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?[1]

Susanne Krüger Kjær,[2] Adriaan J. C. van den Brule, Johannes E. Bock, Paul A. Poll, Gerda Engholm, Mark E. Sherman, Jan M. M. Walboomers, and Chris J. L. M. Meijer

Danish Cancer Society, Division for Cancer Epidemiology, Copenhagen, Denmark [S. K. K.]; Department of Pathology, Section of Molecular Pathology, University Hospital Vrije Universiteit, Amsterdam, the Netherlands [A. J. C. v. d. B., J. M. M. W., C. J. L. M. M.]; Department of Gynecology, Rigshospitalet, Copenhagen, Denmark [J. E. B.]; Department of Pathology, Nykøbing Falster Hospital, Nykøbing Falster, Denmark [P. A. P.]; Centre for Research in Health and Social Statistics, Copenhagen, Denmark [G. E.]; and Department of Pathology, Johns Hopkins Hospital, Baltimore, Maryland [M. E. S.]

Abstract

Most studies of risk factors for human papillomavirus (HPV) DNA detection have focused on overall HPV positivity and have not examined determinants for high-risk and low-risk HPV types separately.

We studied risk determinants for genital HPV infection in 1000 randomly chosen women (20–29 years) with normal cervical cytology from Copenhagen, Denmark. All women had a personal interview, a Pap smear, and cervical swabs for HPV DNA detection using a PCR technique. On the basis of their association with cervical cancer, the HPV types were categorized as belonging to a high-risk group ("oncogenic types") or a low-risk group ("nononcogenic types"). The overall HPV detection rate was 15.4%. Of HPV-positive women, 74% had oncogenic HPV types, and 30% had nononcogenic HPV types. Younger age and lifetime measures of sexual activity (notably, number of partners) were the main risk factors for the oncogenic HPV types. Furthermore, a previous Chlamydia infection was associated with the high-risk HPV types. In contrast, the most important determinants for nononcogenic HPV infection were contraceptive variables related to the physical protection of the cervix (condom or diaphragm) and number of partners in the last 4 or 12 months.

Our study confirms the venereal nature of HPV infection. We hypothesize that the low-risk HPV infection, which correlates with recent sexual behavior, may be more transient than infection with the oncogenic HPV types, which correlates with lifetime exposure measurements of sexual habits.

Introduction

The sexual transmission of genital warts has been recognized since ancient times, and for decades, epidemiological studies have shown that cervical neoplasia behaves like a sexually transmitted disease (1). Over the years, studies examining risk factors for HPV[3] infection have not been entirely consistent (2–8). Factors contributing to this have been suggested to include differences in study populations and especially varying sensitivity and specificity of the detection methods used (9). Possibly, more attention should be paid to characteristics of the study populations such as sexual habits, prevalence of other sexually transmitted diseases, and overall HPV prevalence, as well as prevalence of specific HPV types.

In this paper, "HPV infection" is used as a synonym for HPV DNA detection, even though it is only based on a single measurement. The HPV types have been categorized into two groups, oncogenic (high-risk) types and nononcogenic (low-risk) types, on the background of their association with cervical cancer. We present results concerning the risk determinants for genital HPV infection in women with normal cervical cytology. In particular, we examine whether risk factors for the oncogenic and nononcogenic HPV types differ from each other in young women.

Materials and Methods

Study Population

In an ongoing population-based prospective cohort study, a random sample of 17,949 women (20–29 years of age) was drawn from the general female population living in Copenhagen. During a 1.5 year period, 11,088 women (68%) were included in the study. A random sample of 1000 women was chosen from those 10,758 women who were cytologically normal at enrollment. A detailed description of the study population background and enrollment procedures has been provided elsewhere (10).

Briefly, all women went through a personal interview conducted by trained interviewers (female nurses). The inter-

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[2] To whom requests for reprints should be addressed, at Danish Cancer Society, Kraeftens Bekaempelse, Strandboulevarden 49, Box 839, DK-2100 Copenhagen, Denmark.

[3] The abbreviations used are: HPV, human papillomavirus; GP, general primer; POR, prevalence odds ratio; CI, confidence interval; STD, sexually transmitted disease.

view included questions about demographic and social factors; sexual, contraceptive, and smoking habits; reproductive factors; and previous STDs. The participants also had a gynecological examination, during which material for HPV detection was obtained from the ecto- and endocervix using two cotton-tipped swabs. The swabs were placed in a tube with 2 ml of Tris-EDTA buffer and kept deep frozen at −80°C. Furthermore, a cervical smear was taken by means of another cotton-tipped swab and a cytobrush. Thirty-nine women with a history of cervical neoplasia were excluded from this study because it was our aim to investigate risk determinants for HPV infection independent of presence of correlated risk factors for cervical neoplasia. The information about previous smear history was obtained from the Danish Pathology Register files, where all cytological and histological examinations are registered. Finally, five women were excluded because their cervical samples were inadequate for HPV testing. Consequently, 956 women remained in the final study.

HPV DNA Analysis

The selected samples were sent on dry ice to a laboratory (Department of Pathology, Section of Molecular Pathology, University Hospital Vrije Universiteit, Amsterdam, the Netherlands), marked only with a study number. After thawing and vigorous vortexing, the cotton-tipped swabs were removed.

