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Original Article

Exploration of Potential Gut Microbiota-Derived Biomarkers to Predict the Success of Fecal Microbiota Transplantation in Ulcerative Colitis: A Prospective Cohort in Korea

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Background/Aims: Although fecal microbiota transplantation (FMT) has been proven as one of the promising treatments for patients with ulcerative colitis (UC), potential prognostic markers regarding the clinical outcomes of FMT remain elusive.

Methods: We collected fecal samples of 10 participants undergoing FMT to treat UC and those from the corresponding donors. We categorized them into two groups: responders and nonresponders. Sequencing of the bacterial 16S rRNA gene was conducted on the samples to explore bacterial composition.

Results: Analyzing the gut microbiota of patients who showed different outcomes in FMT presented a distinct microbial niche. Source tracking analysis showed the nonresponder group had a higher rate of preservation of donor microbiota, underscoring that engraftment degrees are not one of the major drivers for the success of FMT. At the phylum level, Bacteroidetes bacteria were significantly depleted ($p<0.003$), and three genera, including *Enterococcus*, *Rothia*, and *Pediococcus*, were enriched in the responder group before FMT ($p=0.003$, $p=0.025$, and $p=0.048$, respectively). Furthermore, we applied a machine learning algorithm to build a prediction model that might allow the prediction of FMT outcomes, which yielded an area under the receiver operating characteristic (ROC) curve of 0.844. Notably, the microbiota-based model was much better at predicting outcomes than the clinical features model (area under the ROC curve=0.531).

Conclusions: This study is the first to suggest the significance of indigenous microbiota of recipients as a critical factor. The result highlights that bacterial composition should be evaluated before FMT to select suitable patients and achieve better efficiency. **(Gut Liver 2022;16:775-785)**

Key Words: Fecal microbiota transplantation; Fecal microbiota transplantation outcome; Machine learning; Ulcerative colitis

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon characterized by periods of disease activity and remission, affecting one in 200 to 400 people in Western countries.[1,2] Although the etiology of UC remains unclear,[3] the combination of genetic and environmental factors is well known as a significant cause that disrupts the balance between intestinal microbial community and immunity of individuals, bringing out exaggeration of the immune response.[4-9] Therapeutic approaches which manipulate inflammation and immune response based on inflammatory cascade were used to cure the multifactorial disease.[10] However, the risk of adverse events and treatment failure even with the most vital drugs (nonresponse) from a substantial proportion of patients has raised significant issues.[11] As the incidence and prevalence of this devastating disease are increasing worldwide,[2,12] there is an urgent

need for alternative methods to address the weaknesses of current therapies.

Fecal microbiota transplantation (FMT) has been paid attention as a novel treatment for a variety of gastrointestinal-related diseases, including inflammatory bowel disease and *Clostridioides difficile* infection.[13-16] Systematic reviews and meta-analyses demonstrated the effectiveness of FMT for the treatment of patients with UC.[11,17-19] However, FMT does not guarantee consistent success or responsiveness in treating inflammatory bowel disease,[13] which has been a significant concern for clinicians. In efforts to solve the issue, previous studies conducted randomized controlled trials. They showed that FMT using healthy donor materials is superior to that of placebo (autologous FMT), proposing the importance of donor microbiota in successful remission.[20,21] While those studies highlighted the donor microbial patterns might be strong indicators in FMT success, considerable variability in FMT-treated subjects remains, suggesting that certain endogenous factors in patients might regulate clinical outcomes after FMT. However, such factors in recipients associated with the post-FMT clinical outcomes have not been explored.

In this context, we hypothesized that the fecal microbial ecology of recipients might be as crucial as the donor microbial community. Hence, we aimed to investigate the fecal microbiota of patients who received FMT and construct a diagnosis model to predict the success of the treatment using a machine learning (ML) algorithm.

MATERIALS AND METHODS

1. Study cohort

This longitudinal prospective study included 10 patients with active UC who received FMT between 2016 and 2019 at Severance Hospital, Seoul, Korea. Participants were followed up for 58 days until August 2019. The following eligibility criteria were used: patients with active UC aged over 10 years with a total Mayo score ≥3 and an endoscopic subscore ≥1; patients who were not responsive to conventional treatment; patients who were dependent on systemic steroids and refused escalation to biologics; patients who were unable to continue medication because of adverse events. FMT was administered following a drug washout period of at least 2 months to exclude the possibility of the effect of the medicines on outcomes. Pregnant patients and patients with previous colonic surgery, current anticoagulant therapy, gastrointestinal infection, antibiotic therapy, and concomitant diseases were excluded. Physicians discussed the procedure's processes and potential adverse events with the patients or their guardians and obtained written informed consent. This study was approved by the Institutional Review Board of Severance Hospital (IRB number: 4-2017-0223) and is registered with ClinicalTrials.gov (NCT03399188).

2. Clinical and biochemical characteristics of UC patients

Partial Mayo (pMayo) scores were assessed at each outpatient visit, and patients were asked to indicate any adverse event related to FMT, such as fever, abdominal pain, and diarrhea.[18] Six blood and fecal samples were obtained before (T0, the day just before FMT) and after FMT up to

