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Human papillomavirus (HPV) cofactors by disease progression and HPV types in the Study to Understand Cervical Cancer Early Endpoints and Determinants (SUCCEED)

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Abstract

HPV cofactors for cervical cancer include smoking, multiparity, and oral contraceptive use, but their mechanisms of action are not fully understood. It is also unknown whether cofactors vary by HPV genotypes. The Study to Understand Cervical Cancer Early Endpoints and Determinants (SUCCEED) is a cross-sectional study comprising women referred to the University of Oklahoma from November 2003 to September 2007 for abnormal cervical screening results. Detailed questionnaire data and liquid cytology specimens were collected and the latter genotyped for HPV using the LINEAR ARRAY® HPV Genotyping Test. The present analysis includes women with both questionnaire and HPV data and diagnosed with <CIN1 (n=535), CIN1 (n=497), CIN2 (n=336), CIN3 (n=292), and cancer (n=80). We evaluated HPV types and cofactors among HPV-infected women by calculating odds ratios (OR) and 95% confidence intervals (95% CI) for CIN3 and CIN2 separately compared to <CIN2 using a polytomous logistic regression model; cancers were excluded from further analysis due to the substantially higher ages of these women. We found that HPV-infected women with minor histologic or cytologic abnormalities (e.g., CIN1, ASCUS, LSIL) were indistinguishable from those with normal histology/cytology, and were thus combined to form the referent group (<CIN2). Among women positive for oncogenic HPV, current smokers had a 2.5-fold increased risk for CIN3 (95% CI=1.8–3.6) compared to nonsmokers. Among HPV16-infected women, current smokers had elevated risk for both CIN2 (OR=1.9, 95% CI=1.1–3.2) and CIN3 (OR=2.7, 95% CI=1.6–4.6). Our data suggest that non-HPV16-related CIN2 likely reflects a combination of CIN1 and CIN3 diagnosis, whereas HPV16-related CIN2 likely indicates a precancerous state. Investigations on the molecular distinctions along the disease continuum of cervical pathogenesis by HPV type are needed.

Keywords

cervical cancer; SUCCEED; HPV; cofactor; epidemiology; molecular

INTRODUCTION

Worldwide, cervical cancer is the second or third leading cancer in women and the leading cause of cancer deaths among women in developing countries (1;2). Persistent infection with one or more of approximately 15 oncogenic human papillomavirus (HPV) types fulfills the epidemiologic criterion for causation and HPV is now accepted as the necessary but not sufficient etiologic agent for cervical neoplasia (3;4). Most HPV infections do not persist. Only about 10% of women found on a cross-sectional screen to be infected with an oncogenic HPV type will progress to precancer. Of these, approximately 20–30% will eventually develop invasive cancer if untreated.

Smoking, multiparity and oral contraceptive use have definitively been shown to be associated with CIN3 and cervical cancer (5–10), but it is not known whether they affect early disease progression (e.g., from normal to CIN1). Determining if these factors have a role in the development of early lesions may provide clues to the biological mechanisms and molecular events important for each progressive transition. Furthermore, it is unknown if these factors confer differential risk by HPV type. For example, it is of particular interest to understand cervical pathogenesis among women infected with HPV16, which conveys a much higher risk of cancer and CIN3 than other oncogenic types (11). If these factors affect CIN3 or cancer risk differentially by HPV type, insight would be gained regarding the synergistic roles of HPV infection and environmental or social factors (HPV co-factors).

The conventional histological schema of cervical carcinogenesis progression from cervical intraepithelial neoplasia grade 1 (CIN1) to CIN 2, then to CIN3 and finally cancer is now yielding to a more molecular-based view that incorporates HPV type. Cytologic classifications as reported according to the Bethesda System (12–14) incorporate the role of HPV and are recognized by fundamental distinctions of normal, equivocal, low-grade, high-grade, and cancer lesions. To better understand the full spectrum of HPV infection leading to cervical cancer, we have integrated molecular, histologic, cytologic, and epidemiologic data in a large cross-sectional study, the Study to Understand Cervical Cancer Early Endpoints and Determinants (SUCCEED). SUCCEED comprises over 1800 women with abnormal screening results referred to the University of Oklahoma Health Sciences Center. On the basis of repeat cytology and colposcopically-directed biopsy, women were classified as having <CIN1, CIN1, CIN2, CIN3, or invasive cancer. Women with <CIN1 were subdivided by the low-grade cytology classifications to evaluate possible differences.

In the present manuscript, we assess the impact of environmental or behavioral factors with HPV infection in the development of cervical cancer.

