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Leading Off

Fecal Microbial Community Profiling Allows Discrimination of Phenotype and Treatment Response in Pediatric Crohn's Disease and Ulcerative Colitis—An International Meta-Analysis

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Background and aims: The pathophysiology of pediatric inflammatory bowel disease (PIBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), is not entirely understood. Dysregulation of the intestinal microbiome is recognized as both a disease-driving and a potential therapeutic target. This study aimed to systematically analyze gut microbiome compositions and its applicability as a biomarker for disease progress and treatment response.

Methods: Bibliographic and nucleotide databases were searched. Raw *16S-rRNA* sequencing reads were subjected to a uniform downstream dada2/phyloseq pipeline to extract taxonomy, community structure, and abundance information. Patient metadata were extracted from publications, and study authors were contacted for further details if required.

Results: Twenty-six studies comprising 3956 stool samples (CD 41%, UC 36%, 23% healthy) were included in the analyses. Median age of individuals was 12 (interquartile range 4). Sex distribution was comparable. Alpha diversity was reduced between the healthy and both UC and CD treatment-naïve groups ($P < .001$) and further reduced with increasing clinical disease activity. Beta diversity revealed altered community structure in treatment-naïve children with PIBD ($P < .001$). This alteration remained in patients in clinical remission ($P < .001$). Machine learning models discriminated between treatment-naïve patients with CD or UC with an area under the receiver operating characteristics curve (AUROC)

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of 98%. Microbial communities differed between patient responders versus nonresponders to treatment ($P < .001$). Further, microbial community profiling distinguished treatment response (eg, steroid, nutrition, or TNFα) with AUROCs of 82%-90%.

Conclusions: Gut microbial community structure is substantially altered in active and inactive PIBD and may be utilized as a biomarker for differentiating PIBD subtype and predicting treatment response.

Lay Summary

We identified 26 studies on the gut microbiome in pediatric patients with IBD compiling a total of 3956 stool samples (CD 41%, UC 36%, 23% healthy) revealing microbial community structures unique to patients with CD and UC. These community patterns allow for the distinction of PIBD type and prediction of treatment response.

Key Words: ulcerative colitis, Crohn's disease, treatment response, 16S metagenomics, microbiome

Key Messages

What is already known? The gut microbiome is affected by inflammatory bowel disease, and alterations of the gut microbiome can fuel intestinal inflammation.

What is new here? This study compiles the largest cohort of pediatric patients with inflammatory bowel disease by systematic review of the literature and collectively analyses the composition of the gut microbiome.

How can this study help patient care? This study highlights that compositional patterns of the gut microbiome may allow distinction of PIBD type and prediction of treatment response.

Introduction

The pathogenesis of inflammatory bowel diseases (IBD) is a complex interplay of a dysregulated immune response, host genetic predisposition, and specific environmental factors.[1] The gut microbiome as an interface between the environment and the immune system plays a key role in this process.[2] The gastrointestinal microbiota is usually described as a postnatally acquired "organ," comprising a wide variety of bacteria, and other microbes, whose composition and activity can have both systemic and local effects.[3] As this is an acquired organ, there are major differences between the adult and infant microbiome.[4] Infant microbial communities are naturally less diverse, often still immature and change in the first years of life in parallel with the development of the immune system.[5] Several studies have added to the understanding of altered microbial community structure, also known as dysbiosis, present in patients with IBD.[2,6–8] As these studies have primarily focused on adults, a direct extrapolation to a pediatric cohort would ignore how the gut microbiome undergoes dynamic changes in diversity and composition during development. A recently published study on IBD across the pediatric age range demonstrated that age and inflammation independently impact microbial community structure in IBD patients.[9] Pediatric studies have also established links between microbiome profiles and disease phenotype,[7,10,11] IBD disease activity,[12–14] and disease course.[15,16] In addition, the influence of diet on the gut microbiome composition, and subsequently the risk of IBD development, is increasingly recognized.[17] To this point, exclusive enteral nutrition (EEN) has been shown to induce remission in pediatric Crohn's disease (CD)[18] with correlating changes in the microbiome including decreased abundance of *Lachnospiraceae*, *Ruminococcaceae*, and *Erysipelotrichaceae*, which are known to be involved in the perpetuation of intestinal inflammation in CD.[19,20] Studies on treatment response have shown that infliximab (IFX) response paralleled an increase in the diversity of gut microbiome.[21,22] In an analysis of absolute abundances of fecal microbiome in relation to response to IFX induction, higher Bifidobacteriales abundance was found to be associated with rapid therapy response.[23]

All these data suggest that the microbiome itself could serve as a biomarker for disease progression, response to treatment and, with deeper insight, a potential therapeutic target.

Here we aimed to systematically expand our current understanding of pediatric IBD (PIBD) by exploring and identifying changes in individuals, specifically with regard to treatment and disease severity. Further, we try to correlate specific outcomes with the microbial change we see within our dataset. We obtained raw *16S*- ribosomal ribonucleic acid (*rRNA*) sequencing reads and clinical data, including fecal calprotectin and disease activity scores, to determine links between the gut microbiome and disease course. Here we describe the role of microbiome abundance in differentiating between PIBD phenotypes, show differences in microbial community structures depending on disease activity, and examine whether the microbiome still differs in patients in clinical remission compared to healthy controls. In addition, we show that treatment responders and treatment nonresponders can be further differentiated based on the microbial composition. Finally, we extend these analyses for different treatment regimens for pediatric CD and pediatric ulcerative colitis (UC).

Materials and Methods

Ethics Statement

This study was conducted in accordance with the guidelines of the Institutional Review Board of the Medical University of Innsbruck and the 1975 Declaration of Helsinki.

Search Strategy and Data Extraction and Harmonization

This systematic review was conducted in accordance with the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) reporting guidelines. Relevant studies to be included in the meta-analysis were identified by a systematic literature search using the following bibliographic databases: PubMed, Scopus and Web of Science, National Center for Biotechnology Information (NCBI) nucleotide archive, the Genome Sequence Archive, and the EMBL European nucleotide archive as data repositories including studies and datasets from the years 2010 until January 2024. The search terms "pediatric," "children," "adolescence," or "childhood"

were combined by the Boolean operator "AND" with "IBD," "PIBD," "Crohn's disease," "Crohn," "ulcerative colitis" or "colitis," "AND" with "microbiota," "microbiome," "16S," or "metagenomic" in all listed databases.

Three investigators (D.A., A.R.P., and G.F.V.) independently performed screening of the mentioned databases via abstract and title search. All papers were categorized and original studies were extracted. Studies from January 2010 until February 2024 were evaluated. Only English studies were included. If an article was considered eligible by either of the 3 reviewers, the full text was retrieved where available. After categorization, a second search was performed on full-text articles to check for methodical quality and fulfillment of inclusion and exclusion criteria (see below). Raw sequencing data were retrieved from the respective archives or study author upon personal contact. Missing metadata or raw sequencing led to study exclusion. In case of any disagreement, the issue was solved by discussion. Patient metadata (eg, age, sex, disease activity, treatment, and fecal calprotectin) were extracted. Anti-tumor necrosis factor alpha (TNF) comprises IFX or adalimumab therapy. Immunomodulators comprise thiopurine or methotrexate therapies. Treatment response was defined by included studies as either a combination of significant improvement in clinical disease activity using international disease activity scores, C-reactive protein, or lastly mucosal healing or normalization of fecal calprotectin levels (threshold 100 µg/g stool). Clinical disease activity scores (eg, pediatric Crohn's disease activity index [PCDAI],[24] weighted PCDAI [wPCDAI],[25] pediatric ulcerative colitis activity index (PUCAI)[26]) were unified by the categorical interval and given as "inactive," "mild," "moderate," or "severe." These ratings were further defined by PCDAI scores: <10 points inactive, 11-29 points mild, 30-39 points moderate, and over 40 severe disease; or for the wPCDAI: <12.5 points inactive, 12.5-40 points mild, 42.5-57.5 points moderate, and over 57.5 points severe disease; or finally for the PUCAI: <10 points inactive, 10-34 points mild, 35-64 points moderate and over 64 points severe disease. Further differentiation of disease subtypes was collected by contacting individual researchers. We used a designation identified by The Paris Classification System of Inflammatory Bowel Disease to separate disease localization. Patients with multiple treatments were excluded in the specific sub-analyses (TNF, EEN, and steroid +/–azathioprine). A period of 3-6 months after treatment initiation was chosen to assess the response to therapy.

Studies that sampled patients longitudinally based on changes in either clinical disease activity or treatment response, or changes in both were included as multiple independent samples as microbial communities seem to change significantly with these features.[15]

Inclusion and Exclusion Criteria

Studies on the gut microbiome in children (age range 6-18 years) with PIBD providing *16S-rRNA* sequencing data were assessed. Pediatric inflammatory bowel disease diagnosis had to be based on clinical, biochemical, endoscopic, and histopathologic findings according to revised Porto criteria. The cohort of healthy individuals was compiled from PIBD studies included. Studies with insufficient raw data annotation, insufficient association of individual microbial sequencing data with corresponding individual patient characteristics, or without *16S-rRNA* sequencing data available (shotgun or ITS sequencing) were excluded.

