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Relationships Between the Vaginal Microbiota and Genitourinary Syndrome of Menopause Symptoms in Postmenopausal Women: The Study of Women's Health Across the Nation

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Abstract

Objective: To describe vaginal microbiota classified by community state types (CST) in a diverse cohort of postmenopausal women and evaluate relationships among genitourinary syndrome of menopause (GSM) symptoms (vaginal dryness, vulvovaginal irritation, sexual pain, dysuria, urinary urgency), CSTs, estrogen, vaginal maturation index (VMI) and vaginal pH.

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Conflicts of interest:
The other authors have nothing to disclose.

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We thank the study staff at each site and all the women who participated in SWAN.

Methods: In the Study of Women's Health Across the Nation (SWAN), 1320 women aged 60.4–72.5 years self-collected (2015–2017) vaginal samples analyzed for: microbiota composition and structure (CSTs) using 16S rRNA gene amplicon sequencing, VMI, and pH. GSM symptoms were collected with self-administered questionnaires; interviewers elicited estrogen use and measured body mass index. Serum estradiol (E2) and estrone (E1) were measured using high performance liquid chromatography. We analyzed data using Pearson chi-square tests, ANOVA, Kruskal Wallis tests and binomial logistic regression.

Results: The most frequently occurring CST was low *Lactobacillus* spp IV-C (49.8%); 36.4% of women had CSTs dominated by *Lactobacillus* spp. Over half of the women with vaginal atrophy biomarkers (VMI<50 and pH>5) had CST IV-C0, while women using estrogen or with higher E1 and E2 levels had higher prevalence of *Lactobacillus crispatus*-dominated CST I (p-values<0.001). Sexual pain was associated with atrophy biomarkers and independently associated with *Streptococcus* spp dominated CST IV-C1 (odds ratio 2.26, 95% confidence intervals 1.20–4.23). For all other GSM symptoms, we found no consistent associations with E1 or E2 levels, atrophy biomarkers or any CST.

Conclusion: While close relationships exist among estrogen, CSTs, VMI and pH, sexual pain was the only GSM symptom associated with the structure of vaginal microbiota and atrophy biomarkers.

Keywords

vaginal microbiome; genitourinary syndrome of menopause; vaginal atrophy; estrogen

Introduction

The genitourinary syndrome of menopause (GSM) is a constellation of signs and symptoms associated with lower estrogen levels after menopause.[1] GSM symptoms include genital dryness, vulvovaginal irritation, sexual pain, dysuria, and urinary urgency. Physical signs of GSM include vaginal pallor, dryness, low rugosity, and tissue fragility. The reason that only a proportion of women develop GSM symptoms is not well-understood, but GSM symptoms have a significant impact on women's lives, including negative effects on intimate relationships, depressive symptoms, and social activities.[2,3]

Because the vaginal microbiome contributes to conditions of premenopausal women, such as bacterial vaginosis[4] and the persistence of human papilloma virus,[5] it is reasonable to hypothesize that the vaginal microbiome plays a role in postmenopausal genitourinary health and disease. Individual variations in the composition of the vaginal microbiota and their relationship to the decline in estrogen after menopause may help to explain the development of GSM. However, few studies have characterized vaginal microbial communities in postmenopausal women from diverse racial/ethnic backgrounds,[6,7,8] and only a small number have investigated associations between individual GSM symptoms or signs and vaginal microbiota composition and structure.[9,10,11] Analysis of the vaginal microbiota composition established using 16S rRNA gene amplicon sequencing have identified five major and 13 sub-types of vaginal microbiota, also termed community state types (CSTs), that have facilitated epidemiological investigations.[12] Four of the major CSTs (CST I, II, III,

and V) are dominated by different *Lactobacillus* species. In contrast, CST IV microbiota are characterized by a paucity of *Lactobacillus* spp. and the presence of a diverse array of strict and facultative anaerobes.[12] Higher resolution within CST IV now comprises the subtypes CST IV-A, IV-B and IV-C; the latter has been further sub-divided into IV-C0,1,2,3 and 4 (Table 1).[13]

In a multi-center, racially/ethnically diverse sample of 1,320 postmenopausal women aged 60 years or older from the Study of Women's Health Across the Nation (SWAN), our objectives were: 1) to describe the distribution of CSTs in the SWAN cohort and evaluate the relationship of CSTs to exogenous estrogen use, serum estrone (E1) and estradiol (E2) levels, and atrophy biomarkers, (vaginal maturation index (VMI)<50 and vaginal pH>5); and 2) to examine the relationships among GSM symptoms (vaginal dryness, vulvovaginal irritation, sexual pain, dysuria and urinary urgency), CSTs, estrogen status, and atrophy biomarkers.

Methods

Study participants:

SWAN is a multi-center, prospective cohort study of the menopausal transition and aging.[14] The study began in 1995 with a cross-sectional survey of 16,065 community-dwelling midlife women, recruited by random digit-dialing and/or list-based sampling[15]. Each of seven clinical sites then recruited about 450 women from this survey for the SWAN cohort (total of 3,302 women). Inclusion criteria for the cohort were: age between 42–52 years and self-identification of race or ethnicity as Black (at the Detroit, MI; Chicago, IL; Pittsburgh, PA; and Boston, MA sites); Hispanic (at the Newark, NJ site), Japanese (at the Los Angeles, CA site), Chinese (at the Oakland, CA site) or White (all sites). Exclusion criteria included: no menstrual period within three months before enrollment, hysterectomy and/or bilateral oophorectomy prior to enrollment, pregnant, lactating or using any reproductive hormone therapy at enrollment, and inability to speak English, Spanish, Japanese, or Cantonese. The cohort has been followed across 16 visits. All women consented to participate in SWAN, and the institutional review boards at each site approved the study protocols.

All women who attended visit 15 between 2015 and 2017 (N = 2,031) were eligible to participate in the "vaginal health cohort;" the only other inclusion criterion for this group was willingness to self-collect vaginal samples, and 1,447 (71.2%) agreed to participate. However, our primary analytic sample comprised 1,320 women whose vaginal microbiota samples (91.2% of vaginal samples) had at least 500 sequence counts. Our secondary analytic sample, used to evaluate relationships with VMI, was smaller (N = 994) due to fewer women collecting VMI samples (Figure 1).

Biological sample collection:

Guided by detailed and illustrated instructions, 1,477 participants self-collected two, and 994 collected three samples from the mid-vagina. Women collected a sample using a nylon swab (FloqSwab, Copan Diagnostics, Murieta CA) that they placed in 1 ml of RNAlater solution for microbiota analysis. For vaginal maturation index (VMI) measurement, each

participant placed a second nylon swab (FloqSwab) in a ThinPrep® solution (Hologic, Mississauga, ON Canada). Women measured their own vaginal pH by placing a cotton-tipped swab into their mid-vaginas and then onto pH paper, circling the matching color change to a reference scale with the assistance of study personnel. We defined a reported pH>5 as a biomarker of vaginal atrophy.[1] The microbiota swab was refrigerated (4°C), shipped on blue ice to the University of Maryland School of Medicine, Institute for Genome Sciences and then stored in −80°C freezers until processing. VMI samples in ThinPrep® solution were stored and shipped at room temperature to the University of Pittsburgh Cytology Lab where VMI was measured.

