bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

Manli Zou[1,2*], Zhuye Jie[2,3,4*], Bota Cui[5*], Honggang Wang[5], Qiang Feng[2,3,6,7,8], Yuanqiang Zou[2,3], Xiuqing Zhang[1], Huanming Yang[2,9], Jian Wang[2,9], Faming Zhang†[5], Huijue Jia†[2,3,4,11]

1. BGI Education Center, University of Chinese Academy of Sciences, Shenzhen 518083 China;
2. BGI-Shenzhen, Shenzhen 518083, China;
3. China National Genebank, Shenzhen 518083, China;
4. Shenzhen Key Laboratory of Human Commensal Microorganisms and Health Research, BGI-Shenzhen, Shenzhen 518083, China;
5. Medical Center for Digestive Disease, the Second Affiliated Hospital of Nanjing Medical University, 121 Jiang JiaYuan, Nanjing 210011, China;
6. Shenzhen Engineering Laboratory of Detection and Intervention of Human Intestinal Microbiome, Shenzhen 518083, China;
7. Department of Biology, Laboratory of Genomics and Molecular Biomedicine, University of Copenhagen, Universitetsparken 13, 2100 Copenhagen, Denmark;
8. Present address: Department of Human Microbiome, School of Stomatology, Shandong
9. James D. Watson Institute of Genome Sciences, Hangzhou 310058, China;
10. Key Lab of Holistic Integrative Enterology, Nanjing Medical University, Nanjing 210011, China.
11. Macau University of Science and Technology, Taipa, Macau 999078, China;

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

*These authors have contributed equally to this study

†Corresponding authors: Faming Zhang, MD, PhD, Medical Center for Digestive Diseases, the Second Affiliated Hospital of Nanjing Medical University, 121 Jiang JiaYuan, Nanjing 210011, China. Tel.: +86-25-58509883; Fax: + 86-25-58509931.

E-mail: fzhang@njmu.edu.cn

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

ABSTRACT

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

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disease characterized by chronic immune-mediated intestinal inflammation, and consists mainly of Crohn's disease (CD) and ulcerative colitis (UC). The etiology of IBD has been proposed to be multifactorial, involving a dysregulated immune response to environmental factors in a genetically susceptible individual *(1)*. Interestingly, given the evidence accumulated in recent years, the gut microbiota is now recognized for playing an important role in IBD. Dysbiosis is a decrease in gut microbial diversity owing to a shift in the balance between commensal and potentially pathogenic microorganisms of the gut microbial ecosystem, and has long been characterized as a trait of IBD patients (*2*,*3*).

Fecal microbiota transplantation (FMT) aims to modify the intestinal microbiota composition and function of the recipients by transferring donor fecal suspension into the gastrointestinal tract of a recipient, and has become a promising method for manipulating the gut microbiota. Its successful application for the treatment of *Clostridium difficile* infection has inspired people to apply it to inflammatory bowel disease patients (*4*,*5*,*6*,*7*,*8*,*9*). However, this application is still in its early stages. According to a recent systematic review and meta-analysis, after minimizing publication bias, IBD patients who received FMT had a remission rate of only 36.2%: 22% for UC and 60.5% for CD (*10*). Moreover, there is a lack of research regarding the efficiency and principles of FMT in treating IBD.

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

MATERIALS AND METHODS

Patient recruitment and sample collection

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

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

Stool sample collection and FMT procedure

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

Metagenomic sequencing and processing methods

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

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

Microbiota taxonomic profiling

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

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

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

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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.

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

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

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

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

Ethical statement

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

RESULTS

Bacteria characterization at a species level

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

(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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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±25.3% of determinant positions across recipients, CD: 11.4±10.3%) and were still present 1 month later (UC: 46.9%, CD: 19.99 ±10.1%). This was in contrast with the much lower rates of variation observed at equivalent time points in autologous FMT recipients (9.5 ± 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 ± 17.1% of shared species, UC: 21.3 ± 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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

6).

