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Disease Features and Gastrointestinal Microbial Composition in Patients with Systemic Sclerosis from Two Independent Cohorts

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Objective. The study objective was to examine alterations in gastrointestinal (GI) microbial composition in patients with systemic sclerosis (SSc) and to investigate the relationship between SSc features and GI microbiota using two independent, international cohorts.

Methods. Prospective patients with SSc from Lund University (LU), Sweden, from the University of California, Los Angeles (UCLA), United States, and control subjects provided stool specimens for 16S ribosomal RNA sequencing. Alpha and beta diversity analyses were performed. Multivariate negative binomial models identified differentially abundant genera between groups.

Results. Patients from LU with SSc (n = 106) with recent SSc diagnosis (median disease duration 2.0 years) had lower abundance of commensal genera (eg, *Faecalibacterium*) and higher abundance of pathobiont genera (eg, *Desulfovibrio*) than LU-controls (n = 85). Patients from UCLA with SSc (n = 71) had a similar prevalence of females, a similar body mass index, and similar age but an increased disease duration (median 7.1 years) compared with patients from LU with SSc. Factors associated with beta diversity in patients with SSc from both LU and UCLA included disease duration ($P = 0.0016$), interstitial lung disease ($P = 0.003$), small intestinal bacterial overgrowth ($P = 0.002$), and immunosuppression use ($P = 0.014$). In multivariable analysis, the UCLA-SSc cohort had higher abundance of specific pathobiont genera (eg, *Streptococcus*) compared with the LU-SSc cohort.

Conclusion. Enrichments and depletions in certain microbial genera were observed in patients recently diagnosed with SSc, suggesting that dysbiosis is present in early SSc. Specific disease features were independently associated with fecal microbial composition in both cohorts. After controlling for these factors, the abundance of several pathobiont bacteria differed between the cohorts, suggesting that environmental factors, along with disease manifestations, should be considered in future SSc studies.

INTRODUCTION

The gastrointestinal (GI) microbiome is a homeostatic organ that modulates immune function and health (1). Dysbiosis (ie, alterations in the composition of commensal microbial communities) is a feature of immune-mediated diseases, including inflammatory bowel disease (IBD) and rheumatoid arthritis (RA) (1). In systemic sclerosis (SSc), studies have demonstrated distinct microbial community differences between patients with SSc and controls (2–4). Specifically, these studies have revealed enrichments in certain genera with pathobiont species and depletions in certain genera with commensal species in patients with SSc (2–4).

Although this emerging research provides compelling evidence that dysbiosis is a feature of the SSc disease state, it is unclear whether the observed microbiota alterations in SSc are due to the disease itself or external factors, such as immunosuppression use (5) or geographic region (6). No prior studies have included patients with recently diagnosed SSc to determine whether compositional shifts are present prior to the progression of disease and/or exposure to therapies. In addition, previous studies have not rigorously evaluated whether patients with SSc with specific disease features possess alterations in GI microbiota. Finally, no studies have examined whether differences exist in SSc cohorts from different geographic areas after controlling for disease features.

To address these questions, the present study aimed to compare GI microbial composition of patients with SSc who had a recent SSc diagnosis with unaffected age- and gender-matched controls. A second aim was to identify the key patient features that are associated with microbial composition in two, independent SSc cohorts. We hypothesized that patients with early-stage SSc have evidence of GI dysbiosis. We also hypothesized that specific SSc disease features, such as the presence of interstitial lung disease (ILD), are associated with alterations in GI microbiota. An exploratory aim was to evaluate whether differences in GI microbial composition exist between patients with SSc from two distinct geographic regions even after the adjusting for disease features. The findings may help to generate hypotheses for future interventional and observational microbiome studies in SSc seeking to explore how specific GI microbiota alterations moderate the development and/or progression of specific SSc manifestations.

PATIENTS AND METHODS

Study participants. Patients fulfilling the 2013 American College of Rheumatology/European Alliance of Associations for Rheumatology Classification Criteria for SSc were consecutively recruited from the rheumatology clinics at Lund University (LU), Sweden, and the University of California, Los Angeles (UCLA), United States. To capture patients with a recent diagnosis of SSc, patients from LU with SSc were eligible if their disease duration from the time of SSc diagnosis was less than 3 years. Unaffected control subjects were recruited from LU and selected for inclusion on the basis of age and sex. UCLA patients with SSc were eligible regardless of disease duration. Study exclusion criteria for all participants (SSc and controls) included IBD, current/prior GI malignancy, use of any antibiotics within 4 weeks of the stool collection, chronic use of antibiotics (defined as the use of antibiotics for any indication more than three times in the preceding year), current use of supplemental probiotics and any prior GI surgery (except for a remote history of appendectomy), and history of fecal microbial transplantation. Patients were allowed to remain on proton pump inhibitors to minimize the risk of unnecessary morbidity during the study. Patients with small intestinal bacterial overgrowth (SIBO) were included to evaluate whether the presence of SIBO affects fecal microbial composition in SSc.

The UCLA Institutional Review Board (#13-001089) and the Regional Ethics Review Board, Lund, Sweden (#2011-596) approved the study protocol, and written informed consent was obtained from each participant. Representatives from the national patient organization for Systemic Sclerosis in Sweden, as well as the Scleroderma Foundation (Southern California Chapter), approved and encouraged patient participation in this study.

