Sexual Behavior and Partner Characteristics Are the Predominant Risk Factors for Genital Human Papillomavirus Infection in Young Women

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Risk factors for cervicovaginal human papillomavirus (HPV) infection were investigated in 604 college women. HPV was detected in 168 (27.8%) of the subjects by L1 consensus primer polymerase chain reaction, Southern blot hybridization, or both. Significant independent risk factors for HPV ($P < .05$) included age (odds ratios [ORs]: 2.6 for 21–23 years old and 1.6 for >23, vs. ≤20), ethnicity (ORs: 3.2 for black, 2.2 for Hispanic, vs. white/other), number of lifetime male vaginal sex partners (ORs: 4.5 for 2, 5.8 for 3 or 4, 10.3 for ≥5, vs. 1), living with smokers (OR: 1.9), male partner's number of lifetime sex partners (ORs: 2.1 for 2 or 3, 3.1 for 4–10, 2.7 for ≥11, vs. 1), duration of sexual relationship for >12 months (OR: 0.6), and male partner currently in college (OR: 0.6). These data demonstrate that the predominant risk factors for genital HPV infection in young women are related not only to their own sexual behaviors but also to those of their male partners.

Cervical cancer is the second most common cancer in women worldwide and accounted for ~5000 deaths in the United States in 1994 [1]. Molecular epidemiologic studies have identified human papillomavirus (HPV) as the major cause of cervical cancer and cervical dysplasia [2–8]. Infection of the female genital tract with mucosal HPV is now recognized as one of the most, if not the most, common sexually transmitted diseases (STDs) [9–14]. However, studies that have examined the role of sexual behavior as a risk factor for HPV infection have yielded inconsistent results. Potential explanations accounting for this discrepancy include limitations in population size, differences in sampling strategies, and varying sensitivity, specificity, and accuracy of HPV detection methods [15–17]. In addition, little is known about how the behavior of the male partner is related to the risk of female genital HPV infection.

This study sought to test the hypothesis that sexual behavior and partner characteristics are the major risk factors for HPV infection in young women. A cohort of predominantly young college women was recruited through advertisement in order to assemble a group with heterogeneous sexual behavior. This investigation used a validated sampling method to collect exfoliated cervicovaginal cells [18, 19], and HPV was detected using both amplified (polymerase chain reaction [PCR]) and direct (Southern blot hybridization) detection methods.

Subjects and Methods

Study population. Between September 1992 and March 1994, women students from a state university were invited to participate in a longitudinal study designed to investigate the natural history of cervicovaginal HPV infection. Women were eligible if they fulfilled the following criteria: first or second year in college and/or planning to stay in the area for at least 2.5 years; not currently pregnant and without plans to become pregnant in the next 3 years; and never had a cervical biopsy or invasive treatment for cervical intraepithelial lesions.

A total of 1090 students responded to advertisements published in local and campuswide newspapers and flyers and were screened for eligibility on the telephone. Of these, 150 (14%) did not fit the eligibility criteria listed above, 332 (30%) were eligible but refused to participate, and 608 (56%) participated. The ethnic distribution of the participants was representative of the ethnic distribution of the total female undergraduate population (i.e., 70% white, 9% Asian, 11% black, 8% Hispanic, and 2% other). Characteristics of the eligible nonparticipants ($n = 308$) and participants ($n = 598$) who completed the telephone screening were compared. Compared with nonparticipants, participants were slightly older (18% of the participants were ≥21 years old compared to 9% of the refusals) and had more lifetime male sex partners (43% of participants and 28% of refusals had ≥3 lifetime partners). Hence, this study sample could overestimate the HPV prevalence in the general female college population.

Data collection. Six hundred eight women were recruited. At the baseline visit, each subject completed a self-administered ques-

Received 14 March 1996; revised 10 June 1996.

Presented in part: 13th International Papillomavirus Workshop, October 1994, Amsterdam (abstract 169).

Informed consent was obtained from all subjects participating in this study and Institutional Review Board approval was obtained, in compliance with human experimentation guidelines of the US Department of Health and Human Services.

Grant support: NIH (AI-31055; DNA oligonucleotides synthesized in Oligo Synthesis facility of Cancer Research Center at Albert Einstein College of Medicine [CA-13330]). R.D.B. and G.Y.F.H. are recipients of a Faculty Research Award and a Junior Faculty Research Award, respectively, from the American Cancer Society.

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The Journal of Infectious Diseases 1996;174:679–89

0022–1899/96/7404–0001$01.00

tionnaire that obtained information on demographic background, sexual history, characteristics of sex partners, smoking history, recreational drug and alcohol use, oral contraceptive usage, and pertinent medical history. The questionnaires were reviewed by the research coordinator, and incomplete answers or inconsistencies were verified with the subjects.

Detailed sexual behavior of the subjects in the 6-month period before the baseline visit was assessed. Two types of sex partners were distinguished in the questionnaire: regular partners were sex partners with whom subjects had ongoing sexual contact for ≥ 1 month, whereas casual partners were defined as partners with whom subjects had sex for <1 month, including "one-night stand" relationships. For each regular partner, subjects provided information on the partner's demographic and lifestyle characteristics, as well as the frequency of having different types of sex, such as vaginal, oral, and anal sex, with that particular partner.

