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Authors

Volkmann, Elizabeth
Hoffmann-Vold, Anna-Maria

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Gastrointestinal tract microbiota modifications in systemic sclerosis

Elizabeth R. Volkmann[1] 🆔, Anna-Maria Hoffmann-Vold[2] 🆔

Abstract

Systemic sclerosis (SSc) is a complex autoimmune disease of unknown etiology. Genetic factors are thought to collude with various environmental triggers to induce SSc and subsequently manifest various SSc disease phenotypes. Emerging evidence suggests that the microbiota of the gastrointestinal tract (GIT) may represent a key pathogenic participant in this disease state. Recent studies have demonstrated specific alterations in the GIT microbial composition in SSc patients, and this article reviews studies that have investigated the GIT microbiota in SSc patients. The focus of this article is to highlight the modifications in the GIT microbiota observed in SSc patients belonging to different cohorts and to demonstrate how these alterations may be associated with specific SSc features. This article presents the results of these SSc microbiota studies in the context of findings from microbiotic studies in other autoimmune states to explore similarities and differences across disease states affecting the immune system. Finally, this article provides insights into potential SSc therapies that target the GIT microbiota. Given the complexity and variability of the SSc disease state, any treatment aimed at modulating GIT microbiota will likely need to be coupled with additional interventions that target other SSc disease components.
Keywords: Systemic sclerosis, scleroderma, microbiota, gastrointestinal tract

Introduction

Systemic sclerosis (SSc) is a complex autoimmune disease characterized by alterations in the immune and vascular systems leading to fibrosis in multiple organs (1). Collective evidence suggests that the aberrant fibroproliferation observed in this disease state results from an interplay between genetic factors, environmental exposures, and epigenetic modifications (2). While specific genetic factors have emerged as strong candidates associated with disease (3), the exact role that environmental factors play in the pathogenesis of SSc remains elusive. Alterations in the gastrointestinal tract (GIT) microbiota may represent one of the key environmental factors contributing to the SSc disease state, as emerging evidence suggests that certain GIT microbiota are associated with specific SSc-related features (4, 5). The present review provides a comprehensive overview of the most recent research studies on the GIT microbiota in SSc. Specifically, this review describes notable GIT microbiota alterations in SSc within the context of our evolving understanding of the pathogenesis of this disease. This review also describes some important research questions that require further investigation in future research conducted on SSc microbiomes.

GIT Microbiota and health

The human GIT microbiota comprises microbial communities, their genome, proteins, and metabolites. Trillions of microbes (e.g., bacteria, viruses, archaea, and fungi) residing in the GIT affect a diverse range of physiological processes, including nutrient update, food metabolism, energy homeostasis, and immune system development (6, 7). The GIT microbiota also functions to create a resilient intestinal barrier (8), inhibit the colonization and proliferation of pathogenic organisms (8), and regulate host immune responses (9, 10).

Alterations in the GIT microbiota causes changes in its metabolic network, leading to perturbations in homeostasis (6). Disruption in the normal balance of gut microbiota (a.k.a. dysbiosis) has been associated with a plethora of disease states, including obesity (11), cancer (12), asthma (13), and autoimmune diseases, such as inflammatory bowel disease (14). Most prior studies have focused on studying bacteria, since the analysis of viruses and fungi is even more challenging. Recently, several groups, including ours, examined the role of gut microbiota in SSc patients by analyzing 16S-rRNA metagenomic sequencing (5). These studies, which are further discussed below, have predominantly assessed microbiota from stool specimens, with

ORCID iDs of the authors:
E.R.V. 0000-0003-3750-6569;
A.M.H. 0000-0001-6467-7422.

[1] University of California, Los Angeles, David Geffen School of Medicine, USA
[2] Oslo University Hospital, Oslo, Norway

Address for Correspondence:
Elizabeth R. Volkmann; University of California, David Geffen School of Medicine, Los Angeles, USA

E-mail: evolkmann@mednet.ucla.edu

the exception of our first study, which assessed microbiota from the human mucosal interface of the cecum and sigmoid regions of the large intestine (15). Collectively, these SSc microbiota studies revealed significant alterations in bacterial phylotypes between SSc patients and healthy controls and highlighted important associations between specific genera and SSc features (4, 5).

Factors affecting microbiota variations

Prior to discussing the unique features of the SSc microbiota, it is important to review the factors that influence variations in GIT microbiota. Understanding the external factors that shape the GIT microbiota is essential for interpreting the results of research studies in this area.

