Human Papillomavirus and Long-term Oral Contraceptive Use Increase the Risk of Adenocarcinoma *in Situ* of the Cervix[1]

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Abstract

We examined United States Surveillance, Epidemiology, and End Results incidence data and conducted a population-based case-control study to examine the role of human papillomavirus (HPV) and oral contraceptive (OC) use in the etiology of adenocarcinoma *in situ* of the cervix (ACIS). One hundred and fifty women diagnosed with ACIS and 651 randomly selected control women completed in-person interviews. The presence of HPV DNA in archival ACIS specimens was determined by E6 and L1 consensus PCR. Serum samples from case and control subjects were collected at interview, and antibodies to HPV-16 L1 and HPV-18 L1 were detected by virus-like particle capture assays. The overall prevalence of HPV DNA was 86.6%, with 39.0% positive for HPV-16 DNA, 52.4% positive for HPV-18 DNA, and 13.4% positive for more than one HPV type. The age-adjusted relative risk of ACIS associated with HPV-18 seropositivity was 3.3 (95% confidence interval 2.2–4.9). No increased risk was associated with antibodies to HPV-16 L1. Among women born after 1945, the relative risk increased with duration of OC use, with the highest risk for 12 or more years of use (odds ratio, 5.5; 95% confidence interval, 2.1–14.6) relative to nonusers. The detection of HPV DNA in 86.6% of ACIS and the strong association of ACIS with HPV-18 L1 seropositivity underscore the importance of HPV, particularly HPV-18, in the etiology of ACIS. In addition, long-term OC use may contribute to the pathogenesis of these tumors in some women.

Introduction

The epidemiology of adenocarcinoma of the cervix has changed substantially over the last two decades. Incidence data indicate a steady increase in the rate of AC,[3] especially in younger women, since the early 1970s in the United States, England, and Norway (1–4). Recent data from the Los Angeles area cancer registry show a continuation of this increasing trend among young women (5) that was also evident for women 30–54 years of age throughout the United States (6). The increasing incidence of invasive cervical adenocarcinoma has been reported from the majority of cancer registries (7) and is seen among white women, but not black women, in the United States SEER data (8).

As with squamous cell lesions, adenocarcinoma of the cervix occurs at the squamocolumnar junction, but in the columnar cells closest to the endocervical canal (9, 10). Invasive and *in situ* adenocarcinoma have been found to coexist, which suggests that ACIS is a precursor of AC (11). The reported prevalence of HPV in ACIS has ranged widely, from 10%–94% (12). However, three studies (13–15), including the worldwide study of invasive cervical cancer (15), showed that HPV DNA was found as frequently in ACIS or AC as in squamous cell carcinoma of the cervix. However, HPV-18 DNA was more prevalent in adenocarcinomas than in squamous cell carcinomas of the cervix.

The population-based epidemiological studies that have been published to date (16–20) combine the *in situ* and invasive forms (19, 20) or include only invasive cases (16–18) of cervical adenocarcinoma. We chose to focus on ACIS in this study for three reasons. First, the potential years of reproductive life lost are especially acute for younger women treated for ACIS, which makes the precursor form of this rare cancer of special interest. Second, the incidence data from other studies highlight the increasing numbers of women diagnosed at young ages with AC, but ACIS incidence trends have not yet been explored. Third, case-control studies suggest an increased risk of cervical adenocarcinoma associated with use of OCs, which are widely used by young women. We conducted a large, population-based study to examine HPV DNA in tumor tissue, HPV antibody prevalence, and OC use associated with ACIS of the cervix. We also examined the United States SEER data for trends in ACIS

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[3] The abbreviations used are: AC, invasive adenocarcinoma of the cervix; ACIS, adenocarcinoma *in situ* of the cervix; HPV, human papillomavirus; OC, oral contraceptive; SEER, Surveillance, Epidemiology, and End Results; CI, confidence interval; OR, odds ratio; HSV, herpes simplex virus; RDD, random digit telephone dialing; CIS, carcinoma *in situ* of the cervix.

incidence rates for evidence of increased incidence similar to those described for AC.

Materials and Methods

Subject Eligibility, Identification, and Recruitment. Case subjects were 18–70-year-old residents of Western Washington diagnosed with incident ACIS between June 1990 and December 1996. The Cancer Surveillance System, a population-based registry that is part of the SEER program of the United States National Cancer Institute, was used to identify the case subjects. All diagnoses were biopsy confirmed by community pathologists at the time of initial diagnosis. One of the 205 cases identified by the registry was found to have benign disease on pathology rereview (described below) and was considered ineligible. We were able to interview 150 of the 204 (73.5%) eligible cases. Reasons for nonparticipation included patient refusal (16.6%), doctor refusal (9.3%), and death (0.5%). The registry assigned the following International Classification of Diseases for Oncology histology codes to the 150 interviewed case subjects used in this analysis: (*a*) 8140, adenocarcinoma *in situ*, $n = 145$; (*b*) 8260, papillary adenocarcinoma *in situ*, $n = 1$; (*c*) 8380, endometrioid adenocarcinoma *in situ*, $n = 1$; (*d*) 8460, papillary serous adenocarcinoma, $n = 1$; (*e*) 8480, mucinous adenocarcinoma *in situ*, $n = 1$; and (*f*) 8560, adenosquamous carcinoma *in situ*, $n = 1$.

Population-based controls were identified using RDD (21, 22). Cases and controls for this study were part of a long-term study (1980–1998) of female anogenital cancers, during which all subjects received the same interview (23). The controls were frequency matched to the age distribution of the anogenital cancer cases in 5-year age intervals. The reference date (the date prior to which risk factor information was collected in the in-person interview) for cases was the month and year of their ACIS diagnosis. For control subjects, reference dates were assigned at random from among the possible case subject diagnosis dates that had occurred prior to the selection of the control through RDD. Thus, at least one control is assigned a reference date that is specifically matched to each case.

