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Leading Off

Exploring the Predictive Value of Gut Microbiome Signatures for Therapy Intensification in Patients With Inflammatory Bowel Disease: A 10-Year Follow-up Study

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Background: Inflammatory bowel diseases (IBDs) pose a significant challenge due to their diverse, often debilitating, and unpredictable clinical manifestations. The absence of prognostic tools to anticipate the future complications that require therapy intensification presents a substantial burden to patient private life and health. We aimed to explore whether the gut microbiome is a potential biomarker for future therapy intensification in a cohort of 90 IBD patients.

Methods: We conducted whole-genome metagenomics sequencing on fecal samples from these patients, allowing us to profile the taxonomic and functional composition of their gut microbiomes. Additionally, we conducted a retrospective analysis of patients' electronic records over a period of 10 years following the sample collection and classified patients into (1) those requiring and (2) not requiring therapy intensification. Therapy intensification included medication escalation, intestinal resections, or a loss of response to a biological treatment. We applied gut microbiome diversity analysis, dissimilarity assessment, differential abundance analysis, and random forest modeling to establish associations between baseline microbiome profiles and future therapy intensification.

Results: We identified 12 microbial species (eg, *Roseburia hominis* and *Dialister invisus*) and 16 functional pathways (eg, biosynthesis of L-citrulline and L-threonine) with significant correlations to future therapy intensifications. Random forest models using microbial species and pathways achieved areas under the curve of 0.75 and 0.72 for predicting therapy intensification.

Conclusions: The gut microbiome is a potential biomarker for therapy intensification in IBD patients and personalized management strategies. Further research should validate our findings in other cohorts to enhance the generalizability of these results.

Lay Summary

Ninety IBD patients were followed-up for 10 years after producing a fecal sample. During this period, 36% of the patients required therapy intensification. We show that the gut microbiome at baseline is associated with, and might hold predictive value for future necessity of therapy intensification.

Key Words: inflammatory bowel disease, therapy intensification, prognostic tools, gut microbiome

Introduction

Inflammatory bowel diseases (IBD) consist of a group of chronic inflammatory diseases with 2 main subtypes: ulcerative colitis (UC) and Crohn's disease (CD), which are characterized by relapsing episodes of inflammation of the gastrointestinal (GI) tract in CD and in the colon in UC.[1] The clinical presentation of IBD varies widely among patients, ranging from a milder disease course marked by long periods of clinical remission, to severe cases characterized by flares of inflammation despite intensive treatment such as immunomodulators and biological therapies,[2] leading to surgical intervention in 47% of the patients with CD and 16% in patients with UC within 10 years of diagnosis.[3,4] Furthermore, the onset of IBD typically occurs between the second and the fourth decade of life.[5] This pattern of onset imposes a significant burden, particularly due to the often debilitating symptoms in a young population,

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Key Messages

- **What is already known?**

The gut microbiome plays a role in the pathogenesis of IBD and has been associated with disease flare-ups and response to IBD therapy.

- **What is new here?**

Our study highlights unique baseline microbiome profiles in patients needing therapy intensification, potentially serving as predictive biomarkers in the clinical care setting.

- **How can this study help patient care?**

To improve health outcomes of patients with IBD, it is necessary to prevent progression of disease. Identifying early indicators of an unfavorable disease course can improve IBD management. The gut microbiome holds potential for predicting the need for therapy intensification.

resulting in adverse consequences for both patients and society, including reduced quality of life and decreased work productivity.[6]

Despite the substantial disease burden, there are currently no established tools that can predict whether a patient with IBD will respond to a specific medication or experience a progressive disease course necessitating surgical intervention. This scarcity of predictive tools results from the highly complex and heterogeneous nature of IBD. This leads to individual therapy success rates of only 20% to 30%.[7]

To date, the precise etiological mechanisms driving IBD and its clinical manifestations remain elusive. Nonetheless, the prevailing hypothesis regarding the pathogenesis of IBD posits that intestinal inflammation arises from an immune-mediated response activated by the commensal gut microbiome, influenced by genetic predisposition, and modulated by environmental factors.[1,8,9] Interestingly, beyond identifying specific gut microbiome signatures in patients already diagnosed with IBD, the composition of the gut microbiome has demonstrated promise as a noninvasive and cost-effective tool for diagnosing IBD,[10] predicting active disease in IBD[11] and predicting response to IBD therapies.[12] Collectively, these findings suggest that specific gut microbiome signatures hold the potential to predict future manifestations of clinical events within the course of IBD patients' disease progression.[13,14]

In this study, our objective was to explore whether the gut microbiome can serve as a predictive biomarker for future IBD disease progression, particularly with regards to the need for therapy intensification. To achieve this, we performed whole-genome metagenomic shotgun sequencing on fecal samples of 90 patients with IBD and subsequently generated taxonomic and functional profiles. Our study cohort comprised individuals who had collected fecal samples in the past (2012-2015) and had been followed by treating physicians until the present day (2023). Our study design allowed us to gather comprehensive clinical data to assess the need for therapy intensification in the 10 years following fecal sample production. This intensification was defined as treatment escalation, intestinal resections, or loss of response to biological medication (Figure 1).

Materials and Methods

Study Cohort

A total of 104 participants from the 1000IBD cohort were included in the present study. The 1000IBD cohort, previously described in detail,[15] comprises patients with IBD who have been recruited from the University Medical Center Groningen (UMCG). Patients in the 1000IBD cohort are followed prospectively, involving meticulous clinical metadata collection and the generation of metagenomic profiles of fecal samples. Patients were diagnosed with IBD based on accepted radiologic, laboratory, and endoscopic findings and were at least 18 years of age. In this study, we selected patients within the 1000IBD cohort who had fecal samples collected between 2012 and 2015. Of these patients, we excluded 11 patients with ileo-anal pouch or an ileostomy prior to sampling, as these conditions are known to significantly affect the composition of the gut microbiome.[16] All patients provided informed consent, and the project was approved by the UMCG Institutional Review Board (IRB number 2008.338).