Table 1 summarizes some key external factors that affect GIT microbiota. Age is a central factor (16). In early life, the human microbiota undergoes ecological maturation, a process that is substantially influenced by various factors, including delivery type (e.g., vaginal versus cesarean section), breastfeeding, and antibiotic exposure (17). Studies have demonstrated that these factors can not only affect GIT microbiota in infancy but may also increase the risk of developing diseases, such as asthma and atopic diseases, later in life (18).

A study of 531 healthy infants, children and adults from Malawi, Venezuela and the US demonstrated that microbiota diversity was largely explained by age, with the greatest compositional differences being noted between infants and adults (16). The same study

found that the next most important factor contributing to microbiota diversity was culture (16). Adults from the US had distinct microbial compositional differences as compared to adults from Malawi and Venezuela. The latter observation illuminates the importance of other factors affecting GIT microbiota, such as diet. For example, increased abundance of *Prevotella* was observed in children consuming a high-fiber, plant-based diet in the rural African village, Burkina Faso (19), and in Malawi and Venezuela (16). In healthy US adults with diets rich in animal protein and saturated fats, *Bacteroides* was found in higher abundance (16).

Adopting specific dietary restrictions, as many of our SSc patients do, can affect GIT microbiota. One small study (N=21) of healthy participants demonstrated that adherence to a gluten-free diet for 4 weeks resulted in a number of bacterial taxonomic shifts within the patients (20). This study collected 9 stool specimens from each patient (1 at baseline, 4 during the gluten-free period, and 4 when they returned to their normal diet). The most remarkable taxonomic shift occurred for the family *Veillonellaceae* (from the class *Clostridia*), which significantly decreased during the gluten-free phase of the study.

In addition to diet, another study of 1135 Dutch participants found that a number of external factors contributed to inter-individual variations in microbial composition (21). These factors included medications, such as antibiotics, but also a variety of other classes of drugs (e.g., oral contraceptives, proton pump inhibitors,

anti-depressants, diuretics, anti-histamines, metformin, benzodiazepines, and anti-hypertensives) (21). Other notable factors affecting the microbiota were alcohol consumption, cigarette smoking, and sugar-sweetened soda consumption (21). In addition to the aforementioned factors, the mode of sampling and the mechanisms for transporting, processing, and preparing the sample for analysis may also affect the sequencing results across studies.

Taken together, the studies highlighted in Table 1, suggest that external factors affecting the GIT microbiota should be considered in research studies seeking to define the unique microbiota features of a particular disease state, including SSc. While it may be impossible to recruit controls for all these factors, efforts should be made to select control populations with similar demographics and diets. Studies with a large enough sample size may be able to soundly adjust for potentially confounding factors, such as use of immunosuppressive drugs and drug-related substances.

Taxonomic features of the SSc disease state

Phyla differences

Recent studies have identified GIT microbiota differences between SSc patients and healthy controls at multiple taxonomic levels. For instance, at the phylum level, our group discovered a decreased relative abundance of *Bacteroidetes* and an increased relative abundance of *Firmicutes* in SSc patients from University of California, Los Angeles (UCLA) and from Oslo University Hospital (OUH) compared with healthy

Main Points

- Accumulating evidence suggests that alterations in gastrointestinal tract microbiota exist in patients with systemic sclerosis and that certain alterations are associated with clinical features of this disease.

- Decreased abundance of specific bacterial species in the lower gastrointestinal tract of patients with SSc is associated with more severe gastrointestinal tract symptoms of systemic sclerosis and may represent a potential target for therapeutic intervention.

- Modifying the gastrointestinal microbiome through dietary manipulation, probiotic/prebiotic supplementation, and possibly fecal microbiota transplantation may improve patient outcomes, particularly for patients with systemic sclerosis-related gastrointestinal tract involvement.

Table 1. Overview of some key non-disease state factors that affect the GIT microbiota.

Factor	Example(s)
Age	Infants have a distinct microbiota as compared to adults (16).
Sex	Males and females have differential abundance of specific bacterial genera (70).
Diet	A plant-based diet is associated with increased *Prevotella* (62, 71).
	An animal-based diet is associated with increase *Bacteroides* (71).
Body mass index (BMI)	BMI is independently associated with GIT microbiota in children (72) and adults (73).
	GIT microbiota during the first 2 years has been found to predict BMI at age 12 (74).
Medications	In addition to antibiotics, a variety of medications can affect the GIT microbiota, including proton pump inhibitors (21).
Smoking	Smoking has been associated with decreased *Bifidobacteria* and *Lactococcus* and decreased microbial diversity (75).
Alcohol	Alcohol consumption is associated with dysbiotic changes in clinical and preclinical studies (76).