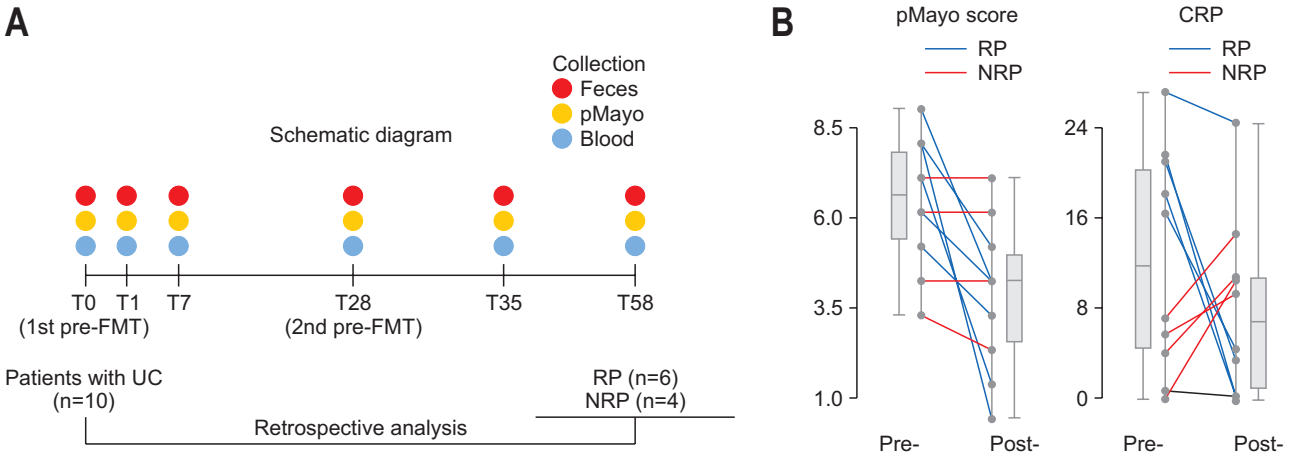

Fig. 1. Decrease in ulcerative colitis (UC) activity after fecal microbiota transplantation (FMT). (A) Schematic diagram of the FMT schedule, assessment of UC severity, and sampling at designated time points (T). (B) At T58 (58 days after the first FMT), patients with UC were classified into RP (decrease in both pMayo and CRP, n=6) and NRP (no change or an increase, n=4) groups.
RP, responder; NRP, nonresponder; CRP, C-reactive protein; pMayo, partial Mayo.

2 months (T1, T7, T28, T35, and T58, respectively). Numbers after T indicate the number of days since FMT. Levels of clinical features including leukocytes (white blood cell), hemoglobin, erythrocyte sedimentation rate, C-reactive protein (CRP), albumin, and fecal calprotectin (FC) were collected from the samples (Fig. 1A).

3. FMT protocol

Donor materials were obtained from healthy donors by GoldBiome (IRB P01-201803-31-009). A detailed history was taken, and an extensive laboratory workup was performed to minimize risks of disease transmission from the donor samples. Fecal specimens were prepared after immediate passage by the donors by homogenizing with normal saline.[22] The fecal homogenate was then passed through a 330-μm filter, and 50 g of the filtered stool in a final volume of approximately 250 mL was utterly sealed and stored at –80℃ until use. Donor materials were thawed in a water bath at 30℃ for 1 hour before use. There was a gap of 1 month between two FMTs performed via colonoscopy, and the patients were followed up at the outpatient clinic.

4. DNA extraction and 16S rRNA gene sequencing

Fecal swab samples were collected in Transwab tubes (Sigma, Dorset, UK) at the time mentioned earlier points and stored at –80℃ until DNA extraction. Total genomic DNA was extracted from all samples using the QIAamp PowerFecal DNA Isolation kits (Qiagen, Hilden, Germany) according to the instructions from the manufacturer. DNA concentration and quality were estimated using Qubit® Fluorometric Quantitation (Life Technologies, Carlsbad, CA, USA) and Bioanalyzer 2100 (Agilent, Satna Clara, CA, USA). DNA libraries were prepared using 515 F (5'-barcode-CGCTCTTCCGATCTGTGNCAGCMGCCGCGGTRA-3') and 907 R (5'-barcode-GTGCTCTTCCGATCCGYCWATTYHTTTRAGTTT-3') and sequenced on the Illumina MiSeq sequencing platform (Illumina, Inc., San Diego, CA, USA) using a MiSeq Reagent Kit v3 (Illumina, Inc.) following the protocol of the manufacturer. Sequencing was performed at the next-generation core facility of Kyungpook National University.

5. Bioinformatics analysis

The raw sequences were introduced to the Quantitative Insights Into Microbial Ecology 2 (QIIME2) v. 2020.8 software for sequence processing.[23] Briefly, the paired-end reads were attempted to be merged using DADA2.[24] However, the merging process leads to the exclusion of many samples due to the poor quality of reverse strands. Thus we opted to select forward ones containing the V4 region. Subsequently, the data were involved in procedures for denoising, chimera filtering, and low-quality filtering. Next, taxonomy information corresponding to each sequence was assigned to make it available to organize the abundance table at the specific taxonomic level on SILVA 138 database.[25] The reads classified as Eukaryota, Archaea, and Unassigned were further excluded for the following analysis to purely focus on the Bacterial community. Lastly, rarefaction steps were set to 9,787, corresponding to the least output quantity among total samples (Supplementary Fig. 1). None of the samples were removed in this step.

6. Statistical analysis

The primary analyses for this study were conducted under the R environment.[26] Alpha diversity indices, including Chao1, Shannon, and Simpson's index, were calculated at the genus level in QIIME2. Statistical differences between groups' alpha diversity indices were calculated using the Wilcoxon rank-sum test or analysis of variance, followed by the Tukey honest significant differences for multiple comparisons, when applicable. Measurement of beta diversity was computed using the Bray-Curtis dissimilarity metrics to investigate microbial distance between groups. Principal-coordinate analysis (PCoA) was conducted using ggplot2 and vegan packages,[27,28] and calculations for categorical differences in PCoA were performed using the Adonis test. To evaluate the bacterial origins that configure donor gut microbiota in post-FMT samples, we applied the SourceTracker. Here, microbial profiles from donors were defined as source communities, and the others were denoted as sink communities as described by Knights *et al.*[29] Bugbase, a computational tool, was further implemented to predict gut-derived bacterial phenotypic traits such as aerobic, anaerobic, facultatively anaerobic, Gram-positive, Gram-negative, biofilm formation, and mobile elements.[30] According to the previous research,[31] the L1 normalized (LASSO) logistic regression model was constructed to test the clinical usefulness of gut microbiota in predicting FMT outcomes. Samples were split into training and testing sets. Specifically, 5-fold cross-validation was applied to compose the sets with resampling number 10. Then, a prediction model was built and assessed using the area under the curve (AUC) of the receiver operator characteristic curve.[32] All steps containing data filtering, normalization, data splitting, model training, and prediction were implemented using the SIAMCAT package.[33]

