The MicroIBioM study: the gut microbiome in inclusion body myositis

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Abstract

Objective

Inclusion body myositis (IBM) is a disorder with features of both inflammation and degeneration yet without effective treatment. Influences of the gut microbiome on degenerative as well as inflammatory disorders and immune treatments are known. We sought to investigate whether the gut microbiome might influence the development or recalcitrance of IBM.

Methods

We appealed to IBM patients and their unaffected spouses/cohabitants for stool samples and data on clinical symptoms, gathering questionnaire data (modified Gastrointestinal Symptom Rating Scale (mGSRS), IBM Functional Rating Scale (IBMFRS) and Bristol Stool Scale) and stool samples for 16S rRNA V3V4 metagenomic analysis from 21 IBM and 20 control probands. Bioinformatic analyses used QIIME2 and MicrobiomeAnalyst software packages. LEfSe and Random Forest analysis aimed to identify group specific biomarkers. PICRUSt was used to perform pathway analysis.

Results

No overall differences of alpha and beta diversity were found between IBM and control group. No impact of immune treatments was found, but a reduction in alpha diversity was identified comparing older (≥ 72 years) IBM and control probands. Increased abundances of some genera, in particular Bacteroides, were detected in the IBM group. Bacteroides, Clostridium CAG 352, and Eggerthella were identified as IBM biomarkers at genus level. Gastrointestinal symptoms (mGSRS) correlated with disease severity (IBMFRS).

Conclusion

General differences of gut microbiome seem unlikely to play a role in the genesis of IBM. Whether the late occurring or the more specific differences detected are part of the disease course needs to be addressed by investigations of further biosamples.

Key words

inclusion body myositis, human microbiome, idiopathic inflammatory myopathies, RNA, ribosomal, 16S, *Bacteroides*

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Introduction

Inclusion body myositis (IBM) is a progressive muscle disorder with typically late onset. Predominant weakness of finger flexion and knee extension are characteristic as are the histopathological findings of endomysial infiltration, MHC-I up-regulation, rimmed vacuoles and pathological protein accumulation (1). Mitochondrial changes, including mitochondrial DNA deletions and duplications (2), are known and age-inadequate numbers of cytochrome c negative fibres are considered supportive for the diagnosis (3). IBM is classified among the idiopathic inflammatory myopathies (IIM) (4), its typical resistance to the usual immunosuppressive treatments was noted early on (5). To integrate these various aspects, a primary inflammatory disease with secondary conversion into a degenerative process was proposed (6). Other explanations for the lack of sustained response of IBM to immunosuppressive therapies focus on T cell exhaustion leading to their dysfunction (7) rather than degeneration. Indeed, highly differentiated cytotoxic T cells (TEMRA), not responding to classical immunosuppression, have been described in IBM (8).

The gut microbiome influences many different processes in humans, including immune tolerance (9). Changes in gut microbiome in comparison to healthy controls were described in different autoimmune diseases including rheumatoid arthritis (10), myasthenia gravis (11), chronic inflammatory demyelinated polyneuropathy (CIDP) (12), but also degenerative diseases such as Parkinson disease (13). Concerning IIM, decreased alpha diversity was found comparing immune-mediated necrotising myopathies (IMNM) (14) and dermatomyositis (DM) (15, 16) to healthy controls. However, no difference was found for a mixed group of IIM patients (17). One study described an increase or decrease of alpha diversity correlating to the myositis-specific/ -associated antibodies in the patients' sera (18). Moreover, influence of the gut microbiome on treatment responses is well described. In epithelial tumours, the composition of gut microbiome effects the response to immune checkpoint therapy (PD1-based) (19).

With its inflammatory as well as degenerative stigmata and populations of hard to target immune cells, we considered IBM a prime candidate for an influence of the gut microbiome on its development and progression. Thus, we analysed the gut microbiome of IBM patients in comparison to healthy subjects from similar living conditions to detect differences associated with IBM.

Methods

Ethics approval

This study conforming with the World Medical Association Declaration of Helsinki was approved by the Ethikkommission der Medizinischen Fakultät Bonn (079/21) and registered as no. 1024 in the RedCap UKB-Studienregister.