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controls (22). The ratio of *Firmicutes* to *Bacteroidetes* was most altered in the UCLA-SSc patients (Figure 1). Another small study from Italy (N=18) also found that members of the *Firmicutes* phylum were more abundant in SSc patients relative to the controls and that the relative abundance of *Bacteroidetes* was reduced in SSc patients (23).

Firmicutes and *Bacteroidetes* phyla represent the most common organisms in the human GIT microbiota, followed by *Actinobacteria, Fusobacteria,* and *Verrucomicrobia*, which are typically less abundant (24). Studies have demonstrated that alterations in the *Firmicutes* and *Bacteroidetes* ratio are associated with other disease states, such as obesity (25, 26) diabetes mellitus (27), and age (28).

We also found that the relative abundances of *Actinobacteria, Fusobacteria,* and *Verrucomicrobia* were significantly increased in the cecum of SSc patients relative to healthy controls using colonic lavage specimens (15). This finding has been observed in patients with inflammatory bowel disease (IBD), using a similar lavage sampling method (29).

Genera differences

Lower level taxonomic differences have also been observed in SSc patients relative to the controls (Table 2). In an analysis of colonic lavage specimens from 17 patients with SSc and 17 health age- and gender-matched controls, we found that SSc patients had decreased abundance of beneficial commensal genera, such as *Faecalibacterium*, *Clostridium*, and *Rikenella* (15). We also observed that SSc patients showed an increased abundance of pathobiont genera, including *Fusobacterium*, *Prevotella*, *Ruminococcus*, *Akkermansia*, and the uncommon γ-*Proteobacteria*, *Erwinia*, and *Trabsulsiella*. The term pathobiont is used to describe resident microbes that possess pathogenic potential and are associated with chronic inflammatory states (30). Pathobionts differ from microbes that cause acute infections because they are typically innocuous to the host under normal conditions (31). It is unlikely that a single pathobiont is fully responsible for the induction of a disease state and is more likely that the unique microbiota environment, in which the pathobiont resides, determines whether the pathobiont will exhibit pathogenic features. On the other hand, commensal microbes are considered symbiotic. Only in certain conditions (e.g. immunodeficiency or impaired function of the intestinal barrier) can commensal microbes cause pathology.

Genus-level differences were also observed in an analysis of stool specimens obtained from 34 patients with SSc (17 from UCLA, 17 from OUH) (22). In this study, the pathobionts *Fusobacterium* (UCLA), *Ruminococcus* (UCLA), and *Akkermansia* (UCLA) were enriched in SSc patients as compared to healthy controls. Increased abundance of *Ruminococcus* (32) and *Akkermansia* (23) has been observed in other SSc cohorts. Interestingly, these two genera have been implicated in the pathogenesis of Crohn's disease, an autoimmune disease that, like SSc, has both inflammatory and fibrosing features (33).

In contrast, the commensal genera, *Faecalibacterium* (UCLA), *Clostridium* (OUH), and *Bacteroides* (UCLA, OUH) were depleted in SSc patients as compared to healthy controls (22). Interestingly, the patients from OUH had a higher abundance of specific commensal genera, including *Faecalibacterium* and *Bacteroides*, as compared to the UCLA-SSc patients (22). This latter finding demonstrates that disease-associated microbiota alterations are likely mediated by the external factors described above, including diet.

