Intestinal factors associated with clinical outcomes after fecal microbiota transplantation in ulcerative colitis

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Intestinal factors associated with clinical outcomes after fecal microbiota transplantation in ulcerative colitis

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ABSTRACT

Intestinal factors associated with clinical outcomes after fecal microbiota transplantation in patients with ulcerative colitis

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Background and Aim

The efficacy of fecal microbiota transplantation (FMT) in the treatment of ulcerative colitis (UC) is inconsistent because of the lack of prognostic factors. Thus, we investigated potential prognostic markers for clinical outcomes of patients with UC after FMT.

Methods

We conducted a prospective study that included 10 patients with active UC who completed two FMTs at a 1-month interval. Intestinal and systemic markers were analyzed for prognostic factors based on clinical outcomes and temporal sequences of samples for 6 months after FMT. Gut microbes and their genes were analyzed using shotgun metagenomic sequencing, followed by analysis using the decision tree classifier method.

Results

At 1 month after the second FMT (T60), we regrouped the patients into remission (R) and non-remission (NR) groups based on their partial Mayo scores and analyzed their pre-FMT (T0) parameters to determine prognostic factors. Remission rate was 40%, and C-reactive protein (CRP) levels significantly decreased from T0 to T60. Moreover, we observed that intestinal factors were more discernible than CRP levels; they were characterized by lower abundance of phylum Bacteroidetes (R *vs.* NR at T0, $p<0.001$; T0 *vs.* T60 in R, $p<0.05$; abundance threshold, 17.8%) and higher copy numbers of genes encoding glycerol dehydrogenase (R *vs.* NR at T0, $p<0.01$; T0 *vs.* T60 in R, $p<0.05$; copy number threshold, 280.88) and mannosyl glycerate hydrolase (R *vs.* NR at T0, $p<0.05$; T0 *vs.* T60 in R, $p<0.05$; gene number threshold, 17.62) at T0.

Conclusions

FMT can be considered as an effective treatment option in patients with active UC, but the post-FMT clinical outcome may vary according to the intestinal environment of recipients. Gut microbes and their genes of the recipients are potential prognostic markers for post-FMT clinical outcome in patients with UC. Therefore, bacterial phyla composition, bacterial genes, and CRP should be evaluated before FMT for the selection of suitable patients to achieve better treatment efficiency.

Keywords: ulcerative colitis, fecal microbiota transplantation, gastrointestinal microbiome, prognosis

Intestinal factors associated with clinical outcomes after fecal microbiota transplantation in ulcerative colitis

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I. INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon characterized by periods of active disease and remission. It is a multifactorial disease caused by genetic and environmental factors, and disrupted homeostasis between the microbial environment and host immunity is regarded as the main cause of disease onset and progression. Therefore, most treatments for UC target inflammation or immune response. However, the risk of adverse events and loss of response due to long-term use of conventional treatments is a major issue; thus, alternative therapeutic treatments for UC are essential.

Fecal microbiota transplantation (FMT) is a promising intervention that works by modulating the gut microbial community, particularly for patients infected with *Clostridium difficile*; moreover, it is under clinical trials for diseases potentially caused by dysbiosis, such as UC.[1] Previous studies have reported improved efficacy of FMT based on several factors, namely donor stool processing,[2, 3] microbial composition of donor stools,[4] administration route,[5, 6] and demographic factors.[7] Although optimization of these parameters has successfully increased the overall efficacy of FMT, considerable variation in outcome of FMT in UC patients is still remaining,[2, 7] demonstrating that certain endogenous factors of patients regulate clinical outcomes after FMT. However, these factors have not been elucidated yet. In this study, we investigated potential prognostic

markers for post-FMT clinical outcomes of patients with UC by focusing on biochemical parameters of stool and blood samples and the gut environment, including gut microbes and their genes.

We elucidated that gut environmental factors are more discernible than systemic parameters for forecasting post-FMT clinical outcomes, and alteration of the gut environment before FMT will generate niches for colonization by exogenous microbes from donor stool samples. Our data will provide physicians and researchers with an initial direction for the personalized treatment of patients with UC.

II. MATERIALS AND METHODS

1. Study cohort

This longitudinal prospective study included 11 patients with active UC who received FMT between 2016 and 2019 at Severance Hospital, Seoul, Republic of Korea. The participants were followed up for 6 months until August 2019. The patients that fulfilled the following eligibility criteria were included in the study: patients with active UC aged ≥10 years with a total Mayo score >3 and an endoscopic subscore >2; patients not responsive to conventional treatment, dependent on systemic steroids, refused escalation to biologics, or 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 an effect of the medication on the outcome. Patients who were pregnant, had undergone colonic surgery, were on anticoagulant or antibiotic therapy, and had gastrointestinal infection and concomitant diseases were excluded. Clinicians discussed the processes of the study and potential adverse events of the procedure and obtained written informed consent from patients or their guardians. This study was approved by the Institutional Review Board (IRB 4-2017-0223) and registered at ClinicalTrials.gov (NCT03399188).

2. Clinical and biochemical characteristics of patients with UC

At each outpatient visit, body mass index and partial Mayo (pMayo) scores of the patients were assessed, and they were asked to indicate any adverse events related to FMT, such as

fever, abdominal pain, and diarrhea.[8] Blood and fecal samples were obtained from the patients before (T0) and after FMT up to 6 months (T60, T120, and T210, i.e., 1, 3, and 6 months after the second infusion, respectively) to examine the levels of white blood cells (WBC), hemoglobin (Hb), C-reactive protein (CRP), albumin, and fecal calprotectin (FC) and erythrocyte sedimentation rate (ESR) (Fig. 1).

