Normal vaginal microbiome in women with primary Sjogren's syndrome-associated vaginal dryness

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Dryness of epithelial surfaces is characteristic for patients with primary Sjögren's syndrome (pSS). Vaginal dryness is frequently reported by pSS-women and is associated with sexual dysfunction.[1 2] Recently, we showed that dysbiosis of the oral microbiome is largely similar between oral dryness patients with and without pSS when compared with healthy controls.[3 4] The objective of our current study was to assess whether the vaginal microbiome of women with pSS-associated vaginal dryness differs from controls.

In a case-control design, we compared the vaginal microbiome of 10 premenopausal pSS-women with that of 10 age-matched premenopausal women without pSS, who underwent general anaesthesia for a laparoscopic procedure. Exclusion criteria were genital inflammatory or infectious comorbidity, endometriosis and use of disease modifying antirheumatic drugs, corticosteroids, vaginal oestrogens or an intrauterine contraceptive device. All patients with pSS fulfilled the 2016 ACR/EULAR classification criteria. All participants completed a questionnaire on vaginal symptoms. Patient-reported vaginal dryness was scored using a Numeric Rating Scale (NRS, range 0–10). Vaginal health was assessed with the Vaginal Health Index (VHI).[5] From all participants, a cervicovaginal lavage (CVL) and endocervical swab (ES) were collected. DNA from all samples was isolated. The V3-V4 region of the bacterial 16S rRNA gene was amplified. Paired-end sequencing was performed on an Illumina MiSeq platform. For details, see online supplementary methods.

After inclusion, one patient with pSS was diagnosed with Chlamydia in the ES and two control women with endometriosis at laparoscopy. These women were excluded, resulting in nine pSS-women and eight controls for further analyses (table 1).

Table 1 Study population characteristics

Characteristic	pSS N=9	Control N=8	P values*
Age, mean (SD)	38 (9)	40 (4)	0.6
anti-SSA antibody positive, n (%)	7 (78)	na	
anti-SSB antibody positive, n (%)	6 (67)	na	
Disease duration in years, mean (SD)	8 (7)	NA	
Smoking, n (%)	3 (33)	4 (50)	0.8
Pack years, mean (SD)	0.7 (2)	0.7 (1)	0.4
Numeric Rating Scale on dryness (0–10)			
Eyes, mean (SD)	7 (1)	2 (2)	**0.001**
Mouth, mean (SD)	7 (1)	1 (2)	**<0.001**
Vagina, mean (SD)	6 (2)	1 (2)	**0.002**
Use of lubricants, n (%)	5 (56)	0 (0)	**0.05**
Dyspareunia, n (%)	9 (100)	2 (25)	**0.01**
Vaginal Health Index† total score, mean (SD)	19 (3)	23 (2)	**0.02**
pH posterior fornix, mean (SD)	4.6 (0.7)	4.7 (0.5)	0.6
Current medication			
Oral contraceptives, n (%)	6 (67)	3 (38)	0.5
Current NSAIDs, n (%)	2 (22)	0 (0)	0.5
ESSDAI—total, mean (SD)	6 (3)	NA	
ESSPRI—dryness, mean (SD)	6 (1)	NA	
ESSPRI—fatigue, mean (SD)	6 (3)	NA	
ESSPRI—pain, mean (SD)	3 (3)	NA	
ESSPRI—total, mean (SD)	5 (2)	NA	
Reason for laparoscopic procedure in controls			
BRCA1 or BRCA2 mutation, n	NA	6	
Refertilisation, n	NA	2	
Mucous cyst of the adnex, n	NA	1	

Bold values indicate a p-value of 0.5 or lower.
*Vaginal Health Index (VHI) scoring system: see online supplementary figure s1.
†χ^2 test and Wilcoxon rank sum test were used for categorical and numerical data, respectively.
ESSPRI, EULAR Sjögren's syndrome patient-reported index; NA, not applicable; na, not assessed; NSAIDs, non-steroidal anti-inflammatory drugs; pSS, primary Sjögren's syndrome; SSA, Sjögren's syndrome antigen A; SSB, Sjögren's syndrome antigen B; SSDAI, EULAR Sjögren's syndrome disease activity index.

As expected, scores for vaginal dryness, dyspareunia and use of lubricants were higher in pSS-women.[2] Furthermore, pSS-women scored significantly lower on the total VHI-score.[5] Vaginal pH-values were normal in patients with pSS. Microbiota composition of CVL and ES samples were highly similar within individuals, with 95% being explained by individuality (*adonis*, $p<0.001$; figures 1A). Disease (pSS vs control) did not affect overall vaginal microbiota composition in both CVL and ES samples (*adonis*, $p>0.05$; figure 1B). Despite the small sample size, we were able to identify in both groups (pSS and controls), four of the five vaginal community state types (CSTs) previously described (figure 1C–E).[6] Distribution of CSTs and distribution of the three most prevalent genera (ie, *Lactobacillus*, *Gardnerella* and *Streptococcus*) showed similar patterns in pSS-women and controls (figure 1F,G). Also, the mean relative abundance of these three genera did not differ between pSS-women and controls ($p>0.05$). Patient-reported vaginal dryness severity (NRS-score) did not correlate with the relative abundance of the three most prevalent genera (Spearman, $p>0.05$). The small number of patients with pSS did not allow us to analyse associations between vaginal microbiota and disease activity.

