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Predictors of Human Papillomavirus Seropositivity in Appalachian Women Aged 18 to 26 Years

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Background: Key informants of the Appalachian community questioned whether their unique environmental stressors would alter their immune response to human papillomavirus (HPV) infections. The primary aim of this study is to determine predictors of HPV seroprevalence to at least 1 of the 4 vaccine-related HPV types before vaccination using a psychoneuroimmunologic model in Appalachian women.

Method: Women aged 18 to 26 years (n = 185) who had not received HPV vaccination provided cervical HPV DNA and blood samples. Human papillomavirus DNA was identified through Hybrid Capture 2 assay and then genotyped for HPV types 6, 11, 16, and 18 by Roche Linear Array. Competitive Luminex Immunoassay measured the type-specific antibodies to HPV types 6, 11, 16, and 18 in milli-Merck units per milliliter. Nine psychoneuroimmunology scales measuring attributes of stress were self-completed.

Results: Human papillomavirus DNA was detected in 50% (92/183) of participants, with only 14% (26/183) positive for HPV-6/11/16/18 DNA. Seropositivity for at least one anti–HPV-6/11/16 or 18, on the other hand, was present in 35% (64/183) of women, with only 10% (19/183) concomitantly infected and seropositive for the vaccine-related types. The Perceived Stress Scale was not a strong predictor of HPV seropositivity.

Conclusions: Both HPV infection and vaccine-related HPV type seropositivity is common among Appalachian women aged 18 to 26 years. The anticipated effect of environmental stressors on HPV seropositivity was not seen when multiple predictors were considered.

Human papillomavirus (HPV) infection is estimated to be the most common sexually transmitted infection in the United States.[1] Risk factors for HPV infection differ from risk factors for HPV seroconversion after natural infection, as only 40% to 60% of those infected mount an immune response. In the immunocompetent, this immune response is limited to approximately 5 years.[2–5] Lower seroconversion rates with a shorter duration of response are seen in the immunocompromised.[6] The risk factors for seropositivity that overlap with HPV infection include lifetime numbers of sexual partners, age of sexual debut, tobacco use, prior herpes (herpes simplex virus type 2) infection, and prior use of oral contraceptives.[2,7–11] Risk factors for seropositivity include persistent HPV infection and high viral load at infection.[4]

Appalachia has the highest rate of cervical cancer mortality among White US women.[12,13] In the Appalachian parts of Kentucky, Maryland, and Ohio, the annual age-adjusted incidence of cervical cancer was higher than for the non-Appalachian parts of each state.[12] This could be due to an increased number of HPV infections via increased numbers of sexual partners and increased viral load at time of infection due to viral load of one or many partners, or due to lack of HPV clearance as a result of immunosuppression from stressors, such as tobacco use and the environment. All of these may reduce the effectiveness of the quadrivalent vaccine against primary infection. In our conversations with key informants from Appalachian Ohio, there is the belief that young women will not benefit from the HPV vaccine because of high rates of existing or past infections and the environmental stressors on the immune system's ability to respond to the vaccine.

The primary aim of this study was to measure the prevalent seropositivity of HPV-6/11/16/18 among women aged 18 to 26 years in Appalachia. Secondarily, we estimated there would be associations between HPV seropositivity and risk factors of psychoneuroimmunologic suppression including the number of sexual partners, perceived stress, depression, and tobacco use. In addition, we explored the reactivation response of past Epstein-Barr virus (EBV) infections as an inverse response theory to perceived stress: higher perceived stress results in immune dysregulation; and