Ten μl of crude cell suspensions of cervical scrapes were subjected to GP 5/6 PCR as described previously (11, 12). Briefly, using a PCR Thermalcycler (Biomed, Theres, Germany), standard PCRs were carried out in 50 μl of a mixture containing 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 200 μM of each dNTP, 3.5 mM $MgCl_2$, 1 unit of thermostable DNA polymerase (Amplitaq; Perkin-Elmer Corp.), and 50 pmol of each of the GP5 and GP6 primers. A 4-min denaturation step at 94°C was followed by 40 cycles of amplification using a PCR processor (PE9600; Perkin-Elmer Corp.). Each cycle included a denaturation step at 94°C for 1 min, a primer annealing 40°C for 2 min, and a chain elongation step at 72°C for 1.5 min. The final elongation step was prolonged by 4 min to ensure a complete extension of the amplified DNA. Ten-μl aliquots of each PCR product were layered on 1.5% agarose gels and transferred onto positively charged nylon membranes (Qiabrane, Westburg) by diffusion blotting in 0.5 N NaOH, 0.6 M NaCl.

Southern Blot Analysis of HPV-specific PCR Products. To detect the presence of HPV, blotted GP5/6 PCR products were hybridized under low-stringency conditions using a random-primed, labeled probe consisting of DNA fragments specific for HPV 6, 11, 16, 18, 31, and 33 as described elsewhere in detail (11, 12).

Subsequently, the HPV-positive samples were subjected to individual typing using HPV type-specific oligoprobes as described previously (13). The high-risk oligonucleotide probes used consisted of the oligoprobes for HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56, 58, 59, 66, and 68. In addition, oligoprobes for HPV 6 and 11 were used. Samples positive for HPV (by low-stringency hybridization) but negative for one of the high-risk HPV types were considered to contain HPV X. Most likely, these HPV X types consist of low-risk HPV types (14).

Statistical Analysis

Association between variables and detection of HPV DNA was assessed by computing the PORs with 95% CIs (using the GENSTAT and SAS software). Unconditional logistic regression was used to assess the association between potential risk factors and HPV while adjusting for confounding factors. Initially, we included all potential confounding factors in the model, but only epidemiological factors with a significant confounding effect were retained in the final statistical model.

We grouped HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56, 58, 59, 66, and 68 together in the so-called oncogenic HPV group (all high-risk types known to date), whereas HPV 6 and 11 were grouped together with the still-unidentified low-risk HPV genotypes (HPV X) in the nononcogenic HPV group.

Results

The participants were evenly distributed over the age range (20–29 years); 30, 32, and 38% were 20–23 years old, 24–26 years old, and 27–29 years old, respectively. The median age of the study population was 25 years. The majority (67%) had ≥11 years of school education, and 46% were current smokers. All in all, 41% of the women had ever been pregnant, and 85% had used or were currently using oral contraceptives. Eighty-five, 22, and 3% had ever used condoms, a diaphragm, or contraceptive foam, respectively. The median lifetime number of sexual partners was 5–9, the median age at first intercourse was 16 years, and the median number of years with sexual activity was 9.

Overall, HPV DNA was detected in 147 women (15.4%). Of these, 108 women (73.5%) had oncogenic (high-risk) HPV types. Low-risk HPV 6, 11, or X was found in 44 women (30.0%; 72% of these were HPV X). Of the positive samples, approximately 10% contained more than one HPV type (only 3% harbored both high-risk and low-risk HPV types).

Prevalence of HPV (any type) was associated with age (Table 1); the relative odds ratio for having HPV DNA detected decreased with increasing age. The sexual history was also important for the risk of HPV infection, and lifetime number of sexual partners was the strongest predictor of the risk. The relative odds reached a peak in women with 10–14 partners (POR, 8.9; 95% CI, 3.0–26.0), after which a falling off was observed in women with ≥15 partners. Likewise, an increasing POR for HPV was observed with increasing number of regular partners (sexual relationships lasting 3 months or more), although this association was much weaker and disappeared after adjustment for confounding variables. The POR for HPV tended to decrease with increasing number of years since first intercourse, whereas virtually no effect was seen for age at first intercourse. No statistically significant relationship was observed between HPV and self-reported gonorrhoea, genital warts, or herpes (data not shown), but in contrast, the relative odds estimate was significantly increased for a history of Chlamydia trachomatis: the adjusted POR was 1.7 (95% CI, 1.1–2.7; Table 1).

The prevalence of HPV infection was related to contraceptive habits (Table 1). Use of condoms significantly increased the POR for HPV infection. The adjusted PORs were 1.7 (95% CI, 0.9–3.3) and 2.2 (95% CI, 1.2–4.3) in former and current users, respectively, compared to never users. In contrast, long-term users of a diaphragm (≥2 years) had a significantly decreased HPV prevalence compared to never users (POR, 0.5; 95% CI, 0.2–0.9), and also, ever use of contraceptive foam seemed to have a protective effect. Long-term use of oral contraceptives was also related to HPV positivity; women with ≥5 years of use had a 2-fold higher POR than never users (95% CI, 1.1–3.6). Women who were currently pregnant had a lower POR for being HPV DNA positive, although the associ-

Table 1 PORs for genital HPV infection (any type) associated with selected variables