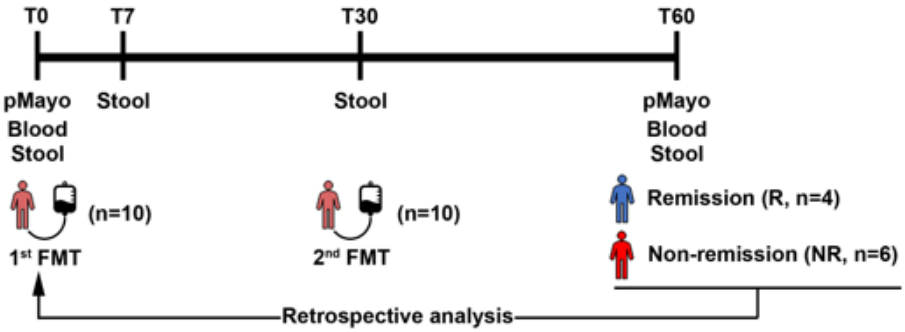


Figure 1. Schematic diagram of FMT schedule, assessment of UC severity, and sampling at designated time points (T). At T60 (60 days after the first FMT), patients with UC were divided into R (pMayo score <2, n=4) and NR (pMayo score ≥ 2, n=6) groups. FMT, fecal microbiota transplantation; NR, non-remission; R, remission; pMayo, partial Mayo; UC, ulcerative colitis.

3. FMT

Fecal samples were obtained from healthy donors using GoldBiome (IRB no.: P01-201803-31-009). Detailed history of each donor was obtained, and extensive laboratory tests were performed to minimize the risk of disease transmission from the donor samples. The fecal specimens were processed immediately after passage by the donors by homogenization with normal saline.[9] The fecal homogenate was passed through a 330-μm filter, and 50 g of the filtered stool in a final volume of approximately 250 mL with normal saline was completely sealed and stored at -80 °C until use. The processed stool samples were thawed in a water bath at 30 °C for 1 h prior to use. Two FMTs were performed at an interval of 1 month using the thawed samples via colonoscopy, and the patients were followed up at the outpatient clinic (Fig. 1).

4. DNA extraction and shotgun metagenomic sequencing

Fecal swab samples were collected in Transwab tubes (Sigma, Dorset, UK) at the aforementioned time points and stored at -80 ℃ until DNA extraction. Total genomic DNA was extracted from all samples using the QIAamp PowerFecal DNA Isolation Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentration and quality were estimated using a Qubit® Fluorometric Quantitation system (Life Technologies, Carlsbad, CA, USA) and a Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA). DNA libraries were prepared using the Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA, USA) and sequenced using the NovaSeq 6000 platform (Macrogen, Seoul, Korea).

5. Data processing and taxonomic analysis

The processing and taxonomic analysis of shotgun metagenomic data were performed as per the Microbiome Helper standard operating procedure.[10]

A. Preprocessing of sequencing data

Shotgun metagenomic reads obtained from the Illumina NovaSeq platform were initially quality-controlled and filtered for human DNA host contamination using Trimmomatic and Bowtie2 as a part of the KneadData pipeline (https://bitbucket.org/biobakery/kneaddata). Raw paired-end reads were obtained and preprocessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), Multiqc, and Trimmomatic (parameters: SLIDINGWINDOW:4:20 MINLEN:50). After the quality-filtering step, FastQC and Multiqc were used to analyze the features of the preprocessing libraries and verify the effectiveness of read trimming. The human (GRCh38/hg38) reference database and Phix genome were downloaded to eliminate sequence contamination using Bowtie2 (options: --very-sensitive --dovetail --phred33). Finally, the separated paired-end FASTQ files were concatenated from identical samples for further analysis.

B. Taxonomic profiling using MetaPhlAn2

Taxonomic distribution of high-quality microbial reads was analyzed using MetaPhlAn2

v2.6.0, which is a profiling algorithm that uses clade-specific marker genes. MetaPhlAn2 was executed using default parameters with a repository of clade-specific markers (ChocoPhlAn database) and whole-shotgun metagenome-based profiles were generated at the strain, species, genus, order, and phylum levels.

6. Functional profiling using HUMAnN2

For functional analysis, filtered reads were processed using HUMAnN2 v0.11.1[11] with the UniRef50 database to create functional annotations and define metabolic molecules of the microbial communities. We obtained a functional profile of complete metabolic pathways using metaCyc[12]. Moreover, two functional profiles were generated by grouping gene IDs into the KO[13] and MetaCyc-reaction functional categories using the humann2_regroup_table command.

7. Random forest classifiers

For both taxonomic and functional profiles, the random forest (RF) algorithm was used to identify and predict variables that significantly discriminated between remission (R) and non-remission (NR) groups. These analyses were performed using the R packages randomForest v4.6-14[14] and caret v6.0-86.[15]

We randomly split the data into training and test sets; the training set comprised 70% of the data and the test set comprised the remaining 30% of the data. The default parameters for the training dataset in the caret package were applied as follows: the number of trees was set to 500 (ntree=500) and the number of variables randomly sampled as candidates at each split was set to 7 (mtry=7). For the training dataset, we performed three repeated iterations of 10-fold cross-validation by applying the following parameters: method=repeatedcv, number=10, and repeats=3 in the trainControl command of the caret package. We separated the training dataset into 10 subsets using 10-fold cross-validation. The models were then trained on a “training dataset” using nine of the 10 subsets as the training set, and the last subset was used as the hold-out test set. The procedure was repeated three times, using a different fold as the hold-out test set for validation. We used the test dataset to assess final error rates after the series of models was tuned. The

"confusionMatrix" function was used to compare the recognized presence/absence of data from the hold-out test set (or final test set) with the predictions made by one-by-one models from the test set of predictor variables. Subsequently, the top-ranking UC-discriminatory variables that led to reasonably good fit were identified based on "rfcv" function in the randomForest package (https://cran.r-project.org/web/packages/randomForest/index.html). To clarify the diagnostic performance of the RF model (https://cran.r-project.org/web/packages/ROCR/index.html) in the training or final test set, the receiver operating characteristic (ROC) curve was plotted, and the area under the ROC curve (AUC) was calculated to summarize the performance of the RF model.