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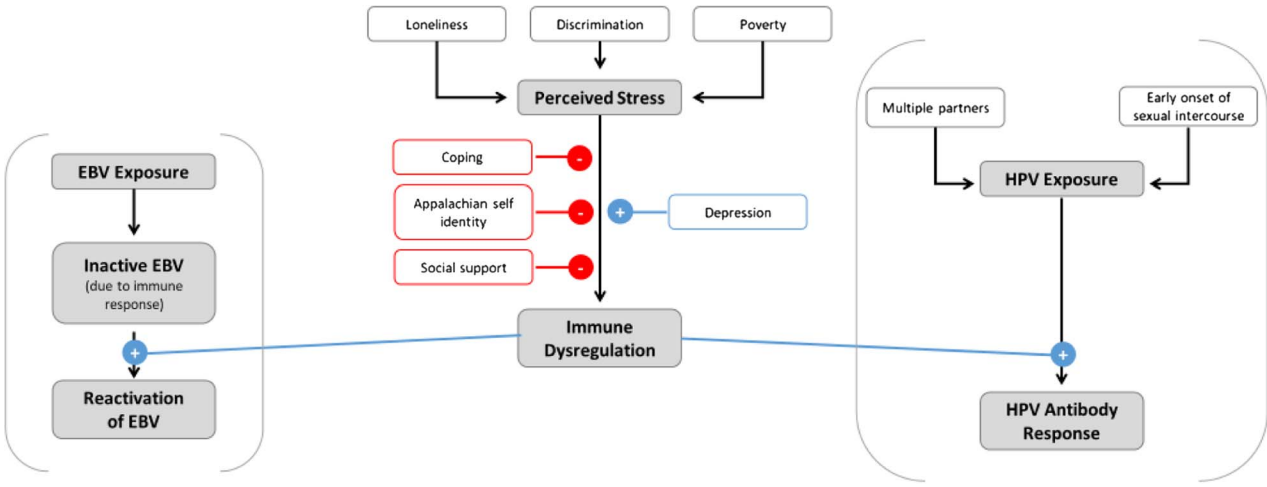

Figure 1. Psychoneuroimmunology model linking psychological stressor to immune dysregulation and failure to develop HPV antibodies after exposure to HPV.

immune dysregulation results in higher EBV titers and lower HPV seropositivity (Fig. 1).

METHODS

Between 2011 and 2013, women aged 18 to 26 years were eligible to participate in the study if they resided in an Appalachian county, had an intact cervix, were able to read and understand English, and were able to provide informed consent. Women were excluded from the study if they had a history of cervical cancer, had cervical lesion treated with cryotherapy or any forms of surgical removal of cervical lesions, were pregnant or planning a pregnancy in the next year, were taking immune suppressive medication, had an autoimmune disorder, had prior exposure to an HPV vaccine of any type, or were planning to move out of the study area in the next year. The product insert for the 4-valent HPV vaccine required participant exclusion because of hypersensitivity to yeast or to a prior 4-valent HPV vaccine dose.

Study participants were recruited from Appalachian areas of Ohio, West Virginia, and Kentucky to study sites along the borders of these 3 states, as the Appalachian identity does not have borders. The recruitment process was a multimedia marketing campaign including ads (radio, billboards, Facebook, craigslist, newspaper, college sidewalk ads, and dining room table tents), interactive Web site, flyers in community sites frequented by the target population, in-person promotions at community medical centers, direct mailing of previous study participants, presentations at relevant community organizations, and community advisory committee promotions.

Eligible women consenting to participate in the study completed a baseline visit. During the baseline visit, each participant completed a series of questionnaires (Supplemental Table 1, http://links.lww.com/OLQ/A652; Supplemental Table 2, http://links.lww.com/OLQ/A653), provided a blood sample for HPV and EBV serology assays, and underwent a pelvic examination for collection of cervical cytology and HPV DNA testing. The questionnaire information included demographics, health, sexual behaviors, Appalachian self-identity, and health care access. The recognized psychological data collected included the Perceived Stress Scale (PSS),[14] the Center for Epidemiologic Studies Depression Scale (CES-D),[15] the Loneliness scale,[14] the Brief COPE scale,[16] the Multidimensional Scale of Perceived Social Support (MPSS),[17] the 36-Item Short Form Health Survey general health scale,[18] and the Daily Discrimination Scale.[19]

Within 1 hour of blood collection, the sera were separated for EBV and HPV antibody analysis. Epstein-Barr virus antibody analysis was performed on site by Euroimmun EBV ELISA plates (Morris Plains, NJ). Epstein-Barr virus VCA IgG antibody titers were assessed and repeated if the end point did not fall within the expected linear range (±15%) per manufacturer's instructions. The sera for HPV antibody analyses were frozen at −80°C as 1 to 5 mL aliquots in 4 separate screw-top cryovials for evaluation by Merck using competitive Luminex Immunoassay (Merck Research Labs, Kenilworth NJ) for IgG antibodies to HPV types 6, 11, 16, and 18, with cutoffs for seropositivity defined as 20, 16, 20, and 24 milli-Merck units/mL.[20]

