Fecal microbiota transplantation brings about bacterial strain displacement in patients with inflammatory bowel diseases

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46 **ABSTRACT**

47 Fecal microbiota transplantation (FMT), which is thought to have the potential to
 48 correct dysbiosis of gut microbiota, has recently been used to treat inflammatory bowel
 49 disease (IBD). To elucidate the extent and principles of microbiota engraftment in IBD
 50 patients after FMT treatment, we conducted an interventional prospective cohort study.
 51 The cohort included two categories of patients: (1) patients with moderate to severe
 52 Crohn's disease (CD) (Harvey-Bradshaw Index ≥ 7 , n = 11, and (2) patients with
 53 ulcerative colitis (UC) (Montreal classification, S2 and S3, n = 4). All patients were
 54 treated with a single FMT (via mid-gut, from healthy donors) and follow-up visits were
 55 performed at baseline, 3 days, one week, and one month after FMT (missing time
 56 points included). At each follow-up time point, fecal samples of the participants were
 57 collected along with their clinical metadata. For comparative analysis, 10 fecal samples
 58 from 10 healthy people were included to represent the diversity level of normal gut
 59 microbiota. Additionally, the metagenomic data of 25 fecal samples from 5 individuals
 60 with metabolic syndrome who underwent autologous FMT treatment were downloaded
 61 from a previous published paper to represent natural microbiota shifts during FMT. All
 62 fecal samples underwent shotgun metagenomic sequencing.

63 We found that 3 days after FMT, 11 out of 15 recipients were in remission (3 out of 4
 64 UC recipients; 8 out of 11 CD recipients). Generally, bacterial colonization was
 65 observed to be lower in CD recipients than in UC recipients at both species and strain
 66 levels. Furthermore, across species, different strains displayed disease-specific

67 displacement advantages under two-disease status. Finally, most post-FMT species (>
68 80%) could be properly predicted (AUC > 85%) using a random forest classification
69 model, with the gut microbiota composition and clinical parameters of pre-FMT
70 recipients acting as the most contributive factors for prediction accuracy.

71 **KEYWORDS** : shotgun metagenomic sequencing; Inflammatory bowel disease; fecal
72 microbiota transplantation; strain level identification; strain displacement; random
73 forest

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91 INTRODUCTION

92 Inflammatory bowel disease (IBD) is a chronic inflammatory disease characterized by
 93 chronic immune-mediated intestinal inflammation, and consists mainly of Crohn's
 94 disease (CD) and ulcerative colitis (UC). The etiology of IBD has been proposed to be
 95 multifactorial, involving a dysregulated immune response to environmental factors in a
 96 genetically susceptible individual (1). Interestingly, given the evidence accumulated in
 97 recent years, the gut microbiota is now recognized for playing an important role in IBD.
 98 Dysbiosis is a decrease in gut microbial diversity owing to a shift in the balance
 99 between commensal and potentially pathogenic microorganisms of the gut microbial
 100 ecosystem, and has long been characterized as a trait of IBD patients (2,3).
 101 Fecal microbiota transplantation (FMT) aims to modify the intestinal microbiota
 102 composition and function of the recipients by transferring donor fecal suspension into
 103 the gastrointestinal tract of a recipient, and has become a promising method for
 104 manipulating the gut microbiota. Its successful application for the treatment of
 105 *Clostridium difficile* infection has inspired people to apply it to inflammatory bowel
 106 disease patients (4,5,6,7,8,9). However, this application is still in its early stages.
 107 According to a recent systematic review and meta-analysis, after minimizing
 108 publication bias, IBD patients who received FMT had a remission rate of only 36.2%:
 109 22% for UC and 60.5% for CD (10). Moreover, there is a lack of research regarding
 110 the efficiency and principles of FMT in treating IBD.