Donor strains showed different transferability under different disease status. Donor-specific strains like *Ruminococcus torques ATCC 27756, Ordoribacter splanchinicus DSM 20712, Klebsiella pneumoniae 342, Intestinaibacter bartlettii DMS 16795, Escherichia coli O26:H11 str. 11368,* and *Erysipelatoclostridium ramosum DSM 1402* only exerted strain displacement in CD patients, while donor-specific strains like *Faecalibacterium prausnitzii SL3/3, Eubacterium ventriosum ATCC 27560, Blautia obeum A2-162, Bifidobacterium longum subsp.infantis ATCC 15697 = JCM 1222 = DSM 20088, Anaerostispes hadrus,* and *Eubacterium rectale M104/1* only exerted strain displacement in UC patients (Figure 5).

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

According to what we have discovered in previous species-level analysis, microbiota of post-FMT recipients are a complex mixture of species from the donor, species from the recipient, and species gained from the environment. We speculated that after accounting for the gut microbiota composition of pre-FMT recipients and donors, along with the corresponding clinical metadata of the recipients, we might be able to predict the post-FMT gut microbiota of the recipients. We, therefore, performed random forest classification and regression analysis, which is non-linear and can accept categorical and continuous predictors simultaneously from our data (*25*).

To investigate whether species compositions of post-FMT patients—that is, the mOTUs profiles—were predictable, we first examined the presence of each mOTU

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

374 across post-FMT recipients using the randomForest classification model, and
375 computed the average area under the curve (AUC) (mean = 74.2%, SD = 16%). We
376 then utilized a randomForest regression model to test the predictability of abundance of
377 each mOTU (rho = 0.478, P < 2.2e-16). Results indicated that the presence of most
378 (>80%) species of post-FMT recipients was highly predictable (AUC > 85%), while a
379 small portion of species was not. The abundance of mOTUs of post-FMT recipients
380 was moderately predictable (Figure 7A). Our results were poorer than a similar study
381 conducted by Christopher *S. Smillie* et al. (*20*) on 19 R-CDI patients. One possible
382 explanation for this discrepancy may be that they included other predictors in their
383 model construction in addition to the ones we used: taxonomy, abundance, clinical
384 metadata, sequencing depth, genome statistics, physiology, and resource utilization.
385 The RandomForest model also provided an algorithm to rank the contribution of each
386 predictor based on variable importance score. According to our analysis, among the top
387 40 most important variables (see Materials & Methods), the IgA score, T-cell, and Th
388 cell-induced of the recipients were the top three clinical-related elements.
389 *Streptococcus.anginosus, Bacteroides.plebeius, Clostridium.bolteae,*
390 *Streptococcus.thermophilus*, and *X.Ruminococcus.gnavus* were the top five species in
391 the classification model (Figure 7B). In terms of species-related factors,
392 *Streptococcus.anginosus* was reported to be associated with colorectal cancer and
393 *Ruminococcus.gnavus* was found to be linked with a certain type of immunological
394 rejection.

395 **Clinical outcomes**

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

Relationship between changes in clinical index and changes in gut microbiota

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

stratification in clinical application.

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

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

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

472 **Contributors:** Conceptualization, Methodology, and Writing – Zhuye Jie, Manli Zou,
473 Bota Cui and Faming Zhang; Revision & Editing – Faming Zhang, Huijue Jia;
474 Acquisition of data – Bota Cui, Honggang Wang, Qiang Feng; Materia Support –
475 Yuanqiang Zou, Xiuqing Zhang, Huanming Yang, Jian Wang; Software and Formal
476 Analysis, Manli Zou and Zhuye Jie; Writing – Original Draft, Manli Zou, and Zhuye
477 Jie; Supervision, Faming Zhang and Huijue Jia.

478

479 **Disclaimer:** The authors declare that they have no competing interests and there is
480 nothing to disclose.

481 **Competing interests:** None declared

482

483 **Funding:**

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

The work was financially supported by grants from the Macau Technology Developme nt Fund (102/2016/A3), the Shenzhen Municipal Government of China (JSGG2016022 9172752028, JCYJ20160229172757249) and the National Natural Science Foundation of China (Grant No.81670606, 81670495).

Patient consent: Obtained

Ethics approval: This study was approved by BGI-IRB (BGI-R004-05)

Availability of supporting data

Datasets are in a publicly accessible repository:

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

REFERENCES AND NOTES :

1. Khor, Bernard, Agnes Gardet, and Ramnik J. Xavier. "Genetics and pathogenesis of inflammatory bowel disease." *Nature* 474.7351 (2011): 307.