Collection of Clinical Data Postfecal Sampling

Clinical information was collected at the time of feces collection, including disease type, disease activity at sampling, medication usage, surgical bowel resections, laboratory measurements such as fecal calprotectin, and demographic details. Additionally, we reviewed patients' medical records to collect extensive clinical follow-up information for the average of 10 years postsampling, including:

1. IBD medication use: all IBD medication used during the follow-up period and the corresponding indications, dates of start and end of treatments, and reason of discontinuation were noted in our database, and response to treatments were determined.
2. Definition of response to IBD medication: we considered a patient to have responded to a biological treatment based on written documentation from the treating gastroenterologist indicating clinical response. The gastroenterologists assess clinical response by considering a combination of the following parameters:

a. Substantial decrease or absence of IBD-related symptoms, which is reflected in significant changes in the Harvey-Bradshaw Index (HBI) or Simple Colitis Activity Index (SCCAI) score compared with scores measured during periods of suspected or endoscopically proven active disease.
b. Biochemical test values: fecal calprotectin levels (FCP) and C-reactive protein (CRP; calprotectin >200 µg/g feces, CRP >5 mg/L)
c. Endoscopic results showing evidence of reduction or absence of intestinal inflammation, which are confirmed by pathological assessments of gut luminal biopsies

3. Definition of loss of response: loss of response to a biological agent was defined as clinical manifestations of increased disease activity in a patient that has been showing signs of clinical or/and endoscopic response to that agent for longer than 1 year.
4. Details of surgical interventions and IBD-related clinical events: all bowel resections are documented in detail, including the type, date, and the indication for the bowel resection. Also, periods of active disease, hospitalization, and emergency department visits were noted.

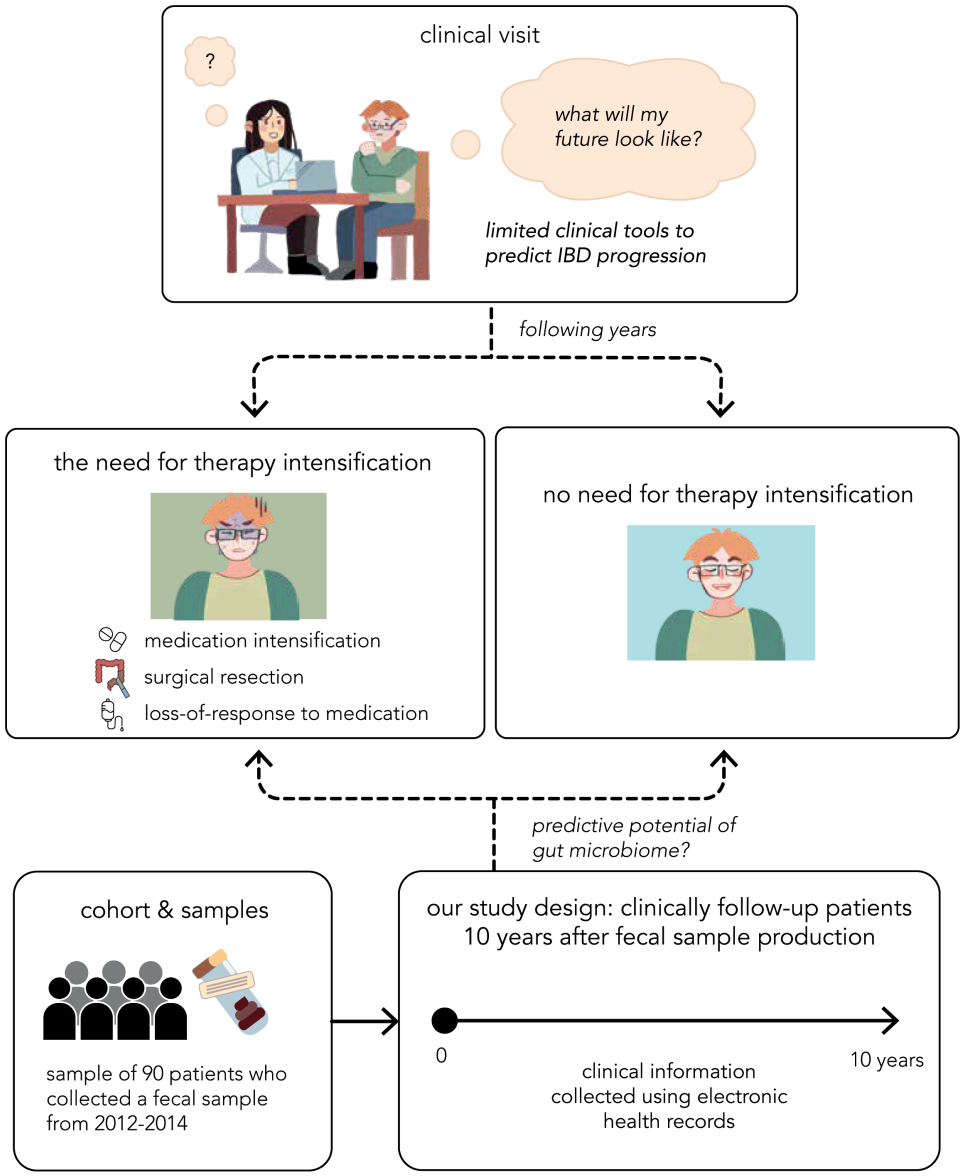

Figure 1. Clinical challenge and study design. An illustration of the clinical challenge faced by patients with IBD who struggle to predict the future course of their disease: whether they need therapy intensification or not. Our study design focuses on exploring how the gut microbiome could serve as a predictive tool in assessing disease course; fecal sample collection occurred at baseline (time point 0) and clinical information on disease course was gathered over a 10-year follow-up period. This Figure was created by PATTERN at the Broad Institute.

Identifying Therapy Intensification Postfecal Sample Collection

To examine the relationship between gut microbiome patterns and the course of disease, we classified the patients into 2 distinct groups based on their disease progression: patients who required therapy intensification after the initial sampling and those who did not require such long-term therapy intensification. We defined long-term therapy intensification as the need for at least one of the following long-term modifications in the treatment approach:

1. Medication intensifications

Medication was considered to be intensified if at least one of the following medication adjustments took place after the sampling:

a. Switching from monotherapy with mesalazine to immunosuppressants (thiopurines or methotrexate)
b. Switching from monotherapy with mesalazine to biologicals
c. Adding an immunosuppressant to monotherapy with mesalazine
d. Adding a biological to monotherapy with mesalazine
e. Switching from an immunosuppressant to a biological
f. Adding a biological to an immunosuppressant

Changes in medications due to side effects are not a part of this definition. Furthermore, we did not classify the following as medication intensification: (1) increasing medication dosages or decreasing treatment intervals[17] (2) temporary courses of corticosteroids without one of the medication intensification methods listed above.[18]

2. Loss of response to a biological (adalimumab, golimumab, infliximab, ustekinumab, vedolizumab)
3. The need for surgical resections of (parts) of the colon or small intestine following the sampling.

