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Original Contribution

Perceived Stress and Molecular Bacterial Vaginosis in the National Institutes of Health Longitudinal Study of Vaginal Flora

Rodman Turpin*, Natalie Slopen, Joanna-Lynn C. Borgogna, Carl J. Yeoman, Xin He, Ryan S. Miller, Mark A. Klebanoff, Jacques Ravel, and Rebecca M. Brotman

* Correspondence to Dr. Rodman Turpin, Department of Epidemiology and Biostatistics, School of Public Health, University of Maryland, College Park, 4200 Valley Dr #2242, College Park, MD 20742 (e-mail: Rturpin@umd.edu).

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Vaginal microbiota provide the first line of defense against urogenital infections primarily through protective actions of *Lactobacillus* species Perceived stress increases susceptibility to infection through several mechanisms, including suppression of immune function. We investigated whether stress was associated with deleterious changes to vaginal bacterial composition in a subsample of 572 women in the Longitudinal Study of Vaginal Flora, sampled from 1999 through 2002. Using Cox proportional hazards models, both unadjusted and adjusted for sociodemographic factors and sexual behaviors, we found that participants who exhibited a 5-unit-increase in Cohen's Perceived Stress Scale had greater risk (adjusted hazard ratio (HR) = 1.40, 95% confidence interval (CI): 1.13, 1.74) of developing molecular bacterial vaginosis (BV), a state with low *Lactobacillus* abundance and diverse anaerobic bacteria. A 5-unit increase in stress score was also associated with greater risks of transitioning from the *L. iners*-dominated community state type (26% higher) to molecular-BV (adjusted HR = 1.26, 95% CI: 1.01, 1.56) or maintaining molecular-BV from baseline (adjusted HR = 1.23, 95% CI: 1.01, 1.47). Inversely, women with baseline molecular-BV reporting a 5-unit stress increase were less likely to transition to microbiota dominated by *L. crispatus*, *L. gasseri*, or *L. jensenii* (adjusted HR = 0.81, 95% CI: 0.68, 0.99). These findings suggest that psychosocial stress is associated with vaginal microbiota composition, inviting a more mechanistic exploration of the relationship between psychosocial stress and molecular-BV.

bacterial vaginosis; community state type; lactobacillus; microbiome; prospective; stress; survival data; transition

Abbreviations: BV, bacterial vaginosis; CST, community state type; HIV, human immunodeficiency virus; LSVF, Longitudinal Study of Vaginal Flora; STI, sexually transmitted infection.

Bacterial vaginosis (BV) is a state of the vaginal microbiota characterized by a low abundance of *Lactobacillus* species and higher abundance of diverse anaerobic bacteria (1). BV affects nearly one-third of US women and is a cause of considerable morbidity as a common reason for vaginal symptoms prompting women to present to primary health care (2). BV has long been associated with serious adverse health outcomes, including the development of pelvic inflammatory disease (3), chorioamnionitis (4), and sexually transmitted infections (STIs) (5–7), including both acquisition and transmission of human immunodeficiency virus (HIV) (8–11). In pregnant women, BV is a well-established risk factor for miscarriages, preterm birth, and postpartum endometritis (12–15), although trials that treated BV have not had consistent success in preventing pregnancy complications (15, 16). The annual economic burden of BV and its comorbidities within the United States is estimated to be over $14 billion (2), and an improved understanding of the factors associated with the development of BV is needed.

While a number of risk factors for BV have been identified, we do not sufficiently understand how an optimal vaginal microbiota dominated by species of *Lactobacillus* is maintained and why a shift to a BV-associated microbiota occurs. Behaviors such as having a new sexual partner, having a greater number of sex partners, smoking, feminine hygiene practices, and lack of condom use are among the strongest risk factors for BV (17–22). Additionally, for reasons that are not known, African American and Hispanic

women have a higher prevalence of BV compared with White women (20, 23), corresponding with marked racial disparities in the prevalence of STIs in population-based US studies (24–27). Individual risk factors, including sexual behaviors, do not fully explain these health disparities (28–34).