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

2. Manichanh C, Borruel N, Casellas F, et al. The gut microbiota in IBD[J]. Nature Reviews Gastroenterology and Hepatology, 2012, 9(10): 599.

3. Halfvarson J, Brislawn C J, Lamendella R, et al. Dynamics of the human gut microbiome in inflammatory bowel disease[J]. Nature microbiology, 2017, 2(5): 17004.

4. Borody T, Torres M, Campbell J, et al. Reversal of inflammatory bowel disease (IBD) with recurrent faecal microbiota transplants (FMT)[J]. American Journal of Gastroenterology, 2011, 106.

5. Zhang F M, Wang H G, Wang M, et al. Fecal microbiota transplantation for severe enterocolonic fistulizing Crohn's disease[J]. World journal of gastroenterology: WJG, 2013, 19(41): 7213.

6. Gutin L, Piceno Y, Fadrosh D, et al. Fecal microbiota transplant for Crohn disease: A study evaluating safety, efficacy, and microbiome profile[J]. United European Gastroenterology Journal, 2019: 2050640619845986.

7. Paramsothy, Sudarshan, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. Lancet Lond Engl 2017; 389: 1218–1228.

8. Moayyedi P, Surette M G, Kim P T, et al. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial[J]. Gastroenterology, 2015, 149(1): 102-109. e6.

9. Rossen N G, Fuentes S, van der Spek M J, et al. Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis[J]. Gastroenterology, 2015, 149(1): 110-118. e4.

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

528 10. Colman R J, Rubin D T. Fecal microbiota transplantation as therapy for inflammatory bowel
529 disease: a systematic review and meta-analysis[J]. Journal of Crohn's and Colitis, 2014,
530 8(12): 1569-1581.
531 11. Angelberger S, Reinisch W, Makristathis A, et al. Temporal bacterial community dynamics
532 vary among ulcerative colitis patients after faecal microbiota transplantation[J]. The
533 American journal of gastroenterology, 2013, 108(10): 1620.
534 12. Gordon H, Harbord M. A patient with severe Crohn's colitis responds to Faecal Microbiota
535 Transplantation[J]. Journal of Crohn's and Colitis, 2014, 8(3): 256-257.
536 13. Cui B, Feng Q, Wang H, et al. Faecal microbiota transplantation through mid-gut for
537 refractory Crohn's disease: Safety, feasibility, and efficacy trial results[J]. Journal of
538 gastroenterology and hepatology, 2015, 30(1): 51-58.
539 14. Suskind D L, Brittnacher M J, Wahbeh G, et al. Faecal microbial transplant effect on
540 clinical outcomes and faecal microbiome in active Crohn's disease[J]. Inflammatory bowel
541 diseases, 2015, 21(3): 556-563.
542 15. Frank D N, Amand A L S, Feldman R A, et al. Molecular-phylogenetic characterization of
543 microbial community imbalances in human inflammatory bowel diseases[J]. Proceedings of
544 the National Academy of Sciences, 2007, 104(34): 13780-13785.
545 16. Hansen R, Russell R K, Reiff C, et al. Microbiota of de-novo pediatric IBD: increased
546 Faecalibacterium prausnitzii and reduced bacterial diversity in Crohn's but not in ulcerative
547 colitis[J]. The American journal of gastroenterology, 2012, 107(12): 1913.
548 17. Bron P A, Van Baarlen P, Kleerebezem M. Emerging molecular insights into the interaction
549 between probiotics and the host intestinal mucosa[J]. Nature Reviews Microbiology, 2012,

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

10(1): 66.

18. Needham B D, Carroll S M, Giles D K, et al. Modulating the innate immune response by combinatorial engineering of endotoxin[J]. Proceedings of the National Academy of Sciences, 2013, 110(4): 1464-1469.

19. Spanogiannopoulos P, Bess E N, Carmody R N, et al. The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism[J]. Nature Reviews Microbiology, 2016, 14(5): 273.

20. Smillie C S, Sauk J, Gevers D, et al. Strain tracking reveals the determinants of bacterial engraftment in the human gut following faecal microbiota transplantation[J]. Cell host & microbe, 2018, 23(2): 229-240. e5.