Fecal Sample Collection, Microbial DNA Extraction and Sequencing

Participants were asked to collect stool samples using a provided stool collection kit that was provided and freeze the samples within 15 minutes. Afterwards, a research member collected the samples for transport. During the transportation, the samples were kept in −80 °C using dry ice before they reached the laboratory. In the laboratory, microbial DNA was isolated using the Qiagen AllPrep DNA/RNA Mini Kit cat. 80204 with the addition of mechanical lysis, followed by randomization and placement on 96-well plates. These samples were sent to and stored at the Broad Institute at −80 °C, where whole genome metagenomic sequencing was performed using the Illumina HiSeq 2000 sequencing platform.[15]

Metagenomic Data Processing

The sequencing facility conducted basic quality control using an in-house pipeline to remove low-quality reads and samples with a read depth of less than 10 million reads. *Trimmomatic* (v.0.32) was used to remove adapters and trim the reads, and *kneaddata* toolkit (v.0.12.0) was used to remove reads that align to human reference genome (hg37dec v0.1). Cleaned metagenomic sequencing reads were used to generate taxonomic abundance profiles using the MetaPhlAn tool (v4.0.6). Our data set consisted of 3826 taxa, including 1218 species. In addition, 485 microbial pathways were identified using the HUMAnN3 pipeline (v3.6) for profiling of microbial pathways.

No filtering steps were applied in the diversity and dissimilarity analyses. For the differential abundance analysis, only species that were present in at least 25% of the samples and with a mean relative abundance of ≥0.01 in the cohort were analyzed ($n = 161$). For microbial functional pathways differential abundance analysis, we included the pathways that were present in 5% of the samples and with a mean relative abundance of 0.001 or higher ($n = 186$). Given the compositional nature of the data, we used the centered log-ratio (CLR) transformation on the data. In order to deal with zero values for the CLR transformation, we calculated a pseudocount as half of the lowest nonzero abundance value present in the data. For the alpha-diversity analyses, we used nontransformed relative abundance data.

Microbiome Diversity Measures

We explored microbiome diversity (α-diversity), using the Shannon diversity Index. This index considers species richness and evenness. We performed these calculations using the *vegan* (v2.6-4) package in R. We compared the baseline α-diversity between therapy intensification groups in a linear regression model considering the following covariates: age, sex, body mass index (BMI), reads depth, diagnosis, disease activity at the time of sampling, history of surgical resections before sampling, PPI use, and antibiotic use within 3 months prior to sampling. We used the *LM* function from the *stat* (v4.3-1) package. Estimated Marginal Means (EMM) were calculated using the *emmeans* package (v1.3-3) based on the linear regression analysis.

To assess differences in microbial communities between groups (the β-diversity) based on clinical characteristics, we calculated the Aitchinson distance, which corresponds to the Euclidean distance of CLR-transformed relative abundances, using the *vegdist* function. To visualize our data and explore any potential clustering of samples per group, we used principal coordinate analysis (PCoA), which is a dimension-reduction technique used for analysis of high dimensional data. To test whether groups had different microbial compositions, proportions of explained variance in microbial β-diversity were calculated using the *adonis2* function from *vegan* package for permutational multivariate analysis of variance (PERMANOVA) with 10 000 permutations. We used the *betadisper* function to test the homogeneity of dispersion. We accounted for multiple testing by applying the Benjamini-Hochberg correction to calculate the false discovery rate (FDR). We set statistical significance at 0.10. Additionally, we examined the nominal P values of the tests to which we applied the FDR corrections, considering tests that were nominally significant ($P < .05$) but did not meet the FDR correction to facilitate further exploration of possible gut microbial signatures.

Differential Abundance Analyses of Microbiome Features

To identify baseline gut microbiome signatures that may correlate with therapy intensification, we explored whether the relative abundance of microbial species and microbial functional pathways differed between groups. We applied a linear regression model using the filtered relative abundance data while taking into account the covariates age, sex, body mass index (BMI), reads depth, diagnosis, disease activity at the time of sampling, history of surgical resections before sampling, PPI use, and antibiotic use within 3 months prior to sampling (Species/pathways in baseline gut microbiome ~ therapy intensification + covariates).

Predicting Therapy Intensification From Baseline Gut Microbiome Features

For the purpose of exploring the predictive value of gut microbiome features, we trained the random forest models using the *RandomForest* package (v4.7-1-1) and calculated receiver operating characteristic (ROC) and the area under the ROC curve (AUC) using the *pROC* package (v1.18.4). We trained 2 separate models for the filtered relative abundance of microbial species and microbial functional pathways using a subset of the cohort (75%, N = 67) and tested the models in the remainder of the data (25%, N = 23). Using the *importance* function from the *RandomForest* R package, we identified the most important predictors of these models.

All statistical analyses were performed using R (version 4.3.1) in RStudio on macOS.

Results

Clinical Characteristics

Our study cohort consisted of 90 patients included in the analyses after excluding samples with either low sequencing depth (<3 million reads, $n = 1$) or identified as outliers in terms of alpha-diversity (>2.5 standard deviations, $n = 2$).

This cohort included 48 patients with CD, 30 with UC, and 12 with inflammatory bowel disease unclassified (IBD-U). These individuals collected a fecal sample between 2012 and 2015. Subsequent clinical follow-up with a mean follow-up time of 9.8 years (SD = 0.49) revealed that 33 patients (36%) required future therapy intensification during their disease course following fecal sample collection, whereas 57 (64%) exhibited a disease course that did not require therapy intensification. Twenty-seven of the 33 patients (82%) who required therapy intensification received pharmacological medication modification as described in the methods, 13 (39%) underwent bowel resections, and 4 (19%) experienced secondary nonresponse to a biological agent. At baseline, only disease activity at sampling was higher in the therapy intensification group (P = .006). We found no other differences between the 2 groups. During the follow-up period there were differences between the groups in terms of medication use and clinical events (Table 1). Additional information about Montreal classification, disease characteristics, and resections can be found in Supplementary Table S1.

Preexisting Clinical Phenotypes Are Associated With the Baseline Gut Microbiome Composition

It has been repeatedly reported that the gut microbiome of IBD patients is influenced by past clinical events and general demographic and disease phenotypes.[16,19,20] Thus, it is essential to identify and adjust for such factors before proceeding to analyze whether baseline composition could serve as a predictive factor for future therapy intensification. In light of this, we first aimed to investigate which clinical phenotypes prior to sampling within our cohort might contribute to baseline changes in the gut microbiome composition.

We examined the relationship between baseline microbiome alpha-diversity (Shannon index) and 16 clinical phenotypes prior to sampling using linear models, correcting for basic covariates (sex, age, BMI, and sequencing read depth). We found that a history of surgical bowel resection significantly altered the Shannon diversity; individuals with a history of resection had a lower Shannon diversity compared with those who did not have resections (β = −0.49, FDR = 0.012), both for ileum/ileocecal resections and colonic resections (Supplementary Figure 2). Furthermore, we found that patients with UC had a higher Shannon diversity compared with patients with CD (β = 0.47, FDR = 0.040; Supplementary Table S2).