One factor that has been understudied and might influence the composition of vaginal microbiota is stress. Stress is a well-known psychosocial factor that can increase disease risk (35) by impairing multiple physiological systems (36, 37), and has been linked to engagement in higher-risk behaviors (38, 39). Elevated stress has been associated with the suppression of immune function, leading to heightened infection susceptibility and increased severity and persistence of infections (19, 35, 40–45).

Epidemiologic studies have identified persistent exposure to stress as a risk factor for BV, independent of confounders (19, 44–48), although some studies have had inconclusive or null findings regarding this association (49–51). However, we are aware of only 2 studies that investigated stress in relation to the vaginal microbiota, one in a mouse model and one with a sample of only 21 participants (47, 48). In 2015, Jašarević et al. (47) reported that prenatal stress altered proteins related to vaginal immunity and decreased the abundance of vaginal *Lactobacillus* species in a mouse model. In 2019, Nunn et al. (48) reported that within a small cohort of 21 adolescent women, 3 measures of psychosocial stress (i.e., depression, perceived stress, and anxiety) were not associated with the vaginal microbiota.

In this study, we prospectively examined the association between changes in perceived stress levels and vaginal microbiota and BV outcomes. These data might contribute a more functional understanding of how psychosocial stress can significantly influence the female reproductive tract.

METHODS

Ethics statement

The University of Alabama School of Medicine and the Jefferson County Department of Health institutional review boards and the Office of Human Subjects Research at the National Institutes of Health approved the study. The University of Maryland Baltimore institutional review board also approved the use of archived samples from this study. All participants provided written informed consent.

Samples

We conducted a secondary analysis of data from the National Institutes of Health's Longitudinal Study of Vaginal Flora (LSVF), a prospective cohort of 3,620 nonpregnant cisgender women (ages 15–44 years) recruited through family planning and general wellness clinics in Birmingham, Alabama, which has previously been described (52). Briefly, data was collected from August 1999 to February 2002. Exclusion criteria at study entry included immunocompromised status and antibiotic therapy greater than 30 days. Participants were followed for approximately 12 months with clinical exams and surveys at quarterly visits. Clinicians collected a vaginal swab, measured pH (ColorpHast; Thermo Fisher Scientific, Waltham, Massachusetts), and a traditional wet mount (microscopy) was performed to assess for clue cells and whiff test. Finally, cervicovaginal lavage was performed and stored for further testing at −80°C; the storage is not expected to affect vaginal microbiota results (53).

From the full LSVF cohort, a subsample was selected to assess the vaginal microenvironment of 397 case observations at the visit before an incident genital STI (chlamydia, gonorrhea, or trichomoniasis) and 1,794 STI-negative control observations (54). Controls were matched to cases on age, race, and follow-up time. We conducted a secondary data analysis of this subsample. First, based on leverages (a measure of an observation's influence on the overall model), 4 excessively influential observations (<1% of the sample) were identified as outliers and removed. Next, participants with only 1 visit ($n = 800$) were excluded, because we could not analyze these prospectively. These exclusions resulted in a final analytical study sample comprising 1,391 observations contributed by 572 participants, with a median follow-up time of 168 days per participant.

Measures

Perceived stress. Perceived stress was measured using Cohen's 10-item Perceived Stress Scale, which measures an individual's appraisal of stress in the past 30 days (55–57). Each item in this scale has a 5-point Likert response format, including questions related to perceptions of control, coping ability, and emotional burden (e.g., "In the last month, how often have you been upset because of something that happened unexpectedly?"). This scale demonstrated excellent internal consistency in our sample (Cronbach's $\alpha = 0.87$), with scores ranging from 4 to 37. Our main measure of interest was change in perceived stress from baseline to last visit, and we also used baseline perceived stress as a covariate. All regression analyses used a continuous measure for perceived stress, but we report parameter estimates that correspond to a 5-point change for ease of interpretability. We chose a range of plus or minus 4 as the range for "consistent stress" because this was within a range of 1 standard deviation rounded.