To be eligible as a control for this study, a woman had to be a resident at reference date of the 13-county area in western Washington State that includes Seattle, have a working telephone at that time, be able to speak English, and have an intact uterus. A household census was successfully conducted for 92.8% of all residential phone numbers called. The interview response rate was 71.2% (1714:2408), based on the number interviewed out of all those who were contacted and found to be eligible. Of the 1714 controls, 844 were not included in this analysis because their reference dates fell outside of the corresponding diagnosis dates for the case subjects (June 1990 through December 1996). From the remaining 870 eligible female controls who were assigned reference dates corresponding to the case subjects' diagnosis dates, we excluded women with a history of cervical cancer ($n = 6$) or hysterectomy ($n = 213$). Data from 651 controls were available for analysis.

All data collection activities were conducted after obtaining written informed consent from each case or control subject. Case and control subjects were offered $10 to participate in the study. The Institutional Review Board of the Fred Hutchinson Cancer Research Center approved the study protocol.

Data Collection and Laboratory Assays. Trained interviewers administered an in-person interview to case and control subjects. Interview topics included demographic characteristics and reproductive, birth control, sexual, and smoking histories. The interviews for cases took place at an average of 15.2 months after diagnosis (median = 10.5 months). The time from the assigned reference date to interview for controls was 18.0 months (median = 16.1 months).

Archived paraffin-embedded tissue blocks from biopsy or surgery specimens were obtained from 101 case subjects. The study pathologist examined each block to determine the histological characteristics of the blocks and chose which blocks were to be tested for the presence of HPV DNA. Detection of HPV nucleic acids was performed using L1 consensus primers (24) and 16E6 and 18E6 type-specific primers (25). The identity of the PCR products was confirmed by Southern hybridizations, and the L1 consensus products were typed by restriction fragment analysis (26) as described previously (25). HPV type was assigned by comparison of restriction patterns with those of HPV recombinant plasmids.

Serum samples were collected at interview and stored at −70°C until they were retrieved for testing of serologic antibody response to HPV and HSV. The laboratories conducting the serologic assays were blinded to all characteristics of the study subjects. The sera were tested for HPV-16 and HPV-18 L1 capsid proteins by using capture ELISAs (27). The monoclonal antibodies were H16V5 and H18J4 used with HPV L1 capsid types 16 or 18, respectively. Six optical density readings were ascertained for each subject from three wells with capsid and three wells without capsid. The results were combined into a single value for each subject by subtracting the average of the natural logarithm of the three wells without capsid from the average of the natural logarithm of the three wells with capsid. A cut point for HPV positivity was determined as the mean plus 2 SDs of ELISA values from a cohort of university women who were virgins and in whom no HPV DNA could be detected from cervical scrapings. These negative controls and a pool of positive controls from a sexually transmitted disease clinic were run on the ELISA plates with the samples (27).

Antibody response to HSV-2 was assessed by using a Western blot assay to discriminate between the immune response to HSV-1 and HSV-2 (28). For the HSV-2 variable, HSV-2-seronegative women included women who were seropositive for HSV-1.

Blood samples were collected from 92.7% of case subjects and 88.3% of control subjects who were interviewed. Samples were sent in batches to the serology laboratories, and all available samples were tested. For some women, all of the sera had been used in previous tests. HSV-2 antibody results were available for 97.1% of case subjects and 99.1% of control subjects who had sera collected. HPV-16 and HPV-18 antibody results were available from 92.9% of case subjects and 96.3% of control subjects who had sera collected.

Data Analysis. The relative risk of cancer was estimated by calculating ORs by using multiple logistic regression. Subjects with missing values for any variables in a model were excluded from that model. Trends were evaluated by a likelihood ratio test performed by adding a continuous, linear exposure variable to the fully adjusted model that did not contain the term of interest. We looked for evidence of interaction between OC use and smoking history. The interaction term was evaluated by a likelihood ratio test for adding the interaction parameter to the fully adjusted multiplicative and additive relative risk models.

The following potential confounders were controlled if they impacted the estimate of the relative risk: lifetime number of sexual partners (1, 2–4, 5+), interval since last screening Pap smear in months (<12, 13–35, 36+), and education in years (<12, 12, 13+). The analyses involving OC use were restricted to women born in 1945 or later, among whom trends

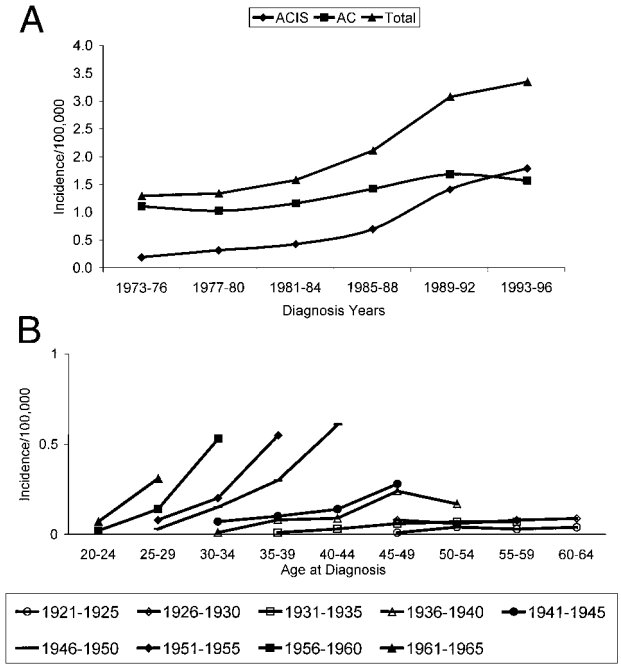

Fig. 1. *A*, Age-adjusted incidence rate of adenocarcinoma of the cervix from SEER data from 1976–1996 among 20–64-year-old white women. *B*, age-specific incidence rates of ACIS among white women born between 1921 and 1965 by year of birth.

in OC use became stable in the United States (29). Additional adjustment for race or age at first intercourse did not affect the results. Age was included as a continuous, linear term in all analyses.