Using dissimilarity assessment, we found that IBD subtype (diagnosis), prior resections, location and indication of prior resection, prior mesalazine use, and sex explained a significantly amount of variance in microbiome composition at species level (FDR < 0.1; Figure 2, Supplementary Table S3). At the functional pathway level, the use of antibiotics prior to sampling and age were also found to significantly associate to the variation (FDR < 0.1; Figure 2, Supplementary Table S4). Principal coordinate analysis plots colored on clinical phenotypes prior to sampling are provided in Supplementary Tables S9 and S10.

To further delve into the impact of prefecal sampling clinical phenotypes on the gut microbiome, we tested if the relative abundances of microbial species and functional pathways correlated significantly with any of these phenotypes. We found a positive correlation between the relative abundance of *Feacalimonas umbilicata* and prior resections (FDR < 0.1). Moreover, patients with prior colonic resections had higher abundances of *Enterocloster clostridiofformis* (FDR = 0.007; Supplementary Table S5). On a functional level, we found 5 clinical phenotypes that significantly correlated with increased or decreased abundance of functional pathways, including the use of biologicals that correlated with a decreased abundance of pathways involved in LPS biosynthesis (3-Deoxy-D-manno-octulosonate) and amino acid degradation (L-histidine degradation; FDR < 0.1; Supplementary Table S7). These findings suggest that gut microbiome composition and function are impacted by surgical resections, prior medication use, and demographic characteristics.

Evaluating Baseline Gut Microbiome Composition Differences in Therapy Intensification Groups

We investigated whether baseline gut microbiome composition showed differences between patients necessitating therapy intensification and those that did not and whether it could therefore hold predictive potential.

Baseline Microbiome Diversity Measures Do No Associate With Need for Therapy Intensification

First, using linear regression, we tested whether α-diversity at baseline significantly associated with therapy intensification, while taking into consideration the covariates that could influence the gut microbiome. The overall regression was not statistically significant for Shannon diversity (P = .07) between therapy intensification groups but showed a trend of a decreased diversity in the group not needing therapy intensification (Figure 3A). Next, for beta-diversity we visualized the dissimilarity in taxonomic and functional microbiome compositions among the therapy intensification groups for the first 5 principal coordinates (PCoA). We did not observe any clear clustering of the groups (Figure 3B, 3C). We then performed PERMANOVA to test the extent to which the variation in the gut microbiome composition could be explained by therapy intensification and other clinical follow-up features. The analysis showed that the proportion of variance explained by the therapy intensification was not significant for microbial composition or function ($R2 < 0.01$; P = .6, P = .74, respectively). The homogeneity of dispersion between groups was not significant for either species or pathways. We also conducted these baseline analyses stratified by IBD subtype, revealing a difference in Shannon diversity among patients with CD (P = .03) showing a decreased Shannon diversity in the group not needing therapy intensification, but not among UC or IBDU patients (P = .77 and P = .30, respectively). Additional details of this stratified analyses are available in Supplementary Table S11.

Baseline Abundances of 12 Microbial Species and 16 Functional Pathways Correlate With Therapy Intensification

Next, we tested whether individual species or pathways were associated with therapy intensification using linear regression. The analyses showed 12 nominally significant ($P < .05$) associations between the relative abundance of microbial species at baseline and the need for intensification of therapy (Figure 4, Supplementary Table S6). Among these findings, we identified 7 species that had increased abundance in patients who needed therapy intensification, while 5 species had decreased abundance. For differential abundance analysis

Table 1. Clinical characteristics.

	IBD Patients Needing Therapy Intensification	IBD Patients Not Needing Therapy Intensification	*P*
No. patients	33	57	
Female sex (%)	25 (76%)	35 (61%)	.2
Age (SD)	52 (15)	55 (16)	.4
Body mass index (SD)	26.2 (5.2)	27.2 (5.6)	.4
Smokers (%)	3 (9.4%)	12 (23%)	.2
IBD characteristics			
Crohn's disease diagnosis (%)	17 (52%)	31 (54%)	>.9
Ulcerative colitis diagnosis (%)	13 (39%)	17 (30%)	.5
IBD-U diagnosis (%)	3 (9.1%)	9 (16%)	.6
Active disease at sampling (%)	19 (58%)	16 (28%)	**.006**
History of bowel resections prior to sampling (colon and ileum/cecum, %)	11 (33%)	19 (33%)	>.9
Medication use prior to sampling			
Mesalazines (%)	12/30 (40%)	23/55 (42%)	>.9
Local Mesalazines (%)	2/30 (6.7%)	0/55 (0%)	.2
Steroids (%)	9/30 (30%)	6/55 (11%)	.056
Anti-TNF (%)	6/30 (20%)	12/55 (22%)	>.9
Biologicals (%)	1/30 (3.3%)	0/55 (0%)	.8
Proton Pump Inhibitors (%)	6/33 (18%)	17/57 (30%)	.3
Antibiotics (<3 months prior to sampling, %)	9/33 (27%)	15/57 (26%)	>.9
Therapy intensification			
Medication Intensification (%)	27/33 (82%)	0 (0%)	-
Resections (%)	13/33 (39%)	0 (0%)	-
Switching Biologcials due to Loss of Response (%)	4/21 (19%)	0 (0%)	-
Follow-up events			
Periods of Active Disease (%)	33/33 (100%)	36/57 (63%)	**<.001**
IBD-related Emergency Department Visits (%)	7/33 (21%)	6/57 (11%)	.3
IBD-related Hospitalizations (%)	17/33 (52%)	10/57 (18%)	**.002**
Medication response			
Anti-TNF Response (%)	7/17 (41%)	12/17 (71%)	.2
Vedolizumab Response (%)	4/10 (40%)	2/2 (100%)	.4
Ustekinumab Response (%)	4/8 (50%)	5/5 (100%)	.2
Medication use after sampling			
Antibiotics (%)	17/33 (52%)	18/57 (32%)	.10
Biologicals (%)	26/33 (79%)	20/57 (35%)	**<.001**
Steroids (%)	32/33 (97%)	32/57 (56%)	**<.001**
Thiopurines (%)	25/33 (76%)	16/57 (28%)	**<.001**
Methotrexate (%)	9/33 (27%)	3/57 (5.3%)	**.008**
Aminosalicylates (%)	16/33 (48%)	27/57 (47%)	>.9
Laxatives (%)	10/33 (30%)	20/57 (35%)	.8
Proton Pump Inhibitors (%)	19/33 (58%)	30/57 (53%)	.8
Metformin (%)	0/33 (0%)	4/57 (7.0%)	.3
Opioids (%)	15/33 (45%)	12/57 (21%)	**.028**
Antiplatelets (%)	4/33 (12%)	10/57 (18%)	.7
SSRI (%)	2/33 (6.1%)	4/57 (7.0%)	>.9
Colecalciferol (%)	10/33 (30%)	6/57 (11%)	**.038**

Table description: Clinical characteristics. Values for categorical variables = counts (proportion, %) and for numerical variables = mean (SD). *P*s are from Pearson's Chi-squared test or Welch Two Sample *t* test. Abbreviations: IBD-U, inflammatory bowel disease unclassified; TNF, tumor necrosis factors; SSRI, selective serotonin reuptake inhibitor.

