



Shared gut, but distinct oral microbiota composition in primary Sjögren's syndrome and systemic lupus erythematosus

Taco A. van der Meulen^{a,*}, Hermie J.M. Harmsen^b, Arnau Vich Vila^{c,d}, Alexander Kurilshikov^d, Silvia C. Liefers^e, Alexandra Zhernakova^d, Jingyuan Fu^d, Cisca Wijmenga^d, Rinse K. Weersma^c, Karina de Leeuw^e, Hendrika Bootsma^e, Fred K.L. Spijkervet^a, Arjan Vissink^a, Frans G.M. Kroese^e

^a Department of Oral and Maxillofacial Surgery, University of Groningen, University Medical Center Groningen, PO Box 30.001, 9700 RB, Groningen, The Netherlands

^b Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, PO Box 30.001, 9700 RB, Groningen, The Netherlands

^c Department of Gastroenterology, University of Groningen, University Medical Center Groningen, PO Box 30.001, 9700 RB, Groningen, The Netherlands

^d Department of Genetics, University of Groningen, University Medical Center Groningen, PO Box 30.001, 9700 RB, Groningen, The Netherlands

^e Department of Rheumatology & Clinical Immunology, University of Groningen, University Medical Center Groningen, PO Box 30.001, 9700 RB, Groningen, The Netherlands

ABSTRACT

Objective: Alterations in the microbiota composition of the gastro-intestinal

tract are suspected to be involved in the etiopathogenesis of two closely related systemic inflammatory autoimmune diseases: primary Sjögren's syndrome (pSS) and systemic lupus erythematosus (SLE). Our objective was to assess whether alterations in gut and oral microbiota compositions are specific for pSS and SLE.

Methods: 16S ribosomal RNA gene sequencing was performed on fecal samples from 39 pSS patients, 30 SLE patients and 965 individuals from the general population, as well as on buccal swab and oral washing samples from the same pSS and SLE patients. Alpha-diversity, beta-diversity and relative abundance of individual bacteria were used as outcome measures. Multivariate analyses were performed to test associations between individual bacteria and disease phenotype, taking age, sex, body-mass index, proton-pump inhibitor use and sequencing-depth into account as possible confounding factors.

Results: Fecal microbiota composition from pSS and SLE patients differed significantly from population controls, but not between pSS and SLE. pSS and SLE patients were characterized by lower bacterial richness, lower Firmicutes/Bacteroidetes ratio and higher relative abundance of *Bacteroides* species in fecal samples compared with population controls. Oral microbiota composition differed significantly between pSS patients and SLE patients, which could partially be explained by oral dryness in pSS patients.

Conclusions: pSS and SLE patients share similar alterations in gut microbiota composition, distinguishing patients from individuals in the general population, while oral microbiota composition shows disease-specific differences between pSS and SLE patients.

1. Introduction

Primary Sjögren's syndrome (pSS) and systemic lupus erythematosus (SLE) are systemic inflammatory autoimmune diseases that share epidemiological, clinical, pathogenic and etiological features [1,2]. The world-wide prevalence rates for pSS and SLE are 0.01–0.09% and 0.02–0.24%, respectively, with a female:male ratio of 10:1 for both diseases [3–6]. pSS is characterized by chronic inflammation of the exocrine glands, in particular the salivary and lacrimal glands, resulting in oral and ocular dryness (sicca) complaints [7,8]. In SLE, a wide range of symptoms can be present, such as skin rash, photosensitivity, arthritis, glomerulonephritis, pericarditis, neurologic and hematological symptoms [2]. Overlap of clinical symptoms is frequently observed in pSS and SLE patients [7,9,10].

Host genetics and environmental factors are important etiological factors in pSS and SLE. pSS and SLE patients share genetic risk loci which predisposes individuals to these diseases. Genetic risk loci for both pSS and SLE include *STAT4* and *IRF5* (involved in innate immunity), *IL12A* and *BLK* (involved in adaptive immunity) and *HLA class II* region [11–14]. Many more genetic risk factors are currently known for SLE than for pSS [11]. The majority of SLE genetic risk factors are involved in innate immune signaling (e.g. TLR7 and TLR9) and lymphocyte signaling (e.g. IL-10, CD80) [15].

Despite increasing knowledge on genetic risk factors, still relatively little is known about environmental factors involved in the development of pSS and SLE. In this respect, the microbial composition in the gut and oral cavity may be important factors in the etiopathogenesis of these two chronic inflammatory autoimmune diseases [16–20]. Several

* Corresponding author. Department of Oral and Maxillofacial Surgery, University Medical Center Groningen, PO Box 30.001, 9700 RB, Groningen, The Netherlands.

E-mail address: t.a.van.der.meulen@umcg.nl (T.A. van der Meulen).

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recent studies reported differences in gut and oral microbiota of pSS and SLE patients compared with healthy- and symptom-controls [17–19,21–24]. However, it is unknown whether gut and oral microbiota of pSS and SLE patients are truly specific for the disease, as there are no direct comparative studies including both diseases. Therefore, the aims of this study were to identify disease-specific differences in gut and oral microbiota of pSS and SLE patients and to assess whether pSS and SLE patients share overlapping signatures in gut microbiota composition. To address these questions, we performed 16S ribosomal RNA (rRNA) sequencing on fecal, buccal swab and oral washing samples from 39 pSS patients and 30 SLE patients that passed quality control. Fecal samples from 965 individuals from the general population, living in the same geographical area as pSS and SLE patients, were processed using the same pipeline and were used to assess shared signatures in pSS and SLE gut microbiota composition.

2. Patients and methods

2.1. Patients

2.1.1. pSS and SLE patients

This study was approved by the Medical Ethical Committee (METc) of the University Medical Center Groningen (UMCG), Groningen, the Netherlands (METc 2015.472). All patients completed a written informed consent according to the declaration of Helsinki. Clinically diagnosed pSS patients (n = 40) and SLE patients (n = 35), living in one of the northern three provinces of the Netherlands, were consecutively selected for participation during a standard follow-up consultation at the UMCG Sjögren's Expertise Center. The UMCG is a tertiary referral center for Sjögren's syndrome (SS) and SS-associated mucosa associated lymphoid tissue (MALT) tumors. No selection based on race or ethnic background was made. Patients who had undergone abdominal surgery with (partial) bowel resection were not selected for participation.

All pSS patients fulfilled the 2016 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria [25]. All SLE patients fulfilled the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria [26]. SLE patients using immunosuppressive drugs, other than hydroxychloroquine and low dose prednisolone (maximum of 7.5 mg daily), were excluded. Furthermore, SLE disease activity index (SLEDAI) was ≤ 4 , in order to select disease controls with stable disease and low disease-activity. Routinely, when there is a clinical suspicion of secondary SS (e.g. sicca complaints), SLE patients are scheduled for a diagnostic workup in the Sjögren's Expertise Center. This workup includes additional blood serology, functional tests of tear production (Schirmer's test, ocular staining score), saliva production (unstimulated whole saliva) and a salivary gland biopsy. Each patient had to complete the Dutch Healthy Diet Food Frequency Questionnaire (DHD-FFQ) [27], the WHO Oral Health Questionnaire for Adults [28], the Xerostomia Inventory questionnaire [29] and the Rome III Diagnostic Questionnaire on irritable bowel syndrome (IBS) [30]. Demographic, clinical, biochemical and medication data were retrieved from standardized electronic medical records. Unstimulated (UWS) and stimulated (SWS) whole salivary flow rates in mL/min, routinely collected in pSS patients, were collected. Patients were required not to have used systemic antibiotics two months before stool and oral sampling.

2.1.2. General population controls

Population controls (n = 974) consisted of individuals participating in a population cohort study in the northern three provinces of the Netherlands, LifeLines DEEP [31,32]. In order to represent the general population as well as possible, population controls were not matched to pSS or SLE patients. Fecal samples and relevant phenotype data were available for 974 individuals. Individuals reported to use systemic antibiotics, anti-malarial or anti-fungal therapy were excluded (n = 9), leaving 965 population controls for analyses.

2.2. Methods

2.2.1. Fecal and oral sample collection and DNA isolation

Fecal and oral (i.e., buccal swab and oral washing) samples were collected from 40 pSS and 35 SLE patients. The required number of patients to include in this study was based on previous studies on the gut microbiome in rheumatoid arthritis (RA), pSS and SLE [18,21,33]. Fecal samples from the 965 population controls were previously collected (LifeLines DEEP) [31]. All fecal samples, from patients and population controls, were collected at home. pSS and SLE patients collected oral samples in the same week as fecal samples, before breakfast and oral hygiene activities. Directly after collection, samples were frozen by participants (i.e., patients and population controls) and stored in the participants' home freezer. Within two weeks after sampling, a research assistant visited each participant to collect the fecal and oral samples. Samples were transported on dry ice to the hospital and stored at -80°C . DNA isolation on all fecal samples was performed exactly the same, using the AllPrep DNA/RNA Mini kit (Qiagen, Venlo, the Netherlands). DNA isolation on buccal swabs and oral washings was performed with the Ultraclean Microbial DNA isolation kit (MO BIO, Carlsbad, California, USA). See Supplementary methods for details.

2.2.2. 16S rRNA gene sequencing, quality control and taxonomy assignment

Illumina Miseq-v2 paired-end sequencing, covering the V4 variable region (Primers: 515F [GTGCCAGCMGCCGCGGTAA] and 806R [GACTACHVGGGTWCTAAT]), was performed the same on all fecal and oral samples using standardized sequencing techniques (see Supplementary Methods). Paired-end read alignment and quality control on samples from pSS and SLE patients was performed using Quantitative Insights Into Microbial Ecology (QIIME) v1.9.1 and for population controls with custom scripts [34], both with a minimum phred-quality score of 33. Rarefaction was performed at 8000 reads/sample for fecal samples and 5000 reads/sample for oral samples. Samples with lower number of reads/sample than these cut-offs were excluded.