GSM symptom measures:

Women in the vaginal health cohort self-completed questionnaires on genitourinary symptoms and recent vaginal practices. We defined *vaginal dryness* as a self-report of any vaginal dryness in the previous two weeks or reporting use of lubricant, vaginal moisturizer, or vaginal estrogen if vaginal dryness was reported in at least one previous SWAN visit. We defined *vulvovaginal irritation/itching/soreness* as a report of experiencing any of these symptoms in the previous two weeks. *Sexual pain* was defined in sexually active women as reporting pain with intercourse at least sometimes in the previous six months and in women who were sexually inactive at visit 15 but who reported pain with intercourse at any previous visit while in the perimenopause, postmenopause or after a bilateral oophorectomy. *Dysuria* was a self-reported external burning sensation with urination at least some of the time in the last month. We defined *urinary urgency* as urgency incontinence or urinary urgency reported at least a few times per week.

Covariate measures:

Self-reported category of race or ethnicity was collected at baseline. Symptom sensitivity[16] (a measure of somatosensory amplification, both a state and a trait associated with a higher likelihood of reporting physical symptoms) was measured at visit 1, while contemporaneous health status and practices that could affect vaginal health at the time of collection (*e.g.*, recent use of antibiotics or vaginal medications) were collected by self-administered questionnaires at the time of vaginal collection. We calculated body mass index (BMI) as weight in kilograms/(height in meters)^2 based on measurements taken by certified staff using calibrated scales and stadiometers.

Exogenous and endogenous estrogen measures:

Trained interviewers obtained systemic and vaginal hormone medication use for each woman. For contemporaneous measures of E1 and E2 at visit 15, assays were performed using high performance liquid chromatography with two sequential mass spectrometry detectors (LC-MS/MS).[17]

VMI measures:

Each sample was transferred to a slide via ThinPrep®−3000 (T-3), which was then stained using a Pap Sakura Slide Stainer with quality control checks on each run. Two cytotechnicians and a pathologist The proportions of parabasal, intermediate and superficial

epithelial cells were counted by one and verified by a second cytotechnologist and a pathologist. The VMI was calculated as 0.5 x [(percent intermediate cells) + (percent superficial cells)]. Within the range of values from 0 to 100%, we defined VMI less than 50% (VMI<50) as a biomarker of vaginal atrophy.[18]

Characterization of the vaginal microbiota composition

***Genomic DNA extraction* and *16S rRNA gene amplification, barcoding and sequencing*:** Amplification, sequencing and analysis of the 16S rRNA gene affords characterization of microbial community composition. DNA was extracted from 300 μL of samples stored in RNAlater using the MagAttract PowerMicrobiome DNA/RNA kit (Qiagen) for high throughput processing automated onto a Hamilton STAR platform that integrated a bead-beating step on a Qiagen TissueLyser II (20 Hz for 20 min) in 96-deep well plates. DNA was eluted in 100 μL of TE buffer. Amplification of the V3-V4 regions of the 16S rRNA gene was performed using a validated two-step PCR.[19] Amplicons were visualized on a 2% agarose gel, quantified, pooled in equimolar concentration and purified prior to loading on an Illumina HiSeq 2500 (San Diego, CA, USA) modified to generate 300 bp paired-end reads.[19] Extraction and PCR negative controls were processed in parallel. In addition, for quality assurance, a positive control composed of a mixture of 20 vaginal specimens of known composition combined into one tube was processed and sequenced on each pool of 90 study samples as per the laboratory standard protocol. A mock community (ZYMObiomics Mock Community, ZYMO research) that comprised eight known bacterial species was also extracted and sequenced. None of the negative controls generated amplicons nor sequences, while the positive controls produced data that matched expectation.

Bioinformatic analysis of 16S rRNA gene sequence dataset.—The sequence reads were de-multiplexed using the dual-barcode strategy, a mapping file linking barcode to samples and split_libraries.py, a QIIME-dependent script.[20] The resulting forward and reverse fastq files were split by sample using the QIIME-dependent script split_sequence_file_on_sample_ids.py, and primer sequences were removed using TagCleaner (version 0.16).[21] Further processing followed the DADA2 Workflow for Big Data and dada2 (v. 1.5.2) (https://benjjneb.github.io/dada2/bigdata.html),[22] and as previously reported.[19]

Taxonomy was assigned to each amplicon sequence variant (ASV) generated by dada2 using the RDP classifier trained on the SILVA (version 1.2.3) database and specific taxa were speciated using SpeciateIT (version 1.0), a rapid per sequence classifier (http://github.com/ravel-lab/speciateit). Read counts for ASVs assigned to the same taxonomy were summed for each sample. A table of read counts assigned to each bacterial taxon for each sample was generated. Bacterial taxa were filtered before analysis if observed in fewer than three samples or present at a frequency of less than 10^{-5} frequency study-wide. Using the relative abundance of bacterial taxa in a sample, we categorized vaginal microbiota into one of 13 of the sub-CSTs (Figure 2) using VALENCIA, a nearest centroid classifier validated for use with vaginal microbiota generated from peri- and post-menopausal women.[23] This approach affords collapsing the hyperdimensional taxonomic profiles into a single

categorical variable, thus facilitating data exploration and statistical modeling efforts. VALENCIA provides for each sample a similarity measure to each CSTs.

Statistical Analyses:

We characterized the vaginal microbiota of 1,320 racially and ethnically diverse postmenopausal women by CST categories. To simplify our analyses, we used the five CSTs based on related phylotypes and frequencies of each category in our SWAN women, at times combining CST I, II, III, and V (N = 481) into a *Lactobacillus* predominant group (the individual four CSTs did not differ in their association with GSM symptoms) and categorizing CST IV into low *Lactobacillus* groups. We combined CST IV-A and B into IV-AB (N = 182) due to low numbers in IV-A (N=12) and combined IV-C2, IV-C3, and IV-C4 into IV-C234 (N=90) due to low numbers in IV-C2 (N = 2) and IV-C4 (N = 7), while IV-C0 (N = 381), IV-C1 (N = 186) had sufficient numbers as a separate category for analysis.

For our first objective to describe the distribution of CSTs in the SWAN cohort and evaluate the relationship of CSTs to exogenous estrogen use, serum estrone (E1) and estradiol (E2) levels, and atrophy biomarkers, we estimated unadjusted associations between CST categories and these factors, using Pearson Chi-square (or Fisher's exact test if expected cell counts were less than five), ANOVA for all continuous variables other than serum estrogens, and Kruskal Wallis tests for E1 and E2 to accommodate right-skewed distributions.

For our second objective to examine the relationships among GSM symptoms (vaginal dryness, vulvovaginal irritation, sexual pain, dysuria and urinary urgency), CSTs, estrogen status, and atrophy biomarkers, we used chi-squared tests for categorical variables or t-tests (Wilcoxon for E1 and E2, Student's t-test otherwise) for continuous variables to estimate the unadjusted associations between the specified risk factors and GSM symptoms, defining GSM symptoms in two ways. First, for *each individual GSM symptom,* we compared characteristics of women reporting versus not reporting each GSM symptom. Second, for *any GSM symptom,* we compared characteristics of women reporting one or more GSM symptoms versus reporting no symptoms. We stratified the second comparison by presence or absence of one or both atrophy biomarkers and assessed statistical significance of effect modification using binomial logistic regression with an interaction term between the characteristic of interest and the presence or absence of atrophy biomarkers when there was a significant association in at least one of the three biomarker strata. Finally, based on our unadjusted findings, we estimated the adjusted association between sexual pain and CST category using binomial logistic regression. In these analyses, we built our regression model with the design variables race/ethnicity and site as covariates and other candidate predictors that were significant at p-value 0.05 in unadjusted analyses (BMI, symptoms sensitivity, current sexual activity, VMI, pH, E1 and E2). Those variables remaining in the final model were retained based on backward elimination. We assessed co-linearity for BMI, race/ethnicity, E1, E2, VMI and vaginal pH to ensure this was not a reason for exclusion from the model and used the Hosmer-Lemeshow statistic to assess model fit. As a sensitivity analysis, we used multiple imputation to include individuals with missing values.