Study Quality and Bias Assessment

Assessment of quality for the included studies was performed with the Joanna Briggs Institute critical appraisal tools for cohort studies, case series, and case reports. Studies were considered with low, moderate, or serious risk of bias if they fulfilled more than 80%, between 60 and 80%, or less than 60% of the requested items.

Bioinformatics and Statistical Analyses

Raw sequences were trimmed for barcodes and primers as provided by the source study or by contacting the study authors using Cutadapt. DADA2 and R (R: A language and environment for statistical computing. R Foundation for Statistical Computing) were used for sequence quality control with filtering of low-quality sequences and chimeras, sequence trimming (according to sequence quality 15-30 nucleotides at both ends), and the construction of amplicon sequence variants (ASVs). Subsequent analyses were carried out on genus level. Taxonomy was assigned to ASV using a naïve Bayesian classifier against Silva operational taxonomic units (version 138.1) and integrated into a phyloseq object using the R package phyloseq for downstream analyses. Revised phylum nomenclature from 2021 was used.[27] Study and sequencing-based variation were controlled for using MMUPHin (Meta-Analysis Methods with a Uniform Pipeline for Heterogeneity in microbiome studies). This adjustment shifts the overall effect seen by specific studies. Alpha diversity was calculated based on richness ("observed") and Shannon diversity measures, and Mann-Whitney U-tests were applied for statistical significance. Beta diversity was visualized as principal coordinates analysis on the Bray-Curtis distance that is calculated after performing variance stabilizing transformation. Beta diversity was tested using a non-parametric multivariate statistical permutation (permutational multivariate analysis of variance [PERMANOVA]) test with the R package vegan for differential taxa abundance.

Differential abundance was tested using zero-inflated negative binominal weighting and DESeq2.[28]

Random forest (RF) and extreme gradient boosting (XGB), both with 10-fold cross-validation and 5 repeats, were used to build prediction models for bacterial composition using the R package caret. Random forest hyperparameter tuning was carried out to define the ideal number of tries and trees. Extreme Gradient Boosting hyperparameter tuning was carried out to define the ideal number of rounds, learning rate, maximum depth of a tree, gamma, minimum child weight, subsamples, and column samples by tree. Receiver operating characteristics curves were plotted using the R package MLeval. Finally, we evaluated features based on the most impactful in identifying the differences between the groups. These discriminating factors were compared by both models and by DEseq2 results.

All statistical analyses were performed using R statistical software. Bonferroni correction was applied to compensate for multiple testing. Spearman correlation was used for correlation analyses. A P-value ≤ .05 was considered significant. Bar, box, and dot plots were generated via the R ggplot2 package. For dendrogram generation, ggtree and ggtreeExtra were used, and the world map was generated using gglot2 and

maps. Schematics and figures were compiled using Illustrator CC 2020 (Adobe).

Results

Study Cohort

The multinational study cohort comprised of 1683 individuals identified through a systematic literature and database search from 26 studies[7,10,15,23,29–50] (Figure 1A and Figure S1). In total, the pediatric cohort comprised 688 individuals with CD, 581 with UC, and 414 healthy individuals as controls (Figure 1B, Table 1, Table S1). The MMUPHin adjustment harmonized the entire data set from an r^2 of 83.6% to an r^2 of 86.1% meaning that variance explained 16.4% of the variance pre-transformation to 13.9% of the post-transformation (Figure 1C). This overall effect is more clearly illustrated in subsets of the population (Figure S2A-C). As some studies included longitudinal sampling or both stool and biopsy samples, the total number of samples (CD 1764, UC 1900, controls 532) was greater than the number of patients (Figure 1B). Due to sample size and variance differences between biopsy and fecal samples (Figure S3A and B), all analyses were done using only fecal samples. Longitudinal data (eg, stool samples of 244 CD and 357 UC patients[7,15,35–37,40,44,45,50]) were included at the specific time points based on disease severity at the moment of collection. The median age of patients with CD, UC, and healthy patients were 12 (interquartile range [IQR] 3.2), 13 (IQR 5), 12 (IQR 3), respectively (Table 1). Seven hundred twenty-six (41%) of the total 1764 CD samples were of treatment-naïve patients and another 371 (21%) of CD samples were of patients in clinical remission. Five hundred seventy-four (30%) of the 1900 UC samples were treatment-naïve and 546 (29%) of these samples were in clinical remission. Detailed clinical disease activity scores and active treatments are listed in Table 1.

Composition of the Gut Microbiome

We first compared mucosal samples with stool samples in treatment-naïve patients with CD and UC and found different clustering of microbiome depending on the sampling method and biopsy site (Figure S3A and B). Hence, for all further analyses, we focused on fecal samples, as fecal samples also represented the larger proportion of included samples (Figure 1B).

Observed richness was significantly reduced for both treatment-naïve UC and CD groups, compared to controls ($P < .001$). In addition, there was a significant difference between the treatment-naïve PIBD groups ($P < .001$). Reduced alpha diversity among treatment-naïve microbiome compared to healthy controls was consistent in the Shannon diversity analysis ($P < .001$) (Figure 1D). Finally, we found no difference in Shannon diversity between the 2 naïve disease groups (Figure 1D). In patients with PIBD, we observed decreasing richness and evenness with increasing clinical disease severity as seen by the comparison of severe to inactive patients (Figure 1E). It is of note that this difference was also consistent when comparing inactive to severe disease severity in patients with CD and UC separately (Figure S3C and D).

Further, beta diversity was statistically different, as calculated by Bray-Curtis distance, for all treatment-naïve groups (eg, healthy, CD, and UC) (PERMANOVA $P < .001$) (Figure 1F) and although it approximated in remission, it still remained different in patients with PIBD in clinical remission (PERMANOVA $P < .001$) (Figure 1G). It should be noted that the effect size of the groups to be studied is clearly different in Figure 1F vs. 1G.

We then characterized the member taxa of CD and UC patient microbiome pre and post treatment. We found *Bacteroides* genus associated with both treatment-naïve and non-naïve CD and patients with UC (Figure 2A). We then further separated these data by disease severity. Due to the gradual nature of the severity grades, we focused on trends that changed as the disease state worsened.

We observed an increase in the *Pseudomonadota* phylum ($P < .001, P < .001$) and a decrease in the *Bacteroidota* phylum ($P < .001, P < .001$) (Figure 2B, Figure S4A). When evaluated based on genera, *Escherichia-Shigella* and *Haemophilus* both showed a higher relative abundance in severe disease compared to healthy states (Figure 2C, Figure S5A). In contrast, *Agathobacter* and *Blautia* showed the reverse trend (Figure 2C, Figure S5A).

Due to the well-established connection between gut inflammation and disease progression within patients with PIBD, we evaluated the association of bacteria relative abundance with the gut-specific inflammatory peptide fecal calprotectin. The relative genus abundance of the commensal bacteria *Bifidobacterium* ($R = –0.2$, $P = 6.3e-06$) and the genus *Subdoligranulum* ($R = –0.22$, $P = 4.9e-05$) both correlated negatively with fecal calprotectin (Figure 2D and E). In contrast, the relative abundance of the 2 pathogenic genera, *Escherichia-Shigella* ($R = 0.25, P = 3.1e-07$) and *Haemophilus* ($R = 0.12, P = 0.016$), showed a positive correlation with the inflammatory marker (Figure 2F and G).

Predictive Analysis of Microbial Communities

In order to further evaluate the applicability of the fecal microbiome composition to aid differentiating disease entities, we first tested our ability to differentiate treatment-naïve populations with CD from UC based on similar localization as designated by the Paris Classification: L2 (colonic) for patients with CD, and E3/E4 (extensive disease—distal to hepatic flexure) and (pan-colitis—proximal to hepatic flexure) for patients with UC. We found that the 2 groups were significantly different when evaluating based on Bray-Curtis distance (PERMANOVA $P < .001$) (Figure 3A). In order to then understand if this distance-based difference was enough for differentiation, we compared these 2 disease subtypes using a machine learning approach. Here, we used 2 separate methodologies to evaluate our sample space and to generate a distribution of predictive area under the curve. This approach allowed us to control for overfitting by any one method. In addition, we used differential abundance testing to further contextualize the biomarkers identified by these machine learning approaches. We found that these 2 subgroups are confidently able to differentiate with an area under receiver operating characteristics curve (AUROC) of 98% (confidence interval [CI], 0.94-0.99, XGB and RF) (Figure 3B, Tables S2 and S3). We found that 5 of the top 10 differentiating factors were the same between our 2 methodologies. We also saw that our top differentiating factor *Haemophilus* is also designated to have a large differential abundance difference between the 2 groups (Figure 3C).