DNA extraction and sequencing. Amplification, sequencing, and bioinformatic analyses of the 16S rRNA gene were generated using widely adopted methods targeting the V3–V4 region that were developed and validated in our laboratory (58, 59). High-quality amplicon sequences were obtained on an Illumina HiSeq 2500 (Illumina, Inc., San Diego, California) modified to generate 300-base-pair paired-end reads (59). An average of 62,354 amplicon sequences per sample were retained following chimera removal. Amplicon sequence variants were generated by DADA2 and taxonomically classified using the Ribosomal Database Project naive Bayesian classifier (60) trained with the SILVA v128 16S rRNA gene database (61). Amplicon sequence variants of major vaginal taxa were assigned species-level annotations using speciateIT (62). Taxa identified as contaminants in extraction and polymerase chain reaction–negative controls were removed from the

overall data set, as well as taxa present at a relative abundance of less than 10^{-5} across all samples.

Community state type. Studies from our group (63–65) and others (66, 67) using 16S rRNA gene amplicon sequencing have demonstrated that vaginal microbiota can be broadly grouped into 6 distinct microbial community state types (CSTs) based on bacterial composition and relative abundance. CSTs were assigned using VALENCIA, a nearest centroid-based classification algorithm based on a reference data set comprising over 13,000 samples (Web Figure 1, available at https://doi.org/10.1093/aje/kwab147) (68). Most (but not all) women with Amsel-BV, BV defined by 3 out of 4 clinical criteria, would have vaginal samples assigned to a CST IV category, in which the vaginal microbiota are characterized by low relative abundance of *Lactobacillus* species and an overrepresentation of a variety of BV-associated bacteria (e.g., *Atopobium, Prevotella, Gardnerella*); this low-*Lactobacillus* state has been termed molecular-BV. Four CSTs are dominated by *Lactobacillus* species: CST I (*L. crispatus*), CST II (*L. gasseri*), CST III (*L. iners*), and CST V (*L. jensenii*).

For our analyses, community state type was collapsed into 3 categories: a category characterized by dominance of *Lactobacillus* species (CST I, II, and V), *L. iners*-dominated as a separate category (CST III), and low-*Lactobacillus* (CST IV). Subtypes for CST IV (CST IV-A, IV-B) were also included in bivariate and regression analyses. CST IV-A and IV-B have a high to moderate relative abundance of *G. vaginalis* and *A. vaginae*, but IV-A also presents with an abundance of *Candidatus* Lachnocurva vaginae (formerly known as bacterial vaginosis–associated bacterium 1, or BVAB1). Samples in CST IV-C, a state dominated by a diverse array of facultative and strictly anaerobic bacteria including *Streptococcus* and *Staphylococcus*, were not used due to exceptionally small sample size (n =15).

Other variables. Based on the existing literature of confounders, we included a set of possible covariates, including self-identified race (Black, White, other), marital status, age (years), high-school education, monthly household income, number of sexual partners, average frequency of condom use (never, seldom, half the time, most of the time, always), and the sexual concurrence of their primary partner (none, unlikely, possible, definite).

Statistical analysis

Sample weighting. Inverse probability weights were constructed to correct for the matching in the original nested case-control design of the subsample (69). These were generated using age, race, and original cohort follow-up time, calculated such that with the application of these weights, the final distribution of age, race, and overall cohort follow-up time is equal between the weighted analytical sample and the original full cohort. Additionally, socioeconomic measures (education, income, marital status) were approximately the same between the weighted analytical sample and the full cohort, with no significant differences in these factors.

Bivariate analysis. First, we compared baseline demographic characteristics and biological and behavioral risk factors across collapsed CST categories using χ^2 tests for categorical variables, and one-way analyses of variance for continuous variables. We used Kruskal-Wallis tests to assess differences in proportions of ordinal variables. We also examined CST proportions at final visit across 3 categories of change in perceived stress score between baseline and the final observation: increasing (+5 units or more), decreasing (−5 units or more), or consistent (change less than 5 units).

Regression models. Cox proportional hazards models were used for all regression analyses. This approach allows for the generation of hazard ratios and accounts for the large range of days between baseline and the final observation across participants (ranging from 49 to 315 days).

