Altered Gut Microbiome in Patients With Dermatomyositis

Sangmee Sharon Bae,1 Tien S. Dong,2 Jennifer Wang,1 Venu Lagishetty,3 William Katzka,3 Jonathan P. Jacobs,2 and Christina Charles-Schoeman1

Objective. The study objective was to compare the microbial composition of patients with dermatomyositis (DM) and healthy controls (HCs) and determine whether microbial alterations are associated with clinical manifestations of DM.

Methods. The 16S ribosomal RNA gene sequencing was performed on fecal samples from patients with DM and HCs. Microbial composition and diversity were compared between subjects with DM and HCs and in association with several DM-specific clinical variables, including myositis-specific autoantibodies (MSAs). Differentially abundant microbial taxa and genes associated with clinical characteristics were identified, and functional analysis was performed using predicted metagenomics. Dietary intake was assessed using a 24-hour dietary recall.

Results. The fecal microbiome of 36 patients with DM and 26 HCs were analyzed. Patients with DM trended toward lower microbial diversity compared with HCs. The higher physician global damage score was significantly correlated with the lower microbial diversity in patients with DM. Patients with interstitial lung disease (ILD)-associated MSA (antisynthetase antibody (ab), anti-melanoma differentiation-associated protein 5 ab, n = 12) had significant differences in microbial composition and lower microbial diversity compared with HCs. Differential abundance testing demonstrated a unique taxonomic signature in the ILD-MSA subgroup, and predictive metagenomics identified functional alterations in a number of metabolic pathways. A significant increase in the relative abundance of Proteobacteria was positively correlated with multiple pathways involved in lipopolysaccharide synthesis and transport in the ILD-MSA group.

Conclusion. Patients with DM, particularly with ILD-associated MSAs, have lower microbial diversity and a distinct taxonomic composition compared with HCs. Further studies are needed to validate our findings and elucidate specific pathogenetic mechanisms that link the gut microbiome to clinical and pathological features of DM.

INTRODUCTION

A growing understanding of the host–microbiome interaction demonstrates that there is a strong dynamic cross talk between resident microbes and the host immune system, in which the immune system shapes and preserves the ecology of the microbiota, and in turn, the microbiota educates and calibrates the immune system (1). The majority of microbiota reside in the intestinal tract, affecting both the local intestinal immune homeostasis as well as systemic inflammatory responses (2).

Alterations in microbiome homeostasis (so-called dysbiosis) can lead to dysregulation of various physiologic functions and enhance susceptibility to chronic inflammatory disorders. Studies in inflammatory bowel disease (IBD), rheumatoid arthritis (RA), spondyloarthritis, and scleroderma have demonstrated that dysbiosis of the gut microbiome is associated with perturbations in immune function, metabolite production, and inflammatory markers (3). The gut microbiome and its derived dietary metabolites may also impact lipid metabolism and lead to metabolic dysfunction and low-grade inflammation, thereby promoting the development of cardiovascular disease (4).

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1Sangmee Sharon Bae, MD, MS, Jennifer Wang, BS, Christina Charles-Schoeman, MD, MS: University of California, Los Angeles; 2Tien S. Dong, MD, PhD, Jonathan P. Jacobs, MD, PhD: David Geffen School of Medicine at University of California, Los Angeles; 3Venu Lagishetty, PhD, William Katzka, BA: David Geffen School of Medicine at University of California, Los Angeles, Los Angeles.

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Address correspondence to Sangmee Sharon Bae, MD, MS, University of California Los Angeles, 1000 Veteran Ave, Rm 32-59, Los Angeles, CA, 90095. Email: sbae@mednet.ucla.edu

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Dermatomyositis (DM) is an autoimmune myopathy associated with marked microvascular dysfunction. As the gut microbiome has been implicated in the pathogenesis of both autoimmune and vascular diseases, we hypothesized that the gut microbiome may impact the development and perpetuation of DM. The current study aims to compare the microbial composition of patients with DM and healthy controls (HCs) and to determine whether microbial alterations are associated with clinical manifestations of DM.

PATIENTS AND METHODS

Study participants and definition of clinical variables. Participants were consecutively enrolled from the University of California, Los Angeles (UCLA). Eligible participants included adults (age ≥ 18 years) with DM or HCs. All patients with DM met European Alliance of Associations for Rheumatology/American College of Radiology Classification Criteria for at least “probable” adult idiopathic inflammatory myopathy (IIM) and DM subclass, which was verified by chart review (5). Exclusion criteria included IBD or other overlapping autoimmune diseases. All subjects gave written informed consent for the study under a protocol approved by the UCLA Human Research Subject Protection Committee (Institutional Review Board #10-001833).