Both of our prior studies demonstrated an increased abundance of *Lactobacillus* in SSc patients relative to their controls (15, 22). This finding has been observed in other cohorts,
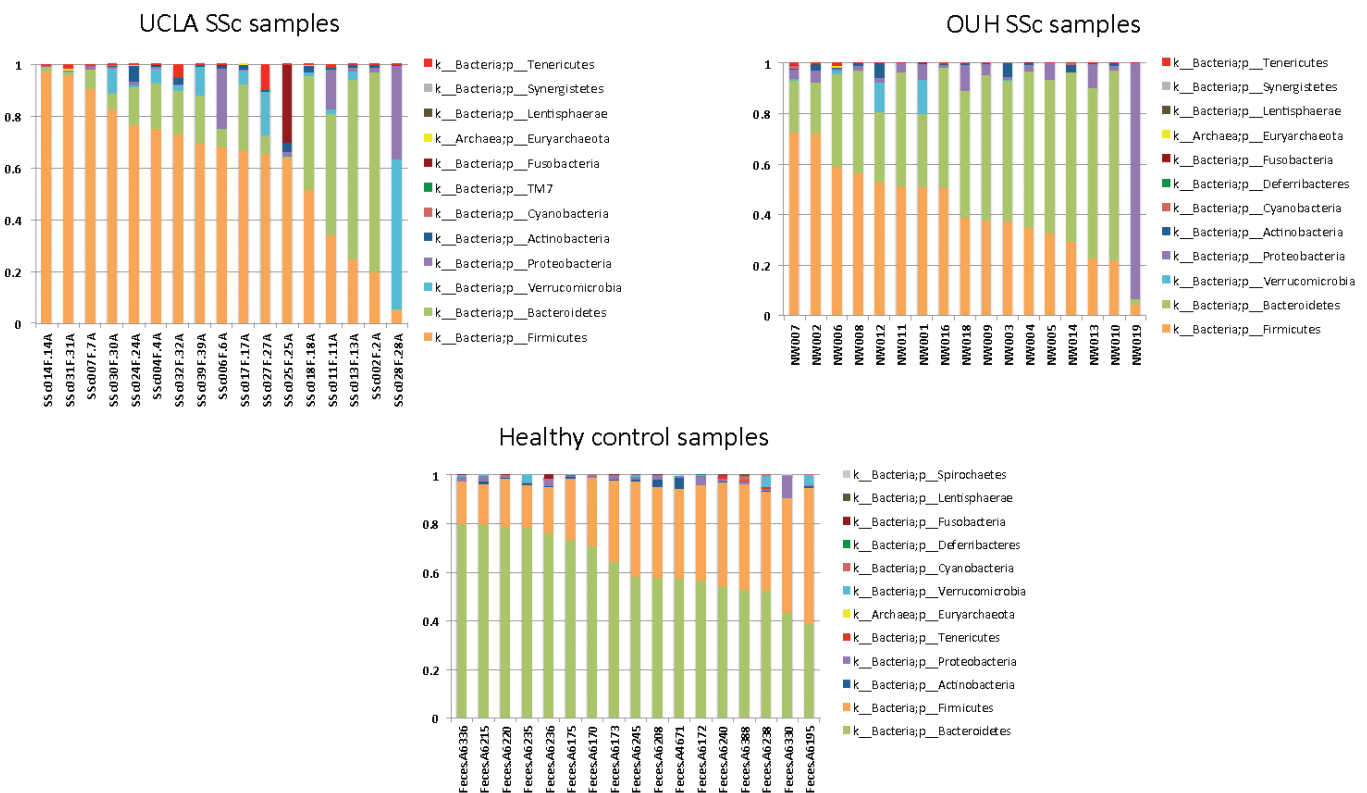
Figure 1. Microbial composition at the phylum level in UCLA-SSc samples (top left), OUH-SSc samples (top right), and healthy samples (bottom left). Color coding specific to each phylum is shown.
Reproduced with permission from BMJ Open Gastroenterol (22).

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Table 2. Taxonomic differences associated with the SSc disease state identified in at least 2 independent SSc cohorts.

Bacterial taxa	Abundance relative to controls	Cohort location	Cohort N
Lactobacillus	Increased	Los Angeles, CA, USA (15, 22)*	17
		Oslo, Norway (22)	17
		Lund, Sweden (34)	98
		Piacenza, Italy (23)	18
		Rome, Italy (32)	66
Ruminococcus	Increased	Los Angeles, CA, USA (15, 22)*	17
		Rome, Italy (32)	66
Akkermansia	Increased	Los Angeles, CA, USA (15,22)*	17
		Piacenza, Italy (23)	18
Bifidobacterium	Increased	Los Angeles, CA, USA (15)	17
		Piacenza, Italy (23)	66
Blautia	Increased	Los Angeles, CA, USA (15, 22)*	17
		Piacenza, Italy (23)	66
Prevotella	Decreased	Piacenza, Italy (23)	18
	Increased	Rome, Italy (32)	66
		Los Angeles, CA, USA (15)	17
Clostridium	Decreased	Los Angeles, CA, USA (15)	17
		Oslo, Norway (22)	17
		Rome, Italy (32)	66
		Lund, Sweden (34)†	98
Bacteroides	Decreased	Los Angeles, CA, USA (22)	17
		Oslo, Norway (22)	17
Faecalibacterium	Decreased	Los Angeles, CA, USA (22)	17
		Oslo, Norway (22)	17
		Lund, Sweden (32)‡	98
		Piacenza, Italy (23)‡	18

