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Gastrointestinal tract involvement in systemic sclerosis: The roles of diet and the microbiome

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Abstract

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Author contributions

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Kristofer Andréasson: study design; data analysis and interpretation; critically reviewed and edited the article; agrees to the journal to which the article will be submitted; reviewed and agreed on article version for submission; agrees to take responsibility and be accountable for article contents.

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Declaration of Competing Interest

None.

Supplementary materials

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Background: Alterations in gastrointestinal (GI) microbial composition have been reported in patients with systemic sclerosis (SSc). However, it is unclear to what degree these alterations and/or dietary changes contribute to the SSc-GI phenotype.

Objectives: Our study aimed to 1) evaluate the relationship between GI microbial composition and SSc-GI symptoms, and 2) compare GI symptoms and GI microbial composition between SSc patients adhering to a low versus non-low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) diet.

Methods: Adult SSc patients were consecutively recruited to provide stool specimens for bacterial 16S rRNA gene sequencing. Patients completed the UCLA Scleroderma Clinical Trial Consortium Gastrointestinal Tract Instrument (GIT 2.0) and the Diet History Questionnaire (DHQ) II and were classified as adhering to a low or non-low FODMAP diet. GI microbial differences were assessed using three metrics of alpha diversity (species richness, evenness, and phylogenetic diversity), as well as beta diversity (overall microbial composition). Differential abundance analysis was performed to identify specific genera associated with SSc-GI phenotype and low versus non-low FODMAP diet.

Results: Of the 66 total SSc patients included, the majority were women ($n = 56$) with a mean disease duration of 9.6 years. Thirty-five participants completed the DHQ II. Increased severity of GI symptoms (total GIT 2.0 score) was associated with decreased species diversity and differences in GI microbial composition. Specifically, pathobiont genera (e.g., *Klebsiella and Enterococcus*) were significantly more abundant in patients with increased GI symptom severity. When comparing low ($N = 19$) versus non-low ($N = 16$) FODMAP groups, there were no significant differences in GI symptom severity or in alpha and beta diversity. Compared with the low FODMAP group, the non-low FODMAP group had greater abundance of the pathobiont *Enterococcus*.

Conclusion: SSc patients reporting more severe GI symptoms exhibited GI microbial dysbiosis characterized by less species diversity and alterations in microbial composition. A low FODMAP diet was not associated with significant alterations in GI microbial composition or reduced SSc-GI symptoms; however, randomized controlled trials are needed to evaluate the impact of specific diets on GI symptoms in SSc.

Keywords

Systemic sclerosis; Gastrointestinal microbiome; FODMAP diet; nutrition

Introduction

Systemic sclerosis (SSc) is a rare, incurable autoimmune disease with the highest cause-specific mortality of all connective tissue diseases [1,2]. The gastrointestinal (GI) tract is one of the most commonly affected internal organs in SSc, [3] and involvement of the GI tract is a leading cause of morbidity and mortality in SSc [4,5].

The pathogenesis of GI involvement in SSc is poorly understood. Recent studies have found significant differences in GI microbial composition between SSc patients and healthy controls, suggesting that gut dysbiosis may contribute to the pathogenesis of this disease

[6-9]. In addition, alterations in GI microbial composition have been observed early in the course of SSc [10]. For example, Andreasson and colleagues found that SSc patients with a disease duration of less than 3 years from the time of diagnosis had increased abundance of genera deemed pathobiont (e.g., *Desulfovibrio*) and decreased abundance of genera deemed commensal (e.g., *Faecalibacterium*) compared with matched controls [10].

Few studies have evaluated the relationship between GI microbial composition and SSc-GI symptoms. A review by Tan et al. [11] reported several studies investigating GI microbial alterations and SSc symptoms, though studies were generally small and none had examined the role of diet. For instance, one small study ($N = 17$) found that higher abundance of *Fusobacterium*, a purported pathobiont genera, was associated with increased GI symptoms, while higher abundance of *Bacteroides fragilis*, a purported commensal species, was associated with decreased GI symptoms [8]. This prior study did not assess the effects of diet on GI microbial composition and did not investigate whether diet is associated with GI symptom severity.

To address these prior limitations and to further our understanding of how the GI microbiome contributes to the SSc-GI phenotype, the present study aimed to examine the relationships between GI symptoms, diet, and the GI microbiome in patients with SSc. A commonly recommended diet for SSc is a diet low in short-chain fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs). One small study has suggested that a diet low in fructose may benefit patients with SSc with fructose malabsorption; however, this study lacked a control arm [12].

