Gut microbiome profiling in systemic sclerosis: a metagenomic approach

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Abstract

Objective

The early gastrointestinal (GI) manifestation of systemic sclerosis (SSc) suggests a possible GI microbiota engagement in the pathophysiology and/or progression of SSc. Previous studies have revealed dysbiosis among Caucasian SSc patients. This study extends these findings to Asian SSc patients.

Methods

Adult SSc patients, stratified according to 1) on immunosuppressive (On-IS) drugs or 2) no immunosuppressive drugs (No-IS), and age-and-sex-matched healthy controls (HC) were recruited. Metagenomic sequencing of stool DNA was compared between SSc patients and HC, and between SSc (On-IS) and (No-IS) patients. Alpha and beta-diversity, taxonomic and functional profiling were evaluated.

Results

Twenty-three female SSc patients (12 On-IS; 11 No-IS; 5 diffuse and 18 limited SSc subtype) and 19 female HC, with median age of 54 years and 56 years, respectively, were recruited. Median SSc disease duration was 3.3 years. Alpha diversity was significantly higher in SSc versus HC ($p=0.014$) and in SSc (No-IS) versus HC ($p=0.006$). There was no significant difference in beta diversity between SSc and HC ($p=0.307$). At the phyla level, there were significantly increased abundance of Firmicutes and Actinobacteria in SSc versus HC, and reduced abundance of Bacteroidetes (all $p<0.001$). At the species level, there were significantly increased abundance of several Lactobacillus, Bifidobacterium, and Coprococcus species in SSc, and increased abundance of Odoribacter, Bacteroides and Prevotella species in HC. KEGG pathway analysis demonstrated distinct differences between SSc versus HC, and between SSc (No-IS) and SSc (On-IS).

Conclusion

Using metagenomic sequencing, our study further underlines distinct alterations in microbiota profiling among Asian SSc patients.

Key words

systemic sclerosis, microbiota, gastrointestinal, Asian

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Introduction

The gastrointestinal (GI) tract is the most common internal organ manifestation in systemic sclerosis (SSc), affecting up to 90% of patients with SSc (1). This significantly impacts patients' quality of life and mortality (2). As GI involvement is an early manifestation of SSc (3), the GI microbiome may play a critical role in the pathogenesis or progression of SSc and its GI manifestations. Accumulating evidence suggests that the GI microbiome of patients with SSc are altered compared to healthy individuals (4). For instance, the commensal bacteria genus Lactobacillus was found in greater abundance and the genus Faecalibacterium in decreased abundance in SSc patients compared to healthy controls (HC) (5-9). Alterations were also detected in patients with more severe compared to less severe GI symptoms, with greater abundance of Akkermansia muciniphila (9), Fusobacterium (6) and Prevotella (7, 9). Majority of studies to date are descriptive and based on the 16S rRNA gene amplification approach which has technical limitations, with resolution reported largely up to the genus level. The choice of primer, PCR biases and the choice of polymerase may affect the results of these studies (4, 10). Shotgun metagenomic sequencing approach is able to overcome these technical limitations, yet limited data is available for SSc (11). In addition to a more comprehensive taxonomic analysis to species and strain level, shotgun metagenomic sequencing enables elucidation of the functional implications of the observed microbiome alterations (11), which our study will address.

There is currently a knowledge gap of the GI microbiome profile in treated versus treatment-naïve patients with SSc. The GI microbiome is able to shape the immune system to maintain homeostasis in healthy individuals or promote inflammation when homeostasis of various T-cell populations is disrupted. In turn, the GI microbiome may modulate the treatment response to immunotherapies. In cancer-bearing mice, the GI microbiome was found to modulate the immunosuppressive effects of cyclophosphamide, a drug commonly used to treat patients with SSc (12). Cancer-bearing mice which were raised in germ-free environment or had been treated with antibiotics to kill gram-positive bacteria showed reduction in peripheral T-helper 17 cells response and their tumours were resistant to cyclophosphamide, compared to those raised in normal environment or untreated cancer-bearing mice (12). Preliminary findings from Hoffman-Vold *et al*. suggested that patients with early SSc who had been treated with cyclophosphamide or mycophenolate mofetil showed altered immune recognition of specific gut bacteria as compared to treatment-naïve early SSc patients (13).

Another knowledge gap is that dysbiosis in SSc has been described in predominantly Caucasian populations, but not in SSc patients of Asian ethnicity. Studies have revealed that heterogeneity in SSc disease manifestations, survival and autoantibody frequencies exist in relation to ethnicity (14, 15), with worse prognosis seen among Asians (16, 17). It is therefore important to understand whether similar disease specific unique dysbiosis occurs in an Asian population beyond dietary influences on the GI microbiome.

Therefore, in this study we aimed to determine the GI microbiome profile using shotgun metagenomic sequencing in an Asian population of SSc patients compared to healthy controls, stratified according to whether patients were on immunosuppressive medications.

Methods

Study population

SSc patients were enrolled from the Scleroderma clinic at Singapore General Hospital, Singapore. Eligibility criteria were adults 18 years-old and older, and SSc fulfilling the American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) 2013 SSc classification criteria (18). Exclusion criteria were (i) on antibiotics or probiotics 30 days prior; (ii) existing infections; (iii) females who were lactating or pregnant; (iv) inflammatory bowel disease or other GI diseases; (v) malignancy and (vi) inability to provide informed consent.