Participant selection

Candidates were recruited via leaflets in our and supporting colleagues outpatient clinics, spread by patient advocacy group. Further information material and consent forms were sent to interested persons, then contacted by telephone. Once written informed consent was given, screening questionnaires were send and IBM participants asked to provide data on clinical and histological diagnosis (unless known in our department) or give written consent to contact their treating colleagues and receive this data from them.

To guarantee correct diagnosis of IBM, we reviewed the clinical and histological reports and included those fulfilling the 2011 ENMC IBM Working Group criteria for 'clinico-pathologically defined' or 'clinically defined' IBM (1) only. Further exclusion criteria for all participants were: (i) diagnosis of a different muscle disorder than IBM, (ii) treatment with antibiotics in the three months prior to sampling, (iii) chemotherapy in the six months prior to sampling, (iv) any chronic disorder of stomach, intestine, gut, liver or pancreas, (v) stomach, intestine or gut surgery in the last five years (excluding appendectomy or cholecystectomy), (vi) COVID19 in the last three months prior to sampling; (vii) participation in

a clinical drug trial in the six months prior to sampling, (viii) the use of laxatives. Other medication, including immunomodulatory/ immunosuppressive drugs were accepted. For the control participants the further condition of not having IBM applied. Throughout we made it clear that we were particularly interested in couples of IBM patients and persons with shared living habits and space to minimise different environmental influences on the microbiome, but this was not an inclusion criterion.

Symptom assessment

To assess disease severity in the IBM participants and similar disability of a different causes in the control participants, the Inclusion Body Myositis Function Rating Scale (IBMFRS) (20) was adapted as a questionnaire. To assess gastrointestinal symptoms, we used the modified Gastrointestinal Symptom Rating Scale (mGSRS) (21) as a further questionnaire. To measure stool consistence, we used the Bristol Stool Scale (BSS) (22)

Exchange of questionnaires, sampling materials and samples

Participants received and returned the questionnaires, sampling materials and material for the return of questionnaires and samples by postal services with exception of a single case with procedures performed in our outpatient clinic. Any questions were resolved by phone.

Stool samples were collected and send in a sterile stool sample tube with 2 ml of Stool DNA Stabilizer (Invitek Molecular Ltd., Germany). To return the sample, the tube was placed in a sealed sample bag (Suesse Labortechnik, Germany) with an activated Anaerocult P sachet (VWR International Ltd., Germany). Upon arrival of samples in the lab, tubes were briefly vortexted and then frozen upright at -80°C until DNA extraction. Stool DNA extraction was carried out using the PSP Spin Stool DNA Basic Kit (Invitek Molecular Ltd., Germany) according to manufacturer's instructions (protocol 3) on 1.4 ml thawed, vortexted sample. Resulting DNA was diluted in TE buffer (pH 7.4) and stored at -20°C. A 5 ng/µl dilution was used for the library preparation during amplicon PCR. In this process, the 16S rRNA gene's V3V4 region underwent amplification using the Bakt_341F (5'-CCTACGGGNGGCWGCAG-3') and Bakt_805R (5'-GACTACHVGGTATCTAATCC-3') primers. This amplification occurred within a 25 µL PCR reaction containing 2.5 µL of template, 12.5 µL of 2×KAPA HiFi HotStart ReadyMix from Roche (Mannheim, Germany) and 5 µL of the corresponding primers (1 µM). PCR was performed in a Mastercycler (Eppendorf, Hamburg, Germany) as follows: denaturation step at 95°C for 3 min, followed by 25 cycles of denaturation (30 s at 95°C), annealing (30 s at 55°C), elongation (30 s at 72°C) and a final elongation step at 72°C for 5 min. In a second PCR phase, dual indexes and an Illumina sequencing adapter were incorporated utilising the Nextera XT v. 2 Index Kit from Illumina, (San Diego, CA, USA). Each sample underwent a second PCR reaction, employing a total volume of 50 µL per sample. This consisted of 25 µL of 2×KAPA HiFi HotStart ReadyMix along with 5 µL of the corresponding Nextera XT Index primer, and 10 µL of PCR grade water. The cycling conditions were: initial denaturation phase at 95°C for 3 minutes, succeeded by 8 cycles involving denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, elongation at 72°C for 30 seconds, and a final elongation step at 72°C for 5 minutes. Amplicon libraries were randomly assessed using Agilent TapeStation 4200 equipped with D1000 ScreenTape (Santa Clara, CA, USA) and subsequently purified using AMPure XP beads. The samples were normalised to a concentration of 4 nM, then pooled equimolarly.