8. Classification and regression tree

To assess the degree of significance of the candidate parameters, the variables were ranked on a 0–100 scale according to their potential importance in binomial clinical outcomes.[16] To determine the split condition for binomial clinical outcomes, the candidate parameters were analyzed using classification and regression tree with Gini measures of goodness of fit, pruning misclassification error for stopping rule, and 10-fold cross-validation.

9. Statistical analysis

Variables that satisfied the following three conditions were defined as prognostic markers: significant cohort difference between R and NR groups at T0, significant change in parameters between T0 and T60, and significant separability using the ROC curve. Repeated-measures analysis of variance, followed by Duncan's post-hoc comparison, was used to test the significance of the cohort-related and temporal changes. All continuous variables are expressed as the mean ± standard deviation. Statistical analyses were performed using STATISTICA 7 (StatSoft, Tulsa, OK, USA) and Prism 5 (GraphPad Software, San Diego, CA, USA).

III. RESULTS

1. FMT improved severity of UC

To determine whether FMT improves intestinal clinical outcomes, 10 patients with UC who

completed their FMT schedules were included. One month after the second FMT (T60), 9 of the 10 patients had at least one point (or 25%) decrease in their pMayo score from that at the baseline (Fig. 2). Four patients achieved remarkable remission, with a pMayo score <2 (decreased from 85.7% to 100% from their baseline, defined as the R group), whereas the pMayo score of the other six patients remained at ≥2 at T60 (decreased from 25% to 75% from their baseline, defined as the NR group) (Fig. 1 and 2).

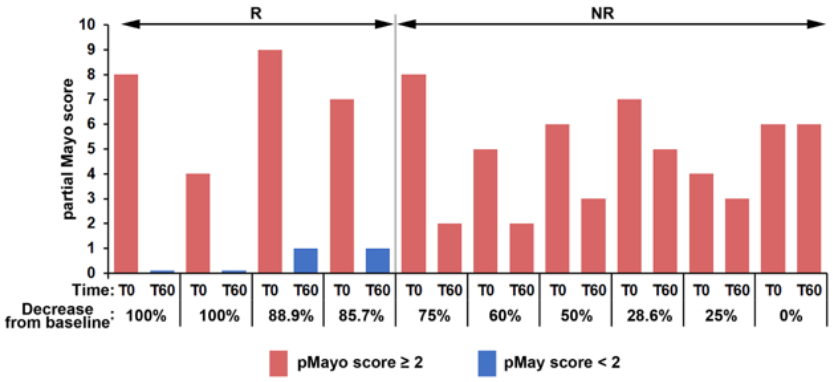


Figure 2. pMayo scores of the R and NR groups at T0 (before FMT) and T60. Percentage decrease in pMayo scores from those at the baseline was calculated as follows: the difference between T0 and T60 pMayo scores was divided by T0, and then multiplied by 100. FMT, fecal microbiota transplantation; NR, non-remission; pMayo, partial Mayo; R, remission; UC, ulcerative colitis.

Baseline demographics, medication, and UC type and severity were well balanced between the R and NR groups before FMT, except for budesonide (Table 1), which was used for treatment but did not exhibit any improvement.

During follow-up at 6 months after the second FMT (T210), the pMayo scores changed most significantly till T60 (p<0.0001 and p<0.001 from T0 to T60 in the R and NR groups, respectively) and then plateaued till T210. This result indicated that the intestinal tract of the recipients underwent dramatic clinical changes and exhibited long-term clinical outcomes from T60. Thus, our FMT schedule ameliorated UC severity, with a 40% remission rate and long-term benefits. Moreover, based on the pMayo score curve, we

focused on the data from T0 and T60 to investigate systemic and intestinal prognostic factors that could indicate post-FMT outcomes.

Table 1. Demographics of the study cohort

Variables (n)	**R (4)**	**NR (6)**	***p*-value**
Age (years)	16.82±3.60	31.18±15.02	0.07
Sex, male (%)	2 (50)	5 (83.3)	0.26
BMI (kg/m^2)	19.92±1.74	21.71±5.39	0.54
Disease duration (years)	4.34±4.43	6.12±5.31	0.60
Disease extent			0.15
Pancolitis % (n)	25.0 (1)	50.0 (3)	
Left sided % (n)	25.0 (1)	50.0 (3)	
Ascending and transverse colon % (n)	50.0 (2)	0.0 (0)	
Total Mayo score	9.75±2.22	8.00±2.00	0.23
Partial Mayo score	7.00±2.16	6.00±1.41	0.40
Medication			
Mesalazine % (n)	100.0 (4)	83.3 (5)	0.39
Systemic steroid % (n)	0.0 (0)	16.7 (1)	0.39
Azathioprine % (n)	75.0 (3)	16.7 (1)	0.07
Biologics % (n)	50.0 (2)	50.0 (2)	1.00
Budesonide % (n)	75.0 (3)	0.0 (0)	0.01

Remission was defined as a partial Mayo score <2 at 1 month after the second FMT (T60).

BMI, body mass index; FMT, fecal microbiota transplantation; NR, non-remission; R, remission.

2. Patients with UC that clustered into the R group had higher CRP levels before FMT

Although all patients underwent the same procedure, FMT gave rise to two different cohorts based on pMayo scores, namely R and NR groups (Fig. 1 and 2), revealing that the

heterogeneity in FMT effectiveness could be because of certain endogenous factors present in patients before FMT. To investigate whether these endogenous factors were strongly associated with post-FMT outcomes, we analyzed blood samples and fecal biochemical markers of both groups at T0 (Fig. 1). We observed that CRP level alone significantly explained the binomial outcomes after FMT. The R group exhibited a significantly higher CRP level than the NR group at T0, and the CRP level of the R group decreased significantly from T0 to T60 (Fig. 3). Although the difference was not statistically significant as per the ROC curve, CRP levels substantially distinguished the two clinical outcomes at T0 (p=0.055) (Fig. 3).