Study assessments. Clinical features of the SSc participants were evaluated at the time of the stool collection (Table 1). Disease duration was defined based on the onset of the first non-Raynaud symptom attributable to SSc. Immunosuppression use was based on any consumption of immunosuppressive medications up until the date of the stool collection. High resolution computed tomography of the chest was used to detect the presence of ILD. Right heart catheterization was used to detect the presence of pulmonary hypertension. The presence of all other disease features was defined based on a physician clinical diagnosis identified through chart review. For example, if a physician documented a history of pseudo-obstruction (based on computed tomography assessment), fecal incontinence (based on clinical symptoms), or SIBO (based primarily on lactulose breath testing) in the medical chart of a patient, this was recorded. All participants were asked whether they adhered to specific dietary patterns, such as a vegetarian diet.

16S ribosomal RNA gene sequencing and microbial composition analysis. Participants at both sites collected stool specimens as previously described (2). LU-SSc samples were shipped overnight on dry ice to UCLA for further processing (2). Microbial DNA was extracted from the stool specimens by bead beating, and the V4 region of the bacterial 16S ribosomal RNA gene was sequenced using an Illumina NovaSeq 6000 (Illumina, Inc.) to a mean sequence depth of 301,239/sample (2). All samples (UCLA-SSc, LU-SSc) were analyzed simultaneously at UCLA to avoid any batch effects. DADA2 was used to perform quality filtering, merge paired end reads, remove chimeras and cluster sequences into exact amplicon sequence variants (ASVs) (7). Taxonomy was assigned for ASVs based upon the SILVA v132 database down to the level of family, genus, or species, depending on the depth of reliable classifier assignments.

Statistical analysis. *Alpha and beta diversity.* Alpha diversity represents the complexity of microbial composition within individual subjects. It was assessed on data rarefied to 26,501 sequences/sample using three metrics including the Chao1 index (a measure of richness, ie, number of different ASVs), Shannon index (which incorporates both richness and evenness, ie, how close the abundances of ASVs are to one another), and Faith's phylogenetic diversity (a measure of the total branch length of a phylogenetic tree present in a subject) (8). Significant differences

in alpha diversity metrics were evaluated by the Mann–Whitney U test (LU-SSc vs. LU-controls; LU-SSc vs. UCLA-SSc).

Beta diversity represents the between-subject similarity of microbial composition and enables the identification of differences between samples within a group. Beta diversity was performed in Quantitative Insights Into Microbial Ecology 2 of the unrarefied genus-level dataset after removing genera present in less than 10% of the samples using robust Aitchison distance implemented with the DEICODE plugin (9). Principal coordinate analysis was performed to visualize the resulting distance matrix (10). Significance was assessed by analysis of variance using distance matrices, a nonparametric test, for each pairwise comparison of sample groups using the Adonis function from the R vegan package (LU-SSc vs. LU-controls; LU-SSc vs. UCLA-SSc).

Univariable Adonis analyses were initially performed to evaluate the relationship between the individual patient features in

Table 1 and beta diversity in the combined SSc cohorts (11). The cohorts were combined for this analysis to improve our statistical power for detecting significant relationships between patient features and beta diversity. Variables significantly ($P < 0.05$) associated with beta diversity in the univariable analysis were combined into a multivariable analysis, adjusting for study cohort.

Genus-level differences. Taxonomic differences at the genus level were evaluated between the early SSc cohort (LU-SSc) versus unaffected controls from LU using differential expression analysis for sequence count data (DESeq2) (12). DESeq2 normalizes the data using size factors estimated by the median-of-ratios method, shrinks dispersion using an empirical Bayesian approach, and fits the data to multivariable negative binomial models. DESeq2 was also used to compare genera abundance between the LU-SSc cohort and the UCLA-SSc cohort. The