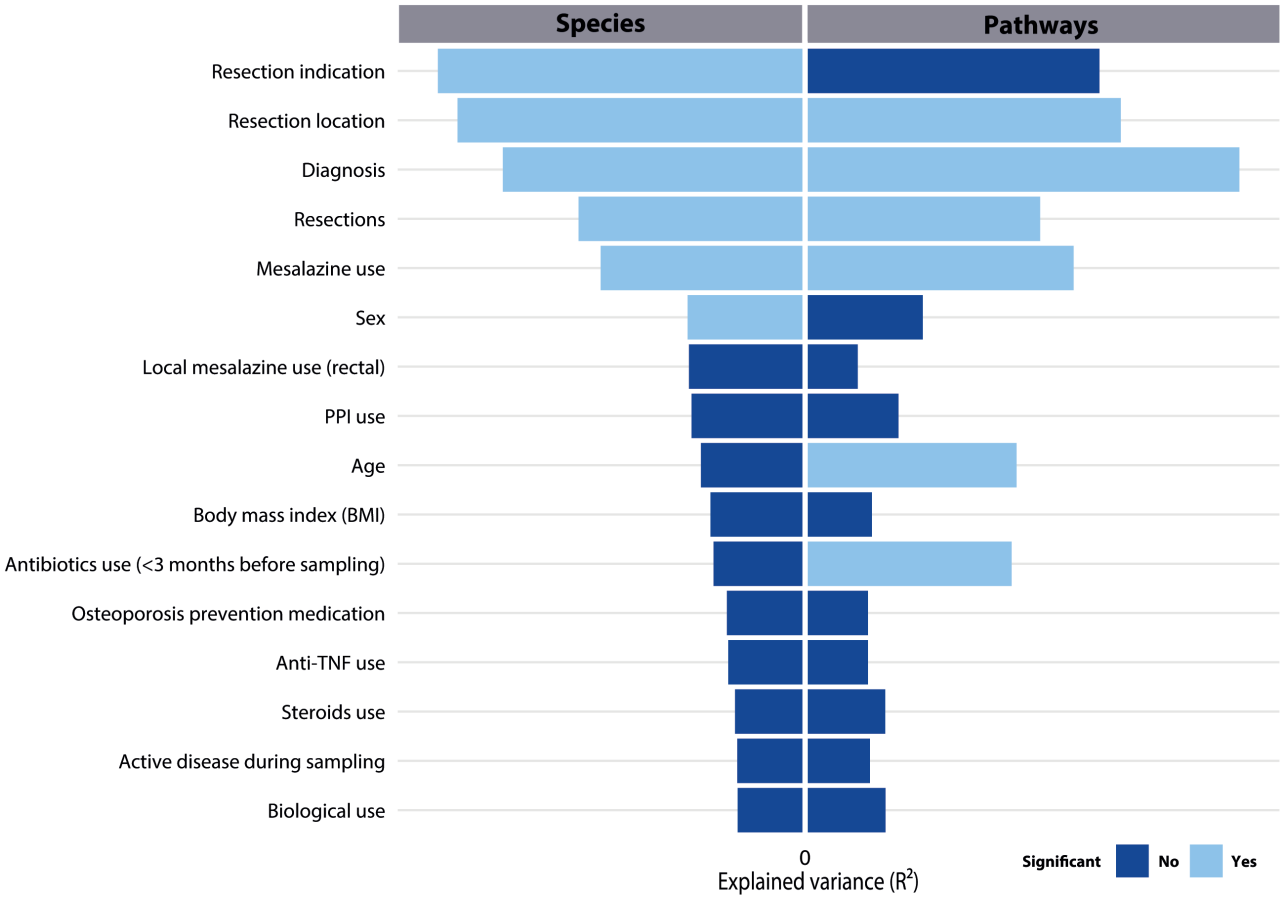

Figure 2. Proportions of variance in species and functional pathway composition explained by clinical phenotypes prior to sampling. The explained variance (R^2) is displayed on the *x* axis and the clinical features prior to the sampling are displayed on the *y* axis. The left side of the plot depicts the variances for species, and the bars to the right show the explained variance for pathways. The light blue color indicates significance at FDR level (<0.1).

of functional pathways, we found 16 pathways to be nominally significantly different ($P < .05$) between therapy intensification groups. We found increased relative abundances in 6 pathways, while 10 pathways were decreased in the therapy intensification group (Supplementary Table S8). After FDR corrections for multiple testing, no microbial species or pathways were significantly differentially abundant between therapy intensification groups (FDR > 0.1).

Utilizing the Gut Microbiome for Prediction of Therapy Intensification

We used random forest analysis to investigate if baseline gut microbial composition can predict long-term therapy intensification. We used the 161 filtered species and 186 microbial pathways to train our model and perform ROC analysis. Our analysis calculated an AUC of 0.75 (standard error [SE] = 0.102) and an AUC of 0.72 (SE = 0.113) using microbial species and functional pathways, respectively (Figure 5). Additionally, we assessed the model's performance by using a curated list of 28 differentially abundant features identified in previous analyses (Supplementary Table S12), resulting in an AUC of 0.78 (SE = 0.102). Furthermore, random forest analysis revealed that the relative abundance of species belonging to *Lachnospiraceae* (*Waltera intestinalis*, *Roseburia hominis*, *Lachnoclostridium sp-An138*), *Ruminococcus bicirculans*, *Barnesiella intestinihominis*, and *Alistipes communis* were the most important for prediction of therapy intensification. On a functional level, our analysis revealed that pathways involved in amino acids (L-arginine, L-alanine, L-cysteine) and polysaccharides (beta-[1,4]-mannan) biosynthesis and degradation, *E. coli* lipid A biosynthesis, and thiamine biosynthesis were important in predicting therapy intensification. The importance of these features, along with less important ones, is shown in Supplementary Figure 1.