Healthy controls (HC) recruited among the health-care worker community and via advertisements around the hospital campus, were age and gender-matched to the SSc patients. Eligibility criteria were adults 18 years-old and older, did not have malignancy or gastrointestinal diseases, and were not taking regular proton pump inhibitors, pro-motility or anti-diarrheal medications. Adults with stable chronic diseases such as hypertension and dyslipidaemia were eligible. Females who were lactating or pregnant and those who were unable to provide consent were excluded.

Written informed consent was provided by all patients and healthy controls. Ethics approval was obtained from the institutional review board of the participating centre. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Data collection

Biodata such as age, sex, ethnicity, weight, height, co-existing medical illnesses and current medications taken were collected from SSc patients and HC. The types of immunosuppression medications, proton pump inhibitors, pro-motility, anti-diarrheal medications taken by SSc patients were recorded. Presence of SSc organ involvement and serology were recorded for each patient.

Gastrointestinal tract symptoms

GI tract symptoms in SSc patients were assessed using the University of California, Los Angeles Scleroderma Clinical Trial Consortium Gastrointestinal Tract 2.0 (UCLA SCTC GIT 2.0). Severity of GI symptoms in 7 categories (reflux, distension, faecal soilage, diarrhoea, constipation, social functioning, and emotional well-being) were assessed by patients' self-rating. A higher GI tract score indicated worse GI symptoms.

Specimen collection and processing

Prior to sample collection, SSc patients were instructed to withhold antacids, proton-pump inhibitors, laxatives, anti-motility and anti-diarrheal medications for 48 hours. Fresh stool samples from SSc patients and healthy controls were collected using sterile containers during their study visit or from their preferred location using a courier service and transported to the laboratory in ice-pack within 6 hours of sample collection for processing. After processing, samples were stored at -80°C until further analysis.

DNA was extracted from all the stool samples in one batch using QIAamp® Fast Stool Mini Kit simultaneously. 250 to 300 mg of stool sample, together with zirconium/silica beads (0.1mm and 1.0 mm respectively), was added to InhibitEx buffer (Qiagen) and homogenised in the QIAgen Tissue Lyser. The obtained supernatant was enzymatically digested by proteinase K (Qiagen) in Buffer AL. 96–100% ethanol was added and the released DNA was absorbed onto QIAamp spin columns (Qiagen). After extensive washing with AW1 and AW2 Buffers, the retained DNA was eluted using ATE Buffer. RNase A (10 mg/ml) was added to the eluted DNA sample to remove 18S and 23S rRNA. Nucleic acid concentration was quantified by measuring the absorbance at 260 nm using a NanoDrop spectrophotometer, and nucleic acid quality was checked using gel electrophoresis. Purified DNA was stored at -80°C prior to further downstream application.

Alpha and beta diversity

Alpha and beta diversity analyses were performed in MEGAN v. 6.17.0. Alpha diversity, a measure of the richness and evenness of microbiome composition of individuals in a group, was determined by the Shannon-Weaver index. Beta diversity, a measure of the differences in microbial composition between groups, was determined using the Bray-Curtis dissimilarity measure and 999 permutations were performed per analysis. Principal coordinates analysis was performed to visualise the resulting distance matrix between groups. Statistical significance was determined using beta dispersion.

Metagenomic sequencing

Library preparation was performed according to Illumina TruSeq Nano DNA sample preparation protocol. The samples were sheared on a Covaris E220 to ~450bp, following the manufacturer's recommendation, and uniquely tagged with one of Illumina TruSeq HT DNA dual barcode combination to enable sample pooling for sequencing.

Finished libraries were quantitated using Promega's QuantiFluor dsDNA assay and the average library size was determined on an Agilent Tapestation 4200. Library concentrations were then normalised to 4nM and validated by qPCR on a QuantStudio-3 real-time PCR system (Applied Biosystems), using the Kapa library quantification kit for Illumina platforms (Kapa Biosystems). The libraries were then pooled at equimolar concentrations and sequenced on the Illumina HiSeq2500 platform at a read-length of 250bp paired-end.

Taxonomic profiling of metagenomic analysis

Raw paired-end reads were trimmed using BBMap/bbduk v. 38.84 with quality cut-off of 20. Reads <20 bp were discarded. Trimmed reads were mapped against human reference genome GRCh38 in Bowtie 2 v. 2.4.1 with "very-sensitive-local" sensitivity setting. Mapped reads were considered as host reads, and unmapped reads produced by "-un-conc" setting were considered as non-host reads, which were then assigned to microbial taxa using Kaiju v. 1.7.3 taxonomic classifier with "nr_2019-11-22" database. MEGAN6 was used to convert the resulting taxonomic classification from Kaiju into abundance normalised against smallest library size of the sequenced samples.

Functional profiling of metagenomic analysis

For functional annotation, protein sequences that could be classified to a microbial taxon were extracted and aligned to EggNOG v. 5.0 database using EggNOG-mapper (19). Genes were annotated with Kyoto Encyclopedia of Genes and Genome (KEGG) KO genes. Heatmaps were used to visualise the KEGG functional pathways. Abundance was normalised against the smallest library size of the sequenced samples.

