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The role of co-factors in the progression from human papillomavirus infection to cervical cancer

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Abstract

Objective—Co-factors for cervical cancer, including oral contraceptive (OC) use, smoking and multiparity have been identified; however, the stage at which they act in cervical carcinogenesis is not clear. We compared established risk factors among women with CIN2 and CIN3 to evaluate the heterogeneity of these factors in precancer and also assessed their role during cervical carcinogenesis.

Methods—The current analysis included 2783 women with various stages of cervical disease who were enrolled in the Study to Understand Cervical Cancer Early Endpoints and Determinants (SUCCEED) and the Biopsy Study. Associations of co-factors within cervical precancer and at different stages of cervical carcinogenesis were estimated using logistic regression.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Results—Long-term OC use (10+ years vs. never: OR=2.42, 95% CI: [1.13–5.15]), multiparity (3+ births vs. nulliparous: OR=1.54 [1.04–2.28]), smoking (ever vs. never: OR=1.95 [1.48–2.58]), and no Pap test in the previous five years (2.05 [1.32–3.17]) were positively associated with CIN3 compared to CIN2. We observed that long-term OC use, parity and smoking were associated with an increased risk of CIN3 compared to <CIN2 (1.97 [1.12–3.46]; 2.233 [1.59–3.11]; 2.60 [2.04–3.30], respectively), whereas associations were not significantly different (OC use, parity) or showed decreased risk (smoking) comparing cancer to CIN3.

Conclusions—Differences in established risk factors suggest that CIN3 is a more specific definition of precancer than CIN2. Hormonally-related factors and smoking play a role in the transition from human papillomavirus infection to precancer.

INTRODUCTION

Cervical carcinogenesis is defined by a multistage progression model where persistent infections with carcinogenic human papillomavirus (HPV) types lead to cervical precancer, which if left untreated can become invasive carcinoma [1–2]. The risk factors that influence the first stage of this model, infection, are well studied and are related to sexual behavior. Pooled analyses of epidemiological studies have established several co-factors for cervical cancer, including long term oral contraceptive (OC) use, smoking, multiparity and lack of cytology-based screening [3–5]; however, with the exception of screening, the mechanisms by which these factors increase risk are not clear. In addition, these studies have focused on comparing women with cancer to those without disease and little is known about how these co-factors potentially influence risk at different stages of cervical carcinogenesis.

Evaluating associations of risk factors during cervical carcinogenesis requires an accurate definition of cervical precancer. This can be challenging when working with the existing framework for histologically classifying precancerous lesions, which combines cervical intraepithelial neoplasia-2 (CIN2) and CIN3. Whereas CIN3 is a clear precursor lesion for cancer, CIN2 suffers from limited reproducibility and frequent misclassification [6–8]. Despite these limitations, recent recommendations for the histological diagnosis of cervical disease proposed a two-tiered approach, combining CIN2 and CIN3 for treatment decisions [9–10]. However, it remains important to understand potential differences in risk profiles among this disease category in order to better define cervical precancer and limit overtreatment.

In the current study, we used cross-sectional data from a large referral population in Oklahoma to compare established cervical cancer risk factors (OC use, smoking, multiparity, and screening history) in women diagnosed with CIN2 and CIN3. In addition, we assessed the role of these factors at the transition stages of the cervical carcinogenesis model.

MATERIALS AND METHODS

Study Population

Women with a recent abnormal Pap smear or biopsy diagnosis of CIN referred to the colposcopy clinic at the University of Oklahoma Health Sciences Center (OUHSC),

Oklahoma City, OK were invited for enrollment in either SUCCEED between 2003 and 2011 or Biopsy Study between 2009 and 2011. Details of the SUCCEED and Biopsy study design, inclusion and exclusion criteria have been described elsewhere [11–12]. Briefly, women were excluded if they were less than 18 years of age, pregnant at the time of their visit, or had been previously treated with chemotherapy or radiation for any cancer. Additionally, women were not eligible for the Biopsy Study if they had received previous treatment for CIN. After completing accrual of the study population for SUCCEED, we continued to enroll women with cancer from the same catchment area to enrich for cancer outcomes. Written informed consent was obtained from all women enrolled in the studies and Institutional Review Board approval was provided by OUHSC and the US National Cancer Institute.