METHODS

Study population

The Study to Understand Cervical Cancer Early Endpoints and Determinants (SUCCEED) was established in November 2003. The catchment population is women referred to colposcopy at University of Oklahoma Health Sciences Center (OUHSC), Oklahoma City, due to an abnormal Pap diagnosis or a biopsy diagnosis of cervical intraepithelial neoplasia. OUHSC is the referral center for the state of Oklahoma, excluding Tulsa. Women were primarily referred to the OUHSC from the OUHSC dysplasia clinic (51%), private physicians (21%), and the OU Medical Center Women's Clinic (10%); remaining referrals originated from Planned Parenthood® clinics or other medical facilities (e.g., Mary Mahoney Memorial Health Center). All women who were scheduled for colposcopy were identified but not contacted prior to their visit; only women who kept their appointments and consented at the time of their scheduled OUHSC visit were offered participation into SUCCEED. SUCCEED is a cross-sectional study;

of the women who kept their appointment, we excluded women under 18 years of age (3%), women pregnant at the time of their visit (10%), women who had prior treatment with chemotherapy or radiation for any cancer (<1%), women identified as HIV-positive (<1%), women with previous hysterectomy (<1%) and women attending the clinic solely for vaginal colposcopy or other reasons (8%). Recruitment was completed September 2007. With the exception of enriching accrual of invasive cancers, the percent disease distribution of women enrolled in SUCCEED is representative of the catchment referral population of Oklahoma. Of eligible women, 55% enrolled in SUCCEED. Written informed consent was obtained from all eligible women enrolled into the study. SUCCEED was approved by the Institutional Review Boards at the OUHSC and the National Cancer Institute.

Questionnaire

All SUCCEED participants were administered an interviewer-based, standardized questionnaire. The questionnaire was designed to capture demographic and essential information on known HPV cofactors previously demonstrated to be associated with cervical neoplasia. Briefly, the questionnaire queried information on gynecologic, sexual, reproductive, medical, sexually transmitted disease, and behavioral (e.g., smoking) history (see Supplemental Methods). All interviews were conducted in a private setting by a nurse or other research staff trained to administer the questionnaire. The average duration of the interview was 10 minutes.

Colposcopy and collection of specimens

A physician conducted the colposcopic examination according to routine practice at OUHSC. Prior to biopsy or LEEP, cervical cell samples were obtained with a Papette™ broom (Wallach Surgical, Orange, CT) and rinsed directly into a PreservCyt™ vial (Cytyc Corporation, Marlborough, MA) as described previously (15). The cytology specimen was used for liquid-based cytology using ThinPrep™ (Cytyc Corporation) and for HPV testing using the LINEAR ARRAY® (LA) HPV Gentoyping Test (Roche Diagnostics, Branchburg, NJ). Cervical cells were also obtained using a Dacron™ swab and placed in a vial containing Universal Collection Medium (UCM) (Qiagen, Gaithersburg, MD). Cervical secretions were collected using an ophthalmic sponge. Following collection of cells and secretions, acetic acid and Lugol's iodine were applied topically to the cervix to identify suspected CIN. Biopsy specimens, obtained for any colposcopically suspected CIN, were placed in separate prelabeled vials containing 10% buffered formalin. An adjacent lesional biopsy was snap-frozen for research purposes as previously described (16). Endocervical curettage was performed according to clinician judgment in cases when the entire transformation zone or extent of a lesion was not visualized adequately. As per standard practice, histologically-confirmed high-grade lesions diagnosed as CIN2 or above (CIN2+) by the institutional pathologists were treated by the loop electrosurgical excision procedure (LEEP) of the transformation zone (*except* women under 21 years old and nulliparous women less than 26 years old, who were followed). The adjacent lesional tissue and a colposcopically normal biopsy were also snap-frozen for research purposes (16;17). The tissue collection team identified the site of the lesion based on the orientation of the cervix and the localization of the most severe abnormality on prior colposcopy. Finally, 10 mL of venous blood was obtained from each woman by a trained phlebotomist for research purposes.

Laboratory methods

Cytology slides—Liquid-based, ThinPrep cytology slides were prepared from PreservCyt vial specimens according to the manufacturer's standard protocol. Cytology diagnosis was based on clinical records; slides were evaluated as previously described by both a

cytotechnologist and cytopathologist (18). Cytologic results were recorded on a standardized data collection form based on the Bethesda System

HPV testing—Each 20 mL PreservCyt vial was vortexed for 15 seconds after which two 1 mL aliquots were removed. One aliquot was processed immediately for DNA isolation using the QIAmp® DNA Blood Mini Kit (Qiagen, Germantown, MD) as described previously (19). Isolated DNA (100 uL total elution volume) was stored at -70°C until subject to amplification using the LINEAR ARRAY® HPV Genotyping Test (Roche Diagnostics, Branchburg, NJ). Pelleted cells from the other aliquot were stored at 70°C for other analysis, as needed. As previously described (19), the LINEAR ARRAY® assay is a multiplexed PCR-based system simultaneously detects up to 37 HPV genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 56, 57, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, CP6108). On each strip, two different concentrations of β -globin probes are present which serve as internal control for assuring adequate amplifiable DNA in each specimen. Beta-globin-negative, HPV-negative samples were considered inadequate and typing was redone. Positive and negative controls (included in the genotyping kit) were processed through all stages of DNA isolation, amplification and detection with each run of clinical specimens. Up to 84 specimens (inclusive of controls) were amplified at one time using 10 uL template DNA in the HPV LINEAR ARRAY® HPV Genotyping Test. Up to 30 specimens were processed at one time during hybridization and detection components of the assay which was accomplished using the Auto-LiPA instrument (Innogenetics N.V., Belgium). This instrument was programmed to reproduce the steps that are detailed in the manual HPV genotyping protocol with the exception that 2.5 mL of each reagent per strip (as compared to 4.0 mL in manual processing), as described previously (20).