111 Clinical research to date has focused more on UC (7,8,9), and there has been
 112 insufficient research on the effects of FMT on CD patients, with only a few case
 113 reports and small-scale case series reported (11,12,13,14). In addition, the majority of
 114 studies conducted so far to investigate the role FMT plays in treating IBD have used
 115 16S rRNA sequencing, which has limited resolution on taxonomic and functional
 116 classification of sequences. Contradictory results were often observed at species-level
 117 resolution, making it hard to determine the exact role of different bacterial agents. For
 118 instance, the abundance of *Faecalibacterium prausnitzii* was found to decrease in one
 119 study and to increase in another (15,16). Thus, it is necessary to be able to appreciate
 120 the whole composition of gut microbiota at a strain level. Strain level variants within
 121 microbial species are crucial in determining their functional capacities within the
 122 human microbiome, such as interaction with host tissues (17), modulation of immune
 123 homeostasis (18), and xenobiotic metabolism (19). Shotgun metagenomic sequencing
 124 with the ability to target all DNA material in a sample can give a base pair level
 125 resolution of the genome that makes single nucleotide analysis possible. Additionally,
 126 promising machine learning methods could enable the establishment of predictive
 127 models to predict the microbiota composition of post-FMT recipients. Recently, S.
 128 Smillie et al. constructed a machine learning model to predict the species profile of
 129 post-FMT recipients for 18 *C. difficile* patients and found that bacterial abundance and
 130 phylogeny were the strongest determinants of engraftment (20). In our study, we utilize
 131 a random forest model to predict the mOTUs profile of IBD recipient 3 days after FMT
 132 and identified the variables that contribute most to model prediction accuracy.

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135 **MATERIALS AND METHODS**

136 **Patient recruitment and sample collection**

137 Patients aged 19–64 years were recruited from the Second Affiliated Hospital of
 138 Nanjing Medical University, China from 2012 to 2014. The dataset was composed of
 139 10 fecal samples from 10 healthy people, among which 6 were FMT donors, and 34
 140 fecal samples from 15 IBD patients. Donor fecal samples were collected prior to FMT.
 141 Stool samples from recipients were collected at baseline, day 3, and day 7 (or day 30)
 142 (Figure 1). Missing points were due to patient discharge. Detailed standards of patient
 143 recruitment and donor screening were previously published (13). Donors were either
 144 related (genetically related family members) or unrelated (screened unrelated family
 145 members). Clinical metadata of IBD patients—including anthropometric index, clinical
 146 parameters, and blood test results—were obtained at each follow-up time point. For
 147 autologous FMT treatment, 25 additional fecal samples from 5 metabolic syndrome
 148 individuals were obtained from the *Vrieze et al.* (21) study with follow-up points on
 149 day 0 and days 2, 14, 42, and 84 after FMT.

150 In summary, 34 samples were used for analysis of the allogenic FMT group, 25 for the
 151 autologous, and 10 for the healthy group.

152 **Stool sample collection and FMT procedure**

153 Fecal samples were obtained from scanned donors and were isolated for microbiota at
 154 lab. Fecal microbiota from the donor was prepared according to the manual method of

155 filtration, centrifugation, washing, discarding, and resuspension and repeated processes.
156 Purified fresh fecal microbiota suspension was input into patients' mid-gut by a tube
157 within gastroscope under anesthesia, and the entire procedure should be done within
158 one hour.

159 Metagenomic sequencing and processing methods

160 DNA extraction and metagenomic sequencing of IBD fecal samples and healthy fecal
161 samples were performed at BGI-Shenzhen, China following HiSeq 2000 sequencing
162 protocol. Metagenomic sequencing of autologous FMT treatment samples was
163 performed at the Genomics Core Facility of the European Molecular Biology
164 Laboratory, Heidelberg using Hiseq 2000.

165 Illumina sequencing reads were quality controlled by trimming low quality bases
166 (quality score < 20), filtering adapter reads, and removing host-related reads after
167 mapping to the human genome database. The reads quality control procedure was
168 conducted using cOMG with default parameters (22). After quality control,
169 1,379,430,125 sequences were obtained, with a mean of 31,350,685 sequences per
170 sample.

171 Microbiota taxonomic profiling

172 Species-level quantification of metagenomic sequencing reads was achieved using
173 mOTUs software with default parameters. mOTUs is a method that establishes
174 metagenomic operational taxonomic units based on single-copy phylogenetic marker
175 genes. It maps the quality-controlled metagenomic sequencing reads against the
176 m-OTUS.v1.padded database, which is composed of 10 MGs extracted from 3,496

177 prokaryotic reference genomes (download from NCBI) and 263 publicly available
178 metagenomes (from the MetaHIT and HMP projects), and then outputs metagenomic
179 OUT linkage groups (m-OTUS) (23).