Differential abundance analysis

Linear discriminant analysis (LDA) was carried out using LDA effect size (LEfSe) tool in Galaxy version 1.0 using the relative abundance from MEGAN for the Species level. Abundance was reported as log-fold change relative to HC, with top 30 significantly increased or reduced abundant species reported. At the phyla level, group comparisons were made using Kruskal-Wallis tests. $p<0.05$ was considered statistically significant.

Results

Clinical characteristics of SSc patients

Systemic sclerosis patients (n=23) and age-and-sex-matched healthy controls (n=19) were included in this study (Table I). All SSc patients and HC were females and non-smokers. There were 12 SSc patients who were on immunosuppression (SSc On-IS), and 11 SSc patients who were not on immunosuppression (SSc No-IS). 78% were of the limited cutaneous subtype and 22% were of the diffuse cutaneous subtype. The median disease duration of all SSc patients was 3.3 years, with shorter disease duration in the On-IS group (2.9 years) v*ersus* No-IS group (6.2 years). Immunosuppressive medications that SSc patients were on included mycophenolate mofetil, cyclophosphamide, azathioprine and methotrexate. SSc (On-IS) were all taking gastrointestinal medications, while 54.5% (n=6) of SSc (No-IS) were taking gastrointestinal medications. The median total UCLA Scleroderma Clinical Trial Consortium Gastrointestinal Tract 2.0 score for SSc (On-IS) was 0.52 (moderate severity), while median total score for SSc (No-IS) was 0.26 (mild). Apart from GI involvement, patients had other organ involvement including arthritis, vasculopathy, cardiac, myositis, interstitial lung disease and pulmonary hypertension (Supplementary Table S1).

Table I. Baseline characteristics of the SSc patients, and healthy controls enrolled in the study.

	Overall SSc (n=23)	SSc On-IS (n=12)	SSc No-IS (n=11)	Healthy controls (n=19)
Median age (range), years	54	50 (33-63)	55 (49-67)	55 (43-67)
Chinese ethnicity, n (%)	21 (91)	10 (83)	11 (100)	19 (100)
Median BMI, kg/m^2	22.7	24.1	21.4	23.8
SSc subtype, n				
DcSSC	5	2	3	NA
LcSSc	18	10	8	
Median SSc duration, years	3.3	2.9	6.2	NA
Serology				
Anti-Scl70 antibody	8	6	2	NA
Anti-centromere antibody	5	1	4	NA
On immunosuppression, n per medication	12	12	0	0
AZA	1	1		
CYC	4	4		
MTX	3	3		
MMF	4	4		
GI medications, n (%)	18 (78.3)	12 (100.0)	6 (54.5)	0
Median total GIT 2.0 score	0.39 (mild)	0.52 (moderate)	0.26 (mild)	NA
Reflux	0.38 (mild)	0.63 (moderate)	0.13 (mild)	
Distension	1.25 (moderate)	1.25 (moderate)	0.50 (mild)	
Faecal soilage	0	0	0	
Diarrhoea	0	0	0	

SSc On-IS SSc: patients on immunosuppression; SSc No-IS: SSc patients not on immunosuppression; DcSSC: diffuse cutaneous SSc patient; LcSSc: limited cutaneous SSc patient; AZA: azathioprine; BMI: body mass index; CYC: cyclophosphamide; GIT: gastrointestinal tract; GIT 2.0 score: UCLA Scleroderma Clinical Trial Consortium Gastrointestinal Tract 2.0 score; MMF: mycophenolate mofetil; MTX: methotrexate; NA: not applicable.

Alpha and beta diversity between SSc and HC

The overall alpha diversity of SSc patients was higher compared to HC ($p=0.014$) (Fig. 1A). SSc (No-IS) patients had significantly higher alpha diversity compared to HC ($p=0.006$, Fig. 1A), but not for SSc (On-IS) compared to HC (Fig. 1A).

Whilst HC was clustered apart from the other 2 SSc groups, there was no significant difference in beta diversity between SSc patients and HC ($p=0.307$), or between SSc (On-IS) and SSc (No-IS) ($p=0.504$). (Fig. 1B).

Differential microbiome abundance in SSc versus HC

The GI microbial composition of SSc (On-IS) and SSc (No-IS) were compared with HC. Comparison of the relative abundance at the phyla level in SSc (On-IS) and SSc (No-IS) with HC are shown in Figure 2A. Compared to HC, the phyla Actinobacteria and Firmicutes showed increased abundance in SSc (On-IS) and SSc (No-IS) ($p=0.00051$ and $p=0.00065$ respectively; Fig. 2B). Bacteroidetes was reduced in abundance in both SSc (On-IS) and SSc (No-IS) compared to HC ($p=0.0004$, Fig. 2B).

Differentially abundant features of bacterial phyla and species in SSc patients compared to HC were identified using the LEfSe method. The top 50 abundant and depleted species compared to HC are shown in Figure 3. There was significantly increased abundance of several Lactobacillus, Bifidobacterium, Coprococcus and Streptococcus species in SSc patients, and increased abundance of Odoribacter, Bacteroides and Prevotella species in HC (Fig. 3).