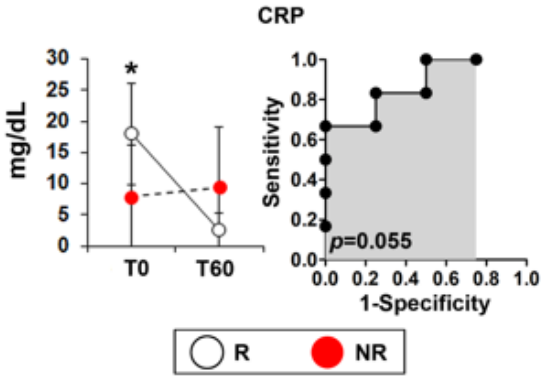


Figure 3. Concentration of CRP in blood is strongly associated with FMT-mediated clinical outcomes. Statistical significance across clinical outcomes and temporal points based on CRP level were tested using repeated-measures ANOVA, followed by Duncan's post-hoc comparison. Data are expressed as means ± standard deviation and test results between temporal points (_____, not significant and ____, p<0.05) and between groups (*, p<0.05). Area under the ROC curve was measured to test separability of CRP level for binomial clinical outcomes (p=0.055). ANOVA, analysis of variance; CRP, C-reactive protein; FMT, fecal microbiota transplantation; NR, non-remission; R, remission; ROC, receiver operating characteristic curve.

Other laboratory parameters (WBC, Hb, ESR, albumin, and FC) were not considered potential markers because the difference in their values were not statistically significant between groups, timepoints, or separability in the ROC curve. Our data revealed that conventional blood parameters were not strongly associated with FMT-mediated clinical outcomes, except for CRP level, which could be a potential prognostic marker for FMT-mediated remission.

3. Gut environmental factors were significantly associated with FMT-mediated

clinical outcomes

As UC is an intestinal pathophysiological event, we hypothesized that gut environmental factors, including gut microbes and their genes, could significantly affect clinical outcomes after FMT. Shotgun metagenomics analysis elucidated the bacterial community composition of stool samples from patients with UC at the phylum level. Four phyla (Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria) constituted approximately 99% of the total gut bacteria across the groups (Fig. 4a). Of the predominant phyla, only Bacteroidetes was significantly associated with the FMT-mediated clinical outcomes; compared with those of the NR group, stool samples from the R group had significantly less abundance of Bacteroidetes at T0, which significantly increased over 30 days after the last FMT (Fig. 4b). Moreover, the AUC demonstrated that the abundance of Bacteroidetes at T0 could significantly differentiate the two clinical outcomes. Other phyla were not considered potential prognostic markers because of the difference in their abundance across groups was not statistically significant.

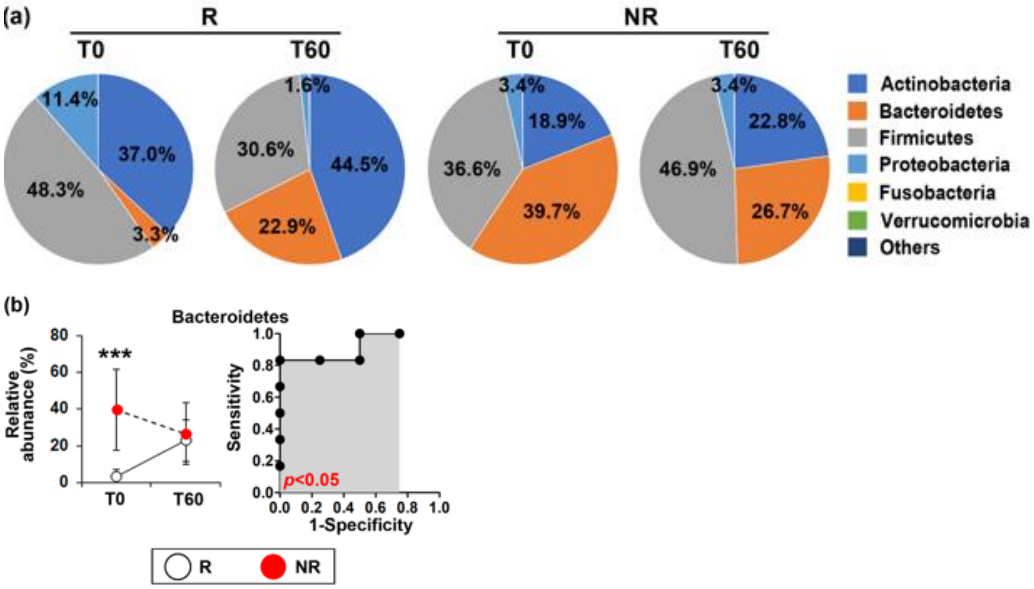


Figure 4. Phylum Bacteroidetes is a potential gut bacterial parameter predicting FMT-mediated clinical outcomes. (a) Phylum level analysis at T0 and T60 in each group. (b) Statistically

significant differences in the abundance of phylum Bacteroidetes between clinical outcomes and temporal points were analyzed using repeated-measures ANOVA, followed by Duncan's post-hoc comparison. Data are expressed as means ± standard deviation and test results between temporal points (_____, not significant and ____, $p<0.05$) and between groups (***, $p<0.001$). AUC was measured to test separability of abundance of Bacteroidetes for binomial clinical outcomes ($p<0.05$). ANOVA, analysis of variance; AUC, area under the receiver operating characteristic curve; CRP, C-reactive protein; FMT, fecal microbiota transplantation; ROC, receiver operating characteristic curve.