180 For strain level profiling, metaSNV was utilized to process quality-controlled
181 metagenomic sequencing reads. metaSNV is a method that is able to disentangle
182 conspecific strains in metagenomic samples using specific single-site allelic variation
183 (SNVs). It uses a collection of microbial reference genomes in which each species is
184 represented by a single representative genome or gene collection (24). To maintain
185 consistency with previous species profiles, we specified the m-OTUS.v1.padded
186 database as our reference genome or gene collection during this procedure. First, we
187 mapped quality-controlled sequencing reads to the m-OTUS.v1.padded database using
188 bwa and Ngless. Next, we ran qaCompute on each sample to determine the average
189 coverage over each reference in each sample and aggregated the coverage information.
190 We then took advantage of the mpileup tool to compute genomic variation, and
191 outputted all the variant positions that met the default-imposed quality criteria. Lastly,
192 we computed per species pairwise distance matrices for the samples.

193 **Quantification and Statistical Analysis**

194 All statistical analyses were performed in R using the following packages: vegan,
195 Hmcc, pROC, and RandomForest. We conservatively used only the baseline and day 3
196 time point samples for each patient when conducting all the two-sided statistical tests.

197 ***Diversity comparisons.*** The diversity of each gut microbiota community per sample
198 was calculated based on its mOTUs profile, referred to as the Shannon index, using the

199 vegan package. The Kruskal-Wallis test was used as a significance test for this
200 multi-group comparison.

201 ***Species-level changes after FMT.*** After species profiling all fecal samples using
202 mOTU, we took only the species with a detected relative abundance of at least 0.001
203 into account to avoid ambiguous results. In order to determine whether donor
204 microbiota could be transferred to recipients, we divided the microbiota composition of
205 post-FMT recipient into 4 groups: donor-specific species, recipient-specific species,
206 common species (shared by donor and recipient), and new species (not found in either
207 the donor or in the pre-FMT recipient). We quantified these 4 groups by comparing the
208 gut microbiota mOTU profiles of the pre-FMT recipient, the post-FMT recipient, and
209 the donor. Results were visualized using bar plots with all available follow-up time
210 points.

211 ***Community-level changes after FMT.*** Community-level changes in gut microbiota
212 composition between pre-FMT and post-FMT recipients were represented by the
213 Bray-Curtis distance, which was computed using the vegan package after applying a
214 logarithmic transformation to mOTU relative abundance with the function $\log(x+x_0)$,
215 where x is the original relative abundance of a certain mOTU and $x_0 = 1e-6$. The cosine
216 dissimilarity was also used to examine the correlations between gut microbiota
217 compositions pre-FMT and post-FMT, and between post-FMT recipients and donors.
218 Results were displayed using scatter plots.

219 ***Strain-level changes after FMT.*** Strain differentiation, which was determined by
220 comparing the presence or absence of donor-specific, recipient-specific, and previously

undetected single-site allelic variations, was monitored in post-FMT recipients based on the output files of metaSNV. Similar to the process of determining species retention and transplantation, the gut microbiota composition of post-FMT recipients was categorized into 3 groups: donor-specific strains, recipient-specific strains, and common strains (shared by donor and recipient). We excluded the newly gained strains because that was not of interest here. Quantification of the three groups was determined according to the frequency per filtered SNVs set.

Species engraftment model. We sought to investigate whether the microbiota composition of post-FMT recipients could be predicted using advanced machine learning models. We therefore applied the Random Forest algorithm in R to predict the presence (random forest classification model) and abundance (random forest regression model) of each mOTU in every post-FMT recipient sample. For a dataset comprised of 15 samples and 123 filtered mOTUs, these models are trained on 15 x 127 total instances. The inputs for these predictions are the gut microbiota composition of each pre-FMT patient and their corresponding donor at a species level, along with clinical metadata of the pre-FMT recipient and donor. Random Forest is a collection or ensemble of classification and regression trees trained on targeted datasets. It is resistant to overfitting and is considered stable in the presence of outliers. The error rate of the classification of all the test sets is the out-of-bag (OOB) estimate of the generalization error (25).

