

Determinants of Genital Human Papillomavirus Detection in a US Population

Cheri L. Peyton,¹ Patti E. Gravitt,² William C. Hunt,¹
 Rosalina S. Hundley,¹ Meifen Zhao,¹
 Raymond J. Apple,³ and Cosette M. Wheeler¹

¹University of New Mexico, Department of Molecular Genetics and Microbiology, Albuquerque; ²Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland; ³Roche Molecular Systems, Alameda, California

This study investigated the association of selected demographic and behavioral characteristics with the detection of low-risk, high-risk, and uncharacterized genital human papillomavirus (HPV) in women attending clinic for routine nonreferral gynecologic health care. Cervical specimens obtained from 3863 women 18–40 years old (mean, 28 years) with no history of high-grade cervical disease were analyzed for 38 HPV types. Overall, HPV prevalence was 39.2%. The prevalence of high-risk, low-risk, and uncharacterized HPV types was 26.7%, 14.7%, and 13.0%, respectively. As expected, the characteristics most strongly associated with overall HPV detection were age and numbers of lifetime and recent sex partners. Low-risk, high-risk, and uncharacterized HPV detection increased with increasing numbers of sex partners. There was a decline in high-risk and low-risk HPV detection with increasing age but little change in uncharacterized HPV detection. These results suggest that the uncharacterized HPV types have a different natural history than either low-risk or high-risk HPV types.

The more than 100 different human papillomavirus (HPV) genotypes identified over the past 20 years [1] can be divided into discrete phylogenetic groups [2–4]. Within the past 5 years, several genital HPV genomes and L1 subgenomic fragments have been cloned, including HPV types 61, 62, 64, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, CP6108, and IS39 [2, 5–9]. To date, >40 HPV types have been detected in the anogenital mucosa, and many have been associated with benign or malignant lesions of the genital tract. Most, if not all, cervical disease can be attributed to HPV infection [10–13]. Disease association and population prevalence have been determined for a number of HPV types [13–15]. On the basis of these studies, HPVs have been grouped as high or low risk for high-grade cervical disease and cancer outcomes.

Epidemiologic studies in diverse populations that consider sexual, behavioral, and demographic factors have concluded that detection of HPV decreases with age and increases with number of sex partners [16–21]. Other risk factors for HPV detection may be population dependent and are probably markers of sexual behavior. Most studies combine all genital HPV types into a single group for analytical purposes. Several recent

studies examined the determinants of high-risk and low-risk HPV types separately [22–28]. Low-risk HPV types are less associated with sex history and age than are high-risk HPV types, although differences in associations have been reported [22, 25, 26]. Few studies have analyzed the HPV types with insufficient data to be assigned to a separate risk group (referred to in this article as uncharacterized HPV types); these types are often included in the low-risk HPV group.

Polymerase chain reaction (PCR) primers that target the highly conserved regions of the HPV L1 open-reading frame are capable of amplifying a broad spectrum of HPV types. Examples of such systems include GP5+/6+ [29, 30], MY09/11 [31–34], PGMY09/11 [35], and the newly developed short-fragment PCR [36]. Here we used the MY09/11 consensus HPV L1 PCR and reverse line blot hybridization systems, to detect 38 genital HPV types in a single assay. To assess differences in risk profiles, analyses were done after grouping the 38 HPV types as low or high risk for cervical disease or as uncharacterized.

Methods

Study subjects and cervical specimens. Subjects attending clinics for routine gynecologic care were recruited consecutively from the Lovelace Women's Health Services and the University of New Mexico Family and Women's Health clinics. Samples for HPV testing and Pap smear cytology were obtained from eligible women.

Subject enrollment eligibility criteria included no history of moderate or high-grade cervical dysplasia, no history of atypical squamous cell of undetermined significance or low-grade squamous intraepithelial lesions within the past year (i.e., not attending clinic for referral abnormal Pap smear), age 18–40 years, Hispanic or non-Hispanic white ethnicity, not currently pregnant, and no hysterectomy. Of the women scheduled to attend clinic for a pelvic examination from July 1996 through May 2000, 4835 met the study

Received 4 August 2000; revised 23 February 2001; electronically published 9 May 2001.

Presented in part: 17th International Papillomavirus Conference, Charleston, South Carolina, 9–15 January 1999 (abstract Epi 9).

All study subjects gave written informed consent for participation on documents approved by the University of New Mexico Human Research Review Committee. The committee reviewed and approved the study.

Financial support: National Institutes of Health (AI-32917 to C.M.W.).

Reprints or correspondence: Dr. Cosette M. Wheeler, Dept. of Molecular Genetics and Microbiology, University of New Mexico, Albuquerque, NM 87131-5276 (cwheeler@salud.unm.edu).

The Journal of Infectious Diseases 2001;183:1554–64

© 2001 by the Infectious Diseases Society of America. All rights reserved.
 0022-1899/2001/18311-0002\$02.00

eligibility criteria. Of these women, 721 (14.9%) refused to participate, 61 (1.3%) reported no or an unknown number of lifetime sex partners, and data were incomplete for 151 women (3.1%). HPV typing was unsuccessful for 38 specimens because of lack of β -globin amplification. HPV testing was successfully done on cervical specimens from 3863 women who agreed to participate in the study. Self-reported reasons for attending clinic were as follows (women may have reported >1 reason): 2905 (75.2%), annual examination; 2106 (54.5%), birth control or family planning; 605 (15.7%), 6-week postpartum checkup; 390 (10.1%), gynecologic problems or infection check; 314 (8.1%), menstrual irregularities; 78 (2.0%), repeat Pap test; 307 (7.9%), "other" reason; and 6 (0.2%), no reason given. Of the women who refused to participate, the most common reason for refusal was lack of time. There was no difference in the refusal rates between Hispanic and non-Hispanic white women. Women who refused to participate in the study were older on average than those who enrolled in the study (22.4% of refusals were in the oldest age group vs. 16.5% of study participants, $P < .05$).

Cervical specimens were collected from consenting women by using a Dacron swab that was placed in 1.0 mL of standard transport medium (STM; Digene). Specimens were held at room temperature after collection at the clinic and were stored the day of collection at -85°C until further processing. Detailed demographic and sexual behavior information was collected from subjects through an interviewer-administered questionnaire. Results of Pap smears taken on the day of the clinic visit were obtained through coordination with the institutional pathology reporting systems. Pap smear and sexually transmitted disease (STD) histories were obtained through self-report and review of each subject's medical chart.

Sample preparation. A new piece of 5.1×5.1 -cm gauze was used to open each specimen tube throughout all sample preparation procedures. Cervical specimens collected in STM were thawed and were processed by adding $30 \mu\text{L}$ of digestion solution (20 mg/mL proteinase K, 10% laurth-12, 20 mM Tris, and 1 mM EDTA [pH 8.5]). Digestion was done at 60°C for 1 h. A $300\text{-}\mu\text{L}$ aliquot of the digested material was added to 1.0 mL of absolute ethanol containing 0.5 M ammonium acetate. The DNA was precipitated at -20°C overnight and then was centrifuged for 30 min at 13,000 g. The supernatant was discarded immediately, and the crude DNA pellet was dried overnight at room temperature. The pellet was resuspended in $150 \mu\text{L}$ of 20 mM Tris and 1 mM EDTA (pH 8.5). A microcentrifuge tube cap lock (GeneMate) was placed on each tube, which was followed by a 15-min incubation at 95°C to inactivate the proteinase K. The crude DNA extracts were amplified immediately or were stored at -85°C until amplification.

