Papanicolaou Test Screening and Prevalence of Genital Human Papillomavirus Among Women Who Have Sex With Women

Jeanne M. Marrazzo, MD, MPH, Laura A. Koutsky, PhD, Nancy B. Kiviat, MD, Jane M. Kuypers, PhD, and Kathleen Stine, NP

ABSTRACT

Objectives. The purpose of this study was to examine frequency of and attitudes toward Papanicolaou (Pap) test screening in women who have sex with women (WSW) and to determine prevalence of genital human papillomavirus (HPV).

Methods. Women were eligible if they reported having engaged in sex with another woman in the preceding year. Medical and sexual histories were obtained. Cervical specimens for Pap tests and cervical and vaginal specimens for HPV DNA testing were collected.

Results. HPV DNA was detected in 31 of 248 WSW (13%). Women who had never had sex with men were less likely to have undergone pelvic examinations and had fewer recent Pap tests. Reasons for not undergoing Pap tests included lack of insurance, previous adverse experiences, and belief that Pap tests were unnecessary.

Conclusions. Despite the occurrence of genital HPV, WSW do not receive adequate Pap test screening. Pap test screening recommendations should not differ for WSW, regardless of sexual history with men. (*Am J Public Health.* 2001;91:947–952)

Specific genital types of human papillomavirus (HPV), most commonly types 16 and 18, are a cause of cervical cancer, a disease that is largely preventable with periodic Papanicolaou (Pap) test screening.[1] Little is known, however, about the epidemiology of HPV or Pap test screening among women who have sex with women (WSW). There are as yet no data on cervical cancer incidence, stage distribution, or mortality among WSW, largely because, until recently, major studies on this topic had not collected information on sexual orientation. Cervical neoplasia, including high-grade squamous intraepithelial lesions, occurs in WSW who report no history of sex with men; HPV DNA is detectable by polymerase chain reaction methods in this group.[2–4]

Some data suggest, however, that routine Pap test screening and other preventive health behaviors among WSW are performed less frequently than national guidelines advise.[4–11] While assumptions about sexual practices between women have contributed to the general opinion that sex between women confers low risk of bacterial transmission of sexually transmitted diseases (including gonorrhea and chlamydia),[12] transmission of HPV requires only skin-to-skin contact. Furthermore, sexual practices among WSW could potentially allow for intravaginal deposition of HPV through digital–vaginal contact and shared "sex toys"[4,13–16]; genital HPV types have also been identified on human fingers.[17]

Perhaps of equal importance, studies have shown that most WSW (53%–99%) have had sex with men and that many (21%–30%) continue to have sex with men.[18,19] Among these women, acquisition of chronic viral sexually transmitted diseases, including HPV, genital herpes, and HIV, from male partners presumably occurs at a rate per contact similar to that in heterosexual populations, and women infected via this route could serve as a source for subsequent viral transmission to their female partners.

Estimates of lifetime same-sex sexual behavior among women range from 8% to 20%, and 1.4% and 4.3% of all women may be sexually active with other women.[20–23] An estimated 2.3 million women in the United States describe themselves as lesbian.[19] In 1999, the Institute of Medicine published a report titled *Lesbian Health: Current Assessment and Directions for the Future*.[24] This document emphasized that more data on Pap test screening and risk of cervical cancer in WSW are needed. We examined the frequency of and attitudes toward routine Pap test screening in a self-referred sample of WSW and sought to determine type-specific prevalence of genital HPV as detected in polymerase chain reaction assays.

Methods

Beginning in February 1998, women in Seattle, Wash, were recruited through advertisements posted in community gathering places (restaurants, bookstores, clubs, and bars), newspaper and magazine articles, and referral from community clinicians. Because self-identification as "lesbian" may not predict actual participation in same-sex sexual behavior or the frequency of such behavior,[12] we oriented recruitment materials toward WSW rather than "lesbians." Neither current symptomatology in regard to sexually transmitted diseases nor previous history of such diseases

The authors are with the University of Washington, Seattle. Jeanne M. Marrazzo and Kathleen Stine are with the Department of Medicine. Laura A. Koutsky is with the Department of Epidemiology. Nancy B. Kiviat and Jane M. Kuypers are with the Department of Pathology.

Requests for reprints should be sent to Jeanne M. Marrazzo, MD, MPH, Harborview Medical Center, Division of Infectious Diseases, 325 9th Ave, Mailbox #359931, Seattle, WA 98104 (e-mail: jmm2@u.washington.edu).