21. Vrieze A, Van Nood E, Holleman F, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome[J]. Gastroenterology, 2012, 143(4): 913-916. e7.

22. Fang C, Zhong H, Lin Y, et al. Assessment of the cPAS-based BGISEQ-500 platform for metagenomic sequencing[J]. GigaScience, 2017, 7(3): gix133.

23. Sunagawa S, Mende D R, Zeller G, et al. Metagenomic species profiling using universal phylogenetic marker genes[J]. Nature methods, 2013, 10(12): 1196.

24. Costea P I, Munch R, Coelho L P, et al. metaSNV: A tool for metagenomic strain level analysis[J]. PloS one, 2017, 12(7): e0182392.

25. Liaw A, Wiener M. Classification and regression by randomForest[J]. R news, 2002, 2(3): 18-22.

26. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

models of biomolecular interaction networks[J]. Genome research, 2003, 13(11): 2498-2504.

27. Zhou Y, Zhi F. Lower level of bacteroides in the gut microbiota is associated with inflammatory bowel disease: a meta-analysis[J]. BioMed research international, 2016, 2016.

28. Li S S, Zhu A, Benes V, et al. Durable coexistence of donor and recipient strains after faecal microbiota transplantation[J]. Science, 2016, 352(6285): 586-589.

29. Neville B A, O'Toole P W. Probiotic properties of Lactobacillus salivarius and closely related Lactobacillus species[J]. Future Microbiology, 2010, 5(5): 759-774.

30. Deng H, Li Z, Tan Y, et al. A novel strain of Bacteroides fragilis enhances phagocytosis and polarises M1 macrophages[J]. Scientific reports, 2016, 6: 29401.

31. Wang H, Cui B, Li Q, et al. The safety of fecal microbiota transplantation for Crohn's disease: Findings from a long-term study[J]. Advances in therapy, 2018, 35(11): 1935-1944.

32. Ding X, Li Q, Li P, et al. Long-Term Safety and Efficacy of Fecal Microbiota Transplant in Active Ulcerative Colitis[J]. Drug safety, 2019: 1-12.

33. Zhang F, Zhang T, Zhu H, et al. Evolution of fecal microbiota transplantation in methodology and ethical issues[J]. Current opinion in pharmacology, 2019, 49: 11-16.

34. Manli Z, Zhuye J, Bota C, et al. Bacterial strain displacement in inflammatory bowel diseases after fecal microbiota transplantation. BioRxiv, 2018, https://doi.org/10.1101/439687

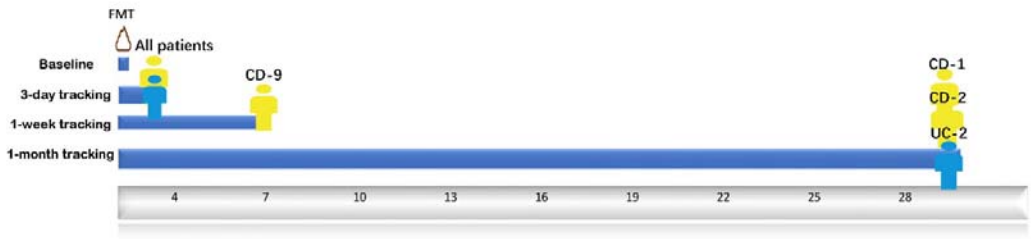
bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Additional files