Questionnaire

Details regarding content and administration of the questionnaire for SUCCEED have been previously described [11]. The questionnaire was designed to capture data on demographic information, HPV exposure and other co-factors that have been previously shown to be associated with cervical cancer. The questionnaire for the Biopsy Study was similar, with the exception of the removal of questions regarding history of medical conditions and sexually transmitted diseases, oral contraceptive (OC) use, and a subset of reproductive history questions. All interviews were conducted in a private setting by a nurse or other trained research staff.

Colposcopy and Specimen Collection

Colposcopy and specimen collection procedures for SUCCEED have been previously described [11, 13–14]. Minor changes in the biopsy protocol were implemented in the Biopsy Study and have been described elsewhere [12]. Histological diagnoses were community-based rather than adjudicated; however, we have previously demonstrated that disease categories in SUCCEED are comparable to general US practice, therefore using community histology diagnoses should not affect our results [11, 15–17]. As per standard practice, all histologically confirmed CIN3 and most CIN2 were treated by loop electrosurgical excision procedure (LEEP) of the transformation zone in both study protocols. Cytology specimens collected prior to colposcopy were used for ThinPrep cytology (Hologic Boxborough, MA) and HPV genotyping using the Linear Array HVP Genotyping Test (Roche Molecular Diagnostics). Analyses presented here were based on the worst overall diagnosis, including biopsy results and LEEP outcomes.

Final Analytic Population

With the exception of a few differences in questionnaire design and biopsy procedure performed during colposcopy (described above), the protocols and populations for the SUCCEED and Biopsy Study were similar; therefore, data were combined for purposes of analyses. A total of 3013 women were enrolled in the SUCCEED and Biopsy Study. Of these, 74 were enrolled in both studies; for the purposes of analyses, these women were attributed to the first study protocol they attended (n=59 for Biopsy Study and n=15 for SUCCEED). Of the 3013 women enrolled, 2956 had histology data from either a LEEP or biopsy. We excluded women with a histological diagnosis of “N/A” (n=9) or “other”

(n=11), and those who received more than 7 Pap tests in the previous 5 years (n=156), as these women are not representative of a typical screening population, leaving a final analytic population of 2783 women. Based on the worst overall diagnosis from either LEEP or biopsy results, the study population consisted of 1453 women with a histological diagnosis of <CIN2, 577 with CIN2, 476 with CIN3 or adenocarcinoma in situ (AIS), and 277 cancer cases [adenocarcinoma (n=46) and squamous cell carcinoma (n=231)]. We did not distinguish between biopsy results of negative and CIN1 because the distinction is poorly reproducible and in the context of molecular evidence of HPV infection, the natural history of the two categories is similar [8, 18]. A sensitivity analysis indicated that removal of the 250 women who were HPV-negative in the population did not change our results; therefore these women were not excluded.

Statistical Analyses

Unconditional logistic regression was used to generate odds ratios and 95% confidence intervals for indicated risk factors comparing a) CIN3 vs. CIN2, to understand the heterogeneity of precancer diagnoses; b) <CIN2 vs. CIN3, to explore the transition between HPV infection and the most definite diagnosis of precancer; c) CIN3 vs. cancer, to study predictors of invasion; and d) <CIN2 vs. cancer, to study the overall risk factors for cancer. We present associations from age-adjusted and multivariable adjusted models (adjusted for age, OC use, parity, frequency of Pap tests, smoking status, number of lifetime sexual partners, and age at sexual debut). Age-adjusted models for associations of OC use with outcomes were also adjusted for number of Pap tests since contraceptive method has been linked to frequency of doctor visits and cancer screening practices [19]. Because the Biopsy Study did not collect information on OC use, results from multivariable models represent estimates based on the SUCCEED population only. All statistical analyses were performed using STATA version 11.2 (StataCorp, College Station, TX).