Gut Microbiome Composition in Treatment Response

After establishing the dynamics of microbial populations between the different diseases and between different severities

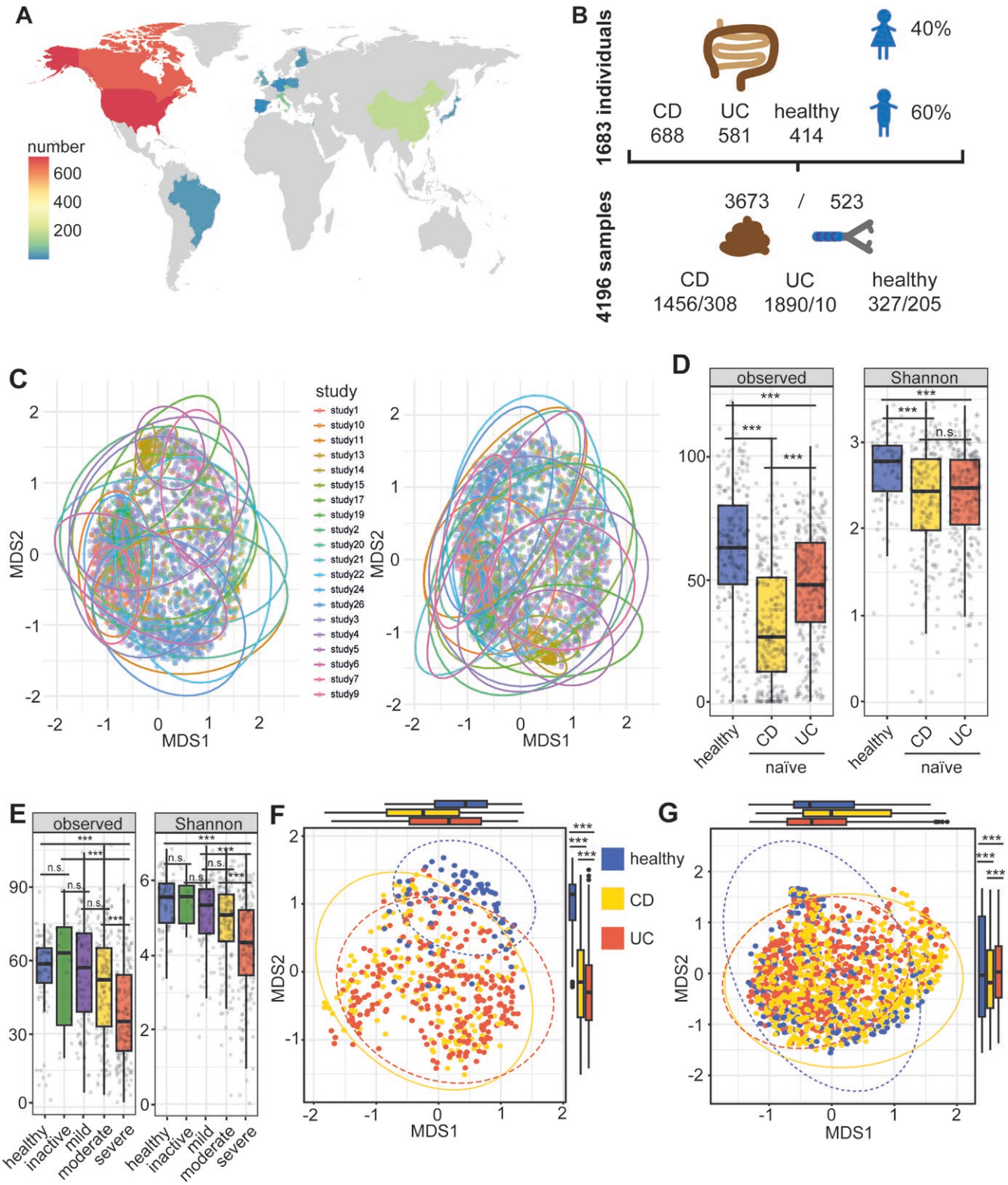

Figure 1. Study design, cohort composition, and diversity metric: (A) World map indicating countries of origin of patient cohort. (B) Schematic overview of the study cohort. (C) Study level comparison of all data pre (left) and post (right) MMUPHin transformation by using a principal coordinate analysis. (D, E) Alpha diversity, expressed as both observed richness and Shannon diversity, of study corrected fecal gut microbiome data in treatment-naïve patients with PIBD compared to healthy individuals; n.s. not significant, $*P < .05$, $**P < .01$, $***P < .001$. (F, G) Beta diversity shown as multidimensional scaling (MDS) using a principal coordinate analysis with Bray-Curtis distance measures. (F) Treatment-naïve patients with UC or CD compared to healthy individuals. (G) PIBD patients in clinical remission with healthy individuals; $***P < .001$.CD, Crohn's disease; MMUPHin, Meta-Analysis Methods with a Uniform Pipeline for Heterogeneity in microbiome studies; PIBD, pediatric inflammatory bowel disease; UC, ulcerative colitis.

Table 1. Composition of study cohort of all included studies compiled into patients with Crohn's disease (CD), ulcerative colitis (UC), and healthy individuals.

Patients	CD (n = 688)	UC (n = 581)	Healthy (n = 414)
Median age (IQR)	12 years (3.2)	13 years (5)	12 years (3)
Female (%)	166 (24), NA = 259	260 (45), NA = 49	91 (22), NA = 206
Samples	CD (n = 1764)	UC (n = 1900)	Healthy (n = 532)
Treatment-naive (%)	726 (41)	574 (30), NA = 22	
Remission (%)	371 (21)	546 (29), NA = 115	
Inactive (%)	321 (18)	531 (28)	
Mild (%)	400 (23)	424 (22)	
Moderate (%)	141 (8)	307 (16)	
Severe (%)	189 (11)	190 (10)	
EEN (%)	336 (19), NA = 87		
TNF (%)	257 (15), NA = 67	107 (5.6), NA = 1264	
Steroids (%)	66 (4), NA = 429	127 (6.7), NA = 1264	
Immunomodulators (%)	708 (40), NA = 94	69 (3.4), NA = 1267	
Antibiotics (%)	286 (16), NA = 87	33 (1.7), NA = 1264	

Patients' characteristics (eg, disease activity and current treatment) at the timepoint of fecal sampling (samples) are further listed.
Abbreviations: CD, Crohn's disease; EEN, exclusive enteral nutrition; IQR, interquartile range; NA, not available; TNF, tumor necrosis factor alpha; UC, ulcerative colitis.

of disease, we evaluated compositional differences in patients with known response to either pharmacologic or nutritional treatment. Microbial communities appeared to not be different between patients who were responsive to treatment as compared to those who were not responsive. No differences were found in alpha diversity metrics in both UC and CD groups based on responsiveness to treatment (Figure 4A and B). Given the lack of observed differences, we evaluated both groups by Bray-Curtis distance to test if beta diversity followed the same trend. In both patient groups with UC or CD, we found a statistical difference between responsive and unresponsive individuals ($P < .001$) (Figure 4C and D). To further clarify these differences, we compared the differential abundance of the bacteriome of responders to nonresponders. Interestingly, we find all top 10 genera that are differentially abundant in both CD and UC responders versus nonresponders share little similarities between patients with CD and UC (Figure 4E and F).

In order to describe the classification power of these data, we again trained 2 machine learning models to test the differentiating power between responsive and unresponsive individuals to all forms of treatment. The models discriminated responsive versus unresponsive CD patients with an AUROC of 89% and 88% in XGB and RF, respectively (XGB CI, 0.86-0.92; RF CI, 0.85-0.91) (Figure 4G, Figure S6A). *Bacteroides* genus (rank 1 in both models), as well as *Holdemanella* genus (rank 2 and 3 in both respective models) were important differentiating factors in both models. Moreover, both markers had very strong differential abundance as well (log2 fold change > 2). It was also of note that *Escherichia-Shigella* genus appeared in the top 6 on both models with a fold change difference of 1.4 (Figure 4H, Tables S4 and S5).

Discerning treatment response in patients with UC yielded an AUROC with XGB of 81% (CI, 0.74-0.88) and with RF of 73% (CI, 0.64-0.82) (Figure S6B and C, Tables S6 and S7). These data also showed a higher variation in predicted outcomes, likely due to the smaller sample size (Table 1). The top genus was *Bacteroides* in both models with *Veillonella* also being in the top 3 (Figure S6D). This was further emphasized by the −3.6 log2 fold change differential abundance of *Bacteroides*. It is also of note that the highest differential abundance change was *Lactobacillus* which is changed by 4.8 in our compared groups. *Lactobacillus* ranks as highly predictive (rank 2) in the XGB methodology, whereas the top 10 in RF.