Bulk master mix components (distilled water and $10\times$ PCR buffer) were tested individually for potential contamination by both direct amplification and amplification of aliquots precipitated with K562 (ATCC) crude cellular DNA, to detect potential low-level HPV contamination. Individual assay sensitivity was assessed by the use of serial dilutions of SiHa (ATCC) crude cell digests, targeting 10^4 , 10^3 , 10^2 , 10^1 , and 10^0 input copies of HPV-16. No-template HPV controls were included with each amplification to monitor potential contamination.

Probe development. Type-specific oligonucleotide probes were designed for HPV types 61, 62, 64, 67, 69, 70, 71, 72, 81, CP6108, and IS39. These genotypes, plus the 27 genotypes included in the standard line blot assay [37], account for >95% of all HPV-positive

samples found in previous studies in the New Mexico population, as determined by generic probe positivity [8]. Potential probes were initially selected by the following criteria: length, 18–22 bp; G/C:A/T ratio, $\sim 50:50$; A/T strings, ≤ 4 bp; temperature, 58°C – 62°C ; and gross visual uniqueness of sequence. Type-specific probes that had ≥ 4 nucleotide mismatches to all genital HPV types by the FASTA program (Genetics Computer Group) were selected for evaluation on HPV genotyping strips. Probes were tested individually on genotyping strips for sensitivity and cross-hybridization with all other HPV types by using serially diluted MY09/11 amplicons derived from cloned plasmid HPV controls. For most HPV types, 2 probes were selected for the final genotyping strip layout. The sequences of the probes are as follows: HPV-61 (AAGCCACAAGCTTTAGGGAA, CCAAGGAGGATCGCTATGC), HPV-62 (GCGACACACGGAGGAATTTG, CCCGTATGCGCAAATGACA), HPV-64 (GGAATCTGAGGATCCATATGC), HPV-67 (TGCAATACATACACACCATGA, CATCCCCTCCAACAGCAAAGG), HPV-69 (GTA-CTGTATCTGCACAATCTGC, GCGATGCCCTGCACAGC), HPV-70 (GCCTGCACCGAAACGGCC), HPV-71 (GTCCATCTGTGCTACCAAAAAC, GCAGATCTTACATTTTGGGAG), HPV-72 (GCCACAGCGTCTCTGTAT, CGTGAGTATCTTCGCCACAC), HPV-CP6108 (TGCTGCTTCCCAGTCTGCC, GCACTGCTGCCCCAGAACC), HPV-81 (GCACAGCTACATCTGCTGC, GCCGACATGTCATTTTGGACA), and HPV-IS39 (GCAGCAACCTCTTGTCAACG, GCACAGACATTCACCTCAAC).

PCR and reverse line blot detection methods. Before amplification, the crude digests were allowed to reach room temperature and were centrifuged briefly to minimize aerosolization. We amplified $6 \mu\text{L}$ of each sample by using the MY09/11 L1 consensus primer system [34] and AmpliTaq gold polymerase (Perkin Elmer). Amplifications were done in a thermal cycler (model 9600; Perkin Elmer) with the following profile: 9-min AmpliTaq gold activation at 95°C followed by 40 cycles of 1-min denaturation at 95°C , 1-min annealing at 55°C , 1-min extension at 72°C , and a 5-min final extension at 72°C .

Details of the reverse line blot detection method have been described elsewhere [37]. Two HPV genotyping strips were used in this study. The first strip detected 27 individual genotypes and β -globin [37], and the second strip detected an additional 11 individual genotypes and β -globin. The design of the second strip was as follows. Two bovine serum albumin (BSA)-conjugated probes per HPV type, each corresponding to a hypervariable region within the amplicon, were deposited together as a single line for each of the following HPV types: 61, 62, 67, 69, 71, 72, 81, CP6108, and IS39. A single probe was deposited for HPV-64 and HPV-70, since alternate probes evaluated resulted in cross-hybridization with other HPV types. The β -globin control line was a single probe.

HPV controls. T. Matsukura provided cloned plasmid HPV types 61, 62, 64, 67, 69, and 71. E.-M. de Villiers provided HPV-72. The HPV-70 genome was cloned previously by our group [38], as were L1 fragments from HPV types 81, IS39, and CP6108 [7, 8].

Statistical methods. All HPV types were classified as low or high risk for high-grade cervical disease or as uncharacterized. HPV assignments were as described elsewhere [13, 37]. HPV types grouped within the high-risk category included 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, 73, 82, and 83. HPV types grouped within the low-risk category included 6, 11, 40, 42, 53,

54, 57, 66, and 84. Uncharacterized HPV types included 61, 62, 64, 67, 69, 70, 71, 72, 81, CP6108, and IS39. Women with multiple HPV types were considered to be in each risk category on the basis of the detection of any 1 of the high-risk, low-risk, or uncharacterized HPV types. Analyses were not restricted to women with only single HPV infections.

The goals of the statistical analysis were to describe the relationship of sex history and age to the detection of high-risk, low-risk, and uncharacterized HPV types, as well as to evaluate additional subject characteristics for their association with the detection of these 3 HPV groups after controlling for sex history and age. Sex history included number of lifetime sex partners, number of sex partners in the past year, age at first intercourse, number of years since first sexual intercourse, and sex history of the current sex partner. Additional subject characteristics included age, ethnicity, marital status, household income, education level, age at menarche, parity, smoking history, history of use of condoms, diaphragm, and birth control pills, history of partner with genital warts, history of STDs (chlamydia, trichomonas, gonorrhea, and herpes), and history of pelvic inflammatory disease.

Defining the relationship among HPV detection, sex history, and age was done graphically and with simple contingency tables. From these results, we concluded that numbers of lifetime sex partners and sex partners in the past year were important determinants of the detection of high-risk, low-risk, and uncharacterized HPV types. It also was apparent that the effect of the number of lifetime sex partners on both high-risk and low-risk HPV detection varied by age. However, this age modification was not observed in the uncharacterized HPV group.

To individually examine other potential predictors of HPV detection after controlling for the effects of sex history and age, logistic regression models for high-risk, low-risk, uncharacterized, and all HPV types were computed separately for each potential predictor variable while controlling for sex history and age, as described above. Both numbers of lifetime and recent sex partners were included in the multivariable models. Although these 2 variables are correlated, in our data there was sufficient overlap in the 2 measures to allow for both to be incorporated as independent factors in the models for HPV detection.

The final step in the analysis was to select variables from these individual regression models that showed a statistically significant effect on high-risk, low-risk, uncharacterized HPV detection, or overall HPV detection for inclusion in the multivariable logistic regression models. Thus, the final regression models have the same set of predictor variables and differ only in the dependent variable. All statistical analyses were computed by SAS procedures [39]. The associations between the detection of specific HPV types were described by using Pearson partial correlation coefficients, adjusting for age and sex history. Throughout this report, we use the terms "detection" and "prevalence" interchangeably.