*Increased in both stool and colonic lavage samples from the same patients (15, 22).
†In the Swedish cohort (34), the family *Clostridiaceae* was decreased in SSc. This family contains the genus *Clostridium*.
‡In the Swedish (34) and Italian cohorts (23), the species *Faecalibacterium prausnitzii* was decreased in SSc. It is the sole known species of the genus *Faecalibacterium*.

including a relatively large cohort in Sweden (N=98) (34) and a smaller cohort (N=18) in Italy (23). Low abundance of *Lactobacillus* has been associated with chronic inflammatory states (35), however, in SSc, this genus appears to be present in higher abundance relative to healthy controls. It is unclear whether the finding of increased abundance of *Lactobacillus* has any effect on the pathogenesis of SSc, but given the association of *Lactobacillus* with GIT motility (36), further investigation of this bacteria in relation to SSc is needed.

Bellocchi et al. (37) discovered an increased abundance of the genus *Desulfovibrio* among SSc patients in Italy. This genus has been implicated in the pathogenesis of IBD (38) and was also elevated in the UCLA-SSc patients as compared with the OUH-SSc patients (22). In the Italian study, which included 59 SSc patients, *Desulfovibrio* was associated with increased GIT symptoms (37). Increased abundance of this genus was also correlated with higher levels of two metabolites, alpha- Nphenylacetyl-l-glutamine and 2,4-dinitrobenzenesulfonic acid (37). The

significance of the latter association is unknown, but it could suggest that metabolites play a key role in moderating the relationship between microbiota alterations and clinical symptoms.

Species-level differences

A few studies have detected species-level differences in SSc patients. For example, two studies found that SSc patients had a decreased abundance of the commensal species, *Faecalibacterium prausnitzii* (23, 34). In the Swedish cohort, patients underwent testing with a dysbiosis indicator (GA-map™ Dysbiosis Test), which evaluates the presence and abundance of 54 bacterial species or clades. The test algorithm was developed and validated using a Scandinavian control population to identify dysbiosis in adults (39), and in the Swedish SSc cohort, 76% had some degree of dysbiosis with 25% found to have severe dysbiosis (34).

Notably, even patients with early disease showed signs of dysbiosis (34). A similar proportion of patients with a disease duration of less than 2 years from the onset of the first non-Raynaud's symptom of SSc had dysbiosis, as compared to those with long-standing SSc (72% versus 76%, respectively). In our longitudinal study of SSc patients at UCLA, we found that although a longer disease duration is associated with increasing GIT symptoms over time, microbiota alterations existed even in early SSc patients (40). These findings suggest that dysbiosis may not necessarily be the result of the intestinal stasis that often evolves over the course of SSc. Instead, dysbiosis may be one of the key drivers of dysmotility. Emerging evidence suggests that alterations in GIT microbiota directly affect GIT motility (41), and that the ingestion of certain species can actually promote motility either directly or through their metabolites (36). Future studies of the SSc microbiome should include an objective measure of motility to further evaluate the connection between GIT motility and dysbiosis in SSc.

Relating taxonomic features of SSc with disease manifestations

SSc-GIT involvement

The studies above provide substantial evidence that alterations exist in the SSc-GIT microbiota. However, the question of how these alterations affect the pathogenesis of this disease and correlate with SSc symptoms still remains. We and others have attempted to evaluate the relationship between specific bacterial taxa and SSc manifestations. In terms of GIT symptoms, there is evidence that increased abundance of certain genera is associated with a reduction in GIT symptoms (Table 3). For instance, increased

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abundance of *Bacteroides fragilis* in both the cecum and sigmoid colon (15) was associated with decreased bloating/distension, decreased diarrhea, and decreased total GIT symptoms as measured by the University of California, Los Angeles Scleroderma Clinical Trial Consortium Gastrointestinal Tract Instrument (UCLA GIT) 2.0 (42). In our longitudinal study of SSc patients, in which we sampled stool microbiota every 3 months over the course of 1 year, we discovered that the abundance of *Bacteroides fragilis* was consistently associated with decreased GIT symptoms over time (40). The latter study provides further evidence that the association between *Bacteroides fragilis* and GIT symptoms observed in our first cross-sectional study also persists with time. *Bacteroides fragilis* appears to play a protective role in other disease states, including IBD (43, 44).