We further separated our data on patients with CD by treatment type to see if the predictive power of our models is able to discriminate responsive from non-responsive based on specific forms of treatment. We first looked at the discernibility of steroidal treatment responders versus nonresponders (Figure S7A and B). The XGB model yielded an AUROC of 82% (CI, 0.74-0.90) and RF an AUROC of 85% (CI, 0.78-0.92) (Figure 5A and Figure S6E, Tables S8 and S9). We saw the most impactful genera, *Bacteroides*, *Bifidobacterium*, and *Fusicatenibacter*, being in the top 4 in both models (Figure 5B). In addition, the aforementioned bacteria were differentially abundant in these 2 groups with all of them having a fold change difference over 1. Differential abundance analysis also pointed to the sixth rank bacteria *Erysipelotrichaceae* UCG-003, as highly differentially abundant between our groups with a log2 fold change difference over 4 (Figure S6F).

Consecutively, we wanted to see if these analyses were applicable to patients with UC (Figure S8A and B). Due to the smaller sample size, the trends were less striking with only one differential model giving an AUROC of 70% (CI, 0.54-0.86) XGB, and RF giving an AUROC of 67% (CI, 0.50-0.84) for response to steroid treatment (Figure S9A and B). The differential abundance showed more differentiation as we saw several bacterial genera with a log2 fold change over 5 (Figures S6G and S9C).

Response to EEN in patients with CD (Figure S7C and D) was effectively discriminated by both XBG and RF with an AUROC of 85% (CI, 0.80-0.90) and 83% (CI, 0.78-0.88), respectively (Figure 5C and Figure S9D). Although the discriminating ability of both models is comparable, the relative

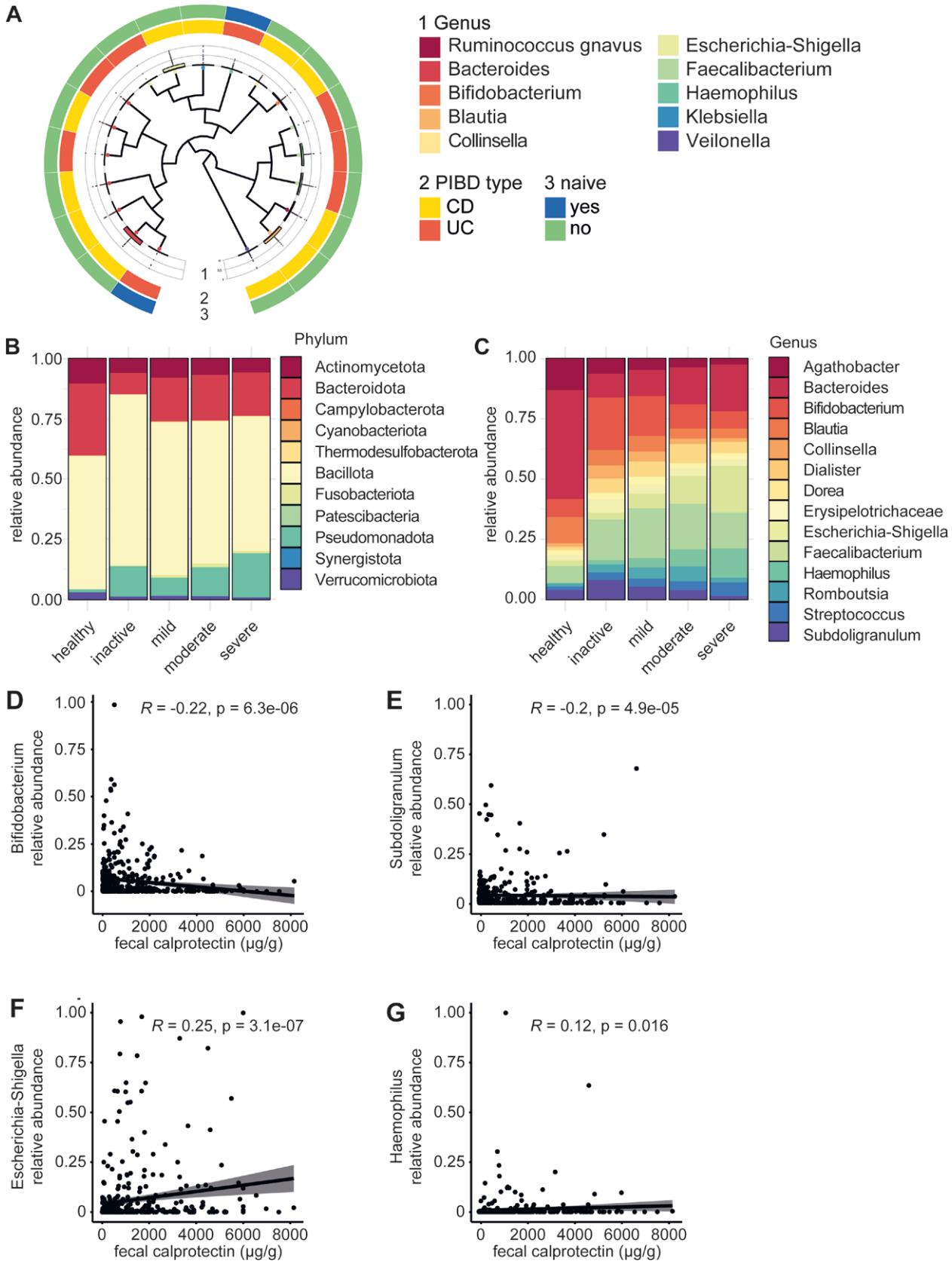

Figure 2. Relative abundance, disease association with differential abundance of taxa: (A) Dendrogram depicting top 10 most abundant species (inner circle/box plots) and associated conditions on the 3 outer circles. (B) Relative abundance of 10 most abundant phyla in healthy individuals and patients with PIBD over different clinical disease activities. (C) Relative abundance of 14 most abundant genera in healthy individuals and patients with PIBD over different clinical disease activities. (D-G) Relative abundance of *Bifidobacterium*, *Subdoligranulum*, *Escherichia-Shigella*, *Haemophilus* genera regression to calprotectin levels. PIBD, pediatric inflammatory bowel disease.