Results

HPV type-specific prevalence. Table 1 shows the prevalence of 38 genital HPV types detected in cervical specimens from 3863 women attending clinics for routine gynecologic care. Also

Table 1. Prevalence of human papillomavirus (HPV) types in 3863 women 18–40 years old, Albuquerque, New Mexico, 1996–2000.

HPV type	No. (%) ^a
HPV negative	2348 (60.8)
HPV positive	1515 (39.2)
Low-risk HPV ^b	567 (14.7)
High-risk HPV ^c	1032 (26.7)
Unknown-risk HPV ^d	503 (13.0)
Multiple HPV types	677 (17.5)
HPV-16	291 (7.5)
HPV-53	202 (5.2)
HPV-62	156 (4.0)
HPV-54	140 (3.6)
HPV-39	129 (3.3)
HPV-61	123 (3.2)
HPV-31	116 (3.0)
HPV-52	115 (3.0)
HPV-51	114 (3.0)
HPV-58	99 (2.6)
HPV-56	95 (2.5)
HPV-45	91 (2.4)
HPV-66	91 (2.4)
HPV-18	89 (2.3)
HPV-70	88 (2.3)
HPV-84	87 (2.3)
HPV-59	82 (2.1)
HPV-6	78 (2.0)
CP6108	69 (1.8)
HPV-81	66 (1.7)
HPV-83	61 (1.6)
HPV-42	48 (1.2)
HPV-68	46 (1.2)
HPV-55	43 (1.1)
HPV-73	39 (1.0)
HPV-33	38 (1.0)
HPV-35	37 (1.0)
HPV-72	33 (0.9)
HPV-82	23 (0.6)
HPV-40	22 (0.6)
HPV-67	20 (0.5)
HPV-11	19 (0.5)
HPV-69	8 (0.2)
IS39	8 (0.2)
HPV-71	7 (0.2)
HPV-26	7 (0.2)
HPV-64	4 (0.1)
HPV-57	1 (0.0)

^a Because of multiple infections, women may be counted more than once.

^b Low-risk HPV types include 6, 11, 40, 42, 53, 54, 57, 66, and 84.

^c High-risk HPV types include 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, 73, 82, and 83.

^d Uncharacterized HPV types include 61, 62, 64, 67, 69, 70, 71, 72, 81, CP6108, and IS39.

shown is the prevalence of low-risk HPV (types 6, 11, 40, 42, 53, 54, 57, 66, and 84), high-risk HPV (types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, 73, 82, and 83), uncharacterized HPV (types 61, 62, 64, 67, 69, 70, 71, 72, 81, CP6108, and IS39), and overall HPV prevalence. Table 2

lists the variables considered in the analyses, with corresponding unadjusted HPV prevalence data. The mean and median age was 28 years. The number of lifetime sex partners and

number of sex partners in the last year were 7 and 1, respectively.

Of women positive for HPV, 838 (55.3%) were infected with a single HPV type, and 677 (44.7%) were infected with >1 HPV type. The distribution of multiple HPV detection among HPV-positive subjects was as follows: 354 (23.4%) with 2 types, 182 (12.0%) with 3 types, 67 (4.4%) with 4 types, 42 (2.8%) with 5 types, and 32 (2.1%) with ≥ 6 types. Detection of multiple HPV types was more common in younger women, women with >2 lifetime sex partners, and women with >2 sex partners in the last year ($P < .01$, Kruskal-Wallis test for each). After adjusting for age and sex history, the correlations among the detection of specific HPV types were small. The median partial correlation coefficient was 0.015, and all were within the range -0.02 to 0.16.

As expected, the concurrent Pap smear report was highly correlated with the detection of high-risk HPV types and less strongly with low-risk and uncharacterized HPV types (table 2). The prevalence odds ratios (pORs) of specific diagnoses given the detection of specific HPV groups, by use of a normal diagnosis as the reference, were as follows. For high-risk HPV types, the pOR and 95% confidence interval of atypical squamous cells of unknown significance (ASCUS) was 2.9 (2.1–3.9), low-grade squamous intraepithelial lesion (LSIL) was 9.5 (6.2–14.6), and high-grade squamous intraepithelial lesion (HSIL) was 22.5 (5.1–98.9). For low-risk HPV types, the pORs of ASCUS was 2.3 (1.6–3.2), LSIL was 4.1 (2.8–6.0), and HSIL was 0.9 (0.2–4.2). For uncharacterized HPV types, the pORs of ASCUS was 1.9 (1.2–2.8), LSIL was 2.5 (1.6–3.8), and HSIL was 2.4 (0.8–7.6). pORs were not reported for atypical glandular cells of unknown significance, because very few women ($n = 10$) had this diagnosis.

Association of sex history and age with the detection of HPV. Figure 1 shows the relationship of age and sex history to the prevalence of low-risk, high-risk, and uncharacterized HPV types unadjusted for other factors. The predominant features were an increasing prevalence of both low-risk, high-risk, and uncharacterized HPV types with the number of lifetime and recent sex partners. A decreasing prevalence of high-risk and low-risk HPV types, but not uncharacterized HPV types, with age was evident. The association of HPV prevalence with the number of sex partners in the past year was comparable for low-risk, high-risk, and uncharacterized HPV types and for all age groups. Women reporting ≥ 2 sex partners within the past year had a prevalence of HPV that was 10%–20% greater than women who reported no sex partners or only 1 sex partner in the past year; a consistent difference for women across all age groups and for both low-risk, high-risk, and uncharacterized HPV types. In contrast, the increase in the prevalence of HPV with the number of lifetime sex partners varied by age and by HPV group. Although an increase in the prevalence of HPV with increasing number of partners was seen for all ages and for high-risk, low-risk, and

uncharacterized HPV types, the increase was greatest for younger women and for high-risk HPV types.

The relationship of HPV prevalence to age was more complex. Younger women (18–22 years old) had a higher prevalence of high-risk and low-risk HPV types than did older women (33–40 years old), and the difference increased as the number of lifetime sex partners increased. High-risk HPV types were detected in 59% of women 18–22 years old who reported ≥ 8 sex partners, compared with 18% of the women 33–40 years old who reported a similar number of lifetime sex partners. In contrast, for women who reported only 1 lifetime sex partner, the prevalence of high-risk HPVs was 20% for women 18–22 years old and 13% for those 33–40 years old. The prevalence of low-risk HPV types varied less with age than did the high-risk HPV types, regardless of the number of lifetime or recent sex partners. These patterns of association between age and HPV prevalence were not seen for uncharacterized HPV types.

Association of other characteristics with the detection of HPV. Characteristics with significant associations with the detection of high-risk, low-risk, uncharacterized HPV types, and/or all HPV types after adjusting for age and number of sex partners included ethnicity, education, income, marital status, number of live births, ever having used birth control pills, ever having used a diaphragm, history of chlamydia, and ever having had a sex partner with genital warts (data not shown). Age at menarche, age at first intercourse, current cigarette smoking, and ever having used condoms were not significant predictors of HPV detection after controlling for age and number of sex partners. The sex history of the current partner was highly associated with the prevalence of all HPV groups but was not included in the multivariate models, because the collection of this information did not begin until May 1997. Thus, these data were obtained for only about half the study participants. The number of years since first sexual intercourse was correlated highly with age and was not included in the multivariate models. The age at first sexual intercourse was not associated significantly with the detection of HPV after adjustment for age and number of sex partners.