Variable	*n*	% HPV positive	Crude POR	Adjusted POR[a] (95% CI)
Age				
20–23	288	19.4	1.0	1.0
24–26	311	14.1	0.6	0.7 (0.4–1.1)
27–29	357	13.1	0.6	0.6 (0.4–1.0)
Lifetime no. of sex partners				
≤2	134	3.7	1.0	1.0
3–4	152	14.5	4.4[b]	4.9 (1.6–14.7)
5–9	284	15.8	4.9[b]	5.5 (1.9–15.9)
10–14	189	23.8	8.1[b]	8.9 (3.0–26.0)
≥15	197	15.2	4.7[b]	5.2 (1.7–15.6)
No. of regular partners (≥3 months)				
≤1	185	9.2	1.0	1.0
2–4	495	16.6	2.0[b]	1.4 (0.8–2.6)
≥5	276	17.4	2.1[b]	1.2 (0.6–2.5)
Age at first intercourse				
≥18	293	14.8	1.0	1.0
16–17	359	15.3	1.0	0.9 (0.6–1.4)
≤15	299	16.1	1.2	0.8 (0.5–1.4)
Years since first intercourse				
≤6	283	17.0	1.0	1.0
7–9	299	18.1	1.1	1.0 (0.6–1.7)
≥10	374	12.0	0.7[b]	0.6 (0.3–1.2)
History of Chlamydia				
No	797	13.7	1.0	1.0
Yes	159	23.9	2.0[b]	1.7 (1.1–2.7)
Condom use				
Never	148	8.1	1.0	1.0
Former	378	14.3	1.9[b]	1.7 (0.9–3.3)
Current	430	18.8	2.6[b]	2.2 (1.2–4.3)
Years of diaphragm use				
Never	752	15.6	1.0	1.0
<2	95	21.0	1.5	1.4 (0.8–2.4)
≥2	108	9.3	0.6	0.5 (0.2–0.9)
Contraceptive foam use				
Never	926	15.8	1.0	1.0
Ever	30	3.3	0.2	0.2 (0.03–1.3)
Years of oral contraceptive use				
Never	152	11.2	1.0	1.0
<2	203	15.3	1.4	1.6 (0.8–3.0)
2–4	262	16.4	1.6	1.6 (0.9–3.0)
≥5	339	16.6	1.6	2.0 (1.1–3.6)
Currently pregnant				
No	921	15.6	1.0	1.0
Yes	40	7.5	0.5	0.6 (0.2–1.9)
No. of live births				
Never pregnant	574	17.1	1.0	1.0[c]
0	226	17.3	1.0	0.9 (0.6–1.5)
≥1	155	6.5	0.3[b]	0.4 (0.2–0.7)
Smoking status				
Never	440	16.6	1.0	1.0
Former	82	7.3	0.4[b]	0.4 (0.2–1.0)
Current	434	15.7	0.9	0.8 (0.6–1.2)

[a] Adjusted for age, lifetime no. of sex partners, no. of live births, condom use, and history of Chlamydia infection.
[b] 95% CI excludes 1.0.
[c] Also adjusted for no. of years with current partner.

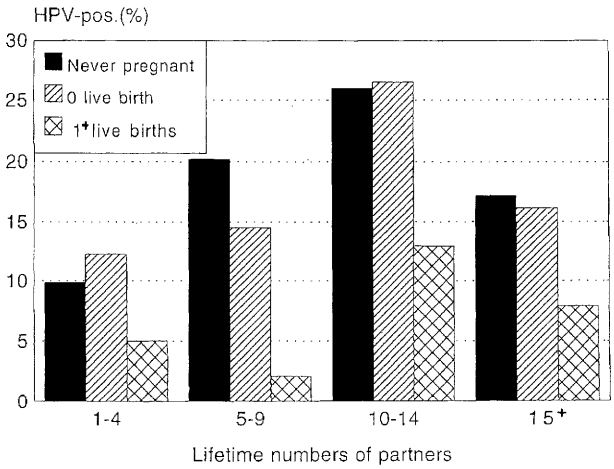

Fig. 1. HPV prevalence according to parity, stratified by lifetime number of partners.

ation was not statistically significant. Number of live births was also associated with lower relative odds of HPV. After adjustment for confounding factors, the relative odds of being HPV positive were 60% lower (95% CI, 0.2–0.7) in women with one or more live birth than in women who had never been pregnant and ever pregnant nulliparous women. The association was observed for all categories of number of sexual partners (Fig. 1).

The prevalence of HPV was nearly the same in current smokers as in never smokers, whereas in former smokers HPV positivity was lower (POR, 0.4; 95% CI, 0.2–1.0; Table 1).

Table 2 shows the stratified analyses of the risk factor pattern for nononcogenic and oncogenic HPV types. None of the sexual habits variables were related to the prevalence of nononcogenic HPV infection, and likewise, no relationship with age was observed. Instead, an important determinant was the use of different contraceptive methods. Compared to women who had never used condoms, both former users (adjusted POR, 1.7; 95% CI, 0.5–5.7) and current users (adjusted POR, 3.8; 95% CI, 1.2–11.6) were more likely to be positive for nononcogenic HPV types. In contrast, long-term users of a diaphragm had decreased relative odds for having these HPV types detected (adjusted POR, 0.3; 95% CI, 0.1–1.1). Infection with the nononcogenic HPV types was not associated with previous Chlamydia infection or history of any other sexually transmitted diseases (STDs) (Table 2).