Table 1. Participant characteristics of the SSc cohorts from LU and UCLA

Characteristics	LU (n = 106)	UCLA (n = 71)	*P* Value
Age, years, mean ± SD	55.31 ± 15.9	54.3 ± 12.6	0.540
Female, n (%)	90/106 (84.9)	61/71 (85.9)	1.000
Diffuse disease, n (%)	20/106 (18.9)	31/70 (44.3)	0.0003
Limited disease, n (%)	86/106 (81.1)	39/71 (55.7)	0.0003
Disease duration, years[a]			0.0001
Median (IQR)	2.0 (4)	7.1 (9.2)	
Mean ± SD	4.5 ± 6.9	9.7 ± 8.5	
History of SIBO, n (%)	5/106 (4.7)	15/69 (21.7)	0.011
History of pseudo-obstruction, n (%)	2/106 (1.9)	5/70 (7.1)	0.116
History of fecal incontinence, n (%)	7/106 (6.6)	11/70 (15.7)	0.074
Current BMI, kg/m^2, mean ± SD	25.5 ± 4.8	25.0 ± 4.2	0.712
Current BMI < 18.5 kg/m^2, n (%)	1/106 (0.9)	4/71 (5.6)	0.159
History of GAVE, n (%)	2/106 (1.9)	2/71 (2.8)	1.000
History of GERD, n (%)	65/106 (61.3)	66/71 (93.0)	0.0001
HRCT-defined ILD, n (%)	35/106 (33.0)	60/69 (87.0)	0.0001
Pulmonary hypertension by RHC, n (%)	7/106 (6.6)	5/71 (7.0)	1.000
History of renal crisis, n (%)	1/106 (0.9)	2/71 (2.8)	0.565
History of SSc cardiac involvement, n (%)[b]	20/106 (18.9)	7/71 (9.9)	0.136
History of inflammatory myopathy, n (%)	9/106 (8.5)	5/71 (7.0)	0.785
Current mRSS (0-51), mean ± SD	5.5 ± 7.6	6.2 ± 6.0	0.0150
History of calcinosis, n (%)	4/106 (3.8)	4/71 (5.6)	0.715
History of digital ulcers, n (%)	12/106 (11.3)	17/71 (23.9)	0.044
Immunosuppression use ever, n (%)[c]	35/106 (33.0)	57/69 (82.6)	0.0001
Current immunosuppression use, n (%)[c]	35/106 (33.0)	38/69 (55.1)	0.008
Ever smoker, n (%)	54/105 (51.4)	17/68 (25.0)	0.0005
Vegetarian, n (%)	5/106 (4.7)	4/47 (8.5)	0.743
Current PPI use, n (%)	62/104 (59.6)	43/70 (61.4)	0.755
Current promotility agent use, n (%)[d]	2/106 (1.9)	7/71 (9.9)	0.031
Scl-70 antibody, n (%)	19/106 (17.9)	19/61 (31.1)	0.190
Anti-centromere antibody, n (%)	42/106 (39.6)	13/57 (22.8)	0.037
RNA polymerase III antibody, n (%)	3/106 (2.8)	1/26 (3.8)	1.000

[a]Based on the onset of the first non-Raynaud symptom attributable to SSc.
[b]Type of cardiac involvement (patients could have more than one cardiac manifestation) in UCLA-SSc cohort: pericardial effusion (n = 2), atrial fibrillation (n = 3), diastolic dysfunction (n = 2), cardiomyopathy (n = 2), reduced right ventricular function (n = 1); and the LU-SSc cohort: right bundle branch block (n = 7), pericardial effusion (n = 4), diastolic dysfunction (n = 3), cardiomyopathy (n = 2), atrial fibrillation (n = 1), AV block (n = 1), left bundle branch block (n = 1).
[c]Immunosuppression use was based on any consumption of immunosuppressive medications up until the date of the stool collection. Please see Supplemental Table 1 for summary of immunosuppressive medications used.
[d]Type of promotility agents used in UCLA-SSc cohort: prucalopride (n = 2), linaclotide (n = 1), metoclopramide (n = 4); and in the LU-SSc cohort: metoclopramide prn (n = 1), domperidone prn (n = 1).
Abbreviations: BMI, body mass index; GAVE, gastric antral vascular ectasia; GERD, gastroesophageal reflux disease; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; IQR, interquartile range; LU, Lund University; mRSS, modified Rodnan Skin Score; PPI, proton pump inihibitor; RHC, right heart catheterization; SIBO, small intestinal bacterial overgrowth; SSc, systemic sclerosis; UCLA, University of California Los Angeles.

patient features found to be significantly associated with microbial composition in the univariable beta diversity analyses were included in the multivariable model.

The LU cohort was divided into training (60%) and test (40%) sets. A random forest classifier was created from genus-level microbiome data to differentiate LU-SSc from LU-controls, using the randomForest package in R with 1,001 trees and mtry of 2 (ie, the number of microbial genera used in each tree). An odd number of trees was used to prevent theoretical ties that may occur from forest generation. Moreover, after various mtry parameters were selected, an mtry of 2 yielded the highest area under the receiver operating curve. Features in the random forest classifier included differentially abundant ASVs. The classifier performance was validated against the test set.

All tests were two-sided. To minimize the risk of type I error due to multiple hypothesis testing, the Benjamini and Hochberg method was used to control for false discovery rate (13), and a significant association was defined as q value less than or equal to 0.05.

RESULTS

Participant characteristics. The LU-SSc cohort (n = 106) had a similar age, body mass index, percentage of females, and percentage of patients consuming a vegetarian diet compared with the UCLA-SSc cohort (n = 71) (Table 1). Although the median disease duration of the LU-SSc cohort was 2.0 years, the UCLA-SSc cohort mainly consisted of patients with established SSc (median disease duration of 7.1 years). As such, the prevalence of ILD, SIBO, and immunosuppression use (Supplementary Table 1) were higher in the UCLA-SSc cohort compared with the LU-SSc cohort.