A pelvic examination was done at the baseline visit. A Pap smear was obtained using a cytobrush for endocervical samples and a spatula for ectocervical samples. Pap smears were classified according to the 1988 Bethesda system for reporting cervical/vaginal cytologic diagnoses [20]. After the Pap smear, exfoliated cervicovaginal cells were obtained by lavage for HPV determination [18, 19, 21]. Screening for other sexually transmitted pathogens included *Chlamydia trachomatis, Neisseria gonorrhoeae,* and *Treponema pallidum*. Briefly, *N. gonorrhoeae* was identified on modified Thayer-Martin agar plates by standard methods. *C. trachomatis* was tested by an EIA (Syva Microtrack; Syva, Palo Alto, CA), with a blocking antibody test for confirmation of positive results. Syphilis serology was assessed by the rapid plasma reagin flocculation test and confirmed by the fluorescent treponemal antibody absorption assay.

Detection of HPV DNA. Lavage samples were processed in a biosafety cabinet in a laboratory physically separated from where the PCR amplification was done. Sedimented cellular material (30 μL) was removed with a disposable, sterile transfer pipette, placed in 100 μL of K buffer with 200 μg/mL proteinase K, and incubated at 55°C for 2 h followed by a 10-min incubation at 95°C [22, 23]. Next, 10 μL of this material was amplified using PCR with the MY09/MY11 L1 consensus primers, including HMB01, which amplifies a 450-bp HPV DNA fragment, and a control primer set (PC04/GH20), which simultaneously amplifies a 268-bp cellular β-globin DNA fragment that serves as an internal control for amplification. PCR reaction mix (10 μL) was analyzed by gel electrophoresis in 3% NuSieve/0.5% SeaKem agarose (FMC BioProducts, Rockland, ME) and transferred to nylon filters. The filters were hybridized overnight with radiolabeled generic probes for HPV and an oligonucleotide for β-globin as described [22, 24]. The filters were washed in 2× SSC (1× SSC = 0.15 mol/L sodium chloride and 0.015 mol/L sodium citrate) with 0.1% SDS at 55°C and exposed to radiographic film.

Samples hybridizing to the β-globin probe but negative for the generic probe were considered HPV-negative. PCR products that were positive with the HPV generic probe were analyzed for HPV DNA type. Aliquots (3 μL) of the initial PCR reaction were denatured in 0.4 *M* NaOH and 25 m*M* EDTA and applied to 10 replicate filters using a 96-well dot blot apparatus (Bio Rad, Hercules, CA). Filters were individually hybridized using biotinylated type-specific oligonucleotide probes for multiple HPV types, including types 2, 6, 11, 13, 16, 18, 26, 31–35, 39, 40, 42, 45, 51–59, 61, 62, 64, 66–70, 72, 73 (PAP238A), AE2, W13B, PAP291, and PAP155, as described [22, 23, 25]. Plasmids containing cloned HPV genomes for use as controls and probes were provided by L. Gissman, E.-M. DeVilliers, and H. ZurHausen (Deutsches Krebsforschungszentrum, Heidelberg, Germany; HPV types 6, 11, 16, 18, 40, 53, 57); G. Orth (Institut Pasteur, Paris; HPV types 32, 33, 34, 39, 42, 54, 55, 66, 68, 70); K. Shah (Johns Hopkins University, Baltimore; HPV-45); T. Matzukura (National Institute of Health, Tokyo; HPV types 58–62, 64–67); S. Silverstein (Columbia University, New York; HPV-51); A. Lorincz (Digene Diagnostics, Silver Spring, MD; HPV-31, -35, -43, -44, -56); R. Ostrow (University of Minnesota, Minneapolis; HPV-26); C. Wheeler (University of New Mexico, Albuquerque; W13B). Samples positive by the generic probe mix but negative by all type-specific probes were considered to represent "uncharacterized" HPV types. Reproducibility of HPV DNA detection by PCR in a subsample of 31 specimens showed a concordance of 96.8% for positive/negative status (κ = .97).

For direct detection of HPV genomes, Southern blot hybridization was used as described [26, 27]. Briefly, DNA was extracted from cervicovaginal cells, 5–10 μg was digested with *Pst*I, and HPV genomes were detected by Southern blot hybridization using ^{32}P-labeled HPV DNA types 11, 16, 18, 51, 52, and 53. Hybridization and the initial wash were done under low-stringency conditions (40°C below melting temperature) to detect the large spectrum of HPV types infecting the cervix. After autoradiography, the filters were rewashed under conditions of high stringency (10°C below melting temperature) and reexposed to radiographic film for 7–14 days. Classification of HPV DNA type was determined by hybridization specificity and the *Pst*I restriction enzyme cleavage pattern [28, 29]. When these data were insufficient to identify a specific type of HPV, the virus was classified as uncharacterized [30]. Southern blot interpretations were made by a single, experienced observer (R.B.) on coded samples. Reproducibility of HPV DNA detection by Southern blot in a subsample of 72 specimens showed a concordance of 97.2% for positive/negative status (κ = .97).

In this report, HPV positivity was defined as detection of HPV DNA by either PCR or Southern blot hybridization. Sixty-one samples (10%) were PCR-positive and Southern blot–negative, and 9 samples (1%) were PCR-negative and Southern blot–positive. These samples were considered HPV-positive. A sample was negative if both PCR and Southern blot hybridization were negative. HPV types identified by dot blot hybridization of PCR products were categorized as follows: high-risk types known to be associated with cervical cancer (types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 [PAP238a], and W13b) and other types (all other types detected in the population, including the uncharacterized) [5]. Low virus load was defined as samples that were Southern blot–negative but PCR positive, whereas high virus load included samples that were positive by Southern blot. Four subjects did not have complete HPV results (2 had no samples, and 2 were Southern blot–negative and had no amplification of cellular DNA by PCR) and were excluded; thus, 604 subjects were included in this analysis.