In contrast, age and most of the variables describing sexual habits were associated with positivity for the oncogenic HPV types (Table 2). Women in the age group 27–29 years had a 60% lower POR than younger women (20–23 years). The relative odds estimate for infection with the oncogenic HPV types in women with many sex partners (≥10) was nearly 4 times as high as in women with ≤4 lifetime partners (POR, 3.9; 95% CI, 2.1–7.3).

In the univariate analysis, time since first intercourse (or presumed first infection) was a significant determinant of the high-risk HPV infection; women with ≥10 years since first intercourse had a 50% decreased POR compared to women with 0–6 years of potential sexual activity (Table 2). After adjustment for confounders, this pattern still existed, although the association was not statistically significant. Age at first intercourse was not associated with HPV positivity (high-risk

Table 2 PORs for genital HPV infection with nononcogenic and oncogenic types according to selected variables[a]

Variable	Nononcogenic HPV		Oncogenic HPV	
	Crude POR	Adjusted POR[b] (95% CI)	Crude POR	Adjusted POR[c] (95% CI)
Age				
20–23	1.0	1.0	1.0	1.0
24–26	0.8	0.8 (0.4–1.9)	0.6[d]	0.5 (0.3–0.9)
27–29	1.1	1.1 (0.5–2.3)	0.4[d]	0.4 (0.2–0.7)
Lifetime no. of sex partners				
≤4	1.0	1.0	1.0	1.0
5–9	1.1	1.0 (0.4–2.2)	2.4[d]	2.6 (1.4–5.1)
10–14	1.7	1.4 (0.6–3.3)	4.3[d]	4.6 (2.3–9.0)
≥15	0.7	0.6 (0.2–1.6)	2.5[d]	2.9 (1.3–6.1)
No. of regular partners (≥3 months)				
≤1	1.0	1.0	1.0	1.0
2–4	1.2	1.1 (0.5–2.5)	2.8[d]	1.8 (0.8–4.1)
≥5	1.2	1.0 (0.4–2.6)	3.1[d]	1.5 (0.6–3.8)
Age at first intercourse				
≥18 or never	1.0	1.0	1.0	1.0
16–17	1.3	1.4 (0.7–2.9)	1.0	0.7 (0.4–1.2)
≤15	1.1	1.1 (0.5–2.5)	1.2	0.7 (0.4–1.3)
Years since first intercourse				
≤6	1.0	1.0	1.0	1.0
7–9	1.1	1.1 (0.4–2.7)	1.0	1.1 (0.6–2.0)
≥10	0.9	0.7 (0.2–2.0)	0.5[d]	0.6 (0.3–1.1)
History of Chlamydia infection				
No	1.0	1.0	1.0	1.0
Yes	1.1	1.0 (0.4–2.3)	2.6[d]	2.1 (1.3–3.4)
History of any other STD				
No	1.0	1.0	1.0	1.0
Yes	0.5	0.6 (0.2–1.4)	1.4	1.3 (0.8–2.0)
Condom use				
Never	1.0	1.0	1.0	1.0
Former	1.7	1.7 (0.5–5.7)	2.0[d]	1.5 (0.7–3.2)
Current	3.8[d]	3.8 (1.2–11.6)	2.4[d]	1.6 (0.8–3.3)
Years of diaphragm use				
Never	1.0	1.0	1.0	1.0
<2	1.1	1.1 (0.4–2.8)	1.6	1.5 (0.8–2.9)
≥2	0.4	0.3 (0.1–1.1)	0.6	0.5 (0.3–1.3)
Years of oral contraceptive use				
Never	1.0	1.0	1.0	1.0
<2	2.9	2.2 (0.7–7.0)	1.3	1.3 (0.6–2.8)
2–4	2.0	2.5 (0.8–7.7)	1.6	1.5 (0.7–3.0)
≥5	2.0	3.0 (1.0–8.9)	1.5	1.6 (0.8–3.1)
No. of live births				
Never pregnant	1.0	1.0[e]	1.0	1.0[e]
0	1.0	1.1 (0.5–2.2)	1.1	0.9 (0.6–1.5)
≥1	0.6	0.6 (0.2–1.5)	0.2[d]	0.3 (0.2–0.7)
Smoking status				
Never	1.0	1.0	1.0	1.0
Former	0.2	0.2 (0.1–3.4)	0.6	0.6 (0.3–1.5)
Current	0.8	0.9 (0.5–1.5)	1.0	0.8 (0.5–1.3)

[a] Nononcogenic HPV types include types 6, 11, and X. Oncogenic HPV types include types 16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56, 58, 59, 66, and 68.
[b] Adjusted for age and condom use.
[c] Adjusted for age, lifetime no. of sex partners, no. of live births, and history of Chlamydia infection.
[d] 95% CI interval excludes 1.0.
[e] Also adjusted for years with current partner.