Pathology outcome and final analytic population

Histologic interpretation of biopsy and LEEP specimens was conducted by the study pathologist at OUHSC (REZ) using CIN terminologies. Histology was the primary determinant of our outcome and no outcomes were defined based on cytology only. Specifically, the main outcomes evaluated here, CIN2, CIN3 and cancer were made regardless of cytology. Of the 1899 women enrolled at the time of the analysis, both questionnaire and HPV typing data were available for 80 cancers, 292 CIN3, 336 CIN2, 497 CIN1 and 535 <CIN1, permitting inclusion in the present manuscript.

Statistical analyses

We calculated the number and percent of women with each of the questionnaire-based (HPV cofactors) and HPV genotyping information by final pathologic diagnosis (<CIN1, CIN1, CIN2, CIN3, cancer) (Supplemental Table 1). We first compared population characteristics and HPV genotype patterns between CIN1 and <CIN1. We also compared population characteristics by cytology (ASCUS, LSIL and LSIL versus <LSIL). Because there was no observable difference between the two histologic groups and between the cytologic definitions with regard to population characteristic, we combined CIN1 and <CIN1 as a comparison group in all HPV-restricted analyses (henceforth referred to as <CIN2).

We compared HPV cofactor characteristics of women diagnosed with CIN3 and CIN2 to those with <CIN2. Cancers were excluded from analysis of cofactors due to the substantially higher ages of these women, as described in the Results section. Comparisons of cofactors were conducted (a) among women infected with any HPV type and (b) among women infected with any oncogenic HPV types (defined as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) to evaluate the comparability of our population with other reported populations and assure that previously confirmed HPV cofactors were also observed in our study population. We calculated odds ratios (OR) and 95% confidence intervals (95% CI) adjusted for age (quintiles).

For exposures where multiple associations were observed with correlated variables (e.g., smoking duration and smoking quantity), we identified the variable(s) that maximally explained disease risk in separate logistic regression models and cluster analysis. Based on these results, we conducted final logistic regression models that included age (quintiles), race (white, black, other), annual income (<\$10K, \$10,001-40K, ≥\$40K), oral contraceptive use (ever, never), BMI (<20, 20–<25, 25–<30, 30+ kg/m²), number of pregnancies (never, 1–3, >4), lifetime number of sexual partners (1, 2–3, 4–10, 11+) and smoking behavior (never, current, former). Final models were polytomous (e.g., CIN3 and CIN2 compared to <CIN2). To further delineate the specific role of HPV cofactors by HPV type, we also conducted all analyses above, restricted to women infected with (a) HPV16, (b) HPV18 (excluding any HPV16 infected women), and (c) all other oncogenic types (excluding women infected with HPV16 or HPV18).

All statistical tests were two-sided and considered to be statistically significant at $p < 0.05$. All logistic regression models were unconditional and conducted using SAS version 9.1.3 (SAS Institute, Cary, NC).

RESULTS

SUCCEED population characteristics

The SUCCEED population was largely white, non-Hispanic and born in Oklahoma (Supplemental Table 1). The median age of the population at enrollment was 25 years. All women self-reported to be sexually active and virtually all reported use of some form of contraception. Pregnancy, oral contraceptive use, smoking, and HPV infection were common. HPV16 infection was the most prevalent HPV type in our population. HPV18 was considerably less common in our population than HPV16. Although HPV18 prevalence was also highest among cancers compared to other histologic diagnoses, a clear increase with disease severity was not observed (Supplemental Table 1). All HPV-negative women were excluded in subsequent analyses.

Of note is the lack of difference regarding population characteristics measured between women with a histological diagnosis of <CIN1 to those with CIN1. All demographic, sexual behavior, reproductive factors, BMI, and smoking behavior characteristics were similar between the two groups. Formal evaluation by Chi square analyses yielded no statistically significant differences between the two groups for any population characteristic.

Not unexpectedly, we also note the striking difference in age between women diagnosed with cancer compared to all other histologies. Median age range was 24 years in <CIN1 to 27 years in CIN3. In cancers, the median age was 45 (range: 27–81). A number of the population characteristics that differed in cancers from the lower grade histologies also reflected this higher age, including higher household income, marital status, number of pregnancies and live births, oral contraceptive use and smoking behavior. In our subsequent analyses of HPV cofactors by histology, we do not present results for cancer due to the statistical inability to adjust for age as a confounder.