No formal adjustment of p-values was made for multiple comparisons; however, results were interpreted in the context of the number of statistical tests conducted.

Results

SWAN microbiome sample from the vaginal health cohort:

At Visit 15, 1,447 women volunteered for the vaginal health cohort. While a higher percentage of these volunteers were sexually active with a partner as compared to the 584 women who declined participation (43.1% of the vaginal health cohort versus 34.1% of those who declined participation, $p < 0.0001$), no difference was observed in reporting of GSM symptoms between women who volunteered and those who declined to provide vaginal samples. Of the vaginal health cohort, 1,320 women with adequate vaginal microbiota samples were included in the analysis (microbiome sample). The average age of the women in our microbiome sample was 65.5 years (range 60.4 to 72.5 years) and were between 2.32 and 19.8 years (mean 13.2 years) from their final menstrual period. While women in the microbiome sample were less likely to report excellent health (10.7% vs 17.5% p=0.03) and less likely to have been in a hot tub within 72 hours of sample collection (10.2% vs 16.5%, p=0.03) compared to the 127 whose microbiota were not included, we observed no significant differences by race/ethnicity, reporting of genitourinary symptoms, or use of oral antibiotics (N = 37) or vaginal medications (N = 12) in the previous 72 hours between women in the full vaginal health cohort and the microbiome sample..

Objective 1: to describe the distribution of CSTs in the diverse SWAN cohort and evaluate the relationship of CSTs to exogenous estrogen use, serum E1 and E2 levels, and atrophy biomarkers (Table 2):

While one-third (36.4%) of the microbiome sample had *Lactobacillus* spp. predominant CSTs (I, II, III, or V), CST IV was the most frequently occurring CST (63.6%), with CST IV-C (49.8% of the primary sample), particularly CST IV-C0 (28.9% of the primary sample), the most prevalent CST. In unadjusted analyses examining differences in CST prevalence by race/ethnicity, we found that Chinese women had the highest proportion of CST IV-C0 and IV-C1 compared to all other racial/ethnic groups. Black women had more than twice the prevalence of CST I compared to all other groups. Meanwhile, we observed no statistically significant variation in the distribution of CSTs across the clinical sites among White women. In contrast, we observed significant variation across the clinical sites in the distribution of CSTs among Black women. Specifically, Black women from the Chicago site (highest median BMI = 35.2 versus 31.6 for all other Black women, p=0.02) had the lowest prevalence of CST IV-A and IV-B and the highest prevalence of CST IV-C0 compared to all other Black women in the cohort. Otherwise, in general, the prevalence of CST I and CST III was higher in women with higher BMIs, while the prevalence of CST IV-C0 was higher in women with lower BMIs. Current sexual intercourse activity was not significantly related to distribution of CSTs. Women using exogenous estrogen (systemic and vaginal), had the highest prevalence of CST I and the lowest prevalence of CST IV-C0 and IV-C1 compared to women not using estrogen. Women with *Lactobacillus* spp. dominant CSTs, particularly CST I, had higher endogenous levels of E1 and E2 compared to women with CSTs IV-C0 and IV-C1. Vaginal pH was lowest in women with CST I and

highest with CST IV-C, while VMI was lowest with CST IV-C0 and IV-C1. More than half the women with both vaginal atrophy biomarkers (VMI<50 and pH >5) had CST IV-C0 compared to 15% among those with no vaginal atrophy biomarkers.

Objective 2: to examine the relationships among GSM symptoms (vaginal dryness, vulvovaginal irritation, sexual pain, dysuria and urinary urgency), CSTs, estrogen status, and atrophy biomarkers (Tables 3, 4, and 5):

The prevalence of GSM symptoms in our microbiome sample was as follows: 925 (63.9%) women reported at least one GSM symptom: 561 (45.3%) reported vaginal dryness, 309 (23.7%) reported vulvovaginal irritation/itching, 380 (39.7%) of ever-sexually active women reported sexual pain, 60 (4.6%) reported dysuria, and 476 (37.6%) reported urinary urgency (Table 3). In unadjusted analyses, while Black women reported vaginal dryness and sexual pain less frequently, they reported urinary urgency more frequently compared to all other women. Chinese women reported urinary urgency infrequently, but more than half reported vaginal dryness and sexual pain. Over half of the Japanese women also reported vaginal dryness and sexual pain. Vaginal dryness and sexual pain were more prevalent in women with normal BMIs compared to women who were overweight or obese, while urinary urgency was more prevalent in women who were obese, and vulvovaginal irritation was not associated with BMI (Table 3).

Individual GSM symptoms, CSTs, atrophy biomarkers, estrogen levels, and estrogen use (Table 3): In unadjusted analyses between CSTs, atrophy biomarkers, estrogen and each individual GSM symptom compared to no report of that symptom, sexual pain was the only GSM symptom associated with a CST. Women with sexual pain had higher prevalence of CST IV-C1 and lower prevalence of all other CSTs. Sexual pain was associated with atrophy biomarkers: VMI<50, pH>5 and both VMI<50 and pH>5 while urinary urgency was inversely associated with these atrophy biomarkers. We found no consistent patterns of association among markers of endogenous estrogen with individual GSM symptoms. For example, vaginal dryness and sexual pain were associated with lower E1 and E2 levels, while urinary urgency was associated with higher levels; vulvovaginal irritation and dysuria were not associated with E1 or E2 levels. Vaginal dryness and vulvovaginal irritation were more prevalent amongst current users of vaginal estrogen but not systemic estrogen.

Any GSM symptom and CSTs, atrophy biomarkers, estrogen levels and estrogen use (Table 4): Because the various GSM symptoms are considered to have a similar etiology related to the lower levels of estrogen after menopause,[1] we evaluated unadjusted relationships among CSTs, E1 and E2 levels, exogenous estrogen use, and reporting of *any GSM symptom* (*i.e.*, one or more GSM symptoms) but found no associations. When we stratified the analyses by presence of one or both atrophy biomarkers (with VMI<50 and pH>5 representing a subgroup with most clearly biomarker defined vaginal atrophy) versus absence of either (VMI≥50 and pH≤5), we found no association between CST categories and *any GSM symptom* (p>0.14 for all three atrophy biomarker strata and p-value for interaction = 0.71). While CST IV-C0 and IV-C1 were more prevalent in women with atrophy biomarkers, especially VMI<50 and vaginal pH>5, the distribution

of CSTs did not vary significantly by reporting of *any GSM symptom* with or without atrophy biomarkers. A higher proportion of all women and women without vaginal atrophy biomarkers using vaginal estrogen reported any GSM symptoms (p = 0.99 for interaction of vaginal estrogen and atrophy biomarkers). But we found no significant association between use of systemic estrogen and serum E1 or E2 levels and the presence of *any GSM symptom*, regardless of atrophy biomarkers, although the number of women in some categories was very small.

Adjusted association between sexual pain and CSTs (Table 5):

Given the unadjusted association of sexual pain with CSTs, biomarkers of atrophy and estrogens, we examined these relationships in multivariable logistic regression for presence/absence of sexual pain that also included demographic and health variables that were significant in our unadjusted analyses, stratified by whether women were ever sexually active in the past (but not currently) or currently sexually active. Pair-wise comparisons indicated that women with CST IV-C1 had over twice the odds of reporting sexual pain compared to CSTs dominated by *Lactobacillus* spp. This finding was independent of VMI, pH, and E1 and E2 levels, which were eliminated from the models during backward stepwise regression and remained significant after Bonferroni correction (the observed p-value of 0.004 was 0.01 after correction for five symptoms). No problems with lack of fit (Hosmer-Lemeshow p-values >.05) or predictor collinearity were observed.