Conversely, increased abundance *Fusobacterium* was associated with increased constipation, increased bloating/distension, and increased total GIT symptoms (15) (Table 4). Various *Fusobacterium* isolates have emerged as compelling candidates for perpetuating inflammation in IBD (45, 46) and colorectal cancer (47, 48). A large study of over 2000 participants from multiple centers across Europe found that *Fusobacterium* abundance could be used to help discriminate between IBD and non-IBD patients (49).

Increased abundance of *Clostridum* was associated with decreased constipation in the cecum of SSc patients (15). In addition, increased levels of *Clostridium* in the stool was associated with decreased GIT symptoms (for the total GIT score and the bloating/distension domain of the GIT 2.0 [42]) in SSc patients from UCLA and OUH (22). Increased *Lactobacillus* abundance was associated with decreased constipation in these two cohorts (22).

On the other hand, increased *Prevotella* abundance was associated with increased diarrhea and increased distension/bloating. Historically, *Prevotella* species are considered commensal bacteria given their prevalence at multiple sites in humans; however, recent evidence suggests that increased abundance of *Prevotella* species at specific mucosal sites is associated with both local and systemic inflammation (50). In rheumatoid arthritis (RA), for instance, *Prevotella copri* was associated with disease severity in patients with new onset RA (51). Moreover, oral adminis-

tration of *Prevotella melanogenica* in humanized HLA-DQ8 mice immunized with CII resulted increased GIT inflammation and RA onset of increased severity (52). As demonstrated in Table 2, SSc studies have found both increased abundance and decreased abundance of *Prevotella* in SSc patients relative to the controls. This disparity highlights a need to look more closely at species-level differences, as some members of this genus may be higher/lower in SSc and only certain bacteria may possess pathogenic potential.

SSc-ILD
Patients with specific SSc features may possess unique microbiotic alterations. In the Swedish cohort, GIT dysbiosis was substantially more prevalent in SSc patients with interstitial lung disease (ILD) than without ILD (34). This analysis did not adjust for potentially confounding variables, such as prior or current immunosuppression use. However, in a subgroup analysis of this cohort, there was no difference in the presence of dysbiosis between SSc patients who were receiving immunosuppression versus those who were not (34). Future studies are needed to assess the impact of immunosuppression on SSc microbiota given prior preclinical and clinical research in this area. For example, cyclophosphamide administration in mice reduced the diversity and shifted the microbiota composition toward a reduction in *Bacteroidetes* (53). Furthermore, Bellocchi et al. (37) found that SSc patients receiving one or more SSc therapies had altered beta diversity as compared to those receiving no therapy.

Supporting the ILD findings from Andreaasen et al. (34), our group found a greater extent of dysbiosis in the UCLA-SSc patients as compared to the OUH-SSc patients. Further, we found that the UCLA-SSc patients had a much higher prevalence of ILD than the OUH-SSc patients (70.6% versus 47.1% of patients, respectively) (22). The two groups were otherwise similar in terms of their baseline disease features (e.g., age, gender, body mass index, auto-antibody profiles, disease duration, presence of diffuse cutaneous disease, use of immunosuppression, and use of prednisone). Larger studies are currently underway to assess the impact of ILD presence and severity on the microbiota in SSc. To our knowledge, no studies have, to date, examined the relationship between lung microbiota and GIT microbiota in SSc.

Clinical and research consequences
The studies highlighted above have taken the first initial steps to understand the GIT microbiota in SSc (15, 22, 23, 32, 34, 37, 40). These studies show that specific bacterial taxa are altered in the SSc-GIT microbiome relative to healthy control subjects. Evidence also seems to indicate that changes

Table 3. Bacterial taxa associated with decreased SSc-GIT symptoms.

Bacterial taxa	SSc-GIT symptoms (42)
B. fragilis	Bloating/Distension, Diarrhea, Total GIT symptoms (15)
Clostridium	Constipation (15)
SMB53 from the *Clostridiaceae* family	Bloating/Distension (22)
Blautia	Total GIT symptoms (22)
Clostridium	Total GIT symptoms (22)
	Bloating/Distension (22)
Lactobacillus	Constipation (22)

Table 4. Bacterial taxa associated with increased SSc-GIT symptoms.