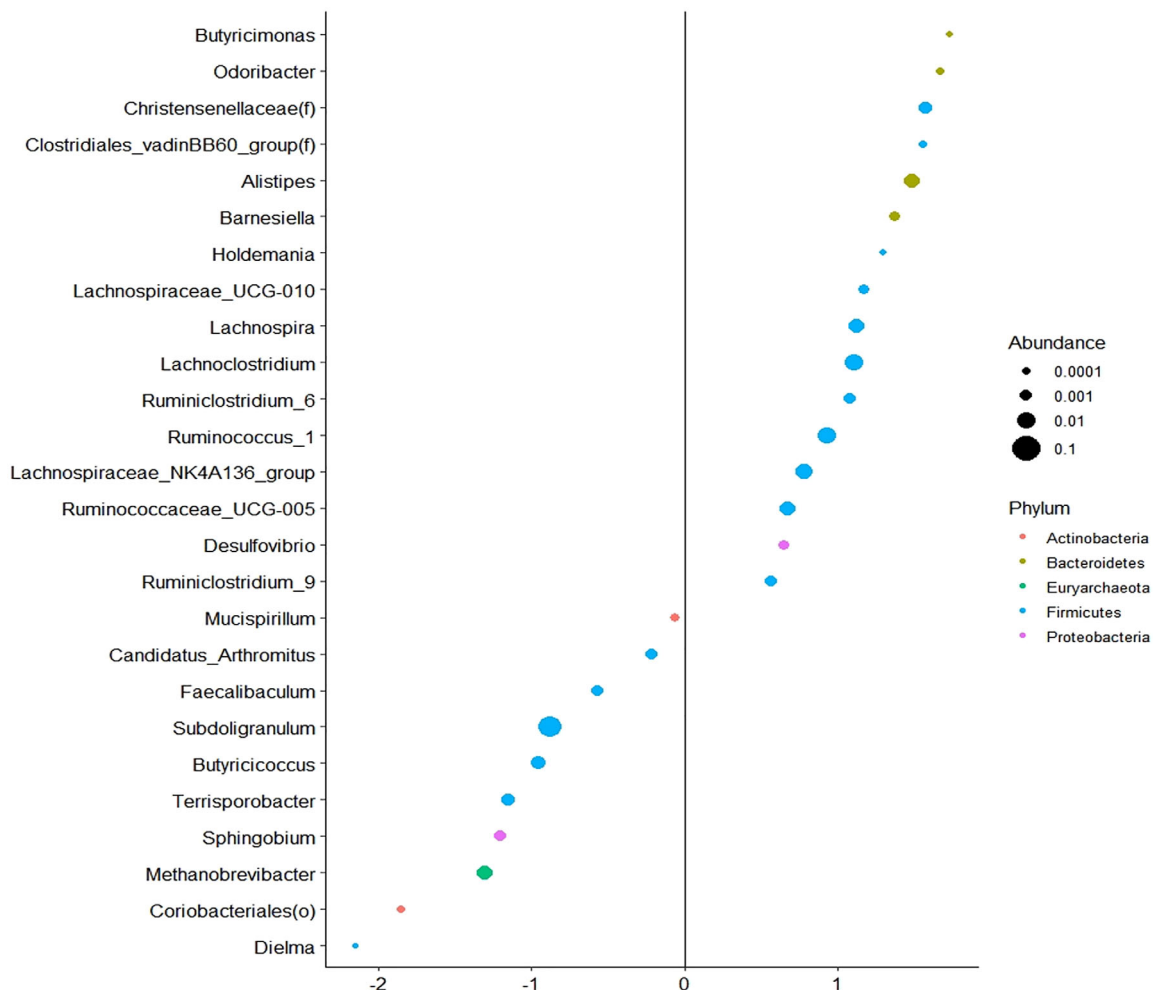The mean age (56.7 [SD 13.5] years) of LU-controls (n = 85) was similar to the mean age of LU-SSc participants (55.3

Figure 1. Genus-level taxa with significant ($q < 0.05$) differential abundance in patients with SSc and unaffected controls from LU in the multivariable analysis adjusting for age and gender. Circles with a positive fold change score represent genera with increased abundance in the LU-SSc cohort, and those with a negative fold change score represent genera with increased abundance in the LU-control cohort. The color of the circle signifies the phylum level of the genus. The size of the circle indicates the relative abundance of the specific genus. LU, Lund University; SSc, systemic sclerosis.
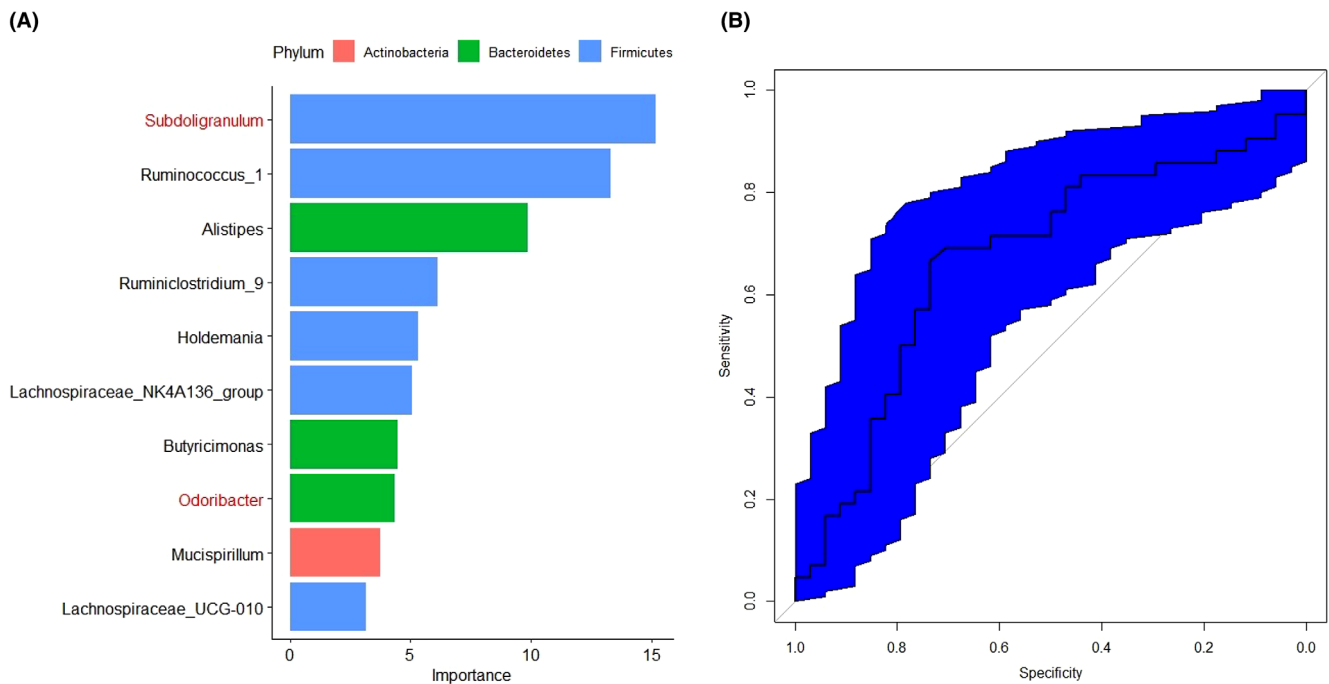
(A)