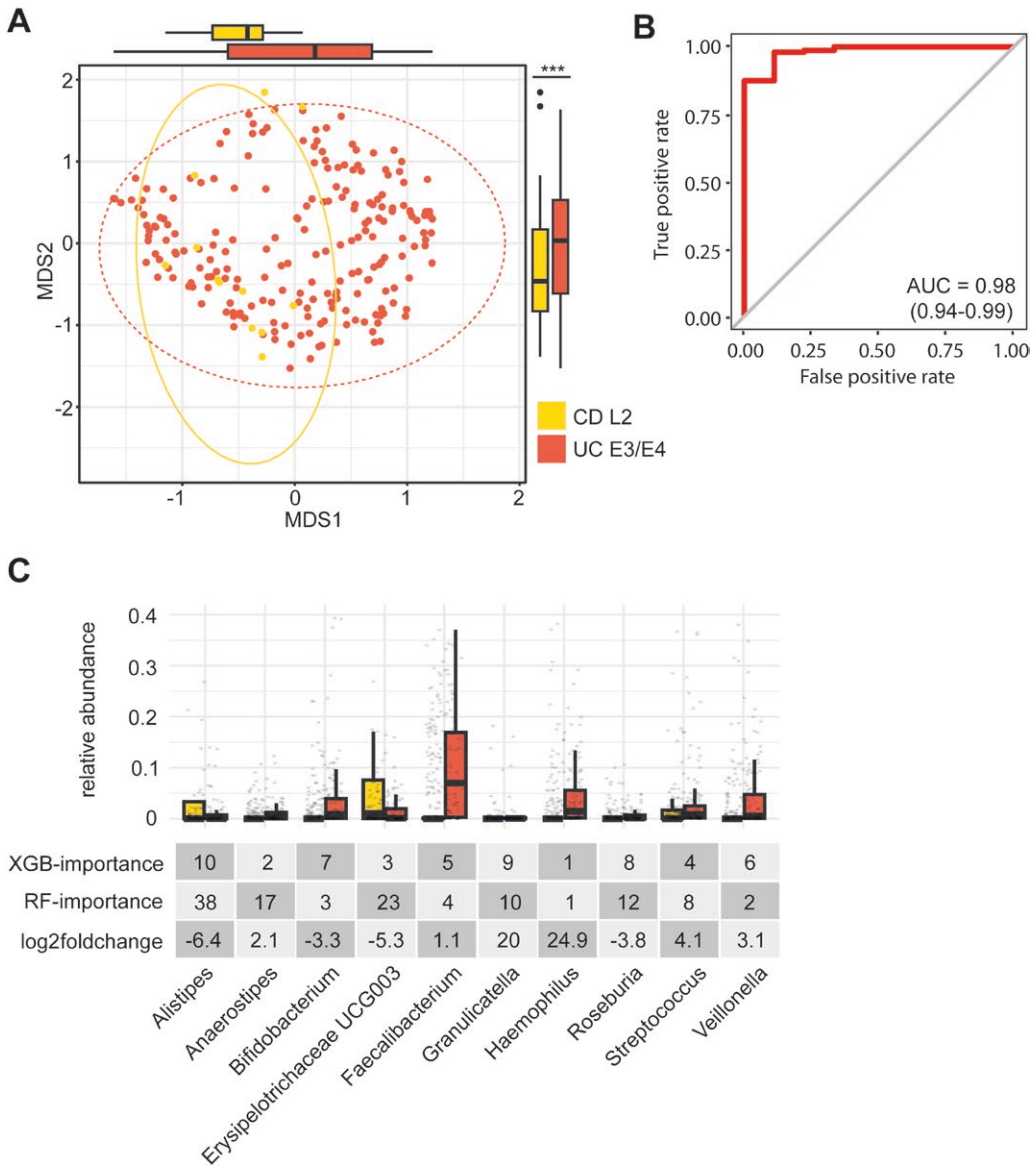

Figure 3. Discrimination of CD and UC treatment response: (A) Beta diversity shown as multidimensional scaling (MDS) using a principal coordinate analysis with Bray-Curtis distance measures comparing treatment-naïve patients with CD (Paris L2) or UC (Paris E3/E4). (B) Area under the receiver operating characteristics curve of XGB and RF models discriminating steroid response treatment-naïve patients with CD (Paris L2) UC (Paris E3/E4) patients. (C) Dotted box-plot of relative genus abundance of top 10 discriminative genus features identified by supervised machine learning with feature ranking and log2 fold change from differential abundance analysis comparing CD (Paris L2) (yellow) with UC (Paris E3/E4) (red). CD, Crohn's disease; RF, random forest; UC, ulcerative colitis; XGB, extreme gradient boosting.

importance of impactful features was different between both models. The relative rankings show 1 of the top 5 most impactful genera (*Bacteroides*), and 3 of the top 10 most impactful genera match (*Dorea*, *Family XII AD3011 group*) (Figure 5D, Tables S10 and S11). We also see that the differential abundance seemed to be more similar to the most discriminatory bacterium identified using the XGB methodology, as 6 of the top 10 factors had log2 fold change over 1 (Figure 5D, Figure S9E).

We further showed an ability to discriminate response to anti-TNF treatment (Figures S7E and F and 8C and D). Here, our 2 models showed an AUROC of 87% for XBG (CI, 0.82-0.92) and 90% for RF (CI, 0.86-0.94) respectively (Figure 5E and Figure S9F). Of note, the rankings were similar between the 2 models' top 5 rank features with the one different being ranked 6 in the other model (Figure 5F, Tables S12 and S13). Moreover, we saw that the differential abundance of 3 of these top 5 organisms is over a log2 fold change of 1. This suggests that the discriminating power is both strong and consistent between evaluating factors (Figure 5F, Figure S9G).

Discussion

The incidence of IBD in Westernized countries has steadily increased over the last decades in both children and adults. Changes in gut microbial community profiles are associated with intestinal inflammation and are increasingly recognized as predisposing, or disease-driving features, rather than a consequence of the inflamed intestinal milieu.[12,15–17,51] We have collected and systematically analyzed a large multinational

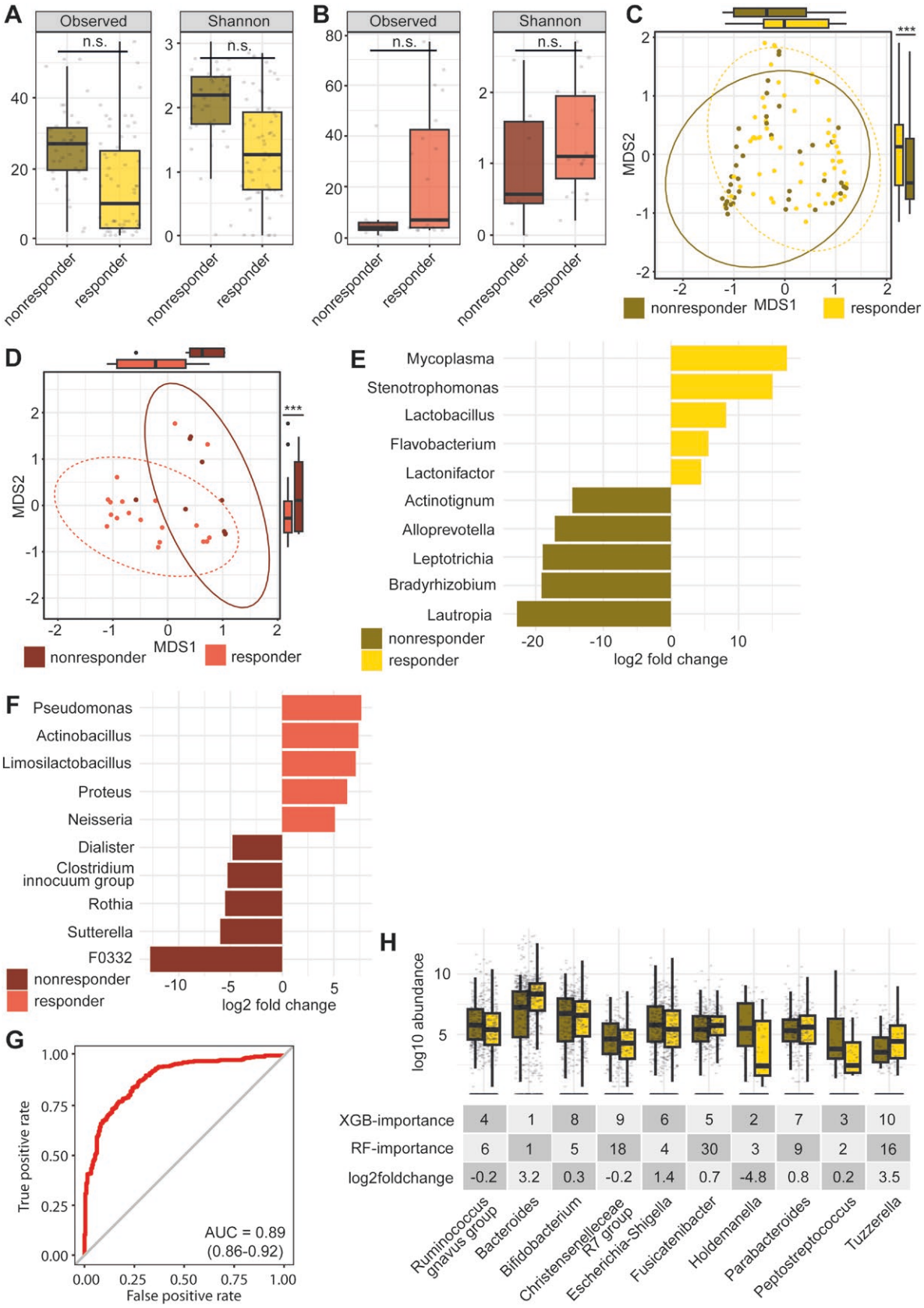

Figure 4. Community diversity and discrimination of treatment response: (A) Alpha diversity of gut microbiome in patients with CD with or without response to treatment (observed); ***$P < .001$, n.s. not significant. (B) Alpha diversity of gut microbiome in patients with UC with or without response to treatment; *$P < .05$, n.s. not significant. (C-D) CD and UC respective beta diversity shown as multidimensional scaling (MDS) using a principal coordinate analysis with Bray-Curtis distance measures; ***$P < .001$. (E) Horizontal bar plot showing the top 10 differential abundant genera comparing patients with CD without (brown yellow) and with (yellow) treatment response. (F) Horizontal bar plot showing the top 10 differential abundant genera

pediatric cohort to study the gut microbiome to evaluate the change in these populations. As *16S* sequencing is currently still the most widely used technique, we have limited the inclusion criteria to *16S* raw data, processed all data via a uniform, state-of-the-art DADA2-based pipeline, and have used a recent database for taxonomic identification. To compensate for the variance in *16S* sequencing loci of the included studies, we have adjusted for study batch effects using MMUPHin, a tool specifically designed to correct for this purpose and validated on IBD cohorts. Together this allowed us to delineate differences in microbial composition in association with PIBD type and disease activity. We were able to further explore the microbial composition as biomarkers to predict treatment response by using machine learning models. Studies on gut microbiome have been investigating both fecal and mucosal, biopsy-derived, samples. Expectedly, this introduces a certain variation in microbial composition which we have also confirmed with our dataset. As fecal sampling is a noninvasive method, integrated in clinical routine visits and easily available, we have focused our analyses on fecal-derived sequencing.