16S rRNA Sequencing

The final pool was quantified using the Qubit dsDNA HS Assay Kit from Thermo Fisher Scientific (Waltham, MA, USA) and fragment size was determined on a D1000 HS ScreenTape. Sequencing was performed on an Illumina MiSeq system using the MiSeq Reagent Kit v. 3 with 2×300 cycles, clustering at 8 pM with a 20% spike-in of PhiX and demultiplexing on the MiSeq system.

Bioinformatics and statistical analyses

The 16S sequencing data underwent processing using DADA2 within the QIIME2 platform (version 2022.8) as described (23). This involved sequence quality control, denoising procedures, and elimination of chimeric sequences. The denoised sequences were classified using SILVA databases to identify distinct variants known as amplicon sequence variants (ASVs) based on sequences exhibiting >99% similarity. A rarefied abundance table was generated, maintaining a sampling depth of 22,986 sequences, and utilised to compute alpha as well as beta diversity metrics QIIME2. A linear discriminant analysis (LDA) coupled with effect size measurement (LEfSe) analysis was performed to identify IBM-specific biomarkers using the MicrobiomeAnalyst platform (24, 25).

Pearson correlation coefficients were determined to test for correlation of age (in years), questionnaire results (IBMFRS, mGSRS, BSS) and alpha diversity metrics (Faith's phylogenetic diversity, Shannon's entrophy, Pielou's evenness index, observed features). Spearman correlation coefficients were calculated for correlation of IBMFRS item 1 (swallowing), items 2 to 4 (hand function) and items 7 to 10 (mobility). Further data of microbiome analyses were tested by unpaired t-test or Mann-Whitney rank sum test. A p-value ≤ 0.05 was defined as statistically significant.

Results

We screened 66 participants (37 IBM and 29 controls). Six participants (three IBM, three controls) failed screening due to other co-existing neuromuscular diseases or gastrointestinal surgery. Nine participants (five IBM, four controls) were re-screened due to antibiotic treatments or COVID19 infection, allowing inclusion of further three IBM and two controls. From these 32 IBM participants, 19 fulfilled 'clinicopathologically defined' and two 'clinically defined' 2011 ENMC research di-

agnostic criteria (1). Two participants fulfilling the 'probable IBM' criteria only were not included. At the time of the study the 2023 ENMC IBM criteria (3) were not yet published, using these new criteria all included IBM participants would be diagnosed with IBM. Three IBM participants failed to send materials, while in the control group one participant withdrew consent and two were lost to follow-up. Isolated DNA of stool samples of one control and one IBM participant, respectively, failed quality standards, leaving us with full datasets for 21 IBM and 20 controls, except for a single control BSS questionnaire (flow chart in Supplementary Fig. S1).

In accord with our inclusion (and the 2011 ENMC diagnostic) criteria, all probands were older than 45 years at the time of inclusion. All IBM probands had a CK <15x ULN. CK values of the control group were not reviewed. Twelve IBM probands were treated with immunomodulatory and/or immunosuppressive therapies. Eight received intravenous immunoglobulins (ivIG), in a wide range of doses and frequencies. Three of these were also taking oral prednisolone. Three were treated with methotrexate (MTX), but not ivIG, one in addition to prednisolone. One proband was treated with oral glucocorticoids only. No control probands received immunomodulatory or immunosuppressive treatment. Proband data are listed in Table I.

Table I. Proband population and questionnaire data.

