The microbiome in primary Sjögren's syndrome

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CHAPTER 6

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

Adapted version of:

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INTRODUCTION

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.

METHODS

This study was approved by the medical ethical committee of the University Medical Center Groningen, Groningen, the Netherlands (METc 2015/039). All participants completed written informed consent according to the declaration of Helsinki.

Patients and controls

In a case-control design, we compared the vaginal microbiome of ten premenopausal pSS-women with that of ten age-matched premenopausal women without pSS, who underwent general anesthesia for a laparoscopic procedure. Exclusion criteria were genital inflammatory or infectious comorbidity, endometriosis and use of disease modifying antirheumatic drugs, corticosteroids, vaginal estrogens or an intrauterine contraceptive device. All pSS-patients 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). The VHI was scored by two gynaecologists (MM and KT). The VHI was first described by Bachmann et al. in 1995 and was developed at the Robert Wood Johnson Medical School (Brunswick, NJ, USA) to assess female urogenital health in a clinically objective manner (see Supplementary figure S1) (6).

Sample collection

From each participant, a gynecologist collected a cervicovaginal lavage (CVL) and an endocervical swab (ES). Cervicovaginal lavages were collected with 10mL sterile phosphate buffered saline (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). Endocervical swab samples were collected with flocked swabs (Eswab, COPAN, Brescia, Italy). Samples were centrifuged at 900 g. The pellet and supernatant of the samples were stored separately at -80°C.

DNA isolation, 16S rRNA gene sequencing and taxonomy assignment

DNA isolation was performed on the supernatant of the CVL and ES samples with a DNeasy UltraClean Microbial kit (QIAGEN Benelux B.V., Venlo, The Netherlands). The V3-V4 region of the 16S rRNA gene was amplified by PCR using modified 341F and 806R primers, as described before (7). Subsequently, paired-end sequencing was performed on a Illumina MiSeq platform. PANDAseq was used to discard reads with a quality score <0.9 (8). Samples were rarefied to 25,000 reads per sample. Taxonomy assignment was performed with the ARB software environment (release 5.5) with SILVA125 as reference database (9,10). The relative abundance of bacterial species was determined by the proportion of reads per species relative to the total number of reads per sample. Species with an overall mean relative abundance <0.01% were removed.

Statistical analysis

QIIME v1.9.1 was used to assess alpha- and beta-diversity (11). Alpha-diversity was measured by the number of observed species and Shannon index. Beta-diversity was assessed by Bray-Curtis distance. *Adonis* function from the R-*vegan* package was used to estimate the explained variance (R^2-value) and significance (p-value) of phenotype data on the variation in microbiota composition between samples using 999 permutations (12). Comparative statistics and clustering analyses were performed in R v3.3.1. A p-value <0.05 and a Benjamini-Hochberg false discovery rate corrected (FDR) p-value (indicated as q-value) <0.10 were used as significance cut-offs.

RESULTS AND DISCUSSION

After inclusion, one pSS-patient was diagnosed with Chlamydia in the ES and two control women with endometriosis at laparoscopy. These women were excluded, resulting in 9 pSS-women and 8 controls for further analyses (Table 1).

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 pSS-patients. Microbiota composition of CVL and ES samples were highly similar within individuals, with 95% being explained by individuality (*adonis*, p<0.001; Figure 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 (Figures 1C-E) (13). Distribution of CSTs and distribution of the three most prevalent genera (i.e., *Lactobacillus*, *Gardnerella* and *Streptococcus*) showed similar patterns in pSS-women and controls (Figures 1F,G). Also,

TABLE 1: Study population characteristics*

Characteristic	pSS n=9	Control n=8	p-value‡
Age, mean (sd)	38 (9)	40 (4)	0.6
SSA positive, n (%)	7 (78)	na	
SSB positive, n (%)	6 (67)	na	
Disease duration in years, mean (sd)	8 (7)	NA	
Smoking, n (%)	3 (33)	4 (50)	0.8
Packyears, 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	
Refertilization, n	NA	2	
Mucous cyst of the adnex, n	NA	1	

*pSS, primary Sjögren's syndrome; sd, standard deviation; na, not assessed; NA, not applicable; NSAIDs, non-steroidal anti-inflammatory drugs; ESSDAI, EULAR Sjögren's syndrome disease activity index; ESSPRI, EULAR Sjögren's syndrome patient reported index.