Discussion

In the diverse SWAN vaginal health cohort of postmenopausal women with an average age of 65 years, we found that CST distributions differed by use of exogenous and levels of endogenous estrogen and atrophy biomarkers, but we found only one GSM symptom associated with CST category: sexual pain with CST IV-C1.

About two-thirds of this sample of postmenopausal women had vaginal microbiota classified as CST IV, including about one-half whose microbiota were classified as CST IV-C. CST IV-C0 (predominance of *Prevotella* spp.) and CST IV-C1 (predominance of *Streptococcus* spp.) were the most frequently occurring subtypes. Meanwhile, about one-third of our microbiome sample had *Lactobacillus* spp. predominated vaginal CST I, II, III and V, a higher proportion than previously described in smaller studies[7]. In pooled premenopausal cohorts, about half (56.2%) have microbiota dominated by *Lactobacillus* spp., and CST IV-C is rare.[23,24] In our postmenopausal cohort, women with higher E1 and E2 levels, estrogen use, VMI>50 and vaginal pH>5, and obesity (which is associated with higher circulating estrogens) [25,26] were more likely to have a vaginal microbiota predominated by *Lactobacillus* spp. (in particular, CST I). Correspondingly, women with lower estrogen levels, biomarkers consistent with vaginal atrophy, and lower BMIs had a higher prevalence of CST IV-C0 and IV-C1. A relationship between estrogen, the higher levels of estrogen in obesity, and the vaginal microbiota has been proposed in which estrogen stimulates the accumulation of glycogen in the most superficial layers of the squamous and mature vaginal epithelium. The glycogen provides nutritional support for *Lactobacillus* spp. which produce

lactic acid, thus lowering the vaginal pH and rendering the vaginal ecosystem inhospitable to other bacteria.[27,28,29]

Distinguishing genitourinary symptoms attributed to menopause (GSM symptoms) from those that may be related to other diagnoses (*i.e.,* dermatological or medical conditions) can be as challenging in epidemiologic studies as in clinical practice. Additionally, reporting of GSM symptoms does not correlate well with physical exam findings of vaginal atrophy or with VMI.[30,31] While we found clear relationships among CSTs, endogenous and exogenous estrogens, atrophy biomarkers and BMI, we found no consistent associations among GSM symptoms and estrogen levels (some symptoms were associated with higher levels, some with lower levels), VMI, vaginal pH or any CST with one exception: sexual pain was associated with biomarkers of atrophy and independently associated with CST IV-C1. Explanations for the independent association between sexual pain and CST IV-C1 could include the possibility of unmeasured overlap between CSTs and atrophy biomarkers, though we found no collinearity between them. A spurious result due to multiple comparisons is unlikely given the association remained significant after a Bonferonni correction. However, CST IV-C has been related to low libido, which is often a consequence of sexual pain.[32] One possible biological explanation that could be explored is that the composition of CST IV-C1, primarily defined by higher proportions of *Streptococcus* spp., may have an inflammatory effect on vulvovaginal tissues, resulting in the experience of insertional sexual pain.

We found variation in the distribution of CSTs both among racial/ethnic groups, but also within Black women at different clinical sites. Racial/ethnic variation in CST distribution may have many explanations. For example, in SWAN, average BMI was highest in Black women and lowest in Chinese women[33] which could, at least partially, explain the prevalence differences of CST I and CST IV-C0 and/or the sexual pain reporting differences between these two groups. Yet, we also found differences in CST distribution amongst Black women by study site which indicates that unmeasured individual, familial, relational, community, and societal factors likely contribute to the establishment and maintenance of specific vaginal microbiota distributions in postmenopausal women, which should be explored further.

This multi-faceted investigation into the relationships among the vaginal microbiota, biological markers of estrogen and GSM symptoms was facilitated by SWAN's large community-based, multi-center, racially/ethnically diverse sample of postmenopausal women with detailed data on behaviors and symptoms from standardized questionnaires, physical and hormone measures, and a standardized vaginal swab collection protocol. Given our large sample size and results similar to other studies, measurement error is not likely to have had a large impact on our findings. The prevalence of recent antibiotic and vaginal medication use that could impact vaginal microbiota was low. The study, however, had limitations that should be considered when interpreting the findings. First, while using CSTs as a means of reducing the dimensionality of the complex microbiota data facilitates epidemiologic investigations and the identification of potentially important biomarkers associated with symptoms, conditions, susceptibility to diseases and/or even responses to treatment,[34,35,36] CST designation may mask signal that could come from

specific bacterial components of the microbiota. Second, SWAN participants collected their own vaginal samples for the microbiota, VMI, and pH analyses; thus, we cannot be certain that all samples were appropriately collected without contamination from other urogenital sites. However, self-sampling of the vagina is a frequently used methodology that has been validated for a number of measures, including microbiota analysis,[37] and is more acceptable than clinical exam for most female study populations.[38,39] Third, this was a cross-sectional analysis and thus, we could only evaluate the associations among CSTs, estrogens, atrophy biomarkers and GSM symptoms, not any temporal and thus potentially causal relationships amongst them. Finally, because the VMI swab collection was not performed on all SWAN vaginal health participants, the analytic sample that included VMI for GSM analysis was smaller than for our main analysis which may have reduced statistical power to detect modest but meaningful associations.

Conclusion

While we found that close relationships exist among the vaginal microbiota, estrogen, and biomarkers of vaginal atrophy, the relationships of these to GSM symptoms in postmenopausal women was not uniform or straightforward. The results of this study raise important and clinically relevant questions about the genitourinary health of postmenopausal women. Further study should explore if subgroups of symptomatic women with specific vaginal biomarker patterns, for example those with sexual pain and CST IV-C1 or those with vaginal dryness and low VMI and/or high pH, can be meaningfully characterized and whether such characterization could result in the development of more targeted, personalized treatment strategies.

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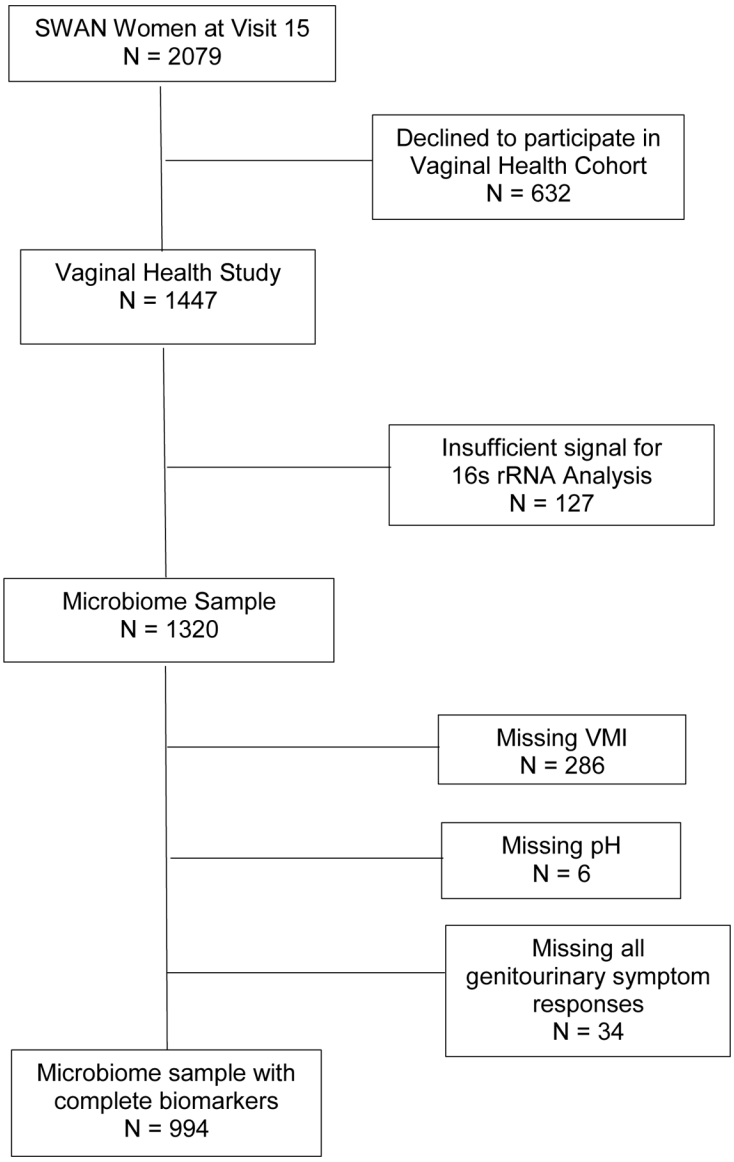