All significant factors were included in the final multivariable logistic regression models along with age, number of lifetime sex partners, and number of sex partners in the past year. Table 3 shows the results of these logistic regression models. All factors, with the exception of ever having used birth control pills, ever having used a diaphragm, and history of chlamydia, remained statistically significant predictors of low-risk, high-risk, uncharacterized, or overall HPV detection. Most pORs were small and not consistent predictors for low-risk, high-risk, or uncharacterized HPV types. The only consistent predictor of increased HPV prevalence was being single (vs. being married). Women who refused to give or did not know their income had significantly increased odds of high-risk HPV and of all HPV types. Hispanic women showed slightly higher odds of high-

Table 2. Study population demographics and human papillomavirus (HPV) prevalence.

Characteristic	No. (%)	Prevalence of HPV, %			
		Any HPV	Low-risk HPV	High-risk HPV	Uncharacterized HPV
Age in years ^{a,b,c,d}					
18–22	910 (23.6)	50.5	21.6	39.1	12.5
23–27	1015 (26.3)	44.8	18.0	31.6	14.8
28–32	848 (22.0)	33.0	8.6	20.6	13.2
33–40	1090 (28.2)	29.4	10.5	16.5	11.7
No. lifetime sex partners ^{a,b,c,d}					
1	735 (19.0)	24.5	8.4	16.1	6.8
2–3	935 (24.2)	35.8	12.1	26.1	10.8
4–7	1204 (31.2)	41.9	16.4	29.3	13.9
≥8	989 (25.6)	50.2	19.6	32.1	18.7
No. of sex partners in past year ^{a,b,c,d}					
0 or 1	3366 (87.1)	35.1	12.4	23.4	11.1
≥2	496 (12.9)	66.9	30.0	49.2	25.8
Unknown	1				
Current partner's no. of previous sex partners ^{a,b,c,d}					
No current partner	343 (8.9)	47.8	19.0	29.4	17.5
1	280 (7.2)	17.5	6.4	12.1	3.6
2–4	803 (20.8)	33.4	14.2	20.9	9.6
5–10	653 (16.9)	39.8	15.0	29.1	12.1
≥10	419 (10.8)	51.3	20.0	34.1	17.7
Unknown	1365 (35.3)	41.0	13.8	29.0	14.9
Age at first intercourse ^{a,b,c,d}					
≤15	1006 (26.1)	47.3	17.9	32.7	16.3
16–17	1427 (37.0)	39.5	15.7	27.7	12.1
18–19	890 (23.0)	36.2	12.4	24.3	12.9
≥20	538 (13.9)	28.3	9.9	16.7	9.7
Unknown	2				
Ethnic group ^c					
Non-Hispanic white	1658 (42.9)	37.6	14.1	23.7	12.8
Hispanic white	2204 (57.1)	40.4	15.1	28.9	13.2
Unknown	1				
Marital status ^{a,b,c,d}					
Never married	917 (23.7)	53.9	23.6	39.0	17.9
Married	1884 (48.8)	27.8	9.3	17.2	8.9
Living with partner	703 (18.2)	44.8	15.9	33.1	12.9
Divorced/separated/widowed	358 (9.3)	50.6	17.9	32.4	22.6
Unknown	1				
Education ^{a,c,d}					
High school or less	1320 (34.2)	40.8	15.6	28.5	13.0
Some college	1565 (40.5)	42.4	15.3	28.8	14.4
College graduate	976 (25.3)	32.0	12.5	20.9	11.0
Unknown	2				
Income ^{a,b,c,d}					
<\$20,000	1280 (33.1)	44.8	17.1	31.2	15.8
≥\$20,000	2342 (60.6)	34.6	12.5	22.5	11.2
Unknown	241 (6.2)	54.8	22.8	44.0	15.8
Parity ^{a,b,c}					
0	1217 (31.5)	44.6	19.4	30.4	14.6
1	1134 (29.4)	41.8	16.1	30.0	13.1
2	1017 (26.3)	33.2	9.5	22.1	11.2
≥3	493 (12.8)	32.3	10.1	19.7	12.4
Unknown	2				
Age at menarche					
<12	682 (17.8)	39.0	15.5	26.2	11.6
12	1120 (29.2)	37.8	13.7	27.3	12.0
13	1070 (27.9)	40.7	15.8	26.7	14.1
≥14	965 (25.1)	39.6	14.2	26.2	14.2
Unknown	26				
Ever smoked ^{a,b,c}					
No	2472 (64.0)	37.1	13.5	24.8	12.8
Yes	1390 (36.0)	42.9	16.8	30.1	13.4
Unknown	1				
Ever used oral contraceptives ^{a,b,c}					
No	402 (10.4)	45.3	18.9	32.3	11.7
Yes	3461 (89.6)	38.5	14.2	26.1	13.2

(continued)

Table 2. (Continued.)

Characteristic	No. (%)	Prevalence of HPV, %			
		Any HPV	Low-risk HPV	High-risk HPV	Uncharacterized HPV
Ever used diaphragm ^{a,b,c}					
No	3385 (87.6)	40.5	15.3	28.0	13.3
Yes	478 (12.4)	30.1	10.5	17.8	10.9
Ever had sexual partner with genital warts ^{a,b,d}					
Unknown	189 (4.9)	44.4	18.5	32.3	15.9
No	3437 (89.0)	38.3	14.2	26.1	12.6
Yes	237 (6.1)	48.5	19.0	30.8	17.3
Self-reported history of STD					
None	2712 (70.2)	36.1	13.5	24.7	10.7
Chlamydia ^{a,b,c,d}	599 (15.5)	51.3	18.7	37.1	19.5
Herpes ^d	222 (5.7)	43.2	14.4	26.1	18.9
Trichomonas ^d	187 (4.8)	41.7	13.9	24.1	20.3
PID	111 (2.9)	45.0	15.3	33.3	18.9
Any ^{a,b,c,d}	1151 (29.8)	46.7	17.4	31.5	18.4
Pap diagnosis ^{a,b,c,d}					
Normal	3283 (85.0)	36.1	13.2	23.8	12.1
ASCUS	188 (4.9)	63.3	25.5	47.3	20.7
AGUS	10 (0.3)	60.0	50.0	50.0	0.0
LSIL	115 (3.0)	88.7	38.3	74.8	25.2
HSIL	16 (0.4)	93.8	12.5	85.7	25.0
Unknown	251 (6.5)	35.5	13.9	22.7	13.9

NOTE. AGUS, atypical glandular cells of unknown significance; ASCUS, atypical squamous cells of unknown significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; PID, pelvic inflammatory disease; STD, sexually transmitted disease.

^a Prevalence of HPV (any type) varies significantly across categories ($P < .05$, Pearson χ^2 test).

^b Prevalence of low-risk HPV types varies significantly across categories ($P < .05$, Pearson χ^2 test).