†Vaginal Hygiene Index (VHI) scoring system: see Supplementary figure S1.

‡*Chi-square test* and *Wilcoxon rank sum test* were used for categorical and numerical data, respectively.

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 pSS-patients 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 (14). The different outcomes may be explained by different underlying causes of vaginal dryness (i.e., pSS in premenopausal versus loss of estrogen in postmenopausal women) (14). Under the influence of estrogen, glycogen is deposited in the epithelium of the vagina (15). 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 (15).

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 (i.e., dental caries and Candida infection). In the vagina, lactobacilli represent a healthy microbiome and are essential for the low vaginal pH (15). Our study suggests that pSS-associated vaginal dryness in premenopausal women does not negatively influence homeostasis of the vaginal ecosystem.

Acknowledgements

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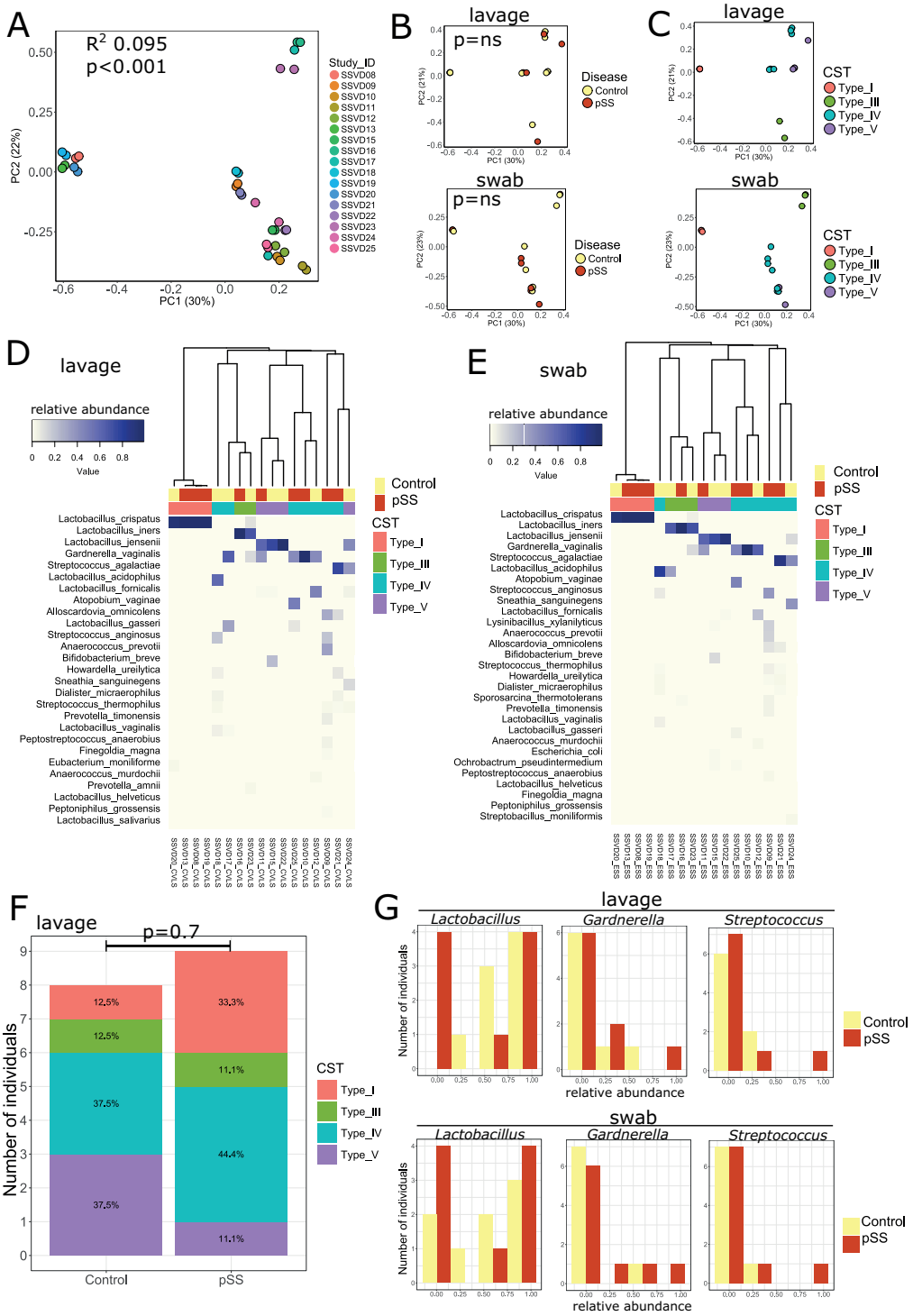
FIGURE 1: Vaginal microbiota composition in premenopausal pSS-women and controls. **(A)** Principal coordinate analysis of CVL and ES samples shows high similarity within individuals (overlapping dots are separated slightly for enhanced clarity, see online supplementary figure S2 for original image). **(B)** No 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 community state types (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.