Differential microbiome functional pathways in SSc versus HC

KEGG pathway and function comparisons were performed to explore differences in the functional proficiencies of the GI microbiome of SSc patients *vs*. HC. The top 50 out of 437 pathways comparing SSc patients, including subsets of SSc (On-IS) and SSc (No-IS) *versus* HC are shown in Figure 4A. There are distinct differences in the KEGG pathways between SSc *versus* HC, and between SSc (No-IS) and SSc (On-IS). Some of these pathways include: biosynthesis of antibiotics, amino acids, aminoacyl tRNA, peptidoglycan; metabolism of carbon,

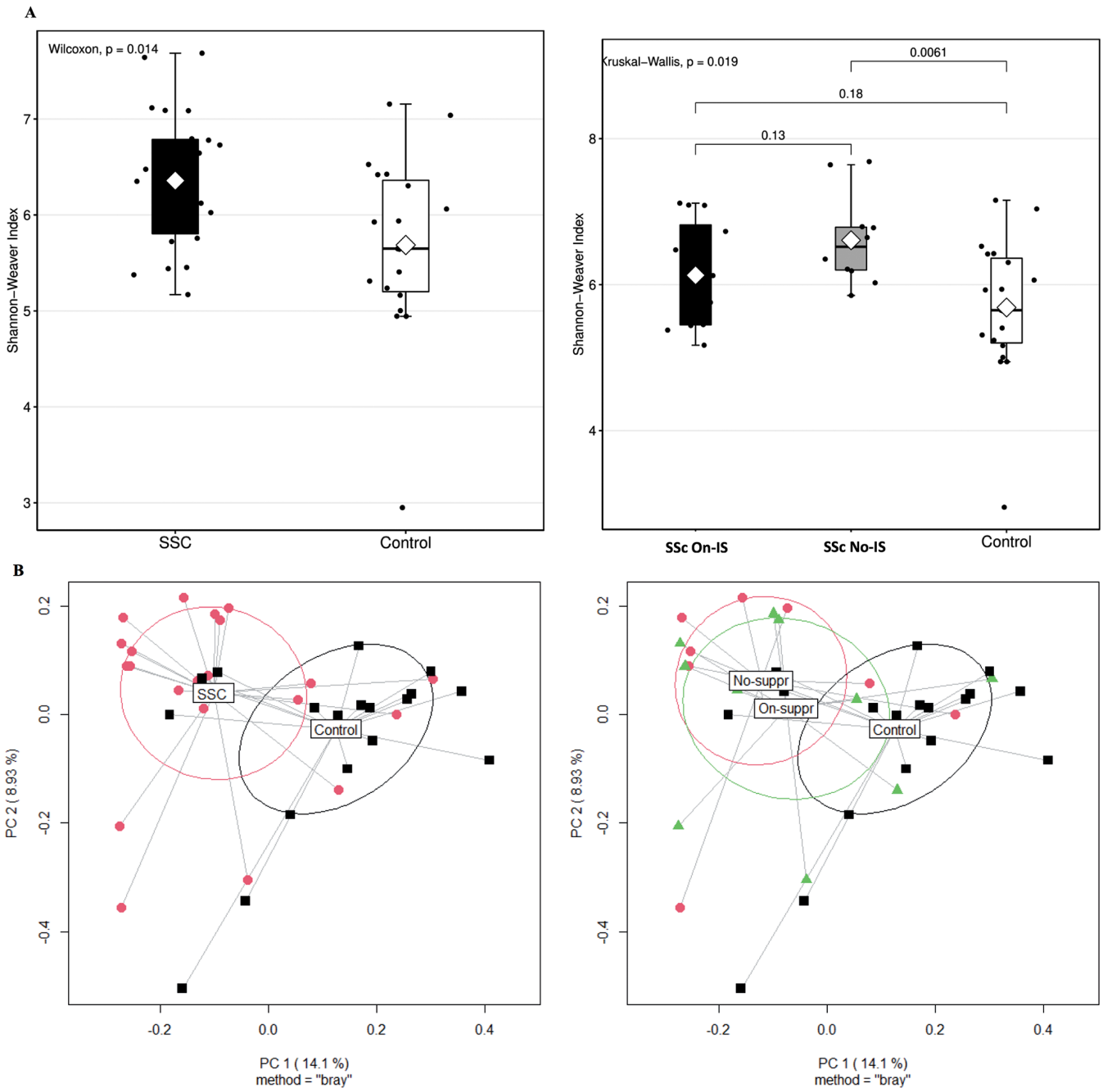

Fig. 1. GI microbiome diversity in systemic sclerosis (SSc) patients compared to healthy controls (HC). **A**. Alpha diversity boxplots indicate that SSc patients have higher alpha-diversity compared with healthy controls (p=0.014). Alpha diversity of SSc patients not on immunosuppression (SSc No-IS) was significantly higher compared with healthy controls (p=0.006). SSc patients who were on immunosuppression (SSc On-IS) did not have significantly different alpha diversity from healthy controls. **B**. Principal coordinate analysis of beta diversity using Bray-Curtis dissimilarity measure. No significant difference in beta diversity between SSc patients and HC (p=0.307), or between SSc (On-IS) and SSc (No-IS) (p=0.504).

purine, pyrimidine, starch, sugar and sphingolipid; DNA replication and drug metabolism pathways. Distinct differences in the KEGG functions between SSc *versus* HC, were also detected. The top 50 out of 939 functions are shown in Figure 4B. Several pathways were downregulated in SSc versus HC (highlighted in yellow boxes), including: DNA mismatch repair protein MutS, DNA topoisomerase III, hexosaminidase, beta-glucosidase.