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was mentioned in recruitment materials. Women who reported having had sex with another woman in the past year were eligible; those who had participated in our earlier pilot study[4] were not. Once enrolled, women were invited to refer their female partners for possible enrollment.

At study entry, women completed a standardized questionnaire that involved detailed questions about their medical and sexual history. Questions regarding beliefs about Pap test screening were developed by modifying a previously validated approach to studying beliefs about breast cancer screening.[25] Pelvic examination was performed. Cervical cells were collected via a Papette broom (Wallach Surgical, Orange, Conn) placed directly into PreservCyt (Cytyc Corp, Boxborough, Mass), for liquid-based cytology (ThinPrep 2000, Cytyc Corp). A combined cervical–vaginal specimen was collected for HPV DNA detection and typing, and colposcopic examination of the cervix was performed.

All subjects were interviewed and examined in the same clinic by a single investigator (Kathleen Stine). All Pap tests were read by the same cytopathologist (Nancy B. Kiviat) using the Bethesda classification system.[26] Histologic specimens were obtained via biopsy if Pap tests showed high-grade squamous intraepithelial lesions.

Polymerase chain reaction amplification and dot blot hybridizations for HPV DNA detection and typing were performed in a single laboratory, as described previously.[27] Briefly, swab samples collected in 1 mL Stuart's transport medium (Digene Diagnostics, Inc, Silver Spring, Md) were digested with proteinase K, and the DNA was ethanol precipitated and suspended in Tris/ethylene diamine tetraacetic acid buffer.

Polymerase chain reaction amplification of HPV DNA was performed with HPV L1 consensus primers MY09, MY11, and HMB01. HPV amplicons were identified via dot blot hybridization performed, as described previously,[27] with a biotin-labeled HPV generic probe and oligonucleotide probes specific for 18 HPV types in 7 hybridization mixes. The probe mixes included low-risk HPV types 6 and 11 and 40, 42, 53, and 54 and high-risk types 16; 18; 31, 33, 35, and 39; 45 and 56; and 51, 52, 55, and 58. Samples positive according to the generic probe and negative according to all 18 type-specific probes were designated "unclassified HPV."

SPSS software (Chicago, Ill) was used in conducting statistical analyses. The Pearson χ^2 test or, if the expected cell frequencies were less than 5, the Fisher exact test was used in making direct comparisons of proportions. Continuous variables were compared between groups with the Student *t* test or the Mann-Whitney test for nonparametric data. Logistic regression techniques were used in conducting multivariate analyses. Tests of significance were 2-tailed ($P < .05$).

The study's procedures were approved by the Human Subjects Research Review Committee of the University of Washington.

TABLE 1—Characteristics of Study Subjects (n = 248): Seattle, Wash, 1998–2000

Characteristic	Sample, No. (%)
Age,[a] y	
<25	69 (27.8)
25–30	65 (26.2)
31–39	65 (26.2)
>39	49 (19.8)
Race/ethnicity	
White	219 (88)
Black	4 (1.6)
Asian/Pacific Islander	2 (0.8)
Native American	2 (0.8)
Other	7 (8.5)
Hispanic[b]	17 (6.9)
Current health insurance	178 (71.8)
Monthly household income, $	
<1800	116 (46)
≥1800	105 (42)
Not reported	27 (10.8)
Education	
High school	11 (4.4)
Some college	65 (26)
College degree or more	172 (69)
Previous pregnancy	66 (27)
Hormonal contraceptive use	
Current	10 (4.0)
Ever (includes current)	132 (53.2)
Never	116 (46.8)
Sex with male, ever	199 (80)
Sex with male, previous year	57 (23)
Lifetime male partners, median	6
Lifetime female partners, median	6
Recruitment source[c]	
Posted advertisements or article	23 (42)
Clinic referral	2 (3.6)
Friend or partner	30 (55)

[a]Mean = 31 years (SD = 9, range = 17–56).
[b]14 of the 17 women who reported Hispanic ethnicity reported no race category in addition to ethnicity.
[c]80 women were specifically queried after questionnaire modification to include this information.