RESULTS

1. Responsiveness after FMT

To determine whether FMT improves intestinal clini-

cal outcomes, 10 UC patients who completed their FMT schedules were included (Fig. 1A). After FMT, seven out of the 10 patients had at least 1 point decrease in their pMayo score from baseline after the second FMT (T58). Notably, six patients responded remarkably to the FMT, dramatically reducing pMayo and CRP scores (defined as RP group). In contrast, the pMayo and CRP scores of the other four patients remained the same or increased (defined as NRP group) (Fig. 1B). Baseline demographics, medication, the extent of disease, and pMayo score were well-balanced between the RP and NRP before FMT, except for budesonide (Table 1), used for treatment but not causing any improvement. In RP group, a significantly higher CRP level was noted compared to the NRP group (p=0.02). Paired t-test revealed a significant decrease of pMayo and CRP level in RP group at T58 (p=0.007 and p=0.047, respectively), and 617.50±796.36 μg/g has decreased in FC without statistical significance (p=0.094). In NRP, a significant increase in CRP level and 544.25±793.41 μg/g of increase in FC without significance at T58 compared to T0 was noted (p=0.016 and p=0.264, respectively). This result indicates that the intestinal tract of the recipients underwent dramatic clinical changes and then appeared to maintain long-term clinical outcomes from T58, 58 days after FMT. Our FMT schedule ameliorated the UC severity, with a 60% response rate and maintenance of long-term benefit.

2. Characterization of the bacterial community

Amplicon sequencing on the V4 region of bacterial 16S rRNA gene granted an overview and dynamics of the microbial ecosystem after FMT. Calculation and comparison of alpha diversity indices in fecal specimens showed no significant alterations in NRP following FMT compared to patients experiencing responses (data not shown). We then assessed the dissimilarity of microbial composition in each group applying the Bray-Curtis based PCoA. Results obtained from the PCoA differed from one another in their microbial composition of each group, with genera explaining the variation between samples (p=0.001, Adonis) (Fig. 2A). Upon closer examination of the Bray-Curtis distance, the NRP group after FMT (post-NRP) was the closest to donor microbiota than the RP group after FMT (post-RP) and pre-FMT, suggesting responsiveness in FMT might not contribute to the overall microbial shift (p<0.001, analysis of variance) (Fig. 2A). Interpreting the phenotypes within independent individuals is essential to understanding microbial change after FMT. To this end, we applied BugBase to surmise high-level phenotypes present in each group. While six phenotypes, including Aerobic, Anaerobic, Stress_Tolerant, Gram-Negative, Mobile Elements, and Facultaive_Anaerobe, were closely resembled as those of donor after FMT in both post-RP and NRP, two containing Forms_biofilm and Gram-Positive were relatively unstable (Fig. 2B). Interestingly, despite non-successful response after FMT, phenotypic recovery toward to donor in post-NRP was observed to be closer to that in post-RP (although statistical significance was not reached). The results concluded that the administration of fecal microbiota from donors conferred a similar phenotype to most recipients regardless of the clinical response from the phenotypic characteristics.

3. Source tracking

Bayesian source tracking analysis was computed on

Table 1. Demographics of the Study Cohort

Variable	RP (n=6)	NRP (n=4)	p-value
Age, yr	19.77±5.33	33.94±18.60	0.25
Male sex	4 (67)	3 (75)	>0.99
Body mass index, kg/m^2	20.31±2.03	22.02±6.69	>0.99
Disease duration, yr	4.17±3.72	7.27±6.20	0.13
Disease extent			
Pancolitis	2 (33)	2 (50)	>0.99
Left sided	3 (50)	1 (25)	0.57
Ascending and transverse colon	1 (17)	1 (25)	>0.99
Partial Mayo score	7.17±1.47	5.00±1.83	0.07
Medications			
Mesalazine	0	1 (25)	0.40
Systemic steroid	6 (100)	3 (75)	0.40
Azathioprine	3 (50)	3 (75)	0.20
Biologics	4 (67)	1 (25)	0.52
Budesonide	3 (50)	4 (100)	0.20

Data are presented as mean±SD or number (%).
RP, responder (remission); NRP, nonresponder (non-remission). Remission is defined as a decrease in partial Mayo score and C-reactive protein 1 month after the second fecal microbiota transplantation (T58).