Discussion

We report that SSc patients display distinct GI microbiome taxa and alpha and beta diversity compared to HC group which correlate with different KEGG pathway patterns in SSC compared to the HC.

SSc patients had higher alpha diversity compared to HC. A similar trend was observed in the GI microbiome of colonic lavage samples from SSc patients in UCLA (6) and Canadian SSc patients with small intestinal bacterial overgrowth (20), suggesting that the GI tract in the SSc disease state could be associated with a potential increase in bacterial richness. Contrary to this,

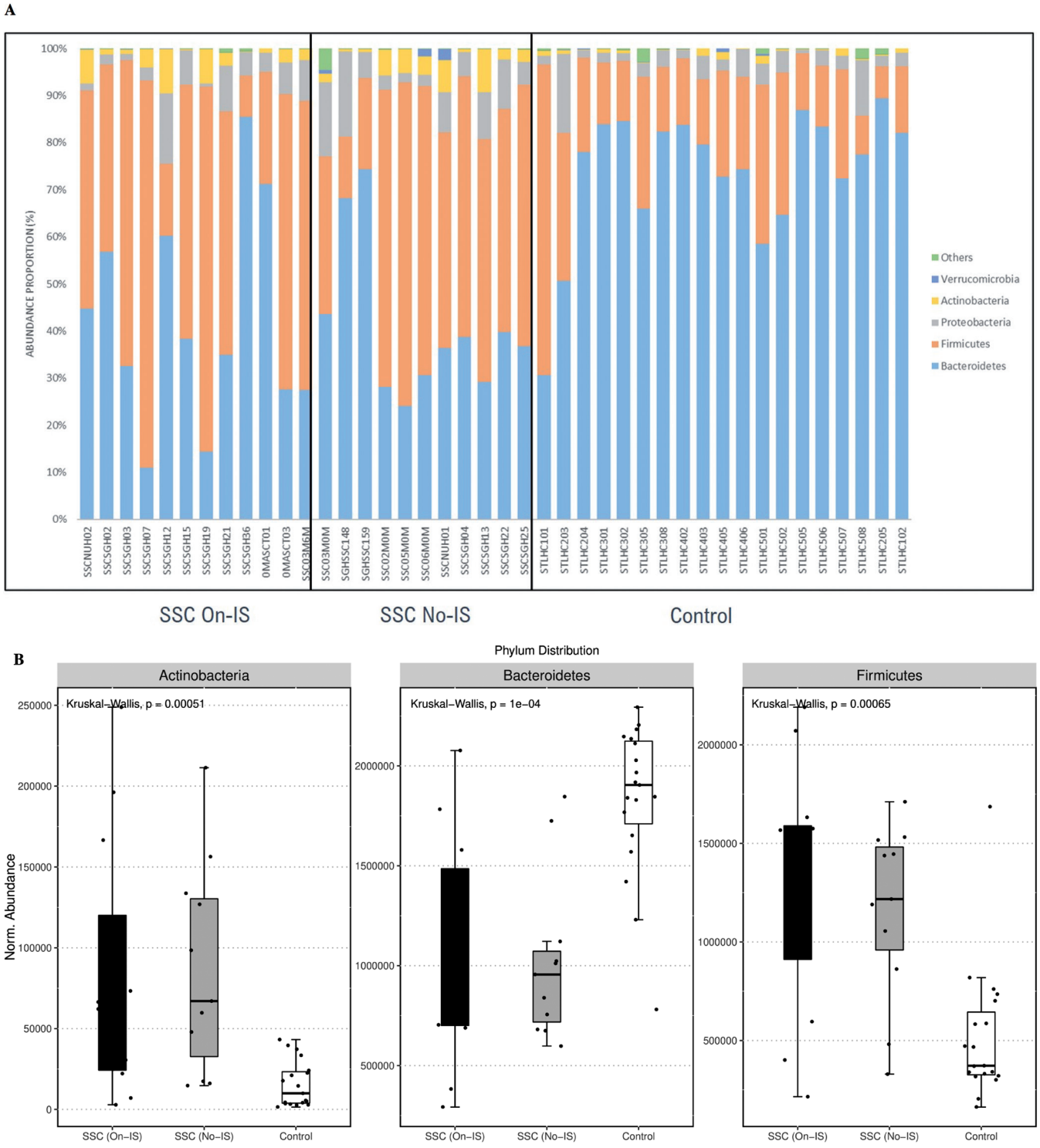

Fig. 2. Microbiome proportions at the phyla level in systemic sclerosis (SSc) patients on immunosuppression (SSc On-IS), not on immunosuppression (SSc No-IS) and healthy controls (HC). **A** Distribution of the five most abundant phyla in SSc and HC. Bar chart shows abundance proportion in percentage. **B** Overabundance of phyla Actinobacteria and Firmicutes in SSc patients and depletion of Bacteroidetes in SSc patients compared to HC. Boxplot show median and upper/lower quartiles.

other studies demonstrated similar alpha diversity in SSc patients compared to HC (8, 9, 21). Apart from geographic, dietary and ethnic differences, longer SSc disease duration may also influence the overall differences in alpha diversity between SSc patients and HC as observed in these studies. We observed that SSc (On-IS) patients showed a trend of lower alpha diversity compared to SSc (No-IS), which is more similar to HC. This may be explained by the presence of immunosuppressive medications altering the GI tract bacterial diversity in SSc patients, resulting in a bacterial richness and evenness that is closer to a non-disease state.