When characterizing the composition of the gut microbiome, we have confirmed significant differences in both abundance and community composition in treatment-naïve patients with PIBD compared to healthy individuals. Interestingly, community structure still diverged in PIBD patients in clinical remission. This is also reflected by the fact that genus composition was strikingly different in patients with clinically inactive disease scoring compared to healthy individuals. This underscores the current efforts to aim for luminal or even histologic remission rather than just clinical remission.[52] In addition to clinical disease activity scoring, we have correlated taxonomic abundance with the luminal inflammation marker fecal calprotectin. With increasing intestinal inflammation, we have observed a decrease in the commensal genus *Bifidobacterium*, bacteria relevant for host immune system interaction.[53] In contrast, abundances of the pathogenic genera *Escherichia-Shigella* and *Haemophilus* were positively correlated with increasing fecal calprotectin.

The large study cohort collected in this meta-analysis allowed for the application of machine learning models. To increase the robustness of the predictions, we have employed 2 different supervised machine learning approaches, RF and XGB. Both algorithms yielded comparable confidence in the tasks applied. Moreover, the pattern of the most important taxonomic features was similar for both models. In both pediatric patients with CD or UC, community structure differed as based on treatment-response status. We were able to predict treatment response to anti-TNF therapy in patients with CD with an accuracy of up to 90%. These results suggest that microbial community profiling can be used to distinguish between treatment -responders and nonresponders. This approach should be pursued in further prospective studies, as it can add significant contribution to the goal of individualized therapy in the age of more and more targeted therapy options. The predictions for patients with CD were more robust than for patients with UC, which is due to fewer UC samples for downstream analyses. In most models for treatment response, *Bacteroides* genus abundance was among the most important model features. *Bacteroides* were also found to have increasing abundance with increasing clinical disease activity. Yet, this is not comparable with healthy individuals, in whom the highest abundance of the genus *Bacteroides* was found. Previous studies have reported that higher abundances of beneficial taxa (eg, *Bifidobacterium* and *Faecalibacterium*) and lower abundances of pro-inflammatory taxa are associated with a response to anti-TNF treatment or EEN in both children and adults.[23,33,54,55] These findings were corroborated by our analyses. Interestingly, the pattern of genus abundance seemed more important than simple differences in abundance, as seen by paralleled differential abundance analyses. However, the limitation of *16S* sequencing data did not allow for proper species-level analysis which might be particularly interesting to understand when thinking about a therapeutic probiotic approach supporting conventional treatment regimens. Moreover, taxonomic patterns precisely distinguished treatment-naïve patients with CD from treatment-naïve patients with UC. In addition to endoscopic and histologic grading, microbial pattern identification could prove useful when a PIBD entity is difficult to classify (IBD-unclassified).[23] It is foreseeable that we may be able to utilize fecal gut microbial composition as a biomarker for disease identification or prediction of treatment response. These findings still need to be validated in large prospective trials.

A major strength of this study is the large multinational sample size covering the most common forms of PIBD, CD, and UC, and allowing analyses across the spectrum of clinical disease activity and treatment. However, our study also has limitations. First, it is retrospective, as our goal was mainly to see the extent to which differences were observed when we expanded our dataset, we are unable to verify the application of this information going forward. Second, there is variability introduced by the different *16S* sequencing techniques employed across the included studies. As we show, we were able to make a correction via a transformation of our dataset, but even after there was still a substantial effect due to study-specific variation. In addition to the variation that might appear due to collection methods, there was potential for variation with regard to subpopulations that were sampled. Our analysis was not intended to identify differences that arose from the continental divide. Third, there are subtypes in both CD and UC which were not fully taken into account in this study due to either missing differentiation or insufficient sample numbers. This is most illustrative in evaluating our cohort with UC as for some samples complimentary metadata was not available. Fourth, while longitudinal sampling allows to monitor changes in the microbiome upon certain treatments, it may also introduce a founder effect that we could not fully quantify. Finally, *16S* provides less precise taxonomic and functional assessment compared to shotgun metagenomics sequencing, but is significantly more affordable and hence still widely used. We therefore focused on *16S*

comparing UC patients without (dark red) and with (red) treatment response. (G) XGB area under the receiver operating characteristics curve of a supervised machine learning model discriminating treatment response in pooled patients with CD. (H) Dotted box-plot of log10-transformed genus abundance of top 10 discriminative genus features identified by both supervised machine learning with feature ranking and log2 fold change from differential abundance analysis comparing patients with CD without (brown yellow) and with (yellow) treatment response. CD, Crohn's disease; UC, ulcerative colitis; XGB, extreme gradient boosting.

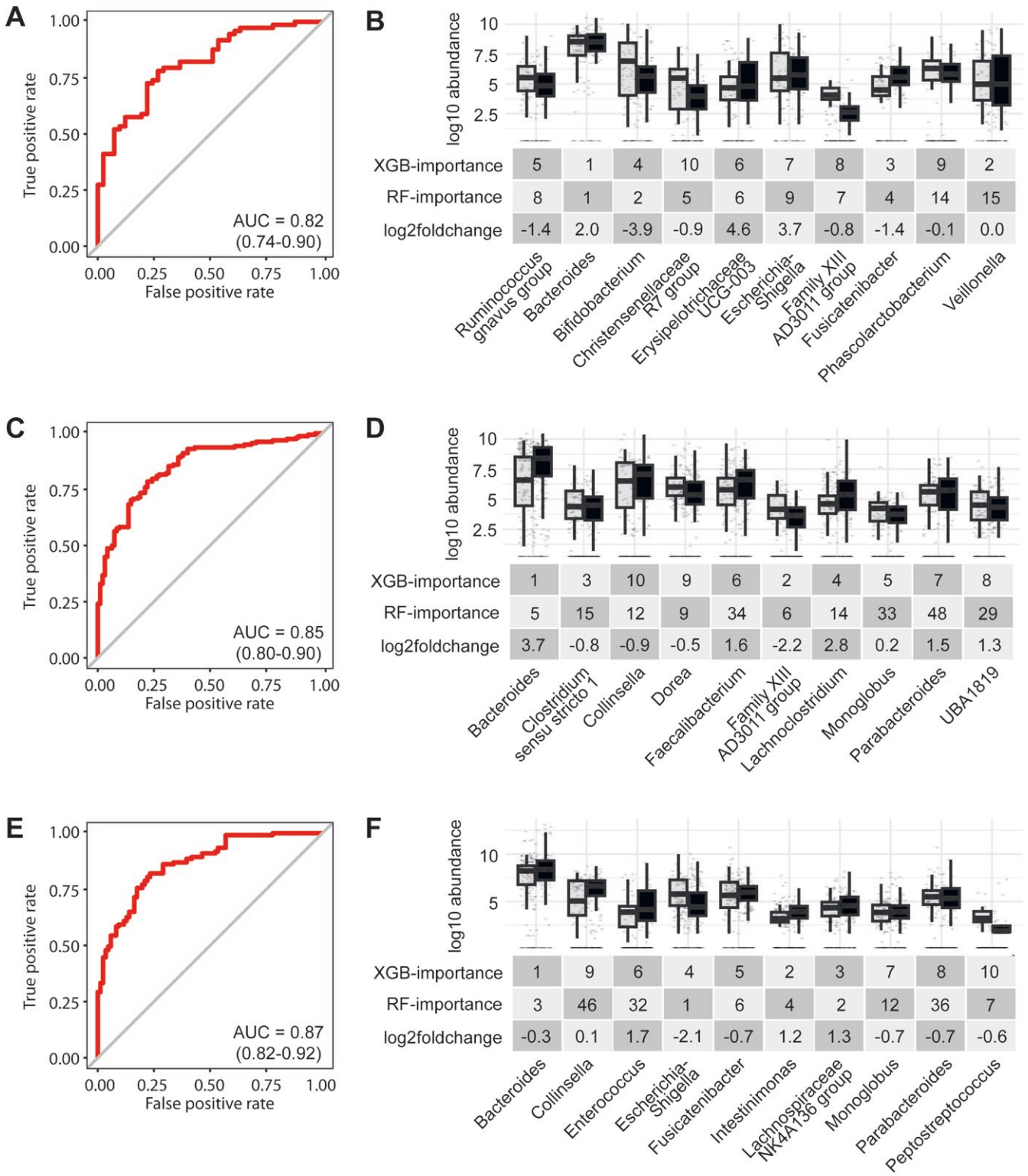

Figure 5. Community diversity and discrimination of treatment response: (A) Area under the receiver operating characteristics curve (AUROC) of XGB model discriminating steroid response in patients with CD. (B) Dotted box-plot of log10-transformed genus abundance of top 10 discriminative genus features identified by supervised machine learning with ranked feature importance of the XGB and RF model, and log2 fold change from differential abundance analysis comparing patients with CD without (gray) and with (black) steroid response. (C) AUROC of XGB model discriminating EEN response in patients with CD. (D) Dotted box-plot of log10-transformed genus abundance of top 10 discriminative genus features identified by supervised machine learning with ranked feature importance of the XGB and RF model, and log2 fold change from differential abundance analysis comparing patients with CD without (gray) and with (black) EEN response. (E) AUROC of RF model discriminating TNF response in patients with CD. (F) Dotted box-plot of log10-transformed genus abundance of top 10 discriminative genus features identified by supervised machine learning with ranked feature importance of the XGB and RF model, and log2 fold change from differential abundance analysis comparing patients with CD without (gray) and with (black) TNF response. CD, Crohn's disease; EEN, exclusive enteral nutrition; RF, random forest; TNF, tumor necrosis factor; XGB, extreme gradient boosting.

raw data. Though this limited our conclusions, given our goal of future application, we felt these data would be more useful for potential clinical application. Despite these limitations, our study contributes to the understanding of the dynamics of gut microbiome in patients with PIBD and offers a novel description of its applicability as a biomarker with potential application to identify treatment response.