Figure 1.
Composition of Microbiome Analytic Sample in SWAN, 2017–2018

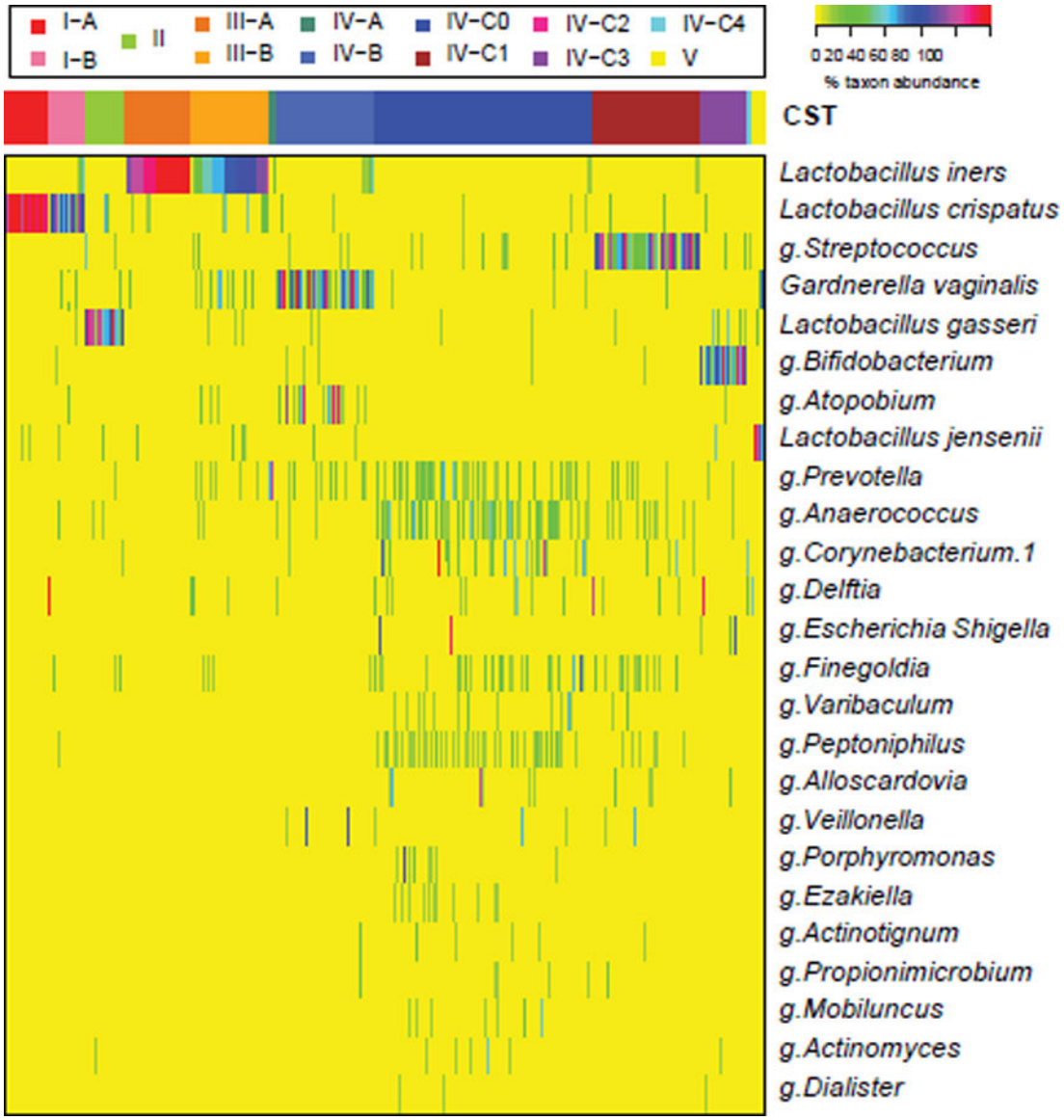

Figure 2:

Heatmap of relative abundance of top 25 bacterial taxa found in the vaginal bacterial communities of 1,320 postmenopausal women from the Study of Women's Health Across the Nation (2015–2017). Vaginal microbiota were assigned to one of 13 community state types (CST) using VALENCIA and are shown grouped by CST as indicated.

Table 1.

Community State Type (CST) Category Definitions[13]

CST I I-A I-B	Dominated by *Lactobacillus crispatus* Close to 100% *Lactobacillus crispatus* Majority of *Lactobacillus crispatus* and other anaerobic bacteria
CST II	Dominated by Lactobacillus gasseri
CST III III-A III-B	Dominated by *Lactobacillus iners* Close to 100% *Lactobacillus iners* Majority of *Lactobacillus iners* and other anaerobic bacteria
CST IV IV-A IV-B IV-C IV-C0 IV-C1 IV C2 IV-C3 IV-C4	Comprises of a diverse array of strict and facultative anaerobes, and lacks any significant amount of *Lactobacillus* High relative abundance of *Candidatus Lachnocruva vaginae* and moderate relative abundance of *Gardnerella vaginalis* High relative abundance of *Gardnerella vaginalis* and low relative abundance of *Candidatus Lachnocruva vaginae* Comprises of a diverse array of strict and facultative anaerobes Moderate relative abundance of *Prevotella* Dominated by Streptococcus species Dominated by Bifidobacterium species Dominated by Enterococcus species Dominated by Staphylococcus species
CST V	Dominated by Lactobacillus jensenii

Table 2.