The cervix was visualized by speculum examination. Cervical sampling was performed with a brush and placed in ThinPrep; a separate brush provided HPV DNA sampling and was placed in a Digene transport tube. Cytology was read according to the Bethesda system.[21] Human papillomavirus DNA was first detected by Hybrid Capture 2, which tests for a cocktail of 14 high-risk HPV types. Of the positives, cervical DNA was purified by using the Qiagen Virus/Bacteria Midi Kit (Qiagen, Inc, Hilden, Germany) on the Qiasymphony SP instrument, and Roche Linear Array HPV Genotyping (Roche Molecular Systems, Inc, Pleasanton, CA) identified HPV DNA 6/11/16/18 along with a positive control.

STATISTICAL METHODS

Demographic, psychological, behavioral, and biological characteristics at baseline by HPV seropositivity status were summarized and univariable associations made by estimating the unadjusted relative risk. Unadjusted and multivariable-adjusted relative risks were estimated through a modified Poisson regression approach.[22] Multivariable models were developed to find the best predictors of HPV seropositivity. Potential predictors were informed by the psychoneuroimmunology model (Fig. 1). Age was considered an a priori confounder and included in all multivariable models. Within each covariate domain, among variables with the strongest associations, the variable with the smallest variance was chosen to first enter the model (e.g., number of partners vs. ever had a Papanicolaou [Pap] test). Interactions of interest were tested based on those of biological interest and included the following: PSS by EBV level (categorical), Appalachian identity by EBV level (categorical), self-rated health by EBV, self-rated health by number of partners, age by PSS, age by EBV, age by self-rated health, and PSS by smoking status. In addition, squared

terms of age and EBV level were also considered. All confidence intervals (CIs) were 2-sided and unadjusted for multiple comparisons. Analyses were performed in STATA (version 13; StataCorp, College Station, TX) and the SAS software (version 9.3 of the SAS System for Windows; SAS Institute Inc, Cary, NC).

RESULTS

The STROBE diagram (Fig. 2) outlines study recruitment. Of the 381 women who responded to advertising, 321 (84%) were eligible and 191 (60%) were finally consented and enrolled in the study. Six women were found to be ineligible after consent, leaving a final cohort of 185 women who responded to the survey questions. Cervical specimens and blood tests were valid in 183 women, of whom 92 were HPV DNA positive (50%) and 64 (35%) were seropositive for 1 or more of the 4 serotypes (6/11/16/18; Table 1). Among HPV 16/18 DNA positives, seropositivities were 63% (7/11) and 75% (3/4), respectively. Of the 92 women with current HPV infections, only 28% (n = 26) were infected with 1 or more of the 4 vaccine-relevant types (HPV-6/11/16/18). Of the