RESULTS

Study Population

Characteristics with regards to demographics and distribution of cervical cancer risk factors based on histological diagnoses of the study population are presented in Table 1.

Risk Factor Associations in CIN2 and CIN3

We evaluated potential differences in risk factor associations to determine whether CIN2 and CIN3 should be combined as precancer in this population. Compared directly to CIN2, CIN3 was positively associated with long-term OC use (10+ years vs never: OR= 2.42, 95% CI [1.13–5.15]), multiparity (3+ live births vs. nulliparous: 1.54 [1.04–2.28]), smoking (ever vs. never: 1.95 [1.48–2.58]) and less frequent cervical cancer screening tests (no previous Pap test in previous 5 years vs. previous test: 2.05 [1.32–3.17]; Table 2). In addition, an increased age at sexual debut was associated with decreased risk of CIN3 compared to CIN2 (18+ vs. <16: 0.67 [0.47–0.96]). Because of the significant differences in risk factor associations between CIN2 and CIN3, we concluded that CIN2 represented equivocal cases of precancer, and excluded women with CIN2 from our analyses of risk factor associations within the multistage carcinogenesis model. However, it should be noted that when CIN2

was included in the precancer diagnosis, the results were largely similar, but clearly attenuated.

Risk Factor Associations in the Progression from HPV Infection to Cervical Cancer

Next, we next examined the associations of known cervical cancer risk factors with the transition from HPV infection (<CIN2) to precancer (CIN3 or AIS) and from precancer to cancer (Table 3). In addition, we present the conventional, overall comparison of women with cancer to those with <CIN2.

Oral Contraceptive Use—Consistent with previous results, we observed an increased risk of cervical cancer among long-term OC users comparing women with cancer to those with <CIN2 ($p_{\text{trend}}=0.02$; Table 3). In the transition from HPV infection to precancer, we observed an increased risk of CIN3 compared with <CIN2 for OC use, and an increasing risk with increasing duration of use (10+ years vs. never: 1.97 [1.12–3.46]; $p_{\text{trend}}=0.02$). In contrast, OC use was associated with a decreased risk of cancer compared with precancer. This association remained significant in women who used OCs for less than 5 years in the age-adjusted and multivariable-adjusted models, which included number of pap tests as a covariate (5+ years vs. never: 0.38 [0.19–0.76]).

Parity—Parity was associated with an increased risk of CIN3 compared to <CIN2, with the strongest association in women with 3+ live births (2.23 [1.59–3.11], $p_{\text{trend}}<0.0001$); however, no association was observed comparing women with cancer to those with CIN3.

Smoking—Current and former smoking was associated with an increased risk of cervical cancer and CIN3 compared with <CIN2. In contrast, a decreased risk of cancer compared to CIN3 was observed for current smoking (0.58 [0.37–0.93]). This association is likely driven by the increased number of women with cancer who are former smokers compared to other disease categories (Table 1). In addition, increased pack-years of smoking was associated with an increased risk of CIN3, but no associations were observed for other comparisons (data not shown).

Cervical Cancer Screening—Infrequent screening (<2 Pap tests in the previous 5 years) was associated with an increased risk of cancer (7.13 [4.38–11.60]) and CIN3 (1.67 [1.24–2.24]) compared with <CIN2. In addition, there was a positive association comparing cancer with CIN3 (2.18 [1.25–3.82]).

Lifetime Sexual Partners—Number of lifetime sexual partners was associated with an increased risk of cervical cancer and CIN3 compared with <CIN2; however, no trend for increasing risk with increasing number of partners was observed. No association of number of lifetime sexual partners was observed comparing cancer with CIN3.

Age at Sexual Debut—Delaying sexual debut until after the age of 18 was associated with a decreased risk of CIN3 (0.44 [0.33–0.60]) compared with <CIN2; however, no association was observed comparing cancer to CIN3.