The primary aim of the present study was to examine the relationship between GI microbial composition and the severity of GI symptoms in SSc. Exploratory aims were to: (1) determine whether a low FODMAP diet is associated with decreased SSc-GI symptoms, and (2) compare GI microbial composition between SSc patients adhering to a low versus non-low FODMAP diet. While previous studies have demonstrated that a low FODMAP diet results in improved GI symptoms in patients with irritable bowel syndrome (IBS), [13,14] the impact of a low FODMAP diet on SSc-GI symptoms is unknown. Therefore, the findings of this study may help to enhance our knowledge of the causes of GI symptoms in SSc and improve our understanding of how diet relates to SSc-GI symptoms. The results of this study may also help to inform the design of future, prospective studies examining the impact of specific interventions (e.g., diet, medications) on the GI microbiome and GI symptoms in SSc.

Materials and methods

Study participants

Patient participants were consecutively enrolled from outpatient rheumatology clinics at the University of California, Los Angeles (UCLA). Inclusion criteria were: (1) adult patients (age ≥18 years); (2) SSc of any disease duration according to the 2013 American College of Rheumatology/European League Against Rheumatism Classification Criteria for SSc [15]. Exclusion criteria were: (1) co-morbid GI condition, including inflammatory bowel disease (IBD), celiac disease and GI malignancy; (2) patients with an inability to withstand from

taking an antibiotic and a probiotic at least three weeks prior to stool collection. Patients were allowed to remain on antacids, histamine H2-receptor antagonists and proton pump inhibitors to minimize the risk of unnecessary morbidity during the study; however, patients had to discontinue their use laxatives, promotility agents and anti-diarrhea medications one week prior to their stool collection, and discontinue any antibiotics and probiotics three weeks prior to their stool collection.

Clinical features of SSc participants from the time of initial presentation to the date of the stool collection were obtained via extensive chart review by three physicians (ERV, NH, HB) (Table 1). The time from the onset of the first non-Raynaud symptom to the date of stool collection was used to define SSc disease duration. The consumption of immunosuppressive medications up until the date of stool collection was used to define immunosuppression utilization. Medication history was self-reported by the patient and verified by our study team using the electronic medical record (ERV, NH, HB). In addition, high resolution computed tomography (HRCT) of the chest was used to identify the presence of interstitial lung disease (ILD). The presence of other disease features was determined according to a physician's clinical diagnosis obtained from chart review. For instance, if a physician documented a history of small intestinal bacterial overgrowth (SIBO) based on lactulose breath testing in the medical chart, this finding was recorded. Missing data on clinical diagnoses, though scarce, were resolved by speaking directly to the treating physician and asking for clarification.

The UCLA Institutional Review Board (#13–001,089) approved the study protocol and written informed consent was obtained from each participant.

Specimen procurement and gene sequencing analysis

Participants collected stool specimens using a previously published home collection method [16]. Specimens were frozen and transferred on ice to the study team, after which they were stored at −80 °C until processing and analysis.

Bead beating was used to extract microbial DNA from stool specimens. The V4 region of the bacterial 16S rRNA gene was then sequenced using the Illumina NovaSeq 6000 (Illumina, San Diego, California, USA) as described in our prior publications [10]. To avoid batch effects, all samples were simultaneously processed at UCLA. Additionally, DADA2 was used for quality filtering, merging paired end reads, deleting chimeras, and clustering sequences into amplicon sequence variants (ASVs) [17]. Lastly, depending on the depth of reliable classifier assignments, taxonomy was determined for ASVs down to the level of family, genus, or species using the SILVA v132 database.

Assessment of GI symptoms and diet history

On the day of stool collection, participants completed the UCLA Scleroderma Clinical Trial Consortium Gastrointestinal Tract (UCLA SCTC-GIT 2.0, or UCLA GIT 2.0) [18]. This 34-item validated scale evaluates the burden of SSc-associated GI symptoms across seven domains: reflux, distension, diarrhea, fecal soilage, constipation, wellbeing, and social functioning. Scores indicate self-reported GI symptom severity and can distinguish patients with none-to-mild, moderate, and severe to very severe symptoms.

In addition, on the day of the stool collection, all participants completed the Diet History Questionnaire (DHQ) II, a validated 142-item questionnaire that assesses dietary recall of specific foods consumed in the preceding four weeks [19]. Forty DHQ II items were characterized as high FODMAP items using the Monash University FODMAP food database [14]. For each of the 40 high FODMAP item questions, patients reported their frequency of consumption. If a patient responded "Never," a score of zero was entered for that item, indicating no consumption. If a patient reported that they consumed the item at least one time per month, a score of one was entered for that item, indicating some consumption. For an individual patient, the mode of their responses to these 40 questions was calculated. If the mode for an individual patient was 0, this patient was categorized as a low FODMAP consumer. If the mode for an individual patient was 1, this patient was categorized as a non-low FODMAP consumer. Due to concerns of recall bias, we did not further subcategorize the non-low FODMAP consumers into high versus moderate consumption of FODMAP items, as we felt it may be difficult for patients to remember the exact number of times they consumed a particular food per month. A brief list of examples of high FODMAP foods and low FODMAP alternatives is shown in Table 2.