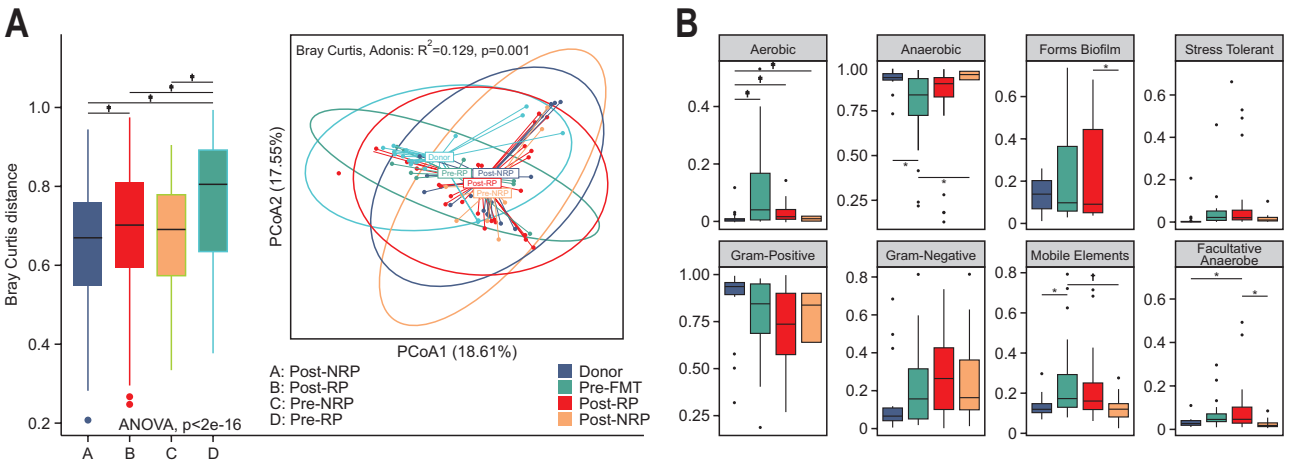

Fig. 2. Variation in gut microbial composition after fecal microbiota transplantation (FMT). (A) Projection displayed by principal coordinates analysis (PCoA) represented significantly differential gut microbiota composition (p=0.001). The compositional variances explained by each axis in PCoA dimensions were shown on the axes. Box plots next to the PCoA indicate a comparison of Bray-Curtis based distance across groups. The distance was calculated in comparison with donor microbiota. (B) Bar chart representation of BugBase analysis shows predicted phenotype. Samples denoted from each grouping were displayed and colored accordingly. Adonis and ANOVA test (Tukey honestly significant difference for multiple comparison) were used where applicable.
ANOVA, analysis of variance; RP, responder; NRP, nonresponder. *p<0.05, †p<0.01, ‡p<0.001.

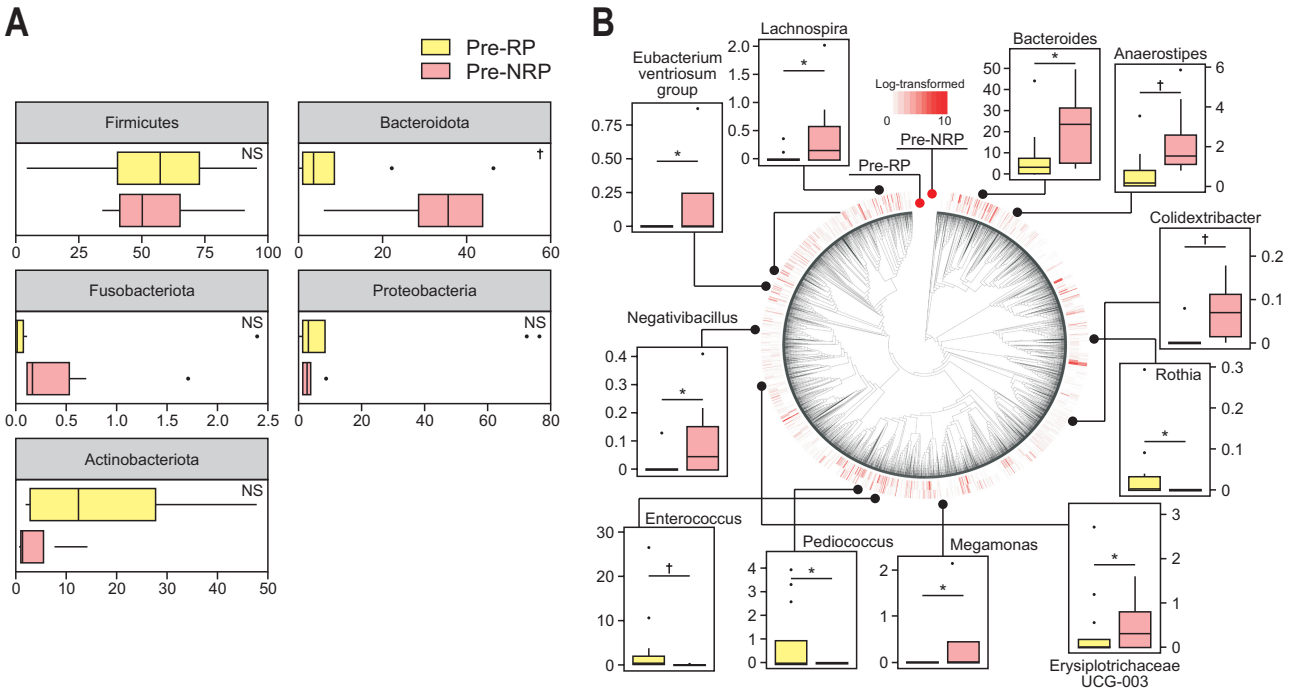

Fig. 3. Identification of indigenous taxa that shapes fecal microbiota transplantation (FMT)-mediated clinical outcomes. (A) Box plots of top five bacterial phyla abundances of each group before FMT (responder [RP] vs nonresponder [NRP]). (B) Phylogenetic tree generated on Amplicon sequence variant (ASV) level to grant overview of the microbial community. Heatmap of outer circle presents log-transformed abundances of individual ASVs. Box plots indicate bacterial genera with significant differences between the RP and NRP groups before FMT (pre-RP and pre-NRP).
NS, not significant. Wilcoxon rank-sum test was used to assess statistical significance, *p<0.05 and †p<0.01.

both post-PR and post-NPR samples to explore whether the engraftment rate of donor microbiota after FMT is a potential prognostic marker for FMT-mediated response. This source tracker allowed us to evaluate the proportion of bacteria from donor microbiota or "unknown" (untraceable sources from the training set) in the post-FMT samples. The donor microbial profiles were denoted as sources. Interestingly, we observed that the post-RP group

maintained about 44% of donor microbiota. The post-NRP group kept 52%, indicating that the degree of engraftment is not one of the barometers affecting FMT success (Supplementary Fig. 2).

4. Distinctive gut microbial profiles between RP and NRP

Since UC is an intestinal pathophysiological event, we hypothesized that gut environmental factors, such as gut microbes, significantly affect the clinical outcomes after FMT. Although no significant heterogeneity in alpha diversity indices was observed, Adonis analysis on beta diversity using the Bray-Curtis dissimilarity showed significant clustering between the RP and NRP groups before FMT (pre-RP and pre-NRP; p=0.009) (Supplementary Fig. 3). Four phyla (Actinobacteriota, Bacteroidota, Firmicutes, and Proteobacteria) made up approximately 90% of the total gut bacteria across the pre-RP and pre-NRP groups. Of the predominant phyla, only Bacteroidota was significantly enriched in the pre-NRP group (Fig. 3A). At the genus level, a total of 11 out of 398 genera were differentially abundant (p<0.04). While three genera, including *Enterococcus*, *Pediococcus*, and *Rothia*, were enriched in pre-RP, eight containing *Bacteroides*, *Lachnospira*, *Colidextribacter*, *Megamonas*, *Eubacterium ventriosum* group, *Negativibacillus*, *Anaerostipes*, and Erysipelotrichaceae UCG-003 were enriched in pre-NRP (Fig. 3B). This comparative analysis suggests a distinctive difference of microbial community might act as unique fingerprints to determine FMT outcome.