^c Prevalence of high-risk HPV types varies significantly across categories ($P < .05$, Pearson χ^2 test).

^d Prevalence of uncharacterized HPV types varies significantly across categories ($P < .05$, Pearson χ^2 test).

risk HPV than did non-Hispanic white women. A lower prevalence of low-risk HPV was found for women having 2 live births, compared with nulliparous women.

Discussion

HPV prevalence provides a measure of the percentage of persons in a population who have new, persistent, or recurring HPV infection at a particular point in time. Prevalence can vary several fold, depending on the method of HPV detection and the demographic and sexual behavior characteristics of the group under study. We detected HPV in 39% of the subjects enrolled in the current study. We believe that the HPV prevalence observed is consistent with the age and sex history of this population. Although the mean and median age of this population was 28 years, previous studies in New Mexico reported a higher number of lifetime sex partners (mean, 6 lifetime sex partners) and HPV prevalence (44.3%) in young women (median age, 23 years) [21]. Other studies reported that, in sexually active young women, HPV prevalence is relatively high [11, 19, 40]. The HPV DNA detection methods used in our report are highly sensitive and target a much broader spectrum of anogenital HPV genotypes than do other methods. We did not use a generic probe to detect HPV genotypes that are amplified with the consensus primers but not targeted by a type-specific oligonucleotide probe. However, the 38 HPV genotypes identi-

fied by the methods presented here are reasonably comprehensive, and inclusion of a generic probe would have resulted in only a minor increase in HPV prevalence.

The predominant HPV types detected in this study were in the high-risk HPV group, which agrees with several PCR-based studies [22, 25–27, 29, 41, 42]. Our study demonstrated a strong correlation between high-risk HPV detection and cytologic abnormalities, as expected, and is consistent with previous findings [10–12, 23, 40, 43–47]. No studies have provided adequate sample sizes from single geographic areas to accurately assign risk to many individual HPV types for high-grade or invasive cervical disease. A separate report from our group will further consider type-specific risk assignment for genital HPVs in the context of carcinoma in situ and invasive cancers collected for an expanded case-control investigation.

Epidemiologic studies have shown that key risk factors for HPV detection include age and sexual behavior [10, 16–21, 26, 45, 48–51]. We saw a decrease in overall HPV prevalence with increasing age and an increase in HPV prevalence with increasing numbers of lifetime sex partners on univariate analysis, which is consistent with previous reports. The positive association with increasing partner number was seen in high-risk, low-risk, and uncharacterized HPV analyses. In fact, recent sexual behavior was associated positively with overall HPV detection in all age categories, emphasizing that opportunity for exposure is a strong predictor of HPV detectability. We found that the prevalence of

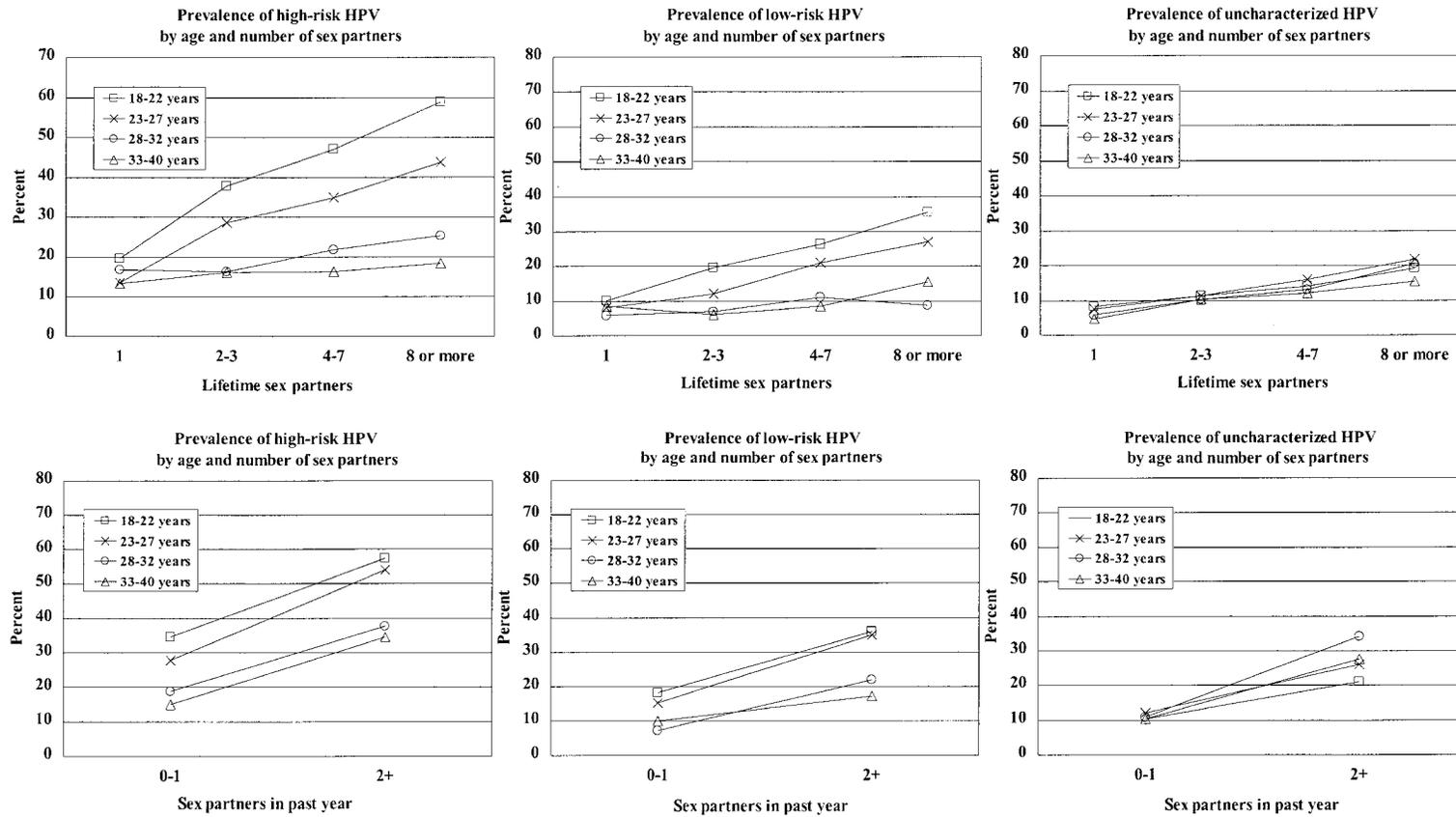


Figure 1. Prevalence of high-risk, low-risk, and uncharacterized human papillomavirus (HPV) types by age, number of lifetime sex partners, and number of sex partners in the past year

Table 3. Multiple logistic regression on prevalence odds ratios (pORs) of human papillomavirus (HPV) detection in 3671 women 18–40 years old, Albuquerque, New Mexico, 1996–2000.