Alpha and beta diversity

To examine differences in GI microbial communities, alpha and beta diversity analyses were performed. Alpha diversity represents the diversity of the microbiome within individual participants and was measured using three metrics: the Chao1 index (measures species richness; number of species), Shannon index (measures richness and evenness; how close the abundances of specific species are to one another), and Faith's phylogenetic diversity (Faith's PD; measure of the total branch length of a phylogenetic tree present in a participant) [20]. The Mann-Whitney U test was used to determine significant differences in alpha diversity metrics between groups.

Beta diversity represents differences in the overall microbial composition between participants, enabling the identification of differences between subjects within a group. Beta diversity was evaluated in QIIME 2 using robust Aitchison distance with the DEICODE plugin of the unrarefied genus-level dataset after filtering out genera present in fewer than 10% of samples [21]. Principal coordinate analysis was performed to visualize the resultant distance matrix [22]. Finally, to assess for statistical significance, analysis of variance using distance matrices was performed for each pairwise comparison of sample groups by using the Adonis function from the R vegan package.

Genus level differences

In order to determine whether taxonomic differences exist at the genus level, differential expression analysis for sequence count data (DESeq2) was used [23]. DESeq2 normalizes the data using size factor estimations, uses an empirical Bayesian approach to diminish dispersion, and generates multivariable negative binomial models.

All statistical analyses were performed using R V.3.1.2. Mean and standard deviations were used to describe continuous parametric data. Median and interquartile ranges were

used to describe continuous non-parametric data. Significance after correction for multiple hypothesis testing (Q-values) was defined as $Q<0.1$.

Results

Participant characteristics

Among the 66 participants enrolled in this study, the majority were women with a mean age of 55 ± 11.8 years (Table 1). The mean disease duration was 9.6 years, and there was a similar balance of patients with diffuse and limited cutaneous sclerosis. Most participants (84%) were taking immunosuppressants at the time of stool collection. No participants had received antibiotics during the four weeks prior to stool collection. No participants received antibiotics more than 2 times in the preceding 12 months prior to the stool collection. No participants were consuming probiotics during the stool collection.

Regarding GI symptoms, mean GIT 2.0 scores reflected moderate severity for the reflux, distension and constipation domains, as well as the total GIT 2.0 score (Table 3). Mean scores for the domains of fecal soilage, diarrhea, social functioning, and emotional well-being indicated mild symptom severity.

Thirty-five participants completed the DHQ II. The predominant reason for participants not completing the DHQ II was forgetting to do so on the day of the stool collection. The low ($N = 19$) and non-low ($N = 16$) FODMAP consumer groups were similar in terms of age,% female, body mass index (BMI), disease duration,% diffuse disease, smoking history, and% SIBO (Supplemental Table 1). There were no significant differences in GIT 2.0 scores including its subdomains between the low and non-low FODMAP groups (Table 3). However, scores for some of the domains (e.g., reflux, distension, diarrhea) were numerically greater in the non-low FODMAP group.

GI microbial composition and SSc-GI symptom severity

Increased GI symptom severity was associated with decreased alpha diversity (e.g., within subject diversity). For example, increased total GIT 2.0 score, increased distension score, and increased social functioning score (indicating worse social functioning) were each significantly associated with decreased alpha diversity based on the Shannon index ($P = 0.043$ for total GIT 2.0 score, $P = 0.039$ for distension score, $P = 0.018$ for social functioning score) ($N = 66$; Fig. 1). Increased diarrhea score was also associated with decreased alpha diversity based on the Chao1 and Faith's PD indices, and this relationship approached statistical significance ($N = 66$; $P = 0.079$ for Chao1 and $P = 0.072$ for Faith's PD) (Fig. 1). No relationships were observed between alpha diversity and scores for the fecal soilage, constipation and the emotional well-being domains.

In terms of overall microbial composition, the total GIT 2.0 score, as well as scores for the domains of diarrhea and social functioning, were significantly associated with alterations in beta diversity ($P = 0.048$, $P = 0.041$, $P = 0.032$, respectively) (Fig. 2). Similar to alpha diversity, no significant relationships were observed between beta-diversity and scores for fecal soilage, constipation and the emotional well-being domains of the GIT 2.0. There was a trend for a significant relationship between distension and beta diversity ($P = 0.077$).