HPV cofactors in the SUCCEED population

CIN3 versus <CIN2—Among oncogenic HPV-infected women, we compared population characteristics between women diagnosed with CIN3 to those diagnosed with <CIN2. In univariate analyses, we observed associations for CIN3 with parity, lifetime number of sexual partners, smoking and current BMI (Table 1). In our final multivariate model, multiparity, current smoking and BMI (≥30 versus <20 kg/m²) remained statistically significantly associated with CIN3 compared to <CIN2 (Table 2). In HPV16-restricted analysis, current

smokers had a 2.7-fold increased risk for CIN3 (95% CI=1.6–4.6) compared to never smokers. The increased CIN3 risk among current smokers was also observed in analyses restricted to both HPV16- and HPV18-positive women. Although this increase in risk was likely driven by the larger number of HPV16-positive women, we note that the association was more pronounced in HPV16/HPV18 restricted analysis thus suggesting a higher magnitude of risk among HPV18-infected women; our sample size precluded a restricted analysis of HPV18 positive women only. Of women positive for all other oncogenic types (non-HPV16 and non-HPV18), higher household income, multiparity and current smoking all conferred elevated risks for CIN3 (Table 2). Results were consistent when a cytologic diagnosis of HSIL or CIN2 was used as an outcome although the magnitude of the risk was in general lower than that observed for CIN3 (data not shown).

CIN2 versus <CIN2—Among oncogenic HPV positive women, a direct comparison of CIN2 to <CIN2 initially yielded smoking behavior (status, duration, and pack-years) associated with increased CIN2 risk (Table 1). Increasing number of sexual partners was also associated with elevated risk for CIN2. However, results of HPV cofactors associated with CIN2 were weaker than those for CIN3, suggesting CIN2 to be a hybrid between CIN3 and CIN1, consistent with the well-documented misclassification of CIN2. In our final multivariate model among all HPV and oncogenic HPV infected women, we observed no statistically significantly elevated risks for CIN2 (Table 2). In general, the same or decreased risk magnitudes observed when comparing CIN3 to CIN2, and CIN3 to CIN1, suggest that the comparison groups of CIN2 and CIN1 are likely similar (Table 1).

One notable exception was that in HPV16 restricted analyses, we observed a statistically significant risk increase for CIN2 among current smokers ($OR_{HPV16}=1.9$, 95% CI=1.1–3.2). Although elevated risk among current smokers was also observed for HPV16 and HPV18 positive women ($OR_{HPV16/18}=1.7$, 95% CI=1.1–2.8), the magnitude of the risk was slightly lower indicating that the increased risk is likely driven by HPV16. In considering cytologic diagnosis, the increased risk among current smokers was statistically significant for CIN2 with or without HSIL (data not shown). Thus, while there are no observable differences between non-HPV16 infections and <CIN2, our data suggest HPV16-associated CIN2 have potentially important similarities to CIN3.

DISCUSSION

The SUCCEED population characteristics by histologic diagnoses are consistent with our understanding of cervical cancer etiology with regard to HPV infection and HPV cofactors. In SUCCEED, the prevalence of oncogenic HPV infection increased with histologic severity. HPV16 infection, which similarly follows this pattern, is the most common HPV type in our population. We demonstrate that multiparity and current smoking are risk factors for CIN3 among HPV-infected women, as previously reported (5;6;8). Interestingly, as risk estimates with multiparity were particularly pronounced among women with non-HPV16/18 infections, we cannot rule out the possibility that different HPV cofactors, such as multiparity, may impart different risks of progression to CIN3 depending on the originating HPV type. Finally, although oral contraceptive use has been identified as a risk factor for CIN3 and cervical cancer (5), the increased risk observed in our population was not statistically significant. However, our analyses of oral contraceptive use was limited by few never users, few long-term users, and few former users with extended time since last use. Interestingly, we also found a pronounced risk increase for CIN3 among those with a higher lifetime number of sexual partners and who were positive for HPV16 or 18. Although our evaluation of women infected or treated with other sexually transmitted diseases (STD) was not informative (data not shown), we cannot exclude the possibility that higher lifetime number of sexual partners may reflect a surrogate

for coinfection with other STDs and have relevance for progression to CIN3 among HPV16/18-positive women.

We observed no difference in any population characteristic between CIN1 and <CIN1 or between LSIL and <LSIL, contrary to previous reports that reported smoking as a specific risk factor for LSIL (20). Prevalence of HPV (any HPV or oncogenic HPV) infection was higher among CIN1 than <CIN1 diagnoses. However, the prevalence of infection with HPV16 and HPV18 did not differ between the two histologic groups; rather, non-HPV16 and non-HPV18 oncogenic types had higher prevalence in CIN1 than <CIN1. Among HPV-infected women, we did not identify smoking or any other HPV-cofactor to be more prevalent in or associated with CIN1 compared to <CIN1. Our data therefore suggest that differences between CIN1 and <CIN1 diagnoses may be attributed to viral differences or other unmeasured host differences (21).