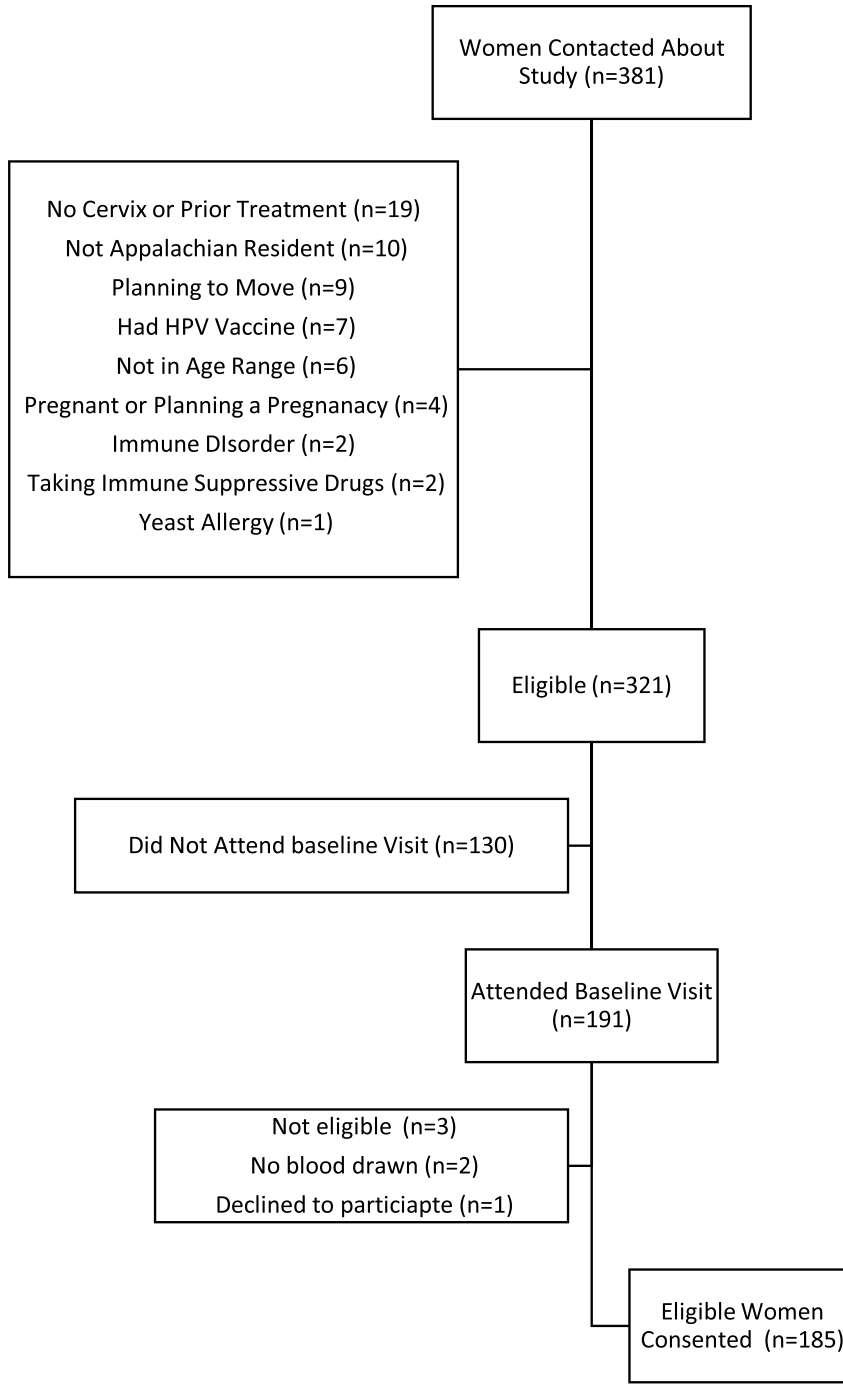

Figure 2. STROBE diagram of study population.

TABLE 1. HPV Serology and DNA Status

		Total (n = 183)	HPV-16 Seropositive (n = 36)	HPV-18 Seropositive (n = 16)	HPV-6 Seropositive (n = 48)	HPV-11 Seropositive (n = 10)	HPV-16, 18, 6, or 11 Seropositive (n = 64)
HPV DNA positive	92	50.3%	22 (61.1%)	10 (62.5%)	33 (68.8%)	5 (50.0%)	43 (67.2%)
Single HPV infections							
HPV-6	6	3.3%	2 (5.6%)	1 (6.3%)	3 (6.3%)	0	3 (4.7%)
HPV-11	0	0	0	0	0	0	0
HPV-16	11	6.0%	5 (13.9%)	2 (12.5%)	5 (10.4%)	0	8 (12.5%)
HPV-18	4	2.2%	2 (5.6%)	1 (6.3%)	2 (4.2%)	0	4 (6.3%)
Multiple HPV infections							
HPV-6/16	3	1.6%	3 (8.3%)	1 (6.3%)	3 (6.3%)	0	3 (4.7%)
HPV-6/18	0	0	0	0	0	0	0
HPV-16/18	2	1.1%	1 (2.8%)	0	1 (2.1%)	0	1 (1.6%)
HPV-6/16/18	0	0	0	0	0	0	0
HPV-6/11/16/18	0	0	0	0	0	0	0

TABLE 2. Demographic Characteristics Predicting Baseline HPV Seropositivity for HPV Types 6, 11, 16, and/or 18

