loss of a putative binding site for

Table 1. Univariate analysis of risk factors for persistence of human papillomavirus type 52 (HPV-52) infection, in 41 women with persistent HPV-52 infection and in 12 women with incident transient HPV-52 infection.

Characteristic	HPV-52 infection		P^a
	Transient (n = 12)	Persistent (n = 41)	
Age, mean ± SD, years	36.6 ± 9.0	32.0 ± 5.5	.04
HIV status			.57
Seronegative	1 (8)	6 (15)	
Seropositive	11 (92)	35 (85)	
CD4 cell count, mean ± SD, no. of cells/mL[b]	369 ± 306	331 ± 214	.99
Ethnicity			.36
White	7 (58)	18 (44)	
African	4 (33)	22 (54)	
Other	1 (9)	1 (2)	
Lifetime sex partners, mean ± SD	106 ± 314	10 ± 19	.57
Coinfection with other HPV types			.12
Present	3 (25)	3 (7)	
Absent	9 (75)	38 (93)	
LCR variant			.003
Prototype	9 (75)	10 (24)	
Nonprototype	3 258)	31 (76)	
E6 variant			.02
Prototype	10 (83)	17 (41)	
Nonprototype	2 (17)	24 (59)	

NOTE. Data are no. (%) of women, unless otherwise noted. LCR, long control region.

[a] Association evaluated by Fisher's exact test, except for intergroup comparison of age and of CD4 cell count, which were evaluated by Kruskal-Wallis test.

[b] Means were determined for 34 women with persistent HPV-52 infections and for 10 women with transient HPV-52 infections.

the C/EBP protein [17]. When this variation was considered individually, 29 (70.7%) of 41 women with persistent HPV-52 infections and 3 (25%) of 12 women with transient HPV infections had an isolate with this variation at the C/EBP binding site (OR, 7.25 [95% CI, 1.67–31.25]; $P = .008$). The presence of nonsynonymous E6 variations was not associated with the persistence of HPV-52 infections—20 (48.4%) of 41 women with persistent HPV infections had an isolate with at least 1 significant E6 mutation, compared with 3 (25%) of 12 women with transient HPV infections ($P = .19$). When the E7 polymorphism was considered, nonsynonymous E7 variations were found in 13 (31.7%) of 41 women with persistent HPV infections, compared with 2 (16.7%) of 12 with transient infections ($P = .47$).

Cytological testing by use of Pap smears was conducted concurrently with HPV detection, for 11 (91.7%) of 12 women with transient infections and for 33 (80.5%) of 41 women with persistent HPV infections ($P = .66$). Of the women so tested, 2 (18.2) of the 11 women with transient HPV infection had smear results suggestive of SIL (2 had low-grade SIL [LSIL]), compared with 17 (51.5%) of the 33 women with persistent HPV infections (15 had LSIL, and 2 had HSIL; $P = .08$); however, nearly all (15/17) HPV-52–positive samples from the latter women also contained another oncogenic HPV type. Because of multiple-type infections, we could not interpret further the association between the persistence of HPV-52 infection and cervical lesions.

We confirmed the association between persistent HPV-52 infection and nonprototypic LCR variants by using the second definition for transient infection, which includes women who had positive specimens at baseline but who were infected for <9 months; according to that definition, there were 29 women with transient HPV-52 infections. As shown in table 3, the only factor significantly associated with the persistence of HPV-52 infection was infection with a nonprototypic LCR variant (OR, 3.32 [95% CI, 1.20–9.17]): 29 (70.7%) of 41 women with persistent HPV-52 infections and 13 (45%) of 29 women with transient infections had an isolate with the variation at the C/EBP binding site (OR, 2.98 [95% CI, 1.10–8.07]; $P = .03$).

DISCUSSION

To our knowledge, our study is, to date, the first extensive investigation of HPV-52 persistence. Very few studies have examined the genetic polymorphism of high-risk types of HPV other than HPV-16 or HPV-18 [6, 18]. Cervical HPV-52 infection was frequently encountered in HIV-seropositive and

Table 2. Risk factors for persistence of human papillomavirus type 52 (HPV-52) infection, in 41 women with persistent HPV-52 infection and in 12 women with incident transient HPV-52 infection.

	HPV-52 infection		Unadjusted data		Adjusted data	
	Transient (n = 12)	Persistent (n = 41)	OR (95% CI)	P[a]	OR (95% CI)	P
Age, mean ± SD, years	36.6 ± 9.0	32.0 ± 5.5	0.90 (0.81–0.99)	.04	0.90 (0.79–1.03)	.11
HIV status						
Seronegative	1	6	1.00		1.00	
Seropositive, no. of CD4 cells/mL						
⩾200	8	23	0.48 (0.05–4.61)	.37	0.69 (0.05–10.00)	.53
<200	2	11	0.92 (0.07–12.32)	.77	1.57 (0.09–28.84)	.55
LCR variant				.003		.04
Prototype	9	10	1.00		1.00	
Nonprototype	3	31	9.26 (2.10–41.67)		14.09 (1.07–200)	
E6 variant				.02		.88
Prototype	10	17	1.00		1.00	
Nonprototype	2	24	7.04 (1.37–37.03)		1.22 (0.08–17.86)	

NOTE. CI, confidence interval; LCR, long control region; OR, odds ratio.