Incidence data for *in situ* adenocarcinoma from the 1973–1996 SEER database was provided by the National Cancer Institute (8). The incidence data are for white women, 20–64 years old, with histology coded as International Classification of Diseases for Oncology code 8140.

Results

Analysis of SEER Data. The incidence of adenocarcinoma (ACIS and AC combined) increased steadily between 1973 and 1996 (Fig. 1*A*). In the 1973–1976 four-year time period, the incidence of ACIS was 0.2 per 100,000 women per year; by the 1993–1996 time point, the incidence of ACIS had increased 9-fold to 1.8 per 100,000 women per year. At the same time points, the rate of AC changed less dramatically, from 1.1 to 1.6 per 100,000 women per year.

When the ACIS incidence data for white women 20–64 years old were examined by age at diagnosis and year of birth (Fig. 1*B*), a strong cohort effect was evident. The age-specific rates begin to increase steadily with women born in 1946 or later for each birth cohort. The age-specific rates for women born in 1945 or earlier, however, increased only slightly for the women born between 1936 and 1945 and did not increase at any age for women born before 1936.

Analysis of Case-Control Data. In this study, 43% of interviewed women diagnosed with ACIS were 30–49 years old at diagnosis (Table 1), and over 90% were white. The income distribution of the cases was similar to the distribution seen in the control population. The number of years of education and the number of women who were unmarried at the time of the interview were slightly higher for control subjects.

Table 1 Demographic characteristics of women with ACIS and population-based controls

	Cases (n = 150)		Controls (n = 651)	
	n	%[a]	n	%[a]
Age (yrs)				
<30	32	21.3	91	14.0
30–49	65	43.3	215	33.0
40–49	34	22.7	172	26.4
50+	19	12.7	173	26.6
Race				
White	136	90.7	601	92.3
Black	3	2.0	19	2.9
Native American	3	2.0	4	0.6
Asian	6	4.0	18	2.8
Other	2	1.3	9	1.4
Income				
$45,000+	64	42.7	266	41.4
$30–$45,000	31	20.7	149	23.2
$15–$30,000	39	26.0	168	26.1
<15,000	16	10.6	60	9.3
Missing			8	
Education (yrs)				
15+	55	36.7	273	41.9
13–14	48	32.0	185	28.4
≤12	47	31.3	193	29.6
Currently married				
Yes	59	39.3	220	33.8
No	91	60.7	431	66.2

[a] Percentages are adjusted to the age distribution of the control population.

Table 2 Prevalence of HPV DNA in cervical biopsies from case subjects with ACIS

HPV DNA	ACIS (n = 82)	
	n	%
Negative, total	11	13.4
Positive, total	71	86.6
Specific HPV types		
HPV-16	22	31.0
HPV-16+18	10	14.1
HPV-18	33	46.5
HPV-18+72	1	1.4
HPV-6	2	2.8
HPV-*X*[a]	3	4.2

[a] HPV type unknown.

During rereview of biopsy samples by the study pathologist (P. P.), the cervical biopsy tissue from 82 subjects was identified as ACIS only; 4 samples were identified as CIS only; 8 samples were identified as ACIS and CIS together; 2 samples were identified as ACIS and AC; and 1 sample was identified as CIS and invasive squamous cell cancer. A 536-bp fragment of the β-globin gene was amplifiable in 97 of 101 (96.1%) of the samples. The four β-globin-negative samples were identified as ACIS only.

PCR for HPV DNA followed by RFLP analysis was performed on tumor tissue from case subjects from whom archived tissue was available (n = 97). Among those tumors identified by the study pathologist as ACIS only (n = 82), the prevalence of any type of HPV DNA was 86.6%. Among the 71 HPV DNA-positive ACIS tumors, 51.5% contained HPV-18

Table 3 Risk of ACIS by sexual history; serology for HPV-16, HPV-18, and HSV-2 antibodies; history of genital warts; and smoking status

	Cases (n = 150)		Controls (n = 651)		OR[a]	95% CI
	n	%	n	%		
No. of partners						
1	22	14.7	182	28.5	1.0	
2–4	47	31.3	181	28.3	1.8	1.0–3.1
5+	81	54.0	276	43.2	1.8	1.1–3.1
Age at first intercourse (yrs)						
20+	32	21.3	230	35.9	1.0	
18–19	38	25.3	167	26.1	1.5	0.9–2.5
<18	80	53.4	243	38.0	1.7	1.1–2.8
Smoking history						
Never	79	52.7	339	52.1	1.0	
Ever	71	47.9	312	47.9	0.9	0.6–1.3
Former	37	24.7	167	25.7	1.0	0.6–1.5
Current	34	22.6	145	22.3	0.8	0.5–1.3
History of genital warts						
Never	123	82.0	586	90.0	1.0	
Ever	27	18.0	65	10.0	1.5	0.9–2.5
HSV-2 antibody						
Negative	95	70.4	424	74.4	1.0	
Positive	40	29.6	146	25.6	1.2	0.8–1.9
HPV-16 antibody						
Negative	99	76.7	388	70.0	1.0	
Positive	30	23.3	166	30.0	0.7	0.5–1.1
HPV-18 antibody						
Negative	63	48.8	414	74.9	1.0	
Positive	66	51.2	139	25.1	3.3	2.2–4.9

[a] ORs are adjusted for age, except for those associated with a history of warts and smoking status, which are adjusted for age and number of partners.