5. Construction of prediction model to predict successful reaction in FMT

We then investigated and constructed a ML model using LASSO logistic regression (L1-regression) to evaluate whether gut microbiota can be considered a prescreening tool to predict responsiveness in FMT. Three genera (*Enterococcus*, *Rothia*, and *Colidextribacter*) were selected as the essential features for constructing an ML model with a decent AUC of 0.844 after 5-fold cross-validation for detecting pre-RP (Fig. 4A and B). Notably, microbiota-based diagnostic power was much higher than clinical features containing white blood cell, hemoglobin, erythrocyte sedimentation rate, CRP, albumin, and FC (Fig. 4B), indicating that gut bacterial consortium can be better served as a noninvasive diagnostic tool to predict responsiveness in FMT. Since a randomized controlled trial study showed that the microbial ecology of the donor is highly associated with patients' therapeutic outcomes,[34] we built an additional prediction model using samples from donors based on the responsiveness of recipients. L1-regression model trained with optimal features resulted in an AUC value of 0.781, which was lower than that of 0.844 obtained from

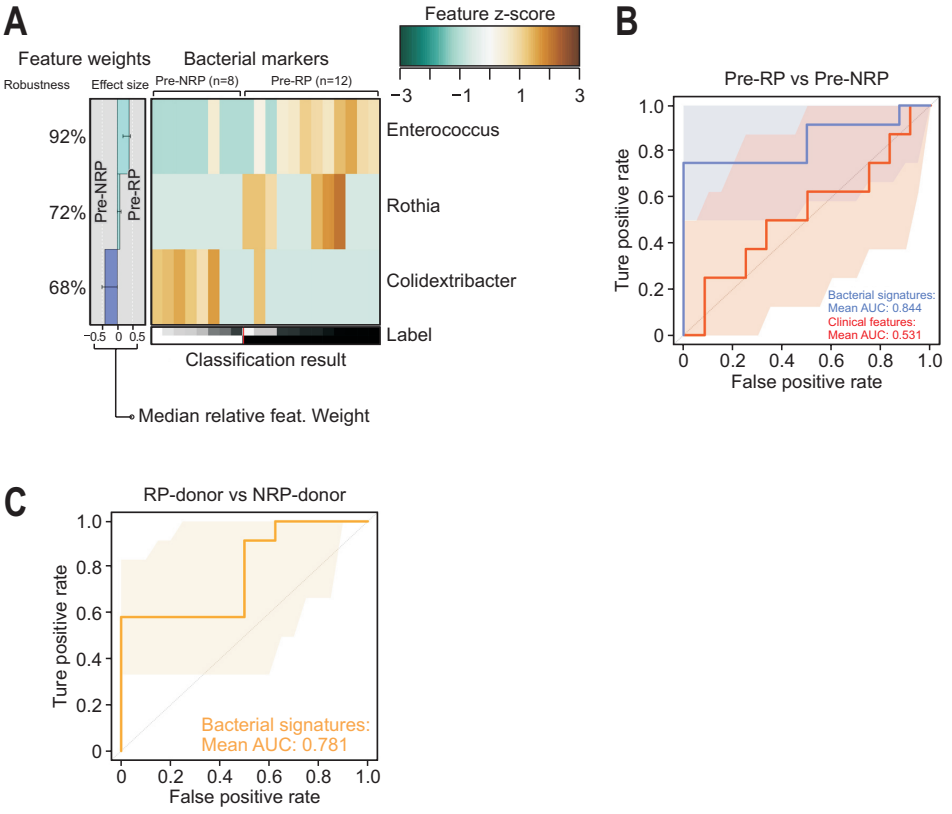

Fig. 4. Construction of a gut microbiota-derived diagnostic model to predict fecal microbiota transplantation (FMT) outcome. A machine learning model was trained to diagnose subjects into responder and nonresponder groups before FMT (pre-RP and pre-NRP). (A) The heatmap plot shows the z scores of selected features across samples, and the bar plots on the left indicate feature weight, including robustness, ordered by effect size. (B, C) The values of area under the curve (AUC) are given under the curve, with the 95% confidence interval shaded to indicate accuracy. Values of 0.844, 0.531, and 0.781 for cross-validation performance were obtained using patients' indigenous microbiota, clinical features, and donor microbiota, respectively.
RP, responder; NRP, nonresponder.

the model that utilized recipients' microbiota (Fig. 4C). Our findings collectively imply that indigenous microbiota of recipients might be one of the critical drivers to determine the effectiveness of FMT and their potential translation of fecal microbiota into clinical application.

DISCUSSION

This study assessed that gut environmental factors are potential prognostic markers for FMT-mediated clinical outcomes through ML. The existence and abundance of three bacterial genera (*Enterococcus*, *Rothia*, and *Colidextribacter*) might affect clinical outcomes in FMT. To our knowledge, this is the first study to analyze gut microbes from the perspective of prognostic factors for post-FMT clinical outcomes.