A
R² 0.095
p<0.001
PC2 (22%)
PC1 (30%)
Study_ID
SSVD08
SSVD09
SSVD10
SSVD11
SSVD12
SSVD13
SSVD15
SSVD16
SSVD17
SSVD18
SSVD19
SSVD20
SSVD21
SSVD22
SSVD23
SSVD24
SSVD25
B
lavage
p=ns
swab
p=ns
Disease
Control
pSS
C
lavage
swab
CST
Type_I
Type_III
Type_IV
Type_V
D
lavage
relative abundance
Value
Lactobacillus_crispatus
Lactobacillus_iners
Lactobacillus_jensenii
Gardnerella_vaginalis
Streptococcus_agalactiae
Lactobacillus_acidophilus
Lactobacillus_fornicalis
Atopobium_vaginae
Alloscardovia_omnicolens
Lactobacillus_gasseri
Streptococcus_anginosus
Anaerococcus_prevotii
Bifidobacterium_breve
Howardella_ureilytica
Sneathia_sanguinegens
Dialister_micraerophilus
Streptococcus_thermophilus
Prevotella_timonensis
Lactobacillus_vaginalis
Peptostreptococcus_anaerobius
Finegoldia_magna
Eubacterium_moniliforme
Anaerococcus_murdochii
Prevotella_amnii
Lactobacillus_helveticus
Peptoniphilus_grossensis
Lactobacillus_salivarius
E
swab
relative abundance
Value
Lactobacillus_crispatus
Lactobacillus_iners
Lactobacillus_jensenii
Gardnerella_vaginalis
Streptococcus_agalactiae
Lactobacillus_acidophilus
Atopobium_vaginae
Streptococcus_anginosus
Sneathia_sanguinegens
Lactobacillus_fornicalis
Lysinibacillus_xylanilyticus
Anaerococcus_prevotii
Alloscardovia_omnicolens
Bifidobacterium_breve
Streptococcus_thermophilus
Howardella_ureilytica
Dialister_micraerophilus
Sporosarcina_thermotolerans
Prevotella_timonensis
Lactobacillus_vaginalis
Lactobacillus_gasseri
Anaerococcus_murdochii
Escherichia_coli
Ochrobactrum_pseudintermedium
Peptostreptococcus_anaerobius
Lactobacillus_helveticus
Finegoldia_magna
Peptoniphilus_grossensis
Streptobacillus_moniliformis
F
lavage
p=0.7
Number of individuals
12.5%
12.5%
37.5%
37.5%
33.3%
11.1%
44.4%
11.1%
Control
pSS
G
lavage
Lactobacillus
Gardnerella
Streptococcus
Number of individuals
relative abundance
swab
Lactobacillus
Gardnerella
Streptococcus
Number of individuals
relative abundance