In our first set of models designed to estimate associations between change in perceived stress level and risk of molecular-BV (CST IV), we fitted stratified models based on CST IV subtypes (CST IV-A, IV-B) at the final observation. A consistent reference group, CST I/II/V, was used in both models. To fit these models, we segmented the data to include only the groups of interest for a given comparison. For these models, we also adjusted for baseline CST subtype.

In our second set of models, designed to show associations between change in perceived stress and transition from *Lactobacillus*-dominated states (CST I/II/V and III) to molecular-BV, and maintenance of molecular-BV, we stratified by baseline CST (CST I/II/V, CST III, CST IV) to show rates of transition from CST I/II/V or CST III to CST IV, as well as rates of maintenance of CST IV. For each CST, we first display bivariate models that include only perceived stress, and then we display models additionally adjusted for covariates. Variance inflation factors were assessed for all models; there was no evidence of multicollinearity (all variance inflation factors <3). We used 2-sided tests of significance for all inferences, and all analyses were conducted in SAS, version 9.4 (SAS Institute, Inc., Cary, North Carolina) (70).

Missing data. There was less than 1% missing data for all variables, with the exception of income (8% missing) and sexual risk factors (up to 7% missing). We imputed 10 data sets using nonmissing data from race, age, educational level, and marital status, because they demonstrated associations with income in our data set. We imputed sexual risk behaviors from nonmissing sexual risk behaviors, because sexual risk factors were all associated with each other, supporting the validity of this approach to imputation.

Results

Sample characteristics. Of the 572 women in our sample, 76% were Black, 80% were unmarried, and the mean age at baseline was 30 years (Table 1). Most women had education beyond high school (62%), and exactly half reported a monthly household income above $3,000 (50%). At the final observations, 18% of samples were classified as CSTs I (11%), II (3%), or V (4%), 35% as CST III, and 47% as

Table 1. Sample Characteristics (%) for the Total Sample and Stratified by Endtime Community State Type Groupings (*n* = 572), Longitudinal Study of Vaginal Flora, Alabama, 1999–2002

Characteristic	Total (*n* = 572)	CST I/II/V[a] (*n* = 97)	CST III[b] (*n* = 186)	CST IV-A[c] (*n* = 112)	CST IV-B[d] (*n* = 150)
Mean change in PSS[e,f]	−0.16[g]	−0.64[g]	−0.64[g]	1.63[g]	−0.36[g]
≥5-unit increase in PSS[h]	23.43[g]	18.48[g]	20.71[g]	33.04[g]	21.05[g]
Baseline PSS[e,f]	16.8	16.5	17.1	16.0	17.0
Educational level[h]					
More than high school	61.81	66.11	63.96	60.22	58.55
High school or less	38.19	33.89	36.04	39.78	41.45
Monthly household income, $[i]					
<500	22.14	21.32	19.62	29.39	23.33
500–3,000	27.53	21.52	34.66	24.46	24.18
>3,000	50.33	57.16	45.72	46.15	52.49
Race[h]					
Black	76.13[g]	72.41[g]	84.20[g]	82.17[g]	73.66[g]
White	23.87[g]	27.59[g]	15.80[g]	17.83[g]	26.34[g]
Mean age[f]	29.76	29.52	29.17	30.31	29.96
Marital status[i]					
Not married	80.05	78.54	81.85	77.66	79.91
Married	19.95	21.46	18.15	22.34	20.09
Condom use[i]					
Never	66.77[g]	51.31[g]	72.47[g]	86.59[g]	68.06[g]
Rarely	3.13[g]	4.52[g]	2.46[g]	2.10[g]	3.61[g]
Sometimes	2.27[g]	3.02[g]	3.11[g]	0.00[g]	1.94[g]
Often	7.38[g]	2.75[g]	6.75[g]	6.24[g]	8.58[g]
Always	20.45[g]	38.39[g]	15.22[g]	5.07[g]	17.81[g]
No. of sexual partners[i]					
0	26.05	21.83	31.55	29.07	19.90
1	68.07	74.01	62.20	66.65	71.33
2 or more	5.87	4.15	6.25	4.27	8.77
Sexual partner concurrence[i]					
None	56.09	58.87	59.51	58.46	45.11
Unlikely	37.84	37.06	33.55	36.17	46.97
Likely	2.65	3.08	3.17	2.63	2.24
Definite	3.4	1.00	3.77	2.73	5.68

Abbreviation: BV, bacterial vaginosis; BVAB1, bacterial vaginosis–associated bacterium 1; CST, community state type; PSS, Perceived Stress Scale.