Discussion

In this study, we aimed to explore the potential of the gut microbiome as a predictive tool to aid predicting future disease course of IBD patients in the clinical practice. We studied baseline gut microbiome features in fecal samples from 90 IBD patients (CD, UC, and IBD-U) who were clinically followed up for 10 years to determine whether their disease course required therapy intensification. We defined therapy intensification as whether patients received long-term intensification of pharmacological agents (excluding short-term remission inductions), underwent surgical resections in the small, or large intestine or/and experienced secondary nonresponse to a biological agent. In our cohort, 33 patients (36%) required therapy intensification in the 10-year period following the fecal sampling, whereas 57 (64%) experienced a disease course that did not necessitate therapy intensification. Our analyses revealed 12 microbial species and 16 functional pathways in baseline gut microbiome samples that

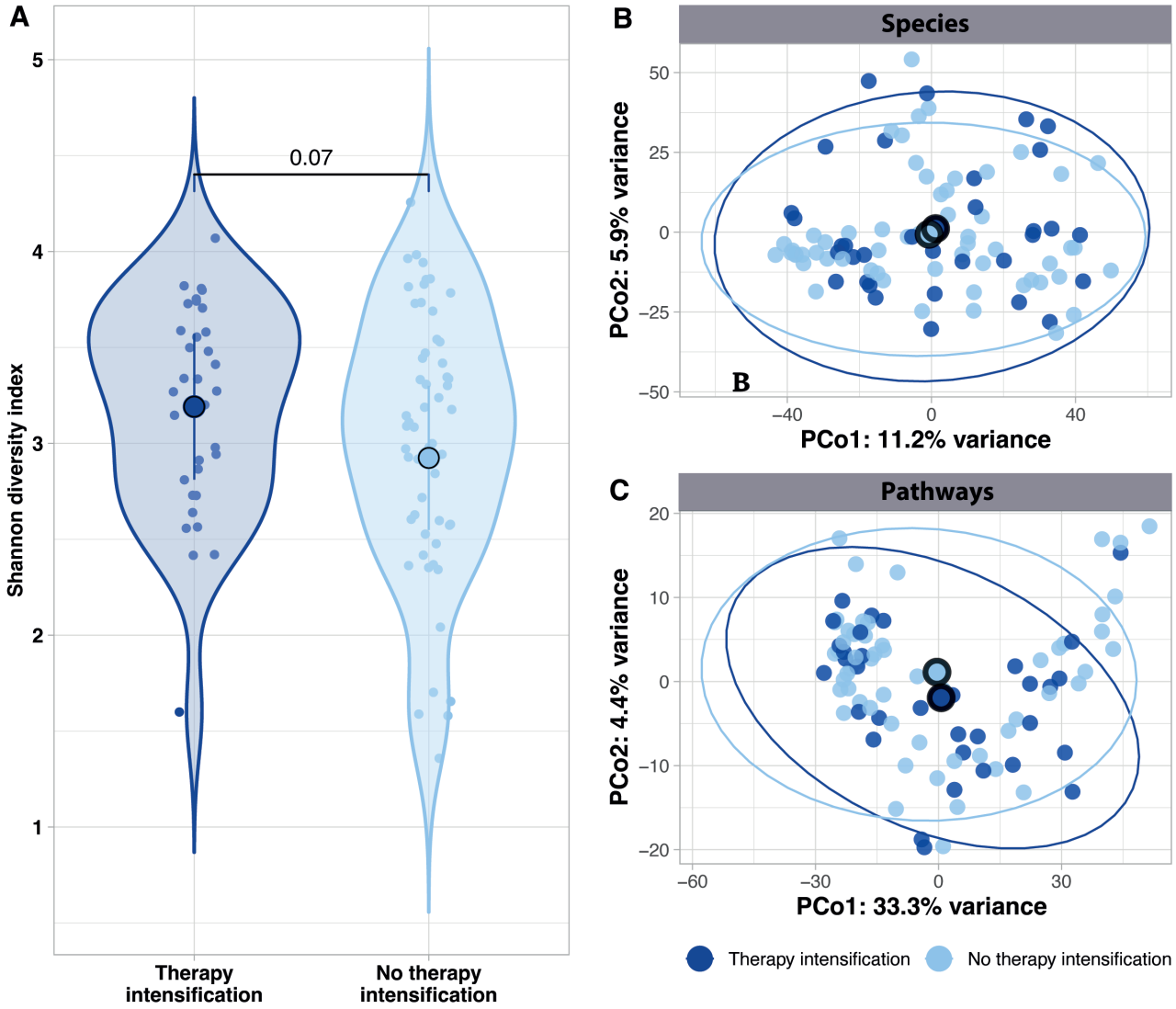

Figure 3. Baseline gut microbiome diversity and dissimilarity. (A) Shannon Diversity Index of fecal samples at baseline. Violin plots show the distribution of the Shannon diversity index. The dot with line represents the estimated marginal mean of the linear regression analysis. (B) Beta diversity depicted using principal coordinates of Aitchison distance for species and (C) for functional pathways. Individual data points are represented by light blue dots for the patients not needing therapy intensification and dark blue dots for the patients needing therapy intensification. The large dot represents the centroid, which represents the mean composition of each group.

nominally correlated with therapy intensification following the sampling. Furthermore, our prediction model calculated an AUC of 0.75 and an AUC of 0.72 using microbial species and functional pathways respectively for predicting therapy intensification.

In our cohort, baseline α-diversity did not differ between individuals receiving therapy intensification up to 10 years after sampling and those who did not. This contradicts previous studies associating reduced α-diversity with more extensive disease behavior in IBD and postoperative endoscopic recurrence of CD.[13,21] Interestingly, patients with a history of bowel resections who needed therapy intensification after sampling had similar diversity to those without prior resections (Supplementary Figure 3). We also found higher *Clostridiales* abundances in patients with a history of bowel resections. This aligns with a prior study indicating increased α-diversity postileocecal resections in Crohn's disease patients achieving remission possibly in relation to increase to beneficial *Clostridiales*.[13] This suggests that in our cohort, some patients might have achieved remission postileocecal resection, possibly in relation to the increased abundances of beneficial *Clostridiales*, consequently increasing their gut microbial diversity postoperatively.