DISCUSSION

Pooled analyses of case-control data have established several risk factors for cervical cancer, including OC use, parity, smoking and lack of cytology-based screening [3–5]. Most studies to date have compared cancer cases with controls, and there is limited data about how these factors act at different stages of cervical carcinogenesis. The few reports that have evaluated co-factors have only reported associations at the early transition to cervical precancer and did not compare precancer to cancer [11, 20–23]. Here, we first assessed the associations of known cervical cancer risk factors in CIN2 and CIN3 to decide whether they should be combined as precancer and then presented associations for the transition stages of the multi-step cervical carcinogenesis model. We showed marked differences in risk factors between women with CIN2 and CIN3, leading us to restrict the precancer category to the more definite diagnosis of CIN3. We then compared CIN3 with <CIN2 as an estimate for risk of progression, and cancer with CIN3 as an estimate for risk of invasion. Our results support a role for hormonally-related factors (OC use and parity) and smoking in the transition of HPV-infected epithelia to cervical precancer.

Recent recommendations for defining cervical precancer proposed combining histological diagnoses of CIN2 and CIN3 [9–10]; however, inclusion of CIN2 in the definition is problematic since this histologic category suffers from poor reproducibility and frequently represents misclassified CIN1 [6–7]. Furthermore, combining these diagnoses could lead to attenuated risk estimates as CIN2 has a higher probability of regressing than CIN3 [24–26]. Our results indicate that there is considerable heterogeneity of cervical precancer with respect to known cervical cancer risk factors, supporting potential differences in risk of progressing to cancer. Specifically, OC use, parity, smoking and lack of cytology-based screening were positively associated with CIN3 compared with CIN2.

Differences in HPV genotype prevalence associated with advancing stages of cervical carcinogenesis have been noted by several studies, with a high prevalence of HPV16 in women with CIN3 compared to women with <CIN3 [14, 27–28]. To account for potential differences in genotype distributions among CIN2 and CIN3, we adjusted for HPV status in two ways: including HPV infection type (HPV16+ vs. other high-risk HPV+) as a covariate in our models as well as conducting a stratified analysis (data not shown). When HPV status was included in our models as a covariate there was no change in the estimates, suggesting the different risk factor associations between CIN2 and CIN3 were not related to different genotype distributions. In our stratified analysis, parity and smoking remained positively associated with CIN3 compared with CIN2 among HPV16+ women, suggesting heterogeneity even among HPV16+ CIN2/3. Other studies have reported that HPV16+ CIN2 is similar to CIN3 with respect to co-factors and age at diagnosis and may represent a more specific diagnosis of precancer than CIN2 that is positive for other HPV types [11, 17, 24]. It is important to note that although these studies showed a similarity between CIN2/HPV16+ and CIN3, the magnitude of the associations were stronger for CIN3, which is in agreement with our findings. Therefore, our data suggest that there are stronger associations of co-factors with CIN3, independent of HPV type.

Several lines of evidence from epidemiological and mouse models suggest a role for hormones in cervical cancer development (reviewed in [29]). Specifically, these data suggest that estrogen plays a role not only in the development of cervical cancer, but also its immediate precursor, CIN3. Although indirect, our data are consistent with a role for hormones in cervical carcinogenesis; we observed a positive association of hormone-related risk factors, such as OC use and parity, with CIN3 compared to CIN2. Furthermore, these risk factors were more strongly associated with progression to CIN3 compared to invasion. This observation is consistent with a previous study of the heterogeneity of CIN3, which showed factors such as parity, OC use, and smoking were not associated with the size of CIN3 lesions, suggesting that they may not play a major role in the later stages of the natural history of cervical cancer [30].

We observed a few unexpected results in our analyses. First, although not significant, there was a positive association of parity with cervical cancer compared with <CIN2 after adjusting for age; however, in the multivariable model, the estimates suggested a non-significant inverse association. The reasons for this are unclear; however, including OC use, number of pap tests and age at sexual debut in the model led to the largest attenuation in the estimates. Second, current smoking was associated with a decreased risk of invasion. This observation was likely driven by the increased number of former smokers in women with cancer compared to other diagnoses. In addition, the median time since smoking cessation for former smokers who had cancer was 5 years and nearly 25% had quit within one year of enrollment, suggesting that most of these women had recently quit (data not shown). Although we cannot assess temporality in our study, these data suggest that the inverse association observed could be due, in part, to reverse causality (i.e., women giving up smoking because they were unwell). Finally, OC use was associated with a decreased risk of invasion. The reasons for this association are not clear. We included number of Pap testing as a potential confounder based on recent results suggesting contraceptive method is related to cervical cancer screening practices in this population; however we may have not fully adjusted for doctor visits in the current analysis [19]. In addition, it is possible that the cancer patients are different from other women included in the study based on referral patterns and other socioeconomic factors which we were unable to account for in our models.