Patients provided a blood sample and completed questionnaires regarding cardiovascular risk and health information. Assessment of creatine phosphokinase (CPK), inflammatory markers including high-sensitivity C-reactive protein (hsCRP), and Westergren erythrocyte sedimentation rate (ESR) were performed using standard methods. Myositis-specific autoantibodies (MSAs) were assessed at the Oklahoma Medical Research Foundation. Patients were divided into the following 3 MSA groups in order to focus on antibodies known to strongly associate with myositis related comorbidities: 1) interstitial lung disease (ILD)-associated ab: antisynthetase antibody (ab) and anti-melanoma differentiation-associated protein 5 (anti-MDA5); 2) cancer-associated ab: anti-transcription intermediary factor 1-gamma (anti-TIF1γ) and anti-nuclear matrix protein 2 (NXP2)/Ju/Ju; and 3) other ab. Disease activity and damage were assessed using physician global myositis disease activity and damage scales by 100 mm visual analog scale (VAS) and 5 point Likert scale (6). Skin disease was assessed using the Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI) activity and damage scores (7). Muscle strength was reported using manual muscle testing (MMT-8) (6). ILD was defined by chest computed tomography (CT) findings consistent with ILD showing at least one of the following features: 1) reticulation and fibrosis, 2) traction bronchiectasis, 3) honeycombing, or 4) ground glass opacification (8).

Fecal sample collection. Consented participants were provided with a toilet hat and prefilled sample containers for home collection. Freshly defecated feces were immediately collected in 95% ethanol and homogenized with a steel ball in the sample containers. Samples were delivered to the laboratory and stored at −80°C.

Microbiome sequencing and microbial diversity and composition analysis. Genomic DNA was extracted from fecal samples using the ZymoBIOMICS DNA Microprep Kit (Zymo Research), and the microbiota were profiled by 16S ribosomal RNA (rRNA) gene sequencing of the V4 region by Illumina MiSeq (Illumina) as previously described (9,10). Amplicon sequence variants (ASVs) were identified using the DADA2 pipeline in R, and bacterial taxonomy was assigned using the SILVA 132 database (11). After preprocessing in R, the data were incorporated into QIIME 2 version 2019.10 (12).

Analysis of diversity, which examines the microbial diversity within each sample, was reported using the Chao1 and Shannon index. Chao1 is a measure of taxon “richness,” or number of different taxa per sample, and Shannon index is a measure of “richness” as well as “evenness,” which is the qualitative amount of taxa of each sample. Analysis of β diversity, which compares the microbial composition between groups, was performed using the robust Aitchison distance metric that accounts for the sparse compositional nature of microbiome data. The robust Aitchison distance metric has been shown to yield higher discriminatory power compared with other common metrics, such as UniFrac or Bray-Curtis (13). Low prevalent ASVs were removed if they were not present in at least 15% of the samples (14). Results of β diversity were visualized using principal coordinate analysis. The relative abundance of microbes was tested at different bacterial taxonomic levels using DESeq2 in R (15). In order to minimize the possibility of placing similar sequences that are, in fact, from different species, we also performed relative abundance testing at the ASV level. Bacteroidetes to Firmicutes ratio (B/F ratio) was calculated using the raw absolute count data in each sample.

Predicted metagenome. In order to increase the insight into changes in the functional capacity of the gut microbiome, metagenomic data were predicted from the 16S rRNA microbiome data using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States version 2 (PICRUSt2) in QIIME2 (16). Differential abundance testing was done using DESeq2 in R. Predicted genes were mapped into biological pathways through the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database.

Assessment of dietary intake. Participants were asked to complete a 24-hour dietary recall using the Automated Self-Administered 24-hour dietary assessment tool (17). The tool has been shown to capture intake with less bias compared with other self-reporting instruments (18).
Table 1. Demographics and clinical characteristics in DM and HCs