The naïve Bayesian Ribosomal Database Project (RDP) Classifier was used to assign bacterial 16S rRNA sequences to genus level with SILVA128 as reference database [35,36]. Scripts on rarefaction and taxonomy assignment are available on https://github.com/alexa-kur/miQTL_cookbook. After taxonomy assignment, taxa observed only once in a sample and taxa with a relative abundance below 0.01% were removed.

Secondly, the ARB software environment (release 5.5) was used to gain more insight in species distribution [37]. ARB aligns 16S rRNA reads based on nucleotide sequence and secondary 16S rRNA gene structure information. To assign 16S rRNA reads to species level, a less stringent cut-off for read assignment was applied. Therefore, ARB was used as secondary taxonomy assignment method solely and not as primary discovery method.

2.2.3. Statistical analyses

QIIME was used to determine alpha-diversity (i.e., bacterial diversity *within* one sample) and beta-diversity (i.e., diversity in bacterial composition *between* samples). Alpha-diversity was determined as bacterial richness (Chao1) and diversity (Shannon index). Beta-diversity was assessed with Bray-Curtis distance matrix and visualized using principal coordinate analysis (PCoA). *Adonis* function from the *vegan* R-package was used to describe how much of the variation in Bray-Curtis distance could be explained by each phenotype, using 999 permutations and the R^2 -value as explanatory estimate.

R (version 3.3.1) was used for comparative statistics [38]. A Benjamini Hochberg false discovery rate (FDR) corrected p-value (q-value) was calculated for comparative tests. A q-value < 0.05 was used as cut-off for comparative statistical tests. Multivariate Association with Linear Models (MaAsLin, version 0.0.4) was used to find bacterial taxa

Table 1
Characteristics of patients and controls included in gut microbiome analyses^a.

	pSS	SLE	Control	p-value ^b	p-value ^b
	N = 39	N = 30	N = 965	pSS vs SLE	pSS, SLE, Control
Age in years, mean ± SD	55 ± 12	47 ± 14	45 ± 13	0.01	< 0.001
Male sex, n (%)	3 [8]	2 [7]	408 [42]	1	< 0.001
White/European ethnic background	36 (92)	28 (93)	NA		
Born in the Netherlands	NA	NA	929 (96)		
Body-mass index, mean ± SD	25 ± 5	27 ± 5	25 ± 4	0.2	0.2
Smoking, n (%)	4 [10]	9 [30]	194 [20]	0.1	0.1
Disease duration in years, mean ± SD	10 ± 6	11 ± 9	NA	0.6	
fulfilling ACR EULAR 2016 criteria for SS, n (%)	39 (100)	1 [3]	NA		
fulfilling SLICC 2012 criteria for SLE, n (%)	0	30 (100)	NA		
Clinically active SLE (%) ^c	NA	6 [20]	NA		
Serological active SLE (%) ^c	NA	4 [13]	NA		
Symptoms, ever reported					
Arthralgia, n (%)	18 [46]	22 (73)	NA	0.03	
Raynaud, n (%)	17 [44]	14 [47]	NA	0.8	
Renal involvement, n (%)	3 [8]	5 [17]	NA	0.3	
Skin involvement, n (%)	11 [28]	20 [67]	NA	0.002	
Neuropathy, n (%)	4 [10]	3 [10]	NA	1	
Oral ulcers, n (%)	NA	9 [30]	NA		
MALT tumor salivary gland, ever diagnosed, n (%)	8 [21]	NA	NA		
Fibromyalgia, n (%)	0	0	23 [2]		0.4
RA, n (%)	0	0	14 [2]		0.6
Diabetes, n (%)	2 [5]	1 [3]	NA	1	
Serum biochemistry					
ANA positive, n (%)	39 (100)	28 (97)	NA ^e	0.4	
Anti dsDNA positive, n (%)	1 [4]	15 [50]	NA	< 0.001	
ENA positive, n (%)	32 (84)	11 [38]	NA	< 0.001	
Ro/SSA positive, n (%)	35 (90)	11 [38]	NA	< 0.001	
La/SSB positive, n (%)	21 [54]	5 [17]	NA	0.003	
Rheumatoid factor positive, n (%)	28 (74)	6 [29]	NA	0.007	
Lupus Anticoagulant positive, n (%)	NA	7 [25]	NA		
Anti dsDNA at inclusion (IU/mL), mean ± SD	NA	15 ± 24	NA		
C3 low at inclusion (< 0.90 g/L), n (%)	4 [10]	13 [43]	NA	0.002	
C4 low at inclusion (< 0.10 g/L), n (%)	4 [10]	3 [10]	NA	1	
IgG high at inclusion (> 16.0 g/L), n (%)	19 [49]	NA	NA		
Current medication					
Proton pump inhibitors, n (%)	13 [33]	18 [60]	68 [7]	0.03	< 0.001
NSAIDs, n (%)	19 [49]	9 [30]	26 [3]	0.1	< 0.001
Corticosteroids, n (%)	2 [5]	5 [17]	2 (0.2)	0.2	< 0.001
Antimalarial, n (%)	0	25 (83)	NA	< 0.001	
Rituximab	2 [5]	0	NA		
Methotrexate	1 [3]	0	NA		
Patient reported disease severity score for SS					
ESSPRI Total, mean ± SD	6 ± 2	NA	NA		
ESSPRI dryness, mean ± SD	6 ± 2	NA	NA		
ESSPRI fatigue, mean ± SD	6 ± 3	NA	NA		
ESSPRI pain, mean ± SD	5 ± 3	NA	NA		
Salivary secretion rate					
UWS (mL/min), mean ± SD	0.07 ± 0.11	NA	NA		
SWS (mL/min), mean ± SD	0.20 ± 0.22	NA	NA		
Questionnaire results ^d					
Irritable Bowel Syndrome, n (%)	17 [46]	6 [23]	165 [22]	0.1	0.004
DHD-FFQ total score, mean ± SD	56 ± 12	52 ± 10	NA ^f	0.1	
DHD fiber score, mean ± SD	8 ± 2	7 ± 2	NA	0.03	
DHD alcohol score, mean ± SD	9 ± 3	9 ± 2	NA	0.3	

^a pSS, primary Sjögren's syndrome; SLE, systemic lupus erythematosus; Control, population controls; NA, not available; SD, standard deviation; ACR EULAR, American College of Rheumatology European League Against Rheumatism; SLICC, Systemic Lupus International Collaborating Clinics; MALT, mucosa-associated lymphoid tissue; RA, Rheumatoid Arthritis; ANA, antinuclear antibody; dsDNA, double-stranded DNA; ENA, extractable nuclear antigen; Ro/SSA, anti-Ro/Sjögren's Syndrome autoantibody A; La/SSB, anti-La/Sjögren's syndrome autoantibody B; C3/C4, complement C3/C4; IgG, Immunoglobulin G; NSAIDs, non-steroidal anti-inflammatory drugs; ESSPRI, EULAR Sjögren's syndrome patient reported index; UWS/SWS, unstimulated/stimulated whole salivary flow; DHD, Dutch healthy diet; FFQ, food frequency questionnaire.

^b P-values for pSS and SLE statistical comparisons were calculated with the *t*-test or Fisher's exact test. P-values for the overall study population were calculated with the one-way ANOVA or Chi-squared test.

^c Clinically and serological SLE activity was clinically determined by the treating physician.

^d Questionnaires were returned by 37/39 (95%) of pSS patients and 26/30 (87%) of SLE patients.

^e Serum biochemistry was not available for population controls. A previous study showed that serum ANA (titer > 1:160) is positive in 6.1% of the general population and that anti-Ro/SSA, anti-La/SSB and anti-dsDNA is positive in 0.2–0.8% of the general population [66].

^f DHD-FFQ data was not available for population controls.

associated with pSS and SLE [39]. In MaAsLin, the following factors were taken into account as possible confounders in the taxa-phenotype association analyses: age, sex, body-mass index (BMI), smoking, diet (i.e., DHD-FFQ score, only available for pSS and SLE patients), proton-pump inhibitor (PPI)-use and sequencing-depth [40,41]. A q -value < 0.10 was used as significance cut-off for taxa-phenotype associations.

3. Results

3.1. Characteristics of the study population

Demographic and clinical characteristics of the study groups are summarized in Table 1. After quality control, 39 fecal samples from pSS patients, 30 fecal samples from SLE patients and 965 fecal samples from population controls were used in the analyses. SLE patients were slightly younger on average than pSS patients, corresponding to peak incidence ages for these diseases [3,4]. Eight out of 39 pSS patients (21%) had developed a mucosa associated lymphoid tissue (MALT) tumor in the course of the disease. Of the eight pSS patients with a history of MALT, seven patients either finished treatment more than one year before inclusion or were on a wait-and-see policy. Only one pSS patient with a MALT history received maintenance therapy with rituximab during the study. The vast majority of clinical characteristics was similar between pSS patients with and without a history of salivary gland MALT tumor (Supplementary Table 1). No differences were observed between pSS patients with and without a MALT tumor in the disease history, regarding the number of reads per fecal sample and the alpha- and beta-diversity of the gut microbiome.