The Firmicutes to Bacteroidetes ratio

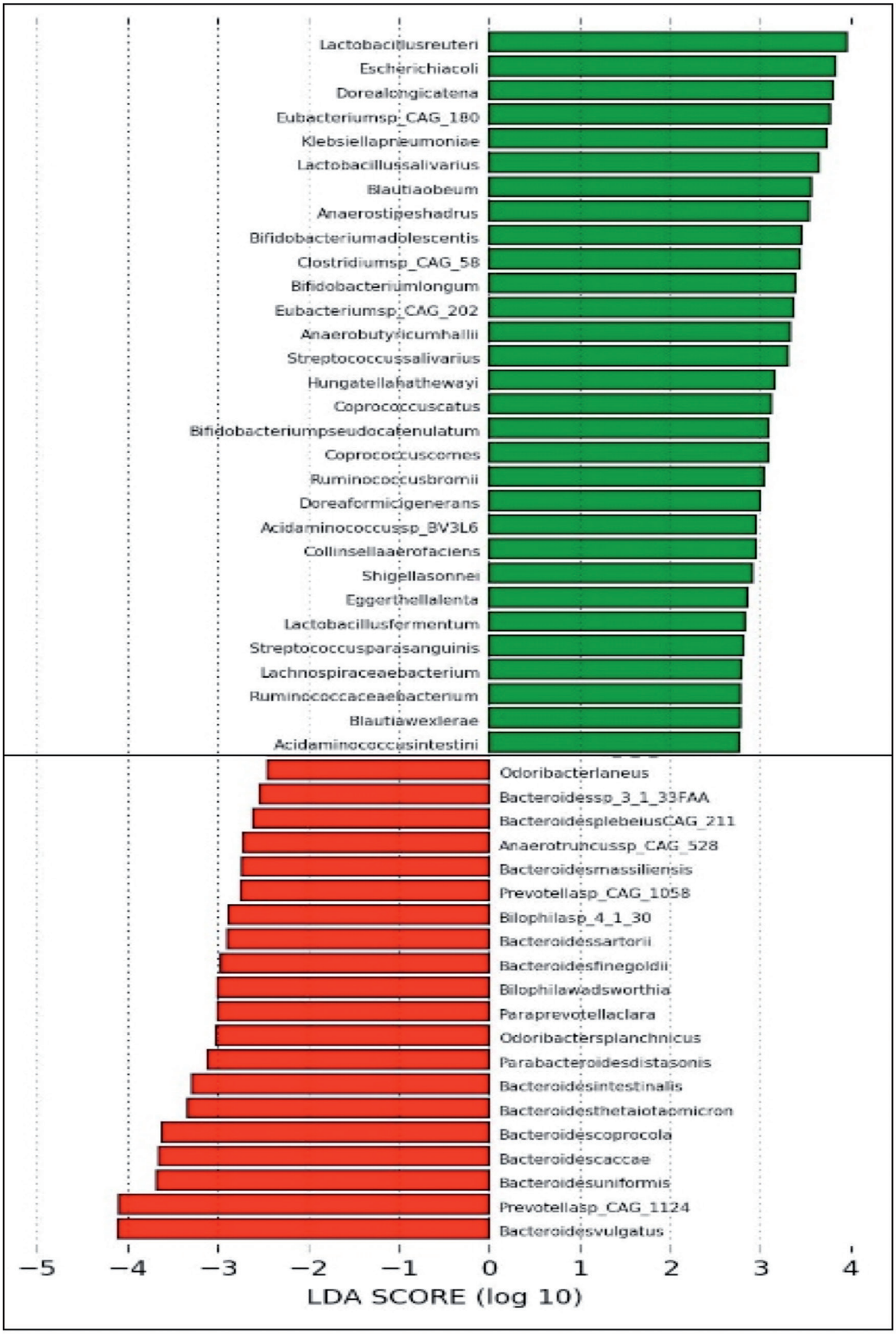

Fig. 3. Top 50 differentially abundant species in SSc patients compared to healthy controls. Linear discriminant analysis was used to calculate effect size. Red denotes decreased effect size and green denotes increased effect size.