CIN3 is histologically well-defined as a precancerous state; CIN2 is not (22–24). In SUCCEED, we therefore classified CIN2 as a separate histologic entity in part to retain the clear distinction between CIN3 from low-grade HPV-infected tissues (<CIN2). This strategy provided an opportunity to determine whether a distinct etiologic profile could be defined for CIN2 or whether it in fact represented a mixture of CIN1–3. Overall, our data indicate that HPV characteristics and HPV cofactors for CIN2 are, in general, attenuated from CIN3, supporting its classification as a hybrid between CIN3 and CIN1.

We conducted restricted analyses within HPV16 infections because HPV16 can confer a much higher risk of CIN3 and cancer than other HPV types (11). We observed that among HPV16-infected women, current smoking was statistically significantly associated with elevated CIN2 and CIN3 risk, consistent with a previous report (25). We believe that based on the strength of association and statistical significance of these data, which demonstrate that the smoking is a cofactor for both CIN3 and specifically for HPV16-related CIN2, our data suggest that HPV16-related CIN2 may reflect precancerous or lesional characteristics. Finally, we also evaluated HPV cofactors among oncogenic HPV types that did not include HPV16 or HPV18 because of the growing interest in understanding the ecological niches of these other HPV types in the absence of HPV16/18 for which vaccines are now available. Current smoking and multiparity remained an HPV cofactor for CIN3.

Study strengths include our large sample sizes for all CIN histologic grades, our validated questionnaire which provided significant detail for evaluating HPV cofactors, and our type-specific HPV data from all women enrolled in SUCCEED which also permitted a number of restricted analyses (e.g., oncogenic HPV, HPV16, oncogenic non-16-, non-18-infection). Because the SUCCEED catchment population comprises a referral population, study strengths also include the ability to evaluate risk factors relevant for cancer progression among HPV-infected women with evidence of cervical abnormalities. Unlike in a screening population, however, our study is not designed to evaluate risk factors for highly transient HPV infections and may not identify factors that mediate risk via early immunologic response to infection. Study limitations include the cross-sectional design of the study; although this design permitted the accrual of large numbers of women into the study for studying disease transitions, evaluating such transitions prospectively remains ideal for determining risk associations. Study limitations also include the use of a single pathologist to render histologic diagnosis of all women, though standardizes our outcome, may be subject to bias in histologic interpretation. The consistency of our results with regard to known HPV cofactors from previous prospective and case-control studies, however, supports the validity and generalizability of our study population. We do note that in our population, BMI was associated with CIN3 but has not been shown to be a risk factor for squamous cell carcinoma of the cervix (26). However, as our

ascertainment of BMI was at the time of enrollment, and not at a specified age, interpretation of our findings is unclear.

In summary, our results support the validity of the use of the SUCCEED population in studying HPV-related risk factors in cervical pathogenesis. We report HPV type differences between CIN1 and <CIN1 diagnosis but no difference by HPV cofactors. We identified current smoking as an HPV16-specific cofactor for both CIN2 and CIN3, and multiparity as an HPV cofactor for progression to CIN3 without HPV type-specificity. Our data are consistent with the evidence that CIN2 is a combination of CIN1-3 with one exception. Among HPV16-infected women, we believe CIN2 may possess precancerous properties and thus warrants further evaluation. Our HPV cofactor and HPV typing results coupled with the intense collection of biologic specimens within SUCCEED provide a framework for understanding epidemiologic and molecular distinctions between each disease transition in cervical pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Among 1,378 oncogenic HPV-infected women enrolled in SUCCEED, univariate analyses (adjusted by age in quintiles) of selected HPV cofactors for (i) CIN3 compared to $\leq$ CIN2, (ii) CIN2 compared to $\leq$ CIN2 and (iii) CIN3 compared to CIN2.