Supplementary data

Supplementary data is available at *Inflammatory Bowel Diseases* online.

Author Contributions

D.A. (Conceptualization [equal], Data curation [lead], Formal analysis [equal], Methodology [equal], Writing—original draft [lead], Writing—review & editing [equal]), A.P. (Conceptualization [equal], Data curation [lead], Formal analysis [equal], Methodology [equal], Writing—original draft [lead], Writing—review & editing [equal]), C.M. (Conceptualization [supporting], Data curation [supporting], Writing—original draft [equal], Writing—review & editing [equal]), K.D. (Recruitment [supporting], Writing—review & editing [equal]), J.P.J. (Recruitment [supporting], Writing—review & editing [supporting]), N.P. (Recruitment [supporting], Writing—review & editing [supporting]), J.C.S. (Recruitment [supporting], Writing—review & editing [supporting]), L.H. (Recruitment [supporting], Writing—review & editing [supporting]), D.T. (Recruitment [supporting], Writing—review & editing [supporting]), F.D.C. (Recruitment [supporting], Writing—review & editing [supporting]), S.C. (Recruitment [supporting], Writing—review & editing [supporting]), Z.G. (Recruitment [supporting], Writing—review & editing [supporting]), L.A.C. (Recruitment [supporting], Writing—review & editing [supporting]), O.C. (Recruitment [supporting], Writing—review & editing [supporting]), C.R.T.(Recruitment [supporting], Writing—review & editing [supporting]), T.S. (Recruitment [supporting], Writing—review & editing [supporting]), E.W. (Recruitment [supporting], Writing—review & editing [supporting]), A.M.G. (Recruitment [supporting], Writing—review & editing [supporting]), T.M. (Writing—review & editing [supporting]), G.F.V. (Conceptualization [equal], Data curation [supporting], Formal analysis [equal], Project administration [lead], Supervision [lead], Visualization [equal], Writing—original draft [equal], Writing—review & editing [equal])

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None declared.

Conflicts of Interest

The authors declare no conflict of interest.

Data availability

Additional data is made available upon reasonable request.

AI Use

No AI was used to write the manuscript or generate data.

Ethical Considerations

This study was conducted in accordance with the guidelines of the Institutional Review Board of the Medical University of Innsbruck and the 1975 Declaration of Helsinki.

References

1. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature*. 2011;474(7351):307-317. doi:10.1038/nature10209
2. Halfvarson J, Brislawn CJ, Lamendella R, et al. Dynamics of the human gut microbiome in inflammatory bowel disease. *Nat Microbiol*. 2017;2:17004. doi:10.1038/nmicrobiol.2017.4
3. Dunne C. Adaptation of bacteria to the intestinal niche: probiotics and gut disorder. *Inflamm Bowel Dis*. 2001;7(2):136-145. doi:10.1097/00054725-200105000-00010
4. Radjabzadeh D, Boer CG, Beth SA, et al. Diversity, compositional and functional differences between gut microbiota of children and adults. *Sci Rep*. 2020;10(1):1040. doi:10.1038/s41598-020-57734-z
5. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol*. 2007;5(7):e177. doi:10.1371/journal.pbio.0050177
6. Frank DN, Robertson CE, Hamm CM, et al. Disease phenotype and genotype are associated with shifts in intestinal-associated microbiota in inflammatory bowel diseases. *Inflamm Bowel Dis*. 2011;17(1):179-184. doi:10.1002/ibd.21339
7. Gevers D, Kugathasan S, Denson LA, et al. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe*. 2014;15(3):382-392. doi:10.1016/j.chom.2014.02.005
8. Tamboli CP, Neut C, Desreumaux P, Colombel JF. Dysbiosis in inflammatory bowel disease. *Gut*. 2004;53(1):1-4. doi:10.1136/gut.53.1.1
9. Conrad MA, Bittinger K, Ren Y, et al. The intestinal microbiome of inflammatory bowel disease across the pediatric age range. *Gut Microbes*. 2024;16(1):2317932. doi:10.1080/19490976.2024.2317932
10. Shah R, Cope JL, Nagy-Szakal D, et al. Composition and function of the pediatric colonic mucosal microbiome in untreated patients with ulcerative colitis. *Gut Microbes*. 2016;7(5):384-396. doi:10.1080/19490976.2016.1190073
11. Haberman Y, Tickle TL, Dexheimer PJ, et al. Pediatric Crohn disease patients exhibit specific ileal transcriptome and microbiome signature. *J Clin Invest*. 2014;124(8):3617-3633. doi:10.1172/JCI75436
12. Olbjørn C, Cvancarova Småstuen M, Thiis-Evensen E, et al. Fecal microbiota profiles in treatment-naïve pediatric inflammatory bowel disease - associations with disease phenotype, treatment, and outcome. *Clin Exp Gastroenterol*. 2019;12:37-49. doi:10.2147/CEG.S186235
13. Michail S, Durbin M, Turner D, et al. Alterations in the gut microbiome of children with severe ulcerative colitis. *Inflamm Bowel Dis*. 2012;18(10):1799-1808. doi:10.1002/ibd.22860
14. Malham M, Lilje B, Houen G, Winther K, Andersen PS, Jakobsen C. The microbiome reflects diagnosis and predicts disease severity in paediatric onset inflammatory bowel disease. *Scand J Gastroenterol*. 2019;54(8):969-975. doi:10.1080/00365521.2019.1644368
15. Schirmer M, Denson L, Vlamakis H, et al. Compositional and temporal changes in the gut microbiome of pediatric ulcerative colitis patients are linked to disease course. *Cell Host Microbe*. 2018;24(4):600-610.e4. doi:10.1016/j.chom.2018.09.009
16. Kugathasan S, Denson LA, Walters TD, et al. Prediction of complicated disease course for children newly diagnosed with Crohn's disease: a multicentre inception cohort study. *Lancet*. 2017;389(10080):1710-1718. doi:10.1016/S0140-6736(17)30317-3