Unadjusted prevalence of community state types (CSTs) by demographic and health characteristics, vaginal atrophy biomarkers, and exogenous and endogenous estrogen exposure in the Study of Women's Health Across the Nation (2015–2017)

N (%)	CST I 139 (10.5)	CST II 69 (5.2)	CST III 250 (18.9)	CST IV 839 (63.6)				CST V 23 (1.7)
				A, B 182 (13.8)	C0 381 (28.9)	C1 186 (14.1)	C2,3,4 90 (6.8)	
Age, mean (SD)	65.0 (2.4)	65.9 (2.5)	65.0 (2.7)	64.9 (2.7)	65.9 (2.7)	65.9 (2.7)	65.4 (2.6)	65.0 (2.8)
p-value <0.0001								
Race/ethnicity by site, N (%)								
Black (overall)	74 (18.8)	27 (6.9)	97 (24.6)	77 (19.5)	56 (14.2)	26 (6.6)	30 (7.6)	7 (1.8)
Boston	11 (14.5)	9 (11.8)	13 (17.1)	16 (21.1)	9 (11.8)	7 (9.2)	10 (13.2)	1 (1.3)
Chicago	20 (21.3)	11 (11.7)	21 (22.3)	10 (10.6)	20 (21.3)	8 (8.5)	4 (4.3)	0 (0.0)
Detroit	32 (21.5)	3 (2.0)	38 (25.5)	36 (24.2)	19 (12.8)	7 (4.7)	11 (7.4)	3 (2.0)
Pittsburgh	11 (14.7)	4 (5.3)	25 (33.3)	15 (20.0)	8 (10.7)	4 (5.3)	5 (6.7)	3 (4.0)
p-value for site = 0.01								
White (overall)	53 (8.2)	34 (5.3)	116(18.0)	67 (10.4)	203(31.6)	112(17.4)	46 (7.2)	12 (1.9)
Boston	13 (10.3)	5 (4.0)	26 (20.6)	14 (11.1)	42 (33.3)	17 (13.5)	5 (4.0)	4 (3.2)
Chicago	6 (7.5)	3 (3.8)	11 (13.8)	8 (10.0)	24 (30.0)	16 (20.0)	11 (13.8)	1 (1.3)
Detroit	10 (11.6)	3 (3.5)	18 (20.9)	9 (10.5)	29 (33.7)	11 (12.8)	6 (7.0)	0 (0.0)
Los Angeles	8 (11.6)	5 (7.3)	15 (21.7)	3 (4.4)	18 (26.1)	15 (21.7)	2 (2.9)	3 (4.4)
Newark	1 (2.5)	0 (0.0)	4 (10.0)	5 (12.5)	15 (37.5)	9 (22.5)	5 (12.5)	1 (2.5)
Pittsburgh	8 (5.6)	12 (8.3)	25 (17.4)	17 (11.8)	48 (33.3)	20 (13.9)	11 (7.6)	3 (2.1)
Oakland	7 (7.1)	6 (6.1)	17 (17.4)	11 (11.2)	27 (27.6)	24 (24.5)	6 (6.1)	0 (0.0)
p-value for site = 0.30								
Hispanic (Newark)	4 (4.7)	2 (2.3)	21 (24.4)	12 (14.0)	28 (32.6)	11 (12.8)	7 (8.1)	1 (1.2)
Japanese (Los Angeles)	6 (9.1)	5 (7.6)	4 (6.1)	9 (13.6)	27 (40.9)	11 (16.7)	4 (6.1)	0 (0.0)
Chinese (Oakland)	2 (1.5)	1 (0.8)	12 (9.2)	17 (13.0)	67 (51.2)	26 (19.9)	3 (2.3)	3 (2.3)
p-value (by race/ethnicity overall) <0.0001								
BMI, N (%)								
<25	16 (4.5)	14 (3.9)	49 (13.7)	51 (14.3)	138 (38.7)	64 (17.9)	18 (5.0)	7 (2.0)
25 – 29.9	39 (10.0)	17 (4.4)	78 (20.0)	47 (12.1)	117 (30.0)	61 (15.6)	26 (6.7)	5 (1.3)
30 – 34.9	32 (12.0)	18 (6.8)	52 (19.6)	36 (13.5)	62 (23.3)	37 (13.9)	21 (7.9)	8 (3.0)
35 – 39.9	28 (16.9)	11 (6.6)	39 (23.5)	23 (13.9)	34 (20.5)	14 (8.4)	16 (9.6)	1 (0.6)
40+	24 (18.2)	9 (6.8)	32 (24.2)	23 (17.4)	26 (19.7)	10 (7.6)	6 (4.6)	2 (1.5)
p-value <0.0001								

N (%)	CST I 139 (10.5)	CST II 69 (5.2)	CST III 250 (18.9)	CST IV 839 (63.6)				CST V 23 (1.7)
				A, B 182 (13.8)	C0 381 (28.9)	C1 186 (14.1)	C2,3,4 90 (6.8)	
Currently Sexually Active, N (%)								
Yes	50 (10.1)	28 (5.7)	100 (20.3)	71 (14.4)	129 (26.2)	80 (16.2)	24 (4.9)	11 (2.2)
No	72 (11.2)	33 (5.1)	117 (18.2)	89 (13.8)	188 (29.2)	77 (12.0)	56 (8.7)	11 (1.7)
p-value = 0.10								
Current Sexual Intercourse Activity, N (%)								
Yes	43 (10.1)	22 (5.2)	89 (20.9)	66 (15.5)	110 (25.8)	68 (16.0)	21 (4.9)	7 (1.6)
No	77 (11.2)	36 (5.2)	122 (17.7)	91 (13.2)	204 (29.6)	86 (12.5)	59 (8.6)	15 (2.2)
p-value = 0.12								
Exogenous or Endogenous Estrogens								
Systemic estrogen use, N (%)								
Yes	11 (39.3)	1 (3.6)	7 (25.0)	4 (14.3)	0 (0.0)	1 (3.6)	2 (7.1)	2 (7.1)
No	128 (9.9)	68 (5.3)	243 (18.8)	178 (13.8)	381 (29.5)	185(14.3)	88 (6.8)	21 (1.6)
p-value = 0.0004								
Vaginal estrogen use, N(%)								
Yes	14 (24.1)	8 (13.8)	12 (20.7)	9 (15.5)	6 (10.3)	3 (5.2)	3 (5.2)	3 (5.2)
No	125 (9.9)	61 (4.8)	238 (18.9)	173 (13.7)	375 (29.8)	181(14.4)	87 (6.9)	20 (1.6)
p-value <0.0001								
E1 level pg/ml, median (IQR)	39 (27, 55)	34 (27, 51)	33 (24, 49)	31 (21, 43)	27 (19, 37)	28 (20, 37)	34 (23, 45)	36 (24, 42)
p-value <0.0001								
E2 level pg/ml, median (IQR)	11 (7, 16)	10 (7, 15)	8 (5, 14)	8 (5, 14)	6 (3, 9)	6 (4,9)	8 (6, 13)	9 (5, 13)
p-value <0.0001								
Vaginal Biomarkers								
Vaginal pH, mean (SD)	5.2 (0.9)	5.8 (0.8)	5.6 (1.0)	6.0 (0.8)	6.6 (0.6)	6.6 (0.6)	6.2 (0.8)	5.3 (0.8)
p-value <0.0001								
Vaginal maturation index, mean (SD)	55.1 (11.2)	55.4 (13.3)	51.5 (13.9)	54.4 (14.4)	39.0 (18.6)	44.8 (17.0)	51.2 (19.0)	57.5 (7.0)
p-value <0.0001								
Vaginal Atrophy Biomarkers								

Menopause. Author manuscript; available in PMC 2024 November 01.

N (%)	CST I 139 (10.5)	CST II 69 (5.2)	CST III 250 (18.9)	CST IV 839 (63.6)				CST V 23 (1.7)
				A, B 182 (13.8)	C0 381 (28.9)	C1 186 (14.1)	C2,3,4 90 (6.8)	
VMI<50 and pH>5	8 (2.1)	13 (3.4)	41 (10.7)	28 (7.3)	201 (52.2)	76 (19.7)	17 (4.4)	1 (0.3)
VMI<50 or pH>5	28 (6.4)	29 (6.7)	72 (16.6)	90 (20.7)	102 (23.5)	66 (15.2)	41 (9.4)	7 (1.6)
VMI≥50 and pH≤5	65 (31.4)	16 (7.7)	86 (41.6)	18 (8.7)	3 (1.5)	3 (1.5)	7 (3.4)	9 (4.4)
p-value <0.0001								

Analytic methods: Pearson Chi-square for categorical variables (Fisher's exact test used if expected cell count<5), Kruskal Wallis test for E1 and E2, ANOVA for all other continuous variables

Table 3.