First, we eliminated the condition of class imbalances by filtering out mOTUs that existed in less than 3 samples to avoid prediction bias in favor of the majority class.

243 Second, the mtry parameter with the lowest error was picked using the rfcv function
 244 with 5-fold cross validation. Third, we applied the randomForest function to perform
 245 classification of post-FMT recipients across all mOTUs. This resulted in 123
 246 randomForest classification models in total, and we computed the auc value for each
 247 model. Finally, we chose important features from those models that had good
 248 prediction performance (auc bigger than 0.9).

249 For the regression model, we also accounted for class balance and then used the rfcv
 250 function with the same predictors that we used in the classification model to perform
 251 prediction.

252 ***Feature Importance.*** Random Forest calculates feature importance by removing each
 253 feature from the model and measuring the decrease in accuracy (for presence) or the
 254 increase in the mean-square error (for abundance). According to these importance
 255 scores, we ranked features in decreasing order across models and picked 40 with the
 256 highest scores to display.

257 ***Correlations between change in mOTUs as well as in clinical parameters.*** Clinical
 258 metadata of patients was collected at baseline and follow-up visits, including physical
 259 parameters, inflammation markers, lymphocyte population, blood fat, and
 260 immunoglobulin. We used the rcorr function in the Hmisc package to compute the
 261 spearman correlation iterating from each mOTU-clinical index pair. The change in
 262 each mOTU was defined as the increase or decrease in its relative abundance 3 days
 263 after FMT treatment compared to baseline. Changes in clinical index were computed
 264 based on the absolute score recipients got at baseline and 3 days after FMT treatment.

265 For multiple comparisons, the Benjamini-Hochberg method was used to adjust the p
266 value to control for false positives. Lastly, we drew a network using Cytoscape based
267 on the pairs with a q-value smaller than 0.05 (26).

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269 Ethical statement

270 *This study was carried out in accordance with the recommendations of good clinical*
271 *research practice (GCP), the Ethical committee of the Second Affiliated Hospital of*
272 *Nanjing Medical University, and BGI-IRB. The protocol was approved by the Ethical*
273 *committee of the Second Affiliated Hospital of Nanjing Medical University and*
274 *BGI-IRB. All subjects gave written informed consent in accordance with the*
275 *Declaration of Helsinki.*

276

277 **RESULTS**

278 **Bacteria characterization at a species level**

279 After profiling sequenced fecal samples using shotgun metagenomics, the Shannon
280 index (alpha diversity of a community) of gut microbiota was measured across IBD
281 recipients. Results showed that the average Shannon index of CD patients was
282 significantly lower than that of healthy controls (P-value = 0.0035). In UC patients,
283 although their Shannon index was lower than the average in healthy controls, dysbiosis
284 was not significant (p-value = 0.57). Three days after FMT treatment, the average
285 Shannon indexes of both CD and UC recipients had not significantly improved

(p-value > 0.01) (Figure 2A). Unexpectedly, CD-6, CD-7, CD-8, and UC-2 had a decreased Shannon index. Among the whole population of the gut microbiota, some bacteria may be more important than others for maintaining a healthy gut environment. For example, 3 days after FMT treatment, there was a universal increase in *Bacteroides* that have been shown to exist at lower levels in IBD patients than in healthy people (27). Some highly individualistic performances were also observed: CD-9 gained an abundant amount of *Lactobacillus*, which was considered to be probiotics, and CD-1 had a great decrease in *Citrobacter*, which was recognized to be pathogenic bacteria (Figure 2B). The amounts of species each recipient gained from their donor after FMT are shown in Figure S1.

Bacterial engraftment at the species level

To investigate the extent to which the gut microbiota of recipients could be altered by FMT treatment, we evaluated both the degree and direction of change. Results showed that microbial communities underwent large compositional changes after FMT, and these changes persisted throughout follow-up visits (Figure 2B).