Three pSS patients used immunosuppressive drugs during the study, of which two rituximab and one methotrexate. None of the pSS patients was treated with antimalarials. This is in accordance with a recent study showing that antimalarials are not effective in reducing symptoms in pSS-patients [42]. Instead, non-steroidal anti-inflammatory drugs (NSAIDs) were prescribed in 49% of pSS-patients, mainly because of arthralgia symptoms. SLE patients were mainly treated with antimalarial therapy (83%). A full history of immunomodulating agents used by pSS and SLE patients, before inclusion in the study, can be found in Supplementary Table 2. PPIs were prescribed more often in SLE-patients (60%) than in pSS patients (33%) and in population controls (7%). To date, no studies are available that show a relation between antimalarial therapy and changes in the gut or oral microbiome. However, PPI-use can affect the gut microbiome [41] and was therefore included as possible confounder in our multivariate statistical model testing for disease-microbiota associations [39,41].

3.2. Alpha- and beta-diversity of the gut microbiome

Alpha-diversity measures of richness and diversity in fecal samples did not significantly differ between pSS and SLE ($q = 0.35$ and $q = 0.93$, respectively; Fig. 1A). Richness was significantly lower in samples from pSS and SLE patients compared with those from population controls (both $q = 0.0004$), but the within-sample diversity did not differ between pSS, SLE and population controls (both $q = 0.7$). Principal coordinate analysis (PCoA) of fecal samples from pSS and SLE patients showed no disease-specific clustering pattern (Fig. 1B). Disease phenotype (i.e., presence of pSS or SLE) did not significantly contribute to the variation in fecal bacterial composition between individuals (*adonis*, $R^2 = 0.026$, $p = 0.07$). In the PCoA including 965 population controls disease phenotype (i.e., presence of pSS, SLE or population control) explained 1.7% of the variation in fecal bacterial composition between individuals (*adonis*, $q < 0.01$; Fig. 1C). Other factors that were shown to contribute to the variation in fecal bacterial composition in the total study population were: age (explaining 1.6%, $q = 0.01$), sex (explaining 0.7%, $q = 0.01$), BMI (explaining 0.5%, $q = 0.01$), smoking (explaining 0.4%, $q = 0.02$), PPI-use (explaining 0.6%, $q < 0.01$) and NSAID-use (explaining 0.2%, $q = 0.03$) as analyzed by *adonis*. The

overall gut microbiota composition of women from the general population was slightly more similar to that of pSS and SLE patients than that of men from the general population (Supplementary Fig. 1). This suggests that a small proportion of the difference in overall gut microbiota composition between pSS/SLE patients and population controls might be related to the higher percentage of males in the population control group (Table 1). In contrast, the gut microbiota composition of population controls with higher age, was slightly less similar to that of pSS and SLE patients (Supplementary Fig. 2). This indicates that the higher average age of pSS patients compared with the average age of population controls (Table 1) did not contribute to the observed difference in overall gut microbiota composition between pSS patients and population controls.

3.3. Individual gut bacteria associated with pSS and SLE compared to population controls

3.3.1. Lower Firmicutes/Bacteroidetes ratio in pSS and SLE patients

Profiles of the average bacterial composition at phylum and genus level in fecal samples were more similar between pSS and SLE than between patients and population controls (Fig. 2A). A lower ratio between phyla Firmicutes and Bacteroidetes has previously been observed in SLE patients compared to control subjects [21,22,43]. We also observed a lower Firmicutes/Bacteroidetes ratio in fecal samples from SLE patients compared to population controls ($q < 0.001$), but found a similarly lower Firmicutes/Bacteroidetes ratio in pSS patients compared with population controls ($q < 0.001$; Fig. 2B).

3.3.2. Individual bacterial taxa associated with pSS and SLE

Next, we assessed whether disease phenotype was associated with individual bacterial taxa ($n = 152$) using MaAsLin. The majority of genera associated with each disease, was shared between pSS and SLE patients compared with population controls (Fig. 2C, Supplementary Tables 3–5). Six genera showed significantly higher and twelve lower relative abundance in both pSS and SLE patients compared with population controls ($q < 0.10$, Fig. 2D). Although pSS and SLE were also associated with different taxa, none of these associations were significant when pSS and SLE patients were compared with each other ($q > 0.10$, Supplementary Table 6). Thus, difference in relative abundance of individual bacteria was much larger between pSS and SLE compared to population controls than the variation between the two diseases.

3.3.3. High relative abundance of *Bacteroides* species is characteristic for pSS and SLE, but not associated with Ro60/SSA-status

Phylum Bacteroidetes and genus *Bacteroides* relative abundance were most evidently higher in pSS and SLE patients compared with population controls (Fig. 2D). Also, genus *Alistipes* (belonging to Bacteroidetes) and phylum Proteobacteria showed evidently higher relative abundance in pSS and SLE patients compared with population controls. We subsequently analyzed which *Bacteroides* species were responsible for the higher relative abundance of genus *Bacteroides* in pSS and SLE patients, using the ARB software package [37]. The relative abundance of *Bacteroides vulgatus*, *Bacteroides uniformis* and *Bacteroides ovatus* was significantly higher in both pSS and SLE patients than in population controls (Wilcoxon, $p < 0.01$, $q < 0.05$; Fig. 2E; Supplementary Table 7). *Bacteroides thetaiotaomicron* (*B. theta*) was significantly higher in SLE patients, but not in pSS patients compared to population controls (Wilcoxon, $q = 0.03$ and $q = 0.17$, respectively). Recently, cross-reactivity has been suggested between the gut commensal *B. theta* and the Ro60-protein [43]. However, we did not observe a different relative abundance of *B. theta* in anti-Ro/SSA-positive pSS/SLE patients ($n = 46$) than in anti-Ro/SSA-negative patients ($n = 22$) (Supplementary Fig. 3). Neither did we observe associations between any other bacterial taxa and serum anti-Ro/SSA-autoantibody presence using the MaAsLin framework (Supplementary Table 8). Also,

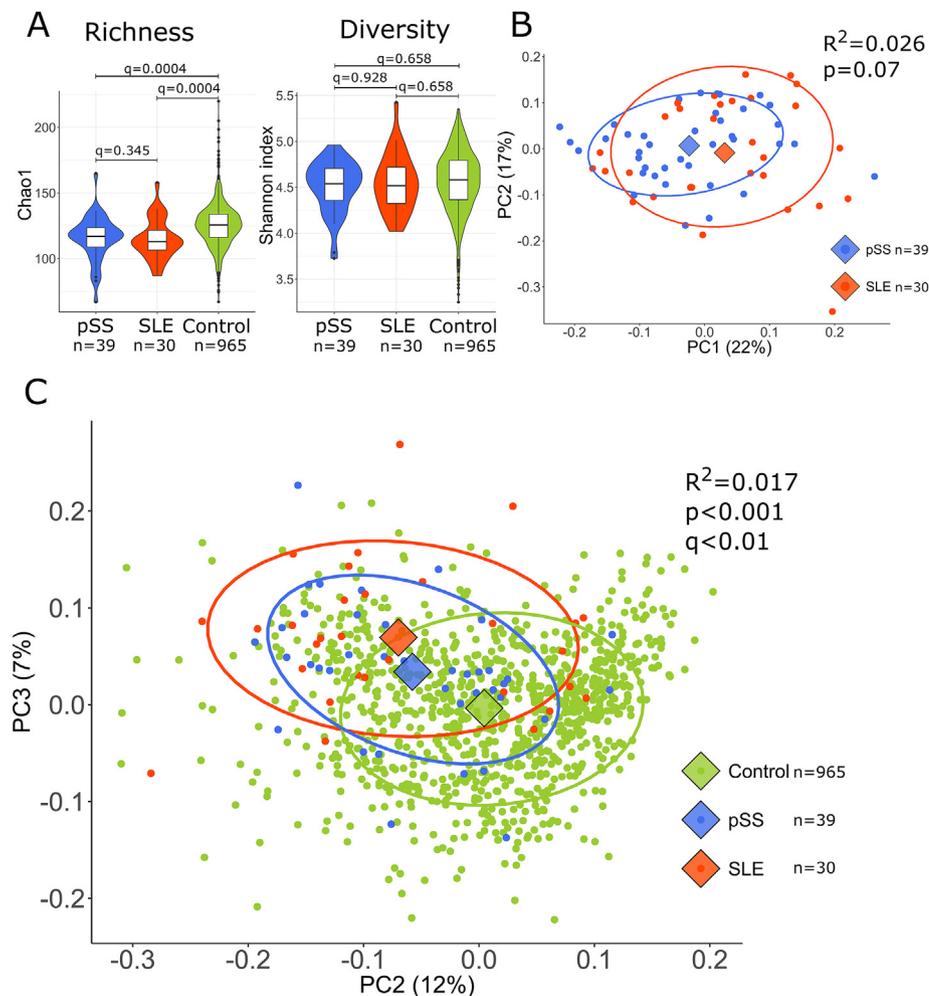


Fig. 1. Significantly lower richness and different bacterial composition in fecal samples from pSS and SLE patients compared with fecal samples from population controls (Control). Q-values represent Benjamini-Hochberg false discovery rate (FDR) corrected p-values. (A) Alpha-diversity measures of richness (Chao1) and diversity (Shannon index) in fecal samples. (B and C) PCoA based on Bray-Curtis distance of fecal bacterial composition in pSS and SLE patients compared to population controls the fecal bacterial composition differs significantly between the three study groups (C). Squares represent centroids per group and ellipses the 75% confidence interval of the centroid.

anti-dsDNA and rheumatoid factor (RF) positivity was not associated with individual bacterial taxa ($q > 0.10$), but anti-La/SSB-autoantibody positivity in pSS/SLE patients was associated with higher relative abundance of genus *Clostridium sensu stricto* ($q = 0.05$) (Supplementary Tables 9–11). However, this genus showed significantly lower relative abundance in pSS and SLE patients than in population controls (Fig. 2D), suggesting that higher *Clostridium sensu stricto* relative abundance cannot directly be linked to positive anti-La/SSB autoantibody status.