|                               | DM (n = 36) | HC (n = 26) |
|-------------------------------|-------------|-------------|
| Age, y                        | 47 ± 15     | 47 ± 17     |
| Gender, Female                | 27 (75)     | 17 (65)     |
| Ethnicity, Hispanic           | 3 (8)       | 4 (15)      |
| Race                          |             |             |
| White                         | 23 (63)     | 18 (69)     |
| Black                         | 5 (14)      | 1 (4)       |
| Asian                         | 8 (22)      | 7 (27)      |
| Current use of antibiotics    | 22 (61)*    | 2 (8)       |
| Use of antibiotics within 3 months | 22 (61)*   | 3 (12)      |
| Current use of probiotic oral supplement | 4 (11)   | 3 (12)      |
| Comorbidities                 |             |             |
| Malignancy                    | 0 (0)       | 0 (0)       |
| Hypertension                  | 9 (25)      | 3 (12)      |
| Dyslipidemia                  | 9 (25)      | 5 (19)      |
| Diabetes                      | 2 (6)       | 1 (4)       |
| History of myocardial infarction | 2 (6)   | 1 (4)       |
| History of stroke/TIA         | 3 (8)       | 1 (4)       |
| Family history of premature myocardial infarction | 4 (11)  | 5 (19)      |
| Ever smoker                   | 9 (25)      | 2 (8)       |
| Hs-CRP, mg/L                  | 2.6 ± 4.6   | 2.0 ± 3.5   |
| Sedimentation rate, mm/h      | 30 ± 25*    | 11 ± 8      |
| Disease duration, mon         | 75 ± 95     |             |
| Disease-specific outcome measures |         |             |
| Physician global activity VAS 0-100 mm | 34 ± 26  |             |
| Physician global activity Likert, median (IQR) | 1.5 (1-2) |             |
| Physician global damage VAS, 0-100 mm | 26 ± 23  |             |
| Physician global damage Likert, median (IQR) | 1 (1-2)  |             |
| CDASI, activity score, median (IQR) | 4.5 (1-8) |             |
| CDASI damage score, median (IQR) | 0 (0-1)  |             |
| MMT-8, 0-150, median (IQR)    | 148.5 (145-150) |         |
| CPK, U/L                      | 207 ± 299   |             |
| LD, U/L                       | 234 ± 137   |             |
| Aldolase, U/L                 | 6.0 ± 6.0   |             |
| Myositis autoantibodies       |             |             |
| Anti-MDA5 ab                  | 7 (19)      |             |
| Anti-Jo1 ab                   | 4 (11)      |             |
| Anti-PL-12 ab                 | 1 (3)       |             |
| Anti-TIF γ ab                 | 8 (22)      |             |
| Anti-NXP2 ab                  | 5 (14)      |             |
| Anti-Mi2 ab                   | 3 (8)       |             |
| Anti-SRP ab                   | 1 (3)       |             |
| Anti-Ro/SSA ab                | 2 (6)       |             |
| Unidentified                  | 1 (3)       |             |
| No Ab                         | 1 (3)       |             |
| ILD, yes                      | 14 (39)     |             |
| Pulmonary function tests      |             |             |
| FVC % predicted               | 94 ± 22     |             |
| FEV1/FVC % predicted          | 95 ± 10     |             |
| TLC % predicted               | 92 ± 28     |             |
| DLCO % predicted              | 77 ± 21     |             |
| Medications                   |             |             |
| Methotrexate                  | 3 (8)       |             |
| Azathioprine                  | 1 (3)       |             |
| Hydroxychloroquine            | 8 (22)      |             |
| Mycophenolate mofetil         | 21 (58)     |             |
| Intravenous immunoglobulin    | 28 (78)     |             |
| Rituximab                     | 5 (14)      |             |
| Cyclophosphamide              | 3 (8)       |             |

(Continued)
Table 1. (Cont'd)

|                      | DM (n = 36) | HC (n = 26) |
|----------------------|-------------|-------------|
| Prednisone           |             |             |
| Low dose (<10 mg/d)  | 28 (78)     |             |
| High dose (≥10 mg/d) | 15 (42)     | 13 (36)     |
| Daily prednisone dose, mg/d | 15 ± 17    |             |

Note: Values reported as mean ± SD or n (%) unless otherwise specified.

Abbreviations: ab, antibody; CDASI, Cutaneous Dermatomyositis Disease Area and Severity Index; CPK, creatine phosphokinase; CRP, C-reactive protein; CT, computed tomography; DLCO, diffusion capacity of lung for carbon monoxide; DM, dermatomyositis; FEV, forced expiratory volume; FVC, forced vital capacity; HC, healthy control; hsCRP, high sensitivity c-reactive protein; ILD, interstitial lung disease; IQR, interquartile range; LD, lactate dehydrogenase; MDA5, melanoma differentiation-associated protein 5; MMT, manual muscle testing; NXP2, nuclear matrix protein 2; PFT, pulmonary function test; SRP, signal recognition particle; TIA, transient ischemic attack; TIF1γ, transcriptional intermediary factor 1γ; TLC, total lung capacity; VAS, visual analog scale.