[a] Association evaluated by Fisher's exact, except for intergroup comparison of age, which was evaluated by Kruskal-Wallis test.

[b] Obtained by multivariate analysis including the variables age, HIV status, LCR variant, and E6 variant.

-seronegative women whom we studied. HPV-52 also had the highest incidence in another cohort of HIV-infected women [16], and a Brazilian cohort study of HIV-seronegative women detected HPV-52 frequently [19]. As we have reported, a majority of the isolates were prototypes; and, as we have shown elsewhere, for HPV-16, all women with persistent HPV-52 infection had the same variant in 2 specimens collected ⩾9 months apart, a finding indicating true persistence [11]. In our study, only women in whom the same variant of HPV-52 was detected in consecutive samples for ⩾9 months were classified as having persistent HPV-52 infection; this 9-months cutoff for persistence of infection, corresponded, in most cases, to 3 positive samples obtained at 6-month intervals. In a recent study of 1425 women living in Brazil, the mean duration of infection with high-risk types was 8.9 months [19].

Our data provide epidemiological evidence that HPV-52 variants differ in their capacity to induce persistent infection and, potentially, cervical SIL. Women with persistent HPV-52 infection were more frequently infected with nonprototypic LCR variants, according to the results of both univariate and multivariate analysis. In fact, after we controlled for age and HIV status, participants infected with nonprototypic HPV-52 variants were 14 times more likely to have persistent HPV-52 infection than were women infected with the prototype. Ethnicity has been associated with HPV variance and also with the risk of either cervical cancer or SIL [6]. In our study, however, race was not associated with the persistence of HPV-52; therefore, we did not control for it in multivariate analysis. Because we were analyzing the persistence of HPV-52 infection, we did not control for the number of visits and the length of follow-up—variables that would be pertinent for analyses of the development and progression of SIL [20]. Women with persistent HPV-52 infection were monitored for longer periods of time than were women with transient infections, because of the study design (data not shown). The association between the persistence of HPV-52 infection and age was opposite to that in previous reports on HPV persistence, in which older age usually predisposed to persistence; however, the association between the persistence of HPV-52 infection and younger age was weak in univariate analysis, with the 95% CI being near 1.00, and it was no stronger in multivariate analysis.

The expression of HPV oncoproteins is mediated by the p97 promoter. The LCR contains binding sites for the HPV regulatory protein E2 and for several cellular transcription factors that modulate the function of the LCR either positively or negatively. Mutations found in nearly all nonprototypic variants modified the interaction between the LCR and cellular proteins, by adding a new site of interaction with a cellular protein or by losing a site. One of the most frequent changes in the LCR was the loss of a binding site for the C/EBP protein. There was an association between persistent infection with HPV-52 and variants with a loss of C/EBP binding site. In HeLa cells, overexpression of C/EBP represses the HPV-18 LCR and, specifically, interferes with binding of the TATA-binding protein to the TATA box of the HPV-18 LCR [21]. No single E6 mutation or E7 mutation was associated with the persistence of HPV-52 infection, and the association between persistent HPV-52 infection and nonprototypic E6 variants was not statistically significant, according to the results of multivariate analysis.

Nonprototypic HPV-16 variants have been associated with the persistence of HPV-16 infection [22, 23] and with anogenital cancer [20, 24–26]. We found the same association between the persistence of HPV-52 infection and nonprototypic LCR variants. Compared with the prototype sequence, variants with

Table 3. Univariate analysis of risk factors for persistence of human papillomavirus type 52 (HPV-52) infection in 41 women with HPV-52–positive samples for ⩾9 months and 29 women with HPV-52–positive samples for <9 months.

Characteristic	HPV-52 infection		
	Transient (n = 29)	Persistent (n = 41)	P[a]
Age, mean ± SD, years	32.6 ± 7.9	32.0 ± 5.5	.78
HIV status			.92
Seronegative	4 (14)	6 (15)	
Seropositive, no. of CD4 cells/mL	25 (86)	35 (85)	
CD4 cell count, mean ± SD, cells/mL[b]	354 ± 243	331 ± 214	.70
Ethnicity			.16
White	10 (34)	18 (44)	
African	16 (55)	22 (54)	
Other	3 (11)	1 (2)	
Lifetime sex partners, mean ± SD	57 ± 200	10 ± 19	.54
Coinfection with other HPV types			.88
Present	8 (30)	3 (7)	
Absent	21 (70)	38 (93)	
LCR variant			.02
Prototype	15 (52)	10 (24)	
Nonprototype	14 (48)	31 (76)	

NOTE. Data are no. (%) of women, unless otherwise noted. LCR, long control region.