		HPV-6/11/16/18 Seropositive		Relative Risk* (95% CI)
	Total (n = 185)	No (n = 120)	Yes (n = 65)	
Demographics				
Age, mean (SD), y	22.8 (2.4)	22.4 (2.5)	23.4 (2.2)	**1.12 (1.03–1.21)**
Race/ethnicity†				
White non-Hispanic	154 (83.2)	101 (65.6)	53 (34.4)	Reference
Non-White or Hispanic	31 (16.8)	19 (61.3)	12 (38.7)	1.12 (0.69–1.85)
Education‡				
High school/GED or less	59 (32.1)	39 (66.1)	20 (33.9)	Reference
Some college or more	125 (67.9)	80 (64.0)	45 (36.0)	1.06 (0.69–1.63)
Marital status‡				
Never married	119 (65.4)	84 (70.6)	35 (29.4)	Reference
Ever married	63 (34.6)	33 (52.4)	30 (47.6)	**1.62 (1.11–2.37)**
Current health insurance				
No/do not know/refused	50 (27.0)	24 (48.0)	26 (52.0)	Reference
Yes	135 (80.0)	96 (71.1)	39 (28.9)	**0.56 (0.38–0.81)**
Socioeconomic status scale				
Relative to community‡, mean (SD)	5.9 (1.8)	6.0 (1.8)	5.8 (2.0)	0.95 (0.85–1.05)
Relative to the United States‡, mean (SD)	5.7 (1.9)	5.8 (1.8)	5.6 (2.1)	0.97 (0.87–1.08)
General health				
<Very good	79 (42.7)	42 (53.2)	37 (46.8)	Reference
Very good or excellent	106 (57.3)	78 (73.6)	28 (26.7)‡	**0.56 (0.38–0.84)**
Sexual Health				
Never had a Pap test				
No	43 (23.2)	37 (86.1)	6 (14.0)	Reference
Yes	142 (76.8)	83 (58.5)	59 (41.6)	**2.97 (1.38–6.64)**
Prior abnormal Pap test result§				
No/do not know	99 (69.7)	63 (63.6)	36 (36.4)	Reference
Yes	43 (30.3)	20 (46.5)	23 (53.5)	**1.47 (1.00–2.16)**
Ever had sex				
No	8 (4.3)	6 (75.0)	2 (25.0)	Reference
Yes	177 (95.7)	114 (64.4)	63 (35.6)	1.42 (0.42–4.82)
Age of first intercourse‡,§, mean (SD), y	16.6 (2.3)	16.8 (2.4)	15.9 (2.1)	**0.90 (0.82–0.98)**
Years of sexual activity‡,§, mean (SD)	6.3 (3.4)	5.6 (3.4)	7.6 (3.0)	**1.11 (1.05–1.17)**
No. male sexual partners‡,§, mean (SD)	6.3 (5.8)	5.0 (5.0)	8.9 (6.4)	
<4	58 (34.7)	49 (84.5)	9 (15.5)	Reference
≥4	109 (65.3)	62 (56.9)	47 (43.1)	**2.78 (1.47–5.27)**
First intercourse ≤18 y, largest age difference among older partners‡,§, mean (SD)	3.4 (4.4)	3.0 (4.5)	4.2 (4.2)	1.03 (0.99–1.08)
First intercourse >18 y, largest age difference among older partners§, mean (SD)	5.6 (5.2)	4.9 (5.4)	6.9 (4.8)	**1.04 (1.00–1.07)**
Partners ever treated for STI§				
Do not know/refused	37 (20.9)	23 (62.2)	14 (37.8)	1.27 (0.77–2.09)
No	114 (64.4)	80 (70.2)	34 (29.8)	Reference
Yes	26 (14.7)	11 (42.3)	15 (57.7)	**1.93 (1.25–2.99)**