Tables S1; Table S2

Figure legends

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

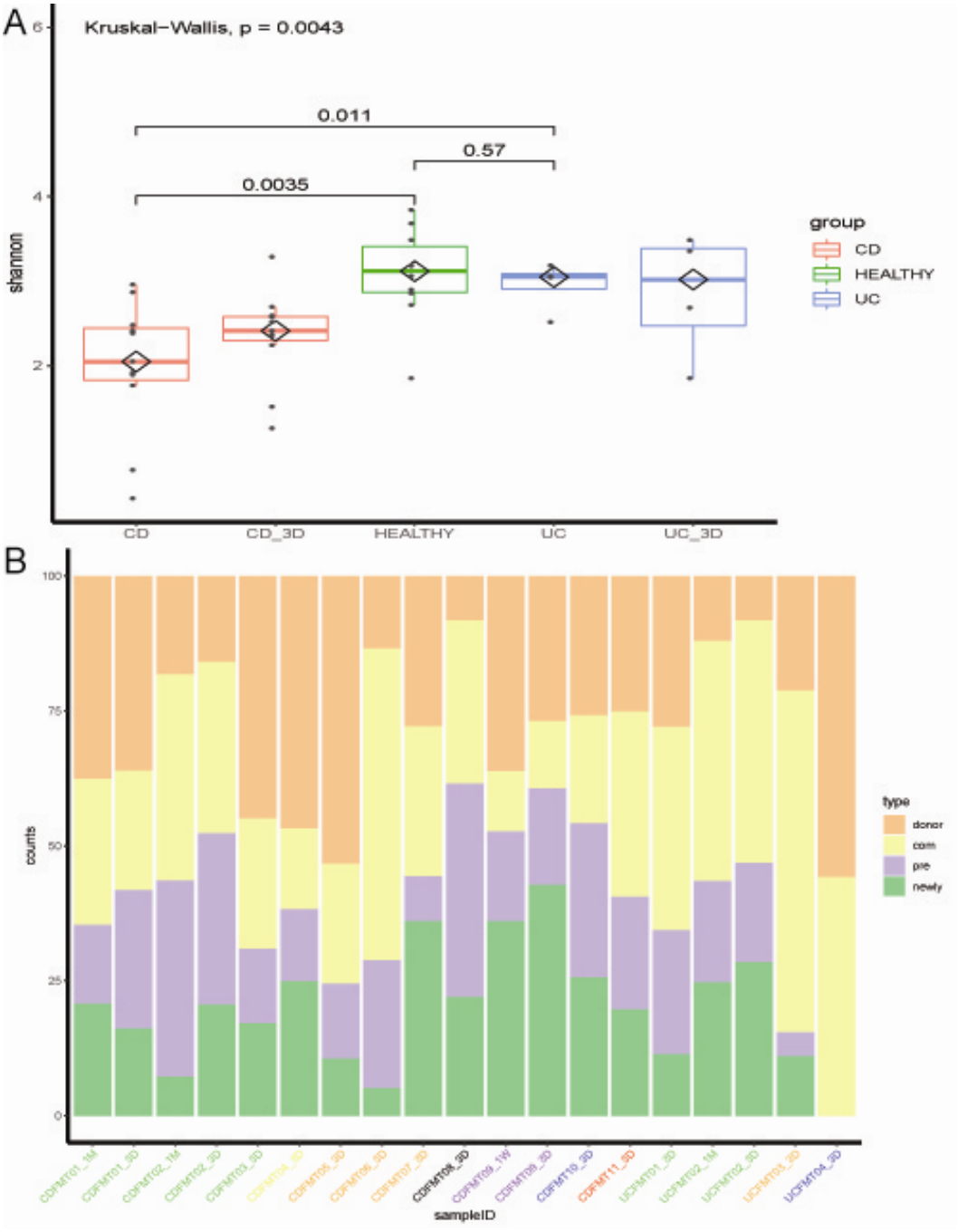


To recognize each patient in simplicity, we labeled each of them with disease subtypes CD- or UC- as prefix plus a random assigned number as suffix.