Results

Study Population

The study recruited 248 women, most of whom responded to posted advertisements in community venues or were referred by a friend or partner (Table 1). The women were predominantly Caucasian, relatively highly educated, and younger than 40 years (mean: 31 years). None had genitourinary complaints at study entry. Most subjects (80%) reported having had sex with a male partner during their lifetime; 23% reported having had sex with a male partner in the past year. The majority (58%) reported having had only 1 female partner during the previous 6 months, and 33% reported 2 or more. Median lifetime number of male partners was 6, as was the median lifetime number of female partners.

Twenty-seven percent of subjects had been pregnant at least once, and 53% had used oral contraceptives. Almost all reported oral–vaginal and digital–vaginal sex with female partners during the past year, and many reported oral–anal and digital–anal sex (34% and 63%, respectively). In the case of 51 couples (102 women, representing 41% of the subjects), both members were enrolled in the study.

Detection of HPV DNA and Pap Test Abnormalities

HPV DNA was detected by polymerase chain reaction in genital tract specimens of 31 subjects (13%; Table 2). Among subjects with detectable HPV DNA, 23 (74%) had oncogenic types (16, 18, 31/33/35/39, 45,56, 51/52/55/58), 9 (29%) had unclassified types, and

TABLE 2—Human Papillomavirus (HPV) and Papanicolaou (Pap) Test Findings, by Sexual History With Men: Seattle, Wash, 1998–2000

	Sex With Women Only, Lifetime (n=49), No. (%)	Sex With Men >1 Year Previously (n=142), No. (%)	Sex With Men and Women, Past Year (n=57), No. (%)	All Subjects With HPV Testing (n=248), No. (%)
Any HPV DNA detected by PCR[a]	3 (6.1)**	14 (10.0)**	14 (24.5)	31 (12.5)
Oncogenic types				
16	0 (0)	5 (3.5)	2 (3.5)	7 (2.8)
18	0 (0)	1 (0.7)	1 (1.7)	2 (0.8)
31/33/35/39	0 (0)	1 (0.7)	2 (3.5)	3 (1.2)
45/56	0 (0)	1 (0.7)	0 (0)	1 (0.4)
51/52/55/58	1 (2.0)*	3 (2.1)	6 (10.5)	10 (4.0)
Nononcogenic types				
6/11	0 (0)	2 (1.4)	2 (3.5)	4 (1.6)
40/42/53/54	0 (0)	3 (2.1)	5 (8.8)	8 (3.2)
Unclassified types	2 (4.1)	4 (2.8)	3 (5.3)	9 (3.6)
Pap test result at study visit				
HSIL	0	2 (2.2)	2 (3.5)	4 (1.6)
LSIL	1 (2.0)	4 (2.8)	2 (3.5)	7 (4.7)
ASCUS	5 (10.2)	5 (3.5)	4 (7.0)	14 (5.6)
Genital warts on exam	0	1 (0.7)	0	1 (0.1)

Note. PCR = polymerase chain reaction; HSIL = high-grade squamous intraepithelial lesion: LSIL = low-grade squamous intraepithelial lesion; ASCUS = atypical squamous cells of uncertain significance.
[a]Refers to HPV positivity by PCR assay of one or more sites (cervix or vagina).
*$P \leq .05$; **$P \leq .01$ (for comparison with women reporting sex with men and women in past year).

12 (39%) had nononcogenic types (6/11, 40/42/53/54). Among the 28 women with HPV DNA who reported having had sex with men, 14 (50%) had not had sex with a male partner in more than a year (range: 1–11 years; median: 2 years). Women who reported having had sex with men in the past year were significantly more likely to have HPV DNA detected. Likelihood of detecting HPV DNA was not affected by age (data not shown); however, only unclassified HPV types were detected in the 4 women 40 years or older (10%) who had HPV detectable by polymerase chain reaction. HPV DNA was detected in 9 women who were part of the 51 couples seen in the study; all 9 infections were detected in only 1 member of the couple.

Twenty-five women (10% of all subjects) had abnormal Pap tests (Table 2). Four women had high-grade squamous intraepithelial lesions, and 7 had low-grade lesions; 7 of these lesions occurred in women who reported either never having had sex with men or having had sex with men more than 1 year before they were tested. HPV DNA was detected in 7 of the 11 women with squamous intraepithelial lesions (HPV 16 in 3 women and other oncogenic types in 4 women). Of the 4 women whose Pap tests showed high-grade squamous intraepithelial lesions, 3 had this finding confirmed through cervical biopsy by the study clinician; 1 other subject with high grade squamous intraepithelial lesions chose to seek further care with her primary provider.