Statistical analysis. Odds ratios (ORs) were used to estimate the association between HPV infection and each of the potential categorical risk factors. In univariate analyses, the statistical significance of the association was assessed by Pearson χ^2 test. For

ordinal risk factors, the Mantel-Haenszel χ^2 test for trend was used. Since multiple risk factors were examined, a univariate association was considered to be statistically significant if $P \leq .002$ and marginally significant if $.002 < P \leq .05$.

Risk factors with $P \leq .05$ in univariate analyses were entered into logistic regression models. Three logistic regression models are presented. The first model included all subjects regardless of their sexual experience, and it aimed to identify demographic and behavioral factors related to the subject as risk factors for HPV infection. Variables were analyzed by groups. For example, all demographic variables with $P \leq .05$ in univariate analyses were assessed simultaneously to determine which of them had the greatest association with HPV infection. Similar procedures were used for drug and alcohol behaviors and sexual behaviors. All variables that were significant at $P < .1$ were entered into a comprehensive logistic regression model. The final model included only variables that were significant at $P \leq .05$. Certain variables, such as age at first coitus and frequency of vaginal sex in the last 6 months, had meaningful values only for subjects who had vaginal sexual intercourse. To include subjects who denied vaginal sex, the logistic regression analysis was done using an analytical approach described by Thompson [31]. Using age at first coitus as an example, two variables were entered in the logistic regression model: X_1 indicated whether a subject had vaginal sex experience, and X_2 indicated the age at first coitus for subjects who had had vaginal sex; for subjects who had never had vaginal sex, X_2 was assigned the median value among nonvirginal subjects. Provided that both of these variables were included in the model, the OR for X_1 was interpreted as the odds of HPV positivity in nonvirginal subjects with the median age at first coitus versus the odds of HPV positivity in the subjects who had never had vaginal sex. The regression coefficient for X_2 was interpretable as the log OR for a 1-year increase in age at first coitus among nonvirginal subjects.

The second model identified characteristics of the male partner as risk factors for HPV infection in women; only subjects who had had at least 1 regular partner in the last 6 months were included in the analysis. Characteristics of the regular sex partner with whom the subject had ongoing sexual activities for at least 1 month were analyzed. If there were multiple regular partners, the most recent partner was chosen; however, if there was >1 recent regular partner, then the partner with whom the subject had the longest sexual relationship was analyzed.

The third model examined the subject's and partner's characteristics simultaneously. This analysis was limited to subjects who had ever had vaginal sex experience and had at least 1 regular partner in the last 6 months. These restrictions were to avoid sparse data: for example, there were no subjects who never had vaginal sex, had a regular partner, and yet were HPV-positive. Moreover, because of the colinearity in age and ethnicity between subjects and their partners, only the subject's age and ethnicity were entered into the model.

Statistical analyses were done using SAS [32]. P values are two-tailed.

Results

Characteristics of study population. Mean age of the 604 study subjects was 20.0 years (SD, 2.7); 83% were recruited through advertisements in the mail, on bulletin boards, or in the campus newspaper and 17% through word of mouth. The majority of subjects were in the first (50.7%) or second (30.6%) year of college. The remaining 18.7% were either in the third to fifth year of college or in graduate or professional school. The study population was predominantly white (57.1%), with 13.1% Hispanic, 12.1% black, 9.6% Asian, and 8.1% other ethnicities. Median annual family income was $40,000–$49,999. Most of the subjects were sexually experienced; of the 76 (12.6%) who denied having had vaginal intercourse, 31 (5.1%) had had oral and/or anal sex. Eighteen subjects (3.0%) reported having had sex with a woman. Among the 528 (87.4%) who had vaginal intercourse, the median age of first coitus was 16, and the median number of lifetime male sex partners was 3. STDs were rare. Seven (2.1%) of 338 subjects tested for *C. trachomatis* were positive, whereas 1 (0.2%) of 570 and 1 (0.2%) of 497 were positive for *N. gonorrhoeae* and syphilis, respectively. Eleven (1.9%) and 31 (5.4%) of 579 subjects had squamous intraepithelial lesions and atypical squamous cells of undetermined significance, respectively.

Prevalence of HPV. HPV DNA was detected by Southern blot, PCR, or both in 168 (27.8%) of 604 subjects. HPV was detected by PCR amplification in 157 (26.0%) and by Southern blot in 107 (17.7%). The distribution of HPV types among HPV-positive subjects is shown in table 1. HPV-16, -53, and -18 were the most common characterized types detected in the population; however, an additional 9 HPV types were present in >5% of infected subjects. In total, 27 different HPV types were detected. Among HPV-positive subjects, 52.2% had infection with high-risk oncogenic HPV types, 63.7% had a high virus load, and 27.4% were infected with multiple HPV types. The prevalence of HPV was 24.4%, 63.6%, and 90.9% among subjects with normal cytology, atypical squamous cells of undetermined significance, and squamous intraepithelial lesions, respectively ($P < .001$).