In the baseline samples of the IBD patients requiring therapy intensification in their disease course, we observed alterations in abundance of specific microbial species compared with those not requiring therapy intensification. We hypothesized that the 7 species that were increased in patients needing therapy intensification might contribute to a detrimental impact on the disease course, possibly due to a pro-inflammatory nature of these species. Indeed, some of these species have been described in relation to inflammation before, including *Ruthenibacterium lactatiformans* that has been described in relation to inflammation in a previous study on COVID-19 patients, showing a positive correlation with inflammatory markers[22] and *Dialister invisus*, which correlated with several inflammatory diseases, including celiac disease.[23] Contrary to our hypothesis, *Bacteroides ovatus*, *Dysosmobacter*

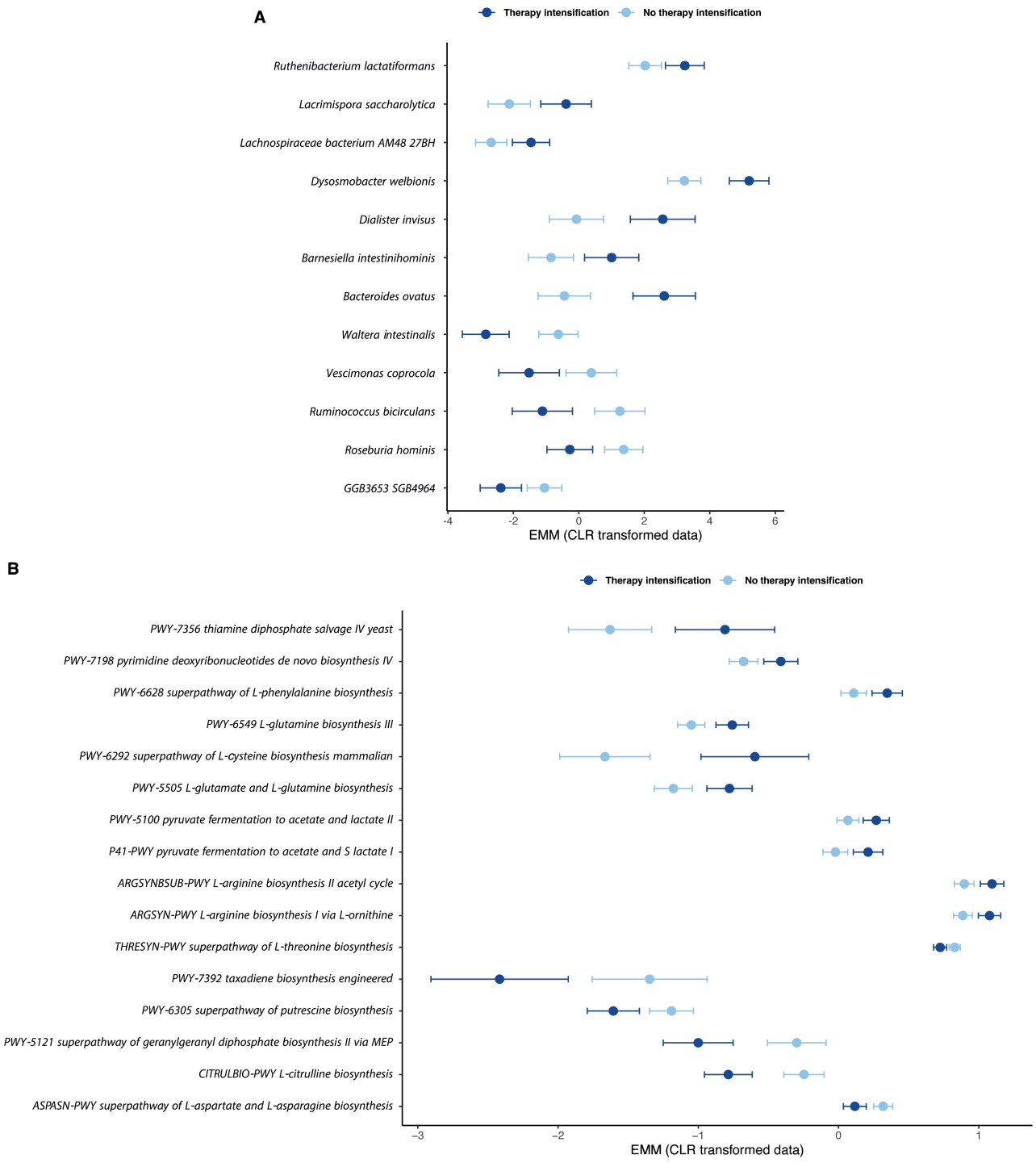

Figure 4. Estimated marginal means (EMM) for the differential abundant species and pathways (nominal significance). (A) Species (B) Pathways. Light blue indicates the CLR-transformed relative abundance of the species for patients not needing therapy intensification and dark blue shows the abundance for the patients needing therapy intensification.

welbionis, and *Barnesiella intestinihominis* have been primarily reported to have anti-inflammatory effects in various studies.[24–27] Additionally, members of the *Lachnospiracea*, typically linked to short-chain fatty acids (SCFA) production, known for its benefits to (gut) health by promoting anti-inflammatory processes, were increased in patients needing therapy intensification.[28,29] However, although SCFA production is generally beneficial to gut health, increased abundance of species of this family has also been associated with diseases such as primary sclerosing cholangitis IBD and metabolic diseases.[30,31] Interestingly, among the 5 species showing decreased abundance at baseline in the therapy intensification group, we hypothesized a favorable impact on disease course. However, a reduced abundance of *Roseburia hominis*, one of the species that we identified, has been linked to increased disease activity in UC, suggesting that a depletion of this species may have an adverse effect on intestinal inflammation,[32] contrary to our hypothesis. Concerning functional pathways, we

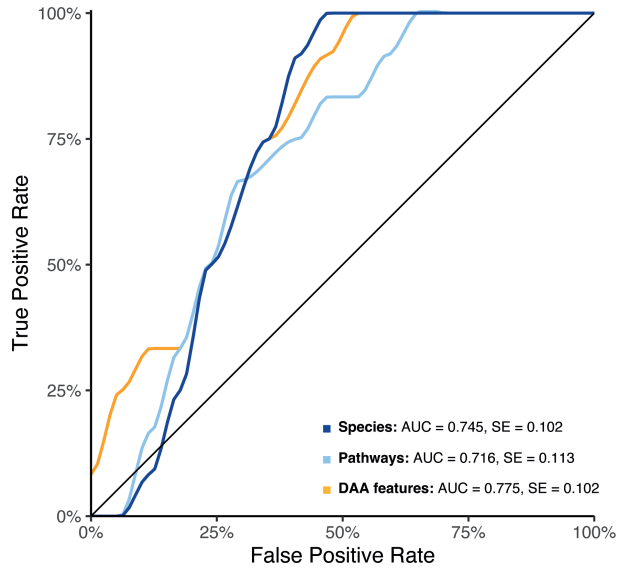

Figure 5. ROC curves for the prediction of long-term therapy intensification using baseline gut microbiome features. Dark blue indicates the model only using species (n = 161), light blue is the model for pathways (n = 186), and the orange line represents the model using the features identified in differential abundance analysis (n = 28).