Because most subjects reported only 1 female sex partner during the past 6 months, we analyzed presence of HPV DNA by duration of subjects' primary partnership with a female partner. This duration was not significantly different for women who had HPV DNA detected and those who did not (means of 16.2 and 25.8 months, respectively; $P=.5$), nor was the frequency of abnormal Pap tests affected. This association did not change when we controlled for number of male partners in the past 6 months, age, race, recruitment method, educational status, or use of sex toys.

Attitudes Toward and Practices Regarding Pap Test Screening

Women's Pap test screening practices differed significantly according to whether they reported ever having had sex with men (Table 3). Relative to subjects who reported sex with both men and women in the past year, subjects who had never had sex with men were significantly less likely to have undergone a pelvic examination. They also had their first Pap test at an older age, had fewer Pap tests in the previous 5 years, and reported a longer interval between their 2 most recent Pap tests. Women who had been sexually active with men at some point, but not in the past year, also reported having their first Pap test at a later age than women currently sexually active with men.

TABLE 3—Papanicolaou (Pap) Test Screening Histories Among Subjects: Seattle, Wash, 1998–2000

	Sex With Women Only, Lifetime (n=49)	Sex With Men >1 Year Previously (n=142)	Sex With Men and Women, Past Year (n=57)	All Subjects (n=248)
No previous pelvic examination, no. (%)	5 (10)*	3 (2.1)	2 (3.5)	10 (4.0)
Mean no. of Pap tests in previous 5 y	2.3**	3.5	3.5	3.3
Mean no. of years to most recent Pap test	2.2**	1.4	1.3	1.5
Mean age, y, at first Pap test	22.5**	19.1*	17.4	19.3
Mean no. of previous abnormal Pap tests	0.2	0.7	0.5	0.6

*$P = .03$; **$P < .001$ (for comparison with women reporting history of sex with men).

TABLE 4—Attitudes Toward Papanicolaou (Pap) Test Screening Among Subjects: Seattle, Wash, 1998–2000

	Sample, No. (%)
How often do you think you should have a Pap test?	
Once a year	200 (80)
Once every 2–3 y after normal one	36 (14.4)
Once every 5 y after normal one	1 (0.4)
Not necessary at all to have one	3 (1.2)
Don't know	10 (4)
If you have not had a Pap test in over 2 years, why not?[a]	
No medical insurance	37 (42)
Believe not necessary if not sexually active with men	20 (22)
Told it wasn't necessary if not sexually active with men	9 (10)
Told by physician it wasn't necessary if not sexually active with men	8 (9)
Don't know where to get one	10 (11)
Previous adverse experiences with screening	23 (26)
Other	23 (26)

[a]89 women (36%) provided at least 1 reason for not having had a Pap test in more than 2 years; 30 of these women (34%) provided more than 1 reason. Percentages refer to the number of women responding to each question.

Reported number of previous abnormal Pap tests did not differ according to women's sexual history with men. Women who had squamous intraepithelial lesions detected through Pap tests as part of the study did not differ significantly in their attitudes toward routine Pap test screening from women with normal Pap tests.

Although an overwhelming majority of subjects (95%) thought that they should receive Pap tests either annually or every 2 years after a normal Pap test, 88 women (36%) provided at least one reason for not having received a Pap test in the previous 2 years (Table 4). Most commonly cited reasons were lack of medical insurance, earlier adverse experiences with Pap test screening, belief that they did not need a Pap test because they were not sexually active with men, and not knowing where to obtain one. Nine women (10%) had been told by a health care provider that they did not need a Pap test because they were not sexually active with men; these providers were identified as physicians in all but 1 case. More than a third of the women provided more than 1 of these reasons for not having undergone a Pap test in the previous 2 years.

Discussion

Using type-specific DNA probes to detect common genital HPV types and a universal probe to detect infection by other related types, we found that 13% of our subjects had genital infection with HPV. We also found that HPV-associated squamous intraepithelial lesions occurred in WSW who had never had sex with men and that women with squamous intraepithelial lesions did not differ from those without such lesions in their attitudes toward Pap test screening.