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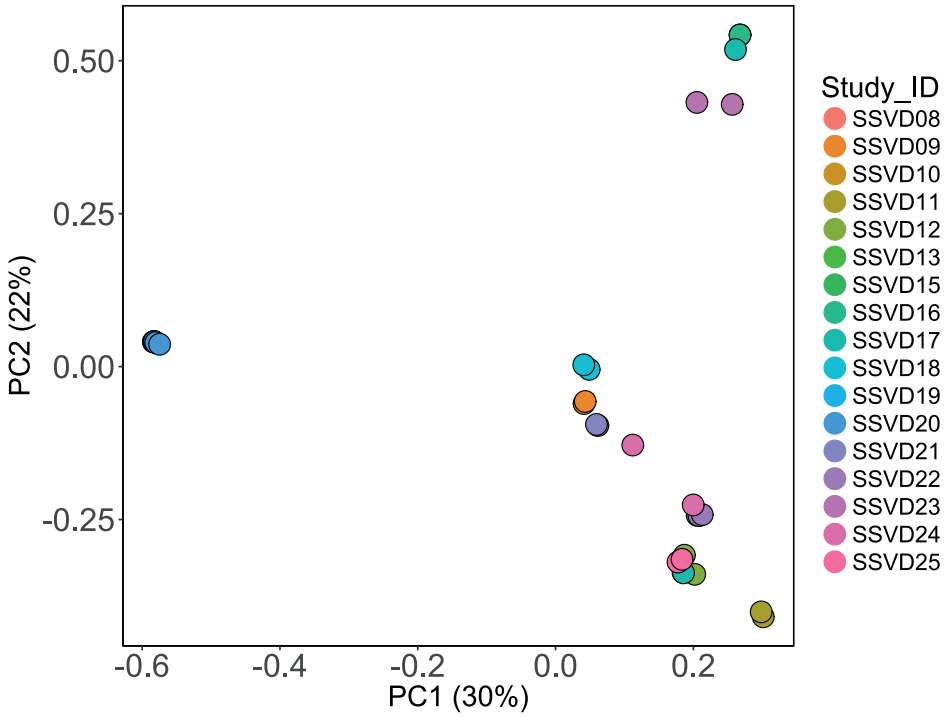
SUPPLEMENTARY FIGURES

Elasticity	Fluid	pH	Mucosa	Moisture
1. None	None	6.1	Petechiae before contact	None, inflamed
2. Poor	Scant, thin, yellow	5.6-6.0	Bleeds with light contact	None, not Inflamed
3. Fair	Superficial, thin, white	5.1-5.5	Bleeds with scraping	Minimal
4. Good	Mod, thin, white	4.7-5.0	Not friable, thin	Moderate
5. Excellent	Normal, white	<4.6	Not friable, normal	Normal

SUPPLEMENTARY FIGURE S1: scoring system of the VHI



SUPPLEMENTARY FIGURE S2: Original version of principal coordinate analysis of CVL and ES samples by study ID. Principal coordinate analysis (PCoA) of cervicovaginal lavage (CVL) and endocervical swab (ES) samples. Each color represents an individual of the study. The microbial composition of many individual samples was highly similar, which resulted in considerable overlap of individual dots (i.e., individual samples) in the visualization of the PCoA, as shown here. Therefore, in Figure 1A this figure was adapted slightly to demonstrate that the microbiota composition from different samples (i.e., CVL and ES) is highly similar within an individual.