	IBM total	IBM_old	Controls total	Ctrl_old
n	21	11	20	9
male:female	17:4	10:1	7:13	3:6
age in years (mean ± SD) range	71.19±9.27 52-83	78.55±3.42 72-83	69.25±9.47 53-82	78±3.46 72-82
Treatment n ivIG (+ GC) MTX (+ GC) GC only	12 8 (3) 3 (1) 1	6 4 (2) 1 (0) 1	0 0 (0) 0 0	0 0 (0) 0 0
IBMFRS (median) range	23 7-40	20 7-40	40 8-40	39 8-40
IBMFRS item 1 (median) range	3 1-4	2 1-4	4 2-4	4 2-4
IBMFRS items 2-4 (median) range	8 3-12	8 3-11	12 4-12	12 4-12
IBMFRS items 7-10 (median) range	8 1-16	7 1-16	16 0-16	15 2-16
mGSRS (median) range	3 0-10	4 0-10	1 0-10	1 0-10
BSS (median) range	4 2-5	4 2-5	4[#] 2-5	4 3-5

BSS: Bristol Stool Scale; controls total: all control probands; Ctrl_old: control probands 72 years or older; GC: glucocorticoids; IBM: inclusion body myositis; IBM total: all IBM probands; IBM_old: IBM probands 72 years or older; IBMFRS: Inclusion Body Myositis Functional Rating Scale; ivIG: intravenous immunoglobulin; mGSRS: modified Gastrointestinal Symptom Rating Scale; MTX: metho-trexate.
[#] n=19, as one proband failed to return the questionnaire.

Questionnaires

We received completed questionnaires from all participants expect for one control IBMFRS BSS. We additionally calculated the scores of the item 1, items 2–4 and items 7–10 only, as these aim to grade swallowing, hand function and overall mobility, respectively, thereby different aspects of the typical impairments in IBM that might affect variably nutrition and gut passage time. Results from the questionnaires are found in Table I.

Microbiome structure in IBM versus control group

Various alpha diversity metrics were assessed in the IBM and control groups. No statistically significant differences were found in their comparisons. Notably, the control group exhibited an inclination towards greater evenness, as indicated by Pielou's evenness index. Additionally, there was a trend towards increased diversity in terms of Shannon entropy and richness (observed features) in the control group, but these tendencies did not reach statistical significance (Fig. 1).

Subsequently, the bacterial composition was evaluated utilising Bray-Curtis, Jaccard index, and both weighted and unweighted UniFrac metrics. This analysis was conducted at the feature level within QIIME2. No statistically significant variances were observed in any of these metrics between the groups as shown in Table II.

Because of the restricted taxonomic detail provided by 16S sequencing, an examination of the composition was further conducted at the genus level. In this analysis, both the Jaccard and Bray-Curtis metrics revealed a distinct inclination for the microbiomes of the IBM and control groups to harbour varying genera (Table II).

The initial taxonomic comparison involved assessing the 10 most abundant genera through a bar chart (Fig. 2A), to identify significant differences. Among these, the most substantial and statistically significant distinction was observed in the abundance of the genus *Bacteroides*, accounting for 18.6% in the control group and 30.4% in the IBM group. Subsequent genera were *Faecalibacterium* (7.2% control, 6.4% IBM), *Prevotella* (6.0%, 2.7%), Alistipes (4.6%, 3.8%), *Subdoligranulum* (4.1%, 3.4%), *Parabacteroides* (4.0%, 3.4%), Lachnospiraceae NK4A136 group (2.9%, 3.9%), Clostridia UCG_014 (2.9%, 2.4%), Oscillospiraceae UCG_002 (2.8%, 2.5%), and Christensenellaceae R-7 group (2.6%, 2.0%). The cumulative abundance of the remaining genera was consolidated under 'other', constituting 44.4% in the control group and 39.1% in the IBM group, respectively.