Thus, altered GI microbial composition was associated with increased severity of certain GI symptom domains.

Differential abundance of microbial genera and SSc-GI symptoms

DESeq2 analyses were conducted to further explore differences in microbial composition and examine microbial genera with differential abundance in participants with greater GI symptom severity. Log fold change scores were computed for domains in which beta diversity was significantly different, including total GIT 2.0 score, diarrhea, and social functioning. Positive fold change scores indicated increased abundance of a specific genera in patients with increased symptoms; negative fold change scores indicated decreased abundance of a specific genera in patients with increased symptoms.

Increased total GIT 2.0 score was associated with increased abundance of genera typically deemed pathobiont (e.g., *Klebsiella, Enterococcus*) and decreased abundance of genera typically deemed commensal (e.g., *Clostridium, Coprococcus*) (Fig. 3). Interestingly, increased total GIT 2.0 score was also associated with greater abundance of *Lactobacillus*, a commensal species that is usually reduced in chronic inflammatory diseases [24]. Furthermore, as shown in Fig. 3, the most differentially abundant genera belong to the phylum Firmicutes, and the enriched genus (*Klebsiella*) belongs to the phylum Proteobacteria, which has been found to be a potential marker of gut dysbiosis and inflammatory disease states [25].

Similar findings were observed for the individual domains of diarrhea and social functioning. Increased abundance of the pathobiont *Enterococcus* and the commensal species *Lactobacillus* was associated with increased scores for the diarrhea domain, representing more severe diarrhea (Supplemental Fig. 1). Increased abundance of typical pathobionts (e.g., *Enterococcus* and *Escherichia/Shigella*), as well as increased commensal *Lactobacillus*, was associated with increased scores for the social functioning domain representing worse social functioning (Supplemental Fig. 2).

GI microbial composition and the low FODMAP diet

No significant differences in alpha diversity were observed between the low and non-low FODMAP groups ($P = 0.605$, $P = 0.534$, and $P = 0.729$ for chao1, Faith's PD, and Shannon index, respectively; Supplemental Fig. 3). Likewise, no significant difference in beta diversity was observed between the low and non-low FODMAP groups ($P = 0.264$; Supplemental Fig. 4).

The DESEq analysis demonstrated differences in the abundance of specific genera in the low versus non-low FODMAP groups. Compared with the low FODMAP group, the non-low FODMAP group had increased abundance of the purported pathobionts, *Enterococcus* and *Klebsiella* (Fig. 4).

Discussion

This study demonstrated that decreased microbial diversity (e.g., alpha diversity) is associated with more severe SSc-GI symptoms. Specifically, patients with less microbial

diversity reported worse GI morbidity and worse symptoms of distension and social functioning. Patients with increased GI symptom severity also exhibited differences in overall microbial composition (e.g., beta diversity) from those with less severe GI symptoms. Moreover, pathobiont genera (e.g., *Enteroccocus*, *Klebsiella*) were enriched in SSc patients with more severe GI symptoms, while typically commensal genera (e.g., *Clostridia*) were decreased. These findings are consistent with a prior smaller study [8] and provide additional evidence supporting the link between GI dysbiosis and the SSc-GI phenotype.

In the present study, the genus *Lactobacillus* (of the phylum Firmicutes) was enriched in patients with more severe GI symptoms. Previous studies of diverse SSc cohorts have reported increased abundance of *Lactobacillus* in SSc patients compared to unaffected controls [7,8,26]. Certain species in the *Lactobacillus* genus may have differing clinical effects and ecological roles in the GI microbiome [27]. For instance, one study showed that SSc patients with GI involvement had increased abundance of *Lactobacillus reuteri* compared to SSc patients without GI involvement and unaffected controls [28]. While *Lactobacillus* is considered a commensal genus in certain chronic inflammatory states, [27] the relationship between certain *Lactobacillus* species and clinical manifestations in specific autoimmune diseases is under investigation. A mouse model of GI microbial composition in systemic lupus erythematosus (SLE) found that increased abundance of *Lactobacillus reuteri* was associated with worsened autoimmunity, which was alleviated by a fiber-rich diet [28]. These findings suggest that whether certain species are commensal appears to be disease-dependent, and longitudinal studies are needed to determine whether increased *Lactobacillus* is the cause or impact of severe GI symptoms in autoimmune disease.