Table 3 PORs for HPV positivity (oncogenic types) in relation to number of sexual partners and history of Chlamydia infection[a]

Lifetime no. of sex partners	History of Chlamydia infection[b]		History of other STDs[b]	
	No	Yes	No	Yes
≤4	1.0[c]	7.3 (2.0–26.9)	1.0[c]	1.6 (0.5–6.6)
5–9	3.0 (1.4–6.2)	9.7 (3.5–27.4)	2.0 (1.0–4.0)	3.3 (1.2–8.7)
≥10	5.4 (2.7–11.0)	7.8 (3.5–17.2)	4.1 (2.1–7.9)	4.3 (2.1–8.9)

[a] Oncogenic types include HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56, 58, 59, 66, and 68.
[b] POR (adjusted for age and no. of live births); range in parentheses, 95% CI.
[c] Reference group.

types). In all statistical models, age was more important than both years since first intercourse and age at first intercourse (data not shown).

The POR for infection with the cancer-associated HPV types was significantly higher in women with a history of *Chlamydia trachomatis* than in women who had not experienced Chlamydia infection, also after adjustment for confounding factors (POR, 2.1; 95% CI, 1.3–3.4). When considering any other STDs as one group, no association was observed with the POR for infection with oncogenic HPV types (Table 2). We found an interaction ($P = 0.05$) between lifetime number of sex partners and a self-reported history of Chlamydia infection (Table 3). The POR for HPV positivity (oncogenic types) increased strongly with number of partners in women without a previous Chlamydia infection. Among women with a history of Chlamydia, the POR for HPV was already strongly increased in women with relatively few sexual partners (≤4 partners), and no further increase was observed with increasing number of partners. In contrast to this, the relative odds estimate for HPV infection was increasing with number of partners independently of a history of other STDs (Table 3).

As for the nononcogenic HPV types, the prevalence of oncogenic HPV types was increased among current condom users and decreased in women who had used a diaphragm for 2 years or more (Table 2). However, the associations were less strong for the high-risk HPV types and did not reach statistical significance. A negative association was observed between high-risk HPV positivity and parity [POR 0.3 (95% CI, 0.2–0.7) for ≥1 live births *versus* never pregnant]. Although it was less strong and statistically nonsignificant, a similar association was seen for the low-risk HPV types (Table 2). For both the oncogenic HPV types and the nononcogenic types, the POR increased with duration of oral contraceptive use, whereas the POR for both groups of HPV was lower in former smokers (*versus* never smokers; Table 2).

Discussion

This study demonstrates that HPV positivity (any type) is associated with the following: (*a*) sexual habits [lifetime number of sexual partners ($P < 0.005$)]; (*b*) contraceptive habits [diaphragm use ($P = 0.02$), condom use ($P = 0.04$), and contraceptive foam use ($P = 0.04$)]; (*c*) exposure to another STD [*Chlamydia trachomatis* ($P = 0.04$)]; (*d*) reproductive/hormonal factors [parity ($P = 0.01$) and oral contraceptive use ($P = 0.06$)]; and (*e*) age ($P = 0.05$). In this study, the most significant risk factor was number of sexual partners. The trend is not linear, but rather the POR curve forms a peak and subsequent a falling off. Immunity has been suggested to explain this phenomenon (15). This pattern of risk factors fits with the expected epidemiology of a STD. Our results are in line

Table 4 PORs for HPV 6/11 and HPV X according to selected variables

Variable	HPV 6/11		HPV X	
	Crude POR	Adjusted POR[a] (95% CI)	Crude POR	Adjusted POR[a] (95% CI)
Age				
20–23	1.0	1.0	1.0	1.0
24–26	0.9	1.0 (0.2–4.2)	1.0	1.1 (0.4–3.1)
27–29	0.9	0.9 (0.2–4.0)	1.3	1.3 (0.6–3.9)
Lifetime no. of sex partners				
≤4	1.0	1.0	1.0	1.0
5–9	0.4	0.4 (0.1–2.1)	1.2	1.0 (0.4–2.6)
10–14	1.4	1.2 (0.3–5.1)	0.9	0.7 (0.2–2.2)
≥15	0.3	0.4 (0.03–2.6)	0.8	0.6 (0.2–1.8)
Condom use				
Never	1.0	1.0	1.0	1.0
Former	1.7	1.7 (0.2–16.2)	1.5	1.6 (0.3–7.8)
Current	2.7	2.9 (0.3–24.8)	4.3	4.7 (1.1–20.5)

[a] Adjusted for other variables in this table.

with others' findings of an association with age (3, 5, 6, 8), reproductive/hormonal factors (3, 5), barrier contraceptives (3, 8), and STDs (8). Previously, a debated issue has been the lack of association with lifetime number of sexual partners in some studies, leading to hypotheses about other (nonsexual) transmission routes of HPV (16). Reasons for the discrepancies undoubtedly include the use of HPV detection methods less sensitive and specific than, *e.g.*, the PCR technique. However, it is also likely that some of the lack of consistency is caused by the study populations being different. Indeed, we found another pattern of risk factors for HPV detection in two high-risk groups of women (STD patients and prostitutes) than in the present study of women from the general population (17). In neither of these two high-risk population groups was lifetime number of partners associated with HPV positivity. Instead, number of partners in the last year and number of (noncommercial) partners in the last 4 months were the most important HPV determinants in STD patients and prostitutes, respectively. Although these findings support the venereal nature of genital HPV infection, they also point to the role of different characteristics of the study population in relation to the identification of specific HPV risk factors. These results may also partly explain why not all studies have found the generally expected association between HPV and lifetime number of sexual partners.