Table 4 Risk of ACIS associated with OC use among women born in 1945 or later

	Cases (n = 133)		Controls (n = 478)		OR[1] (95% CI)[a]	OR[2] (95% CI)[a]
	n	%	n	%		
OC use						
Never	8	6.1	74	16.2	1.0	
Ever	124	93.9	384	83.8	2.7 (1.2–5.8)	
Duration of use (mo)						
0–<1	8	6.1	74	16.2	1.0	
1–71	64	48.5	250	54.6	2.1 (1.0–4.8)	
72–143	40	30.3	101	22.1	3.4 (1.5–8.0)	
144+	20	15.2	33	7.2	5.5 (2.1–14.6)	
					$P < 0.001$[b]	
Age at first use (yrs)						
Never	8	6.1	74	16.2	1.0	
20+	49	37.1	168	36.7	2.6 (1.1–5.8)	1.0
18–19	31	23.5	110	24.0	2.4 (1.0–5.5)	0.8 (0.5–1.4)
16–17	31	23.5	79	17.2	2.9 (1.2–6.9)	0.9 (0.5–1.7)
<15	13	9.8	27	5.9	3.7 (1.3–10.4)	1.1 (0.5–2.5)
					$p = 0.02$[b]	
Interval since last use (yrs)						
Never	8	6.1	74	16.2	1.0	
10+	51	38.6	190	41.4	2.2 (0.9–5.0)	1.0
<10	38	28.8	124	27.1	2.4 (1.0–5.6)	0.7 (0.4–1.3)
Current	35	26.5	70	15.3	4.5 (1.9–10.9)	1.0 (0.5–2.1)
					$P < 0.001$[b]	

[a] OR^1 is adjusted for age (continuous, linear), lifetime number of sex partners (1, 2–4, 5+), and interval since last screening Pap (<12 months, 12–35 months, 36+ months). OR^2 is adjusted as described for OR^1 but is also adjusted for duration of OC use, and the comparison is restricted to OC users.
[b] Test for trend.

DNA, and 40.2% contained HPV-16 DNA (Table 2). HPV DNA types 6 and 72 were also identified in three specimens. Three other specimens were HPV DNA late consensus primer positive with an unrecognizable pattern on RFLP. Multiple HPV DNA types were present in 13.4% of the typed case specimens.

All case subjects reported a history of sexual intercourse, but nine of the control subjects were virgins, and three refused to provide information about their lifetime number of sexual partners. Compared to women reporting only one sexual partner, the relative risk of ACIS was increased for women with two to four sexual partners (OR, 1.8; 95% CI, 1.0–3.1) and women with five or more partners (OR, 1.8; 95% CI, 1.1–3.1); Table 3. Having been less than 18 years old at first intercourse was also associated with a 70% increased relative risk of ACIS (OR, 1.7; 95% CI, 1.1–2.8). Smoking cigarettes was not associated with an increased risk of ACIS because the smoking histories of case and control subjects were similar. When we examined smoking duration, there was no evidence of a trend by number of years smoked.

A history of genital warts (OR, 1.5; 95% CI, 0.9–2.5) was marginally associated with an increased relative risk of ACIS. The seroprevalence of HPV-16 in control subjects, 30.0%, was somewhat higher than the seroprevalence of HPV-16 in the ACIS case subjects, (23.3%; age-adjusted OR, 0.7; 95% CI, 0.5–1.1). The seroprevalence of antibodies to HPV-18 was 25.1% in controls and 51.2% in cases. In contrast to the lack of association between ACIS and antibodies to HPV-16, the relative risk associated with seropositivity to HPV-18 was 3.3 (95% CI, 2.2–4.9). Among women positive for HPV-16 DNA, the seroprevalence of HPV-16 L1 was 35.3%, and the seroprevalence of HPV-18 L1 was also 35.3%. In contrast, among women with HPV-18 DNA in their tumor tissue, 26.7% were HPV-16 L1 seropositive, and 60.0% were HPV-18 L1 seropositive. Among women with HPV-18 DNA in their tumor tissue, compared to controls, the relative risk of ACIS associated with seropositivity was 5.5 (95% CI, 2.3–13.6).

There was an increased relative risk of ACIS associated with ever use of OCs (Table 4; OR, 2.7; 95% CI, 1.2–5.8). The risk was not affected by adjustment for interval since last Pap smear or frequency of Pap smears, which may be due to the high prevalence of OC use in both the case and control groups (94% and 84%, respectively). The relative risk associated with duration of OC use increased linearly ($P < 0.001$ for trend). Women who used OCs for 12 or more years had a relative risk of 5.5 associated with ACIS (95% CI, 2.1–14.6) compared with women who never used OCs. There were also significant trends for early age at first OC use and recent OC use. These trends were no longer apparent after adjustment for duration of use (Table 4). There was an increased relative risk (OR, 2.5; 95% CI, 0.9–6.7) of ACIS among women who had used OCs for 1–6 months compared to women who had never used them (data not shown).

In an analysis restricted to women who had regular Pap screening in the 36 months prior to reference date, the relative risk estimates were similar to those seen in Table 4. Among these recently screened women, the risk of ACIS associated with ever use of OCs was 3.2 (95% CI, 1.2–8.3) adjusted for age, interval since last screen, and number of sexual partners. The relative risks were slightly elevated compared to those shown in Table 4 when adjustment was made for HPV-18 serology instead of number of partners (OR, 4.0; 95% CI, 1.7–9.4 for ever use adjusted for age, interval since last Pap, and HPV-18 serology). Alternatively, when sex partners and

HPV-18 were included in the model, the relative risk of ACIS associated with ever use of OCs was 3.2 (95% CI, 1.4–7.6) adjusted for age, interval since last Pap, number of sexual partners, and HPV-18 serology.