Moreover, as many commercially-available probiotic supplements contain *Lactobacillus*, these study findings suggest that a more personalized probiotic may be beneficial depending on the underlying autoimmune disease [29]. This could also explain why two small randomized controlled trials assessing the safety and efficacy of probiotic supplementation containing *Lactobacillus* for SSc failed to demonstrate a treatment effect on overall GI symptoms [30,31]. Notably, increased abundance of *Lactobacillus* has been associated with lower exposure to metabolites of mycophenolate mofetil in SSc, suggesting its relationship with alterations in drug metabolism [32]. Further studies are needed to explore the clinical relevance of increased or decreased abundance of specific microbial genera in treatment considerations for SSc.

In our study cohort, genera that are typically deemed pathobiont (e.g., *Klebsiella, Enterococcus*) were enriched in SSc patients with increased GI symptom severity. Increased abundance of these genera is associated with other chronic inflammatory disease states, including IBD, chronic kidney disease, and atopic asthma [33,34]. In fact, this genus has been associated with increased intestinal inflammation in mouse models of IBD and was found to be the most strongly enriched microbial species in the colonic mucosa of patients with Crohn's disease [35]. In addition, increased abundance of *Enteroccocus* species was associated with increased gut translocation and the development of autoimmunity in mouse models of SLE [36,37]. While altered GI microbial composition is associated with diverse autoimmune conditions, such as rheumatoid arthritis, type I diabetes, and IBD, [33] the

mechanism underlying the relationship between GI dysbiosis and clinical symptoms in SSc needs further investigation.

This study also explored the relationship between diet and SSc-GI symptoms. While a low FODMAP diet has been associated with GI symptom relief in other autoimmune conditions, such as IBD, [38] this study did not demonstrate any differences in GI symptoms between patients consuming a low versus non-low FODMAP diet. A recent systematic review of randomized controlled trials examining the effects of the low FODMAP diet in patients with IBD and functional gastrointestinal disorders reported inconsistent results for reduced GI symptoms and no significant differences in gut microbial composition [39]. In SSc, a systematic review of three studies examining the role of probiotics, low FODMAP diet, and individualized nutrition counseling reported improvements in patient-reported GI manifestations with probiotic treatment and a low FODMAP diet, but no improvement with customized dietary counseling. However, the studies included in this review were small, non-randomized studies that lacked control groups [40]. Thus, whether the low FODMAP diet improves GI symptoms in SSc remains unclear. Given the relatively small sample size of the present study and relatively low number of patients with significant SIBO and diarrheal symptoms, it is possible that a significant association was not detected due to inadequate statistical power. Larger, prospective studies are therefore needed to better understand the relationship between the low FODMAP diet and SSc-GI symptoms, particularly because this dietary approach can be burdensome for the patient, affect their psychosocial functioning, and may cause and/or exacerbate micronutrient deficiencies [41].

Although this study did not find differences in GI symptoms between the low and non-low FODMAP groups, there were several differences in the enrichment of bacteria between groups. For example, the pathobiont *Enterococcus*, which has been associated with autoimmunity in mouse models of SLE, [36,37] was enriched in the non-low FODMAP group of our study cohort. *Klebsiella*, a marker of GI dysbiosis, [25] was also enriched in the non-low FODMAP group. The relationship between FODMAP diet, microbial composition, and autoimmune conditions is under investigation. Previous studies have suggested that a low FODMAP diet may be associated with elevated abundance of bacteria associated with dysbiosis and decreased abundance of the commensal species *Bifidobacterium*, while high FODMAP diets are also associated with altered abundance of microbial species [42,43]. While these findings suggest that diet can potentially modify GI microbial composition, few studies have examined the specific species identified in the present study and their potential pathogenic role in SSc patients consuming a non-low FODMAP diet. Larger studies are needed to elucidate the relationship between dietary modifications and alterations in GI microbial composition.

The findings of the present study should be interpreted in the context of several limitations. The small sample size limits the generalizability of findings. Despite the sample size, significant associations were still observed, making it less likely that group differences were due to chance alone. Second, this study relied on self-report of dietary habits and GI symptoms. This methodology may introduce recall bias and measurement error associated with dietary questionnaires, as participants were asked to retrospectively recall food intake [44]. Future studies might consider alternative dietary assessment methods, such as food

diaries, 24-hour dietary recall, or dietary biomarkers [44]. Furthermore, confounding factors may affect the relationship between FODMAP consumption and GI symptoms as the volume, preparation, and texture of FODMAP foods may affect their digestibility and alter their associated symptoms. In addition, not all patients completed the dietary recall portion of this study (e.g., DHQ II survey). However, the baseline characteristics between those patients who completed the DHQ II and those who did not were reassuringly similar (Supplemental Table 1). Lastly, because this study was a cross-sectional analysis, it is unknown whether SSc-GI symptoms were driven by alterations in the microbiome or vice versa. To address this question, future prospective studies are needed to investigate how alterations in the microbiome affect GI symptoms in SSc over time.