The significant clinical relevance of our study can be emphasized by less frequency of FMT and recipient factors for FMT effectiveness. Attempts have been made to increase response rates using multiple FMTs.[35] However, donor stools are not always readily available to the clinical field. Our FMT schedule (two infusions with a 1-month interval) resulted in a 60% response rate, comparable to results of other studies using multisession FMT per week.[17,20] To study the factors that affect FMT effectiveness, we focused on gut-derived factors of recipients, a fundamental concept of our study to consider the gut environment as a potential mechanism for personalized treatment for UC. The positive outcomes of FMT in terms of the gut microbiome were significantly associated with the species of Bacteroidota phylum.[36,37]

Furthermore, our findings revealed that a low level of Bacteroidota in the recipient's intestinal tract was required for successful colonization by gut microbes from donor stools. Previous studies showed that pretreatment with antibiotics (2-week combination therapy of amoxicillin, fosfomycin, and metronidazole) before FMT significantly reduced the abundance of Bacteroidota to almost 0%.[38] FMT treatment after 2 days the pretreatment replenished the altered gut microbial community with Bacteroidota, resulting in significant clinical response at 4 weeks after FMT.[38] On the other hand, a higher abundance of indigenous Bacteroidota in the pre-NPR seems to inhibit the beneficial effect of exogenous Bacteroidota. The antagonistic ability of the indigenous Bacteroidota has been demonstrated with commensal colonization factor to prevent super-colonization[39] of an antimicrobial toxin, called bacteroidetocins, to kill the same bacterial lineage.[40] According to the oxygen hypothesis, the intestinal lumen of UC patients is characterized by inflammatory immune cells, epithelial cells, and hemoglobin (carrying oxygen).[41-43] So, the antagonism of the Bacteroidota is likely associated with aerotolerance. Taken together, we suggest that the mechanism for FMT-mediated improvement is the recruitment of exogenous commensal Bacteroidota into the intestinal niches without competition.

Several studies have emerged the selection of donor material as one of the most critical factors in FMT outcome.[44,45] However, our results support previous studies that highlighted the success of autologous FMT; Kalla *et al.*[46] reported endoscopic and histological evidence of remission by conducting autologous FMT to a patient who suffered from treatment-resistant diversion colitis. Holster *et al.*[47] concluded that allogenic fecal sources obtained from healthy donors did not guarantee an advantage compared to the autologous source in their randomized controlled study. Likewise, Rossen *et al.*[48] also observed no differences in both clinical and endoscopic remission according to fecal sources in UC patients. A previous clinical trial conducted by Costello *et al.*[34] consistently showed a higher remission rate by using "healthy" donor material. Still, their successful records of autologous FMT also showed that donor material might not be the primary driver to determine and diagnose FMT outcome, which supports our hypothesis.

Phenotype prediction showed interesting aspects; after FMT, six out of eight phenotypes (Aerobic, Anaerobic, Stress_Tolerant, Gram-Negative, Mobile Elements, and Facultaive_Anaerobe) of recipient gut microbiota showed the similarities with the donor's. This result alludes that the engraftment of donor microbiota into UC patients drove the shift of the bacterial community in aspects of the physiological trait. However, three phenotypes (Biofilm formation, Gram-Negative, and Gram-Positive) showed relatively unstable trends. In detail, the abundance of biofilm forming bacteria showed consistence after FMT, and Gram-positive bacteria showed decreased abundance in post-NRP group. Deepening our insight into the results, the biofilm forming bacteria might acquire resistance in the environment shift after the FMT, showing no decline in abundance after FMT.

According to previous work, some intestinal bacteria tend to resist in stress conditions utilizing biofilm formation, supporting our theory that the endogenous biofilm forming bacteria in patients' gut maintained their abundance via utilizing their biofilm, which strengthened the ability to endure environmental alteration after FMT.[49] Even though the other five phenotype predictions indicated the moving trends of recipient's microbiome toward donor's, furthermore, we could not assert that the successful engraftment of microbiota guaranteed the prognosis of

FMT. For example, the proportion of facultatively anaerobic bacteria and bacteria with mobile elements which were pointed as a potential pathogen in previous study[50,51] were higher in RP group. Additionally, aerobic bacteria represented as a biomarker of colitis[52] was more common in RP compared to NRP. These results suggest the limitation of forecasting the prognosis of FMT solely on the degree of engraftment. At the same time, it emphasizes the plausibility of our theory that the endogenous bacterial community of recipients is critical in predicting the prognosis of FMT.

Among three genera assessed as the contributors in ML model, *Enterococcus* and *Rothia* showed a significant dominance in RP compared to NRP. Interestingly, several previous studies have reported the antimicrobial activity of those two genera. For example, *Enterococcus* has been known to suppress pathogenic bacteria by secreting antimicrobial metabolites[53] and provoking host immune system specifically targeting pathogen such as *Giardia intestinalis*.[54] Rosier *et al.*[55] demonstrated that *Rothia* possesses an enzyme converting nitrite into nitric oxide, which has a bactericidal power. Considering that human cells do not have such capability, the authors implied the capacity of *Rothia* in inhibiting gut pathogens. Those previous research suggest that the abilities inhibiting pathogenic gut microorganisms from those genera might contribute to the stabilized gut environment thus guaranteeing a good prognosis of FMT.

On the other hand, the other meaningful feature derived from our model, *Colidextribacter*, showed significant enrichment in NRP. In the study investigating an association of inflammation-related serum metabolites and depressive disorder, Bai *et al.*[56] reported that Firmicutes including *Colidextribacter* showed a significant correlation with the inflammation-related serum metabolites derived from gut microbes, implying that *Colidextribacter* might produce inflammatory metabolites thus to potentially deteriorate the prognosis of FMT. With the linkages of previous studies related to the contribution of three genera defined by ML in this study, our result manifests the importance of patient microbial communities in assessing the prognosis of FMT again. At the same time, the result suggests a plausible usage of ML in selecting the meaningful bacteria as the contributor of robust classifier and as the indicator of their biological state.

In light of the current study, we built an ML-based non-invasive tool and illustrated specific bacterial markers that may contribute to diagnosis for FMT success. To be mentioned, the ML-based prediction model successfully classified RP among patients before FMT, with achievement of 0.844 of AUC. Notably, utilizing indigenous microbiota of recipients showed higher predictive capability than other factors such as clinical features and donor microbiota. This result provides a new strategy towards personalized therapeutics in treating FMT, suggesting that evaluating recipients' microbial community should be recommended before selecting suitable patients and achieving better efficiency.