Characteristics of subjects that were risk factors for HPV infection. The subjects' demographic and behavioral characteristics were examined for their associations with HPV positivity (table 2). In univariate analyses, prevalent HPV infection was strongly associated ($P \leq .002$) with the subject's demographic characteristics—age, ethnicity, and year in college; sexual behavior—experience with vaginal sex, number of lifetime male partners for vaginal sex, frequency of douching after sexual intercourse, concern of having been exposed to an STD, and sexual activities in the last 6 months as indicated by the number of male partners for vaginal sex, number of regular sex partners, and having had casual sex; and other lifestyle and behavior characteristics, including frequency of attending religious service and number of smokers in the household. Marginally significant variables ($.002 < P \leq .05$) included current smoking status, current use of oral contraceptives, frequency of using seat belt, age at first coitus, and frequencies of the following activities (in the last 6 months): vaginal sex, sex under the influence of alcohol or drugs, condom use, and

Table 1. Characteristics of genital HPV infection among HPV-positive subjects.

	No.	%
HPV type with prevalence >5% by PCR (n = 157)*		
16	23	14.7
53	14	8.9
18	12	7.6
PAP155	11	7.0
59	10	6.4
39	9	5.7
51	9	5.7
61	9	5.7
73 (PAP238a)	9	5.7
45	8	5.1
58	8	5.1
66	8	5.1
Other†	46	29.3
Uncharacterized	33	21.0
HPV type by PCR (n = 157)		
High-risk types only	67	42.7
High-risk and other types	15	9.5
Other types only	75	47.8
Multiple types by PCR (n = 124)‡	34	27.4
Virus load (n = 168)§		
Low	61	36.3
High	107	63.7

* Among 157 HPV-positive subjects with HPV DNA detected by polymerase chain reaction (PCR). Percentages total >100% because of subjects with multiple types.

† HPV types with prevalence ≤5% were types 6, 11, 26, 31, 32, 33, 35, 40, 52, 54, 55, 56, 68, 70, and PAP291.

‡ Among 124 subjects with specific HPV types detected by PCR; 33 subjects with uncharacterized HPV type were excluded.

§ Among 168 HPV-positive subjects with HPV DNA detected by PCR or Southern blot (or both).

alcohol or recreational drug use. Variables not significantly associated with HPV infection included annual family income, frequency of giving or receiving oral sex in the last 6 months, and having had anal intercourse in the last 6 months.

Multivariate logistic regression analyses were used to distinguish risk factors that were independently associated with HPV infection (see table 2). Increased risk for HPV infection was correlated with age, but the relationship was not linear; compared with subjects ≤20 years old, the OR for HPV infection was 2.41 (95% confidence interval [CI], 1.32–4.40) among subjects 21–23 years old, and the OR dropped off to 1.76 (95% CI, 0.82–3.79) among those who were >23 years old. Black and Hispanic subjects were more likely to be HPV-positive than the other subjects, who were predominantly white. HPV positivity increased proportionately with lifetime as well as more recent (6 months) numbers of male sex partners. None of the lifestyle or behavioral risk factors identified in the univariate analyses were significant, except the numbers of smokers in the household. Subjects who lived with people who smoked were more likely to have HPV infection than those who did not.

Characteristics of subjects' male sex partners that were risk factors for HPV infection in subjects. To identify the male partners' characteristics that were associated with increased risk of genital HPV infection in women, 468 subjects who had at least 1 regular male sex partner in the last 6 months were included in the analysis. In univariate analyses (table 3), HPV positivity in subjects was significantly ($P \leq .002$) associated with the male partner's age, ethnicity, college status, and estimated number of lifetime sex partners. Female genital HPV infection was marginally ($.002 < P \leq .05$) associated with the male partners' frequencies of attending religious services, using automobile seat belts, and alcohol use, the duration of their sexual relationship, and frequencies of the couple having vaginal sex and sex under the influence of alcohol or drugs in the last 6 months. Multivariate analysis (table 3) identified the following regular male partner's characteristics to be independently associated with an increased risk of genital HPV infection in women: age >20 years, black or Hispanic ethnicity, currently not attending college, and increased number of lifetime female sex partners. Subjects who had sex with their regular partner under the influence of alcohol or drugs and those who had a sexual relationship with their partner for <12 months also had an increased risk of HPV.

The characteristics of the subjects and their regular male partners were combined and examined by multivariate analysis. Only those subjects who had had vaginal sex and had at least 1 regular male partner in the last 6 months were analyzed (table 4). In this model, the subject's age (21–23 years), ethnicity (black and Hispanic), number of smokers in subject's household, lifetime number of male sex partners, duration of the sexual relationship, whether subject's partner was currently in school, and lifetime number of partners of the subject's regular male partner were significant risk factors for HPV infection in the subject.

Discussion

Cervicovaginal HPV infection was detected in 27.8% of a population-based group of young women participating in a study advertised throughout a college campus. Over 29 different HPV DNA types were identified; HPV-16 and -53 were the two most common, and 27.4% of positive subjects were infected with multiple HPV types. In addition to information about the subjects themselves, detailed information was obtained about their male sex partners. Univariate analyses identified a number of variables associated with a high HPV prevalence (>50%), including age 21–23 years, black ethnicity, >4 lifetime sex partners, douching after sex, having a male partner who had >10 female partners, and having a regular male partner not currently in college. In contrast, HPV was detected in only 3% of subjects denying vaginal sex and in 9% of sexually active subjects whose regular male partner had only 1 lifetime female sex partner. Increasing numbers of sex partners for either the female subject or her regular male partner were the

Table 2. Risk factors for female genital HPV infection: characteristics and behaviors of subjects.