Bacterial taxa	SSc-GIT symptoms (42)
Fusobacterium	Constipation, Bloating/Distension, Total GIT symptoms (15)
Actinobacillus	Constipation, Bloating/Distension (15)
Actinomyces	Total GIT symptoms (22)
Ruminococcus	Constipation, Total GIT symptoms (22)
Dorea	Total GIT symptoms (22)
Parabacteroides	Constipation (22)
Undefined genus from *Enterobacteriaceae* family	Constipation (22)
Prevotella	Diarrhea, Bloating/Distension (22)
Sutterella	Bloating/Distension (22)

in microbiota composition are present early in the course of the disease, although more extensive work is needed to confirm this observation. In addition, associations identified between specific taxa and SSc-GIT symptoms suggest that changes in microbiota composition may cause or perpetuate inflammation/fibrosis in these patients. Understanding the pathophysiological effects of altered GIT microbiome composition in SSc could potentially lead to the development of novel therapeutic strategies. For example, an effective therapeutic approach targeting microbiota may involve introducing an intervention that counteracts dysbiosis, attempts to selectively eradicate the pathobiont species, or possibly augments the abundance of commensal species that reduce inflammation.

To advance this field in SSc, more research is needed to understand how the disease itself affects the GIT microbiome in SSc. The pathology of the GIT tract (e.g. smooth muscle atrophy, collagen deposition, vascular changes, etc.) in SSc may have a substantial impact on GIT microbiome composition. Dysbiosis may not only be a *driver* of disease progression, but may also be a *consequence* of disease progression in SSc. To adequately address this research question, longitudinal studies are needed to evaluate how the GIT microbiota changes evolve in SSc as clinical symptoms and manifestations. In our longitudinal microbiota analysis, we found no change in the presence or abundance of bacterial taxa over the course of 1 year within a small group of patients with SSc (40). Longer studies are needed, especially those which focus on the long-term follow up of patients with very early SSc, in order to understand the likely bi-directional relationship between GIT microbiota and SSc disease progression.

SSc microbiota-based therapeutics
Targeting the GIT microbiome with therapeutics could help manage SSc symptoms and could even have potential disease-modifying effects. GIT microbiome-based interventions have been studied in other autoimmune disease states and may play a future therapeutic role in SSc.

Antibiotic therapy
Antibiotics are frequently employed to acutely manage lower GIT symptoms in SSc (54). However, it is unknown how repeated cycles of antibiotics can affect GIT microbiota over time in SSc. Recent studies suggest that multiple courses of antibiotics can lead to dysbiosis (55, 56). Furthermore, the use of broad spectrum antibiotics, often employed in the management of SSc-GIT symptoms, can cause structural changes in the GIT microbiome that increase the risk of developing opportunistic infections from *Clostridium difficile* and other enteric pathogens (57). Therefore, the long-term risks associated with recurrent broad spectrum antibiotic use in SSc could potentially outweigh the short-term benefits of this approach.

Fecal microbiota transplantation
Fecal microbiota transplantation (FMT) theoretically acts to replace the entire GIT microbiome in the hope of correcting dysbiosis. This approach is now an accepted and effective therapeutic option for patients with treatment-refractory *C. difficile* infection (58, 59). In autoimmune disease, FMT has been studied extensively in IBD, particularly in patients with active ulcerative colitis (UC). A recent Cochrane review assessed the efficacy of FMT in the treatment of IBD and concluded that although FMT may increase the proportion of patients achieving clinical remission UC, the quality of the few studies reviewed was low and no studies assessed the long-term maintenance of remission in IBD patients (60). In addition, there were concerns about serious adverse events in the FMT-treatment group, including infections with *C. difficile* and *cytomegalovirus*, as well as small bowel perforation. A small pilot study of FMT over 16 weeks in patients with SSc was recently conducted at OUH, and it demonstrated changes in relative abundance of fecal microbiota and reduced lower GI-symptoms in the FMT group (N=5) relative to the placebo group (N=4) (61). A larger trial on FMT in SSc is planned in the future. However, numerous questions regarding the safety of this approach, the optimal route of administration, frequency of

applications, preparation protocol for the donor stool sample, and the antibiotic regimen for the recipient prior to FMT remain to be answered.

