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Microbiota of Inflammatory Bowel Disease Models

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Abstract

Gut microbiome plays an important role in inflammatory bowel disease (IBD), a group of intestinal chronic inflammation conditions that affect a large population. The animal models of IBD have long been established on basis of pathological features, but their ability to recapitulate patient gut microbiota is unknown. We investigated and compared the composition and biodiversity of bacterial population in the fecal samples from rat models of the two IBD subtypes, and compared them with patient samples. Our analyses revealed that inflammation reduces overall microbiome diversity and increased variation between individuals. We identified specific microbial signatures associated with the two IBD subtypes that were consistent between the animal models and human IBD patients, suggesting that the animal models can partially recapitulate the microbiota in human diseases. Furthermore, metagenome prediction analysis suggested microbial functions that were likely altered by host-microbiota interactions in IBD models.

I. Introduction

IBD is a group of intestinal disorders entailing chronic and relapsing inflammation of the lower digestive tract[1]. There are two major types of human IBDs: Crohn's disease (CD)

and ulcerative colitis (UC), with very different signs and symptoms[2]. Gut microbiota plays an important role in maintaining the integrity of the intestinal mucosa, the alteration of which contributes to the chronic inflammation conditions[3–5].

Dextran Sulfate Sodium (DSS) and 2,4,6-trinitrovebzebe sulfonic acid (TNBS) are widely used to induce UC and CD in animal model respectively[6, 7]. Although the DSS and TNBS treated animals present similar pathological features as human IBD specimens, whether they recapitulate patients' microbiota patterns have not been examined.

We performed 16S ribosome RNA sequencing (16S rRNA-seq) to examine the microbiomes of DSS and TNBS models and compared them with those of UC and CD patients. The biodiversity and microbial signature profiling analyses revealed that microbiota patterns in disease models were significantly altered from health control, and partially overlapped with human IBD.

II. Material and methods

A. IBD rats model

Male rats weighed around 250 grams were used. They were fed with drinking water containing 4% DSS to induce UC (n=10). 22.5mg TNBS in 40% ethanol (total volume 0.75ml) was given via enema once at baseline to induce CD (n=13). Fecal samples were collected at baseline (Day 0) and the end points of chemical induction (TNBS: Day5, DSS: Day7).

B. Sequencing and statistical analysis

The 16s rRNA V3 region of each sample was sequenced via Illumina MiSeq[8, 9]. We processed the raw sequencing data by Qiime 1.9.1, with open reference OTUs picked against Greengenes 13_5[10, 11]. The R package ggplot2 was utilized to visualized the results of taxonomy, alpha and beta diversity analyzed by Qiime[12]. Differential analysis of taxonomies was performed using LEfSe[13]. Morpheus[14] was used to process heatmaps showing relative abundance of corresponding bacterial.

C. Metagenome prediction

The functional contents were computed through metagenome prediction analysis via Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt)[15]. The predicted functions were assigned against Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology database[16]. Differential functions analysis was done by applying limma package at KO level 2[17].

III. Results

A. α-Diversity significantly altered in inflammation models

We first performed α-diversity analysis on 16s rRNA data to investigate the microbiota diversity within individual samples (Figure 1). In UC and CD models, the shannon index

decrease remarkably after the treatment of DSS and TNBS, which suggests the microbiota diversity was greatly reduced by inflammation.

B. Gut inflammation induces shift in biodiversity

We calculated the unifrac distance matrix from beta diversity to display the distance between samples[18]. Diversity describes the extent that individuals differ within each condition, while variation represents for the difference among samples. The distance within each condition increased after inflammation was induced, suggesting greater microbiome variation between individual rat. The distances between baseline and inflammation were highest (Figure 2 A,B). The principle coordinate analysis (PCoA) plots show how the beta-diversity patterns vary in the two IBD models. The region of the fitted ellipse expanded after DSS or TNBS treatment, and the distribution of samples shift to a non-overlapping pattern when IBDs induced (Figure 2 C,D). These results suggest that inflammation causes global changes of microbiome in guts, reducing diversity in each sample and increasing s between samples.

C. Taxonomy abundance analysis

We next performed taxonomy analysis to investigate the abundance of microbiota in control and DSS/TNBC treated rats (Figure 3). The abundance of *Clostridia* and *Bacteroidia*, which constitutes majority of the microbiota (approximately 80%), altered significantly after drug treatment in both the UC and CD models. The rest of the classes made up nearly 20% of the whole microbiota. The ratio of the majority to the minority remained consistent after induction of inflammation in both models. Although most of the shifted patterns are overlapped by two models, some abundance alternations were specific to each treatment. In the UC model, *Alphaproteobacteria* was enriched after DSS treatment, while in the CD's disease model, *Erysipelotrichi, Deltaproteobactria* and *Actinobacteria* were enriched after TNBS treatment.