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

610 **after FMT.**



611

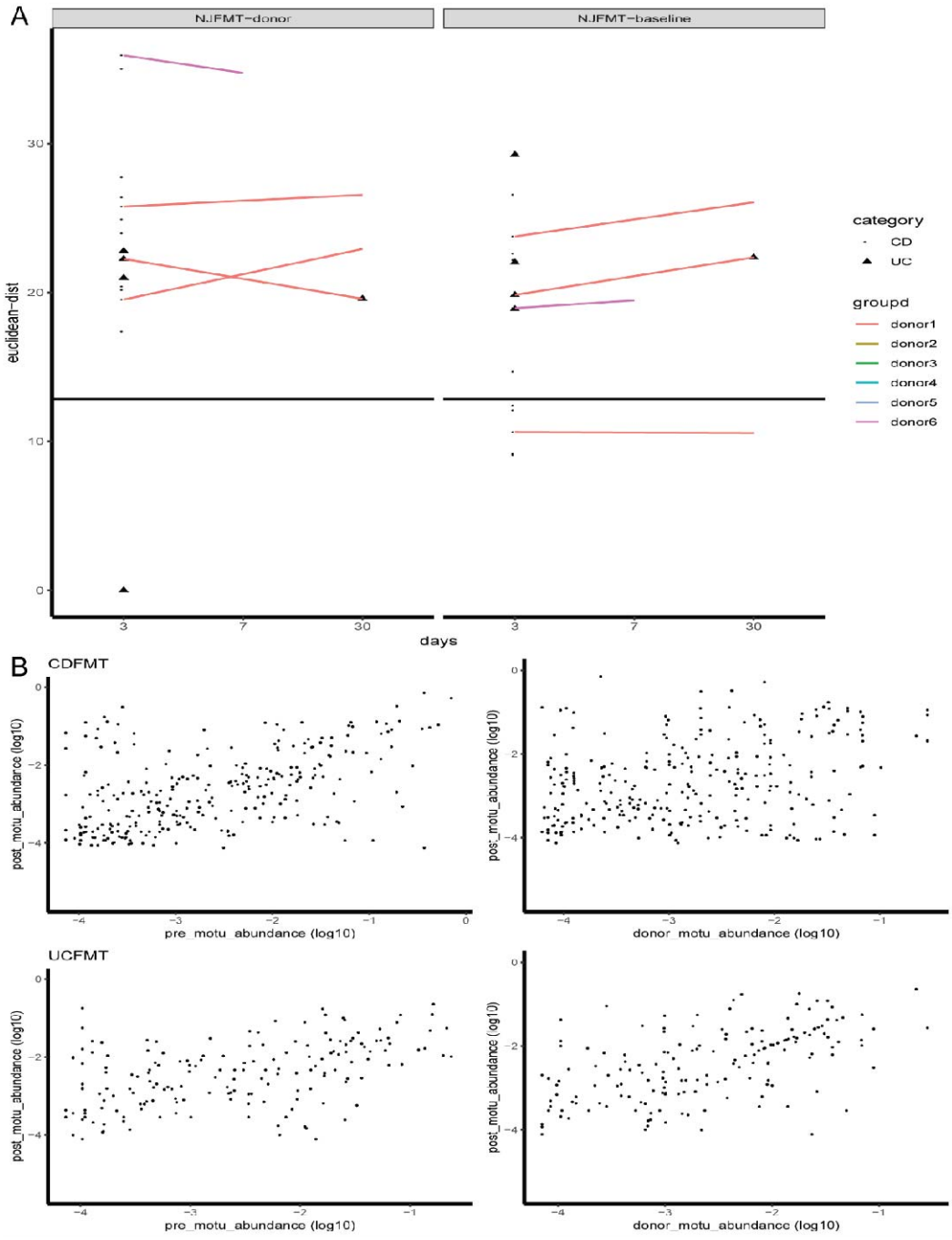
bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

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

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

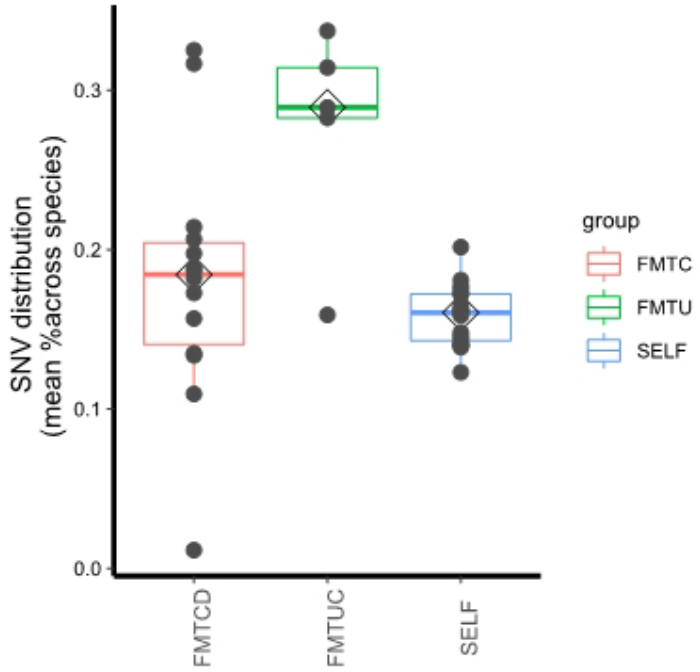


(A) After FMT, the microbiota composition of most patients is further from their initial status than natural shift observed in placebo (solid black line). Additionally, recipients with the same donor (lines of the same color) may vary in their shifting tendency.