[a] Association evaluated by Fisher's exact test, except for intergroup comparison of age and of CD4 cell count, which were evaluated by Kruskal-Wallis test.

[b] Means were determined for 34 women with persistent HPV-52 infections and for 24 women with transient HPV-52 infections.

mutations either in the LCR or elsewhere in the genome may have a higher oncogenic potential [24]. The results of in vitro studies have demonstrated that HPV-16 variants have different biochemical and biological properties [6]. HPV protein variations may reduce the host immune system's ability to eradicate HPV, thus leading to persistence of HPV infection, or they could alter protein function, resulting in a greater or lesser potential to induce persistent HPV-52 infection and disease [6, 9]. A Brazilian study reported an association between the persistence of HPV infection and nonprototype variants, but, to obtain statistically significant results, it had to analyze types HPV-16 and HPV-18 together [23]. Some researchers have found that the HPV-16 E6 variant 350G (L83V) has been associated with the persistence of HPV-16 infection and cervical intraepethelial neoplasia [22]. We found the latter variation in 1 HPV-52 isolate, but it was not associated with the persistence of HPV-52 infection. None of the E6 mutations or E7 mutations that we studied were located at the presumed sites of activity of these oncoproteins [15].

Our study is limited by the small number of women with transient HPV-52 infections. Nevertheless, we did obtain significant differences between women with transient HPV-52 infections and women with persistent HPV-52 infections. The exclusion of women who had initial samples testing positive for HPV-52 but who rapidly lost their HPV-52 infections resulted in a reduced sample size when the first definition of transient HPV-52 infection was used. However, these women may have been misclassified—they could represent women with persistent HPV-52 infections who cleared it immediately after enrollment in the study. A subsequent analysis, using the second, stricter definition for transient HPV-52 infection—in which only women with incident HPV-52 infections were considered to have transient infection—confirmed the results of the initial analysis. Because of the high rate of multiple types of HPV infection in HIV-seropositive women, it was impossible to evaluate the effect that HPV-52 variance and the persistence of HPV-52 infection might have had on cervical SIL.

We have demonstrated that nonprototype HPV-52 variants are associated with the persistence of HPV-52 infection in a population of women at risk for HIV infection. Further studies, which recruit women at lower risk for STDs, should be conducted to assess whether this association can be found in other populations of women. Mutations in the LCR could be responsible for this association or could have cosegregated with significant mutations elsewhere on the HPV-52 genome. Further studies of mutations in the other genes of HPV-52 are necessary to determine whether this association is explained by a direct effect that LCR variations have on HPV-52 infection or by linkage disequilibrium with variations present in other regions of the genome. Functional studies of mutated isolates would permit the evaluation of the impact that these mutations have during HPV infection. Our findings demonstrate that,

during the course of the infection, genetic polymorphism in HPV types other than HPV-16 is important. This notion is of considerable significance, given that persistence of HPV is a surrogate marker for cervical disease [3].

THE CANADIAN WOMEN'S HIV STUDY GROUP

The Canadian Women's HIV Study Group comprises the following individuals (coinvestigators are denoted by an asterisk [*]):

Principal investigator—Catherine Hankins
Halifax—Janet Conners,* Rob Grimshaw, David Haase, Lynn Johnston,* Wally Schlech,* and Arlo Yuzicappi-Fayant*
Hamilton—Stephen Landis and Fiona Smaill
London—Tom Austin, Ole Hammerberg, and Ted Ralph
Montréal—François Coutlée,* Julian Falutz, Alex Ferenczy,* Marina Klein,* Louise Labrecque, Normand Lapointe, Richard Lalonde, John Macleod, Grégoire Noël,* Chantal Perron,* Jean-Pierre Routy, and Emil Toma
Ottawa—Claire Touchie and Garry Victor*
Québec—Louise Coté, Hélène Senay, and Sylvie Trottier*
Saskatoon—Kurt Williams*
Sherbrooke—Alain Piché*
Sudbury—Roger Sandre
Toronto—Louise Binder,* Donna Keystone, Anne Phillips, Anita Rachlis,* Irving Salit, Cheryl Wagner, and Sharon Walmsley*
Vancouver—Paula Braitstein,* David Burdge,* Marianne Harris, Deborah Money,* and Julio Montaner*

Acknowledgments

We thank Diane Gaudreault and Diane Bronsard for processing the genital samples.

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