To elucidate the species or genes of gut bacteria that were significantly different between the R and NR groups, 410 bacterial species and 7,101 genes were analyzed using RF with cross-validation error and a minimal number of top-ranking species or genes. The RF algorithm estimated 13 species and seven genes as the top predictors to differentiate between the R and NR groups (Fig. 5a). Of the 20 candidates, three bacterial genes, namely K00005: glycerol dehydrogenase; K01223: 6-phospho-beta-glucosidase; and K15524: mannosyl glycerate hydrolase, were significantly associated with FMT-mediated clinical outcomes.

Compared with that in the NR group, the copy numbers of these three genes were detected significantly higher in the R group at T0, followed by a decrease in their copy numbers over 30 days after the last FMT (Fig. 5b). The separability test revealed that the copy number of genes encoding glycerol dehydrogenase and mannosyl glycerate hydrolase at T0 significantly discriminated between the R and NR groups (Fig. 5b). At T0, the gene encoding 6-phospho-beta-glucosidase substantially differentiated the clinical outcomes, but the difference was not statistically significant ($p=0.055$) (Fig. 5b). Other bacterial species and genes were not considered potential markers because of the difference in their copy numbers and levels, respectively, were not statistically significant. Thus, the abundance of phylum Bacteroidetes and the expression of genes encoding glycerol dehydrogenase, 6-phospho-beta-glucosidase, and mannosyl glycerate hydrolase at T0 are candidate markers that may predict post-FMT clinical outcomes of patients with UC.