This study also has some strengths. To our knowledge, this is the first study which performed an integrative analysis of diet, GI symptoms and the GI microbiome in SSc. Valid measures of GI symptoms and extensive dietary history were prospectively collected in a well characterized SS cohort. Multiple objective dimensions of GI microbiome were also explored, including species richness and composition, as well as the differential abundance of bacterial genera. The present findings may help inform the design of future clinical and translational studies aiming to modulate the GI microbial flora through dietary modifications.

Conclusions and future directions

In summary, SSc patients reporting more severe GI symptoms exhibited signs of GI microbial dysbiosis characterized by reduced species richness and altered microbial composition. While a low FODMAP diet was not associated with reduced symptom severity or altered microbial composition compared to a non-low FODMAP diet, certain pathobiont phylotypes were enriched in the non-low FODMAP group. Understanding the relationships between alterations in GI microbial composition and SSc may help identify new therapeutic targets and guide clinical and nutritional management. Larger, prospective studies are warranted to determine whether dietary changes and treatments targeting gut microbial alterations may reduce GI symptoms in SSc.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

SSc	systemic sclerosis

FODMAP	fermentable oligosaccharides, disaccharides, monosaccharides, and polyols
GI	gastrointestinal

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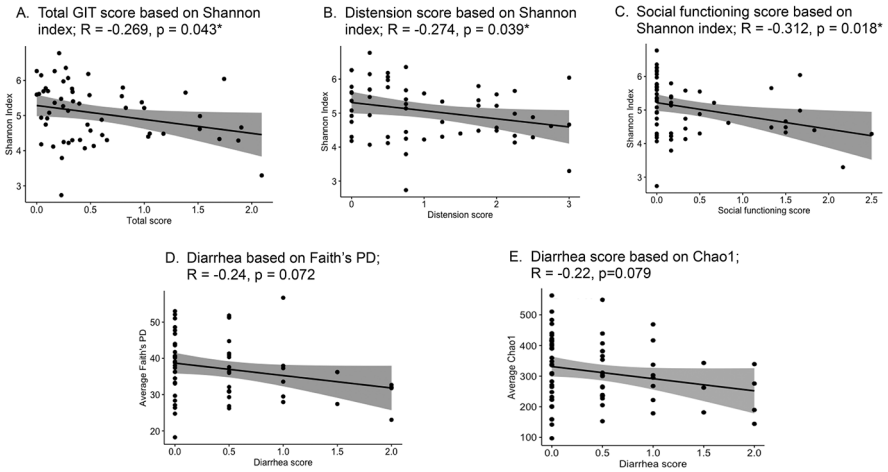

Fig. 1. The relationship between alpha diversity metrics and GI symptoms based on univariate analyses.

Decreased alpha diversity was associated with increased total GIT score (Fig. 1A), distension score (Fig. 1B), and social functioning score (Fig. 1C) according to the Shannon index and with increased diarrhea score according to Faith's PD (Fig. 1D) and Chao1 (Fig. 1E).

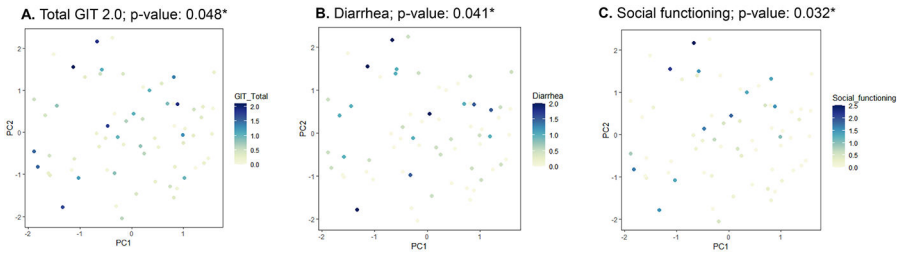

Fig. 2. Significant differences in beta diversity based on GI symptoms in all participants.

Beta diversity analyses were performed using robust Aitchison distance, and the differences between groups are visualized by principal coordinate analysis plots. Each dot represents an individual patient. Increased GIT symptom severity was associated with alterations in beta diversity for total GIT 2.0 (Fig. 1A), diarrhea (Fig. 1B), and social functioning (Fig. 1C). GI symptom severity is represented by a color gradient, with yellow indicating less severe symptoms and blue indicating more severe symptoms.

Note to Journal: If possible, color should be used for Fig. 2 in print.