Discussion

This study found evidence of a cohort effect in ACIS incidence, a trend of increasing risk with longer duration of OC use, and an increased risk associated with HPV, especially HPV-18. Taken together, these results suggest that there has been a potential increase in ACIS incidence but do not completely rule out an increase due solely to detection bias.

The increasing incidence of ACIS and AC may be attributed to improved detection with the addition of endocervical brushes to cytologic screening. However, the endocervical brush was introduced in the early 1990s, and the incidence rates have been increasing since the 1970s. Another source of potential improved detection could be that more ACIS has been detected as it has become more widely discussed in the literature, bringing it to the attention of more pathologists. Further evaluation of the impact of screening on ACIS incidence may be possible in the setting of a population-based cytology registry.

If OC users are overrepresented in our case group because they receive more Pap smear screening, the risk estimates for OC use could be inflated. Women who do not receive cytologic screening as often as OC users may escape diagnosis until an invasive, potentially symptomatic stage of disease or may regress without having been diagnosed. In an attempt to mitigate this potential bias, we assessed OC exposures in an analysis that was restricted to women who have had regular Pap screening in the 36 months before reference date. Interval since last screening cytology was controlled for in the analysis. The relative risk estimates for the OC use variables were similar to those reported in Table 4 among women with an equal opportunity for diagnosis of asymptomatic disease via screening [*e.g.,* the risk of ACIS associated with ever use of OCs was 3.2 (95% CI, 1.2–8.3) adjusted for age, interval since last screen, and number of sexual partners]. Therefore, it does not appear that the increased risk of ACIS associated with OC use is solely the result of opportunity for screening. Also, the significant trend seen with duration of use suggests a dose-response effect, which strengthens the plausibility of the OC association.

The diagnosis of ACIS is somewhat controversial. Endocervical abnormalities such as dysplasia, tubal metaplasia, cervical endometriosis, and pathological or histological changes due to previous biopsy, cervicitis, and use of OCs have been misdiagnosed as ACIS (30–34), and others have reported on the difficulty of distinguishing ACIS from microinvasive lesions (35) and even invasive disease (36). We were able to include an expert rereview of the tumor tissue for 101 of the 150 case subjects. Among the cases rereviewed by the study pathologist, 86 of 101 (85.1%) were confirmed to contain ACIS only, 10 contained ACIS and CIS (9.9%), and 5 contained only CIS (5.0%). When the OC analyses were restricted to the subset of women with ACIS only on rereview by the study pathologist, the results were similar to the results for all interviewed cases.

Another limitation of this study may be the somewhat low response rate. If case or control subjects who did not participate in the study differed from those who did respond in a consistent way, a bias would be introduced. Also, if case of control subjects were more or less likely to recall specific exposures, then our estimate associated with that exposure could possibly be biased. Efforts were made to enhance recall of sexual relationships, reproductive, contraceptive, and Pap smear history by use of a calendar of life events during the in-person interview. Data on age at diagnosis and smoking were available from the registry for women who were not interviewed. The age distribution of the noninterviewed cases was similar to that of the cases interviewed [38.3 years (median, 38) and 37.5 years (median, 37)]. Smoking information, which was collected from only 27 of 54 nonrespondent cases by the registry, indicated that 77.8% were never smokers compared to 52.7% never smokers among those interviewed. Without the same information from controls, it is unclear how the relative risk for smoking would be affected. Smoking information from the registry and from the interview was in agreement for 83.0% of the interviewed women. First course of cancer-directed therapy as recorded on the registry was identical for all interviewed and noninterviewed cases (*i.e.,* treatment was by surgery alone without radiotherapy or chemotherapy).

In this study, elevated relative risks of ACIS were associated with two or more lifetime number of sex partners and early age at first intercourse. These data confirm that ACIS, like squamous cell cervical neoplasms, has a sexually transmitted etiology. Furthermore, there were marginally increased relative risks of ACIS associated with a history of genital warts, which may be a marker of exposure to a sexually transmitted pathogen.

HPV has been implicated in the etiology of AC (12, 15). We found that 86.6% of the ACIS tumors were HPV DNA positive, which may be an underestimate because we used archived tissue that may be degraded. Therefore, HPV is most likely to be integral to the causal mechanism for ACIS.

Several studies have reported that a higher prevalence of HPV-16 antibodies is a risk factor for squamous cell cervical and vulvar cancers (23, 27, 37, 38). Because many ACIS lesions are reportedly found next to squamous cell lesions, and because we found oncogenic HPV-16 DNA and HPV-18 DNA to be prevalent among ACIS cases, one might expect to find an association between HPV-16 seroprevalence and ACIS. However, antibodies to HPV-18 L1, but not HPV-16 L1, were associated with an increase in the risk of ACIS. There may be some immunological interference in the presence of HPV-18 infection that allows only one type of antibody to be formed. Only 11% of the control subjects and 18% of the case subjects were seropositive for both HPV-16 and HPV-18. Using the same antibody test, Carter *et al.* (39) provided evidence in a different population that antibody response was type specific. The data presented here support a strong role for HPV-18 infection in the etiology of ACIS.

Smoking has been reported to be a risk factor for squamous cell carcinomas but not adenocarcinomas of the cervix (16, 17, 40, 41). There was no increased risk for ACIS among women who smoked cigarettes. This finding is consistent with the hypothesis that glandular cells are exposed to carcinogens during periods of increased ectopy and that ectopy is diminished in women who smoke (42). Furthermore, it has been reported that nicotine stimulates growth of epithelial cells in healthy women (43) and that nicotine stimulates the growth of HPV-immortalized ectocervical cells (44). In the latter *in vitro* study, this effect was not seen in endocervical cell lines. Thus, a lack of exposure to the carcinogens of cigarette smoke through decreased ectopy and a lack of stimulation of endocervical cell proliferation but enhancement of ectocervical cell growth by nicotine may account for the observed lack of an association of smoking in ACIS as compared to the generally increased risks associated with smoking for squamous cell cervical cancer.