On average, post-FMT CD recipients gained 29.4% of mOTUs from donors (n = 11, SD = 14.4%), while post-FMT UC recipients gained 28.2% of mOTUs from donors (n = 4, SD= 20%). Our results were analogous to a previous study that found that FMT recipients gained 35% of mOTUs from donors (n = 436, SD = 27%) (28).

By measuring the distance between donor-recipient pairs using Euclidean distance, we determined the direction of microbiota change. Results varied between different donor-recipient pairs. Out of the 4 patients that had 2 follow-up time points, we found

that CD-9 and UC-2 tended to be closer to their donors and further from their pre-FMT status. CD-2 showed a slightly tendency to return to their initial status, but the disturbance was small enough to be ignored (a shift from 10.628 to 10.57). Surprisingly, CD-1 showed an increased distance from both their donor and their pre-FMT status, which could be attributed to environmental factors. Though CD-1, CD-2, and UC-2 all shared the same donor, the direction of their gut flora shift after the treatment varied (Figure 3A). In addition, we explored the abundance consistency of mOTUs of recipients before and after FMT. mOTUs of the recipient post-FMT were highly correlated with mOTUs of the recipient pre-FMT (median cosine similarity of UC patient mOTUs = 0.93, CD patients = 0.95). More importantly, the results showed that mOTUs of post-FMT recipients had high similarity to mOTUs of their donors (median cosine similarity of UC patient mOTUs = 0.95, that of CD patients = 0.91) (Figure 3B).

Bacterial engraftment at the strain level

To investigate the extent of strain level changes in our study groups, we monitored SNVs identified at baseline over all available time points. Higher levels of single-site allelic variations were observed in UC FMT recipients and CD FMT recipients compared to autologous FMT recipients from a previous paper (21) ($P = 0.0056$ and 0.148 , respectively). Moreover, SNVs were found to be higher in UC FMT recipients than in CD FMT recipients ($P = 0.070$) (Figure 4).

To investigate whether this increased variation was due to the transfer and establishment of donor microbiota, we followed methods described in a previously

published paper (28), defining a set of determinant genomic positions (containing both donor- and recipient-specific SNVs) and monitoring them over time (Figure 5). For the credibility of SNVs detection, we chose species with sufficient abundance that were consistently detected in at least one donor-recipient pair. Donor-specific SNVs were most highly retained 3 days after FMT (UC: $62.8 \pm 25.3\%$ of determinant positions across recipients, CD: $11.4 \pm 10.3\%$) and were still present 1 month later (UC: 46.9% , CD: $19.99 \pm 10.1\%$). This was in contrast with the much lower rates of variation observed at equivalent time points in autologous FMT recipients ($9.5 \pm 1.8\%$) (Figure S1), showing that the increased variations of gut microbiota in post-FMT patients could be attributed to donor strain transfer instead of temporal variability.

Furthermore, marked differences in colonization success were observed between UC and CD recipients who shared a donor (subjects CD-1,2,3,8, and UC-1,2). 3 days after treatment, UC-1,2 retained a higher amount of donor-specific SNVs compared to CD-1,2,3,8 (48.9% , 44.4% , 11.9% , 3.4% , 1.5% , and 9.3% , respectively). Extensive coexistence of donor and recipient strains (CD: in $44.1 \pm 17.1\%$ of shared species, UC: $21.3 \pm 14.1\%$) was found in all other recipients, and persisted for at least one month. This suggests that novel strains can colonize the gut without replacing the indigenous strain population of the recipient. It appeared that introduced strains were more likely to be established in a new environment if the species was already present, and a pattern of donor strains establishing alongside indigenous strains of the recipient was observed.

While the phenomenon of donor strain establishment occurred in both CD and UC recipients, UC patients were more susceptible to external sources of microbiota (Figure

352 6).

353 Donor strains showed different transferability under different disease status.

354 Donor-specific strains like *Ruminococcus torques* ATCC 27756, *Ordoribacter*

355 *splanchnicus* DSM 20712, *Klebsiella pneumoniae* 342, *Intestinaibacter bartlettii* DMS

356 16795, *Escherichia coli* O26:H11 str. 11368, and *Erysipelatoclostridium ramosum*

357 DSM 1402 only exerted strain displacement in CD patients, while donor-specific

358 strains like *Faecalibacterium prausnitzii* SL3/3, *Eubacterium ventriosum* ATCC 27560,

359 *Blautia obeum* A2-162, *Bifidobacterium longum* subsp. *infantis* ATCC 15697 = JCM

360 1222 = DSM 20088, *Anaerostipes hadrus*, and *Eubacterium rectale* M104/1 only

361 exerted strain displacement in UC patients (Figure 5).