observed that 4 pathways associated with anti-inflammatory properties (involved in biosynthesis of amino acids, amides, and enzymes) were less abundant in the group who received therapy intensification, supporting our hypothesis. However, the increased abundance of pathways involved in thiamine biosynthesis and pyruvate fermentation (a precursor of SCFA) in patients needing therapy intensification contradicts our hypothesis, given that numerous studies emphasize the anti-inflammatory properties of these pathways.[33,34]

Comparing our findings to the existing literature underscores the inconsistency within the field; some studies suggest the increased abundance of certain species to be beneficial, while others indicate a detrimental effect. The variability in the associations between microbiome features and clinical outcomes poses a significant challenge. To enable the implementation of microbiome-based tools in clinical practice, consistent identification of "bad" and "good" gut microbiome signatures across IBD microbiome studies is crucial. However, the substantial inter-individual variability is an important factor to consider. Adding another layer of complexity is the diversity in sample processing methods used in the field, where each method introduces biases toward specific microbial communities, leading to discrepancies in study findings. Additionally, postsample processing steps (including bioinformatic pipeline) further contribute to variability. Our study recognizes these challenges, highlights the need for further standardization of methodology and bioinformatics in translation of microbiome research into clinical practice, and aims to provide a preliminary exploration, setting the stage for further investigations into the potential use of gut microbiome profiles in the specific context of disease progression in IBD patients.

We examined the potential of using baseline gut microbiome features for predicting therapy intensification. Our AUC values in the range of 0.72 to 0.77 are modest, indicating predictive potential primarily within our cohort. In our study, we found that multiple species belonging to genus *Lachnospiraceae*, *Barnesiella intestinihominis*, and *Ruminococcus bicirculans* were among the most important taxa in classifying patient samples as patients who need therapy intensification or who do not require therapy intensification. According to our RF-algorithm, pathways involved in amino acids (L-arginine and L-alanine) and polysaccharides (beta-[1,4]-mannan) biosynthesis and degradation, and *E. coli* lipid A biosynthesis were among the most important pathways for the prediction of therapy intensification based on baseline gut microbiome microbial functional pathways. *E. coli* lipid A promotes inflammation through aiding the production of inflammation mediators, such as tumor necrosis factor-α and interleukin-1β.[35] The β-mannans is one of the mannans that is polymers of mannose residues. The ingestion of mannans has been shown to trigger the development of inflammatory conditions such as rheumatoid arthritis, psoriasis, and psoriatic arthritis in mice, suggesting that exposure to mannans may cause similar effects in humans.[36] While our study reports comparable AUC values as previous studies aiming to utilize the gut microbiome for prediction (eg, predicting host responsiveness to IBD treatment),[12,37] the translation of microbiome-based disease prediction models into clinical practice remains a substantial challenge. Despite promising insights, further validation and replication of these models are needed before their potential integration into routine clinical decision-making processes.

While our study provides promising exploratory insights into gut microbiome features that hold potential for prediction of disease progression, some limitations need consideration. Firstly, our definition of therapy intensification may have played a significant role in shaping the obtained results and should be taken into account when interpreting the outcomes of this study. There is a need for a standardized and universally accepted definitions in future research to enhance comparability between studies. Secondly, the cross-sectional design of our sampling poses a challenge for capturing the dynamic and individual-specific nature of gut microbiomes. Longitudinal studies could aid in the identification of a set of microbiome-based signatures that can contribute to patient-tailored therapies. A recent multi-omic study aimed to explore gut microbiome dynamics associations with disease activity in IBD and concluded that gut microbiome dynamics require close monitoring (ie, weekly sampling in order to detect consistent changes associated with IBD disease course).[11] Thirdly, although compositional and functional gut microbiome analyses are informative, understanding the role of the gut microbiome in IBD disease course requires consideration of additional factors such as intraspecies genetic variance and metabolites resulting from microbes-host environment interactions.[38] Thus, in-depth high-resolution gut microbiome studies that examine gut metabolites are required for better understanding of the functionality of gut microbiome. Lastly, our relatively small sample size and the absence of an independent validation set for our prediction models limits the generalizability of the results to a broader population. To establish the reliability of the identified predictive features within our study, validation in an external independent cohort is needed. Consequently, caution should be taken in drawing conclusions based solely on the findings of this study. Our prediction model results do not provide sufficient evidence to claim that baseline gut microbiome data can reliably predict therapy intensification in different IBD patient populations, which is the ultimate goal.

While acknowledging these limitations, our study also has several strengths; the use of shotgun metagenomic sequencing offers more detailed insights into the functional aspects of the gut microbiome in intestinal inflammation, which surpasses the commonly used 16S sequencing. Additionally, our clinical follow-up period of 10 years makes this a unique study offering valuable insights into long-term outcomes in IBD patients.

Overall, our study of 90 IBD patients revealed gut microbiome features that hold a predictive value for the future necessity to initiate therapy intensification. These findings contribute to the growing potential of predictive microbiome analysis in personalizing IBD management strategies. It would be beneficial to explore the longitudinal intra-individual changes in gut microbiome and their association with disease behavior to further advance the field of precision medicine.

Supplementary Data

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

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Author Contributions

Study concept and design—M.A.Y.K., R.K.W.; analysis and interpretation of data—Z.M.A.A.R., F.M.P., M.A.Y.K., R.G.; drafting of the manuscript—Z.M.A.A.R., F.M.P., M.A.Y.K.; critical revision of the manuscript—all authors; study supervision—R.W.K., M.A.Y.K., R.G.

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Conflicts of Interest

R.K.W. has acted as a consultant for Takeda and received unrestricted research grants from Takeda, Johnson & Johnson, Tramedico, and Ferring; and received speaker fees from MSD, AbbVie, and Janssen Pharmaceuticals. E.A.M.F. has received an unrestricted research grant from Takeda. G.D. has received research grants from the Royal DSM and speaker fees from Janssen Pharmaceuticals, Takeda, Pfizer, and AbbVie. R.G. has received funding from Janssen Pharmaceuticals and consulting fees from Esox Biologicals Ltd. (for unrelated research projects). All other authors affirm that they do not have any relevant competing financial interests or relationships that could have influenced the findings presented in this study.

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Data Availability

Supplementary data and figures are available. The data and codes used for the analyses in this study will be shared upon reasonable request to the corresponding author. Metagenomic data are available at EGA (study EGAS00001002702).

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