Characteristic	No.	Any HPV pOR (95% CI)	Low-risk HPV pOR (95% CI)	High-risk HPV pOR (95% CI)	Uncharacterized HPV pOR (95% CI)
Lifetime no. of sex partners, age group in years					
1					
18–22	227	1.0	1.0	1.0	1.0
23–27	183	1.0 (0.6–1.5)	1.1 (0.5–2.1)	0.9 (0.5–1.5)	1.2 (0.6–2.5)
28–32	133	1.5 (0.9–2.5)	0.9 (0.4–2.2)	1.4 (0.8–2.5)	1.0 (0.4–2.5)
33–40	184	1.0 (0.6–1.7)	1.4 (0.7–2.8)	1.0 (0.6–1.8)	0.9 (0.4–2.0)
2–3					
18–22	286	1.9 (1.3–2.8) ^a	2.0 (1.2–3.4) ^a	2.3 (1.5–3.5) ^a	1.1 (0.6–2.1)
23–27	221	2.1 (1.4–3.1) ^a	1.6 (0.9–2.9)	1.9 (1.2–3.0) ^a	1.6 (0.8–3.0)
28–32	205	1.1 (0.7–1.7)	0.9 (0.5–1.9)	1.1 (0.6–1.8)	1.5 (0.8–3.1)
33–40	190	1.3 (0.8–2.1)	0.9 (0.4–2.0)	1.2 (0.7–2.0)	1.7 (0.8–3.5)
4–7					
18–22	231	3.1 (2.0–4.6) ^a	2.8 (1.6–4.8) ^a	3.1 (2.0–4.9) ^a	1.3 (0.7–2.4)
23–27	350	2.7 (1.9–4.0) ^a	2.8 (1.7–4.8) ^a	2.4 (1.6–3.7) ^a	2.1 (1.2–3.8) ^a
28–32	262	1.6 (1.0–2.4) ^a	1.5 (0.8–2.8)	1.4 (0.8–2.2)	1.7 (0.9–3.3)
33–40	311	1.3 (0.8–1.9)	1.2 (0.6–2.2)	1.1 (0.7–1.8)	1.8 (1.0–3.4)
≥8					
18–22	130	5.0 (3.0–8.3) ^a	4.2 (2.3–7.6) ^a	4.2 (2.5–6.9) ^a	1.6 (0.8–3.2)
23–27	214	3.3 (2.2–5.2) ^a	3.1 (1.8–5.6) ^a	3.0 (1.9–4.7) ^a	2.2 (1.2–4.1) ^a
28–32	211	2.1 (1.4–3.3) ^a	0.9 (0.5–1.8)	1.5 (0.9–2.5)	2.6 (1.4–4.8) ^a
33–40	333	1.9 (1.2–2.8) ^a	2.1 (1.2–3.8) ^a	1.2 (0.7–1.9)	2.0 (1.1–3.8) ^a
No. of partners in past year					
0 or 1	3210	1.0	1.0	1.0	1.0
≥2	461	1.9 (1.5–2.5) ^a	1.7 (1.3–2.2) ^a	1.8 (1.4–2.2) ^a	1.9 (1.4–2.4) ^a
Ethnicity					
Non-Hispanic white	1552	1.0	1.0	1.0	1.0
Hispanic	2119	1.1 (0.9–1.3)	1.1 (0.9–1.4)	1.3 (1.1–1.5) ^a	1.0 (0.8–1.3)
Education					
College graduate	914	1.0	1.0	1.0	1.0
Some college	1486	1.1 (0.9–1.4)	0.9 (0.7–1.2)	1.0 (0.8–1.2)	1.2 (0.9–1.6)
High school or less	1271	1.1 (0.8–1.4)	1.0 (0.8–1.5)	0.9 (0.7–1.1)	1.1 (0.8–1.6)
Income					
≥\$20,000	2218	1.0	1.0	1.0	1.0
<\$20,000	1221	1.1 (0.9–1.3)	1.1 (0.9–1.4)	1.0 (0.9–1.3)	1.2 (0.9–1.5)
Refused or unknown	232	1.5 (1.1–2.1) ^a	1.4 (1.0–2.1)	1.6 (1.2–2.3) ^a	1.4 (0.9–2.2)
Marital status					
Never married	859	1.0	1.0	1.0	1.0
Married	1795	0.6 (0.5–0.8) ^a	0.7 (0.5–0.9) ^a	0.6 (0.5–0.8) ^a	0.7 (0.5–0.9) ^a
Living with partner	679	0.8 (0.7–1.1)	0.8 (0.6–1.0)	0.9 (0.7–1.2)	0.9 (0.6–1.2)
Divorced/widowed	338	1.1 (0.8–1.5)	1.0 (0.7–1.4)	1.1 (0.8–1.5)	1.3 (0.9–1.9)
No. of live births					
0	1141	1.0	1.0	1.0	1.0
1	1089	1.0 (0.8–1.2)	0.9 (0.7–1.2)	1.1 (0.9–1.4)	1.0 (0.7–1.2)
2	964	0.8 (0.7–1.0)	0.6 (0.4–0.8) ^a	0.9 (0.7–1.2)	0.8 (0.6–1.1)
≥3	477	0.9 (0.7–1.2)	0.7 (0.5–1.1)	1.0 (0.7–1.4)	0.9 (0.6–1.4)
Ever had chlamydia					
No	3118	1.0	1.0	1.0	1.0
Yes	553	1.1 (0.9–1.4)	0.9 (0.7–1.2)	1.2 (1.0–1.5)	1.3 (1.0–1.6)
Ever used oral contraceptives					
No	391	1.0	1.0	1.0	1.0
Yes	3280	0.8 (0.7–1.1)	0.8 (0.6–1.1)	0.9 (0.7–1.2)	1.0 (0.7–1.4)
Ever used diaphragm					
No	3223	1.0	1.0	1.0	1.0
Yes	448	0.8 (0.7–1.1)	0.9 (0.6–1.2)	0.9 (0.6–1.1)	0.9 (0.6–1.3)
Ever had partner with genital warts					
No	3436	1.0	1.0	1.0	1.0
Yes	235	1.4 (1.1–1.9) ^a	1.4 (1.0–2.1) ^a	1.3 (0.9–1.7)	1.3 (0.9–1.8)

NOTE. These models were based on 3671 subjects with complete data. pORs were adjusted for all variables. CI, confidence interval.

^a pOR significantly different from 1.0, $P < .05$.

high-risk, low-risk, uncharacterized, and all HPV types was significantly lower in married than in single women. This difference remained after adjusting for age and number of lifetime sex partners. One possible explanation is that the time since last exposure to HPV is longer in married women than in single women. We suggest that future models include marital status to more completely adjust for sex history.

Factors that were significantly associated with HPV detection when examined individually were not associated or were weakly associated after controlling for confounding by age and numbers of lifetime and recent sex partners. We believe that most of the other factors associated with HPV prevalence in previous studies were markers of age or sex history. Accurate measurement of both the HPV status and the exposure variables in this study probably reduced residual confounding that may have led to prior inconsistent associations with age and sexual behavior-related variables reported in other studies.