(B)

Figure 2. Random forests classifier for differentiating LU-SSc from LU-controls based upon the fecal microbiome. (**A**) Importance scores for nine genera from three phyla (Bacteroidetes, Firmicutes, and Proteobacteria) that contributed significantly to the random forest classifier distinguishing patients from LU with SSc versus LU-controls. Black-colored genera on the y-axis were enriched in SSc, whereas red-colored genera were depleted when compared with controls. (**B**) ROC curve for the random forest classifier. The AUC is 0.67 (95% confidence interval 0.56-0.78). AUC, area under the curve; LU, Lund University; ROC, receiver operating characteristic; SSc, systemic sclerosis.

[SD 15.9]). Fifty-nine (69.4%) of the LU-controls were female, which was slightly lower than the percentage of females in the LU-SSc cohort (84.9% female).

Microbial community differences present in patients with SSc with early disease stage. Patients with LU-SSc had higher microbial alpha diversity (within-subject diversity of microbial species) compared with LU-controls by the Shannon index ($P = 0.0003$), which incorporates both richness (ie, number of species) and the evenness of their relative abundances. There were also trends for differences between LU-SSc and LU-controls using metrics of species richness only (Chao1; $P = 0.134$) and phylogenetic diversity (Faith's Phylogenetic diversity; $P = 0.087$) (Supplementary Figure 1). Taken together, these results suggest a more heterogenic GI microbial compositional profile in individuals with recent-onset SSc. Patients with SSc from LU had a trend toward significant differences microbial composition (beta diversity) compared with LU-controls ($P = 0.083$ for unadjusted analysis; $P = 0.107$ for analysis adjusted for age and gender) (Supplementary Figure 2).

After adjusting for age and gender, patients with SSc from LU had increased abundance of several genera deemed pathobiont (eg, *Desulfovibrio*, *Ruminococcus*) and decreased abundance of several genera deemed commensal (eg, *Faecalibacterium*) compared with LU-controls (Figure 1). Using these

differentially abundant genera, a random forest classifier was created to differentiate LU participants with SSc from controls, and the contribution of each significant taxa was expressed with
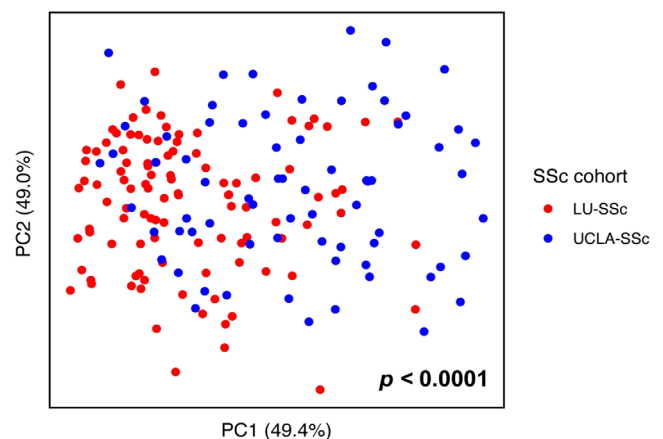

Figure 3. Significant difference in microbiome composition between LU-SSc participants and UCLA-SSc participants. Beta diversity analyses were performed using robust Aitchison distance, and the differences between groups are visualized by principal coordinate analysis plots. Each dot represents a patient sample. The *P* value (<0.0001) was calculated by univariate Adonis. LU, Lund University; PC1, first principle coordinate; PC2, second principle coordinate; SSc, systemic sclerosis; UCLA, University of California Los Angeles

a variable importance score. Nine genera from three phyla (Bacteroidetes, Firmicutes, and Proteobacteria) contributed significantly to the classifier (Figure 2A). The area under the receiver operating curve for classifier performance validated against the test set was 0.67 (95% confidence interval 0.56-0.78) (Figure 2B), demonstrating that fecal microbial profiles distinguished patients with SSc from controls with good sensitivity and specificity.

SSc disease features and microbial composition. UCLA-SSc participants had significantly lower alpha diversity scores ($P < 0.0001$ for all three metrics) compared with the LU-SSc participants (Supplementary Figure 3), suggesting a less diverse GI microbial flora in the established patients with SSc from UCLA. In addition, significant differences in beta diversity were observed between the UCLA-SSc and LU-SSc cohorts ($P < 0.0001$) (Figure 3).