D. Reconstitution of microbiota pattern

To find out the key species that contribute to the microbiome pattern shifting in IBD rat's model, we did the differential analysis to compare the microbiome signature between the health and induced IBD status. Linear differential analysis (LDA) is a method for characterizing two or more categories of objects. It was applied to the abundance of microbiome at genus or the lowest level by LDA Effect Size (LEfSe) after filtering out the least 0.001% genus. Using LDA scrore >=3.0 as the cutoff ($p<=0.05$), the remaining taxonomies are considered as the biomarkers for their corresponding condition (Baseline and the end day at DSS and TNBS treatment). Ranked by the LDA score of the biomarkers under health and inflammation status, the heatmaps show clear signature that biomarkers for health status get more enriched at baseline, and biomarkers for induced IBD become more abundant after DSS or TNBS treated.

Bacteriodes and *Sutterella* were enriched by DSS or TNBS treatments (Figure 4), consistent with previous reports that they were enriched in IBD patients.[19, 20] The abundance of *RF39*, *Lachonospiraceae*, *Ruminococcus*, *Rikenellaceae*, and *Clodtridiales* commonly reduced on the two IBD models. (Figure 4). In the DSS model, the abundance signature of

Bacteroides, Peptostreptococcaceae, Enterobaceriaceae, Prevotella, Akkermansia, Lachnospiraceae, Ruminococcus, Rikenellaceae is identical to that of UC patients.[19, 21–28] In the TNBS model, the abundance signature of *Blautia, Fusobacterium, Odoribacter, Lactobacillus, Lactobacillus, Rikenellaceae, Ruminococcus, Lachnospiraceae, Oscillospira, Clostridiales* is similar to that of the CD patients.[21, 26, 27, 29–33]

E. Functional Content Prediction

We performed metagenome prediction analysis based on the close-reference picked up OTUs to infer the microbial functions in IBD, testing 7 functional categories from KEGG Orthology (KO) level 1 and 40 from level 2. The abundance of functional contents on level one only shows slight difference between the two models. Metabolism consist to near half of the whole contents, while organismal systems take the smallest part. Differential analysis was applied to the KO level 2 groups by limma.

We selected the two most significantly different functions of each condition to demonstrate how the functions were regulated with the alteration of microbiota. (Figure 5) In both TNBS and DSS models, membrane transport (Level1: Environmental Information Processing) and cell motility (Level 1: Cellular Processes) were downregulated while glycan biosynthesis and metabolism (Level1: Metabolism) was upregulated compared with the healthy controls. In the DSS (UC) model, the level of carbohydrate metabolism (Level 1: Metabolism) increased most significantly among all the functions at KO Level 2, while in the TNBS (CD) model, energy metabolism had the most significant increase.

IV. Discussion

Animal models have been widely used to study human IBDs, and it is critical to understand how the models recapitulate the pathological features from IBDs. DSS (UC) and TNBS (CD) treatment are two of the most common models for IBD. Our study characterized the microbiome in the DSS and TNBS induced IBD rat models. The α-diversity of gut microbiome in individual samples was significantly reduced by inflammation. DSS and TNBS rat models recapitulate microbiome signatures consistent with clinical samples from human IBD patients. The functional analyses suggested possible microbial functional changes in response to gut inflammation. Therefore, IBD might cause profound changes in host-microbiome interactions.

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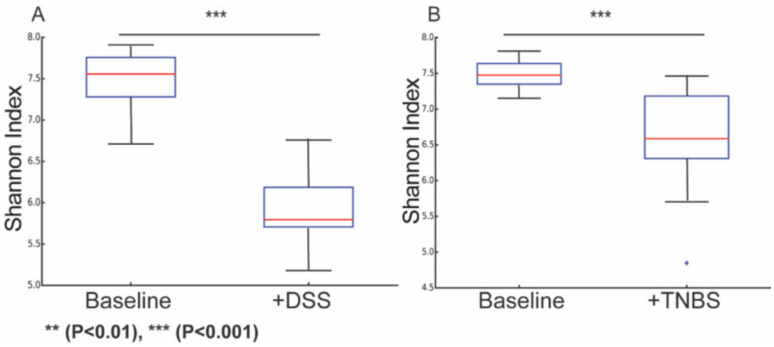

Figure 1.

α-diversity was calculated through shannon index of each sample. The boxplots depicted the shannon index within each group.