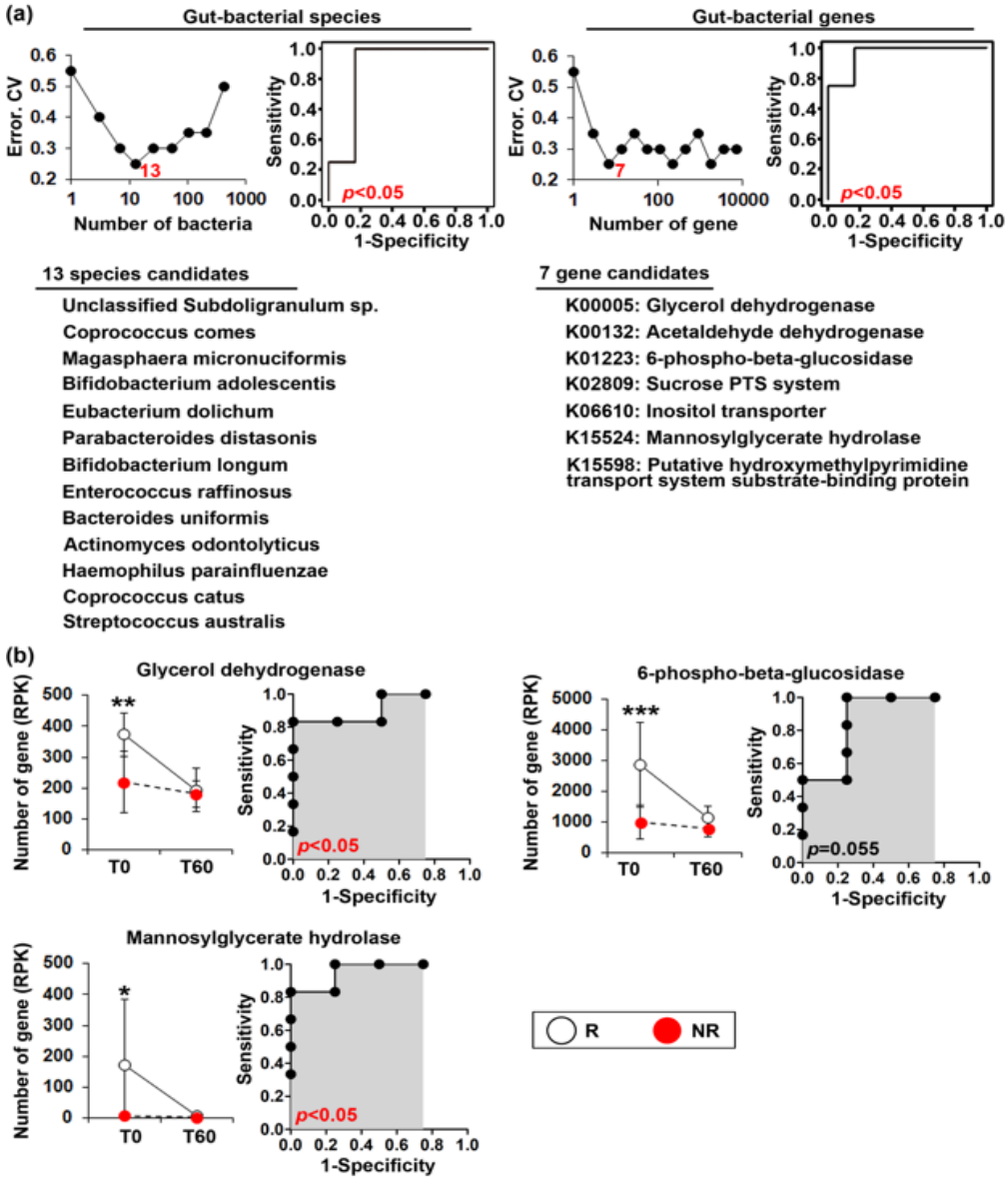


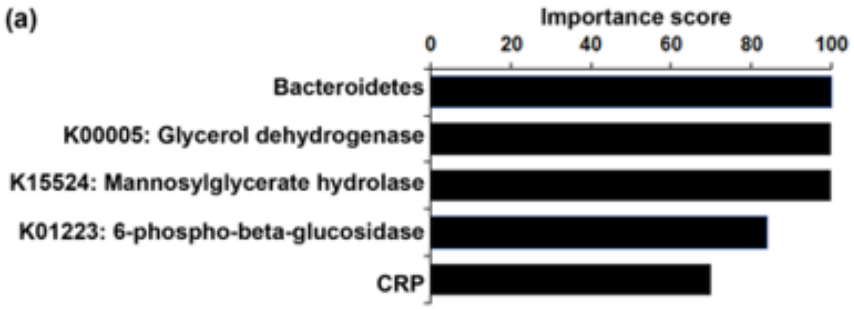
Figure 5. Glycerol dehydrogenase, 6-phospho-beta-glucosidase, and mannosyl glycerate hydrolase genes significantly explain FMT-mediated clinical outcomes. (a) Candidate parameters from bacterial species or genes that differentiated the clinical outcomes were selected by the lowest cross-validation error and minimal number of top-ranking candidates using the RF algorithm: 25% cross-validation error on 13 species and 7 genes. Area under the ROC curve was

measured to test the separability of bacterial species or genes for binomial clinical outcomes (p<0.05). (b) Statistical analyses of copy number of K00005: glycerol dehydrogenase, K01223: 6-phospho-beta-glucosidase, and K15524: mannosyl glycerate hydrolase with respect to clinical outcomes and temporal points were performed using repeated-measures ANOVA, followed by Duncan's post-hoc comparison. Data are expressed as means ± standard deviation and test results between temporal points (_____, not significant and ____, p<0.05) and between groups (*, p<0.05; **, p<0.01; ***, p<0.001). Area under the ROC curve was measured to test separability of each gene for binomial clinical outcomes (p=0.055; p<0.05).

ANOVA, analysis of variance; FMT, fecal microbiota transplantation; RF, random forest; ROC, receiver operating characteristic curve.

4. Abundance of phylum Bacteroidetes and levels of glycerol dehydrogenase, and mannosyl glycerate hydrolase at T0 predicted post-FMT clinical outcome of patients with UC

To determine the most discernible factors for FMT-mediated clinical outcomes, significant blood and gut candidate markers (CRP, Bacteroidetes, glycerol dehydrogenase, 6-phospho-beta-glucosidase, and mannosyl glycerate hydrolase genes) were subjected to classification and regression tree analyses. Based on the relative importance of each candidate, intestinal factors, namely Bacteroidetes, glycerol dehydrogenase, and mannosyl glycerate hydrolase, were found to be the most significant decision variables for FMT-mediated clinical outcomes (Fig 6a); only one patient was misclassified by each variable (Fig. 6).



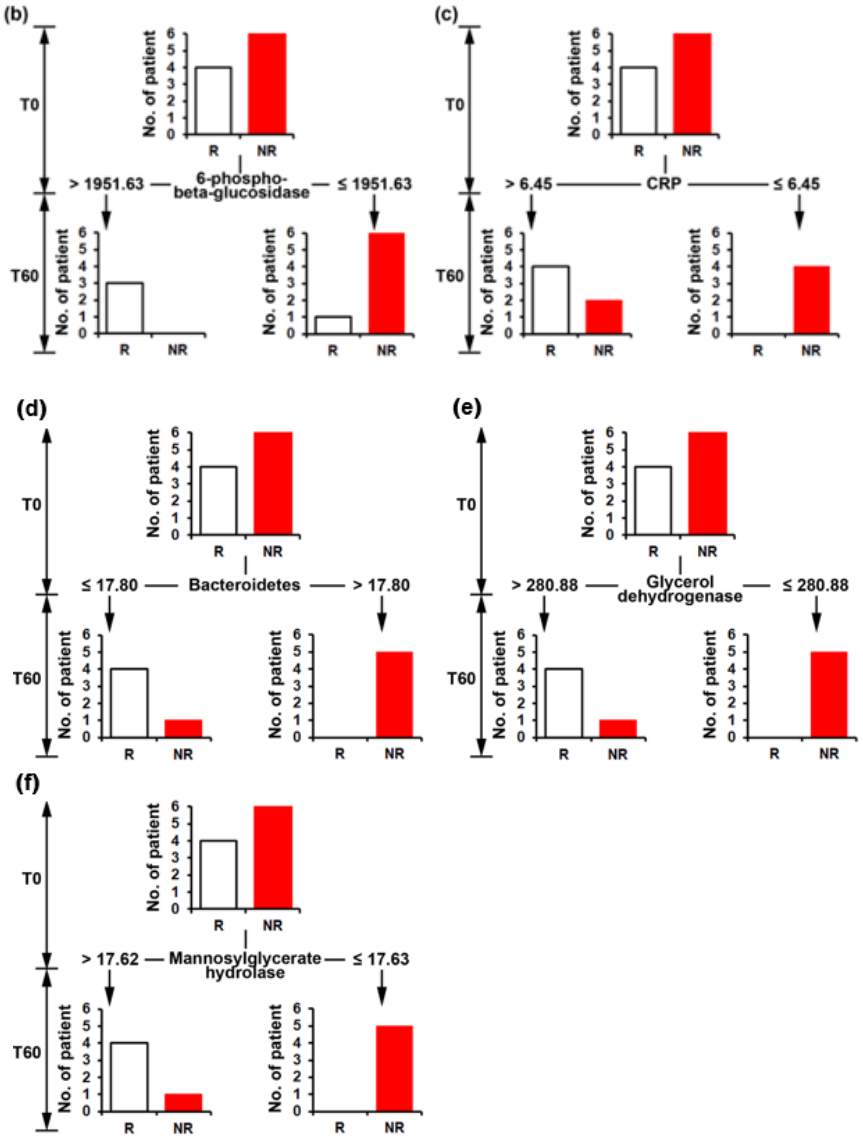


Figure 6. Bacteroidetes phylum and genes encoding glycerol dehydrogenase and mannosyl glycerate hydrolase are significant prognostic markers for clinical outcomes of patients with UC after FMT. (a) Importance scores from classification and regression tree analyses. Classification and regression tree analyses were used to calculate split conditions of (b) CRP level (c) 6-phospho-betaglucosidase gene expression, (d abundance of phylum Bacteroidetes, (e) number of glycerol dehydrogenase and (f) mannosyl glycerate hydrolase genes at T0 to forecast the FMT-

mediated clinical outcomes at T60. CRP, C-reactive protein; FMT, fecal microbiota transplantation.