While the presence of HPV DNA as shown by polymerase chain reaction was strongly associated with a history of sex with men, HPV DNA was also detected among women who reported no history of sex with men or last sex with men up to 11 years earlier. These data provide further support that HPV is sexually transmitted between women. In a pilot study published in 1998,[4] we found a higher prevalence of HPV (30%) than that detected in the present study. The explanation may be in part that we did not perform vulvar sampling in the present study. HPV DNA was detectable only at the vulva in 9 (21%) of the 43 women with any HPV detected in the pilot study. We did not collect vulvar samples in the present study because we wanted to focus specifically on oncogenic HPV types at the cervix or in the vagina.

Furthermore, subjects in the pilot study had a significantly higher number of partners (male and female) in the 6 months before study entry, which could have contributed to an increased likelihood of HPV detection. Omission of vulvar sample collection may also in part explain the lack of HPV DNA detection in partners of women who had HPV DNA detected as part of this study.

Despite relatively high levels of education and income, subjects who had never had sex with men were less likely to have ever received a pelvic examination, received their first Pap test at a later age, and had less frequent Pap tests than subjects who also reported a history of sex with men. Among subjects who had not undergone a Pap test in the preceding 2 years, multiple reasons were often cited, including lack of medical insurance, earlier adverse experiences with Pap test screenings, belief that they did not need a Pap test because they were not sexually active with men, and not knowing where to obtain a Pap test.

While this study confirms that HPV prevalence among WSW is probably modified by recent number of male sex partners, it also constitutes the fourth report of squamous intraepithelial lesions with documentation of associated HPV infection in WSW who report no history of sex with men. These findings corroborate the results of earlier studies involving fewer subjects[6,28,29] and support the recommendation that WSW should undergo routine Pap test screening according to standard guidelines.[30–33]

Our data suggest that self-reported frequencies of routine Pap test screening among WSW are lower than population-based estimates for predominantly heterosexual women and that screening practices among WSW are strongly influenced by previous or current sex with men. In our study, 10% of subjects who had never had sex with men reported never having had a Pap test, and 23% had not had a Pap test in more than 3 years. The 1998 Behavioral Risk Factor Surveillance Survey showed that 5.6% of women 18 years or older with an intact cervix reported never having had a Pap test (state-specific range: 2.7% to 19.7%) and that 15.1% had not had a Pap test in 3 years (range: 6.1% to 32.4%).[34]

Clinic-based studies and surveys have produced data suggesting that WSW undergo routine Pap test screening less frequently than heterosexual women of similar ages. In one study, mean intervals between routine Pap tests were longer among WSW than among age-matched heterosexual women attending the same clinic (21 months vs 8 months).[6] Data from our pilot study of 149 WSW suggested that routine Pap test screening was affected by a history of sex with men: 57% of subjects who had never had sex with a male partner (14% of all subjects) reported having had 2 or fewer routine Pap tests in the preceding 5 years, as compared with 21% of women who had ever had sex with a male partner ($P=.01$).[4]

The findings just described are similar to those noted in a recent study, conducted in 2 lesbian health clinics in London,[5] in which 23% of 606 women queried believed that they had less need for Pap test screening than heterosexual women, a belief that was significantly more prevalent among those who had never had sex with men (41% vs 19%; $P<.001$). In that study, beliefs about need for cervical cytology were highly correlated with screening behavior; furthermore, 17% of all subjects and 42% of subjects who had never been sexually active with men had never had a Pap test.[5]

Findings from surveys are similar. Twenty-three percent of 1925 respondents in the Na-

tional Lesbian Health Care Survey reported an interval of more than 2 years since their most recent Pap test; 5% of all women and 23% of women aged 17 to 24 years had never had a Pap test.[35] In a recent study in which 6935 WSW were surveyed, receipt of Pap tests among younger respondents was well below the goals set in *Healthy People 2000*.[36,37] However, Pap test screening history is probably strongly influenced by educational level and correlates with other indices of preventive health care.[36,38]

For example, investigators in the Boston Lesbian Health Project used snowball sampling to query a national sample of 1633 WSW, most of whom were White, highly educated, and relatively young. Thirty-eight percent of the group had had annual Pap tests in the previous 2 years. The authors concluded that overall screening rates were similar to the general estimates obtained by others.[39] However, 39% of respondents younger than 20 years and 16% of those aged 20 to 29 years had never had a Pap test. Furthermore, 28% of women aged 20 to 29 years and 29% of those 30 to 39 years had not had a Pap test in 3 or more years.