3.4. Oral microbiota composition differs between pSS and SLE patients

3.4.1. Significant differences in alpha- and beta-diversity

Study population characteristics of pSS and SLE patients used for oral microbiota analyses are summarized in Table 2. Buccal swab samples from 33 pSS patients and 34 SLE patients and oral washings from 34 pSS patients and 34 SLE patients passed quality control and were used in statistical analyses. Richness and diversity were significantly higher in buccal swabs and oral washings from SLE patients than in the same samples from pSS patients ($q < 0.05$, Fig. 3A). The bacterial composition in buccal swabs, oral washings and merged oral samples differed significantly between pSS and SLE patients ($p < 0.01$, *adonis*; Fig. 3B and C). Disease phenotype explained 7.8–8.8% of the variation in oral microbiota composition. Other factors significantly contributing to the variation in overall oral microbiota composition (i.e., in merged oral samples) were: number of own teeth (14.8%, $q = 0.002$), self-reported condition of gums (15.8%, $q = 0.01$), Xerostomia Inventory-score (9.8%, $q = 0.002$) and severity of oral dryness

sensation (11.3%, $q = 0.049$, *adonis*; Supplementary Fig. 4). These results indicate that oral dryness contributes more to oral microbiota composition than disease phenotype, which corresponds with our previous findings [23,24]. Smoking was not a factor affecting the overall oral microbiota composition in pSS and SLE patients ($R^2 = 0.016$, $p = 0.4$, *adonis*).

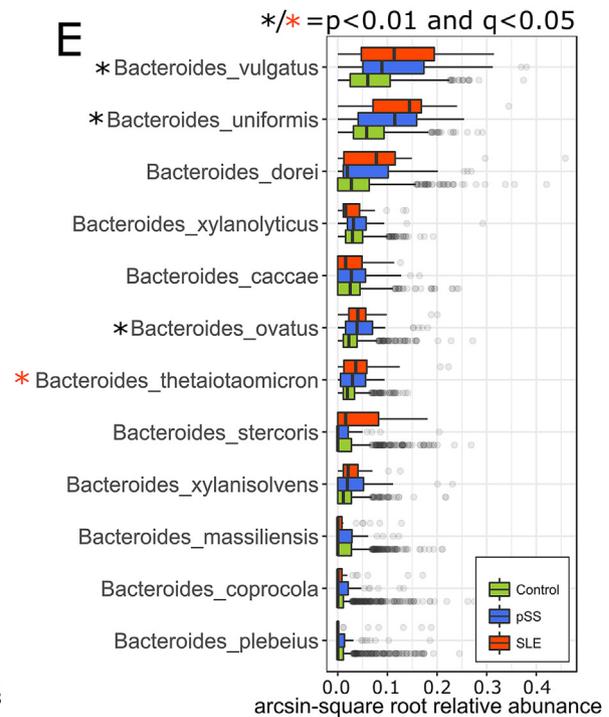
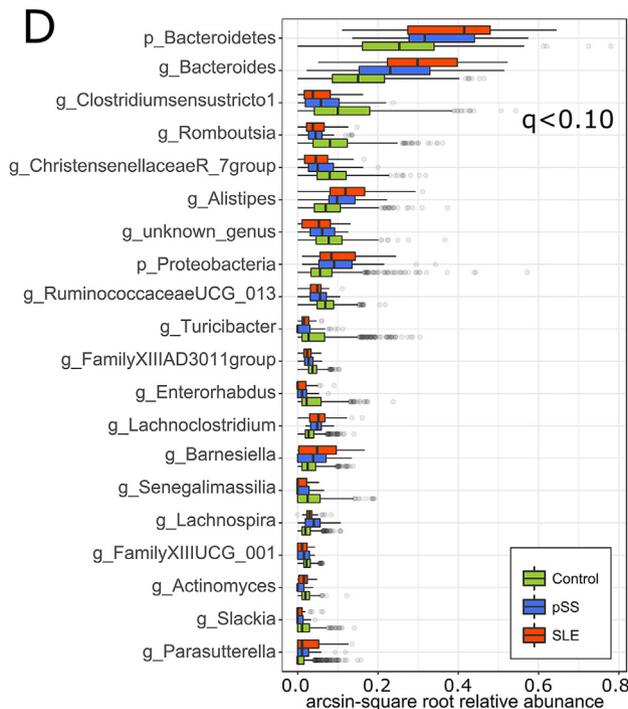
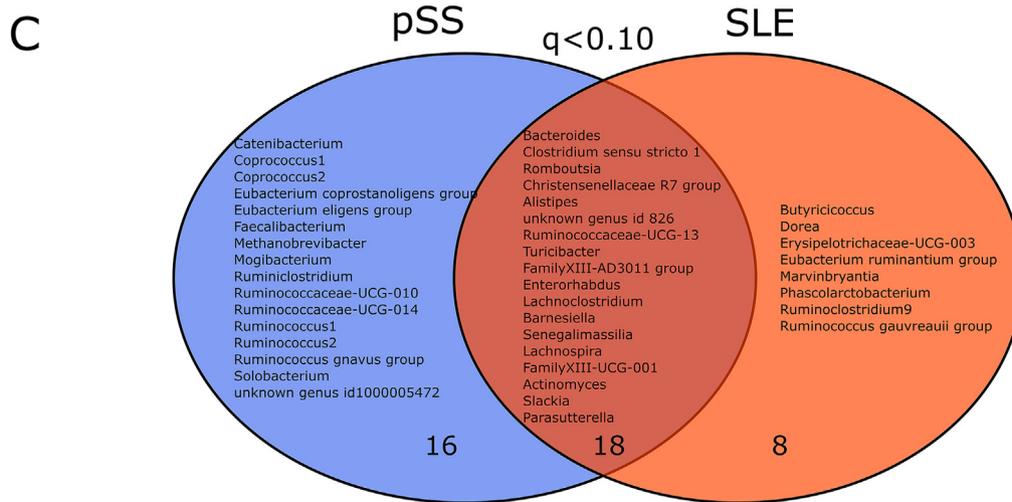
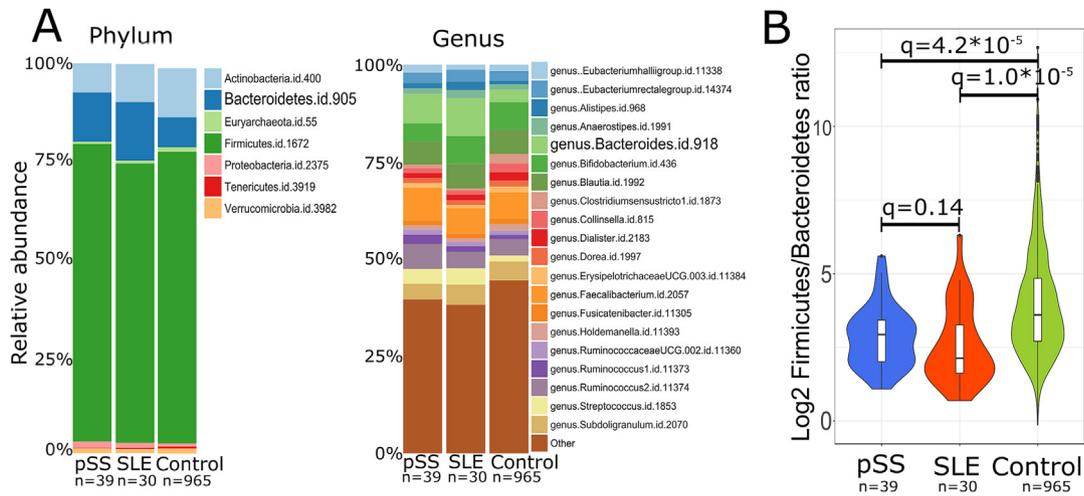
3.4.2. No individual taxa associated with disease-phenotype or Ro60/SSA-status

Profiles of the average bacterial composition at phylum and genus level in buccal swabs and oral washings showed disease specific patterns (Fig. 3D). However, no individual bacterial taxon in buccal swabs or oral washings was significantly associated with pSS or SLE (MaAsLin, $q > 0.1$). When all samples (i.e., one buccal swab and one oral washing from each patient) were included in the analysis, 36 taxa showed statistically significant differences in relative abundance ($q < 0.10$, see Supplementary Table 12).

Previously, epitope mimicry between the oral species *Capnocytophaga ochracea* and the Ro60/SSA protein has been reported [16]. However, we did not observe a difference in relative abundance of genus *Capnocytophaga* in anti-Ro/SSA-positive patients (pSS and SLE patients together) compared with anti-Ro/SSA-negative patients (Supplementary Fig. 5). Neither did we observe associations with other taxa and anti-Ro/SSA-positive pSS/SLE patients in MaAsLin.