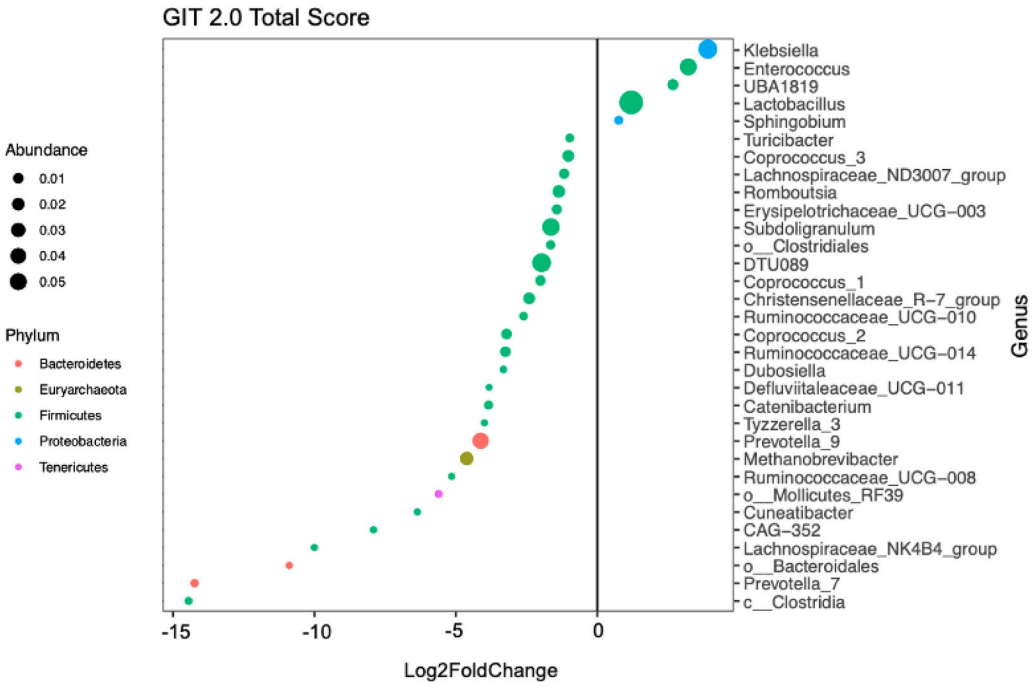

Fig. 3. Differential abundance of specific genera based on GIT 2.0 Total Score.

Positive fold change scores indicate genera with increased abundance in patients with increased GI symptoms. Negative fold change scores represent genera with decreased abundance in patients with increased GI symptom severity. For example, genera deemed pathobiont (e.g., *Klebsiella* and *Enterococcus*) were significantly more abundant in patients with increased GI symptom severity. The color of the circles signifies the phylum and the size represents the relative abundance.

Note to Journal: If possible, color should be used for Fig. 3 in print.

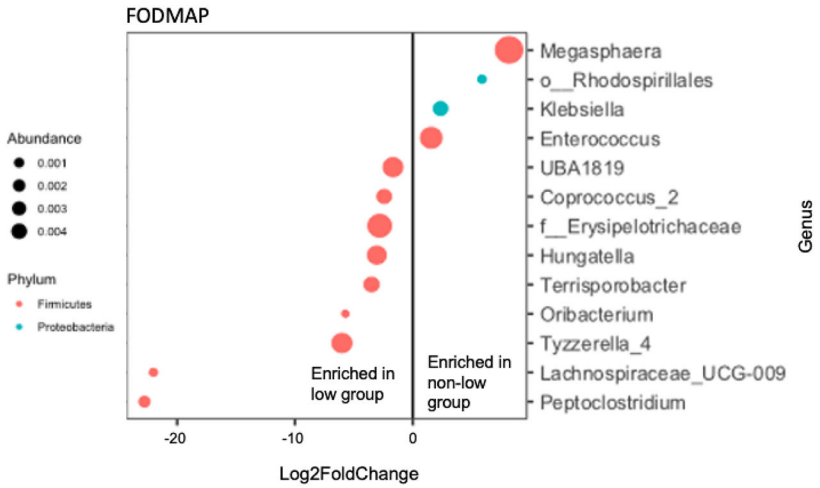

Fig. 4. Differential abundance of specific genera between low versus non-low FODMAP groups.

Positive fold change scores indicate genera with increased abundance in the non-low FODMAP group. Negative fold change scores represent genera with increased abundance in the low FODMAP group. For example, genera deemed pathobiont (e.g., *Klebsiella* and *Enterococcus*) were more abundant in the non-low FODMAP group. The circle color signifies the phylum and the size represents the relative abundance.

Note to Journal: If possible, color should be used for Fig. 4 in print.

Table 1

Patient characteristics of study cohort.