[a] *Lactobacillus crispatus*–, *L. gasseri*–, *L. jensenni*–dominated.

[b] *L. iners*–dominated.

[c] Molecular-BV, BVAB1–dominated.

[d] Molecular-BV, *Atopobium vaginae*–dominated.

[e] Values are expressed as mean.

[f] Tested using 1-way analysis of variance.

[g] Statistically significant ($P < 0.05$). Analyses use inverse probability weights to correct for original case-control selection.

[h] Tested using χ^2 test.

[i] Tested using Kruskal-Wallis test.

CST IV (20% CST IV-A and 27% CST IV-B). Changes in stress score ranged from decreases of 26 units to increases of 30 units. Approximately half of the samples stayed within 4 units of their baseline perceived stress, while a quarter had decreases of 5 units or more, and a quarter had increases of 5 units or more.

Bivariate analysis. The greatest increases in perceived stress were associated with CST IV-A microbiota at the final observation, while the greatest decreases were associated with CST I/II/V and CST III at the final observation (Table 1). While only 18.5% of those in CST I/II/V at baseline had a stress increase greater than 4 units, 33.0% of those in CST IV-A did. Black racial group was associated with significantly higher proportions of CST III and IV-A microbiota and the lowest proportions of CST I/II/V. A lack of condom use was also associated with CST IV-A.

Regression models. Both before and after covariate adjustment, increases in perceived stress across the observation period were associated with molecular-BV outcomes, with a 5-unit increase in perceived stress score associated with a 26% greater risk of CST IV-B at the final observation (adjusted HR = 1.29, 95% CI: 1.05, 1.59), and a 40% greater risk of CST IV-A at the final observation (adjusted HR = 1.40, 95% CI: 1.13, 1.74) (Table 2). Web Table 1 shows transition ratios and maintenance ratios stratified by baseline CST. After adjusting for confounders, a 5-unit increase in perceived stress score was associated with 26% greater risk of transition from CST III to CST IV (adjusted HR = 1.26, 95% CI: 1.01, 1.56) and a 33% increased risk of transition from CST I/II/V to CST IV (adjusted HR = 1.33, 95% CI: 0.87, 2.04), although the latter was not statistically significant. A 5-unit increase in perceived stress score was also associated with 23% increased risk of maintaining CST IV from baseline. Inversely, this means that a 5-unit increase in perceived stress score was associated with 19% lower rates of clearance of CST IV (adjusted HR = 0.81, 95% CI: 0.68, 0.99). Examination of adjusted CST proportions also demonstrated that increases in stress were associated with greater transitions from optimal to less optimal CSTs (Web Figure 2). Among those with decreased stress (≥5-unit reduction in score), 47% of those starting in CST I/II/V transitioned to CST IV, while among those with increased stress (≥5-unit increase in score), 69% of those starting in CST I/II/V transitioned to CST IV.

DISCUSSION

Stress can modulate immunological function and has also been shown to influence the microbiota of other body sites, playing a role in disorders such as irritable bowel syndrome and acute pathogenic diarrhea (71–75). Accordingly, stress could be important to a woman's genitourinary health. In this prospective cohort of women, increases in perceived stress were associated with elevated risk of molecular-BV. This included both a greater risk of transitioning from a *Lactobacillus*-dominated state to molecular-BV and a greater risk of maintaining molecular-BV. Inversely, reductions in stress were associated with greater rates of clearing molecular-BV.