3.5. Connection between oral and gut microbiota

Recent clinical and experimental studies have suggested that



(caption on next page)

Fig. 2. Taxonomic differences in gut microbiota composition between pSS patients, SLE patients and population controls. (A) Profiles of the average relative abundance per group at phylum and genus level. (B) Log 2 Firmicutes/Bacteroidetes ratio was significantly lower in pSS and SLE patients compared to population controls, but similar between diseases (Wilcoxon). (C) Venn diagram of genera significantly associated with pSS and SLE compared to population controls using MaAsLin ($q < 0.10$). (D) Phyla (p_) and genera (g_) significantly associated with both pSS and SLE compared to population controls (MaAsLin, $q < 0.10$). (E) The top 12 *Bacteroides* species with a relative abundance $> 0.10\%$ observed in fecal samples from patients and population controls. Black asterisks indicate significant results for both pSS and SLE versus Control, red asterisk indicates significant for SLE vs Control (Wilcoxon test, $p < 0.01$ and $q < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Characteristics of pSS and SLE patients included in oral microbiome analyses^a.

	pSS	SLE	p-value ^b
	N = 34	N = 34	
Disease duration, mean \pm sd	10 \pm 7	10 \pm 9	1
Age, mean \pm sd	54 \pm 13	48 \pm 14	0.07
Male sex, n (%)	2 [6]	4 [12]	0.7
Smoking, n (%)	3 [9]	9 [27]	0.1
SLE oral ulcers, n (%)	NA	9 [27]	
UWS mL/min, mean \pm sd	0.08 \pm 0.11	NA	
SWS mL/min, mean \pm sd	0.20 \pm 0.21	NA	
Questionnaire results			
Total Xerostomia Inventory score, mean \pm sd ^c	38 \pm 9	23 \pm 8	< 0.001
<i>Xerostomia Inventory questionnaire: feeling dry mouth</i>			
Always, n (%)	12 [36]	2 [7]	< 0.001
Very often, n (%)	7 [21]	3 [10]	
Often, n (%)	11 [33]	9 [29]	
Rarely, n (%)	3 [9]	10 [33]	
Never, n (%)	0	7 [23]	
<i>Dentition/number of own teeth</i>			
Complete, n (%)	18 [55]	21 [66]	0.4
10 to 19, n (%)	2 [6]	3 [9]	
1 to 9, n (%)	5 [15]	1 [3]	
Edentulous, n (%)	8 [24]	7 [22]	
<i>Self-reported condition teeth</i>			
Excellent, n (%)	1 [4]	5 [20]	0.02
Very good, n (%)	1 [4]	5 [20]	
Good, n (%)	10 [39]	11 [44]	
Moderate, n (%)	12 [46]	3 [12]	
Bad, n (%)	1 [4]	0	
Very bad, n (%)	1 [4]	1 [4]	
<i>Self-reported condition gums</i>			
Excellent, n (%)	1 [3]	7 [23]	0.06
Very good, n (%)	2 [6]	4 [13]	
Good, n (%)	17 [52]	11 [36]	
Moderate, n (%)	11 [33]	5 [16]	
Bad, n (%)	1 [3]	3 [10]	
Very bad, n (%)	1 [3]	1 [3]	

^a pSS, primary Sjögren's syndrome; SLE, systemic lupus erythematosus; UWS/SWS, unstimulated/stimulated whole salivary secretion rate.

^b P-values were calculated using either the Fisher's exact test or *t*-test.

^c Total xerostomia inventory score in a normal (elderly) population is estimated at 20 [67].

increased relative abundance of oral bacteria in the gut may induce intestinal inflammation [34,44]. Local intestinal inflammation may subsequently predispose individuals to systemic inflammatory disorders, such as pSS and SLE [45,46]. Therefore, we assessed whether genera observed in oral samples from pSS and SLE patients were more prevalent and/or abundant in fecal samples from pSS and SLE patients than in fecal samples from population controls. The cumulative relative abundance of oral genera did not differ in fecal samples from pSS patients, SLE patients or population controls (Fig. 4A). Furthermore, none of the individual oral genera was more prevalent or abundant in fecal samples from pSS or SLE patients than in samples from population controls (Fig. 4B and C). However, *Actinomyces* presence and relative abundance was significantly lower in fecal samples from pSS patients than in those from population controls ($q = 2.2 \cdot 10^{-8}$ and $q = 4.1 \cdot 10^{-8}$, respectively). The relative abundance of *Actinomyces* and *Lactobacillus* in oral samples significantly correlated with their relative abundance in fecal samples ($q < 0.05$, Kendall's rank

correlation, $\tau = 0.35$ and $\tau = 0.24$, respectively) (Fig. 4D). Also, fecal *Actinomyces* relative abundance correlated negatively with oral *Lactobacillus* relative abundance ($q < 0.05$, $\tau = -0.31$). Furthermore, we observed higher *Actinomyces* relative abundance in both oral and fecal samples from SLE patients compared to samples from pSS patients (Fig. 4E). The difference in *Lactobacillus* relative abundance between diseases was significant in oral samples, but not in fecal samples. Together, these results suggest that specific oral microbiota can affect the gut microbiota composition.

4. Discussion

Alterations in gut and oral microbiota composition have previously been suggested as possible environmental factors in the etiology of pSS and SLE [16–19,21,43]. However, until now, it was unknown whether the gut and oral microbiome are specific for pSS and SLE or that these diseases share common characteristics in microbiota composition. Here we show that gut microbiota composition of pSS and SLE patients are very similar to each other but differ significantly from individuals in the general population. In contrast, oral microbiota composition differs between pSS and SLE patients.

Living-area, ethnic background, sex, age, technical variations, diet and medication use can influence the outcome of gut microbiota analyses [40,47–51]. Furthermore, ethnicity and living-area also influence the phenotypic expression of pSS and SLE patients [52–54]. In this study, we included pSS and SLE patients from the same geographical area and with a similar ethnic background as population controls. We showed that the strong female predisposition in pSS and SLE patients may have explained a small proportion of the difference in overall gut microbiota composition between pSS/SLE patients and population controls. However, the effect of age on the overall gut microbiome composition in the total study population was smaller and even opposed to the age-difference observed between pSS patients and population controls. Methods for fecal sampling, sequencing and taxonomy assignment were the same for pSS/SLE patients and population controls. Dutch-Healthy-Diet Food Frequency Questionnaire (DHD-FFQ)-scores were not available for our general population cohort, but another Dutch population based study (i.e., Nutrition Questionnaires plus study, including randomly selected individuals from the central part of the Netherlands, aged 20–70 years, $n = 1235$) showed very similar DHD-FFQ scores as in our pSS and SLE patient cohort [27].

None of the patients or population controls received antibiotic treatment at time of sampling. As expected, medication use differed between pSS/SLE patients and population controls. Also, the difference in use of antimalarials between pSS and SLE is not surprising, since antimalarial therapy has not been proven effective in pSS, but is a standard treatment for SLE [42,55]. Chloroquine and hydroxychloroquine have been reported to inhibit intracellular growth of bacteria in vitro, but these drugs do not seem to have any antibacterial activity or effect on extracellular bacterial growth [56,57]. Despite the difference in use of antimalarial therapy, no significant differences were observed in gut microbiota composition between pSS and SLE patients. This may indicate that antimalarial therapy has no significant effect on the gut microbiota composition. Only three pSS patients received treatment with immunomodulating agents during the study, of which two rituximab and one methotrexate. No evidence is available that rituximab has an effect on the oral or gut microbiome. No significant

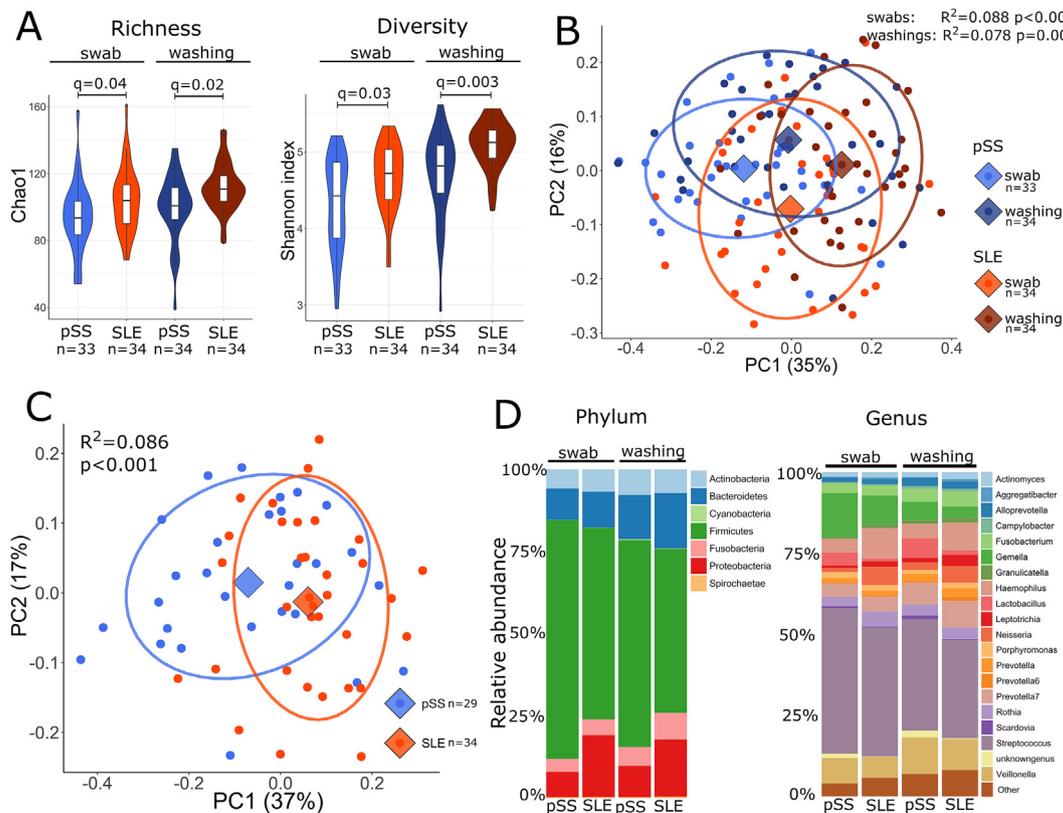


Fig. 3. Oral microbiota composition differs significantly in buccal swabs and oral washings between pSS and SLE patients. (A) Significantly lower richness (Chao1) and diversity (Shannon index) in buccal swab and oral washing samples from pSS patients compared with SLE patients (Wilcoxon, q-value = FDR corrected p-value). (B) Bray-Curtis based PCoA on buccal swabs and oral washings shows significant difference in composition between pSS and SLE patients. Squares represent centroids per group and sample type, ellipses correspond with the 75% confidence interval of the centroid. (C) Disease phenotype explains 8.6% of the variation in overall bacterial composition between pSS and SLE. (D) Profiles of the average relative abundance at phylum and genus level per group show disease specific differences in buccal swabs and oral washings.