Risk factor of subject	No. HPV-positive/ total (%)	Univariate analysis COR (95% CI)	Multivariate analysis AOR (95% CI)
Demographic factors			
Age			
≤20	115/486 (24)	1.00	1.00
21–23	36/71 (51)	3.32 (1.99–5.53)	2.41 (1.32–4.40)
>23	17/47 (36)	1.83 (0.97–3.43)	1.76 (0.82–3.79)
Ethnicity			
White/Asian/other	102/452 (23)	1.00	1.00
Hispanic	29/79 (37)	1.99 (1.20–3.31)	2.77 (1.49–5.12)
Black	37/73 (51)	3.53 (2.12–5.87)	4.24 (2.31–7.79)
Annual family income			NA
<$40,000	71/221 (32)	1.00	
≥$40,000	66/268 (25)	0.69 (0.47–1.03)	
Year in college			NA
1	69/306 (23)	1.00	
2	52/185 (28)	1.34 (0.88–2.04)	
3–5	29/63 (46)	2.93 (1.67–5.15)	
Postgraduate	18/50 (36)	1.93 (1.02–3.65)	
Sexual behavioral factors			
Sexual experience			NA
Never had oral/anal/vaginal sex	2/45 (4)	1.00	
Had oral/anal sex only	0/31 (0)	0.28 (0.01–5.95)	
Had vaginal sex	166/528 (31)	9.86 (2.36–41.18)	
No. of male vaginal sex partners in lifetime			
0	2/76 (3)	1.00	1.00
1	9/137 (7)	2.60 (0.55–12.36)	2.19 (0.45–10.70)
2	30/108 (28)	14.23 (3.28–61.66)	12.39 (2.78–55.33)
3–4	49/135 (36)	21.08 (4.96–89.66)	17.52 (3.98–77.15)
≥5	78/148 (53)	41.23 (9.76–174.21)*	29.67 (6.67–131.90)*
Ever douched after sex†			NA
Never	144/485 (30)	1.00	
Rarely–all the time	22/42 (52)	2.61 (1.38–4.92)	
Age at first coitus†			NA
>16	75/278 (27)	1.00	
≤16	91/250 (36)	1.55 (1.07–2.24)	
No. of male vaginal sex partners in last 6 months†			
0	6/48 (13)	1.00	1.00
1	85/317 (27)	2.56 (1.05–6.25)	3.80 (1.36–10.63)
≥2	75/163 (46)	5.97 (2.40–14.81)*	4.89 (1.68–14.21)‡
Condom use in last 6 months†			NA
Never/rarely/sometimes	74/200 (37)	1.00	
Most of the time	52/146 (36)	0.94 (0.60–1.47)	
All of the time	34/134 (25)	0.58 (0.36–0.94)‡	
Had casual sex in last 6 months§			NA
No	92/374 (25)	1.00	
Yes	74/185 (40)	2.04 (1.41–2.97)	
No. of regular sex partners in last 6 months§			NA
0	20/91 (22)	1.00	
1	108/386 (28)	1.38 (0.80–2.38)	
≥2	38/82 (46)	3.07 (1.59–5.93)	

predominant risk factors for HPV infection, providing compelling evidence for the sexual transmission of HPV infection mediated through sexual promiscuity.

Association between sexual behavior and female HPV infection in previous studies has not been reported consistently in the literature [17, 33–38]. For example, Rohan et al. [39] did not identify number of sex partners as a risk factor for genital HPV in a student health clinic population. The lack of association could be attributed, in part, to differences in sample collection [18, 19], virus detection methods lacking adequate sensitivity and specificity [16], or population characteristics. To maximize sampling of the cervicovaginal area, cervicovaginal

Table 2. *(Continued)*

Risk factor of subject	No. HPV-positive/ total (%)	Univariate analysis COR (95% CI)	Multivariate analysis AOR (95% CI)
Maximum frequency in last 6 months of			
Vaginal sex[‖]			NA
0–5 times	16/83 (19)	1.00	
≥6 times	130/383 (34)	2.15 (1.20–3.86)	
Giving oral sex[‖]			NA
0–5 times	45/151 (30)	1.00	
≥6 times	101/315 (32)	1.11 (0.73–1.69)	
Receiving oral sex[‖]			NA
0–5 times	41/151 (27)	1.00	
≥6 times	105/314 (33)	1.35 (0.88–2.07)	
Receiving anal sex[‖]			NA
None	129/424 (30)	1.00	
≥1	17/42 (40)	1.56 (0.82–2.97)	
Sex under influence of alcohol or drugs[‖]			NA
0–5 times	105/373 (28)	1.00	
≥6 times	41/93 (44)	2.01 (1.27–3.20)	
Lifestyle factors			
Frequency of using seat belt			NA
Never/rarely/sometimes	32/80 (40)	1.00	
Most of the time/all of the time	136/523 (26)	0.53 (0.33–0.85)	
Frequency of attending religious service			NA
Never	40/106 (38)	1.00	
1–5 times/year	73/234 (31)	0.75 (0.46–1.21)	
≥6 times/year	55/263 (21)	0.44 (0.27–0.71)*	
Currently using oral contraceptives			NA
No	118/461 (26)	1.00	
Yes	49/141 (35)	1.55 (1.03–2.32)	
Current smoker			NA
No	132/503 (26)	1.00	
Yes	36/100 (36)	1.58 (1.01–2.48)	
No. of smokers in household			
0	95/400 (24)	1.00	1.00
≥1	73/197 (37)	1.89 (1.31–2.73)	2.03 (1.30–3.15)
Frequency of alcohol use in last 6 months			NA
<1 time/week	116/455 (25)	1.00	
≥1 time/week	52/149 (35)	1.57 (1.05–2.33)	
Frequency of drug use in last 6 months			NA
None	104/422 (25)	1.00	
<1 time/month	37/117 (32)	1.41 (0.90–2.21)	
≥1 time/month	27/65 (42)	2.17 (1.27–3.73)‡	
Self-perceived possibility of having been exposed to a sexually transmitted disease[¶]			NA
Very unlikely	52/309 (17)	1.00	
Unlikely	59/175 (34)	2.51 (1.63–3.87)	
Somewhat likely or likely	57/120 (48)	4.47 (2.81–7.13)*	