Table 1. (Cont’d)

|                      | DM (n = 36) | HC (n = 26) |
|----------------------|-------------|-------------|
| Prednisone           |             |             |
| Low dose (<10 mg/d)  | 28 (78)     |             |
| High dose (≥10 mg/d) | 15 (42)     | 13 (36)     |
| Daily prednisone dose, mg/d | 15 ± 17    |             |

Dietary intake was reported using the healthy eating index (HEI) score, a measure of dietary quality according to the Dietary Guidelines for Americans (19). The HEI is reported by a total score, indicative of overall dietary quality, and separate component scores with higher scores for a healthier diet pattern.

Statistical analysis. Microbiome diversity and composition were examined in DM compared with controls and in DM subgroups. Associations of microbial diversity and composition with DM-specific disease measures (MSA groups, physician global VAS activity/damage, skin disease activity/damage by CDASI, and muscle disease by MMT-8 and CPK) were assessed using Spearman’s correlation for continuous variables and analysis of variance (ANOVA) for categorical variables. Disease outcome measures were analyzed both as continuous and categorical (tertiles for VAS, dichotomized at the median for skin scores) variables. Differences in α diversity between groups were evaluated using multivariate analysis (MVA) of variance (MANOVA) and β diversity were evaluated using permutational multivariate analysis of variance (PERMANOVA) in order to control for confounding covariates. Each of the following covariates was tested by bivariate ANOVA (for α diversity) or PERMANOVA (for β diversity) comparison between groups and included in the final MVA model when significant: demographics (age, gender, and race and ethnicity), comorbidities including cardiovascular risk factors (malignancy, hypertension, diabetes, dyslipidemia, history of myocardial infarction/stroke, family history of premature myocardial infarction, and smoking history), and antibiotic use. In patients with DM, additional covariates were tested and included in MVA when significant: MSA group, presence of ILD, immunosuppressive medications, labs (ESR, hsCRP, CPK, and aldolase), and global and skin disease activity/damage scores.

All tests were two-sided with an α level of 0.05, except for exploratory correlations where significance level was set at 0.1. For differential abundance testing, we used the false discovery rate (FDR) of Benjamini and Hochberg (20) to correct for multiple hypothesis testing, and a significant association was defined at the FDR q-value threshold of less than or equal to 0.05.

RESULTS

Participant characteristics. A total of 36 patients with DM and 26 HCs were analyzed (Table 1). The HC group had similar demographics as the DM group. Global disease scores were mild to moderate in most patients with DM; 39% had ILD on chest CT, and no patients had concurrent malignancy. Use of antibiotics was significantly higher in the DM group because of *pneumocystis jiroveci* pneumonia prophylaxis for patients with DM on immunosuppressive therapy (20/22 patients with antibiotic use [Table 1]; 17 with trimethoprim/sulfamethoxazole, 3 with atovaquone).

Altered gut microbial diversity in patients with DM compared with HCs. A total of 1615 ASVs were generated, and after filtering to remove low-prevalence ASV, 212 ASVs were used for the final analysis. The overall composition of microbial communities based on β diversity was not significantly different in patients with DM compared with HCs (P = 0.52, Figure 1A).

Microbial α diversity was significantly lower in patients with DM compared with HCs by the Shannon index (P = 0.01) and Chao1 (P = 0.04). MVA was performed to adjust for confounders that were significant in bivariate analysis (current antibiotic use), which demonstrated that patients with DM still trended toward lower α diversity compared with controls by the Shannon index (P = 0.08, Figure 1B). Also, when patients with DM were stratified by antibiotic use, there was a similar trend for differences between patients with DM not taking antibiotics (n = 14) and HCs (P = 0.09, Supplementary Figure 1). These results suggest that the DM disease state may be associated with a potential decrease in microbial species diversity independent of the effects of antibiotic use.

The relative abundance of microbial taxa in patients with DM and HCs are summarized in Figure 1C and D. At the phylum level, patients with DM had a significant shift to increased Bacteroidetes
relative to Firmicutes (B/F ratio 0.33 [0.23] in DM vs. 0.22 [0.18] in HCs, mean [SD], P = 0.038), which is a similar trend as reported in systemic lupus erythematosus (SLE) (21). The relative abundance of the Proteobacteria phylum was also increased, whereas Actinobacteria was decreased in patients with DM compared with HCs (P = 0.03 and 0.04, respectively). At the genus level, DM samples demonstrated a nominally significant expansion of Streptococcus, Lachnoclostridium, and Tyzzerella 3 and 4 and a reduction of Bifidobacterium, Christensenellaceae R-7 group, and Anaerostipes compared with HCs. However, once the significance level was adjusted for multiple hypothesis testing, there were no microbial genera that were significantly associated with DM disease state, indicating that community-level differences detected may be driven by subtle changes in multiple taxa, rather than a marked change in select microbial taxa.