Unadjusted prevalence of individual genitourinary syndrome of menopause symptoms* by key demographic and health characteristics, sexual intercourse activity, community state types (CSTs), exogenous estrogen use, estrogen levels, and vaginal atrophy biomarkers in the Study of Women's Health Across the Nation (2015–2017)

	Vaginal dryness		Vulvovaginal Irritation/itching		Sexual pain (ever sexually active)		Dysuria		Urinary urgency	
	No 54.7% N=678	**Yes 45.3% N=561**	**No 76.3% N=992**	**Yes 23.7% N=309**	**No 60.3% N=576**	**Yes 39.7% N=380**	**No 95.4% N=1243**	**Yes 4.6% N=60**	**No 62.4% N=791**	**Yes 37.6% N=476**
Age										
Mean (SE)	65.58(0.10)	65.29(0.11)	65.42 (0.08)	65.59 (0.16)	65.27 (0.11)	65.46 (0.14)	65.46 (0.08)	65.35 (0.34)	65.36 (0.09)	65.57 (0.13)
p-value	0.06		0.32		0.30		0.76		0.18	
Race/ethnicity %(N)										
White	50.3 (306)	49.8 (303)	76.2 (482)	23.9 (151)	59.1 (277)	40.9 (192)	96.2 (612)	3.8 (24)	65.3 (406)	34.7 (216)
Black	64.8 (237)	35.3 (129)	77.9 (303)	22.1 (86)	69.9 (197)	30.1 (85)	95.9 (373)	4.1 (16)	49.6 (190)	50.4 (193)
Chinese	46.6 (55)	53.4 (63)	73.9 (96)	26.2 (34)	44.9 (40)	55.1 (49)	95.4 (125)	4.6 (6)	82.4 (108)	17.6 (23)
Japanese	48.5 (32)	51.5 (34)	64.6 (42)	35.4 (23)	45.1 (23)	54.9 (28)	95.4 (62)	4.6 (3)	63.9 (39)	36.1 (22)
Hispanic	60.0 (48)	40.0 (32)	82.1 (69)	17.9 (15)	60.0 (39)	40.0 (26)	86.6 (71)	13.4 (11)	68.6 (48)	31.4 (22)
p-value	**<0.0001**		0.11		**<0.0001**		**0.003**		**<0.0001**	
BMI %(N)										
<25	43.7 (148)	56.3 (191)	75.9 (268)	24.1 (85)	47.3 (132)	52.7 (147)	95.8 (339)	4.2 (15)	77.8 (270)	22.2 (77)
25 – 29.9	54.0 (197)	46.0 (168)	76.9 (296)	23.1 (89)	63.1 (178)	36.9 (104)	96.1 (369)	3.9 (15)	69.8 (261)	30.2 (113)
30 – 34.9	55.7 (141)	44.3 (112)	77.7 (205)	22.4 (59)	66.0 (126)	34.0 (65)	94.7 (251)	5.3 (14)	51.8 (132)	48.2 (123)
35 – 39.9	68.2 (103)	31.8 (48)	73.2 (120)	26.8 (44)	69.7 (76)	30.3 (33)	94.5 (155)	5.5 (9)	44.4 (71)	55.6 (89)
40+	66.4 (81)	33.6 (41)	76.6 (98)	23.4 (20)	67.4 (62)	32.6 (30)	94.6 (122)	5.4 (7)	44.8 (56)	55.2 (69)
p-value	**<0.0001**		0.87		**<0.0001**		0.86		**<0.0001**	
Symptom sensitivity score										
Mean (SE)	9.47(0.15)	9.79(0.15)	9.48(0.12)	10.02(0.21)	9.36(0.15)	9.94(0.18)	9.55 (0.10)	10.68 (0.53)	9.37(0.13)	10.02 (0.17)
p-value	0.13		**0.02**		**0.02**		**0.02**		**0.002**	
Sexual intercourse activity: %(N)										
No	72.3 (497)	27.7 (190)	78.5 (540)	21.5 (148)	58.7 (318)	41.3 (224)	94.9 (651)	5.1 (35)	58.3 (386)	41.7 (276)

	Vaginal dryness		Vulvovaginal Irritation/itching		Sexual pain (ever sexually active)		Dysuria		Urinary urgency	
	No 54.7% N=678	**Yes 45.3% N=561**	**No 76.3% N=992**	**Yes 23.7% N=309**	**No 60.3% N=576**	**Yes 39.7% N=380**	**No 95.4% N=1243**	**Yes 4.6% N=60**	**No 62.4% N=791**	**Yes 37.6% N=476**
Yes	32.7 (138)	67.3 (284)	74.2 (313)	25.8 (109)	62.3 (258)	37.7 (156)	96.5 (409)	3.5 (15)	69.1 (289)	30.9 (129)
p-value	**<0.0001**		0.10		0.25		0.22		**0.0003**	
Community state types %(N)										
IV-C0	54.2 (193)	45.8 (163)	75.3 (283)	24.7 (93)	55.8 (149)	44.2 (118)	95.0 (358)	5.0 (19)	65.6 (240)	34.4 (126)
IV-C1	48.8 (84)	51.2 (88)	78.7 (144)	21.3 (39)	45.3 (58)	54.7 (70)	94.5 (173)	5.5 (10)	65.0 (117)	35.0 (63)
IV-C2/C3/C4	59.3 (51)	40.7 (35)	68.5 (61)	31.5 (28)	64.1 (41)	35.9 (23)	93.3 (83)	6.7 (6)	62.4 (53)	37.7 (32)
IV-A/B	53.3 (90)	46.8 (79)	73.7 (132)	26.3 (47)	61.3 (84)	38.7 (53)	95.6 (172)	4.4 (8)	58.1 (101)	42.0 (73)
I/II/III/V	57.0 (260)	43.0 (196)	78.5 (372)	21.5 (102)	67.8 (244)	32.2 (116)	96.4 (457)	3.6 (17)	60.6 (280)	39.4 (182)
p-value	0.37		0.23		**0.0001**		0.64		0.39	
Exogenous and endogenous estrogens										
Systemic estrogen use %(N)										
No	55.0 (667)	45.0 (546)	76.4 (972)	23.6 (301)	60.3 (564)	39.7 (371)	95.4 (1216)	4.6 (59)	62.2 (770)	37.9 (469)
Yes	42.3 (11)	57.7 (15)	71.4 (20)	28.6 (8)	57.1 (12)	42.9 (9)	96.4 (27)	3.6 (1)	75.0 (21)	25.0 (7)
p-value	.20		0.54		0.77		1.00		0.16	
Vaginal estrogen %(N)										
No	57.2 (674)	42.8 (505)	77.1 (957)	22.9 (284)	60.7 (549)	39.3 (355)	95.3 (1185)	4.7 (58)	62.3 (753)	37.7 (456)
Yes	6.9 (4)	93.1 (54)	58.6 (34)	41.4 (24)	52.9 (27)	47.1 (24)	96.6 (56)	3.5 (2)	67.9 (38)	32.1 (18)
p-value	**<0.0001**		**0.001**		0.27		1.00		0.40	
Estrone (pg/ml)										
Median (Q1,Q3)	33 (23, 46)	29 (20, 40)	30 (22, 43)	31 (21, 46)	31 (22, 45)	29 (21, 40)	30 (22, 43)	31 (23, 45)	29 (21, 41)	32 (22, 50)
p-value	**<.0001**		.43		**.009**		.72		**.002**	
Estradiol (pg/ml)										
Median (Q1,Q3)	8 (5, 13)	7 (4, 11)	7 (4, 12)	7 (4, 13)	8 (4, 12)	7 (4, 10)	7 (4, 12)	8 (5, 10)	7 (4, 11)	8 (5, 14)
p-value	**.0001**		.98		**.002**		.90		**.0001**	
Vaginal atrophy biomarkers										
Vaginal maturation index (VMI) <50 %(N)										
No	53.8 (322)	46.2 (277)	76.1 (478)	23.9 (150)	63.2 (292)	36.8 (170)	95.9 (602)	4.1 (26)	59.4 (364)	40.6 (249)