The main strength of this study was the large number of women diagnosed with a wide range of cervical disease and the range in age at diagnosis. This allowed for the evaluation of risk factor associations at each stage of cervical carcinogenesis, especially the later stage of invasion (i.e. cancer vs. precancer), which has not been well addressed previously. Furthermore, results from our case-control analysis were generally consistent with previous literature, supporting the validity of our findings. Although the cross-sectional design of this study may be considered a limitation in determining the temporality of the exposures studied, current treatment guidelines make it unethical to conduct longitudinal studies to evaluate associations at the later stages of cervical carcinogenesis. It should also be noted that the questionnaire data used for this analysis was self-reported; however, it was administered as a private interview as opposed to self-administered, which should limit potential misclassification. In addition, our study population is comprised of women referred to colposcopy due to an abnormal Pap test result, thus our results may not reflect differences

that would be seen in a general population; however, our findings are consistent with what has been previously reported in the same and other populations [3–5, 11]. Finally, the average age of women with cancer was greater than that of other disease categories, potentially leading to incomplete adjustment for age in our models. Restricting to age groups with more overlap and adjusting for age in different ways did not meaningfully change our results. Nevertheless, we cannot rule out residual confounding by age in our comparisons of cancer with other disease groups.

In conclusion, we demonstrate that co-factor associations differ between CIN2 and CIN3, suggesting that CIN3 is a more specific definition of precancer compared to CIN2 and CIN3 combined. Our data indicate that most known co-factors that are not related to HPV acquisition act at the transition from HPV infection to cervical precancer, specifically hormonal factors and smoking.

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Research Highlights

- CIN2 and CIN3 are heterogeneous with respect to known cervical cancer risk factors.
- Hormonal factors are important for the progression from HPV infection to precancer.
- Smoking promotes progression from HPV infection to precancer.

Table 1

Characteristics of the study population

		<CIN2 (n=1453)	CIN2 (n=577)	CIN3 ^a (n=476)	Cancer (n=277)
Age, mean (SD) ^b		28.1 (9.88)	27.3 (7.35)	29.8 (9.39)	48.5 (13.4)
Oral contraceptive use ^c , n (%)					
	Never	154 (16.21)	47 (13.31)	42 (12.03)	37 (22.42)
	<5 years	639 (67.26)	251 (71.10)	236 (67.62)	79 (47.88)
	5–10 years	87 (9.16)	38 (10.76)	34 (9.74)	15 (9.09)
	10+ years	70 (7.37)	17 (4.82)	37 (10.60)	34 (20.61)
Number of live births, n (%)					
	0	473 (33.59)	172 (31.22)	96 (21.15)	26 (11.93)
	1	347 (24.64)	136 (24.68)	106 (23.35)	32 (14.68)
	2	307 (21.80)	131 (23.77)	119 (26.21)	55 (25.23)
	3+	281 (19.96)	112 (20.33)	133 (29.30)	105 (48.17)
Smoking status, n (%)					
	Never	665 (47.81)	226 (41.17)	115 (25.78)	78 (36.62)
	Former	205 (14.74)	56 (10.20)	65 (14.57)	54 (25.35)
	Current	521 (37.46)	267 (48.63)	266 (59.64)	81 (38.03)
	Never	665 (47.81)	226 (41.17)	115 (25.78)	78 (36.62)
	Ever	726 (52.19)	323 (58.83)	331 (74.22)	135 (63.38)
History of Pap test ^d , n (%)					
	Yes	1242 (91.32)	491 (92.29)	358 (82.68)	101 (47.87)
	No	118 (8.68)	41 (7.71)	75 (17.32)	110 (52.13)
Number of Pap tests ^d , n (%)					
	4–5	631 (46.40)	257 (48.31)	169 (39.03)	30 (14.22)
	2–3	382 (28.09)	145 (27.26)	122 (28.18)	36 (17.06)
	6–7	119 (8.75)	42 (7.89)	33 (7.62)	5 (2.37)
	<2	228 (16.76)	88 (16.54)	109 (25.17)	140 (66.35)
Number lifetime sex partners, n (%)					
	<4	452 (32.97)	144 (26.82)	102 (23.34)	62 (32.63)
	4–6	403 (29.39)	169 (31.47)	146 (33.41)	73 (38.42)
	7+	516 (37.64)	224 (41.71)	189 (43.25)	55 (28.95)
Age at sexual debut, n (%)					
	<16	460 (33.19)	206 (37.94)	185 (41.29)	45 (21.23)
	16–17	508 (36.65)	215 (39.59)	182 (40.63)	88 (41.51)
	18+	418 (30.16)	122 (22.47)	81 (18.08)	79 (37.26)