	Total *N* = 66
Age, Mean (SD)	55.4 (11.8)
Female, N (%)	56 (85%)
Limited cutaneous disease, N (%)	36 (55%)
Disease duration (first non-Raynaud phenomenon symptom)	
Mean (SD)	9.6 (8.6)
Median (IR)	7.1 (3.6, 12.5)
BMI, Mean (SD)	25.1 (4.2)
SIBO, N (%)	13 (20%)
HRCT defined ILD, N (%)	58 (91%)
MRSS (0–51), Mean (SD)	6.3 (6.1)
Ever smoker, N (%)	17 (26%)
PPI use, N (%)	44 (67%)
Scl-70 Ab positive, N (%)	19 (33%)
Centromere Ab positive, N (%)	13 (23%)
RNA Polymerase III Ab positive, N (%)	1 (4%)
Any current/prior immunosuppression, N (%)	54 (84%)
Low FODMAP	19/35 (54%)

ANA, anti-nuclear antibody; BMI, body mass index; FODMAP: fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; HRCT, high-resolution CT; MRSS, Modified Rodnan Skin Score; PPI, proton pump inhibitor.

Table 2

Examples of foods high in FODMAPs, along with potential low FODMAP alternatives.

FODMAP Component	High FODMAP Foods	Low FODMAP Alternatives
Fermentable oligosaccharides (e.g., galactans, fructans)	Artichoke, cabbage, Brussels sprout, garlic Rye and wheat cereals, pasta Watermelons, persimmons Legumes (e.g., chickpea, lentil)	Bok choy, carrot, eggplant, pumpkin, lettuce Gluten-free cereals Tomato
Disaccharides (e.g., lactose)	Milk Ice cream Soft cheeses (e.g., brie, burrata, feta) Yogurts	Lactose-free milk and yogurts Sorbet, gelato Hard cheeses (e.g., cheddar, gruyere)
Monosaccharides (e.g., fructose)	Apple, mango, pear High-fructose corn syrup sweeteners Fruit concentrate (e.g., fruit juices)	Banana, orange, strawberry, grapefruit Maple syrup
Polyols	Apple, apricot, lychee, plum, pear Mushroom, cauliflower Sweeteners (e.g., sorbitol, mannitol, “-ol” sweeteners)	Banana, orange, strawberry, grapefruit Bok choy, carrot, eggplant, pumpkin, lettuce Alternative sweeteners (e.g., sucrose)

Table 3

GIT symptoms as measured by GIT 2.0 score for all participants, and for those participants consuming a low versus non-low FODMAP diet.

GIT Symptoms Mean ± SD Median (IQR)	All participants (*N* = 66)	FODMAP Non-Low (*N* = 16)	FODMAP Low (*N* = 19)	P-value[#]
Total GIT 2.0	0.56 ± 0.54 [†]	0.49 ± 0.35 [*]	0.37 ± 0.40 [*]	0.349
	0.34 (0.65)	0.37 (0.33)	0.23 (0.40)	
Reflux	0.75 ± 0.65 [†]	0.67 ± 0.47 [†]	0.5 ± 0.38 [†]	0.245
	0.56 (0.75)	0.5 (0.53)	0.44 (0.56)	
Distension/Bloating	1.03 ± 0.91 [†]	0.89 ± 0.56 [*]	0.65 ± 0.84 [*]	0.343
	0.75 (1.5)	0.75 (0.75)	0.25 (0.94)	
Constipation	0.57 ± 0.63 [†]	0.44 ± 0.65 [*]	0.42 ± 0.51 [*]	0.917
	0.50 (0.75)	0.13 (0.56)	0.25 (0.75)	
Fecal Soilage	0.26 ± 0.59 [*]	0.19 ± 0.54 [*]	0.22 ± 0.55 [*]	0.854
	0 (0)	0 (0)	0 (0)	
Diarrhea	0.45 ± 0.58 [*]	0.5 ± 0.61 [†]	0.36 ± 0.45 [*]	0.449
	0.25 (0.5)	0.5 (0.63)	0 (0.88)	
Social Functioning	0.40 ± 0.63 [*]	0.33 ± 0.52 [*]	0.19 ± 0.41 [*]	0.363
	0.08 (0.5)	0.17 ± 0.38	0 ± 0.17	
Emotional Well-being	0.47 ± 0.79 [*]	0.35 ± 0.59 [*]	0.28 ± 0.47 [*]	0.679
	0.11 (0.56)	0.06 ± 0.5	0 (0.31)	

◆ severe to very severe symptom severity based on predetermined thresholds[18]

[*] none-to-mild symptom severity based on predetermined thresholds[18]

[†] moderate symptom severity based on predetermined thresholds[18]

[#] P-value indicates differences between low versus non-low FODMAP groups.