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

(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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

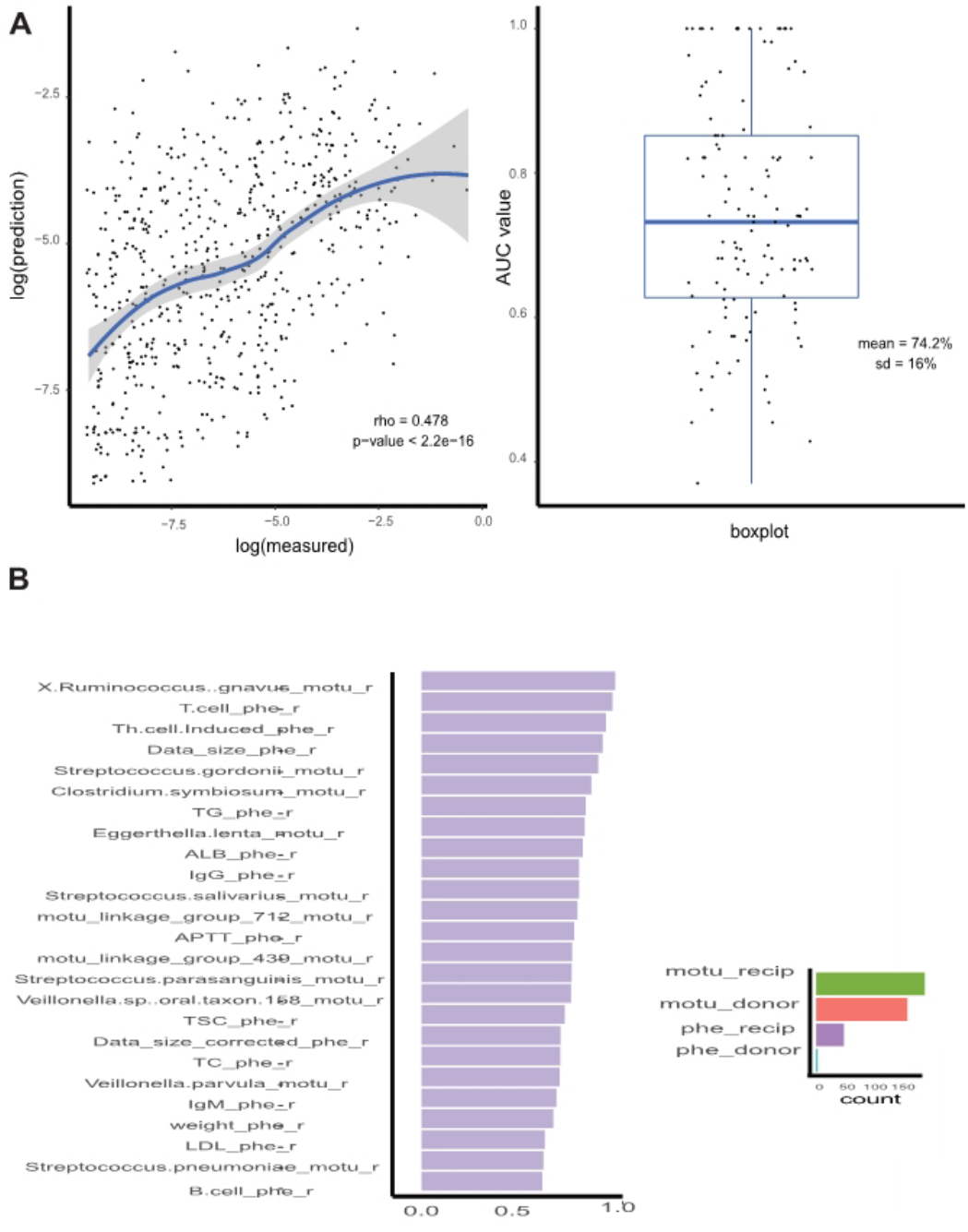
645 ±25.3%, CD: 11.4 ±10.3%), and a portion of them persists in recipients 1 month later

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

Figure 6. Random forest models have the ability to predict the gut microbiota composition of post-FMT patients.