We also compared risk factors for the so-called high-risk or oncogenic HPV types and for the low-risk or nononcogenic HPV types. It is a limitation of this study that only 26% (n = 12) of women with the low-risk HPVs were positive for HPV 6 or 11. However, when we examined the risk factors for HPV 6/11, and HPV X separately, similar patterns appeared (data shown for age and number of partners; Table 4).

An important finding of the present study is the observed differences in the risk factor pattern between the oncogenic HPV group and the nononcogenic HPV group. Whereas age and sexual behavior, notably lifetime number of sexual partners, were highly significant risk determinants for the oncogenic HPV types, no association with these variables was observed for the low-risk HPV types.

The observed difference between the two HPV groups could be explained if infection with the oncogenic types tended to be of a longer duration and infection with the nononcogenic HPVs were of a more transient nature. If this hypothesis is true, lifetime measurements of exposure would probably correlate more strongly with the oncogenic HPV types than with the low-risk HPV types (as observed in this study). Consequently, if we still assume a sexual route of transmission of the low-risk HPV types, it could be anticipated that more recent sexual behavior would correlate better with these HPV types. To address this hypothesis, we analyzed results from a subgroup of women in our investigation (n = 235), who were reexamined 2 years later and on whom we collected information on recent sexual activity (data not shown). Preliminary results show no association between low-risk HPV types and lifetime number of partners, but both number of partners in the last 4 months [POR, 2.7 (95% CI, 1.0–7.9) for ≥2 partners *versus* 0 or 1 partner], and partners in the last year [POR, 4.9 (95% CI, 1.6–14.9) for ≥4 partners *versus* ≤1 partner) were related to nononcogenic HPV positivity. Additional support for this hypothesis may be offered by the finding of Hildesheim *et al.* (18) that high-risk HPV types are more likely to persist than the low-risk types. Only one previous study has reported on risk factors separately for nononcogenic and oncogenic HPV types (9). Our results are in line with those findings by Franco *et al.* (9); however, that report did not include information on recent sexual behavior.

Increased risk was associated with a history of Chlamydia, but only for the oncogenic types. In a recent study (8), the presence of Chlamydia trachomatis antibodies was a significant determinant of HPV positivity in middle-aged women. No information on the distribution of specific HPV types among HPV-positive cases or their relationship with potential risk factors was provided in that study. From our data, it appears that once a woman is infected with Chlamydia (reports a history of Chlamydia), the relative odds for being infected with oncogenic HPV types increases strongly, even in women with relatively few lifetime partners (≤4). It should be emphasized that in our study no definite conclusion can be made regarding the association between Chlamydia and HPV infection because the temporal relationship is not known. However, our results are interesting in the light of the suggested hypothesis of Chlamydia playing a role in the development of cervical neoplasia (19, 20). Support for a specific effect of Chlamydia may be that we exclusively observe this association with Chlamydia and not with any of the other STDs. Whether Chlamydia acts as a cofactor for HPV (*e.g.*, by facilitating infection with high-risk HPV types) or as an independent risk factor through a yet unidentified mechanism is still not known.

We also found similarities between high-risk and low-risk HPV groups in risk factors, although statistical significance was not always achieved. The fact that use of condoms consistently increased the PORs for both HPV groups may seem contradictory to what could be expected for a barrier contraceptive method. However, it may be explained if the condom is not used during the entire intercourse (*i.e.*, used primarily to avoid pregnancy and not to avoid STDs). It could also be hypothesized that women with a history of genital warts would be more likely to use condoms because of concerns about disease transmission. However, when we restricted our analysis to women who had never had genital warts, the association between condom use and HPV infection remained unchanged in both the oncogenic and nononcogenic HPV group (data not shown). This also applied when we excluded women who reported ever having had Chlamydia.

In contrast, we found that for both HPV groups, use of the other barrier contraceptive method (diaphragm) offered protection, indicating that barrier contraceptives can prevent transmission of HPV (provided that they are used during the entire intercourse). Also, use of contraceptive foam decreased the

HPV prevalence, perhaps supporting a viricidal effect of foam, which was suggested by some authors (21) but not by others (22).

In our study population, a lower HPV detection rate was found in currently pregnant women and particularly in women with ≥1 live birth (for both the oncogenic and the nononcogenic types). Although this has also been found in other studies (5), still others have found the opposite (6). In addition, inconsistencies also exist concerning the prevalence of HPV in women during pregnancy; some investigators find a higher prevalence in pregnant women (23–25), whereas others find no differences between pregnant and nonpregnant women (26, 27). It may be hypothesized that currently pregnant women, in particular, could have a recent history of monogamy. However, the association observed between HPV and parity (number of live births) remained unchanged when currently pregnant women were excluded. We had no information on recent sexual activity; instead, we used the duration (years) of the relationship with the current partner as a surrogate measure. Compared to single women, women with increasing number of years with the partner had decreasing risk of HPV (data not shown). However, additional adjustment of parity for this variable did not change the estimates. Furthermore, in our study, the association between HPV and parity was observed in all categories of lifetime sexual partners, including women with only a few partners.

Finally, we found that currently smoking women had a similar prevalence of HPV positivity as never smokers, whereas former smokers had a decreased prevalence. Others have produced similar results (5, 6); however, the explanation for this association remains obscure.