Dietary modifications
As discussed earlier, dietary patterns have a tremendous impact on GIT microbiota (62). Manipulating the diet represents a promising therapeutic strategy for ameliorating dysbiosis and may have lower associated risks as compared with the aforementioned approaches. A landmark study demonstrated that short-term consumption (5 days) of an entirely plant-based diet or animal-based diet led to rapid shifts in the GIT microbiota (63). In this study, the plant-based diet was comprised of vegetables, fruits, grains and legumes, while the animal-based diet was comprised of meats, eggs, and cheeses. Consumption of the animal-based diet resulted in more changes in species-level bacterial phylotypes. Interestingly, the one subject who was a vegetarian and was assigned to the animal-based diet group showed a decrease in the abundance of *Prevotella* during the consumption of an animal-based diet (63). Dietary modifications could also potentially affect a patients' health in other positive ways in terms of reducing symptoms of gastroesophageal reflux or lowering the risk of cardiovascular disease. Future studies that assess the effects of diet on GIT microbiota and health outcomes in SSc are needed.

Probiotics and prebiotics
Probiotics are live microorganisms in food or supplements that are thought to be present in sufficient quantities to reach the lower GIT in an active state. They are primarily composed of *Bifidobacterium* and *Lactobacillus* species. Studies have found that probiotics do not consistently colonize the host GIT (64) and can actually impair the host GIT microbiome from returning to its normal state following a course of antibiotics (65). A number of patients with SSc consume probiotics, even though only two studies have investigated their safety and efficacy in this disease state as described further below (Table 5).

Table 5. Clinical trials assessing the safety and efficacy of probiotics in SSc.

Study	N	Probiotic	Design	Duration	Outcome
Frech et al. 2011 (66)	10	Align (*Bifidobacterium infantis;* 109 CFU per capsule) or Culturelle (*Lactobacillus* GG; 109 Colony-forming units (CFU) per capsule	Open-label	8 weeks	Improvement in total GIT 2.0 score and 3 individual domains (reflux, bloating, emotional)
Marighela et al. 2019 (67)	73	*Lactobacillus paracasei, Lactobacillus rhamnosus, Lactobacillus acidophillus* and *Bifidobacterium lactis;* 109 CFU per capsule	RCT	8 weeks	No improvement in GIT 2.0 scores; decrease in circulating Th17 cells in probiotic group; no difference in HAQ-DI, circulating Th1, Th2, or regulatory T cells between groups

GIT 2.0: UCLA Scleroderma Clinical Trial Consortium Gastrointestinal Tract (UCLA SCTC GIT 2.0); HAQ-DI: Health Assessment Questionnaire-Disability Index.

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A small, open-label study administered a probiotic comprised of either *Bifidobacterium* or *Lactobacillus* to 10 patients with SSc with moderate to severe bloating (66). Over the course of 2 months, patients reported improved GIT symptoms, however, without a control group, this study could not properly test for a treatment effect. A more recent randomized, placebo-controlled study (N=73) found that a 2-month course of a probiotic (*Lactobacillus paracasei, Lactobacillus rhamnosus, Lactobacillus acidophilus* and *Bifidobacterium lactis*, 109 colony-forming units per capsule) did not improve GIT symptoms in SSc patients, although a reduction in circulating Th17 cells was noted in the probiotic-treated arm (67).

Prebiotics are non-digestible oligosaccharides, such as inulin, that possess the potential to stimulate the growth of selective and beneficial GIT bacteria. The very nature of their chemical composition allows them to reach the large intestine unabsorbed, where they can then undergo fermentation by specific bacteria into small chain fatty acids and lactate (68). Animal studies have demonstrated that ingestion of prebiotics has a greater impact on GIT microbiota than probiotics (69). Future studies are needed to assess the effects of prebiotics on SSc microbiota.

Conclusion

SSc is a complex and incurable autoimmune disease. While over the last 2 decades the therapeutic options for treating SSc have increased, these therapies largely target symptoms of the disease and do not consistently modify the course of the disease (2). Exploring the GIT microbiota in SSc represents a promising avenue of clinical investigation, which could potentially reveal new treatment targets. Understanding how the GIT microbiome evolves over the course of the disease could also shed light on the pathogenesis of SSc and may lead to the discovery of biomarkers that can predict the development of specific SSc features (e.g., ILD). Future collaborative research efforts in this area are needed to advance microbiota research in SSc.

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