The Linear Discriminant Analysis Ef-

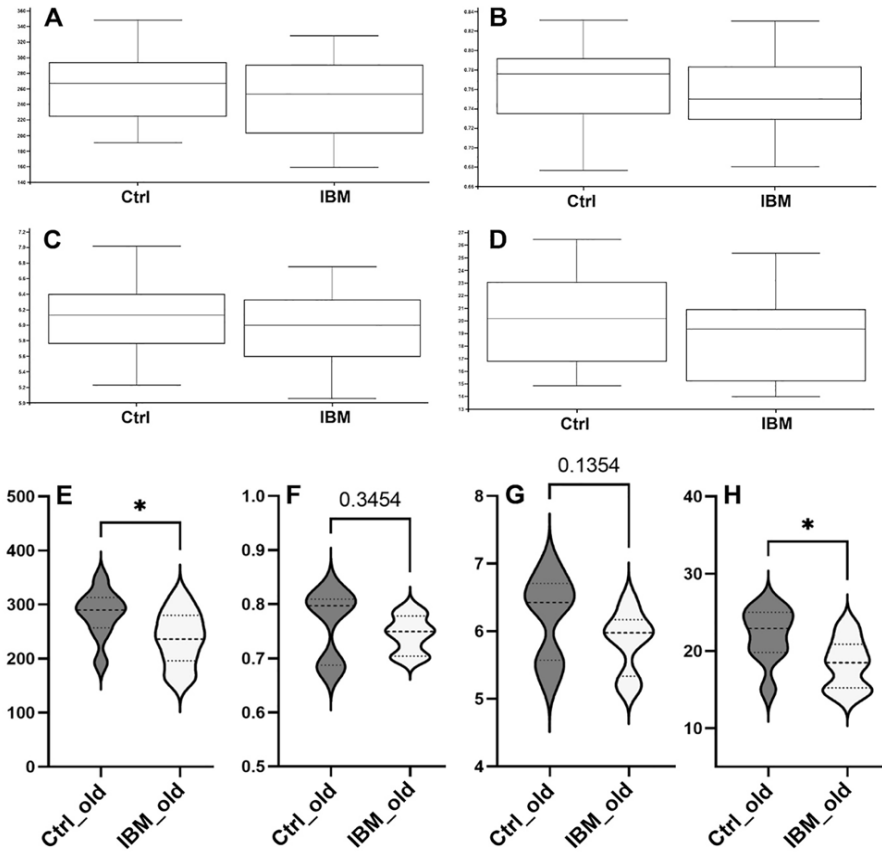

Fig. 1. Comparing alpha diversity metrics: observed features (**A, E**), Pielou evenness (**B, F**), Shannon entropy (**C, G**) and Faith's phylogentic diversity (**D, H**). Box plots in upper part (**A-D**) show comparisons between entire control (Ctrl) and IBM groups. Violin plots in lower part (**E-H**) show comparisons between the older subgroups (Ctrl_old and IBM_old) with * indicating differences at the first significance level (p<0.05).

Table II. Beta-diversity metrics on feature and genus level for comparisons of total and older groups.

Level	ß-diversity metric	Group1-Group2	n	F-value	*p*-value
Feature	Unweighted UniFrac	IBM-Ctrl	41	0.885	0.583
Feature	Weighted UniFrac	IBM-Ctrl	41	1.144	0.278
Feature	Jaccard	IBM-Ctrl	41	0.901	0.908
Feature	Bray-Curtis	IBM-Ctrl	41	0.866	0.837
Genus	Jaccard	IBM-Ctrl	41	1.489	0.057
Genus	Bray-Curtis	IBM-Ctrl	41	1.736	0.059
Feature	Jaccard	IBM_old-Ctrl_old	20	1.211	0.116
Feature	Bray-Curtis	IBM_old-Ctrl_old	20	1.303	0.143
Genus	Jaccard	IBM_old-Ctrl_old	20	1.704	0.041
Genus	Bray-Curtis	IBM_old-Ctrl_old	20	1.987	0.049

Ctrl: control; IBM: inclusion body myositis; Ctrl_old: control probands 72 years or older; IBM_old: IBM probands 72 years or older.

fect Size (LEfSe) method identified three biomarkers at genus level, all of which showed elevated levels within the IBM group, exceeding the specified significance threshold of p<0.05. These were, along with their respective LDA scores, *Bacteroides* (-5.69), *Clostridium* CAG 352 (-4.27), and *Eggerthella* (-3.02).