According to split conditions in the decision trees, low abundance of Bacteroidetes at T0 was significantly associated with improved clinical phenotype; the prognosis of patients with less than or equal to 17.8% proportion of Bacteroidetes in stool samples at T0 was FMT-remissive UC (Fig. 6d), indicating that an intestinal tract populated with a low number of indigenous Bacteroidetes is favorable for FMT. However, a high number of genes at T0 also led to amelioration of UC phenotypes; thus, the prognosis of patients with more than 280.88 reads per kilobase of glycerol dehydrogenase gene (Fig. 6e) or 17.62 reads per kilobase of mannosyl glycerate hydrolase gene in stool samples at T0 (Fig. 6f) was FMT-remissive UC. This indicates that an intestinal tract with higher copy numbers of glycerol dehydrogenase and mannosyl glycerate hydrolase genes makes the recipients more responsive to FMT. In a meta-analysis, lower abundance of *Bacteroides* species was significantly associated with gut inflammation.[17] Moreover, dihydroxyacetone produced from glycerol catalyzed by glycerol dehydrogenase and compatible solutes, such as mannosyl glycerate, causes mucosal inflammation[18-20] and exhibits anti-inflammatory properties,[21] respectively. Thus, FMT could be more effective in UC patients with a highly inflamed intestinal tract. Our data suggest that low abundance of Bacteroidetes and higher copy numbers of glycerol dehydrogenase and mannosyl glycerate hydrolase genes are barometers of gut inflammation, and they lead to higher effectiveness of FMT.

IV. DISCUSSION

We suggest that gut environmental factors are potential prognostic markers of FMT-mediated clinical outcomes of patients with UC. Low abundance of *Bacteroides* and high levels of glycerol dehydrogenase and mannosyl glycerate hydrolase genes enable the colonization of exogenous gut microbes from donor stools. To the best of our knowledge, this is the first study to analyze gut microbes and genes from the perspective of prognostic factors for post-FMT clinical outcomes of patients with UC.

The significant clinical relevance of our study can be emphasized by the lower frequency of FMT, *i.e.*, only twice, and the recipient factors for FMT effectiveness. Scientists have attempted to increase remission rates by performing multiple FMTs.[22] However, donor stool samples are not always readily available in the clinical field. Our FMT schedule (two infusions with a 1-month interval) resulted in a 40% remission rate, which is comparable to the results of other studies using multisession FMT per week.[23, 24] To study the factors that affect the efficacy of FMT, we focused on gut-derived factors of the recipients, which is the key concept of our study, and considered the gut environment as a potential strategy for personalized treatment of UC.

The effect of FMT on UC phenotypes has been investigated from the perspective of the gut microbiome and is significantly associated with the members of phylum Bacteroidetes.[7, 25] Our findings revealed that low abundance of Bacteroidetes in the intestinal tract of the recipient is required for successful colonization of gut microbes from donor stool samples. Previous studies reported that pre-treatment with antibiotics (combination therapy of amoxicillin, fosfomycin, and metronidazole for 2 weeks) before FMT significantly decreased the abundance of Bacteroidetes to approximately 0%.[26] FMT performed 2 days after the pre-treatment replenished the altered gut microbial community with Bacteroidetes, resulting in significant clinical remission at 4 weeks after FMT.[26] Thus, we suggest that the mechanism for FMT-mediated improvement is the recruitment of exogenous commensal Bacteroidetes into the intestinal niches without competition.

On the other hand, a higher abundance of indigenous Bacteroidetes in the NR group inhibited the beneficial effects of exogenous Bacteroidetes. The antagonistic ability of indigenous Bacteroidetes has been demonstrated as per the commensal colonization factor (*ccf*) to prevent supercolonization of the bacteria[27] and anti-microbial toxins, called bacteroidetocins, to kill the same bacterial lineage.[28] According to the oxygen hypothesis, the intestinal lumen of patients with UC is characterized by the presence of inflammatory immune cells, epithelial cells, and hemoglobin (carrying oxygen);[29-31] thus, the antagonism exhibited by the phylum Bacteroidetes is most likely associated with aerotolerance. Some

clinical isolates of *Bacteroides* species exhibit oxygen-enabled (*oxe*) polymorphisms; thus, they can survive under microaerobic conditions[32] and induce superoxide dismutase that is resistant in oxygenated tissues.[33] However, the link between aerotolerance and antagonistic capacity of *Bacteroides* species is unexplored.

Two bacterial genes, i.e., those encoding glycerol dehydrogenase and mannosyl glycerate hydrolase, were selected as the intestinal prognostic candidates. Glycerol dehydrogenase is a catalytic enzyme that produces dihydroxyacetone from glycerol. Dihydroxyacetone modulates epithelial function and induces apoptosis; dihydroxyacetone-treated primary human tracheobronchial epithelial cells significantly decrease cilia motility, mucin secretion, and release of matrix metalloproteinase-10 and -13 *in vitro*.[18] It also induces apoptosis through cell cycle arrest and DNA damage in keratinocyte[19] and melanoma[20] cell lines.