362 **Construction of a prediction model for gut microbiota composition of post-FMT**

363 **patients**

364 According to what we have discovered in previous species-level analysis, microbiota

365 of post-FMT recipients are a complex mixture of species from the donor, species from

366 the recipient, and species gained from the environment. We speculated that after

367 accounting for the gut microbiota composition of pre-FMT recipients and donors,

368 along with the corresponding clinical metadata of the recipients, we might be able to

369 predict the post-FMT gut microbiota of the recipients. We, therefore, performed

370 random forest classification and regression analysis, which is non-linear and can accept

371 categorical and continuous predictors simultaneously from our data (25).

372 To investigate whether species compositions of post-FMT patients—that is, the

373 mOTUs profiles—were predictable, we first examined the presence of each mOTU

across post-FMT recipients using the randomForest classification model, and computed the average area under the curve (AUC) (mean = 74.2%, SD = 16%). We then utilized a randomForest regression model to test the predictability of abundance of each mOTU ($\rho = 0.478$, $P < 2.2e-16$). Results indicated that the presence of most (>80%) species of post-FMT recipients was highly predictable (AUC > 85%), while a small portion of species was not. The abundance of mOTUs of post-FMT recipients was moderately predictable (Figure 7A). Our results were poorer than a similar study conducted by Christopher S. Smillie et al. (20) on 19 R-CDI patients. One possible explanation for this discrepancy may be that they included other predictors in their model construction in addition to the ones we used: taxonomy, abundance, clinical metadata, sequencing depth, genome statistics, physiology, and resource utilization. The RandomForest model also provided an algorithm to rank the contribution of each predictor based on variable importance score. According to our analysis, among the top 40 most important variables (see Materials & Methods), the IgA score, T-cell, and Th cell-induced of the recipients were the top three clinical-related elements. *Streptococcus.anginosus*, *Bacteroides.plebeius*, *Clostridium.bolteae*, *Streptococcus.thermophilus*, and *X.Ruminococcus.gnavus* were the top five species in the classification model (Figure 7B). In terms of species-related factors, *Streptococcus.anginosus* was reported to be associated with colorectal cancer and *Ruminococcus.gnavus* was found to be linked with a certain type of immunological rejection.

Clinical outcomes

396 Out of all 15 patients, 8 out of 11 CD patients and 3 out of 4 UC patients were relieved
397 3 days after FMT treatment. Clinical improvement was defined as a decrease in the
398 Harvey-Bradshaw Index > 3 for CD, and a decrease in the Mayo score > 3 for UC
399 (Table S1).

400 **Relationship between changes in clinical index and changes in gut microbiota**

401 Potential antigens in the microflora could have pro- or anti-inflammatory effects, and it
402 could be argued that by reacting to these antigens, an organism is mounting an
403 autoimmune response; by extension, the chronic mucosal inflammation of IBD could
404 be thought of as an autoimmune disease. Given this perspective, it would make sense
405 to relate the change in clinical parameters to the abundance change of gut microbiota in
406 response to FMT treatment. We established the relationships between the change in
407 clinical indexes as well as in mOTUs of recipients using Spearman's correlation. We
408 found that defecation changes were significantly positively correlated with
409 *Selenomonas.artemidis* and two unclassified species, and negatively correlated with
410 *Enterococcus.casseliflavus* and *Prevotella.bivia*. Changes in CD4+CD8+, which have
411 been identified to be higher in IBD patients than in normal people in previous studies,
412 were significantly positively correlated with *Streptococcus.sp..C150*,
413 *Streptococcus.infantis*, *Streptococcus.parasanguinis*, and *Streptococcus.australis*, and
414 negatively correlated with changes in *Streptococcus.gordonii* and
415 *Lactobacillus.salivarius*, a probiotic bacterium that lives in the gastrointestinal tract
416 and has a range of therapeutic properties including suppression of pathogenic bacteria
417 (29). Changes in TSC were significantly positively correlated with changes in