There was a greater than 2-fold increased risk of ACIS associated with ever use of OCs and a more than 5-fold risk for 12 or more years of OC use, consistent with the reports of most (5, 17, 18, 20, 45, 46) but not all (16) studies of AC and ACIS. In a recent case-control study, Lacey *et al.* (20) found OC use to be associated with an increased risk of ACIS but not of invasive adenocarcinoma. That study included 33 women with ACIS, and current use was found to be the most important OC risk factor (OR, 12.6; 95% CI, 2.5–64.2) after controlling for HPV and many other factors (20). Among current users in this study, the relative risk was 3.4 (95% CI, 1.1–10.2) for 6–11 years of OC use and 6.1 (95% CI, 1.4–27.2) for 12 or more years of OC use compared to women with less than 6 years of OC use. There was not a significant trend because the data were limited to 35 cases and 70 controls, but the increasing estimates may indicate that duration of use is the strongest risk factor even among current users.

We did not have the opportunity to determine the presence of HPV DNA in control subjects for this study. Residual confounding by HPV could lead to overestimation of the risks associated with OC use. In an attempt to make the controls more comparable to cases on exposure to HPV, we repeated the OC analysis but used only HPV-18 L1-seropositive controls compared to all cases. We include all cases because we assume that most if not all cases of ACIS are HPV related. The relative risk of ACIS was 3.3 (95% CI, 1.2–9.3) for ever use of OCs and 3.8 (95% CI, 1.2–11.5) for 6 or more years of OC use, comparable to the results we presented in Table 4. Furthermore, the relative risk of ACIS associated with ever use of OCs was 3.2 (95% CI, 1.4–7.6) adjusted for age, interval since last screen, number of sexual partners, and HPV-18 serology. These additional surrogates of HPV increased the point estimates and the width of the confidence intervals but may indicate that additional control for HPV does not eliminate the risk associated with OC use.

We chose to restrict the OC analyses to women born in 1945 or later because these women represent the first cohort that had OCs available from the beginning of their reproductive life (29). Coinciding with the availability of OCs, age-specific incidence rates of ACIS appear to be increasing among women born in 1946 or later. These women, as opposed to women born in 1945 or earlier, were becoming sexually active during a period (the 1960s) that is generally characterized by less restricted sexual behavior in the United States. In these data, the percentage of control women with five or more partners was 21.9% among those born before 1945 and 52.3% among those born after 1946. Likewise, the percentage of case subjects with five or more partners was 38.9% and 56.4% in those birth cohorts, respectively. Whether the increasing age-specific incidence of ACIS in 5-year age cohorts since 1945 is due to a detection bias, increased exposure to HPV from having more partners, or OC use is difficult to separate. The data presented here would support a role for OC use as a promoter of HPV-related carcinogenesis.

There are two lines of evidence to support an etiological pathway by which OCs may promote accumulation of endocervical changes necessary to promote carcinogenesis. OCs may affect the risk of cervical cancer by enhancing HPV infection of endocervical cells and/or oncogenic transformation of infected cells. A prospective study of young women (42) showed that OC use increases the prevalence of ectopy, perhaps magnifying the risk of HPV infection of endocervical cells. Others have proposed a directly carcinogenic effect of OCs on the actively phagocytic metaplastic glandular cells found in adolescent women (1, 47). Evidence from *in vitro* studies of Pater *et al.* (48, 49) show that cells became transformed and tumorigenic in the presence of HPV and progestins. In another study, Mittal *et al.* (50) showed that HPV expression was enhanced when progesterone or glucocorticoids were added to human ectocervical cell lines. It is possible that any promotional effect of OCs could act at more than one step in the accumulation of changes that lead to ACIS.

If smoking cigarettes decreases ectopy and OC use promotes ectopy, nonsmokers and OC users may be at greater risk of HPV infection of endocervical cells because of having more endocervical cells in the transformation zone. If so, smoking and OC use could be important for the initial HPV infection of endocervical cells. They could also act as promoters that could aid transformation, surveillance evasion, and accumulation of carcinogenic changes. There was no evidence of an interaction between smoking and OC use in this study.

By studying the risk factor data with an understanding of the historical context provided by the incidence data, a picture of the potential cofactors leading to the increase in ACIS incidence is beginning to emerge. It may be that increased exposure of glandular epithelium to HPV, promoted by OC use, may be such a factor. It is therefore important to continue to closely monitor young women who use OCs by cytologic screening, to continue population-based epidemiological studies to refine risk factors, and to track trends in incidence of ACIS and AC.

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References

1. Peters, R. K., Chao, A., Mack, T. M., Thomas, D., Bernstein, L., and Henderson, B. E. Increased frequency of adenocarcinoma of the uterine cervix in young women in Los Angeles County. J. Natl. Cancer Inst. (Bethesda), *76:* 423–428, 1986.

2. Schwartz, S., and Weiss, N. Increased incidence of adenocarcinoma of the cervix in young women in the United States. Am. J. Epidemiol. *124:* 1045–1047, 1986.

3. Chilvers, C., Mant, D., and Pike, M. C. Cervical adenocarcinoma and oral contraceptives. Br. Med. J., *295:* 1446–1447, 1987.

4. Eide, T. J. Cancer of the uterine cervix in Norway by histologic type, 1970–1984. J. Natl. Cancer Inst. (Bethesda), *79:* 199–205, 1987.

5. Ursin, G., Peters, R. K., Henderson, B. E., d'Ablaing, G., III, Monroe, K. R., and Pike, M. C. Oral contraceptive use and adenocarcinoma of cervix. Lancet, *344:* 1390–1394, 1994.