Among all of the individual variables from Table 1, the following were significantly associated with beta diversity in the univariable analysis: disease duration ($P = 0.0016$), presence of SIBO ($P = 0.002$), presence of ILD ($P = 0.003$), and any history of immunosuppression use ($P = 0.014$) (Figure 4). The aforementioned variables were combined into the multivariable analysis, which adjusted for cohort, and the following remained significantly associated with beta diversity: ILD ($P = 0.0004$), SIBO ($P = 0.0002$), and SSc cohort ($P < 0.0001$).

Differential abundance of bacterial genera between two SSc cohorts. The unadjusted DESeq2 analysis demonstrated differentially abundant bacteria genera between the SSc cohorts (Supplementary Figure 4). For example, the pathobionts *Streptococcus* and *Enterococcus* were more abundant in the UCLA-SSc cohort, whereas *Bifidobacterium*, a known commensal, was more abundant in the LU-SSc cohort. *Lactobacillus* was also more abundant in the UCLA-SSc cohort.

After adjusting for disease duration, SIBO, ILD, and any immunosuppression use, the number of differentially abundant
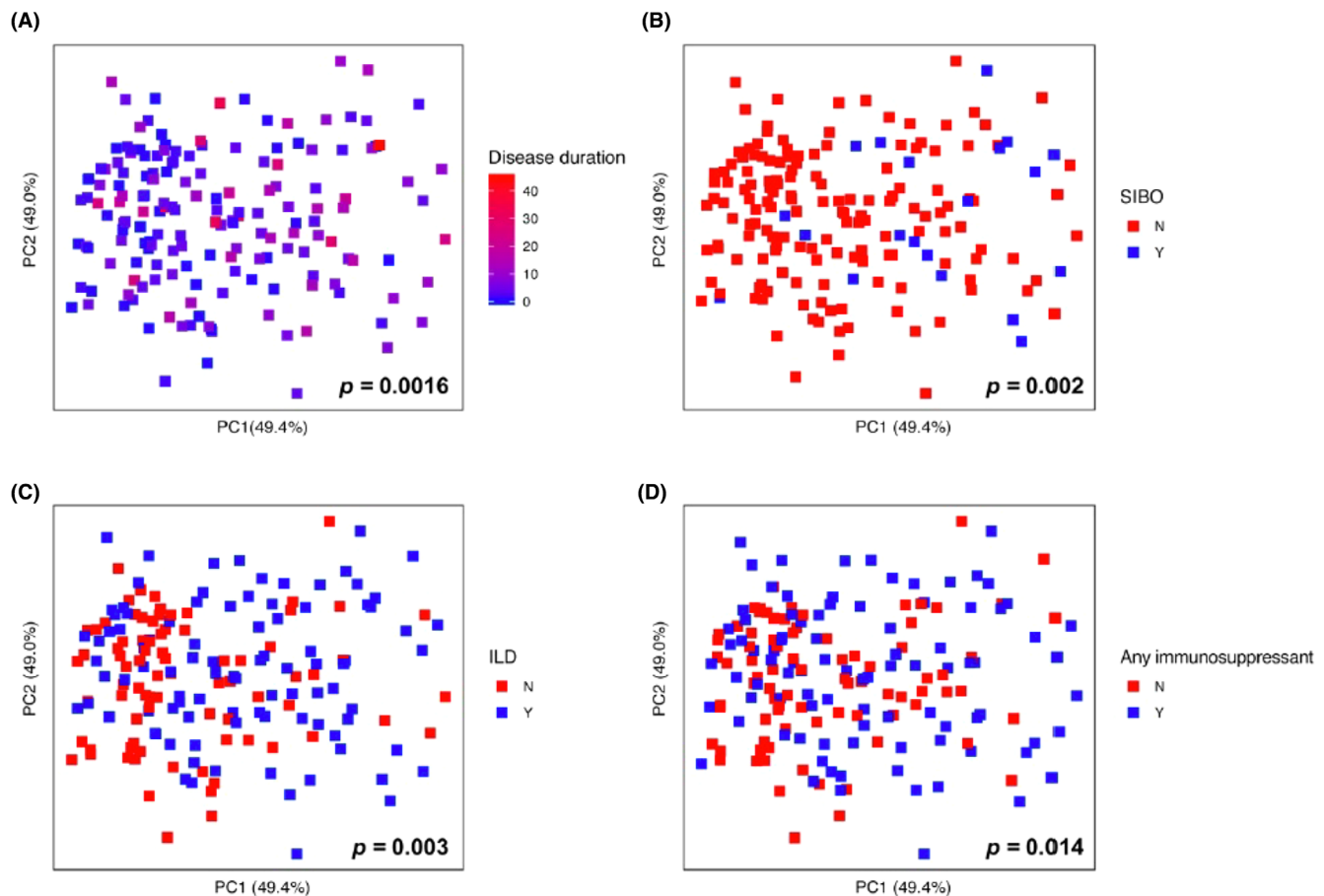