Moreover, increased osmolarity in the colon has been characterized as a pathogenic event of inflammatory bowel disease (IBD). The extent of fecal osmotic gap has been demonstrated to be closely related to clinical classification in patients with IBD.[34] Under osmotic stress, microorganisms produce compatible solutes that act as osmolytes to protect hosts.[35] Interestingly, bacteria-derived osmolytes ectoine and 5α-hydroxyectoine ameliorate experimental IBD, characterized by an improved histological phenotype and lower myeloperoxidase, TNF-α, and IL-1β levels in colon-tissue homogenates of rats treated with 2,4,6-trinitrobenzenesulfonic acid.[21] Mannosyl glycerate hydrolase produces mannose and glycerate from mannosyl glycerate, which is a compatible solute produced by bacteria under osmotic stress. However, its preventive or protective role in gastrointestinal pathogenesis has not been investigated. We hypothesized that the elevated mannosyl glycerate hydrolase gene expression could degrade mannosyl glycerate molecules, leading to failed osmoadaptation of the gut microbial community and induction of anti-inflammatory molecules in patients with UC. The link between gut-derived osmolytes, including mannosyl glycerate, and UC phenotypes before and after FMT needs to be elucidated.

Since the trend of copy numbers in bacterial genes is the same as that of CRP concentration over time, the intestines of patients in the R group were found to be more inflamed than those of patients in the NR group. Therefore, further studies should be performed to validate the effect of the inflammatory gut environment on post-FMT clinical outcomes; stool samples from new patients with UC should be quantitatively characterized using primer sets targeting intestinal inflammation, including abundance of Bacteroidetes and expression of glycerol dehydrogenase and mannosylglycerate hydrolase genes, and the levels of the inflammatory molecules before FMT should be analyzed to understand their ability to predict clinical outcomes after FMT. Thus, our study will help physicians in selecting patients suitable for FMT treatment and provide a background for personalized treatment of UC (Fig. 7).

V. CONCLUSIONS

We elucidated that FMT can be considered as an effective treatment option in patients with active UC, with the remission rate of 40%. Gut microbes and their genes of the recipients are potential prognostic markers for post-FMT clinical outcome in patients with UC. Therefore, bacterial phyla composition and bacterial genes should be evaluated before FMT for patient selection and better efficiency of the treatment.

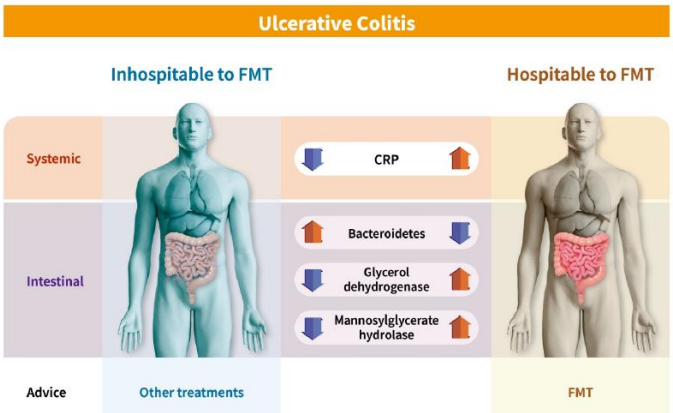


Figure 7. Schematic presentation of treatments tailored according to gut environment of patients with UC. UC, ulcerative colitis.

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ABSTRACT(IN KOREAN)

궤양성대장염에서 분변미생물이식술의 결과에 영향을 미치는 요인 분석

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궤양성대장염에서 분변미생물이식술의 효과는 상이하며, 이는 결과에 영향을 미치는 요인에 대한 이해의 부족에서 기인한다. 따라서 본 연구에서는 분변미생물이식술의 결과에 영향을 미치는 요인을 평가하고자 한다.

10명의 활동성 궤양성대장염 환자에서 1개월 간격으로 두 차례 분변미생물이식술을 시행하였다. 분변미생물이식술 전과 후의 임상적인 지표와 혈액학적인 지표, 대변 칼프로텍틴 등의 변화를 분석하였으며, 장내미생물과 유전정보의 발현을 샷건 메타지노믹스 분석과 의사결정나무 분석법으로 분석하였다.

분변미생물이식술 한 달 경과 후(T60) 부분 메이요 점수를 기반으로 하여, 환자들을 관해군(R) 과 비관해군(NR)로 분류하였고, 각 군에서 분변미생물이식술 전(T0)에 차이를 보이는 요인을 분석하였다.

분변미생물이식술 후 관해율은 전체의 40%였고, T0와 T60 사이에 C반응단백(C-reactive protein, CRP)이 감소하였다. 관해군에서 박테로이데테스문의 상대적 수(relative abundance)는 T0에서 비관해군에 비해 유의미하게 적었으며, T60에서 T0에 비해 유의미학 수적 증가를 보였다. (R *vs.* NR at T0, $p<0.001$; T0 *vs.* T60 in R, $p<0.05$; abundance threshold, 17.8%) 유전 정보의 발현에서도 유의미한 차이를 보였으며, T0에 glycerol dehydrogenase (R *vs.* NR at T0, $p<0.01$; T0 *vs.* T60 in R, $p<0.05$; gene number threshold, 280.88) 와 mannosylglycerate hydrolase (R *vs.* NR at T0, $p<0.05$; T0 *vs.* T60 in R, $p<0.05$; gene number threshold, 17.62) 의 발현이 관해군에서 유의미하게 높았고, T60에서 T0에 비해 유의미하게 감소하였다.

장내미생물과 유전정보는 분변미생물이식술의 결과에 영향을 미치는 요인으로

분석되었다. 분변미생물이식술 전에 박테리아의 조성과 장내 염증 정도를 평가하여, 이식술을 시행할 환자군을 분별한다면 그 효과가 더 좋을 것으로 기대한다.

핵심되는 말 : 궤양성대장염, 분변미생물이식술, 장내미생물, 예후

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