The negative association with age was not consistent when we examined determinants of high-risk and low-risk HPV detection separately from uncharacterized HPV detection. Several studies reported findings that suggest that high-risk HPV types have a different association with age than do low-risk HPV types [23, 25–28]; however, these studies often included uncharacterized HPV types in the low-risk group. High-risk HPV types followed the predicted natural history profile of HPV infection that has been reported in many previous studies in that prevalence was highest among the youngest most sexually active women and lowest among older sexually monogamous women. Franco et al. [42] found that the average duration of high-risk HPV infection was constant across age categories, whereas the incidence rate of high-risk HPV infection was 2 times higher in women <35 years old than in women \geq 35 years old. Given the relationship of the prevalence odds, incidence, and duration of infection, [$\text{prevalence}/(1 - \text{prevalence}) = \text{incidence} \times \text{duration}$], our observations of higher prevalence among younger women is consistent with the high-risk HPV incidence and duration data measured in the Brazilian cohort. In this same cohort, when patterns of low-risk HPV infection were examined, there was a constant incidence of low-risk HPV across age categories but there was \sim 2 times longer duration of low-risk HPV infection in women <35 years old than in women \geq 35 years old. Our observations are generally consistent with the expectation of somewhat higher low-risk HPV prevalence among younger women; however, the magnitude of prevalence differences by age was diluted relative to the age–high-risk HPV association.

The explanation of the prevalence differences by age and sexual behavior within each HPV group is not clear but is potentially strongly influenced by differences in immune response to HPV [17, 20, 24, 40, 51]. For example, the high-risk and low-risk HPV prevalence patterns reported here are consistent with a historical model of HPV natural history that proposes that HPV is acquired near the onset of sexual activity,

is relatively transient in that most infections resolve within about 1 year, and provides lifetime protection against reinfection. One hypothesis that allows for this model to fit with the observed data assumes that every detectable high-risk or low-risk HPV infection is a result of first exposure to that HPV genotype. Duration would remain constant, independent of the age at which exposure is incurred. The incidence of high-risk or low-risk HPV infection appears to decrease with age, which may be a function of both less frequent exposures and, importantly, a decrease in susceptibility to infection given exposure due to completely protective acquired immunity. Cross-sectional data cannot measure the true number of susceptibles in the population; therefore, the denominator in the incidence rate would be overestimated, which would result in an apparent decrease in incidence rate, as has been observed.

The uncharacterized HPV patterns do not support this model in that prevalence does not vary by age. HPV types 61, 62, 71, 72, 81, and CP6108 form a phylogenetic branch separate from either the low-risk or high-risk HPV branches [3, 4]. Because this phylogenetic branch has not been well studied independently of the other HPV branches, little is known in regard to persistence and immunogenicity of these types. The remaining HPV types in the uncharacterized group (HPV types 64, 67, 69, 70, and IS39) are related to other established high-risk HPV types, but they have not been studied adequately to determine their relationship to cervical disease outcomes.

Prevalence rates that are critically dependent on unmeasured factors such as prevalence of HPV among the male partners of the women studied leave the differences between high-risk, low-risk, and uncharacterized HPV natural histories still incompletely explained. However, they suggest that longitudinal studies should examine potential markers of immune response and susceptibility, potential for reinfection of HPV a decade or more after first infection, and the prevalence of HPV in males to allow for a more precise understanding of the natural history of type-specific HPV infections. The present data suggest that the natural history of uncharacterized HPV types differ from those of both high-risk and low-risk HPV types. This difference may reflect combined influences of molecular mechanisms of virus-host interactions, including immunity, viral fitness, and persistence characteristics.

Acknowledgments

We thank T. Matsukura (Laboratory of Tumor Viruses, Dept. of Virology II, Tokyo, Japan) for providing cloned plasmid HPV types 61, 62, 64, 67, 69, and 71 and E.-M. de Villiers (Deutsches Krebsforschungszentrum, Heidelberg, Germany) for providing HPV-72. We also thank Valerie Venghiattis, Carrie Gurule, Robin Mack, Cathryn Cunningham, Gilda de la Garza, Mary Jane Rodriguez, Marilyn Lebednik, and Dana Shock for recruiting the study subjects and for administering the questionnaires; the health care providers at the University of New Mexico Family and Women's Health clinics and Lovelace Women's Health Services for help and cooperation with recruitment and sample

collection efforts; Sharon Wayne for data management and statistical support; Lori Lambert for initial study coordination activities; and Michele Manos for critical review of this manuscript.