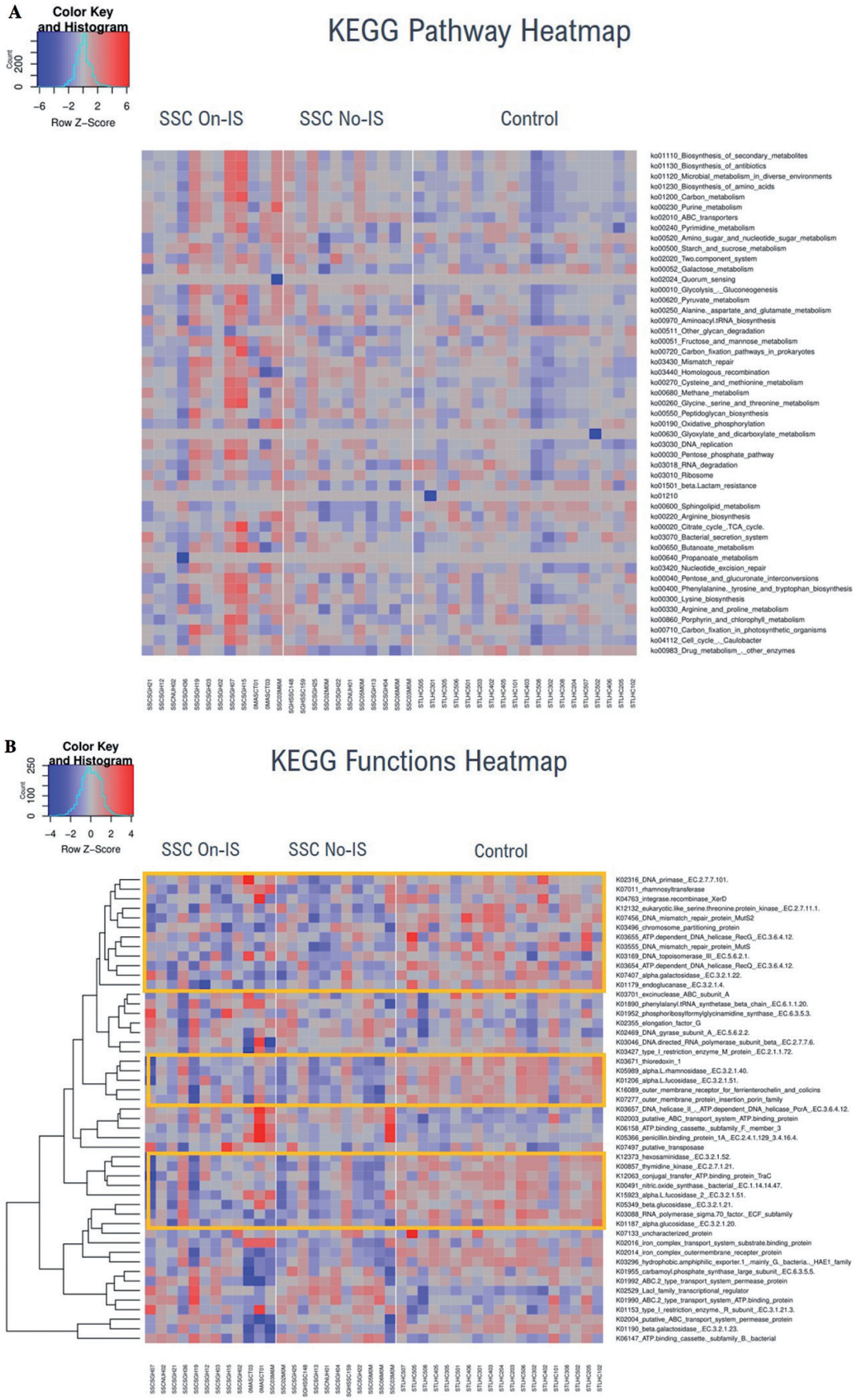

Fig. 4. Functional microbiome signature in SSc patients and healthy controls. Comparison of SSc patients on immunosuppression (SSc On-IS) and SSc patients not on immunosuppression (SSc No-IS) with healthy controls are shown in (**A**) KEGG pathways and (**B**). KEGG functions heatmap. Top 50 differential pathway and functions are shown.

was found to be increased in our SSc patients, as similarly observed in other SSc cohorts (7–9, 21). Actinobacteria was more abundant in our cohort and in caecal colonic lavage samples of SSc patients from UCLA (6). Abundance of Actinobacteria was observed in colonic lavage samples from patients with inflammatory bowel disease, suggesting common inflammatory mechanisms in the GI tract (22).

Several bacterial genera were observed to be more abundant in our SSc patients and in other SSc cohorts: Lactobacillus (5-9), Streptococcus (9), Coprococcus (8), Bifidobacterium (6), Lachnospiraceae (8), Ruminococus (7, 9), Dorea (8), Blautia (9). Increased abundance of Lactobacillus in several different SSc cohorts may suggest a pathobiont role by this genus. In particular, Lactobacillus reuteri was most abundant in our SSc patients compared to HC. This species was increased in SSc patients with GI involvement as compared to those without GI involvement (8), and in SSc patients with low constipation scores (7). Increased abundance of Lactobacillus, Bifidobacterium and Actinobacteria had been observed in wild-type mice exposed to high-dose dexamethasone (23). In another paradigmatic autoimmune disease, increased abundance of Lactobacillus spp. was observed in systemic lupus erythematosus (SLE) patients when compared to HC (24), and in SLE mice models associated with more severe disease (24, 25). Furthermore, Lactobacillus salivarius was more abundant in the gut and saliva of rheumatoid arthritis patients, and present in increased amounts in very active rheumatoid arthritis (26). Thus, further investigation in the relationship between Lactobacillus and SSc patients with more severe GI involvement and the effect of immunosuppression is needed.