Factors that may explain reduced use of general health care by WSW include alienating behavior among health care providers,[8,19,40–42] inability to pay for care (owing to lack of health care coverage, overall lower earnings in households without an adult man, or both),[8,43] and perceptions of low levels of risk for sexually transmitted diseases and cervical cancer on the part of both women and providers.[10,44] Among respondents to the National Lesbian Health Care Survey, 16% stated that they did not receive health care because they could not afford it, 27% had health providers who assumed heterosexuality, and 16% reported that they would not feel comfortable disclosing same-sex sexual behavior to providers.[8]

Other studies have noted that a majority of lesbians (53%–72%) do not disclose their sexual behavior to physicians when they seek medical care.[43,45] Few data are available on economic barriers to routine health care among WSW, but in a survey of 1681 lesbians attending a music festival in Michigan, the average annual income was $10 000 lower than the Michigan average for women.[7] One study reported a direct relationship between the quality of subjects' experiences with health care providers and the likelihood of a recent Pap test being performed.[40] Among WSW, self-perception of low risk for sexually transmitted diseases and cervical cancer may also contribute to reduced frequency of Pap test screening.[8–10,15,44]

In one survey of 1086 WSW, perceptions of risk for HIV acquisition were discordant with the HIV-related risk behaviors reported: only 43% of women with a clear HIV risk factor perceived themselves to be at risk for HIV acquisition.[44] Finally, WSW who do not have sex with men are not likely to access venues providing reproductive health care for the sole purpose of obtaining birth control; this effectively eliminates another "routine" opportunity for Pap test screening.

Our study has important limitations. Subjects were self-referred and therefore may not be representative of all WSW or self-identified lesbians. Most were Caucasian, and although median income was relatively high, 28% were uninsured. Also, in the case of several associations, such as duration of partnership with female partners among women with and without HPV, our sample size may have been too small for subgroup analyses to detect statistical significance. Most subjects reported only 1 female sex partner in the preceding 6 months. The median age (31 years) of subjects was also relatively high for peak exposure to many sexually transmitted diseases, including HPV; a study focusing on younger women might yield different results.

Although guidelines for routine Pap test screening vary,[31–33] a consensus recommendation adopted by the American Cancer Society, the National Cancer Institute, the American College of Obstetricians and Gynecologists, the American Medical Association, the American Academy of Family Physicians, and other organizations is that women who are or have been sexually active or have reached the age of 18 years undergo routine annual Pap tests until 3 normal tests have been documented. At that point, the duration between tests may be increased to 2 years "at the discretion of the physician."[31]

This recommendation permits Pap testing less frequently after results of 3 or more annual tests have been normal. Data published since our study was undertaken suggest that undergoing routine Pap tests every 3 years may be adequate.[46,47] Obviously, an accurate assessment of a woman's Pap test screening history would entail access to documentation of normal and abnormal test results, an effort that was beyond the scope of this study. Finally, definitive conclusions about Pap test screening practices are limited by the absence of a control group of heterosexual women who could be compared with the WSW enrolled in our study.

Because data indicate that HPV is sexually transmitted between female partners and may not require previous or recent sex with men, erroneous assumptions about HPV acquisition from female partners may place WSW at increased risk for delayed detection of cervical cancer through less frequent or no Pap test screening. The prevalence of genital HPV infection, squamous intraepithelial lesions, and suboptimal Pap test screening observed in our study among WSW reporting no previous sex with men support the need for investigation in a larger number of women. Such information will help clarify messages provided to WSW about their risk of cervical cancer and their need for Pap test screening and protective sexual practices. It should also direct efforts to educate providers about appropriate screening guidelines among WSW. In the meantime, all WSW should undergo Pap test screening in accordance with standard guidelines. □

Contributors

J. M. Marrazzo designed and obtained funding for the study, designed instruments, oversaw all clinical activities, and analyzed all data. L. A. Koutsky provided ongoing input into study and questionnaire design, specimen collection, and data analysis and presentation and assisted in writing the manuscript. N. B. Kiviat read all of the Pap tests, oversaw the work of the laboratory personnel who performed the HPV polymerase chain reaction testing, and assisted in writing the manuscript. J. M. Kuypers performed all of the type-specific HPV polymerase chain reaction testing, provided advice and assistance regarding specimen collection and processing, and assisted in writing the manuscript. K. Stine conducted the interviews and performed clinical evaluations. She also worked to recruit subjects and assisted in study design, the development of the study questionnaire, and the writing of the manuscript.

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