associations were found between gut microbiota and methotrexate use in a recent study in the British TwinsUK cohort (a nation-wide registry of volunteer twins, $n = 2737$, 89% female, mean age 60 ± 12 SD, mean BMI 26 ± 5 SD) [49]. Regarding the use of NSAIDs, we found that NSAID use explained 0.2% of the overall gut microbiota composition in the total study population (i.e., patients and population controls, $n = 1034$), suggesting that NSAID use has a very small effect on the overall gut microbiota composition. In both the TwinsUK cohort (testing for topical NSAID use) [49] as well as in the LifeLines DEEP cohort ($n = 1135$, 58% female, mean age 45 range 18–81, mean BMI 25 range 17–49, testing for oral NSAID use) [40], no significant effect of NSAIDs on the gut microbiome was observed. Also, oral corticosteroid use did not significantly affect the gut microbiome in these two large studies [40,49]. Thus, current evidence indicates that the effect of NSAID and corticosteroid use on the gut microbiome in humans is absent or very small. In contrast, PPI-use showed a stronger effect on the gut microbiome in both the LifeLines DEEP and TwinsUK cohort studies, and explained 0.6% of the overall gut microbiome composition in our study. PPI use has been associated with an increased relative abundance of oral microbiota in the gut microbiota composition [41]. Although more pSS and SLE patients used PPIs than population controls, pSS/SLE patients did not have a higher cumulative relative abundance of oral microbiota in the gut.

Thus, many possible confounding factors were considered in our study and the most important factors (i.e., sex, age, smoking, BMI, sequencing depth, PPI use) were taken into account in the multivariate statistical framework MaAsLin [39]. Therefore, the results of this study implicate that the observed differences in fecal microbiota composition between pSS/SLE patients and population controls are related to

biological variations rather than geographical, ethnic, sex, age, technical, dietary or medication differences.

Lower richness in gut microbiota composition was present in pSS and SLE patients compared to population controls. Individuals with low richness in gut microbiota have higher inflammatory markers in blood (i.e., number of leucocytes and high-sensitivity C reactive protein) than in individuals with high richness [58]. Furthermore, in pSS patients, elevated levels of fecal calprotectin are observed, which suggests that (low grade) intestinal inflammation may be present in these patients [45]. Together, these results suggest a connection between lower gut microbial richness and local and systemic inflammation. However, the sequence of events remains to be further investigated.

A lower Firmicutes/Bacteroidetes ratio has been observed before in SLE patients, but not in spondyloarthritis or RA patients [33,59,60]. Therefore, our results suggest that low Firmicutes/Bacteroidetes ratio is shared between two diseases with significant overlap in pathogenesis (viz. pSS and SLE), but not with systemic rheumatic diseases in general.

Bacteroides species are commensal gut bacteria and are well-known for their glycan degrading ability and short-chain fatty acid production [61,62]. These metabolic processes are considered beneficial to the host and for this reason high *Bacteroides* relative abundance is not directly associated with dysbiosis of the gut microbiome. However, recently the species *Bacteroides thetaiotaomicron* (*B. theta*) has been reported as a potential gut pathobiont. Greiling et al. showed that lysates of *B. theta* can bind to serum from anti-Ro60-positive patients [43]. Moreover, B and T cell responses to the Ro60-protein occurred after monoclonization of mice with *B. theta* and this monoclonization lead to enhanced lupus-like disease [43]. We did not observe an association between anti-Ro/SSA-positivity in patients and *B. theta* relative

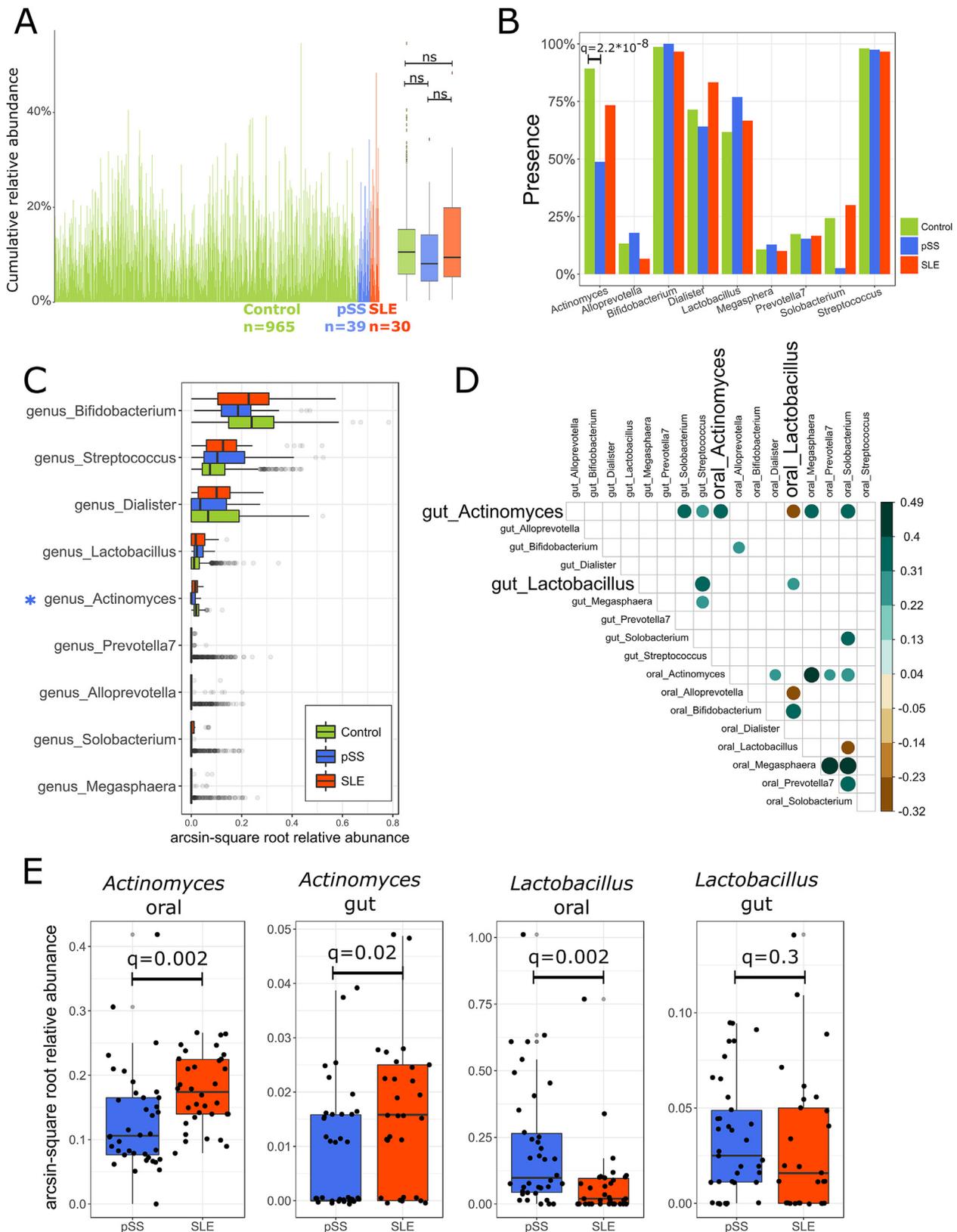


Fig. 4. Connection between oral genera observed in fecal samples. (A) Cumulative relative abundance of nine oral genera observed in fecal samples. Each line is one study subject. Boxplots summarize the cumulative relative abundance per group and correspond with the y-axis (Wilcoxon). (B) Presence of oral genera in fecal samples. Absence was defined as relative abundance $< 0.0125\%$. Fisher's exact tests were performed on genera with an overall presence $> 25\%$. (C) Relative abundance of oral genera in fecal samples. Blue asterisk indicates $q = 4.1 \times 10^{-8}$ for pSS vs population control (Wilcoxon). (D) Correlations between genera observed in oral and fecal samples. Relative abundances of buccal swab and oral washing samples were merged per individual. The color and size of the dots correspond with the strength of the correlation (Kendall's tau, $q < 0.05$). (E) Differences in *Actinomyces* and *Lactobacillus* relative abundance in oral and fecal samples from pSS and SLE patients (Wilcoxon). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

abundance in fecal samples, similar to the results reported by Greiling et al. [43]. Possibly, the low relative abundance of *B. theta* in fecal samples (< 1%) requires larger numbers of anti-Ro60/SSA-positive and -negative patients to detect a significant difference between these groups. To overcome this problem, quantitative microbiome profiling, in contrast to relative microbiome profiling, should be applied in future studies assessing the possible connections between *B. theta*, SLE and anti-Ro60/SSA autoantibodies [63]. Interestingly, *B. theta* relative abundance was significantly higher in SLE patients than in population controls. Thus *B. theta* may also be involved in SLE, independent of anti-Ro60/SSA autoantibody status.