Functional prediction (Tax4Fun) to identify differences in functional capacity between IBM and control group

Comparison between the IBM and control group was conducted using the 'Shotgun Data Profiling' plugin within MicrobiomeAnalyst 2.0. Initially, a functional prediction was executed utilising Tax4fun Silva. Subsequently, this output was utilised as input for the Shotgun Data Profiling Plugin. This facilitated the comparison of the functional potential between the groups within the 'Shotgun Data Profiling' plugin. A DeSeq2 analysis revealed 272 notable Kyoto Encyclopedia of Genes and Genome (KEGG) orthologs (KO) highlighted by MicrobiomeAnalyst. The initial six KOs are presented in Supplementary Table S1. Across all significant findings, decreased levels were observed primarily within the enzymes/KOs associated with the IBM contrast to the control group.

Network mapping involving the 272 noteworthy KOs aimed to uncover significant enrichment pathways. Table III presents the findings, highlighting pathways that display significant differences. Following the False Discovery Rate (FDR) correction, seven pathways exhibited significant differences. Although this table lacks specifics regarding the direction or magnitude of these changes within pathways, as previously mentioned, the majority of pathways displayed had reduced levels in the IBM compared to the control group.

Spearman correlations between bacterial genera and IBMFRS for items 1, 2 to 4 and 7 to 10 did not result in significant findings following FDR correction. Supplementary Fig. S2 shows the comparison of the top 25 correlated genera for the three groups.

Microbiome structure in IBM vs. control group in elderly patients

Various stratification approaches were employed to identify potential stronger associations between IBM and the microbiome. These stratifications were based on factors such as gender, age, IBMFRS, and the use of immunosuppressive medication. Notably, the most pronounced difference was observed within an older subgroup when participants were divided into two equal age brackets (using a cut-off of 72 years) (Table I).

Comparing the older control group (Ctrl_old) to the older IBM group (IBM_old), there was a noticeable trend. The alpha diversity characteristics, specifically the observed fea-