Perceived stress has been shown to have an adverse impact on immune regulation by increasing release of adrenal corticotropic hormone from the pituitary gland, which induces cortisol secretion from the adrenal cortex (76). Cortisol can impair the immune response by activating type-2 cytokine-driven and pro-inflammatory responses and suppressing lymphocyte proliferation (76, 77). These changes can result in a shift from Th1-associated cytokines, which are required for active cellular immunity, to pro-inflammatory Th2-associated cytokines (78). Increased inflammation can lead to destruction of epithelial cells and inhibit deposition of vaginal glycogen, the byproducts of which are the major carbon source utilized by vaginal *Lactobacillus* species (79)

These changes can lead to greater susceptibility to colonization by anaerobic bacteria associated with BV. Stress can also directly affect sexual risk behaviors, in that various forms of sexual risk-taking can represent a form of stress coping; however, our results were maintained independent of differences in sexual risk behaviors. When examining CST IV subtypes, we found similar associations between perceived stress and risk of CST IV-A and CST IV-B. While both subtypes are associated with an absence of protective *Lactobacillus* species, they are compositionally distinct. We did not detect a significant difference in association with stress between these 2 CST subtypes. These findings build upon our previous work in the LSVF (80) identifying a positive association between stress and BV measured using both Nugent's score (Nugent-BV) and Amsel criteria (Amsel-BV), consistent with our current findings that increased stress is associated with molecular-BV. Molecular-BV is a broad term that describes a nonoptimal vaginal microbiota; it broadly overlaps with BV defined by Amsel and Nugent methods (8) and is a relevant risk factor for adverse outcomes, including HIV/STIs (10). Additionally, the VALENCIA classifier was validated with test data sets (68).

Similar to previous research on factors associated with BV, race was highly associated with CST, with White women having a prevalence of CST IV-A one-fifth that of Black women after adjusting for all other covariates. Several factors affected by racial discrimination, including sexual network structure, access to health care, and certain types of severe stressful events or traumas (unmeasured in this study) are disproportionately common for Black women as compared with White women, and thus might have an impact on this disparity (81). Our finding that a reduction in stress is associated with increased odds of clearing molecular-BV complements prior research in a longitudinal cohort of Black and White women documenting that Black women take longer to clear high-risk cervical human papillomavirus infection relative to White women (82); future research should examine the role of perceived stress, as well as other social and structural factors that place excess burden on Black women, to understand and address this disparity. This line of research is important for addressing health disparities, given the role of the vaginal microbiota in protection against STIs, adverse birth outcomes, and cervical cancer (8, 14, 83–85). Black women are disproportionately affected by BV, with 51% prevalence compared with 23% among White women (20). Moreover, perceived stress might have implications for HIV disparities through BV; this is salient to Black women, who are disproportionately at risk for HIV (86). BV is thought to affect susceptibility to HIV by numerous mechanisms, including the induction of pro-inflammatory cytokines and disrupting mucosal barrier function (87).

Table 2. Hazard Ratios for Changes in Perceived Stress Associated With Suboptimal Community State Type Outcomes (n = 572), Longitudinal Study of Vaginal Flora, Alabama, 1999–2002

Measure	CST IV-A[a]		CST IV-B[a]	
	HR	95% CI	HR	95% CI
Changes in PSS, continuous[b]	1.40[c,d]	1.13, 1.74	1.29[d,e]	1.05, 1.59
Baseline PSS, continuous	1.13	0.91, 1.41	1.14	0.93, 1.41
Educational level				
Above high school	1.00	Referent	1.00	Referent
High school and under	1.71	0.77, 3.78	0.84	0.38, 1.84
Monthly household income, $				
<500	1.00	Referent	1.00	Referent
500–3,000	0.51	0.12, 2.13	1.64	0.83, 3.21
>3,000	0.86	0.28, 2.65	0.87	0.45, 1.65
Race				
Black	1.00	Referent	1.00	Referent
White	0.20[d]	0.08, 0.47	1.15	0.54, 2.41
Age, continuous	1.00	0.95, 1.05	1.02	0.98, 1.06
Marital status				
Not married	1.00	Referent	1.00	Referent
Married	0.47	0.16, 1.38	0.46[f]	0.21, 1.04
Condom use				
Never	1.00	Referent	1.00	Referent
Rarely	0.76	0.05, 10.71	0.23	0.04, 1.25
Sometimes	1.42	0.19, 10.79	0.56	0.10, 3.13
Often	0.78	0.15, 3.97	0.80	0.40, 1.61
Always	0.48	0.18, 1.26	0.59	0.32, 1.07
No. of sexual partners				
0	1.00	Referent	1.00	Referent
1	0.41	0.16, 1.04	1.54	0.70, 3.39
2 or more	0.52	0.07, 3.81	2.19	0.74, 6.52
Sexual partner concurrence				
None	1.00	Referent	1.00	Referent
Unlikely	0.88	0.40, 1.95	1.92[d]	1.17, 3.16
Likely	2.06	0.34, 12.46	3.23	0.80, 12.97
Definite	12.69[d]	1.67, 98.50	0.85	0.28, 2.64