Figure 4. Significant differences in microbiome composition based on (**A**) disease duration (years), (**B**) SIBO presence, (**C**) ILD presence, and (**D**) immunosuppression use. Beta diversity analyses were performed using robust Aitchison distance, and the differences between groups are visualized by principal coordinate analysis. Each dot represents a patient sample. The *P* values were calculated by univariate Adonis. ILD, interstitial lung disease; PC1, first principle coordinate; PC2, second principle coordinate; SIBO, small intestinal bacterial overgrowth.
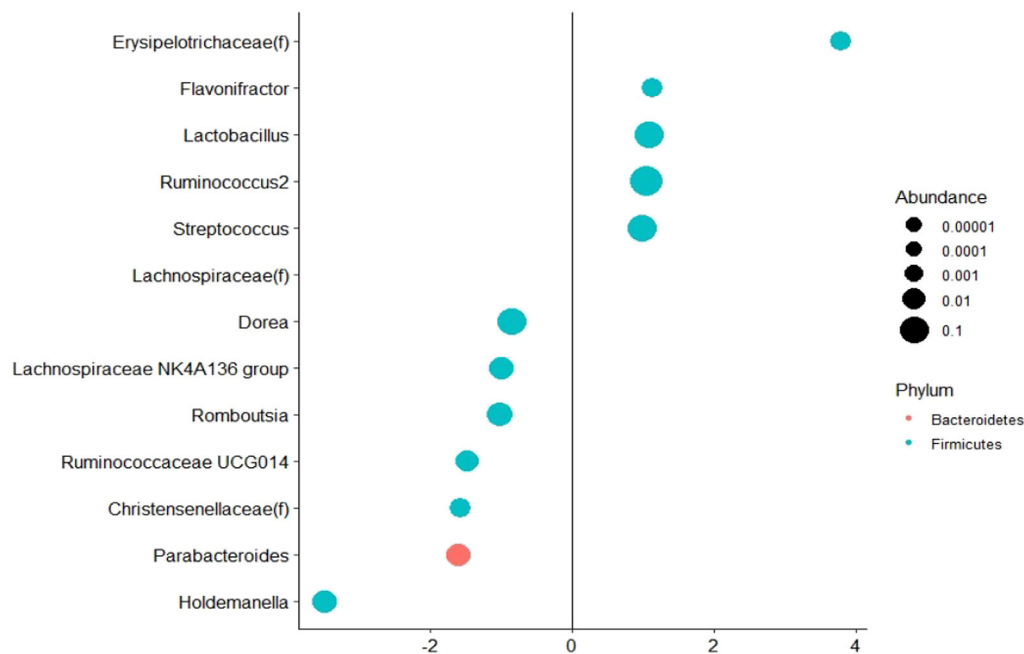
Figure 5. Genus-level taxa with significant ($q < 0.05$) differential abundance in patients with SSc from LU compared with those from UCLA adjusting for disease duration, SIBO, ILD, and immunosuppression use. Circles with a positive fold change score represent genera with increased abundance in the UCLA-SSc cohort, and those with a negative fold change score represent genera with increased abundance in the LU-SSc cohort. The color of the circle signifies the phylum level of the genus. The size of the circle indicates the relative abundance of the specific genus. ILD, interstitial lung disease; LU, Lund University; SIBO, small intestinal bacterial overgrowth; SSc, systemic sclerosis; UCLA, University of California Los Angeles.

genera between the two cohorts decreased from 27 to 13 (Figure 5). *Lactobacillus*, *Streptococcus*, *Erysipelotrichaceae*, and *Ruminococcus* remained significantly more abundant in the UCLA-SSc cohort.

DISCUSSION

In the first two-center study to investigate the relationship between SSc features and the GI microbiome, the SSc manifestations of ILD and SIBO were independently associated with microbial composition. This study also found that dysbiosis was present in recently diagnosed patients with SSc. These findings suggest that perturbations to the GI microbiota are present early in the SSc disease course and may underlie/develop in parallel with the evolution of this systemic disease.

The observation that patients with SSc with earlier disease stage have microbial community differences compared with unaffected controls is a novel finding. Previous SSc microbiome studies included patients with established SSc (more than 3 years from the onset of the first non-Raynaud symptom of SSc) with average disease durations ranging from 6 to 12.5 years (2–4,14,15). In these prior studies, it was unclear to what degree dysbiosis was due to the SSc disease state versus external factors, such as SSc-targeted therapies or dietary modifications adopted to address SSc GI symptoms. However, consistent with

these prior studies, enrichments in specific genera deemed pathobiont (eg, *Desulfovibrio* [4] and *Ruminococcus* [2,15]) and depletions in specific genera deemed commensal (eg, *Faecalibacterium* [2,15]) were observed in the patients with SSc of earlier disease stage. The present findings support the hypothesis that GI microbes and/or their metabolic products may contribute to early pathogenic processes in SSc and warrant further investigation in additional early and very early SSc cohorts.

Among SSc features, the presence of ILD was independently associated with microbial composition, which is consistent with a single-center study that found that patients with SSc and ILD had increased dysbiosis scores compared with patients with SSc without ILD (3). Previous studies have evaluated how external factors affect the composition and functionality of the GI microbiome in relation to the development of respiratory disorders (16). For instance, a small study demonstrated that the patients with silica-induced pulmonary fibrosis had unique alterations in the abundance of specific bacterial taxa, including increased Lachnospiraceae (17). It is conceivable that the upper GI microbiota interface directly with the lung in SSc through aspiration from esophageal dysmotility. In addition, GI microbial input (eg, priming of immune cells trafficking through the gut that return to circulation) may modulate inflammatory/fibrotic responses in distant organs, such as the lungs (18). Future studies are needed to understand how the

abundance of specific bacteria and their metabolites contribute to ILD pathogenesis in SSc.