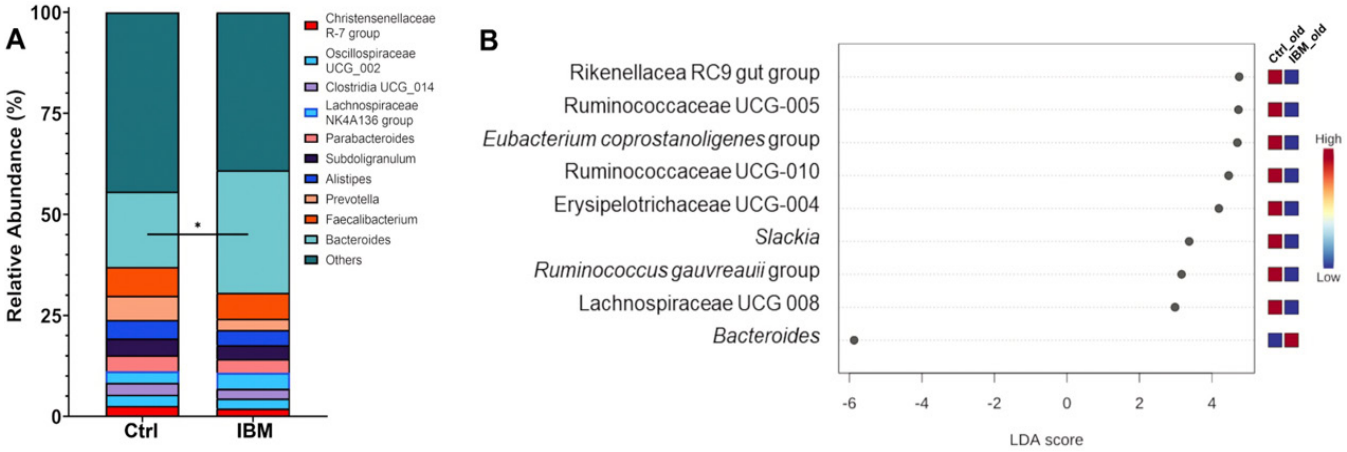

Fig. 2. A: Bar plots visualising the distribution of genera among both the control (Ctrl) and IBM group highlight observable distinctions in abundance with * indicating $p<0.05$.
B: Comparison of old control (Crtl_old) and IBM (IBM_old) groups using LEfSe (Linear Discriminant Analysis Effect Size) at genus level. Graphical representation of the outcomes with $p<0.05$ was generated using the LDA (Linear Discriminant Analysis) score.

Table III. Expected microbiome pathways where expression changed significantly between control and IBM group.

Pathway	Hits	*p*-value	FDR
Porphyrin metabolism	26	2.24e-15	3.36e-13
Histidine metabolism	9	1.94e-05	0.00124
Citric acid cycle (TCA cycle)	11	2.49e-05	0.00124
C5-Branched dibasic acid metabolism	7	7.07e-05	0.00217
Valine, leucine and isoleucine biosynthesis	6	7.23e-05	0.00217
O-Antigen nucleotide sugar biosynthesis	12	0.000491	0.0123
Streptomycin biosynthesis	5	0.000785	0.0168
Carbon fixation pathways in prokaryotes	11	0.00462	0.0832
Oxidative phosphorylation	12	0.00499	0.0832
Amino sugar and nucleotide sugar metabolism	12	0.0187	0.281
Phenylalanine, tyrosine and tryptophan biosynthesis	6	0.0497	0.677

Identified by one-way statistical analysis using DeSeq2, with a specified *p*-value cut-off of 0.05.
FDR: false discovery rate; TCA: tricarboxylic acid.

tures, exhibited a notably higher level of diversity within Ctrl_old compared to IBM_old. Furthermore, the sum of branch lengths within the phylogenetic tree encompassing all species (Faith's phylogenetic diversity) also highlighted significant differences between these two groups. The analysis of Shannon entropy indicated a trend towards reduced diversity within IBM_old compared to Ctrl_old (Fig. 1, violin plots).
Beta diversity analysis conducted at both the feature and genus levels revealed a more pronounced dissimilarity in microbial composition between Ctrl_old and IBM_old, shown in Table II. Statistically significant differences were observed for the Bray-Curtis metric (p=0.049) and the Jaccard metric (p=0.041).
Stratification resulted in an increased number of biomarkers detected in the LEfSe analysis. These biomarkers serve as distinctive features, aiding in the differentiation of microbiomes between IMB_old and Ctrl_old. Remarkably, among the nine identified biomarkers, only one could be distinctly attributed to the IBM_old group. *Bacteroides* stood out as the most prominent difference between the compared groups (LDA=-5.88). Conversely, the remaining biomarkers primarily linked to the Ctrl_old group exhibited reduced representation in the IBM_old group. These biomarkers, listed in descending order based on their LDA score are shown in Figure 2B.

Correlation analyses of alpha diversity metrics, proband age and questionnaire results

No significant correlation between alpha diversity metrics and age, IBMFRS, mGSRS or BSS were found. However, the mGSRS score correlated negatively with the IBMFRS (total and item 7-10, respectively) and the total IBMFRS (but not its items 7-10) correlated negatively with age, (p>0.05), respectively. Pearson correlation coefficients listed in Supplementary Table S2.

Discussion

Overall, no significant difference of alpha or beta diversity of the gut microbiome were evident between IBM and control probands. This argues against marked gut microbiome changes as an early event in the pathobiology of IBM. Investigations into the gut microbiome of other IIM frequently reported differences. Decreased alpha diversity has been reported in DM and IMNM (14-16) but not in mixed IIM groups (17, 18), where some subgroups even showed increased alpha diversity (18). In comparison to these studies, we chose an approach in the selection of our study population that favoured controlling for dietary and environmental influences (26) by preferring cohabitant controls to matching by sex or clinical characteristics as most other IIM gut microbiome studies did (14, 15, 18). Due to the predominance of males in IBM (27) and of heterosexual cohabitation, we thereby accepted a disparity of sexes between the groups. While various sex differences of the gut microbiome have been described, these ought to be relatively small in

the age group we included here, as sex hormone levels seem to have a crucial influence (28).