NOTE. COR, crude odds ratio; CI, confidence interval; AOR, adjusted odds ratio. Final logistic regression model included age, ethnicity, no. of lifetime male vaginal sex partners, no. of male vaginal sex partners in last 6 months, and no. of smokers in household. NA, not applicable, since variable not included in final model.

* $P < .001$, Mantel-Haenszel χ^2 test for linear trend.

† Among subjects who had vaginal sex.

‡ $P < .05$, Mantel-Haenszel χ^2 test for linear trend.

§ Among subjects who had any type of sex (vaginal, oral, and/or anal).

‖ Frequency of different sexual activities with regular partner in last 6 months was assessed among subjects who had at least 1 regular partner. If subject had multiple partners, partner with whom subject had the maximal frequency of sexual activities was analyzed.

¶ Possibility of having been exposed to sexually transmitted disease was rated on 5-point scale: 1 = very unlikely, 2 = unlikely, 5 = very likely. Ratings ≥3 were grouped for analysis as somewhat likely or likely.

Table 3. Risk factors for genital HPV infection in women: male partners' characteristics and behaviors.

Risk factor of regular male partner*	No. HPV-positive/ total (%)	Univariate analysis COR (95% CI)	Multivariate analysis AOR (95% CI)
Demographic factors			
Age			
≤20	60/261 (23)	1.00	1.00
21–23	56/138 (41)	2.29 (1.47–3.57)	1.82 (1.10–3.04)
>23	29/65 (45)	2.70 (1.53–4.76)†	1.73 (0.90–3.35)
Ethnicity			
White/Asian/other	82/338 (24)	1.00	1.00
Hispanic	25/55 (47)	2.80 (1.56–5.02)	2.84 (1.50–5.41)
Black	37/70 (53)	3.50 (2.06–5.95)	2.76 (1.49–5.13)
Currently in college			
No	62/120 (52)	1.00	1.00
Yes	82/343 (24)	0.29 (0.19–0.45)	0.59 (0.39–0.90)
Sexual behavioral factors			
No. of lifetime sex partners estimated by subject			
1	9/99 (9)	1.00	1.00
2–3	33/136 (24)	3.20 (1.45–7.06)	2.46 (1.09–5.57)
4–10	68/162 (42)	7.23 (3.41–15.36)	4.32 (1.96–9.52)
≥11	32/61 (52)	11.03 (4.72–25.81)†	5.21 (2.09–12.96)†
Duration of sexual relationship with subject			
≤6 months	71/191 (37)	1.00	
7–12 months	32/99 (32)	0.81 (0.48–1.35)	1.00
13–18 months	12/46 (26)	0.60 (0.29–1.23)	
>18 months	30/125 (24)	0.53 (0.32–0.88)‡	0.59 (0.36–0.96)§
Frequency of vaginal sex with subject in last 6 months			NA
0–5 times	21/93 (23)	1.00	
≥6 times	124/370 (34)	1.73 (1.02 2.94)	
Frequency of sex with subject under influence of alcohol or drugs in last 6 months			
0–5 times	109/380 (29)	1.00	1.00
≥6 times	36/83 (43)	1.90 (1.17–3.10)	1.84 (1.06–3.20)
Lifestyle factors			
Frequency of using seat belt			NA
Never/rarely/sometimes	64/165 (39)	1.00	
Most of the time/all of the time	73/267 (27)	0.59 (0.39–0.90)	
Frequency of attending religious service			NA
Never	56/155 (36)	1.00	
1–5 times/year	48/150 (32)	0.83 (0.52–1.34)	
≥6 times/year	23/119 (19)	0.42 (0.24–0.74)‡	
Frequency of alcohol use			NA
<1 time/week	76/288 (26)	1.00	
≥1 time/week	64/167 (38)	1.73 (1.16–2.60)	
Current smoker			
No	91/313 (29)	1.00	
Yes	54/141 (38)	1.49 (0.98–2.27)	

NOTE. COR, crude odds ratio; CI, confidence interval; AOR, adjusted odds ratio. Final logistic regression model included partner's age, ethnicity, current school attendance, length of sexual relationship with subject, no. of lifetime female sex partners as estimated by subject, and sex with subject while under influence of alcohol or drugs. NA, not applicable, since variable not included in final model.