Patients with DM by MSA subgroups have distinct microbial taxonomic signatures. Because MSAs are important in the classification of DM disease types, we investigated differences in microbial signatures between DM subgroups by MSA. We compared patients with DM with ILD-MSA, those with cancer-MSA, and HCs (n = 26) (Table 2). Demographics, medications, and global and skin disease activity/damage scores were similar between the MSA subgroups (Table 2).

Significant differences in microbial communities of patients with DM with ILD-MSA, those with cancer-MSA, and HCs were observed using PERMANOVA (P = 0.02). β diversity analyses showed that the microbial community of patients with DM with ILD-MSA grouped separately from HCs as well as from patients with DM with cancer-MSA (Figure 2A).

Microbial α diversity was also significantly reduced in ILD-MSA and cancer-MSA patients with DM compared with HCs by both Shannon index and Chao1 (P = 0.02 by ANOVA for both). The difference in Shannon index remained significant after multivariate adjustment (P = 0.046 for Shannon index; P = 0.14 for Chao1) (Figure 2B). Similar trends were observed when only patients with DM without antibiotics were compared with HCs. Patients with ILD-MSA had the lowest α diversity by the Shannon index (P = 0.01 by ANOVA, Supplementary Figure 2C).

To further define compositional differences between MSA groups and HCs, the relative abundance at different taxonomic levels was computed. In the ILD-MSA subgroup, the phylum level profile demonstrated a significant expansion of Proteobacteria (Figure 2C). Relative abundance of Proteobacteria in
patients with DM with antibiotics versus without antibiotics were similar ($P = 0.97$), suggesting that the difference in microbiome between patients with DM and HCs was not primarily driven by the difference in antibiotic use. The B/F ratio was also significantly higher in ILD-MSA compared with HCs (0.45 [0.30] vs. 0.22 [0.18], mean [SD], $P = 0.006$). At the family level, Bacteroidaceae, Christensenellaceae, and Ruminococcaceae families were enriched compared with HCs ($P = 0.01$, 0.02, and 0.04, respectively), and Bacteroidaceae maintained significance after multiple hypothesis testing ($q = 0.003$, Figure 1D). At the genus level, patients with DM with ILD-MSA showed nominally significant enrichment of Bacteroides, Streptococcus, Lachnoclostridium, and Escherichia/Shigella and depletion of Christensenellaceae R-7 group, Eisenbergiella compared with HCs (Figure 1E, $P < 0.05$ for all), although no genera remained significant after adjusting for multiple hypothesis testing.

At the ASV level, extreme differences were observed in several ASVs within the Firmicutes and Actinobacteria phyla that were depleted in patients with ILD-MSA as well as in patients with cancer-MSA compared with HCs. Patients with DM with ILD-MSA also had significant depletion of the Christensenellaceae R-7 group (within the Christensenellaceae family), and multiple ASVs within the Ruminococcaceae family (Ruminococcus 1, Ruminococcaceae UCG-002, and Subdoligranulum) compared with controls and the cancer-MSA group. These differences remained significant after adjustment for multiple hypothesis testing (Figure 2F-H).