17. Kaplan GG, Ng SC. Understanding and preventing the global increase of inflammatory bowel disease. *Gastroenterology*. 2017;152(2):313-321.e2. doi:10.1053/j.gastro.2016.10.020
18. Gong D, Yu X, Wang L, Kong L, Gong X, Dong Q. Exclusive enteral nutrition induces remission in pediatric Crohn's disease via modulation of the gut microbiota. *Biomed Res Int*. 2017;2017:8102589. doi:10.1155/2017/8102589
19. MacLellan A, Moore-Connors J, Grant S, Cahill L, Langille MGI, Van Limbergen J. The impact of exclusive enteral nutrition (EEN) on the gut microbiome in Crohn's disease: a review. *Nutrients*. 2017;9(5):447. doi:10.3390/nu9050447
20. Kaakoush NO, Day AS, Leach ST, Lemberg DA, Nielsen S, Mitchell HM. Effect of exclusive enteral nutrition on the microbiota of children with newly diagnosed Crohn's disease. *Clin Transl Gastroenterol*. 2015;6(1):e71. doi:10.1038/ctg.2014.21
21. Wang Y, Gao X, Ghozlane A, et al. Characteristics of faecal microbiota in paediatric Crohn's disease and their dynamic changes during infliximab therapy. *J Crohns Colitis*. 2018;12(3):337-346. doi:10.1093/ecco-jcc/jjx153
22. Kowalska-Duplaga K, Kapusta P, Gosiewski T, et al. Changes in the intestinal microbiota are seen following treatment with infliximab in children with Crohn's disease. *J Clin Med*. 2020;9(3):687. doi:10.3390/jcm9030687
23. Höyhtyä M, Korpela K, Saqib S, et al. Quantitative fecal microbiota profiles relate to therapy response during induction with tumor necrosis factor α antagonist infliximab in pediatric inflammatory bowel disease. *Inflamm Bowel Dis*. 2023;29(1):116-124. doi:10.1093/ibd/izac182
24. Hyams JS, Ferry GD, Mandel FS, et al. Development and validation of a pediatric Crohn's disease activity index. *J Pediatr Gastroenterol Nutr*. 1991;12(4):439-447.
25. Turner D, Griffiths AM, Walters TD, et al. Mathematical weighting of the pediatric Crohn's disease activity index (PCDAI) and comparison with its other short versions. *Inflamm Bowel Dis*. 2012;18(1):55-62. doi:10.1002/ibd.21649
26. Turner D, Otley AR, Mack D, et al. Development, validation, and evaluation of a pediatric ulcerative colitis activity index: a prospective multicenter study. *Gastroenterology*. 2007;133(2):423-432. doi:10.1053/j.gastro.2007.05.029
27. Oren A, Garrity GM. Valid publication of the names of forty-two phyla of prokaryotes. *Int J Syst Evol Microbiol*. 2021;71(12). doi:10.1099/ijsem.0.005130
28. Calgaro M, Romualdi C, Waldron L, Risso D, Vitulo N. Assessment of statistical methods from single cell, bulk RNA-seq, and metagenomics applied to microbiome data. *Genome Biol*. 2020;21(1):191. doi:10.1186/s13059-020-02104-1
29. Douglas GM, Hansen R, Jones CMA, et al. Multi-omics differentially classify disease state and treatment outcome in pediatric Crohn's disease. *Microbiome*. 2018;6(1):13. doi:10.1186/s40168-018-0398-3
30. Zuo W, Wang B, Bai X, et al. 16S rRNA and metagenomic shotgun sequencing data revealed consistent patterns of gut microbiome signature in pediatric ulcerative colitis. *Sci Rep*. 2022;12(1):6421. doi:10.1038/s41598-022-07995-7
31. Assa A, Butcher J, Li J, et al. Mucosa-associated ileal microbiota in new-onset pediatric Crohn's disease. *Inflamm Bowel Dis*. 2016;22(7):1533-1539. doi:10.1097/MIB.0000000000000776
32. Liu TC, Gurram B, Baldridge MT, et al. Paneth cell defects in Crohn's disease patients promote dysbiosis. *JCI Insight*. 2016;1(8):e86907. doi:10.1172/jci.insight.86907
33. Wang Y, Gao X, Zhang X, et al. Microbial and metabolic features associated with outcome of infliximab therapy in pediatric Crohn's disease. *Gut Microbes*. 2021;13(1):1-18. doi:10.1080/19490976.2020.1865708
34. Diederen K, Li JV, Donachie GE, et al. Exclusive enteral nutrition mediates gut microbial and metabolic changes that are associated with remission in children with Crohn's disease. *Sci Rep*. 2020;10(1):18879. doi:10.1038/s41598-020-75306-z
35. Ijaz UZ, Quince C, Hanske L, et al. The distinct features of microbial "dysbiosis" of Crohn's disease do not occur to the same extent in their unaffected, genetically-linked kindred. *PLoS One*. 2017;12(2):e0172605. doi:10.1371/journal.pone.0172605
36. Jones CMA, Connors J, Dunn KA, et al. Bacterial taxa and functions are predictive of sustained remission following exclusive enteral nutrition in pediatric Crohn's disease. *Inflamm Bowel Dis*. 2020;26(7):1026-1037. doi:10.1093/ibd/izaa001
37. Sprockett D, Fischer N, Boneh RS, et al. Treatment-specific composition of the gut microbiota is associated with disease remission in a pediatric Crohn's disease cohort. *Inflamm Bowel Dis*. 2019;25(12):1927-1938. doi:10.1093/ibd/izz130
38. Jacobs JP, Goudarzi M, Singh N, et al. A disease-associated microbial and metabolomics state in relatives of pediatric inflammatory bowel disease patients. *Cell Mol Gastroenterol Hepatol*. 2016;2(6):750-766. doi:10.1016/j.jcmgh.2016.06.004
39. Alipour M, Zaidi D, Valcheva R, et al. Mucosal barrier depletion and loss of bacterial diversity are primary abnormalities in paediatric ulcerative colitis. *J Crohns Colitis*. 2016;10(4):462-471. doi:10.1093/ecco-jcc/jjv223
40. Turner D, Bishai J, Reshef L, et al. Antibiotic cocktail for pediatric acute severe colitis and the microbiome: the PRASCO randomized controlled trial. *Inflamm Bowel Dis*. 2020;26(11):1733-1742. doi:10.1093/ibd/izz298
41. Pai N, Popov J, Hill L, Hartung E, Grzywacz K, Moayyedi P; McMaster Pediatric Fecal Microbiota Transplant Research Collaboration. Results of the first pilot randomized controlled trial of fecal microbiota transplant in pediatric ulcerative colitis: lessons, limitations, and future prospects. *Gastroenterology*. 2021;161(2):388-393.e3. doi:10.1053/j.gastro.2021.04.067
42. Nusbaum DJ, Sun F, Ren J, et al. Gut microbial and metabolomic profiles after fecal microbiota transplantation in pediatric ulcerative colitis patients. *FEMS Microbiol Ecol*. 2018;94(9):fiy133. doi:10.1093/femsec/fiy133
43. Wang X, Xiao Y, Xu X, et al. Characteristics of fecal microbiota and machine learning strategy for fecal invasive biomarkers in pediatric inflammatory bowel disease. *Front Cell Infect Microbiol*. 2021;11:711884. doi:10.3389/fcimb.2021.711884
44. Connors J, Dunn KA, Allott J, et al. The relationship between fecal bile acids and microbiome community structure in pediatric Crohn's disease. *ISME J*. 2020;14(3):702-713. doi:10.1038/s41396-019-0560-3
45. Hart L, Farbod Y, Szamosi JC, et al. Effect of exclusive enteral nutrition and corticosteroid induction therapy on the gut microbiota of pediatric patients with inflammatory bowel disease. *Nutrients*. 2020;12(6):1691. doi:10.3390/nu12061691
46. Cortez RV, Moreira LN, Padilha M, et al. Gut microbiome of children and adolescents with primary sclerosing cholangitis in association with ulcerative colitis. *Front Immunol*. 2021;11:598152. doi:10.3389/fimmu.2020.598152
47. Iwasawa K, Suda W, Tsunoda T, et al. Characterisation of the faecal microbiota in Japanese patients with paediatric-onset primary sclerosing cholangitis. *Gut*. 2017;66(7):1344-1346. doi:10.1136/gutjnl-2016-312533
48. Del Chierico F, Cardile S, Baldelli V, et al. Characterization of the gut microbiota and mycobiota in Italian pediatric patients with primary sclerosing cholangitis and ulcerative colitis. *Inflamm Bowel Dis*. 2024;30(4):529-537. doi:10.1093/ibd/izad203
49. Hoelz H, Heetmeyer J, Tsakmaklis A, et al. Is autologous fecal microbiota transfer after exclusive enteral nutrition in pediatric Crohn's disease patients rational and feasible? Data from a feasibility test. *Nutrients*. 2023;15(7):1742. doi:10.3390/nu15071742
50. Hurych J, Mascellani Bergo A, Lerchova T, et al. Faecal bacteriome and metabolome profiles associated with decreased mucosal inflammatory activity upon anti-TNF therapy in paediatric Crohn's disease. *J Crohns Colitis*. 2024;18(1):106-120. doi:10.1093/ecco-jcc/jjad126

51. Raygoza Garay JA, Turpin W, Lee SH, et al.; CCC GEM Project Research Consortium. Gut microbiome composition is associated with future onset of Crohn's disease in healthy first-degree relatives. *Gastroenterology.* 2023;165(3):670-681. doi:10.1053/j.gastro.2023.05.032
52. Turner D, Ricciuto A, Lewis A, et al.; International Organization for the Study of IBD. STRIDE-II: an update on the selecting therapeutic targets in inflammatory bowel disease (STRIDE) Initiative of the International Organization for the Study of IBD (IOIBD): determining therapeutic goals for treat-to-target strategies in IBD. *Gastroenterology.* 2021;160(5):1570-1583. doi:10.1053/j.gastro.2020.12.031
53. O'Callaghan A, van Sinderen D. Bifidobacteria and their role as members of the human gut microbiota. *Front Microbiol.* 2016;7:925. doi:10.3389/fmicb.2016.00925
54. Ventin-Holmberg R, Eberl A, Saqib S, et al. Bacterial and fungal profiles as markers of infliximab drug response in inflammatory bowel disease. *J Crohns Colitis.* 2021;15(6):1019-1031. doi:10.1093/ecco-jcc/jjaa252
55. Dijk S, Jarman M, Zhang Z, et al.; Canadian Children IBD Network. Pre-diagnosis diet predicts response to exclusive enteral nutrition and correlates with microbiome in pediatric Crohn disease. *Nutrients.* 2024;16(7):1033. doi:10.3390/nu16071033