Characteristic	CIN3 n	CIN2 n	$\leq$ CIN2 n	CIN3 v $\leq$ CIN2 OR (95% CI)	CIN2 v $\leq$ CIN2 OR (95% CI)	CIN3 v CIN2 OR (95% CI)
Race						
White	215	230	508	1.00 (ref)	1.00 (ref)	1.00 (ref)
Black	22	39	112	0.46 (0.29–0.75)	0.77 (0.52–1.14)	0.60 (0.35–1.05)
Indian	14	21	35	0.95 (0.50–1.79)	1.33 (0.76–2.33)	0.71 (0.35–1.44)
Other	11	11	29	0.90 (0.44–1.83)	0.84 (0.41–1.71)	1.07 (0.45–2.52)
Ethnicity						
Hispanic	37	39	136	1.00 (ref)	1.00 (ref)	1.00 (ref)
Non-Hispanic	245	273	611	1.47 (1.00–2.18)	1.56 (1.06–2.29)	0.95 (0.58–1.53)
Income (\$)						
5,000 or less	53	72	170	1.00 (ref)	1.00 (ref)	1.00 (ref)
5,001–10,000	38	42	125	0.98 (0.61–1.57)	0.79 (0.51–1.24)	1.23 (0.70–2.16)
10,001–15,000	53	52	114	1.49 (0.95–2.34)	1.08 (0.70–1.65)	1.39 (0.82–2.33)
15,001–20,000	29	37	80	1.16 (0.69–1.97)	1.09 (0.68–1.76)	1.07 (0.58–1.94)
20,001–30,000	41	46	79	1.67 (1.02–2.71)	1.38 (0.87–2.17)	1.21 (0.70–2.10)
30,001–40,000	14	20	40	1.12 (0.57–2.22)	1.18 (0.65–2.16)	0.95 (0.44–2.05)
40,001+	21	17	32	2.11 (1.12–3.96)	1.25 (0.66–2.40)	1.68 (0.81–3.49)
Marital Status						
Single	96	150	402	1.00 (ref)	1.00 (ref)	1.00 (ref)
Married	137	123	264	2.17 (1.60–2.94)	1.25 (0.94–1.66)	1.74 (1.22–2.48)
Divorced	48	44	96	2.09 (1.39–3.16)	1.23 (0.82–1.84)	1.71 (1.05–2.76)
Widowed	6	2	2	12.56 (2.50–63.19)	2.68 (0.37–19.19)	4.69 (0.93–23.70)
Insurance						
Employer	14	23	57	1.00 (ref)	1.00 (ref)	1.00 (ref)
Health dept	91	68	170	2.18 (1.15–4.12)	0.99 (0.57–1.74)	2.20 (1.05–4.59)
Medicare/aid	100	149	329	1.24 (0.66–2.31)	1.12 (0.67–1.89)	1.10 (0.54–2.25)
None	77	76	195	1.61 (0.85–3.05)	0.97 (0.56–1.68)	1.66 (0.80–3.48)
Self	3	3	9	1.36 (0.32–5.68)	0.83 (0.21–3.33)	1.64 (0.29–9.29)
Ever Pregnant						

Characteristic	CIN3 n	CIN2 n	<CIN2 n	CIN3 v <CIN2 OR (95% CI)	CIN2 v <CIN2 OR (95% CI)	CIN3 v CIN2 OR (95% CI)
No	45	92	242	1.00 (ref)	1.00 (ref)	1.00 (ref)
Yes	243	228	526	2.48 (1.75–3.54)	1.14 (0.86–1.52)	2.18 (1.46–3.25)
<i>Number of pregnancies</i>						
0	45	92	242	1.00 (ref)	1.00 (ref)	1.00 (ref)
1	66	65	188	1.89 (1.24–2.89)	0.91 (0.63–1.32)	2.08 (1.27–3.40)
2	57	67	147	2.09 (1.34–3.24)	1.20 (0.82–1.75)	1.74 (1.05–2.87)
3	51	50	94	2.92 (1.83–4.65)	1.40 (0.92–2.13)	2.09 (1.23–3.54)
4	36	25	42	4.61 (2.67–7.97)	1.57 (0.90–2.72)	2.94 (1.58–5.49)
5+	33	21	53	3.35 (1.95–5.74)	1.04 (0.60–1.82)	3.21 (1.67–6.17)
<i>Number of live births</i>						
0	15	21	60	1.00 (ref)	1.00 (ref)	1.00 (ref)
1	74	68	193	1.53 (0.82–2.87)	1.01 (0.57–1.78)	1.52 (0.73–3.19)
2	72	75	159	1.81 (0.96–3.40)	1.35 (0.76–2.38)	1.34 (0.64–2.81)
3+	82	63	113	2.90 (1.54–5.47)	1.59 (0.89–2.86)	1.82 (0.87–3.82)
Years between menarche and first sexual intercourse						
<2	65	54	147	1.00 (ref)	1.00 (ref)	1.00 (ref)
2, 3	96	110	235	0.92 (0.63–1.35)	1.27 (0.87–1.87)	0.73 (0.46–1.14)
4, 5	82	90	205	0.91 (0.61–1.33)	1.20 (0.80–1.78)	0.76 (0.47–1.21)
6+	38	58	161	0.53 (0.34–0.84)	0.98 (0.64–1.51)	0.54 (0.32–0.94)
Age at sexual debut (years)						
≤16	198	191	440	1.00 (ref)	1.00 (ref)	1.00 (ref)
17	41	54	124	0.74 (0.50–1.09)	1.00 (0.70–1.44)	0.73 (0.47–1.15)
18	21	32	92	0.51 (0.31–0.84)	0.80 (0.52–1.24)	0.63 (0.35–1.14)
19–20	15	23	64	0.52 (0.29–0.94)	0.83 (0.50–1.37)	0.63 (0.32–1.24)
21+	9	14	37	0.54 (0.26–1.14)	0.87 (0.46–1.65)	0.62 (0.26–1.47)
Lifetime # sexual partners						
1	15	16	66	1.00 (ref)	1.00 (ref)	1.00 (ref)
2 to 3	45	69	167	1.19 (0.62–2.27)	1.70 (0.92–3.15)	0.70 (0.31–1.55)
4 to 5	74	77	165	1.97 (1.06–3.68)	1.93 (1.05–3.54)	1.03 (0.47–2.22)
6 to 10	79	99	217	1.60 (0.86–2.97)	1.88 (1.04–3.41)	0.85 (0.40–1.83)
11+	61	49	130	2.07 (1.09–3.91)	1.56 (0.82–2.94)	1.33 (0.60–2.95)