References

- de Villiers EM. Human pathogenic papillomavirus types: an update. *Curr Top Microbiol Immunol* **1994**;186:1–12.
- Bernard HU, Chan SY, Manos MM, et al. Identification and assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length polymorphisms, nucleotide sequence, and phylogenetic algorithms. *J Infect Dis* **1994**;170:1077–85.
- Chan SY, Delius H, Halpern AL, Bernard HU. Analysis of genomic sequences of 95 papillomavirus types: uniting typing, phylogeny, and taxonomy. *J Virol* **1995**;69:3074–83.
- Myers G, Bernard HU, Delius H, et al., eds. *Human papillomaviruses 1994: a compilation and analysis of nucleic acid and amino acid sequences*. Los Alamos, NM: Los Alamos National Laboratory, **1994**.
- Manos MM, Waldman J, Zhang TY, et al. Epidemiology and partial nucleotide sequence of four novel genital human papillomaviruses. *J Infect Dis* **1994**;170:1096–9.
- Ong CK, Bernard HU, Villa LL. Identification of genomic sequences of three novel human papillomavirus sequences in cervical smears of Amazonian Indians. *J Infect Dis* **1994**;170:1086–8.
- Peyton CL, Jansen AM, Wheeler CM, et al. A novel human papillomavirus sequence from an international cervical cancer study. *J Infect Dis* **1994**;170:1093–5.
- Peyton CL, Wheeler CM. Identification of five novel human papillomavirus sequences in the New Mexico triethnic population. *J Infect Dis* **1994**;170:1089–92.
- Tachezy R, Van Ranst MA, Cruz Y, Burk RD. Analysis of short novel human papillomavirus sequences. *Biochem Biophys Res Commun* **1994**;204:820–7.
- Schiffman MH, Bauer HM, Hoover RN, et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. *J Natl Cancer Inst* **1993**;85:958–64.
- Bauer HM, Ting Y, Greer CE, et al. Genital human papillomavirus infection in female university students as determined by a PCR-based method. *JAMA* **1991**;265:472–7.
- Kjaer SK, van den Brule AJC, Bock JE, et al. Human papillomavirus: the most significant risk determinant of cervical intraepithelial neoplasia. *Int J Cancer* **1996**;65:601–6.
- Bosch FX, Manos MM, Muñoz N, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. *J Natl Cancer Inst* **1995**;87:796–802.
- Lörincz AT, Reid R, Jenson AB, Greenberg MD, Lancaster W, Kurman RJ. Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. *Obstet Gynecol* **1992**;79:328–37.
- Van Ranst M, Kaplan JB, Burk RD. Phylogenetic classification of human papillomaviruses: correlation with clinical manifestations. *J Gen Virol* **1992**;73:2653–60.
- Bauer HM, Hildesheim A, Schiffman MH, et al. Determinants of genital human papillomavirus infection in low-risk women in Portland, Oregon. *Sex Transm Dis* **1993**;20:274–8.
- Burk RD, Kelly P, Feldman J, et al. Declining prevalence of cervicovaginal human papillomavirus infection with age is independent of other risk factors. *Sex Transm Dis* **1996**;23:333–41.
- Hildesheim A, Gravitt P, Schiffman MH, et al. Determinants of genital human papillomavirus infection in low-income women in Washington, DC. *Sex Transm Dis* **1993**;20:279–85.
- Ley C, Bauer HM, Reingold A, et al. Determinants of genital human papillomavirus infection in young women. *J Natl Cancer Inst* **1991**;83:997–1003.
- Melkert PWJ, Hopman E, van den Brule AJC, et al. Prevalence of HPV in cytomorphologically normal cervical smears, as determined by the polymerase chain reaction, is age-dependent. *Int J Cancer* **1993**;53:919–23.
- Wheeler CM, Parmenter CA, Hunt WC, et al. Determinants of genital human papillomavirus infection among cytologically normal women attending the University of New Mexico student health center. *Sex Transm Dis* **1993**;20:286–9.
- Franco EL, Villa LL, Ruiz A, Costa MC. Transmission of cervical human papillomavirus infection by sexual activity: differences between low and high oncogenic risk types. *J Infect Dis* **1995**;172:756–63.
- Herrero R, Hildesheim A, Bratti C, et al. Population-based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. *J Natl Cancer Inst* **2000**;92:464–74.
- Hildesheim A, Schiffman MH, Gravitt PE, et al. Persistence of type-specific human papillomavirus infection among cytologically normal women. *J Infect Dis* **1994**;169:235–40.
- Jacobs MV, Walboomers JMM, Snijders PJF, et al. Distribution of 37 mucosotropic HPV types in women with cytologically normal cervical smears: the age-related patterns for high-risk and low-risk types. *Int J Cancer* **2000**;87:221–7.
- Kjaer SK, van den Brule AJC, Bock JE, et al. Determinants for genital human papillomavirus (HPV) infection in 1000 randomly chosen young Danish women with normal Pap smear: are there different risk profiles for oncogenic and nononcogenic HPV types? *Cancer Epidemiol Biomarkers Prev* **1997**;6:799–805.
- Richardson H, Franco E, Pintos J, Bergeron J, Arella M, Tellier P. Determinants of low-risk and high-risk cervical human papillomavirus infections in Montreal university students. *Sex Transm Dis* **2000**;27:79–86.
- Rousseau MC, Franco EL, Villa LL, et al. A cumulative case-control study of risk factor profiles for oncogenic and nononcogenic cervical human papillomavirus infections. *Cancer Epidemiol Biomarkers Prev* **2000**;9:469–76.
- Jacobs MV, Snijders PJF, van den Brule AJ, Helmerhorst TJ, Meijer CJ, Walboomers JM. General primer GP5+/6(+)-mediated polymerase chain reaction–enzyme immunoassay met detection of 14 high-risk and 6 low-risk human papillomaviruses genotypes in cervical scrapings. *J Clin Microbiol* **1997**;35:795–5.
- de Roda Husman AM, Walboomers JMM, van den Brule AJC, Meijer CJLM, Snijders PJF. The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *J Gen Virol* **1995**;76:1057–62.
- Bauer HM, Greer CE, Manos MM. Determination of genital human papillomavirus infection by consensus PCR amplification. In: Herrington CS, McGee JO'D, eds. *Diagnostic molecular pathology, a practical approach*. Oxford: Oxford University Press, **1992**:131–52.
- Gravitt P, Hakenewerth A, Stoerker J. A direct comparison of methods proposed for use in widespread screening of human papillomavirus infections. *Mol Cell Probes* **1991**;5:65–72.
- Gravitt PE, Manos MM. Polymerase chain reaction–based methods for the detection of human papillomavirus DNA. In: Muñoz N, Bosch FX, Shah KV, Meheus A, eds. *The epidemiology of cervical cancer and human papillomavirus*. Lyon, France: International Agency for Research on Cancer, **1992**:121–33.
- Manos MM, Ting Y, Wright DK, Lewis AJ, Broker TR, Wolinsky SM. Use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. *Cancer Cells* **1989**;7:209–14.
- Gravitt PE, Peyton CL, Alessi TQ, et al. Improved amplification of genital human papillomaviruses. *J Clin Microbiol* **2000**;38:357–61.
- Klefer B, van Doorn LJ, ter Schegget J, et al. Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. *Am J Pathol* **1998**;153:1731–9.
- Gravitt PE, Peyton CL, Apple RJ, Wheeler CM. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. *J Clin Microbiol* **1998**;36:3020–7.
- Stewart ACM, Gravitt PE, Cheng S, Wheeler CM. Generation of entire

- human papillomavirus genomes by long PCR: frequency of errors produced during amplification. *Genome Res* **1995**;5:79–88.
39. SAS Institute. SAS/STAT software: changes and enhancements through release 6.11. Cary, NC: SAS, **1996**:219–30.
 40. Ho GYF, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus: infection in young women. *N Engl J Med* **1998**;338:423–8.
 41. Kotloff KL, Wasserman SS, Russ K, et al. Detection of genital human papillomavirus and associated cytological abnormalities among college women. *Sex Transm Dis* **1998**;25:243–50.
 42. Franco EL, Villa LL, Sobrinho J, et al. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. *J Infect Dis* **1999**;180:1415–23.
 43. Davidson M, Schnitzer PG, Bulkow LR, et al. The prevalence of cervical infection with human papillomavirus and cervical dysplasia in Alaska native women. *J Infect Dis* **1994**;169:792–800.
 44. Kiviat NB, Koutsky LA, Critchlow CW, et al. Prevalence and cytologic manifestations of human papillomavirus (HPV) types 6, 11, 16, 18, 31, 33, 35, 42, 43, 44, 45, 51, 52, and 56 among 500 consecutive women. *Int J Gynecol Pathol* **1992**;11:197–203.
 45. Koutsky LA, Holmes KK, Critchlow CW. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. *N Engl J Med* **1992**;327:1272–8.
 46. Sherman ME, Schiffman MH, Lörincz AT, et al. Toward objective quality assurance in cervical cytopathology: correlation of cytopathologic diagnoses with detection of high-risk human papillomavirus types. *Am J Clin Pathol* **1994**;102:182–7.
 47. Liaw KL, Glass AG, Manos MM, et al. Detection of human papillomavirus DNA in cytologically normal women and subsequent cervical squamous intraepithelial lesions. *J Natl Cancer Inst* **1999**;91:954–60.
 48. Moscicki AB, Palefsky J, Gonzales J, Schoolnik GK. Human papillomavirus infection in sexually active adolescent females: prevalence and risk factors. *Pediatr Res* **1990**;28:507–13.
 49. Muñoz N, Kato I, Bosch X, et al. Risk factors for HPV DNA detection in middle-aged women. *Sex Transm Dis* **1996**;23:504–10.
 50. Reeves WC, Gary HE, Johnson PR, et al. Risk factors for genital papillomavirus infection in populations at high and low risk for cervical cancer. *J Infect Dis* **1994**;170:753–8.
 51. Figueroa JP, Ward E, Luthi TE, Vermund SH, Brathwaite AR, Burk RD. Prevalence of human papillomavirus among STD clinic attenders in Jamaica: association of younger age and increased sexual activity. *Sex Transm Dis* **1995**;22:114–8.