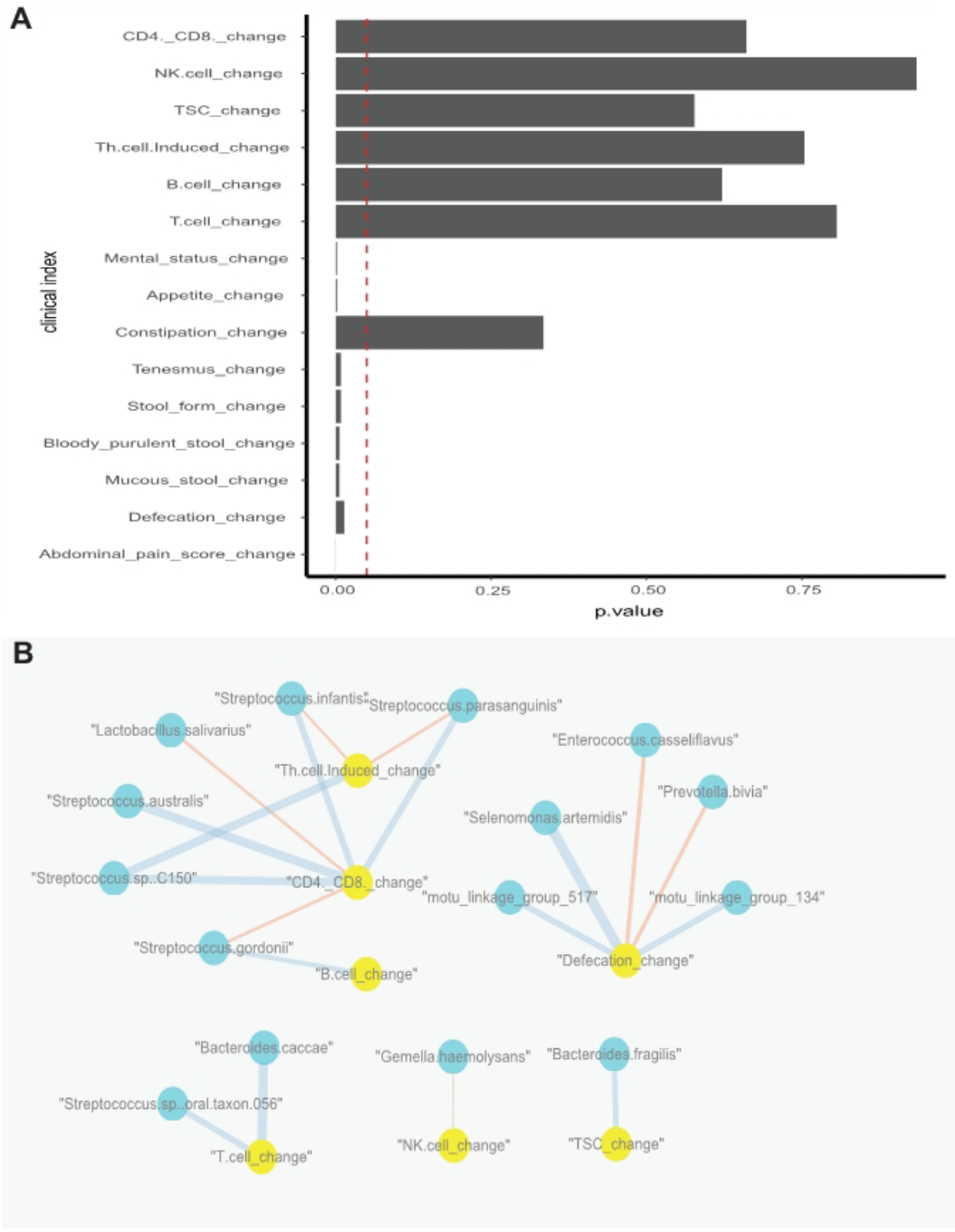
bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

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

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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



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

bioRxiv preprint doi: https://doi.org/10.1101/439687; this version posted August 5, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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.
681
682
683
684
685