Characteristic	CIN3 n	CIN2 n	<CIN2 n	CIN3 v <CIN2 OR (95% CI)	CIN2 v <CIN2 OR (95% CI)	CIN3 v CIN2 OR (95% CI)
OC use						
Never	31	39	109	1.00 (ref)	1.00 (ref)	1.00 (ref)
Ever	250	271	632	1.39 (0.91–2.13)	1.20 (0.81–1.77)	1.16 (0.70–1.92)
OC status						
Current	93	98	282	1.16 (0.73–1.84)	0.97 (0.63–1.50)	1.19 (0.69–2.07)
Former	153	172	341	1.58 (1.01–2.46)	1.41 (0.94–2.12)	1.12 (0.67–1.88)
Smoking status						
Never	69	127	343	1.00 (ref)	1.00 (ref)	1.00 (ref)
Current	174	156	309	2.80 (2.04–3.85)	1.36 (1.03–1.80)	2.05 (1.43–2.96)
Former	37	34	105	1.75 (1.11–2.76)	0.88 (0.57–1.35)	2.00 (1.16–3.47)
Smoking duration (years)						
<10	93	104	272	1.00 (ref)	1.00 (ref)	1.00 (ref)
10+	115	85	136	2.47 (1.76–3.48)	1.64 (1.15–2.33)	1.51 (1.02–2.25)
Smoking by pack-years						
<5 pack-yr	90	86	252	1.00 (ref)	1.00 (ref)	1.00 (ref)
5 to <15 pack-yr	72	70	102	1.98 (1.34–2.91)	2.01 (1.36–2.97)	0.98 (0.63–1.53)
15+ pack-yr	40	29	41	2.73 (1.66–4.49)	2.07 (1.21–3.54)	1.32 (0.75–2.31)
BMI (kg/m²)						
<20	46	42	83	2.06 (1.34–3.17)	1.13 (0.74–1.72)	1.82 (1.11–2.99)
20 to <25	86	143	319	1.00 (ref)	1.00 (ref)	1.00 (ref)
25 to <30	69	65	176	1.45 (1.01–2.10)	0.82 (0.58–1.17)	1.77 (1.15–2.72)
30+	83	66	176	1.75 (1.23–2.49)	0.84 (0.59–1.18)	2.09 (1.38–3.18)
Pap test (frequency)						
2+ times per year	34	50	139	1.00 (ref)	1.00 (ref)	1.00 (ref)
Every year	48	47	136	1.44 (0.88–2.38)	0.96 (0.61–1.53)	1.50 (0.83–2.72)
Every 2 years	132	168	375	1.44 (0.94–2.20)	1.25 (0.86–1.81)	1.16 (0.71–1.89)
Every 3+ years	21	21	58	1.48 (0.79–2.76)	1.01 (0.56–1.82)	1.47 (0.70–3.10)

Table 2

Final multivariate logistic regression model (polytomous) of HPV cofactors among women enrolled in SUCCEED restricted to women infected with (i) any HPV type, (ii) oncogenic-HPV types, (iii) HPV16 only, (iv) HPV16 or -18, (v) oncogenic HPV types excluding HPV16 and HPV18. Final models include all variables listed and age (quintiles) for comparisons of: (a) CIN3 compared to <CIN2, and (b) CIN2 compared to <CIN2.