6. Beral, V., Hermon, C., Munoz, N., and Devesa, S. S. Cervical cancer. Cancer Surv., *19/20:* 65–285, 1995.

7. Vizcaino, A., Moreno, V., Bosch, F. X., Munoz, N., Barros-Dios, X. M., and Parkin, D. M. International trends in the incidence of cervical cancer. I. Adenocarcinoma and adenosquamous cell carcinomas. Int. J. Cancer, *75:* 536–545, 1998.

8. Surveillance, Epidemiology, and End Results (SEER) Program Public-Use CD-ROM (1973–1996). National Cancer Institute, DCPC, Surveillance Program, Cancer Statistics Branch, released April 1999, based on the August 1998 submission.

9. Teshima, S., Shimosato, Y., Kishi, K., Kasamatsu, T., Ohmi, K., and Uei, Y. Early stage adenocarcinoma of the uterine cervix. Cancer (Phila.), *56:* 167–172, 1985.

10. Bertrand, M., Licklish, G. M., and Colgsn, T. J. The anatomic distribution of cervical adenocarcinoma *in situ*: implications for treatment. Am. J. Obstet. Gynecol., *157:* 21–25, 1987.

11. Kashimura, M., Shinohara, M., Oikawa, K., Hamasaki, K., and Sato, H. An adenocarcinoma *in situ* of the uterine cervix that developed into invasive adenocarcinoma after 5 years. Gynecol. Oncol., *36:* 128–133, 1990.

12. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Human Papillomaviruses. IARC Mongographs, Vol. 64. Lyon, France; IARC, 1995.

13. Tase, T., Okagaki, T., Clark, B. A., Manias, D. A., Ostrow, R. S., Twiggs, L. B., and Faras, A. J. Human papillomavirus types and localization in adenocarcinoma and adenosquamous carcinoma of the uterine cervix: a study by *in situ* DNA hybridization. Cancer Res., *48:* 993–998, 1988.

14. Farnsworth, A., Laverty, C., and Stoler, M. H. Human papillomavirus messenger RNA expression in adenocarcinoma *in situ* of the uterine cervix. Int. J. Gynecol. Pathol., *8:* 321–330, 1989.

15. Bosch, F. X., Manos, M. M., Sherman, M. E., Jansen, A., Peto, J., Schiffman, M. H., Moreno, V., and Shah, K. V. Prevalence of HPV DNA in cervical cancer in 22 countries. J. Natl. Cancer Inst. (Bethesda), *87:* 796–802, 1995.

16. Parazzini, F., La Vecchia, C., Negri, E., Fasoli, M., and Cecchetti, G. Risk factors for adenocarcinoma of the cervix: a case-control study. Int. J. Cancer, *57:* 201–204, 1988.

17. Brinton, L. A., Tashima, K. T., Lehman, H. F., Levine, R. S., Mallin, K., Savitz, D. A., Stolley, P. D., and Fraumeni, J. F. Epidemiology of cervical cancer by cell type. Cancer Res., *47:* 1706–1711, 1987.

18. Brinton, L. A., Reeves, W. C., Brenes, M. M., Herrero, R., de Britton, R. C., Gaitan, E., Tenorio, F., Garcia, M., and Rawls, W. E. Oral contraceptive use and risk of invasive cervical cancer. Int. J. Epidemiol., *19:* 4–11, 1990.

19. Ursin G., Pike, M. C., Preston-Martin, S., D'Ablaing, G., III, and Peters, R. K. Sexual, reproductive, and other risk factors for adenocarcinoma of the cervix: results from a population-based case-control study (California, United States). Cancer Causes Control, *7:* 391–401, 1996.

20. Lacey, J., Brinton, L. A., Abbas, F. M., Barnes, W. A., Gravitt, P. E., Greenberg, M. D., Greene, S. M., Hadjimichael, O., McGowan, L., Mortel, R., Schwartz, P. E., Silverberg, S. G., and Hildesheim, A. Oral contraceptives as risk factors for cervical adenocarcinomas and squamous cell carcinomas. Cancer Epidemiol. Biomark. Prev., *8:* 1079–1085, 1999.

21. Hartge, P., Brinton, L. A., Rosenthal, J. F., Cahill, J. I., Hoover, R. A., and Waksberg, J. Random digit dialing in selecting a population-based control group. Am. J. Epidemiol., *120:* 825–833, 1984.

22. Harlow, B. L., and Davis, S. Two one-step methods for household screening and interviewing using random digit dialing. Am. J. Epidemiol., *127:* 857–863, 1988.

23. Daling, J. R., Madeleine, M. M., McKnight, B., Carter, J. J., Wipf, G. C., Ashley, R., Schwartz, S. M., Beckmann, A. M., Hagensee, M. E., Mandelson, M. T., and Galloway, D. A. The relationship of human papillomavirus-related cervical tumors to cigarette smoking, oral contraceptive use, and prior herpes simplex virus type 2 infection. Cancer Epidemiol. Biomark. Prev., *5:* 541–548, 1996.

24. Manos, M. M., Ting, Y., Wright, D. K., Lewis, A. J., and Broker, T. R. Use of the polymerase chain reaction for detection of genital human papillomavirus. Cancer Cells (Cold Spring Harbor), *7:* 209–214, 1989.

25. Anderson, S., Shera, K., Ihle, J., Billman, L., Goff, B., Greer, B., Tamimi, H., McDougall, J., and Klingelhutz, A. Telomerase activation in cervical cancer. Am. J. Pathol., *151:* 25–31, 1997.

26. Longu, O., Wright, T. C., and Silverstein, S. Typing of human papillomavirus by polymerase chain reaction amplification with L1 consensus primers and RFLP analysis. Mol. Cell Probes, *6:* 145–152, 1992.

27. Carter, J. J., Koutsky, L. A., Wipf, G. C., Christensen, N. D., Lee, S-K., Kuypers, J., Kiviat, N., and Galloway, D. A. The natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. J. Infect. Dis., *174:* 927–936, 1996.