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TABLE 2. *(Continued)*

	Total (n = 185)	HPV-6/11/16/18 Seropositive		Relative Risk* (95% CI)
		No (n = 120)	Yes (n = 65)	
Self ever treated for STI				
Do not know	8 (4.3)	5 (62.5)	3 (37.5)	1.15 (0.46–2.89)
No	168 (90.8)	113 (67.3)	55 (32.7)	Reference
Yes	9 (4.9)	2 (22.2)	7 (77.8)	**2.38 (1.57–3.59)**
Ever use condoms				
No	33 (17.8)	18 (54.6)	15 (45.6)	Reference
Yes	152 (82.2)	102 (67.1)	50 (32.9)	0.72 (0.47–1.12)
Substance abuse behaviors				
Smoking				
Never	127 (68.7)	93 (73.2)	34 (26.7)	Reference
Ever	58 (31.4)	27 (46.6)	31 (53.5)	**2.00 (1.37–2.91)**
Alcohol use in last week				
No/do not know	51 (27.6)	36 (70.6)	15 (29.4)	Reference
Yes	134 (72.4)	84 (62.7)	50 (37.3)	1.27 (0.79–2.05)
Binge drinking in last month				
No/do not know	119 (64.3)	78 (65.6)	41 (34.5)	Reference
Yes	66 (35.7)	42 (63.6)	24 (36.4)	1.06 (0.70–1.58)
Any drug use				
No	145 (78.4)	97 (66.9)	48 (33.1)	Reference
Yes	40 (21.6)	23 (57.5)	17 (42.5)	1.29 (0.84–1.97)
Surrogate of immune dysregulation				
EBV (natural log scale)				
Mean (SD)	5.8 (2.0)	5.6 (2.1)	6.2 (1.8)	
Negative/low, n (%)	56 (30.3)	44 (78.6)	12 (21.4)	Reference
Medium/high, n (%)	120 (69.7)	76 (58.9)	53 (41.1)	**1.92 (1.11–3.30)**

Bold indicates statistical significance.

*Relative risk for a 1-unit increase.

†Only 5 women indicated Hispanic ethnicity.

‡Participants are missing responses for the following: age (n = 1), education (n = 1), marital status (n = 2), socioeconomic scale relative to the community (n = 1) and to the United States (n = 1), age of first intercourse (n = 6), years of sexual activity (n = 7), number of sexual partners (n = 10), largest difference in partner age ≤18 years (n = 12), and largest difference in partner age >18 years (n = 15).

§For those who indicate a history of Pap testing (n = 142) or a history of sexual activity (n = 177).

64 women seropositive for HPV-6/11/16/18, the corresponding current HPV infections were low. For instance, among the 36 women seropositive for HPV-16, only 9 women were HPV-16 DNA positive (25%), with 14% having corresponding HPV-6 infections and antibodies and 8% having corresponding HPV-18 infections and antibodies. Although 10 women were HPV-11 seropositive, no women in the study were HPV-11 DNA positive.

Table 2 describes the demographics and health characteristics of the study cohort by HPV serostatus at baseline. The majority of the cohort was White non-Hispanic (85%), with at least some college education (68%), never married (65%), with health insurance (80%), and self-reported good to excellent health (57%). Older age and ever being married were significantly positive predictors of seropositivity for at least 1 of the 4 HPV vaccine types at baseline (odds ratios, 1.12 [95% CI, 1.03–1.21] and 1.62 [1.11–2.37], respectively). Having current health insurance and self-reporting good to excellent health were significantly negative predictors of HPV seropositivity (0.56 [0.38–0.81] and 0.56 [0.38–0.84], respectively). Specifically, women with health insurance had a low proportion of HPV seropositivity (29%), similar to the general population versus 52% without (or unknown) health insurance. A low proportion of women with self-rated very good or excellent health were HPV seropositive, with rates of 27% versus 47% for those with poorer health.[9,23,24]

For our sexual health indicators, the majority of the cohort had never had a Pap test (77%), had sexual intercourse (96%), had 4 or more partners (65%), had used condoms (82%), but never had been treated for an STI (91%). The average (SD) age of first intercourse was 16.6 (2.3) years, and the average (SD) number of years of sexual activity were 6.3 (3.4). Of those having had a Pap test, 30% had an abnormal test result. Significant predictors of HPV seropositivity included never having had a Pap test (2.97 [1.38–6.64]), having a prior abnormal Pap test result (1.47 [1.00–2.16]), an increasing number of years of sexual activity (1.11 [1.05–1.17]), having 4 or more male sexual partners (2.78 [1.47–5.27]), being treated for an STI (2.38 [1.57–3.59]), having a partner treated for an STI (1.93 [1.25–2.99]), and having a sexual partner after the age of 18 years who on average was more than 5.6 years older (1.04 [1.00–1.07]).