Several species of Bacteroides are reduced in our SSc patients, which is in agreement with other diverse SSc populations (7, 9). In addition, decreased abundance of Bacteroides fragilis was associated with more severe GI symptoms in SSc patients (6). Bacteroides is thought to protect the host against mucosal inflammation and colonisation of pathogenic species (27), and had been observed to be decreased in abundance in Crohn's disease as compared to HC (28, 29). Such GI bacteria profile may be helpful to identify SSc patients at risk of developing severe GI manifestations. Increased abundance of Lactobacillus, Streptococcus and decreased Bacteroides were also observed in our SSc patients. This bacteria profile has also been observed in a cohort of patients with primary immune thrombocytopenia as compared to HC (30). Identifying unique gut bacteria profile may help to predict and stratify patients into disease phenotypes, for which future research may shed light on choice of therapeutics based on disease phenotypes. A previous study showed that SSc patients with high GIT scores had reduced abundance of Bifidobacterium, Odoribacter, Coprococcus and Blautia and increased Parabacteroides in their stool compared to SSc patients with low GIT score (31). The use of a high dose multi-strain probiotic in these SSc patients with significant GI symptoms demonstrated improved GIT score at the end of this double-blind, placebo-controlled trial (31).

Similar to another study that investigated functional proficiencies of the GI microbiota in SSc (6), we found that genes involved in sphingolipid metabolism (such as beta-galactosidase) and amino acid and nucleotide sugar metabolism (such as beta-hexosaminidase), were decreased in abundance in SSc patients versus HC. Alterations in sphingolipid metabolism have been implicated in SSc, and shown to contribute to SSc fibrosis in dermal fibroblast cultures (32), with deficiency of acid sphingomyelinase contributing to resistance of SSc fibroblasts to apoptosis (32). Specifically, our data showed reduced abundance of genes linked to regulation of hexosaminidase and beta-galactosidase functions, compared to HC (Fig. 4B). Beta-hexosaminidase A enzyme deficiency is associated with Tay-Sachs disease (33), while lysosomal acid beta-galactosidase deficiency causes G_{M1} gangliosidosis, a neurologic disorder and Morquio disease type B, a skeletal-connective tissue disorder (34). Faecal samples from our SSc patients had reduced gene level of DNA mismatch repair protein MutS compared to HC. MutS is an enzyme within the DNA mismatch repair pathway. Autoantibodies to other DNA mismatch repair enzymes such as PMS1 autobody had been detected in patients with autoimmune myositis (35), and autoantibodies to MLH1, MSH2, PMS1 and PMS2 had been detected in patients with idiopathic inflammatory myositis and SLE (36). Interestingly, faecal samples from our SSc patients demonstrated downregulation of DNA topoisomerase III function. Autoantibody to DNA topoisomerase I, otherwise known as anti-Scl-70 antibody is specific for SSc (37), and skin biopsies of early DcSSc patients with positive anti-topoisomerase I antibody seemed to suggest more refractory immune cell-driven fibrosis (38). Anti-DNA topoisomerase IIα autoantibody levels were increased in Japanese SSc patients compared to HC, and interstitial lung disease was more frequently observed in the SSc patients who had anti-DNA topoisomerase IIα antibody compared to SSc patients without the antibody (39). The aforementioned downregulated functions of GI bacteria communities in SSc patients will require future research to understand their significance and effects.

In our study, SSc patients had differences in pathways related to metabolism of carbon, purine, pyrimidine, starch and sugar compared to HC. Such diverse observations may be due to geographic, dietary or ethnic differences between different cohorts from USA and Asia and will require larger samples sizes to elucidate the significance of these pathways.

Our study adds to current knowledge on the profile of GI microbiome profile difference between SSc patients and HC. Specifically, by deploying a metagenomic sequencing approach, we avoid genomic sequence amplification and thus provide better structural data from the microbiome population (40). While there are similarities and differences in our results compared to previous studies (4), our results provide evidence of differences between On-IS and No-IS SSc patients. Like many

human genomic profiling analyses, our study, has limitations: the cross-sectional design did not allow us to analyse the GI microbiome in SSc patients over time, nor take into account changes in the GI microbiome as the SSc disease evolves. Second, although our HC were age- and sex-matched, lifestyle and dietary habits were not standardised or accounted for. Lastly, faecal calprotectin, previously shown to correlate with increased GI symptom score (41), was not measured in our study. Trends in faecal calprotectin values may help to shed light on the differences in GI microbiome in our SSc patients. Future studies with longitudinal data of the GI microbiome profile and functional investigations in a larger group of SSc patients, at various time points of the disease are therefore highly warranted.

Conclusion

This study report that faecal samples from Asian SSc patients display a unique GI microbiome profile and exhibit differences in KEGG pathways implying possible functional pathway variation between SSc and HC.

Take home messages

- Previous studies have revealed gastrointestinal dysbiosis in Caucasian SSc patients, by using 16S profiling.
- Asian SSc patients have distinct alterations in gastrointestinal microbiota profile by using metagenomic sequencing.
- This study may further support the changes in microbiome profiling to the pathophysiology of SSc.

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