Part of our results concerning differences between oncogenic and nononcogenic HPV types could emerge if the HPV X group contained a high proportion of false positives. However, this is not likely to be the case with the PCR products generated by the GP5/6 primers. It has been shown that the GP5/6 PCR allows the detection of at least 27 mucosotropic HPV types, including 15 high-risk types and at least 12 low-risk types (13, 14, 28). In these studies, in which the same method was used as in the current study, HPV positive samples, found by Southern blot hybridization under low-stringency conditions, were successfully further typed into low-risk types HPV 6, 11, 40, 42, 43, and 44. Thus, the low-risk HPVs are not likely to be underestimated in our study but are contained in the HPV X group. On the basis of this conclusion, we emphasize that the HPV positivity (both the oncogenic HPVs and the HPV X group; the latter most likely consist of low-risk types) is reliably measured in this study. Future studies should be initiated to further identify the individual low-risk HPV types in this HPV X group.

In conclusion, our study confirms the venereal nature of the HPV infection (for both oncogenic and nononcogenic types). The data also lend some support to a protective effect of barrier contraceptives that are used during the entire intercourse. Furthermore, the results suggest a role of previous Chlamydia infection for the oncogenic HPV types. Finally, based on our findings of differences in the risk factor pattern for viruses belonging to the oncogenic and nononcogenic groups, respectively, it is hypothesized that the low-risk HPV infection, which seems to correlate only with recent sexual activity, may be of a more transient nature than the high-risk infection, which correlates more closely to lifetime sexual exposure measurements.

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References

1. Brinton, L. A. Epidemiology of cervical cancer: overview. *In:* N. Munoz, F. X. Bosch, K. Shah, and A. Meheus (eds.), The Epidemiology of Human Papillomavirus and Cervical Cancer, pp 3–23. IARC Scientific Publ. No. 119. Lyon, France: IARC, 1992.

2. Rohan, T., Man, V., McLaughlin, J., Harnish, D. G., Yu, H., Smith, D., Davis, R., Shier, R. M., and Rawls, W. PCR-detected genital papillomavirus infection: prevalence and association with risk factors for cervical cancer. Int. J. Cancer, *49:* 1–5, 1991.

3. Ley, C., Bauer, H. M., Reingold, A., Schiffman, M. H., Chambers, J. C., Tashiro, C. J., and Manos, M. M. Determinants of genital human papillomavirus infection in young women. J. Natl. Cancer Inst., *83:* 997–1003, 1991.

4. Wheeler, C. M., Parmenter, C. A., Hunt, W., C. Becker, T. M., Greer, C. E., Hildesheim, A., and Manos, M. M. Determinants of genital human papillomavirus infection among cytologically normal women attending the University of New Mexico student health center. Sex. Transm. Dis., *20:* 286–289, 1993.

5. Bauer, H. M., Hildesheim, A., Schiffman, M. H., Glass, A. G., Rush, B. B., Scott, D. R., Cadell, D. M., Kurman, R. J., and Manos, M. M. Determinants of genital human papillomavirus infection in low-risk women in Portland, Oregon. Sex. Transm. Dis., *20:* 274–278, 1993.

6. Hildesheim, A., Gravitt, P., Schiffman, M. H., Kurman, R. J., Barnes, W., Jones, S., Tchabo, J-G., Brinton, L. A., Copeland, C., Epp, J., and Manos, M. M. Determinants of genital human papillomavirus infection in low-income women in Washington, DC. Sex. Transm. Dis., *20:* 279–285, 1993.

7. Kjaer, S. K., de Villiers, E-M., Caglayan, H., Svare, E., Haugaard, B. J., Engholm, G., Christensen, R. B., Møller, K. A., Poll, P., Jensen, H., Vestergaard, B. F., Lynge, E., and Jensen, O. M. Human papillomavirus, herpes-simplex virus and other potential risk factors for cervical cancer in a high-risk area (Greenland) and a low-risk area (Denmark): a second look. Br. J. Cancer, *67:* 830–837, 1993.

8. Muñoz, N., Kato, I., Bosch, F. X., Eluf-Neto, J., de Sanjosé, S., Ascunce, N., Gili, M., Izarzugaza, I., Viladiu, P., Tormo, M. J., Moreo, P., Gonzalez, L. C., Tafur, L., and Walboomers, J. M. M. Risk factors for HPV DNA detection in middle-aged women. Sex. Transm. Dis., *23:* 504–510, 1996.

9. Franco, E. L., Villa, L. L., Ruiz, A., and Costa, M. C. Transmission of cervical human papillomavirus infection by sexual activity: differences between low and high oncogenic risk types. J. Infect. Dis., *172:* 756–763, 1995.

10. Kjaer, S. K., van den Brule, A. J. C., Bock, J. E., Poll, P. A., Engholm, G., Sherman, M. E., Walboomers, J. M. M., and Meijer, C. J. L. M. Human papillomavirus: the most significant risk determinant of cervical intraepithelial neoplasia. Int. J. Cancer, *65:* 601–606, 1996.

11. Van den Brule, A. J. C., Meijer, C. J. L. M., Bakels, V., Kenemans, P., and Walboomers, J. M. M. Rapid detection of human papillomavirus in cervical scrapes by combined general primer-mediated and type-specific polymerase chain reaction. J. Clin. Microbiol., *28:* 2739–2743, 1990.