^a CIN3 includes Adenocarcinoma In Situ (AIS).^b SD: Standard deviation.^c SUCCEED population only.

^dIn the previous 5 years.

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Table 2

Risk factor associations among women with CIN2 and CIN3

		CIN3 ^a vs. CIN2 OR (95% CI)	
	Cases/Controls	Age adjusted	MV ^{b,c} adjusted
Oral contraceptive use ^c			
Never	42/47	Ref (1.00) ^e	Ref (1.00)
<5 years	236/251	1.09 (0.68–1.74) ^e	1.09 (0.66–1.81)
5–10 years	34/38	1.09 (0.57–2.09) ^e	1.06 (0.53–2.12)
10+ years	37/17	2.42 (1.13–5.15) ^e	2.55 (1.13–5.76)
Number of live births			
0	96/172	Ref (1.00)	Ref (1.00)
1	106/136	1.34 (0.93–1.93)	1.24 (0.77–1.98)
2	119/131	1.44 (1.00–2.08)	1.38 (0.85–2.22)
3+	133/112	1.54 (1.04–2.28)	1.60 (0.93–2.73)
Smoking status			
Never	115/226	Ref (1.00)	Ref (1.00)
Former	65/56	2.12 (1.37–3.27)	2.00 (1.13–3.54)
Current	266/267	1.92 (1.44–2.55)	1.86 (1.27–2.74)
Never	115/226	Ref (1.00)	Ref (1.00)
Ever	331/323	1.95 (1.48–2.58)	1.88 (1.29–2.74)
History of Pap test ^d			
Yes	358/491	Ref (1.00)	Ref (1.00)
No	75/41	2.05 (1.32–3.17)	1.76 (1.02–3.06)
Number of Pap tests ^d			
4–5	169/257	Ref (1.00)	Ref (1.00)
2–3	122/145	1.24 (0.90–1.70)	1.68 (1.12–2.52)
6–7	33/42	1.26 (0.76–2.09)	1.13 (0.62–2.06)
<2	109/88	1.57 (1.09–2.26)	1.58 (0.97–2.58)
Number lifetime sex partners			
<4	102/144	Ref (1.00)	Ref (1.00)
4–6	146/169	1.30 (0.92–1.84)	1.08 (0.69–1.71)
7+	189/224	1.26 (0.91–1.75)	0.84 (0.53–1.35)
Age at sexual debut			
<16	185/206	Ref (1.00)	Ref (1.00)
16–17	182/215	0.95 (0.71–1.27)	1.00 (0.68–1.47)
18+	81/122	0.67 (0.47–0.96)	0.98 (0.58–1.65)

^a CIN3 includes Adenocarcinoma In Situ (AIS).^b Multivariable model includes all variables in the table and age (continuous).

^cEstimates are based on the SUCCEED population only.

^dIn the previous 5 years.

^eModels were adjusted for age and frequency of Pap testing. Statistically significant associations are shown in bold.