Self-reported substance use was predominantly alcohol use, with 72% of the population using within the last week and 36% binge drinking in the last month. A history of tobacco smoking and drug use were less common at 31% and 22% of the population, respectively. A history of tobacco use was the only predictor of HPV seropositivity, with a significantly higher likelihood of baseline seropositivity for at least 1 of the 4 vaccine HPV types (2.00 [1.37–2.91]).

Surrogate immune dysregulation was measured by EBV antibody titers, which reactivated at medium/high levels in 70% of the responders and was a significant predictor of HPV seropositivity with a relative risk at 1.92 (1.11–3.30).

The psychological scales used to characterize the study population are summarized in Table 3. The PSS mean was 23, with 46% of study population having a CES-D score of 16 or greater, the identifying threshold for depression.

The only psychological scale measure significantly associated with baseline HPV serology was behavioral disengagement (1.18 [1.06–1.32]), indicating that the more the woman was likely to give up trying as a coping strategy, the more likely she had antibodies to at least 1 of the 4 vaccine HPV types.

TABLE 3. Psychological Scale Predictors of Baseline HPV Seropositivity for HPV Types 6, 11, 16, and/or 18

		HPV-6/11/16/18 Seropositive		
	Total (n = 185)	**No (n = 120)**	**Yes (n = 65)**	**Relative Risk* (95% CI)**
PSS				
Mean (SD)	23.5 (8.1)	23.0 (7.5)	24.4 (9.1)	1.01 (0.99–1.04)
Median (min, max)	23 (7, 52)	23 (5, 43)	25 (7, 52)	
CES-D†, mean (SD)	11.8 (10.1)	11.2 (9.4)	13.0 (11.2)	
<16	133 (72.3)	86 (64.7)	47 (35.3)	Reference
≥16	51 (27.7)	33 (64.7)	18 (35.3)	1.00 (0.64, 1.55)
MPSS†				
Mean (SD)	5.7 (1.2)	5.7 (1.1)	5.8 (1.4)	1.01 (0.79–1.28)
Median (min, max)	6.0 (1, 7)	5.9 (1, 7)	6.1 (1, 7)	
Daily discrimination score				
Mean (SD)	9.6 (7.8)	9.6 (7.2)	9.8 (8.9)	1.00 (0.98–1.03)
Median (min, max)	9 (0, 35)	9 (0, 31)	9 (0, 35)	
Loneliness scale†				
Mean (SD)	4.7 (1.6)	4.6 (1.6)	4.8 (1.7)	1.04 (0.93–1.17)
Median (min, max)	4 (3, 9)	4 (3, 9)	4.5 (3, 9)	
Active Coping Scale				
Mean (SD)	5.5 (1.6)	5.4 (1.5)	5.7 (1.8)	1.09 (0.95–1.25)
Median (min, max)	6 (2, 8)	5 (2, 8)	6 (2, 8)	
Use of emotional support scale				
Mean (SD)	5.2 (1.8)	5.2 (1.7)	5.1 (2.0)	0.98 (0.88–1.10)
Median (min, max)	5 (2, 8)	5 (2, 8)	5 (2, 8)	
Use of instrumental support				
Mean (SD)	4.7 (1.7)	4.6 (1.6)	5.0 (2.0)	1.09 (0.97–1.23)
Median (min, max)	5 (2, 8)	4 (2, 8)	5 (2, 8)	
Behavioral disengagement				
Mean (SD)	2.5 (1.1)	2.3 (0.8)	2.7 (1.4)	**1.18 (1.06–1.32)**
Median (min, max)	2 (2, 8)	2 (2, 7)	2 (2, 8)	

Bold indicates statistical significance.

*Relative risk for a 1-unit increase.

†Participants are missing responses for the following: CES-D (n = 1), MPSS (n = 1), Loneliness scale (n = 3).