* Sex partner with whom subject had ongoing sexual activities for ≥1 month in last 6 months. If there were multiple regular partners, most recent was analyzed.

† $P < .001$, Mantel-Haenszel χ^2 test for linear trend.

‡ $P < .05$, Mantel-Haenszel χ^2 test for linear trend.

§ OR was derived from >12 months vs. ≤12 months.

lavage was used [21]. This technique has the advantage of collecting cells exfoliated from the cervix in the recent past and washed off during the procedure. The technique is simple and universally accepted by patients and provides abundant cellular material for molecular tests. To detect a large spectrum of HPV DNA genotypes, we used a low-stringency Southern blot hybridization method [28] in addition to the MY09/MY11 PCR amplification system. This latter system has been exten-

Table 4. Multivariate logistic regression analysis of subjects' and regular male sex partners' risk factors for genital HPV infection in women.

Risk factor	Adjusted OR (95% CI)	*P*
Subject's age		
≤20	1.00	
21–23	2.62 (1.29–5.31)	.008
>23	1.58 (0.63–3.98)	.329
Subject's ethnicity		
White/Asian/other	1.00	
Hispanic	2.16 (1.06–4.23)	.034
Black	3.17 (1.58–6.37)	.001
Subject's lifetime no. of male vaginal sex partners		
1	1.00	<.001*
2	4.46 (1.83–10.87)	
3–4	5.82 (2.48–13.70)	
≥5	10.31 (4.39–24.23)	
No. of smokers in subject's household		
0	1.00	
≥1	1.91 (1.15–3.19)	.013
Regular partner currently in school		
No	1.00	
Yes	0.57 (0.36–0.89)	.013
Duration of sexual relationship between subject and regular partner		
≤12 months	1.00	
>12 months	0.56 (0.34–0.93)	.028
Male partner's lifetime no. of sex partners†		
1	1.00	.018*
2–3	2.11 (0.89–4.99)	
4–10	3.13 (1.37–7.15)	
≥11	2.73 (1.05–7.11)	

NOTE. Analysis was restricted to subjects who had vaginal intercourse and regular male partner within past 6 months. Regular male partner was sex partner with whom subject had ongoing sexual activities for ≥1 month in last 6 months. If there were multiple regular partners, most recent was analyzed.

**P* value for linear trend.

† Estimated by subject.

sively used in epidemiologic studies and has proved to be highly sensitive in detecting a large spectrum of genital HPV types [5, 12, 23]. In addition, this approach allows a broad range of quantitative assessment of HPV load and complements the detection of HPV genomes poorly amplified by the MY09/MY11 system (e.g., HPV-42 and -43).

An important feature of this study was the establishment of the cohort through advertisement. This attracted a diverse spectrum of the female college population with greater heterogeneity in sexual behaviors than women attending the health service for gynecologic exams. This latter point is relevant since demonstration of sexual behavior as the quintessential risk factor for HPV is analytically a comparison between groups in a cohort. The importance of how a population has been recruited becomes relevant when interpreting risk factors for HPV infection. Populations that tend to be more homogeneous in their or their partners' level of sexual promiscuity will diminish findings on the relationship between sexual behavior and HPV. For instance, two previous studies from our group that recruited young women requiring a gynecologic exam had higher prevalences of HPV and showed a lower association between sexual behavior and HPV detection than the current study [40, 41]. In fact, we detected a higher prevalence of HPV infection and a smaller association with sexual behavior in a pilot study of women coming to the university student health center for gynecologic care.

Three studies using a sensitive, validated PCR detection system have reported significant association with lifetime number of sex partners and HPV infection in young women [12, 33, 34, 37]. The Berkeley study [12, 33] reported 33% of 467 subjects with cervical HPV infection and 46% with HPV detected in the vulvar or cervical swab specimen. The New Mexico study [34] reported that 44.3% of 357 women attending the University Student Health Center for routine gynecologic care were HPV-positive on cervical swabs. In both studies, HPV positivity was independently correlated with increasing numbers of sex partners. Consistent with the higher prevalence of HPV detected in these latter two studies, two differences in the populations should be noted. In the current study, a population-based cohort was obtained by advertisement, in contrast to the Berkeley and New Mexico studies, which both recruited women coming in for gynecologic care. The mean age of the women in both studies was 23 years, ~3 years older than the current population.

A population-based Swedish study [37] detected cervical HPV infection in 20% of 581 women who were 19–25 years old. Lifetime number of male sex partners was the only independent risk factor for cervical HPV infection and showed a linear trend, similar to the current report. Moreover, in the Swedish study, 4% of 55 non–sexually active women were HPV-positive compared to 33% of women with >5 lifetime sex partners.

Taken together, the three previous studies [33, 34, 37] and the current report all show a significant association with lifetime number of sex partners and HPV infection in young women. The relationship between lifetime partners and prevalent HPV infection is not as dramatic in older populations of women, in whom recent numbers of sex partners is more strongly associated with HPV [17, 26]. This may reflect differences in the sexual experience of college-aged women, whose lifetime number of sex partners reflects sexual encounters in the recent time period. In contrast, for women >30 years of age, the period of time encompassing numbers of lifetime sex partners may span decades, thus accentuating differences between recent and lifetime number of partners. Moreover, the differences in risk for prevalent HPV infection in distinct age groups of women from lifetime versus recent partners may reflect the transient nature of most cervical HPV infections [13,

23, 42]. Discrepant with the predominant sexual transmission of HPV, however, is the detection of HPV in virginal or non–sexually active women, albeit at a much lower prevalence. In this study, 2 of 76 women who denied vaginal sex were HPV-positive. In each case, the HPV was untypeable and may have been acquired by nonpenetrant sexual contact, as previously reported [43]. Alternatively, sexual behavior might have been inaccurately reported, since other studies have failed to detect cervical HPV in virginal women [44, 45].