| Table 2. Demographics and clinical characteristics in patients with DM by MSA subgroup |
|--------------------------------------|-----------------|-----------------|-----------------|
|                                      | ILD-associated  | Cancer-associated | Other |
|                                      | MSA (n = 12)    | MSA (n = 13)     | (n = 8)         |
| Age, y                               | 53 ± 10         | 39 ± 15          | 49 ± 20         |
| Gender, female                       | 8 (67)          | 12 (92)          | 4 (50)          |
| Ethnicity, Hispanic                  | 2 (17)          | 0 (0)            | 1 (13)          |
| Race                                  |                 |                 |                 |
| White                                 | 5 (42)          | 10 (77)          | 6 (75)          |
| Black                                 | 2 (17)          | 2 (15)           | 1 (13)          |
| Asian                                 | 5 (42)          | 1 (8)            | 1 (13)          |
| Global activity VAS, 1-100 mm        | 34 ± 24         | 36 ± 26          | 45 ± 29         |
| Global damage VAS, 1-100 mm          | 38 ± 18         | 22 ± 23          | 26 ± 24         |
| CDASI activity                        | 4.6 ± 5.4       | 6.5 ± 6.3        | 4.9 ± 4.7       |
| CDASI damage                          | 0.2 ± 0.4       | 1.6 ± 2.9        | 1.5 ± 3.5       |
| MMT-8, 0-150                          | 148 ± 3         | 146 ± 6          | 142 ± 11        |
| ILD, yes$^a$                          | 11 (92)         | 1 (8)            | 2 (15)          |
| FVC % predicted                      | 88 ± 26         | 97 ± 15          | 101 ± 23        |
| TLC % predicted                       | 74 ± 35$^b$     | 102 ± 11         | 98 ± 35         |
| DLCO Hg % predicted                  | 65 ± 22$^b$     | 93 ± 11          | 80 ± 15         |
| Antibiotic use within 3 months       | 10 (83)         | 6 (46)           | 4 (50)          |
| Trimethoprin/sulfamethoxazole         | 9 (90)          | 3 (50)           | 4 (100)         |
| Atovaquone                            | 1 (10)          | 1 (17)           | 0               |
| Voriconazole                          | 0               | 1 (17)           | 0               |
| Doxycycline                           | 0               | 1 (16)           | 0               |
| Medications                           |                 |                 |                 |
| Methotrexate                          | 0               | 0                | 1 (13)          |
| Azathioprine                          | 0               | 1 (8)            | 0               |
| Hydroxychloroquine                    | 4 (33)          | 3 (23)           | 0               |
| Mycophenolate mofetil                 | 9 (75)          | 8 (62)           | 3 (38)          |
| Intravenous immunoglobulin            | 10 (83)         | 10 (77)          | 6 (75)          |
| Rituximab                             | 3 (25)          | 0                | 2 (25)          |
| Cyclophosphamide                      | 1 (8)           | 1 (8)            | 1 (13)          |
| Prednisone                            |                 |                 |                 |
| Low dose (≤10 mg/d)                   | 6 (50)          | 5 (38)           | 3 (38)          |
| High dose (>10 mg/d)                  | 5 (42)          | 4 (31)           | 3 (38)          |
| Daily prednisone dose, mg/d           | 15 ± 18         | 12 ± 14          | 20 ± 23         |

Note: Patients that did not have myositis antibody results available were excluded. Values are reported as mean ± SD or n (%). ILD-associated MSA includes antisynthetase ab and anti-MDAS ab. Cancer-associated MSA includes anti-TIF1γ ab and anti-MJ ab. Abbreviations: ab, antibody; ANOVA, analysis of variance; CDASI, Cutaneous Dermatomyositis Disease Area and Severity Index; DLCO Hg, diffusion capacity of lung for carbon monoxide Hg; DM, dermatomyositis; FVC, forced vital capacity; ILD, interstitial lung disease; MDAS, melanoma differentiation-associated protein 5; MMT-8, manual muscle testing; MSA, myositis-specific autoantibody; TIF1, transcription intermediary factor 1-gamma; TLC, total lung capacity; VAS, visual analog scale.

$^aP < 0.05$ between groups by $\chi^2$ test.

$^bP < 0.05$ between groups by ANOVA.
The cancer-MSA subgroup of patients with DM also had a significantly higher B/F ratio compared with HCs (0.35 [0.17] vs. 0.22 [0.18], respectively, mean [SD], \( P = 0.04 \)). The Lachnospiraceae family was significantly depleted compared with HCs, particularly the genus CAG-56 in the Lachnospiraceae family, which was absent in patients with cancer-MSA compared with HCs. No other genera were significantly different compared with the ILD-MSA subgroup or HCs. At the ASV level, patients with cancer-MSA showed depletion of the Christensenellaceae R-7 group compared with HCs, similar to the ILD-MSA group (Figure 2F).
Higher disease damage in patients with DM associates with lower microbial diversity. Patients with ILD had significantly lower microbial diversity when compared with patients without ILD ($P = 0.03$, Figure 3A). Patients with ILD were older and less likely to be White compared with patients without ILD; however, there were no significant differences in medication use between the groups (Supplementary Table 1). To further investigate the altered microbial diversity in DM disease...
subgroups, we analyzed its association with the following DM-specific disease outcome measures: global disease activity/damage scores, clinical and laboratory measures of muscle disease, and skin disease activity/damage scores.

Higher physician global disease damage scores in patients with DM were significantly correlated with lower microbial diversity measured both by the Shannon index \(r = -0.33, P = 0.048\) and Chao1 \(r = -0.37, P = 0.01\) (Figure 3D and F). Similar correlations were noted when patients with DM with antibiotic use were excluded \(n = 14\), Shannon index \(r = -0.57, P = 0.03\); Chao1 \(r = -0.55, P = 0.04\) (Supplementary Figure 3A and C). The opposite trend was seen for skin disease activity, as patients with more active skin disease demonstrated higher microbial diversity compared with patients with lower CDASI scores (CDASI activity divided at the median) by Chao1 index \((P = 0.03)\) (Figure 3B). Significant correlations were not observed between microbial diversity and global disease activity or muscle disease (MMT-8, CPK).