Multivariable regression models investigated predictors of HPV seropositivity for types 6/11/16/18, based on the psychoneuroimmunology model (Table 4, Fig. 1). The risk of HPV seropositivity for at least 1 of the 4 vaccine HPV types was significantly influenced by smoking status (1.48 [1.00–2.18]), having 4 or more male sexual partners (1.96 [1.04–3.68]), and medium/high EBV titers (1.79 [1.00–3.21]) when modeled together. Those who self-rated their health as very good to excellent had a 43% significantly lower risk of being HPV seropositive at baseline (0.57 [0.38–0.86]) than did those who rated themselves in worse health. The PSS was not a strong predictor of HPV seropositivity. No significant interactions were observed.

DISCUSSION

Our study was driven by local community and primary care providers' concerns that Appalachian women aged 18 to 26 years have high rates of exposure to HPV and exposure to the Appalachian specific environmental factors that would impair the immune system's ability to respond to HPV vaccination at a time when HPV vaccine

TABLE 4. Multivariable Model Estimating Associations of Demographic, Behavioral, Psychosocial, and EBV Level With Seropositivity for HPV-6, HPV-11, HPV-16, or HPV-18

	Adjusted Relative Risk (95% CI)	
	Model 1: PSS Retained	**Model 2: Final**
Age (1-y increase)	1.07 (0.98–1.18)	1.07 (0.98–1.17)
Smoking status		
Never	Reference	Reference
Ever	1.47 (1.00–2.16)	1.48 (1.00–2.18)
No. partners		
<4	Reference	Reference
4+	1.94 (1.03–3.65)	1.96 (1.04–3.68)
EBV		
Negative/low	Reference	Reference
Moderate/high	1.80 (1.00–3.23)	1.79 (1.00–3.21)
PSS (10-unit increase)	1.06 (0.86–1.31)	
Self-rated health		
Good or worse	Reference	Reference
Very good or better	0.58 (0.38–0.89)	0.57 (0.38–0.86)

rates were very low. We developed a psychoneuroimmunologic theoretical model incorporating these community beliefs about environmental stressors, common HPV exposure, and impaired immune response. We have confirmed the community leaders' belief that HPV exposure was common, but no more so than in other populations.[25] We also showed that women continued to be infected with HPV, but with much less infectivity of the 6/11/16/18 types possibly because of the high natural seroconversion rates to these types earlier in life. The seroconversion may be providing women with short-term natural immunity, as the incidence of cancers at a later age exceeds the general population. In addition, the local perception of high levels of perceived stress, depression, and loneliness was confirmed.

Despite these higher measures of stress, we showed that the significant positive predictors of seropositivity to at least 1 of the 4 HPV vaccine-related types (having ever smoked, having 4 or more sexual partners, and having a resurgence in EBV titers) did not include stress markers. The reactivation of EBV titers indicates that there was immune dysregulation, but the effects of depression, perceived stress, and Appalachian self-identity, although potential minor contributors to the immune dysregulation, were not found to contribute to HPV seropositivity. We hypothesize that perhaps the reactivation of EBV titers is an indicator that HPV, which usually evades immune detection,[26] is now detected and seropositivity ensues. In addition, the predictors for HPV seropositivity were independent of age in the final adjusted model, something that has not been reported in other less stressed populations.

The strength of this study is also the community-based input for the study design and their acceptance of the measures used to identify stress and other environmental modifiers hypothesized to suppress immune status. The community leaders supported the biological specimen collections and maintained enthusiasm for the study throughout the study period. Although this study was conceived and implemented in 2011 to 2013, the conditions of Appalachia have not changed and the inferences from this work remain relevant to understanding vaccine seroconversion.

The limitations of the study include a sample size, which may limit our ability to detect small differences that may still be clinically meaningful for HPV seroconversion and seropositivity as well as EBV titers. The study population is representative of the Appalachian population of women aged 18 to 26 years. The results may not be the same if populations from other Appalachian areas were examined. In addition, our study was cross-sectional without the ability to follow EBV or HPV serostatus over time or with precise titers. We did not explore positive potential networks of family/friends/associates in this work.

Appalachian women have high baseline seropositivity rates for at least 1 of the 4 HPV vaccine-related types but low vaccine-relevant type infections, indicating natural HPV-6/11/16/18 exposure has induced sufficient antibodies for short-term protection. In addition, Appalachian women have elevated EBV titers indicating an immune dysregulation that we hypothesize assists in the high natural seroconversion rates.

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