418 *Bacteroides fragilis*, which is found in most anaerobic infections and can promote the
 419 induction of type 1 T helper (TH1) cells, suppress IL-17 production, and improve
 420 experimental colitis (30). Additionally, we tested whether the physical characteristics
 421 of patients, such as BMI, age, and disease duration, (Table S2) could affect clinical
 422 outcomes. Changes in CD4+CD8+, Th.cell.Induced (counted by Flow cytometry), and
 423 abdominal pain score were found to significantly negatively correlate with the disease
 424 start age of patients ($p < 0.05$), which could reflect disease duration. In addition,
 425 changes in CD4+CD8+ and Th.cell.Induced were significantly negatively correlated
 426 with the age of patients (when patients received FMT treatment). Disease duration and
 427 the age of patients were also discovered to be important features in the random forest
 428 classification model. As a result, we speculated that disease duration and age could be
 429 used as stratifying factors for IBD patients in future therapy plans (Figure 7B).

430

431 **DISCUSSION**

432 Consistent with previous findings, our study found reduced bacterial diversity in CD
 433 and UC patients. Strain level analysis monitored across samples revealed that 3 days
 434 after FMT treatment, a certain amount of species had noticeable strain replacements.
 435 Moreover, donor-specific strains belonging to different species demonstrated
 436 differentially competitive advantages during the process of displacement, measured by
 437 their relative abundance in recipients after FMT. We also observed that same-donor
 438 recipients undergo varying degrees of gut microbiome shifts, implying that the FMT
 439 treatment effect may be patient-specific, and raising the possibility of patient

440 stratification in clinical application.

441 We also aimed to identify factors that could contribute to the accurate prediction of
 442 post-FMT gut microbiota composition of the recipients. The moderate predictability of
 443 the classification and regression model suggests that the gut microbiota composition of
 444 post-FMT recipients can be recognized not through sequencing methods but through
 445 algorithms, indicating a promising future towards FMT precision treatment. In our
 446 model, we only take the species composition of the donor and pre-FMT patient, along
 447 with clinical indexes of the pre-FMT patient as predictors. There is space left to
 448 enhance the resolution of prediction accuracy. Based on previous studies concerning
 449 the etiology of IBD, factors like genetic background, non-bacterial components
 450 (virome, fungi), metabolites profile, and dietary records have the potential to account
 451 for the unexplainable part of our model.

452 Associations between immunological factors and clinical outcomes provide us with
 453 some limited but intriguing perspectives. CD4+CD8+, TSC, and Th.cell.Induced have
 454 been found to be associated with certain bacterial species, implying that bacteria have
 455 the potential to affect the adaptive immunity of patients. However, there are many
 456 intermediate issues to be dealt with before making a cohesive interpretation of this
 457 assumption. Combining the information from functional metagenomes and
 458 metabolomics will minimize the gap between gut microbiota and immunological
 459 responses of the recipients.

460 The present attractive clinical findings are mainly based on our one-hour FMT protocol
 461 for providing fresh FMT, which means the time from defecation of stool to deliver

462 purified microbiota to patient's intestine within one hour (31,32). Another factor
 463 contributing to this positive clinical response, according to our experience, might be
 464 the criteria of donor screening which is based on young age population, generally
 465 cover children and college students under 24-year-old (33). However, the small sample
 466 size of our study and the incomplete follow-up visits inevitably limited the scope of
 467 our results. Future studies need to include a larger study cohort and longer tracking
 468 times for the explicit identification of specific bacterial strains that may play a role in
 469 FMT treatment efficacy, and to uncover a comprehensive principle of strain
 470 displacement.