28. Ashley, R., and Militoni, J. Use of densitometric analysis for interpreting HSV serologies based on western blot. J. Virol. Methods, *18:* 159–168, 1987.

29. Dawson, D. A. Trends in use of oral contraceptives. Data from the 1987 national health interview survey. Family Plann. Perspect., *22:* 169–172, 1990.

30. Valente, P. T., and Hanjani, P. Endocervical neoplasia in long-term users of oral contraceptives: clinical and pathologic observations. Obstet. Gynecol., *67:* 695–704, 1986.

31. Yeh, I-T., LiVolsi, V. A., and Noumoff, J. S. Endocervical carcinoma. Pathol. Res. Pract., *187:* 129–144, 1991.

32. Pacey, F., Ayer, B., and Greenberg, M. The cytologic diagnosis of adenocarcinoma *in situ* of the cervix uteri and related lesions. III. Pitfalls in diagnosis. Acta Cytol., *32:* 325–330, 1988.

33. Lee, K. R. False positive diagnosis of adenocarcinoma *in situ* of the cervix. Acta Cytol., *32:* 276–277, 1988.

34. Novotny, D. B., Maygarden, S. J., Johnson, D. E., and Frable, W. J. Tubal metaplasia: a frequent potential pitfall in the cytologic diagnosis of endocervical glandular dysplasia on cervical smears. Acta Cytol., *36:* 1–10, 1992.

35. Kudo, R., Sagae, S., and Hayakawa, O. Morphology of adenocarcinoma *in situ* and microinvasive adenocarcinoma of the uterine cervix: a cytologic and ultrastructural study. Acta Cytol., *35:* 109–116, 1991.

36. Fu, Y. S., Berek, J. S., and Hilborne, L. H. Diagnostic problems of *in situ* and invasive adenocarcinoma of the uterine cervix. Appl. Pathol., *5:* 47–56, 1987.

37. Shah, K., Viscidi, R. P., Alberg, A. J., Helzlsouer, K. J., and Comstock, G. W. Antibodies to human papillomavirus 16 and subsequent *in situ* or invasive cancer of the cervix. Cancer Epidemiol. Biomark. Prev., *6:* 233–237, 1997.

38. Nonnemacher, B., Hubbert, N. L., Kirnbauer, R., Shah, K. V., Munoz, N., Bosch, F. X., de San Jose, S., Viscidi, R., Lowy, D. R., and Shiller, J. T. Serologic response to human papillomavirus type 16 (HPV-16) virus-like particles in HPV-16 DNA: positive invasive cervical cancer and CINIII patients and controls from Colombia and Spain. J. Infect. Dis., *172:* 19–24, 1995.

39. Carter, J. J., Koutsky, L. A., Hughes, J. P., Lee, S. K., Kuypers, J., Kiviat, N., and Galloway, D. A. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody following incident infection. J. Infect. Dis., *181:* 1911–1919, 2000.

40. Hopkins, M. P., and Morley, G. W. A comparison of adenocarcinoma and squamous cell carcinoma of the cervix. Obstet. Gynecol., *77:* 912–917, 1991.

41. Daling, J. R., Sherman, K. J., Hislop, T. G., Maden, C., Mandelson, M. T., Beckmann, A. M., and Weiss, N. S. Cigarette smoking and the risk of anogenital cancer. Am. J. Epidemiol., *135:* 180–189, 1992.

42. Critchlow, C., Wolner-Hanssen, P., Eschenbach, D. A., Kiviat, N. B., Koutsky, L. A., Stevens, C. E., and Holmes, K. K. Determinants of cervical ectopia and of cervicitis: age, oral contraception specific cervical infection, smoking, and douching. Am. J. Obstet. Gynecol., *173:* 534–543, 1995.

43. Basu, J., Mikhail, M. S., Paraudeau, P. H., Palan, P. R., and Romney, S. L. Smoking and the antioxidant ascorbic acid: plasma leukocyte and cervicovaginal cell concentrations in normal healthy women. Am. J. Obstet. Gynecol., *163:* 1948–1952, 1990.

44. Waggoner, S. E., and Wang, X. Effect of nicotine on proliferation of normal, malignant, and human papillomavirus-transformed human cervical cells. Gynecol. Oncol., *55:* 91–95, 1994.

45. Thomas, D. B., Ray, R. M., and the WHO Collaborative Study of Neoplasia and Steroid Contraceptives. Oral contraceptives and invasive adenocarcinomas and adenosquamous carcinomas of the uterine cervix. Am. J. Epidemiol., *144:* 281–289, 1996.

46. Brinton, L. A., Herrero, R., Reeves, W. C., de Britton, R. C., Gaitan, E., and Tenorio, F. Risk factors for cervical cancer by histology. Gynecol. Oncol., *51:* 301–306, 1993.

47. Coppelson, M. The origin and nature of premalignant lesions of the cervix uteri. Int. J. Gynaecol. Obstet., *8:* 539–550, 1970.

48. Pater, M. M., Hughes, G. A., Hyslop, D. E., Nakshatri, H., and Pater, A. Glucocorticoid-dependent oncogenesis by type 16 but not type 11 human papillomavirus DNA. Nature (Lond.), *335:* 832–835, 1988.

49. Pater, A., Bayatpour, M., and Pater, M. M. Oncogenic transformation by human papillomavirus type 16 deoxyribonucleic acid in the presence of progesterone or progestins from oral contraceptives. Am. J. Obstet. Gynecol., *162:* 1099–1103, 1990.

50. Mittal, R., Pater, A., and Pater, M. M. Multiple human papillomavirus type 16 glucocorticoid response elements functional for transformation, transient expression, and DNA-protein interactions. J. Virol., *67:* 5656–5659, 1993.