It should be further noted that half of our IBM probands received intravenous immunoglobulin (ivIG) treatments in a wide range of doses and intervals and a number of them also had immunosuppressive treatments, while most other IIM studies used samples from untreated patients. Drugs, including immunomodulating therapies, can change the gut microbiome (29, 30), but we failed to detect a difference in treated probands in general and the ivIG subgroup in particular. This is in contrast with some studies in autoimmune diseases (31), but in line with the absence of changes in CIDP after ivIG infusion (12). Our sub-analyses comparing patients and controls older than 72 years at the time of sampling showed more pronounced changes. One might have expected that differences in this subgroup were rather smaller due to the fact that ageing itself is associated with changes in the gut microbiome including reduced diversity (32, 33). Thus, our results might point to differences developing over the course of the disease. An obvious parameter of interest here was disease duration. Unfortunately, due to different diagnostic definitions used and frequently reported symptoms and signs (*e.g.* elevated CK, dysphagia, foot drop) prior to making the diagnosis, we were unable to reliably extract this from the patients' reports used for this study. However, one of the effects of IBM that typically increases over the disease's course is a reduction in mobility. We considered reduced physical activity as a cause of prolonged colonic transit time leading to decreased diversity (34) and tried to address this by a subanalysis of the IBMFRS items 7-10, intending to capture generally reduced mobility rather than other impairments. However, we found no correlation between these items and the alpha diversity. Altered passage time should also manifest with different stool consistence (35), assessed here by the BSS, that did not show difference between both groups either. Likewise, while altered nutrition due to dysphagia could influence the gut microbiome, correlations of bacterial genera to IBMFRS sub-scores for item 1 (swallowing), items 2–4 (hand function) and items 7–10 (s. above) were not significant (Supplementary Fig. S2).

Despite the lack of significant differences in the overall diversities, some of the differences of microbiota genera appear remarkable. In particular, *Bacteroides* show the largest increase in abundance and are identified as a biomarker in IBM, prominent in the older population. *Bacteroides* is one of the most abundant bacterial genera in human gut microbiota, important for the metabolism of dietary and host glycans (36). Lack of sphingolipids produced by *Bacteroides* species plays a role in inflammatory bowel disease (37). *Bacteroides* was found in lower abundance in IMNM (14), higher abundance in the mixed IIM group of Luo *et al*. (17) and not among the genes differing markedly in the DM groups (15, 16). Li *et al*. found *Bacteroides* overall increased but noted different regulations of species comparing patients with rapid progressive to those with chronic interstitial lung disease (18). *Eggerthella* have not been mentioned in the IIM studies discussed above (14-18). However, *Eggerthella* have been found enriched in the gut microbiome of patients with multiple sclerosis, systemic lupus erythematosus and rheumatoid arthritis, respectively, and some strains have been shown to activate Th17 cells (38).

It needs to be stressed that we performed an exploratory 16S rRNA analysis of V3 and V4 hypervariable regions in a relatively small sample size. To determine whether any of these characteristics or any of the pathways affected theoretically by the microbiome changes we found might play roles in IBM, these findings need to be confirmed in more in-depth analysis, preferably in an independent sample. In particular the hypothetical metabolic effects of the changes described here (Table III), of which only the changes in oxidative phosphorylation have so far been linked directly to IBM (2), need to be correlated to metabolic measurement in further biological samples.

Our study contributes to a slowly emerging picture of the gut microbiome in IIM. Undoubtedly, this is a complex matter, and we are a long way from establishing cause-effect relations. If the pattern of study results known so far holds true, gut microbiome changes in IIM may be as diverse as, *e.g.* these diseases' histopathological pattern.

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