471

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488

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492

493 **Availability of supporting data**

494 Datasets are in a publicly accessible repository:

495 The quality-controlled sequencing reads are available in the CNGB Nucleotide
496 Sequence Archive (CNSA: <https://db.cngb.org/cnsa> ;accession number CNP0000134)

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594 Additional files

595 Tables S1; Table S2

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603 Figure legends

604 **Figure 1. Study design and follow-up visits of the patients**

605

606 To recognize each patient in simplicity, we labeled each of them with disease subtypes

607 CD- or UC- as prefix plus a random assigned number as suffix.

608

609 **Figure 2. Bacterial communities undergo compositional changes in IBD recipients**
 610 **after FMT.**

612 (A) The Shannon index of gut microbiota was lower in IBD patients than in healthy
 613 controls, and was not significantly improved 3 days after FMT (p-value > 0.01).
 614 Different groups are represented by different colored boxes.

615 (B) The proportion of species gained from the donor in post-FMT recipients lasts
 616 during follow-up visits. However, the proportions varied among recipients, even
 617 those who shared a donor (labels with the same color). Gut microbiota composition
 618 per patient was divided into four parts: orange represented donor-specific species,
 619 yellow represented species shared by donor and recipient, purple represented
 620 recipient-specific species and green represented newly gained species.

621

622 **Figure 3. High compositional resemblance of the gut microbiomes of post-FMT**
 623 **recipients and their pre-status, as well as post-FMT recipients and their donors.**

(A) After FMT, the microbiota composition of most patients is further from their initial status than natural shift observed in placebo (solid black line). Additionally, recipients with the same donor (lines of the same color) may vary in their shifting tendency.

(B) High consistency (median cosine similarity > 0.9) is found between post-FMT IBD patients (3 days after treatment) with their pre-FMT status, as well as with their donors.

Figure 4. UC recipients display higher strain level variations than CD recipients 3 days after FMT treatment.

Single-site allelic variations of UC and CD recipients after FMT treatment are a bit higher than autologous FMT recipients (p-value = 0.148 and 0.234, respectively). Single-site allelic variations of UC recipients are significantly higher than CD recipients after FMT treatment (p-value = 0.00056).

Figure 5. Some donor-specific strains undergo transfer, and the existence of donor

642 strains are highest 3 days after FMT.

643

644 The rate of donor strain transfer is greatest in recipients 3 days after FMT (UC: 62.8

645 $\pm 25.3\%$, CD: $11.4 \pm 10.3\%$), and a portion of them persists in recipients 1 month later

646 (UC: 46.9%, CD: $19.99 \pm 10.1\%$). Proportions of donor- and recipient-specific strains

647 across 50 species are shown in orange and purple, respectively.

648

649 **Figure 6. Random forest models have the ability to predict the gut microbiota**

650 **composition of post-FMT patients.**

651

652 (A) Left panel shows the classification result: predicted values have a moderate
 653 consistency with true values ($\rho = 0.478$ and $p\text{-value} < 2.2e-16$). Right panel
 654 shows the regression result: a boxplot of all the AUC values of each mOTU in
 655 post-FMT recipients (median AUC value = 74.2%, SD = 16%).

656 (B) Important variables are computed across those models, defined as those with an
 657 AUC value greater than 0.90. Important variables are divided into different
 658 categories (represented by different colors). The top 25 variables are classified as
 659 the clinical parameters of recipients.

660

661 **Figure 7. Some clinical indexes of IBD recipients have significantly changed 3**
 662 **days after FMT, and several clinical indexes correlated with changes in the**
 663 **mOTUs profiles of recipients.**

664

665 (A) Mental status, appetite, tenesmus, etc. significantly changed 3 days after FMT

666 (p-value < 0.05). Vertical dotted line indicates a p value of 0.05.

667 (B) Defecation changes and CD4+CD8+ changes have relationships with several

668 mOTUs. Blue represents a significant positive correlation, while red indicates a

669 significant negative correlation (p-value < 0.01). The width of the lines indicates
670 the weight of correlation.

671

672 **Figure S1. The amount of donor-specific species gain after FMT differs, even for**
673 **same-donor recipients.**

674 Recipients that share a donor are colored the same.

675

676 **Figure S2. A certain number of donor species display apparent transfer after FMT**
677 **treatment in IBD patients.**

678 Heatmap and hierarchical clustering of mOTU profiles for all samples. Pre- and
679 post-FMT CD recipients, pre- and post-FMT UC recipients, and healthy controls are
680 separated by space.

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