Differential taxa abundance analysis demonstrated that higher CDASI activity was significantly correlated with enrichment of the Dorea genus within the Lachnospiraceae family \((r = 0.50, P = 0.002)\). No further associations were seen with other DM disease outcome measures in differential taxa abundance or \(\beta\) diversity analysis.

**Functional alterations in gut microbiome of patients with DM with ILD-MSA.** Microbial functional prediction using PICRUSt2 indicated a total of 970 genes with differential representation in patients with DM with ILD-MSA compared with controls \((q < 0.05\) with multiple hypothesis correction). We mapped biological pathways of 97 genes which were present in

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**Figure 4.** Associations between enriched genes in LPS biosynthesis/transport pathways and Proteobacteria abundance in DM patients with ILD-MSA. Associations between Y axis: enriched genes in LPS biosynthesis/transport pathways \((A)\) UDP-O-[3-hydroxyymistoyl] N-acetylgulosamine deacylase, \((B)\) heptosyltransferase II, \((C)\) heptosyltransferase I, \((D)\) ADP-L-glycer-D-manno-heptose 6 epimerase, \((E)\) LPS-assembly protein, \((F)\) lipopolysaccharide export system protein LptA counts and X axis: Proteobacteria abundance in patients with DM with ILD-MSA. Spearman’s correlation showing significant correlations between relative abundance of Proteobacteria and 4 out of the 6 LPS pathways identified as significantly enriched in ILD-MSA compared to HC using predictive metagenomics. Proteobacteria abundance was log transformed due to skewness. DM, dermatomyositis; HC, healthy control; ILD-MSA, interstitial lung disease-myositis-specific autoantibody; LPS, lipopolysaccharide.
more than 0.001% of total predicted bacterial gene counts using the KEGG database (Supplementary Figure 4).

Patients with DM with ILD-MSA displayed a higher potential for metabolism of carbohydrates, amino acids, and vitamins. Also, the ILD-MSA subgroup had higher abundance of genes involved in signal transduction, cellular transporters, and genetic information processing including aminoacyl-transfer RNA (tRNA) synthetases compared with controls, whereas genes involved in cell growth pathways were depleted.

Genes required for the synthesis and transport of lipopolysaccharides (LPSs) were enriched in patients with DM with ILD-MSA. As Proteobacteria is a known source of potent forms of bacterial LPS and was also significantly enriched in this subgroup (Figure 2C), we analyzed the correlations between identified LPS pathways and Proteobacteria. Significant correlations were demonstrated between Proteobacteria and four of six LPS pathway genes (P < 0.1, Figure 4).

**Dietary differences in DM subgroups and HCs.** Dietary questionnaires were answered by 21 patients with DM and 15 controls with similar demographics (Supplementary Table 2). HEI scores showed that patients with DM had higher consumption of whole grains compared with HCs. Total HEI scores were low in both groups and were not significantly different (Supplementary Table 1).

To assess whether dietary differences existed between MSA subgroups that may contribute to microbiome differences, HEI scores were compared between MSA subgroups and HC subjects who answered the questionnaire (n = 6 ILD-MSA, n = 6 cancer-MSA; Supplementary Figure 5). Patients with ILD-MSA had a higher consumption of whole grains, but otherwise, HEI scores were similar to HCs. Patients with cancer-MSA had more significant differences compared with HCs: a lower consumption of total vegetables and grains compared with HCs. Total HEI scores were low in both subgroups that may contribute to microbiome differences, HEI scores were compared between MSA subgroups and HC subjects.

The patients with ILD-MSA also had extreme depletion of Christensenellaceae R-7 group ASV and multiple ASVs within the Ruminococcaceae family compared with HCs and the
cancer-MSA group. Reduction of Christensenellaceae and Ruminococcaceae has been associated with inflammatory diseases, metabolic dysfunction, and lipid metabolism. In particular, patients with inflammatory intestinal diseases had lower abundance of Christensenellaceae R-7 group compared with controls (38), and a large Chinese population study demonstrated that depletion of the Christensenellaceae family is correlated with more pathologic features of metabolic syndrome (obesity, hypertriglyceridemia, and lower high-density lipoprotein) (39,40). Depletion of the Ruminococcaceae family was also associated with higher hsCRP, IL-6, and LPS binding protein levels and noted more frequently in individuals with overweight (41). The functional significance of the depletion of these organisms in patients with DM with ILD-MSA warrants further investigation.