The information on sexual behavior of the women in the current study indicates that cervical HPV infection is associated with vaginal intercourse and not oral or anal sex (see table 2). A variety of sexual behaviors was associated with HPV in univariate but not in multivariate analyses, including douching after sex, age at first coitus, recent casual sex, number of recent regular male partners, frequency of recent vaginal sex, and frequency of recent sex under the influence of alcohol or drugs. After multivariate analysis, only lifetime and recent number of male vaginal sex partners remained significant, suggesting exposure to different men as the predominant risk, in contrast to frequency of sex. Alternatively, these other risk factors may just be correlated with number of partners. Although condom use provided some protection in the univariate analysis, this effect was lost after the multivariate analysis. Surprisingly, subjects who claimed to use condoms all of the time still had a relatively high rate of HPV infection (25%). Thus, this study did not provide compelling evidence that condoms offer adequate protection from transmission of HPV infection; further study is needed to address this point. In addition, subjects who believed they had been exposed to an STD had a higher prevalence of HPV than those not anticipating such a risk.

A number of characteristics of women not associated with sexual behaviors were also risk factors for HPV infection. Subject's age, ethnicity, year in college, frequency of seat belt use, frequency of attending religious services, smoking, living with smokers, and alcohol or drug use were all associated with HPV in the univariate analysis. However, only age, ethnicity, and number of smokers in the household were independently associated with HPV. Similar age trends have been seen in other studies of college-aged women [33, 46]. These trends probably reflect the sexual behavior patterns of college-aged women who enter college relatively sexually quiescent and become more sexually active as they expand their social networks in their upper years of college. In support of this notion, the prevalence of HPV went from ~25% in the first 2 years of college to 46% in the later years (see table 2). Different ethnic groups display varying prevalences of HPV in college populations [33, 34]. Similar to our findings, the Berkeley study identified being black as an independent risk factor for HPV infection [33]. Hispanic women were at increased risk in our study but not in the New Mexico study [34]. Reasons for ethnic differences as risk factors for prevalent HPV infection may include genetic predisposition for acquisition or persistence of HPV infection. One such mechanism might be HLA haplotype differences related to immunologic reactivity [47, 48]. Alternatively, different levels of endemic HPV may exist in given groups, thus yielding a higher risk on exposure to a group member. This later mechanism has been proposed as an important variable in the high rate of cervical cancer in Latin America [49]. Similarly, the unexpected independent risk factor of a women living in a household with smokers might be construed as placing the subject at risk through association with friends who engage in risky sexual behaviors [50]. Additional studies will be needed to determine the importance of this association.

This is the first report to investigate the behaviors of the regular male sex partners of women as risk factors for HPV infection in the women. We did an analysis focusing on the characteristics of the subjects' male partners alone. Many of the characteristics were similar to those identified in the subjects and likely represent similarities in age, race, and behavior among partners. The significant factors among male partners imparting risk to the subjects included older age, black and Hispanic ethnicity, educational status, increasing number of sex partners, a short-term relationship, frequent sex while intoxicated, rarely using seat belts, and less attendance at religious service (see table 3). Taken together, these risk factors paint the picture of male partners of HPV-infected women as being more sexually promiscuous, as indicated by lifetime number of partners, duration of relationship, and frequency of sex. In addition, male partners of infected women exhibit characteristics of risk-taking behaviors, as evidenced by lack of seat belt use and substance abuse. These observations strengthen the importance of the "male factor" in HPV infection in women and are consistent with the recent observation of HPV type–specific concordance in sex partners [51].

To evaluate the contributions of both the subject and her male partners' characteristics as risk factors for HPV infection in women, a logistic regression model was developed. The lifetime numbers of both female and male partners were independently associated with HPV in subjects, as was ethnicity, college status of partner, duration of sexual relationship, and number of smokers in household.

Certain limitations apply to this study and should be appreciated. The cohort of women studied is relatively young and early in their sexual behavior patterns and thus is not likely representative of older women. The information on male partners was obtained from the subjects and may be biased by each subject's own sexual behaviors and assumptions concerning her partner. In addition, differences in geographic or ethnic variation of endemic HPV could be significant variables influencing risk factors for HPV infection.

In summary, our data suggest three main areas of risk for college-aged women to have a cervicovaginal HPV infection. The first and most significant is sexual exposure through multiple male sex partners. The second is their partners' level of promiscuity as evidenced by his lifetime number of partners. Last, the probability of a woman having HPV infection was related to the prevalence of HPV in her social/sexual contact

pool (i.e., endogenous HPV prevalence). Characteristics of contact groups appear to be associated with ethnicity, college status, having short-term relationships, and living with persons who smoke.

Acknowledgments

We acknowledge the HPV DNA analyses by Ruth Tachezy, Renee Lewis, Weimin Qu, Yvette Cruz, and Gang Jiang; special thanks to Anna Kadish and Robert Klein for critical review.

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