Characteristic	CIN3 v <CIN2				CIN2 v <CIN2					
	All HPV OR (95% CI)	Oncogenic HPV OR (95% CI)	HPV16 OR (95% CI)	HPV16 or 18 OR (95% CI)	Non-HPV16, 18 OR (95% CI)	All HPV OR (95% CI)	Oncogenic HPV OR (95% CI)	HPV16 OR (95% CI)	HPV16 or 18 OR (95% CI)	Non-HPV16, 18 OR (95% CI)
Race										
White	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Black	0.6 (0.3–1.0)	0.7 (0.4–1.1)	0.9 (0.4–2.1)	1.0 (0.4–2.1)	0.9 (0.4–2.1)	0.8 (0.5–1.2)	0.9 (0.6–1.3)	1.5 (0.7–3.1)	1.6 (0.8–3.1)	0.7 (0.4–1.3)
Other	0.9 (0.5–1.5)	0.9 (0.6–1.6)	1.1 (0.5–2.4)	1.1 (0.5–2.2)	0.7 (0.2–2.3)	1.1 (0.7–1.8)	1.2 (0.7–1.8)	1.2 (0.5–2.7)	1.0 (0.5–2.2)	1.4 (0.7–2.6)
Income (\$)										
<10,000	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
10,001–40,000	1.2 (0.9–1.7)	1.3 (0.9–1.8)	1.6 (1.0–2.6)	1.5 (1.0–2.4)	1.0 (0.6–2.0)	1.2 (0.9–1.6)	1.2 (0.9–1.7)	1.9 (1.1–3.0)	1.6 (1.0–2.4)	0.9 (0.6–1.4)
40,000+	1.9 (1.0–3.6)	2.0 (1.0–3.8)	2.4 (0.9–6.5)	2.0 (0.8–4.9)	3.9 (1.2–12.6)	1.3 (0.7–2.4)	1.3 (0.7–2.5)	2.6 (1.0–7.2)	2.3 (0.9–5.7)	0.8 (0.3–2.1)
Lifetime number of sexual partners										
1	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
2–3	1.1 (0.6–2.3)	1.1 (0.5–2.3)	2.9 (0.9–9.2)	2.2 (0.7–6.3)	0.3 (0.1–1.0)	1.6 (0.9–2.9)	1.5 (0.8–2.9)	2.2 (0.7–6.7)	1.9 (0.7–5.3)	1.2 (0.5–2.8)
4–10	1.4 (0.7–2.7)	1.3 (0.7–2.6)	2.6 (0.9–7.8)	1.9 (0.7–5.4)	0.6 (0.2–1.7)	1.5 (0.8–2.7)	1.4 (0.8–2.7)	1.9 (0.7–5.6)	1.6 (0.6–4.3)	1.1 (0.5–2.7)
11+	1.4 (0.7–2.8)	1.4 (0.6–2.9)	2.8 (0.8–8.9)	1.7 (0.6–5.1)	0.7 (0.2–2.2)	1.2 (0.6–2.3)	1.1 (0.5–2.2)	1.2 (0.4–3.9)	0.9 (0.3–2.6)	1.2 (0.5–3.1)
Number of pregnancies										
0	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1–3	1.6 (1.1–2.3)	1.5 (1.0–2.3)	1.2 (0.7–2.1)	1.2 (0.7–1.9)	3.2 (1.2–8.4)	1.0 (0.8–1.4)	1.0 (0.7–1.3)	0.7 (0.4–1.3)	0.8 (0.5–1.3)	1.1 (0.7–1.9)
4+	2.1 (1.3–3.6)	2.0 (1.2–3.3)	1.7 (0.8–3.6)	1.7 (0.9–3.4)	3.3 (1.1–10.2)	1.1 (0.7–1.8)	0.9 (0.5–1.5)	0.7 (0.3–1.6)	0.7 (0.3–1.5)	1.3 (0.6–2.7)
Smoking										
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Current	2.5 (1.8–3.6)	2.5 (1.8–3.6)	2.7 (1.6–4.6)	2.9 (1.8–4.7)	2.0 (1.0–3.8)	1.3 (0.9–1.7)	1.3 (0.9–1.8)	1.9 (1.1–3.2)	1.7 (1.1–2.8)	1.0 (0.6–1.5)
Former	1.4 (0.9–2.3)	1.5 (0.9–2.4)	1.6 (0.8–3.2)	1.8 (0.9–3.4)	1.4 (0.6–3.4)	0.8 (0.5–1.2)	0.8 (0.5–1.3)	0.8 (0.3–1.8)	0.9 (0.4–1.9)	0.8 (0.4–1.4)
Oral contraceptive use										
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Ever	1.1 (0.7–1.6)	1.1 (0.7–1.7)	0.9 (0.5–1.7)	1.0 (0.6–1.8)	2.0 (0.7–6.1)	1.0 (0.7–1.5)	1.1 (0.7–1.6)	1.4 (0.7–2.8)	1.7 (0.9–3.2)	0.7 (0.4–1.3)
BMI										

Characteristic	CIN3 v < CIN2					CIN2 v < CIN2				
	All HPV OR (95% CI)	Oncogenic HPV OR (95% CI)	HPV16 OR (95% CI)	HPV16 or 18 OR (95% CI)	Non-HPV16, 18 OR (95% CI)	All HPV OR (95% CI)	Oncogenic HPV OR (95% CI)	HPV16 OR (95% CI)	HPV16 or 18 OR (95% CI)	Non-HPV16, 18 OR (95% CI)
20-25	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
25-30	1.4 (0.9-2.0)	1.4 (0.9-2.1)	1.5 (0.9-2.7)	1.5 (0.9-2.4)	1.7 (0.8-3.5)	0.9 (0.6-1.2)	0.8 (0.6-1.2)	1.0 (0.6-1.8)	1.1 (0.7-1.8)	0.6 (0.4-1.1)
30+	1.5 (1.0-2.2)	1.6 (1.1-2.3)	1.6 (0.9-2.7)	1.5 (0.9-2.6)	2.0 (1.0-4.2)	0.8 (0.6-1.1)	0.8 (0.6-1.2)	0.7 (0.4-1.4)	0.8 (0.5-1.4)	0.7 (0.4-1.2)
<20	2.0 (1.3-3.1)	2.0 (1.3-3.2)	2.3 (1.2-4.3)	2.5 (1.4-4.6)	1.3 (0.5-3.7)	1.1 (0.7-1.7)	1.2 (0.8-1.8)	1.2 (0.6-2.3)	1.4 (0.8-2.7)	0.9 (0.5-1.8)