Our results indicate that the vaginal microbiome in pSS-women with vaginal dryness is similar to that of controls, which contrasts the observed difference in vaginal microbiota composition between postmenopausal women with and without vaginal dryness.[7] The different outcomes may be explained by different underlying causes of vaginal dryness (ie, pSS in premenopausal vs loss of oestrogen in postmenopausal women).[7] Under the influence of oestrogen, glycogen is deposited in the epithelium of the vagina.[8] Lactobacilli use the breakdown products of glycogen to produce lactic acid, which contributes to the low vaginal pH and thereby inhibits the growth of other bacteria.[8]

Apparently, the unique vaginal microbiome—dominated by acid producing lactobacilli—is less dependent on dryness than the oral microbiome. Oral dryness is associated with higher *Lactobacillus* relative abundance, which contributes to oral diseases (ie, dental caries and Candida infection). In the vagina, lactobacilli represent a healthy microbiome and are essential for the low vaginal pH.[8] Our study suggests that pSS-associated vaginal dryness in premenopausal women does not negatively influence homeostasis of the vaginal ecosystem.

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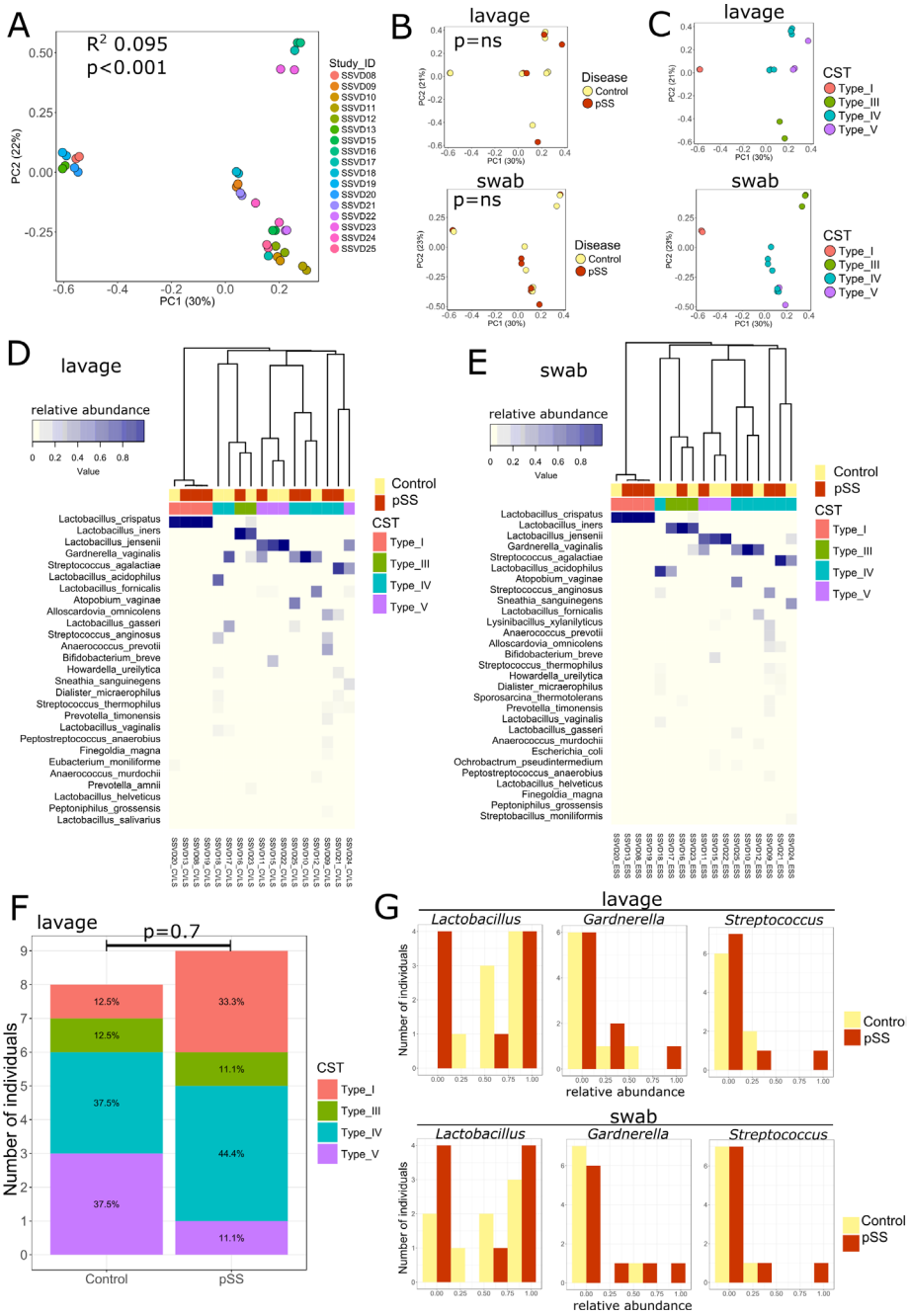

Figure 1 Vaginal microbiota composition in premenopausal pSS-women with and controls. (A) Principal coordinate analysis of CVL and ES samples shows high similarity within individuals (overlapping dots are separated slightly for enhanced clarity, see online supplementary figure S2 for original image). clustering of pSS-women or control women is observed based on vaginal microbiota composition in CVL (lavage) or ES (swab) samples. (C) CVL and ES samples show evident clustering based on the four CSTs. (D and E) CST-I, dominated by *Lactobacillus crispatus*, CST-III, dominated by *Lactobacillus iners*, CST-IV, a heterogeneous non-lactobacilli dominated type and CST-V, which is dominated by *Lactobacillus jensenii* were identified using Bray-Curtis distance clustering, based on the relative abundance of bacterial species with a relative abundance >0.1%. (F) Distribution of CSTs did not differ between pSS-women and controls (Fisher's exact test). (G) Histograms of the three most abundant genera show similar patterns in pSS-women and controls. CST, community state type; CVL, cervicovaginal lavage; ES, endocervical swab; pSS, primary Sjögren's syndrome.

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