12. Van den Brule, A. J. C., Snijders, P. J., Gordijn, R. L., Bleker, O. P., Meijer, C. J. L. M., and Walboomers, J. M. M. General primer-mediated polymerase chain reaction permits the detection of sequenced and still unsequenced human papillomavirus genotypes in cervical scrapes and carcinomas. Int. J. Cancer, *45:* 644–649, 1990.

13. Jacobs, M. V., de Roda Husman, A. M., van den Brule, A. J. C., Snijders, P. J. F., Meijer, C. J. L. M., and Walboomers, J. M. M. Group-specific differentiation between high- and low-risk human papillomavirus genotypes by general primer-mediated PCR and two cocktails of oligonucleotide probes. J. Clin. Microbiol., *33:* 901–905, 1995.

14. Jacobs, M. V., Snijders, P. J. F., van den Brule, A. J. C., Helmerhorst, T. J. M., Meijer, C. J. L. M., and Walboomers, J. M. M. A general primer PG5/6+ mediated PCR-enzyme immunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. J. Clin. Microbiol., *35:* 791–795, 1997.

15. Schiffman, M. H. Epidemiology of cervical human papillomavirus infections. *In:* Current Topics in Microbiology and Immunology, Vol. 184, pp. 55–81. Berlin: Springer-Verlag, 1994.

16. Fairley, C. K., Tabrizi, S. N., Mcneil, J. J., Chen, S. J., Borg, A. J., and Garland, S. M. Is HPV always sexually acquired? Med. J. Aust., *159:* 724–726, 1993.

17. Kjaer, S. K., Svare, E. I., Worm, A-M., Meijer, C. J. L. M., Walboomers, J. M. M., and van den Brule, A. J. C. Genital human papillomavirus infection in

prostitutes from Copenhagen. Fourteenth International Papillomavirus Conference, p. 11. Quebec City, Quebec, Canada. 1995.

18. Hildesheim, A., Schiffman, M. H., Gravitt, P. E., Glass, A. G., Greer, C. E., Zhang, T., Scott, D. R., Rush, B. B., Lawler, P., Sherman, M. E., Kurman, R. J., and Manos, M. M. Persistence of type-specific human papillomavirus infection among cytologically normal women. J. Infect. Dis., *169:* 235–240, 1994.

19. Koutsky, L. A., Holmes, K. K., Critchlow, C. W., Stevens, C. E., Paavonen, J., Beckmann, A. M., Derouen, T. A., Galloway, D. A., Vernon, D., and Kiviat, N. B. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. New. Engl. J. Med., *29:* 1272–1278, 1992.

20. Muñoz, N., Bosch, F. X., de Sanjosé, S., Vergara, A., del Moral, A., Muñoz, M. T., Tafur, L., Gili, M., Izarzugaza, I., Viladiu, P., Navarro, C., Alonso de Ruiz, P., Aristizabal, N., Santamaria, M., Orfila, J., Daniel, R. W., Guerrero, E., and Shah, K. V. Risk factors for cervical intraepithelial neoplasia grade III/carcinoma *in situ* in Spain and Colombia. Cancer Epidemiol. Biomark. Prev., *2:* 423–431, 1993.

21. Postic, B., Singh, B., Squeglia, N. L., and Guevara, L. Inactivation of clinical isolatis of Herpesvirus hominis types 1 and 2, by chemical contraceptives. Sex. Transm. Dis., *1:* 22–24, 1978.

22. Hermonat, P. L., Daniel, R. W., and Shah, K. V. The spermicide nonoxynol-9 does not inactivate papillomavirus. Sex. Transm. Dis., *19:* 203–205, 1992.

23. Schneider, A., Hotz, M., and Gissman, L. Increased prevalence of human papillomaviruses in the lower genital tract of pregnant women. Int. J. Cancer, *40:* 198–201, 1987.

24. Czegledy, J., Gergely, L., and Endrodi, I. Detection of human papillomavirus deoxyribonucleic acid by filter *in situ* hybridization during pregnancy. J. Med. Virol., *28:* 250–254, 1989.

25. Rando, R. F., Lindheim, S., Hasty, L., Sedlacek, T. V., Woodland, M., and Eder, C. Increased frequency of detection of human papillomavirus deoxyribonucleic acid in exfoliated cervical cells during pregnancy. Am. J. Obstet. Gynecol., *161:* 50–55, 1989.

26. de Roda Husman, A. M., Walboomers, J. M. M., Hopman, E., Bleker, O. P., Helmerhorst, T. M. J., Rozendaal, L., Voorhorst, F. J., and Meijer, C. J. L. M. HPV prevalence in cytomorphologically normal cervical scrapes of pregnant women as determined by PCR: the age-related pattern. J. Med. Virol., *46:* 97–102, 1995.

27. Kemp, E. A., Hakenewerth, A. M., Laurent, S. L., Gravitt, P., and Stoerker, J. Human papillomavirus prevalence in pregnancy. Obstet. Gynecol., *79:* 649–656, 1992.

28. de Roda Husman, A. M., Walboomers, J. M. M., Meijer, C. J. L. M., Risse, E. K. J., Schipper, M. E. I., Helmerhorst, T. M., Bleker, O. P., Van den Brule, A. J. C., and Snijder, P. J. F. Analysis of cytomorphologically abnormal cervical scrapes for the presence of 27 mucosotropic human papillomavirus genotypes using polymerase chain reaction. Int. J. Cancer, *56:* 802–806, 1994.