Abbreviation: CI, confidence interval; CST, community state type; HR, hazard ratio; PSS, Perceived Stress Scale.

[a] Referent is *Lactobacillus*-dominated CST I/II/V. All models adjust for baseline CST category and use inverse probability weights to correct for original case-control selection.

[b] Hazard ratios use continuous change in perceived stress from baseline to final observation and report a 5-unit increase in PSS score for interpretation.

[c] Unadjusted HR = 1.22, 95% CI: 1.05, 1.43.

[d] Statistically significant ($P < 0.05$) estimates.

[e] Unadjusted HR = 1.20, 95% CI: 1.02, 1.40.

[f] Marginally statistically significant ($0.05 < P < 0.10$) estimates.

In interpreting our results, there are limitations to consider. The original study participants were recruited in Birmingham, Alabama, and consisted primarily of Black women, reducing our study's generalizability. However, this is an important population to study, given the increased burden of STIs in Black populations in the southern United States (88). In addition, social desirability bias is a concern for the reporting of some measures, particularly the measures of

sexual risk behaviors. Due to relatively small sample sizes within CST categories, some estimates were imprecise. We were, however, able to detect relatively large magnitudes of association considering the small scale of units, with implications for how both decreases and increases in stress relate to molecular-BV. Reports of perceived stress rely on subjective assessments; therefore, in the future, it will be valuable to examine whether objective assessments of stressors, such as reports of stressful events or traumas, display associations with the composition of the vaginal microbiota that align with the results of this study. Finally, while Cohen's Perceived Stress Scale is a well-validated measure of stress, it is limited to stress in the past 30 days. As such, it does not fully capture chronic stress, nor is it a specific measure of acute stress. Because unique forms of stress might have different psychological effects, including on the vaginal microbiota, future research investigating distinct forms of chronic and acute stress is recommended. Additionally, this measure does not parse specific sources of stress, such as stigma and discrimination; this is particularly important given that our sample consisted primarily of Black women.

In conclusion, in one of the first studies to explore an association between a psychosocial characteristic and molecularly defined BV, increases in perceived stress were associated with greater risk of molecular-BV, including transition to a molecular-BV state and maintenance of molecular-BV. These results have relevance for the perpetuation of health disparities for poor women and women of color (89). Future studies are needed to clarify the mechanisms by which stress affects the vaginal microbiota, including access to care, sexual networks, and the body's immune response.

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Author affiliations: Department of Epidemiology and Biostatistics, University of Maryland, College Park, Maryland, United States (Rodman Turpin, Xin He); Department of Social and Behavioral Sciences, T. H. Chan School of Public Health, Harvard University, Boston, Massachusetts, United States (Natalie Slopen); Department of Microbiology and Immunology, Montana State University, Bozeman, Montana, United States (Joanna-Lynn C. Borgogna, Carl J. Yeoman); Division of Pediatric Endocrinology, University of Maryland School of Medicine, Baltimore, Maryland, United States (Ryan S. Miller); Center for Perinatal Research, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, United States (Mark A. Klebanoff); Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, Maryland, United States (Jacques Ravel); Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, Maryland, United States (Jacques Ravel, Rebecca M. Brotman); and Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, Maryland, United States (Rebecca M. Brotman).

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Data availability: The Longitudinal Study of Vaginal Flora data can be found at the National Center for Biotechnology Information Database of Genotypes and Phenotypes (dbGaP) under accession number phs002367.v1.p1.

Any interpretations and opinions expressed herein are solely those of the authors and may not reflect those of the Centers for Disease Control and Prevention.

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