	Vaginal dryness		Vulvovaginal Irritation/itching		Sexual pain (ever sexually active)		Dysuria		Urinary urgency	
	No 54.7% N=678	**Yes 45.3% N=561**	**No 76.3% N=992**	**Yes 23.7% N=309**	**No 60.3% N=576**	**Yes 39.7% N=380**	**No 95.4% N=1243**	**Yes 4.6% N=60**	**No 62.4% N=791**	**Yes 37.6% N=476**
Yes	54.6 (204)	45.5 (170)	76.4 (297)	23.7 (92)	53.4 (156)	46.6 (136)	95.6 (371)	4.4 (17)	71.2 (267)	28.8 (108)
p-value	0.81		0.93		**0.008**		0.85		**0.0002**	
pH>5: %(N)										
No	55.1 (147)	44.9 (120)	76.3 (212)	23.7 (66)	68.4 (143)	31.6 (66)	96.4 (268)	3.6 (10)	60.8 (163)	39.2 (105)
Yes	54.8 (529)	45.2 (437)	76.4 (776)	23.6 (240)	57.9 (430)	42.1 (313)	95.2 (969)	4.8 (49)	62.9 (624)	37.1 (368)
p-value	0.93		0.97		**0.006**		0.39		0.53	
Both VMI<50 and pH>5 %(N)										
No	55.0 (368)	45.0 (301)	76.5 (537)	23.5 (165)	64.0 (331)	36.0 (186)	96.0 (674)	4.0 (28)	57.5 (393)	42.5 (291)
Yes	54.4 (199)	45.6 (167)	76.4 (291)	23.6 (90)	53.7 (153)	46.3 (132)	95.5 (363)	4.5 (17)	72.2 (265)	27.8 (102)
p-value	0.84		0.97		**0.004**		0.70		**<.0001**	

*Each symptom compared to absence of that symptom

Table 4.

Relationship among any genitourinary syndrome of menopause symptom, community state type category, and estrogen exposure stratified by the presence or absence of vaginal atrophy biomarkers in the Study of Women's Health Across the Nation (2015–2017)

	Any GSM symptom		Any GSM symptom women with both biomarkers of vaginal atrophy* (VMI<50 and pH>5)		Any GSM symptom, women with one biomarker of vaginal atrophy* (VMI<50 or pH>5)		Any GSM Symptom women with no biomarker of vaginal atrophy (VMI≥50 and pH≤5)	
	None N (%) N=258	Any N (%) N=975	None N (%) N=87	Any N (%) N=276	None N (%) N=73	Any N (%) N=337	None N (%) N=46	Any N (%) N=147
Community State Type								
IV-C0	73 (28.3)	280 (28.7)	43 (49.4)	141 (51.1)	16 (21.9)	81 (24.0)	1 (2.2)	2 (1.4)
IV-C1	34 (13.2)	140 (14.4)	15 (17.2)	59 (21.4)	12 (16.4)	49 (14.5)	1 (2.2)	2 (1.4)
IV-C2,3,4	20 (7.8)	65 (6.7)	6 (6.9)	11 (4.0)	8 (11.0)	31 (9.2)	1 (2.2)	5 (3.4)
IV-AB	26 (10.1)	145 (14.9)	3 (3.5)	24 (8.7)	15 (20.6)	70 (20.8)	3 (6.5)	14 (9.5)
I, II, III, V	105 (40.7)	345 (35.4)	20 (23.0)	41 (14.9)	22 (30.1)	106 (31.5)	40 (87.0)	124 (84.4)
p-value**	0.24		0.14		0.97		0.93	
Systemic estrogen use								
Yes	5 (1.9)	21 (2.2)	0 (0.0)	0 (0.0)	2 (2.7)	2 (0.6)	1 (2.2)	14 (9.5)
No	253 (98.1)	954 (97.9)	87 (100.0)	276 (100.0)	71 (97.3)	335 (99.4)	45 (97.8)	133 (90.5)
p-value	0.83		--		0.15		0.13	
Vaginal estrogen use								
Yes	1 (0.4)	57 (5.9)	0 (0.0)	9 (3.3)	0 (0.0)	12 (3.6)	1 (2.2)	27 (18.4)
No	257 (99.6)	916 (94.1)	87 (100.0)	267 (96.7)	73 (100.0)	324 (96.4)	45 (97.8)	120 (81.6)
p-value	**0.0002**		0.12		0.14		**0.004**	
E1 pg/ml, median (IQR)	31 (23, 44)	30 (21, 44)	27 (21, 34)	26 (19, 37)	30 (23, 44)	32 (22, 43)	39 (30, 55)	38 (26, 55)
p-value***	0.51		0.50		0.97		0.69	
E2 pg/ml, median (IQR)	7 (4, 12)	7 (4, 12)	7 (4, 10)	6 (4, 10)	8 (4, 12)	8 (5, 12)	11 (9, 15)	10 (7, 18)
p-value***	0.67		0.50		0.69		0.65	

Numbers in Any GSM Symptom, any GSM Symptom with biomarkers of vaginal atrophy and any GSM symptom without biomarkers of vaginal atrophy are different due to missing values for VMI and pH

* Vaginal atrophy defined as both VMI greater than 50 and pH less than 5; no vaginal atrophy defined as having VMI 50 or greater and pH 5 or less)

** Chi-square test

*** Wilcoxon rank sum test

Table 5.

Adjusted associations between community state types (CSTs) and sexual pain at least sometimes, stratified by ever, past and current sexual activity in the Study of Women's Health Across the Nation (2015–2017).

	Odds Ratio (95% CI)*		
	Ever sexually active (N=948)	**Sexually active in the past, not currently (N=537)**	**Currently sexually active (N=411)**
CST:			
IV-C0	1.31 (0.92–1.87)	1.25 (0.79–2.00)	1.42 (0.81–2.48)
IV-C1	**2.17 (1.40–3.37)**	**2.19 (1.17–4.12)**	**2.26 (1.20–4.23)**
IV-C2,3,4	1.14 (0.64–2.02)	1.11 (0.54–2.27)	1.39 (0.52–3.72)
IV-A,B	1.26 (0.82–1.94)	1.24 (0.69–2.22)	1.27 (0.65–2.46)
I, II, III, V	Reference	Reference	Reference
p-value	**0.02**	0.20	0.16

*All odds ratios adjusted for race/ethnicity, site, BMI, symptom sensitivity and current sexual activity for ever sexually active model.

Other predictors eliminated by backwards, step-wise regression and thus not included in this model were: VMI, pH, E1 and E2 levels

Adding interaction between CST and current sexual intercourse for "ever sexually active" model: p = 0.94; the association between CST and sexual pain does not differ by current sexual activity.

No collinearity or correlations noted between BMI, race/ethnicity, E1, E2, VMI, vaginal pH