Predictive metagenomics identified many genes in a number of metabolic and cellular pathways that were significantly altered in the ILD-MSA gut microbiome compared with HCs. In addition to the expansion of LPS pathways, this group also had significantly increased abundance of genes encoding aminoacyl-tRNA synthetases compared with HCs. Although host aminoacyl-tRNA synthetases are a well-known autoantibody target in patients with IIM with ILD-MSA gut microbiome compared with HCs. In addition to the dance of Christensenellaceae R-7 group compared with controls (42), and a large Chinese population study demonstrated that depletion of the Christensenellaceae family is correlated with more pathologic features of metabolic syndrome (obesity, hypertriglyceridemia, and lower high-density lipoprotein) (39,40). Depletion of the Ruminococcaceae family was also associated with higher hsCRP, IL-6, and LPS binding protein levels and noted more frequently in individuals with overweight (41). The functional significance of the depletion of these organisms in patients with DM with ILD-MSA warrants further investigation.

Damage of the microvasculature is strongly implicated in DM disease pathogenesis (43), and increasing evidence supports that the gut microbiome may influence vascular disease (44,45). Menni et al used carotid-femoral pulse wave velocity (PWV) to demonstrate that lower gut microbiome diversity and depletion of the Ruminococcaceae family were both associated with increased arterial stiffness, which is highly predictive of future vascular events (46). In fact, the variance in PWV was explained more by microbiome-related factors than by well-known markers of metabolic syndrome. Lower microbiome diversity and depletion of Ruminococcaceae family were observed in our ILD-MSA subgroup. Vascular damage has been suggested to play a role in myositis-associated ILD, evidenced by increased expression of adhesion molecules and higher blood levels of markers of endothelial damage in these patients (47). Further studies may investigate the association between dysbiosis and vascular damage in DM and its associated ILD.

In our current study, patients with less active skin disease activity had lower gut microbial diversity. Zakostelska et al demonstrated that psoriasis murine models treated with antibiotics to reduce gut bacterial diversity had reduction in Th17 cells and developed less severe imiquimod-induced skin inflammation than conventional mice, suggesting that reduced gut microbial diversity may alter the proinflammatory T cell response and result in less severe skin inflammation (48). Higher DM skin disease activity in our study was also correlated with abundance of the Dorea genus. Dorea species are common members of the gut microbiome that can use host mucin as a source of energy and increase gut permeability. Certain Dorea species, such as Dorea longicatena and Dorea formicigenerans, have been connected with higher stimulus-induced interferon γ response in large population studies (49), which is of interest given the strong interferon signatures in DM skin disease (50,51).

Diet plays a significant role in shaping the microbiome (52). Overall dietary composition of patients with ILD-MSA and HCs in our study were similar, other than higher whole grain intake in patients with ILD-MSA, suggesting that the differences of the gut microbiome in our study are more likely related to the underlying disease state rather than due to dietary differences.

Our findings should be interpreted with several limitations in mind. First, our study includes a relatively small sample size, as DM is a rare disease with an estimated annual incidence of 1 per 100,000 persons (53). Despite the small sample size, we observed several significant associations, suggesting that our findings are unlikely to be due to chance alone. Second, patients with DM were on immunosuppressive treatments, whereas HCs were not, which is an expected limitation for data from an observational disease cohort. Environmental exposures such as antibiotics are known to alter the gut microbiome (54). We used MVA to adjust for antibiotics when they were significant in bivariate analyses. We also demonstrated similar results when analysis was performed after excluding all patients with DM with antibiotic use. Further work is needed to determine whether the intestinal microbial conditions are more dependent on disease or drug effects. Acute dietary changes are also known to rapidly alter the gut microbiome for a short term (55), which may not be accounted for in our cross-sectional study. In order to address this, future studies will include repeat collections of this cohort to account for intrasubject variation. Dietary data were collected in 36 of 62 (58%) of the subjects, which introduces the possibility of a biased dietary assessment. Lastly, the gut microbiome was determined from fecal samples alone. As the gut microbial composition varies throughout the gastrointestinal tract, future studies may consider obtaining mucosal microbiota from different locations along the gut to obtain a more complete assessment.

In summary, the current study reports the results of a fecal microbiome analysis of 36 patients with DM showing that patients with DM, particularly those with ILD-MSAs, have lower microbial diversity and a distinct taxonomic composition compared with HCs. Further studies in larger DM cohorts are needed to validate our findings and elucidate specific pathogenetic mechanisms that link the gut microbiome to clinical and pathological features of DM.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Bae had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Bae, Jacobs, Charles-Schoeman.

Acquisition of data. Bae, Wang, Lagishetty, Katzka, Charles-Schoeman.

Analysis and interpretation of data. Bae, Dong, Jacobs, Charles-Schoeman.

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