The differences in oral microbiota composition between pSS and SLE patients are similar to what we previously observed between pSS patients and healthy controls, but also show new insights [23,24]. Previously, we did not observe differences in alpha-diversity between pSS patients and healthy controls in buccal swabs or oral washings, which suggests that the alpha-diversity of the oral microbiome in pSS patients is relatively normal [23,24]. Here we show that SLE is associated with a higher alpha-diversity in both buccal swabs and oral washings than in pSS patients. Higher alpha-diversity of the subgingival microbiome has previously been associated with periodontitis [64]. Furthermore, periodontitis prevalence is higher in SLE patients than in controls, while pSS patients do not have a higher risk of periodontitis [17,65]. Thus, although we did not clinically assess patients for periodontitis, higher alpha-diversity in SLE patients might be explained by a higher periodontitis prevalence in the SLE group.

In this study we confirm our previous observation that oral dryness explains more of the variation in oral microbiota composition than underlying disease [23,24]. Additionally, we show that self-reported number of own teeth and condition of gums explain large proportions (15% and 16%, respectively) of overall oral microbiota composition. This indicates that questionnaires on oral health can provide valuable information relevant to oral microbiome analyses.

The unique simultaneous sampling of the oral cavity and the gut allowed us to find correlations between oral and gut relative abundances of *Actinomyces* and *Lactobacillus*. This indicates that the oral microbiota composition influences that of the gut, supporting the idea that the oral cavity can serve as reservoir for potential pathobionts involved in intestinal inflammation [44]. Bacterial species- and strain-level identification is needed to further assess whether this oral-gut microbiota connection plays a role in intestinal dysbiosis and inflammation.

A limitation of this study is that the results were not replicated in an independent cohort, but our results confirm previously reported alterations in gut microbiota composition in SLE patients compared to (healthy) controls [21,22]. Furthermore, by including a large population control group, instead of healthy controls, we reduced the risk of introducing selection bias in assessing whether the gut microbiome is truly associated with a disease-phenotype.

The results of our study provide an important and solid basis for future studies on the role of the gut and oral microbiome in pSS and SLE. Elucidating the cause and effect relationship between the gut microbiome and pSS/SLE should be the main focus of future studies investigating the etiopathogenesis of pSS and SLE.

5. Conclusion

In conclusion, we show that pSS and SLE patients share a very similar gut microbiota composition that distinguishes patients from a large group of controls from the general population. The main characteristics of the gut microbiota composition in pSS and SLE patients are lower bacterial richness, lower Firmicutes/Bacteroidetes ratio and higher relative abundance of *Bacteroides* species. In contrast to the gut microbiota, the oral microbiota composition in pSS and SLE patients is determined by disease-related changes in the oral environment.

Conflicts of interest

No financial support or other benefits from commercial sources were received for this study. None of the authors have any financial interest that could potentially form a conflict of interest with regard to the work presented.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jaut.2018.10.009>.

References

- [1] M. Wahren-Herlenius, T. Dörner, Immunopathogenic mechanisms of systemic autoimmune disease, *Lancet* 382 (2013) 819–831.
- [2] F. Goldblatt, S.G. O'Neill, Clinical aspects of autoimmune rheumatic diseases, *Lancet* 382 (2013) 797–808.
- [3] F. Rees, M. Doherty, M.J. Grainge, P. Lanyon, W. Zhang, The worldwide incidence and prevalence of systemic lupus erythematosus: a systematic review of epidemiological studies, *Rheumatology (Oxford)* 56 (2017) 1945–1961.
- [4] B. Qin, J. Wang, Z. Yang, M. Yang, N. Ma, F. Huang, et al., Epidemiology of primary Sjögren's syndrome: a systematic review and meta-analysis, *Ann. Rheum. Dis.* 74 (2014) 1983–9.
- [5] C. Maldini, R. Seror, O. Fain, R. Dhote, Z. Amoura, M. De Bandt, et al., Epidemiology of primary Sjögren's syndrome in a French multiracial/multiethnic area, *Arthritis Care Res (Hoboken)* 66 (2014) 454–463.
- [6] I. Gergianaki, A. Fanouriakis, A. Repa, M. Tzanakakis, C. Adamichou, A. Pompieri, et al., Epidemiology and burden of systemic lupus erythematosus in a Southern European population: data from the community-based lupus registry of Crete, Greece, *Ann. Rheum. Dis.* 76 (2017) 1992–2000.
- [7] X. Mariette, L.A. Criswell, Primary Sjögren's syndrome, *N. Engl. J. Med.* 378 (2018) 931–939.
- [8] M. Ramos-Casals, R. Solans, J. Rosas, M.T. Camps, A. Gil, J. Del Pino-Montes, et al., Primary Sjögren syndrome in Spain: clinical and immunologic expression in 1010 patients, *Medicine (Baltim.)* 87 (2008) 210–219.
- [9] I. Gilboe, T.K. Kvien, T. Uhlig, G. Husby, Sicca symptoms and secondary Sjögren's syndrome in systemic lupus erythematosus: comparison with rheumatoid arthritis and correlation with disease variables, *Ann. Rheum. Dis.* 60 (2001) 1103–1109.
- [10] M. Ramos-Casals, P. Brito-Zerón, R. Seror, H. Bootsma, S.J. Bowman, T. Dörner, et al., Characterization of systemic disease in primary Sjögren's syndrome: EULAR-SS Task Force recommendations for articular, cutaneous, pulmonary and renal involvements, *Rheumatology* 54 (2015) 2230–2238.
- [11] M. Teruel, M.E. Alarcón-Riquelme, Genetics of systemic lupus erythematosus and Sjögren's syndrome: an update, *Curr. Opin. Rheumatol.* 28 (2016) 506–514.
- [12] C.J. Lessard, H. Li, I. Adrianto, J. A. Ice, A. Rasmussen, K.M. Grundahl, et al., Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjögren's syndrome, *Nat. Genet.* 45 (2013) 1284–1292.
- [13] K.E. Taylor, Q. Wong, D.M. Levine, C. McHugh, C. Laurie, K. Doheny, et al., Genome-wide association analysis reveals genetic heterogeneity of Sjögren's syndrome according to ancestry, *Arthritis Rheum.* 69 (2017) 1294–1305.
- [14] Y. Li, K. Zhang, H. Chen, F. Sun, J. Xu, Z. Wu, et al., A genome-wide association study in Han Chinese identifies a susceptibility locus for primary Sjögren's syndrome at 7q11.23, *Nat. Genet.* 45 (2013) 1361–1365.
- [15] C. Mohan, C. Putterman, Genetics and pathogenesis of systemic lupus erythematosus and lupus nephritis, *Nat. Rev. Nephrol.* 11 (2015) 329–341.
- [16] A. Szymula, J. Rosenthal, B.M. Szczerba, H. Bagavant, S.M. Fu, U.S. Deshmukh, T cell epitope mimicry between Sjögren's syndrome Antigen A (SSA)/Ro60 and oral, gut, skin and vaginal bacteria, *Clin. Immunol.* 152 (2014) 1–9.
- [17] J.D. Corrêa, D.C. Calderaro, G.A. Ferreira, S.M.S. Mendonça, G.R. Fernandes, E. Xiao, et al., Subgingival microbiota dysbiosis in systemic lupus erythematosus: association with periodontal status, *Microbiome* 5 (2017) 34.
- [18] T. Mandl, J. Marsal, P. Olsson, B. Ohlsson, K. Andréasson, Severe intestinal dysbiosis is prevalent in primary Sjögren's syndrome and is associated with systemic disease activity, *Arthritis Res. Ther.* 19 (2017) 237.
- [19] C.S. de Paiva, D.B. Jones, M.E. Stern, F. Bian, Q.L. Moore, S. Corbiere, et al., Altered mucosal microbiome diversity and disease severity in sjögren syndrome, *Sci. Rep.* 6