Being mainly bioinformatics-based analysis, we acknowledge the weakness associated with this study. First, the number of samples was too small to validate whether our prediction model was over- or under-estimated. Future studies in larger cohorts should be done to validate microbial markers suggested in this study. Second, our results provide only a potential association between gut microbiota and the outcome of FMT. Therefore, additional studies are required to unravel how gut-derived bacteria affect FMT outcomes in molecular mechanisms. Third, although the results of this study fulfilled the hypothesis that gut microbiota can affect the outcome of FMT, this does not mean causality. Thus, the generalization of findings from this study should be cautious. Fourth, the results of this study were derived from amplicon sequencing, which is mainly regarding prediction. To validate the result of the current study, shotgun metagenomics are required to identify bacterial markers at species level and metabolomics are also essential to answer biological and scientific questions related to the results. Lastly, we could not evaluate endoscopic response or remission after the second FMT during the study period due to the invasive nature of the colonoscopy, which could be another limitation of our study. Instead, we have followed up with pMayo score, CRP, and FC, which could be the clinical and surrogate marker of the disease activity. We could observe a dramatic decrease in FC and pMayo score and CRP in the RP group compared to the NRP group.

Notwithstanding these weaknesses, the ML-based predictive model in this study reflected that fecal microbiota of baseline in FMT was associated with therapeutic response. Furthermore, we identified three biomarkers regarding FMT responses. More importantly, the novelty of our study is to highlight the potent of bridging microbiome study with clinical diagnosis.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

Conceptualization and participants enrollment: J.H.S., H.K. Data collection: S.P., H.K. Data analysis: G.U.K., S.P., J.J.J. Data curation: G.U.K., S.P., Y.J., M.S.K., J.J.J. Writing - original draft: G.U.K., S.P., J.J.J. Writing - review & editing: G.U.K., D.W.L., S.L., H.K., J.H.S. Funding acquisition: H.K., J.H.S. All authors approved the final version of the manuscript.

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SUPPLEMENTARY MATERIALS

Supplementary materials can be accessed at https://doi.org/10.5009/gnl210369.

REFERENCES

1. Singh S, Murad MH, Fumery M, Dulai PS, Sandborn WJ. First- and second-line pharmacotherapies for patients with moderate to severely active ulcerative colitis: an updated network meta-analysis. Clin Gastroenterol Hepatol 2020;18:2179-2191.
2. Head KA, Jurenka JS. Inflammatory bowel disease Part 1: ulcerative colitis: pathophysiology and conventional and alternative treatment options. Altern Med Rev 2003;8:247-283.
3. Chan SS, Luben R, van Schaik F, et al. Carbohydrate intake in the etiology of Crohn's disease and ulcerative colitis. Inflamm Bowel Dis 2014;20:2013-2021.
4. Kuhnen A. Genetic and environmental considerations for inflammatory bowel disease. Surg Clin North Am 2019;99:1197-1207.
5. Cleynen I, González JR, Figueroa C, et al. Genetic factors conferring an increased susceptibility to develop Crohn's disease also influence disease phenotype: results from the IBDchip European Project. Gut 2013;62:1556-1565.
6. Lerner A. Aluminum is a potential environmental factor for Crohn's disease induction: extended hypothesis. Ann N Y Acad Sci 2007;1107:329-345.
7. Chiba M, Nakane K, Komatsu M. Westernized diet is the most ubiquitous environmental factor in inflammatory bowel disease. Perm J 2019;23:18-107.
8. Lopez J, Grinspan A. Fecal microbiota transplantation for inflammatory bowel disease. Gastroenterol Hepatol (N Y) 2016;12:374-379.
9. Mizoguchi E, Low D, Ezaki Y, Okada T. Recent updates on the basic mechanisms and pathogenesis of inflammatory bowel diseases in experimental animal models. Intest Res 2020;18:151-167.
10. Hibi T, Inoue N, Ogata H, Naganuma M. Introduction and overview: recent advances in the immunotherapy of inflammatory bowel disease. J Gastroenterol 2003;38 Suppl 15:36-42.
11. Paramsothy S, Nielsen S, Kamm MA, et al. Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. Gastroenterology 2019;156:1440-1454.
12. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet 2017;390:2769-2778.
13. Khanna S, Vazquez-Baeza Y, González A, et al. Changes in microbial ecology after fecal microbiota transplantation for recurrent C. difficile infection affected by underlying inflammatory bowel disease. Microbiome 2017;5:55.
14. Kelly CR, Kahn S, Kashyap P, et al. Update on fecal microbiota transplantation 2015: indications, methodologies, mechanisms, and outlook. Gastroenterology 2015;149:223-237.
15. Khanna S. Management of Clostridioides difficile infection in patients with inflammatory bowel disease. Intest Res 2021;19:265-274.
16. Sehgal K, Khanna S. Gut microbiome and checkpoint inhibitor colitis. Intest Res 2021;19:360-364.
17. Paramsothy S, Kamm MA, Kaakoush NO, et al. Multidonor

intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. Lancet 2017;389:1218-1228.
18. Paramsothy S, Paramsothy R, Rubin DT, et al. Faecal microbiota transplantation for inflammatory bowel disease: a systematic review and meta-analysis. J Crohns Colitis 2017;11:1180-1199.
19. Allegretti J, Eysenbach LM, El-Nachef N, Fischer M, Kelly C, Kassam Z. The current landscape and lessons from fecal microbiota transplantation for inflammatory bowel disease: past, present, and future. Inflamm Bowel Dis 2017;23:1710-1717.
20. Moayyedi P, Surette MG, Kim PT, et al. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. Gastroenterology 2015;149:102-109.
21. Costello SP, Waters O, Bryant RV, et al. Short duration, low intensity, pooled fecal microbiota transplantation induces remission in patients with mild-moderately active ulcerative colitis: a randomised controlled trial. Gastroenterology 2017;152:S198-S199.
22. Cammarota G, Ianiro G, Tilg H, et al. European consensus conference on faecal microbiota transplantation in clinical practice. Gut 2017;66:569-580.
23. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;37:852-857.
24. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13:581-583.
25. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 2013;41(Database issue):D590-D596.
26. R Core Team. R: A language and environment for statistical computing. R package version 2013:2-0. Vienna: The Comprehensive R Archive Network, 2013.
27. Wickham H. ggplot2. Wiley Interdiscip Rev Comput Stat 2011;3:180-185.
28. Oksanen J, Blanchet FG, Kindt R, et al. The vegan package: community ecology package. R package version. 2013. [Internet]. Vienna: The Comprehensive R Archive Network [cited 2021 Nov 23]. Available from: https://cran.r-project.org/web/packages/vegan/index.html.
29. Knights D, Kuczynski J, Charlson ES, et al. Bayesian community-wide culture-independent microbial source tracking. Nat Methods 2011;8:761-763.
30. Ward T, Larson J, Meulemans J, et al. BugBase predicts organism-level microbiome phenotypes. BioRxiv:133462 [Preprint]. 2017 [cited 2020 May 2]. Available from: https://doi.org/10.1101/133462.
31. Schmidt TS, Hayward MR, Coelho LP, et al. Extensive transmission of microbes along the gastrointestinal tract. Elife 2019;8:e42693.
32. Lobo JM, Jiménez-Valverde A, Real R. AUC: a misleading measure of the performance of predictive distribution models. Glob Ecol Biogeogr 2008;17:145-151.
33. Wirbel J, Zych K, Essex M, et al. Microbiome meta-analysis and cross-disease comparison enabled by the SIAMCAT machine learning toolbox. Genome Biol 2021;22:93.
34. Costello SP, Hughes PA, Waters O, et al. Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: a randomized clinical trial. JAMA 2019;321:156-164.
35. Siegmund B. Is intensity the solution for FMT in ulcerative colitis? Lancet 2017;389:1170-1172.
36. Ishikawa D, Sasaki T, Takahashi M, et al. The microbial composition of bacteroidetes species in ulcerative colitis is effectively improved by combination therapy with fecal microbiota transplantation and antibiotics. Inflamm Bowel Dis 2018;24:2590-2598.
37. Okahara K, Ishikawa D, Nomura K, et al. Matching between donors and ulcerative colitis patients is important for long-term maintenance after fecal microbiota transplantation. J Clin Med 2020;9:1650.
38. Ishikawa D, Sasaki T, Osada T, et al. Changes in intestinal microbiota following combination therapy with fecal microbial transplantation and antibiotics for ulcerative colitis. Inflamm Bowel Dis 2017;23:116-125.
39. Lee SM, Donaldson GP, Mikulski Z, Boyajian S, Ley K, Mazmanian SK. Bacterial colonization factors control specificity and stability of the gut microbiota. Nature 2013;501:426-429.
40. Coyne MJ, Béchon N, Matano LM, McEneany VL, Chatzidaki-Livanis M, Comstock LE. A family of anti-Bacteroidales peptide toxins wide-spread in the human gut microbiota. Nat Commun 2019;10:3460.
41. Rigottier-Gois L. Dysbiosis in inflammatory bowel diseases: the oxygen hypothesis. ISME J 2013;7:1256-1261.
42. Henson MA, Phalak P. Microbiota dysbiosis in inflammatory bowel diseases: in silico investigation of the oxygen hypothesis. BMC Syst Biol 2017;11:145.
43. Zhu H, Li YR. Oxidative stress and redox signaling mechanisms of inflammatory bowel disease: updated experimental and clinical evidence. Exp Biol Med (Maywood) 2012;237:474-480.
44. Basson AR, Zhou Y, Seo B, Rodriguez-Palacios A, Cominelli F. Autologous fecal microbiota transplantation for the treatment of inflammatory bowel disease. Transl Res 2020;226:1-11.
45. Wilson BC, Vatanen T, Cutfield WS, O'Sullivan JM. The super-donor phenomenon in fecal microbiota transplanta-

tion. Front Cell Infect Microbiol 2019;9:2.
46. Kalla R, Pitt M, Sharma A. The role of autologous fecal microbiota transplantation in diversion colitis: a case report. Inflamm Bowel Dis 2019;25:e29-e30.
47. Holster S, Lindqvist CM, Repsilber D, et al. The effect of allogenic versus autologous fecal microbiota transfer on symptoms, visceral perception and fecal and mucosal microbiota in irritable bowel syndrome: a randomized controlled study. Clin Transl Gastroenterol 2019;10:e00034.
48. Rossen NG, Fuentes S, van der Spek MJ, et al. Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. Gastroenterology 2015;149:110-118.
49. Srivastava A, Gupta J, Kumar S, Kumar A. Gut biofilm forming bacteria in inflammatory bowel disease. Microb Pathog 2017;112:5-14.
50. Stecher B, Hapfelmeier S, Müller C, Kremer M, Stallmach T, Hardt WD. Flagella and chemotaxis are required for efficient induction of Salmonella enterica serovar Typhimurium colitis in streptomycin-pretreated mice. Infect Immun 2004;72:4138-4150.
51. Pedraz L, Blanco-Cabra N, Torrents E. Gradual adaptation of facultative anaerobic pathogens to microaerobic and anaerobic conditions. FASEB J 2020;34:2912-2928.
52. Toiyama Y, Araki T, Yoshiyama S, Hiro J, Miki C, Kusunoki M. The expression patterns of Toll-like receptors in the ileal pouch mucosa of postoperative ulcerative colitis patients. Surg Today 2006;36:287-290.
53. Nami Y, Vaseghi Bakhshayesh R, Mohammadzadeh Jalaly H, Lotfi H, Eslami S, Hejazi MA. Probiotic properties of enterococcus isolated from artisanal dairy products. Front Microbiol 2019;10:300.
54. Benyacoub J, Pérez PF, Rochat F, et al. Enterococcus faecium SF68 enhances the immune response to Giardia intestinalis in mice. J Nutr 2005;135:1171-1176.
55. Rosier BT, Moya-Gonzalvez EM, Corell-Escuin P, Mira A. Isolation and characterization of nitrate-reducing bacteria as potential probiotics for oral and systemic health. Front Microbiol 2020;11:555465.
56. Bai S, Xie J, Bai H, Tian T, Zou T, Chen JJ. Gut microbiota-derived inflammation-related serum metabolites as potential biomarkers for major depressive disorder. J Inflamm Res 2021;14:3755-3766.