The finding that SIBO was associated with changes in fecal microbial composition is in line with a recent small study that demonstrated differences in GI bacterial diversity and richness in patients with SSc with and without SIBO [19]. A pivotal study comparing microbial composition from duodenal aspirates of patients with and without SIBO (defined as more than 10^3 colony forming units per milliliter) demonstrated that patients with SIBO had increased abundance of Proteobacteria and decreased abundance of Firmicutes [20]. In the present study, SIBO was primarily diagnosed based on indirect assessment using carbohydrate (eg, lactulose) breath testing. The present results suggest that a SIBO diagnosis in SSc may serve as an indicator of microbial alterations in other regions of the GI tract.

Similar to an earlier study comparing patients with SSc from UCLA and Oslo University [15], the abundance of specific genera differed between the present cohorts. Although the prior comparator analysis [15] was not adequately powered to control for potentially confounding variables, the present study was able to adjust for the patient-related factors associated with microbial composition (eg, SIBO, ILD, immunosuppression, disease duration). After adjusting for these factors, 13 genera were differently abundant in the two cohorts. Compared with the early disease stage SSc cohort from LU, the more established SSc cohort from UCLA had an increased abundance of known pathobionts, such as *Streptococcus*. This genus has been found in higher abundance in IBD, RA, multiple sclerosis [21], and fibrotic liver disease [22]. Our prior studies (2,15) demonstrated significant enrichments in *Streptococcus* in SSc compared with controls.

An unidentified genus from the Lachnospiraceae family was also more abundant in the UCLA-SSc cohort. In addition to the aforementioned association with silica-induced pulmonary fibrosis [17], Lachnospiraceae measured in the bronchoalveolar lavage (BAL) fluid of patients with idiopathic pulmonary fibrosis was positively associated with epidermal growth factor BAL levels [23], suggesting that members of this family may moderate profibrotic processes. More research is needed to understand whether species of this family play a role in the pathogenesis of autoimmune lung disease.

Lactobacillus was also found in greater abundance in the LU-SSc cohort compared with LU-controls and in the UCLA-SSc cohort compared with the LU-SSc cohort. *Lactobacillus* was enriched in patients with SSc compared with controls in previous studies (2,3,14,15). Although species of this genus are deemed commensals in IBD [24], the precise pathogenic role that *Lactobacillus* plays in SSc is unclear. Interestingly, animal studies have demonstrated that ingestion of *Lactobacillus reuteri* slows GI motility [25], suggesting a potential link between *Lactobacillus* and GI dysmotility in SSc. Also, SSc-specific pharmacokinetics of the commonly used agent mycophenolate mofetil have been associated with intestinal levels of *Lactobacilli* [26].

If the associations between the abundance of specific genera and SSc features are affirmed in future studies, specific genera and/or their metabolic products could serve as potential therapeutic targets. In particular, if certain bacteria are deficient in patients with SSc from different geographic regions, interventions (eg, probiotics) could be developed to replete these bacteria and studied further to determine whether this personalized approach to bacterial repletion improves SSc GI symptoms. Understanding how specific genera interface with and/or affect immune function could also help improve our overall understanding of the multifactorial processes that contribute to fibrosis in SSc.

Strengths of this study include the relatively large sample size (n = 262), the study of two independent SSc cohorts, the inclusion of patients with SSc with earlier disease stage, as well as the careful clinical characterization of study participants. Limitations include our inability to measure the effects of specific immunosuppressive therapies on microbial composition given the variability in the type, duration, and temporal sequence of these therapies. In addition, detailed information on nutrient consumption was not collected in both cohorts. Furthermore, although the LU-control participants were matched by age and gender to the LU-SSc participants, other factors (eg, diet, medications) may contribute to the differences observed between controls and patients with SSc. Another limitation was that the UCLA-SSc cohort did not have enough early-stage patients (only seven patients had a disease duration of less than 3 years) to evaluate differences in microbial composition between early-stage UCLA patients with SSc and controls. We and others have previously demonstrated microbial community differences between patients with established SSc and controls (2–4,14,15); therefore, we opted to perform the control comparator analysis with the recently diagnosed Swedish cohort.

In summary, the present study demonstrated that microbial alterations are present in newly diagnosed patients with SSc compared with controls and that patients with SSc have distinct differences in GI microbial composition based on clinical features, including the presence of ILD. Moreover, this study found cohort-specific differences in microbial composition that are associated with SSc disease features and also likely associated with environmental factors. Future studies endeavoring to evaluate or modulate the GI microbiota in SSc should consider disease duration and SSc features, including SIBO and ILD. Understanding the unique microbiota perturbations associated with SSc features may reveal distinct pathobiological underpinnings of diverse clinical manifestations of SSc and lay the groundwork for future clinical trials aiming to restore GI microbiota homeostasis in SSc.

AUTHOR CONTRIBUTIONS

Acquisition of data. Andréasson, Wu, Howlett, English, Clements, Volkmann.

Analysis and interpretation of data. Andréasson, Lee, Lagishetty, Hesselstrand, Jacobs, Volkmann.

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