- (2016) 23561.
- [20] B. Chen, L. Sun, X. Zhang, Integration of microbiome and epigenome to decipher the pathogenesis of autoimmune diseases, *J. Autoimmun.* 83 (2017) 31–42.
- [21] A. Hevia, C. Milani, P. López, A. Cuervo, S. Arboleya, S. Duranti, et al., Intestinal dysbiosis associated with systemic lupus erythematosus, *mBio* 5 (2014) e01548-14.
- [22] Z. He, T. Shao, H. Li, Z. Xie, C. Wen, Alterations of the gut microbiome in Chinese patients with systemic lupus erythematosus, *Gut Pathog.* 8 (2016) 64.
- [23] T.A. van der Meulen, H.J.M. Harmsen, H. Bootsma, S.C. Liefers, A. Vich Vila, A. Zernakova, et al., Reduced salivary secretion contributes more to changes in the oral microbiome of patients with primary Sjögren's syndrome than underlying disease, *Ann. Rheum. Dis.* 77 (2018) 1542–1544.
- [24] T.A. van der Meulen, H.J.M. Harmsen, H. Bootsma, S.C. Liefers, A. Vich Vila, A. Zernakova, et al., Dysbiosis of the buccal mucosa microbiome in primary Sjögren's syndrome patients, *Rheumatology* (2018), <https://doi.org/10.1093/rheumatology/key215> [Epub ahead of print].
- [25] C.H. Shiboski, S.C. Shiboski, R. Seror, L.A. Criswell, M. Labetoulle, T.M. Lietman, et al., American College of rheumatology/European League against rheumatism classification criteria for primary Sjögren's syndrome, *Ann. Rheum. Dis.* 76 (2017) 9–16.
- [26] M. Petri, A.M. Orbai, G.S. Alarcón, C. Gordon, J.T. Merrill, P.R. Fortin, et al., Derivation and validation of the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus, *Arthritis Rheum.* 64 (2012) 2677–2686.
- [27] L. Van Lee, E.J.M. Feskens, S. Meijboom, E.J.C. Hooft Van Huysduynen, P. Van'T Veer, J.H.M. De Vries, et al., Evaluation of a screener to assess diet quality in The Netherlands, *Br. J. Nutr.* 115 (2015) 517–526.
- [28] P.E. Petersen, J.B. Ramon, Oral health questionnaire for Adults [internet]. Fifth. World-Health-Organization, Oral Health Surveys: Basic Methods, WHO, 2013, pp. 111–115.
- [29] G.J. van der Putten, H.S. Brand, J.M.G. Schols, C. de Baat, The diagnostic suitability of a xerostomia questionnaire and the association between xerostomia, hyposalivation and medication use in a group of nursing home residents, *Clin. Oral Invest.* 15 (2011) 185–192.
- [30] D.A. Drossman, D.L. Dumitrascu, Rome III: new standard for functional gastrointestinal disorders, *J. Gastrointest. Liver Dis.* 15 (2006) 237–241.
- [31] J. Fu, M.J. Bonder, M.C. Cenit, E.F. Tigchelaar, A. Maatman, a M. Dekens, et al., The gut microbiome contributes to a substantial proportion of the variation in blood lipids, *Circ. Res.* 117 (2015) 817–824.
- [32] E.F. Tigchelaar, A. Zernakova, J. a M. Dekens, G. Hermes, A. Baranska, Z. Mujagic, et al., Cohort profile: LifeLines DEEP, a prospective, general population cohort study in the northern Netherlands: study design and baseline characteristics, *BMJ Open* 5 (2015) e006772.
- [33] J.U. Scher, A. Sczesnak, R.S. Longman, N. Segata, C. Ubeda, C. Bielski, et al., Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis, *Elife* 2 (2013) e01202.
- [34] D. Gevers, S. Kugathasan, L. a Denson, Y. Vázquez-Baeza, V. Van Treuren, B. Ren, et al., The treatment-naïve microbiome in new-onset Crohn's disease, *Cell Host Microbe* 15 (2014) 382–392.
- [35] Q. Wang, G.M. Garrity, J.M. Tiedje, J.R. Cole, Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy, *Appl. Environ. Microbiol.* 73 (2007) 5261–5267.
- [36] C. Quast, E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, et al., The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, *Nucleic Acids Res.* 41 (2013) 590–596.
- [37] W. Ludwig, O. Strunk, R. Westram, L. Richter, H. Meier, A. Yadukumar, et al., ARB: a software environment for sequence data, *Nucleic Acids Res.* 32 (2004) 1363–1371.
- [38] R Core Team, R: a Language and Environment for Statistical Computing, R Found Stat Comput Vienna, Austria, 2017 <https://www.r-project.org/>.
- [39] X.C. Morgan, T.L. Tickle, H. Sokol, D. Gevers, K.L. Devaney, D.V. Ward, et al., Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment, *Genome Biol.* 13 (2012) R79.
- [40] A. Zernakova, A. Kurilshikov, M.J. Bonder, E.F. Tigchelaar, M. Schirmer, T. Vatanen, et al., Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity, *Science* 352 (2016) 565–569.
- [41] F. Imhann, M.J. Bonder, A. Vich Vila, J. Fu, Z. Mujagic, L. Vork, et al., Proton pump inhibitors affect the gut microbiome, *Gut* 65 (2016) 740–748.
- [42] J.E. Gottenberg, P. Ravaut, X. Puéchal, V. Le Guern, J. Sibilia, V. Goeb, et al., Effects of hydroxychloroquine on symptomatic improvement in primary sjögren syndrome: the JOQUER randomized clinical trial, *JAMA, J. Am. Med. Assoc.* 312 (2014) 249–258.
- [43] T.M. Greiling, C. Dehner, X. Chen, K. Hughes, A.J. Iñiguez, M. Boccitto, et al., Commensal orthologs of the human autoantigen Ro60 as triggers of autoimmunity in lupus, *Sci. Transl. Med.* 10 (2018) eaan2306.
- [44] K. Atarashi, W. Suda, C. Luo, T. Kawaguchi, I. Motoo, S. Narushima, et al., Ectopic colonization of oral bacteria in the intestine drives TH1 cell induction and inflammation, *Science* 358 (2017) 359–365.
- [45] K. Andréasson, B. Ohlsson, T. Mandl, Elevated levels of faecal calprotectin in primary Sjögren's syndrome is common and associated with concomitant organic gastrointestinal disease, *Arthritis Res. Ther.* 18 (2016) 1–6.
- [46] T.A. van der Meulen, H.J.M. Harmsen, H. Bootsma, F.K.L. Spijkervet, F.G.M. Kroese, A. Vissink, The microbiome–systemic diseases connection, *Oral Dis.* 22 (2016) 719–734.
- [47] Y. He, W. Wu, H.-M. Zheng, P. Li, D. McDonald, H.-F. Sheng, et al., Regional variation limits applications of healthy gut microbiome reference ranges and disease models, *Nat. Med.* 24 (2018) 1532–1535.
- [48] M. Deschasaux, K.E. Bouter, A. Prodan, E. Levin, A.K. Groen, H. Herrema, et al., Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography, *Nat. Med.* 24 (2018) 1526–1531.
- [49] M.A. Jackson, S. Verdi, M.-E. Maxam, C.M. Shin, J. Zierer, R.C.E. Bowyer, et al., Gut microbiota associations with common diseases and prescription medications in a population-based cohort, *Nat. Commun.* 9 (2018) 2655.
- [50] D.I. Bolnick, L.K. Snowberg, P.E. Hirsch, C.L. Lauber, E. Org, B. Parks, et al., Individual diet has sex-dependent effects on vertebrate gut microbiota, *Nat. Commun.* 5 (2014) 4500.
- [51] M. Kumar, P. Babaei, B. Ji, J. Nielsen, Human gut microbiota and healthy aging: recent developments and future perspective, *Nutr. Healthy Aging* 4 (2016) 3–16.
- [52] P. Brito-Zerón, N. Acar-Denizli, M. Zeher, A. Rasmussen, R. Seror, E. Theander, et al., Influence of geolocation and ethnicity on the phenotypic expression of primary Sjögren's syndrome at diagnosis in 8310 patients: a cross-sectional study from the Big Data Sjögren Project Consortium, *Ann. Rheum. Dis.* 76 (2017) 1042–1050.
- [53] M.J. Lewis, A.S. Jawad, The effect of ethnicity and genetic ancestry on the epidemiology, clinical features and outcome of systemic lupus erythematosus, *Rheumatology (Oxford)* 56 (2017) i67–77.
- [54] A.T. Borchers, S.M. Naguwa, Y. Shoenfeld, M.E. Gershwin, The geoepidemiology of systemic lupus erythematosus, *Autoimmun. Rev.* 9 (2010) A277–A287.
- [55] B. Thong, N.J. Olsen, Systemic lupus erythematosus diagnosis and management, *Rheumatology (Oxford)* 56 (2017) i3–13.
- [56] J.M. Rolain, P. Colson, D. Raoult, Recycling of chloroquine and its hydroxyl analogue to face bacterial, fungal and viral infections in the 21st century, *Int. J. Antimicrob. Agents* 30 (2007) 297–308.
- [57] R. Wolf, A. Baroni, R. Greco, G. Donnarumma, E. Ruocco, M.A. Tufano, et al., Quinine sulfate and bacterial invasion, *Ann. Clin. Microbiol. Antimicrob.* 5 (2002) 1–5.
- [58] E. Le Chatelier, T. Nielsen, J. Qin, E. Prifti, F. Hildebrand, G. Falony, et al., Richness of human gut microbiome correlates with metabolic markers, *Nature* 500 (2013) 541–546.
- [59] X. Zhou, C. Nardini, X. Morgan, T. Tickle, H. Sokol, D. Gevers, et al., A method for automated pathogenic content estimation with application to rheumatoid arthritis, *BMC Syst. Biol.* 10 (2016) R79 101 2016.
- [60] M. Breban, J. Tap, A. Leboime, R. Said-Nahal, P. Langella, G. Chiochia, et al., Faecal microbiota study reveals specific dysbiosis in spondyloarthritis, *Ann. Rheum. Dis.* 76 (2017) 1614–1622.
- [61] A.G. Wexler, A.L. Goodman, An insider's perspective: Bacteroides as a window into the microbiome, *Nat. Microbiol.* 2 (2017) 17026.
- [62] L.E. Comstock, Importance of glycans to the host-Bacteroides mutualism in the mammalian intestine, *Cell Host Microbe* 5 (2009) 522–526.
- [63] D. Vandeputte, G. Kathagen, K. D'hoë, S. Vieira-Silva, M. Valles-Colomer, J. Sabino, et al., Quantitative microbiome profiling links gut community variation to microbial load, *Nature* 551 (2017) 507–511.
- [64] L. Abusleme, A.K. Dupuy, N. Dutzan, N. Silva, J. a Burleson, L.D. Strausbaugh, et al., The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation, *ISME J.* 7 (2013) 1016–1025.
- [65] B. Lugonja, L. Yeo, M. Milward, D. Smith, T. Dietrich, I. Chapple, et al., Periodontitis prevalence and serum antibody reactivity to periodontal bacteria in primary Sjögren's syndrome: a pilot study, *J. Clin. Periodontol.* 43 (2016) 26–33.
- [66] C. Selmi, A. Cerbelli, E. Generali, C.A. Scirè, F. Alborghetti, G. Colloredo, et al., Serum antinuclear and extractable nuclear antigen antibody prevalence and associated morbidity and mortality in the general population over 15 years, *Autoimmun. Rev.* 15 (2016) 162–166.
- [67] W.M. Thomson, S.M. Williams, Further testing of the xerostomia inventory, *Oral Surg. Oral Pathol. Oral Radiol. Endod.* 89 (2000) 46–50.