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Table 3

Risk factor associations at the transitions of the cervical carcinogenesis progression model

Case-Control (Cancer vs. <CIN2)				
	OR (95% CI)	Progression (CIN3 ^a vs. <CIN2)		
	OR (95% CI)	Invasion (Cancer vs. CIN3 ^a)		
OR (95% CI)				
	Age Adjusted	MV ^{b,c} Adjusted	Age Adjusted	MV ^{b,c} Adjusted
Oral contraceptive use ^c				
Never	Ref (1.00) ^d	Ref (1.00)	Ref (1.00) ^e	Ref (1.00)
<5 years	0.75 (0.40–1.39) ^d	0.92 (0.47–1.82)	1.43 (0.97–2.10) ^e	1.37 (0.91–2.07)
5–10 years	1.22 (0.49–3.05) ^d	1.15 (0.41–3.22)	1.57 (0.91–2.72) ^e	1.52 (0.84–2.75)
10+ years	2.09 (0.94–4.62) ^d	2.50 (1.08–5.82)	1.97 (1.12–3.46)^e	2.06 (1.12–3.79)
Number of live births				
0	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
1	1.15 (0.62–2.13)	0.85 (0.37–1.99)	1.48 (1.08–2.03)	1.59 (1.08–2.34)
2	1.18 (0.66–2.10)	0.91 (0.41–2.01)	1.89 (1.37–2.59)	1.94 (1.29–2.90)
3+	1.22 (0.70–2.14)	0.94 (0.42–2.10)	2.23 (1.59–3.11)	2.63 (1.68–4.10)
Smoking status				
Never	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
Former	1.91 (1.17–3.10)	1.29 (0.66–2.50)	1.71 (1.21–2.43)	1.24 (0.80–1.93)
Current	1.56 (1.04–2.35)	0.65 (0.34–1.23)	2.95 (2.30–3.80)	2.24 (1.60–3.13)
Never	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
Ever	1.67 (1.15–2.43)	0.87 (0.50–1.53)	2.60 (2.04–3.30)	1.92 (1.39–2.64)
History of Pap test ^d				
Yes	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
No	5.49 (3.68–8.19)	7.00 (4.05–12.10)	2.08 (1.49–2.88)	2.46 (1.57–3.83)
Number of Pap tests ^d				
4–5	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
2–3	1.57 (0.89–2.78)	1.67 (0.81–3.43)	1.13 (0.86–1.48)	1.16 (0.84–1.60)
6–7	0.80 (0.27–2.33)	0.90 (0.26–3.06)	0.99 (0.64–1.51)	0.97 (0.59–1.58)
<2	7.13 (4.38–11.60)	10.38 (5.48–19.65)	1.67 (1.24–2.24)	2.02 (1.39–2.94)
Number lifetime sex partners				
<4	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
4–6	2.06 (1.29–3.30)	2.30 (1.18–4.49)	1.64 (1.22–2.19)	1.36 (0.94–1.99)
7+	1.37 (0.82–2.22)	1.45 (0.72–2.93)	1.69 (1.28–2.24)	0.99 (0.67–1.46)
Age at sexual debut				
<16	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
16–17	1.38 (0.87–2.16)	1.24 (0.66–2.31)	0.89 (0.70–1.14)	0.92 (0.67–1.26)

Case-Control (Cancer vs. <CIN2)				
OR (95% CI)		Progression (CIN3 ^a vs. <CIN2)		
OR (95% CI)		Invasion (Cancer vs. CIN3 ^a)		
OR (95% CI)				
	Age Adjusted	MV ^{b,c} Adjusted	Age Adjusted	MV ^{b,c} Adjusted
18+	0.87 (0.54–1.41)	0.68 (0.34–1.39)	0.44 (0.33–0.60)	0.63 (0.41–0.96)

^a CIN3 includes Adenocarcinoma In Situ (AIS).

^b Multivariable model includes all variables in the table and age.

^c Estimates are based on the SUCCEED population only.

^d In the previous 5 years.

^e Models were adjusted for age and frequency of Pap testing. Statistically significant associations are shown in bold.