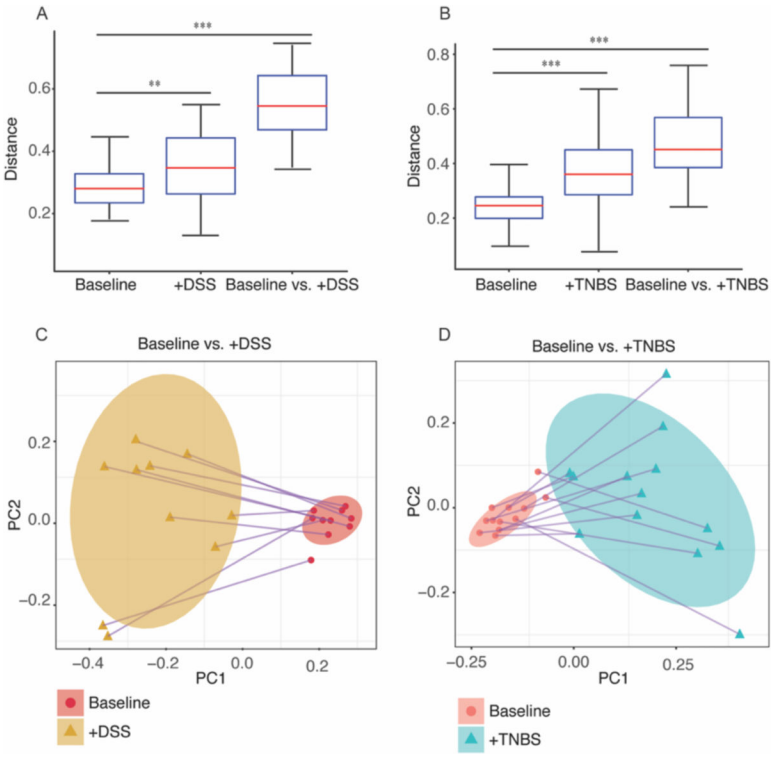

Figure 2.

Beta-diversity of microbiota in IBD model rats.

A, B. Boxplots of the unifrac distances within and between baseline and inflammation groups. **(p-value <0.01), *** (p-value <0.001)

C, D. Positions of paired samples in UC and CD model are shown by the PCoA plots. The statistical ellipses are fitted to the range of scatters in each condition. The paired samples at baseline and inflammation condition are linked by purple lines.

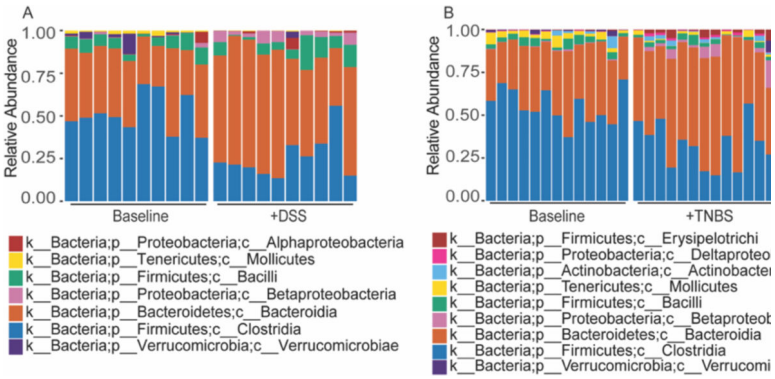

Figure 3.

Relative abundance of taxonomy composition at class level. Each color of the stacked bar represents a class. with the least 0.5% of the total removed. Each column represents the sample at baseline or after IBD induced, the paired samples are in the same order under both conditions.

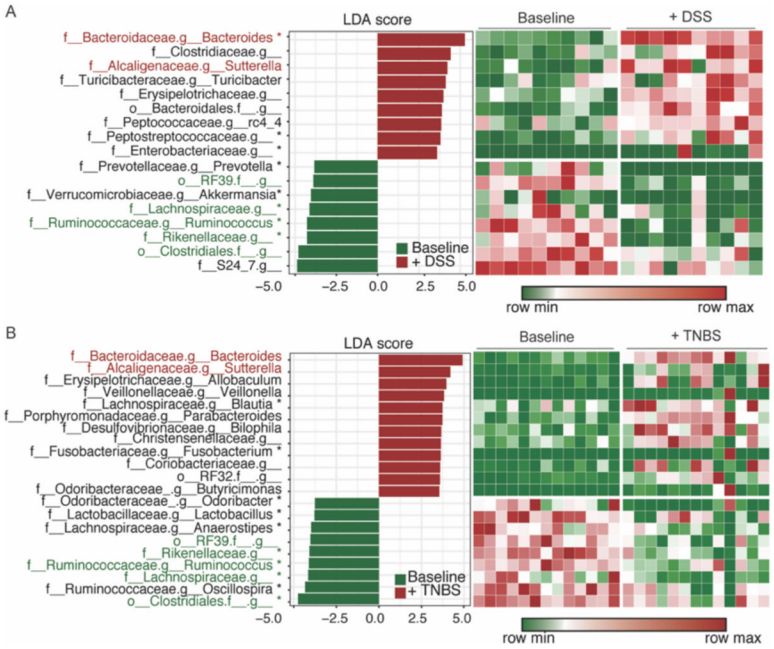

Figure 4.

Microbiome signature of significantly differential taxonomies

The results of differential analysis on taxonomies are displayed as LDA score (threshold is set as 3, p<=0.05). Positive values represent the taxonomies are enriched when inflammation induced, while negative values represent that at baseline. The relative abundance of each taxonomy is quantile normalized by rows, shown in heatmaps as the color range between row min and row max. The taxonomy names assigned to red or green are intersected by inflammation or baseline enhanced bacteria respectively. In addition, the taxonomy names marked with '*' are consistent with clinical reports, sharing the same signature with the corresponding disease (UC and CD).

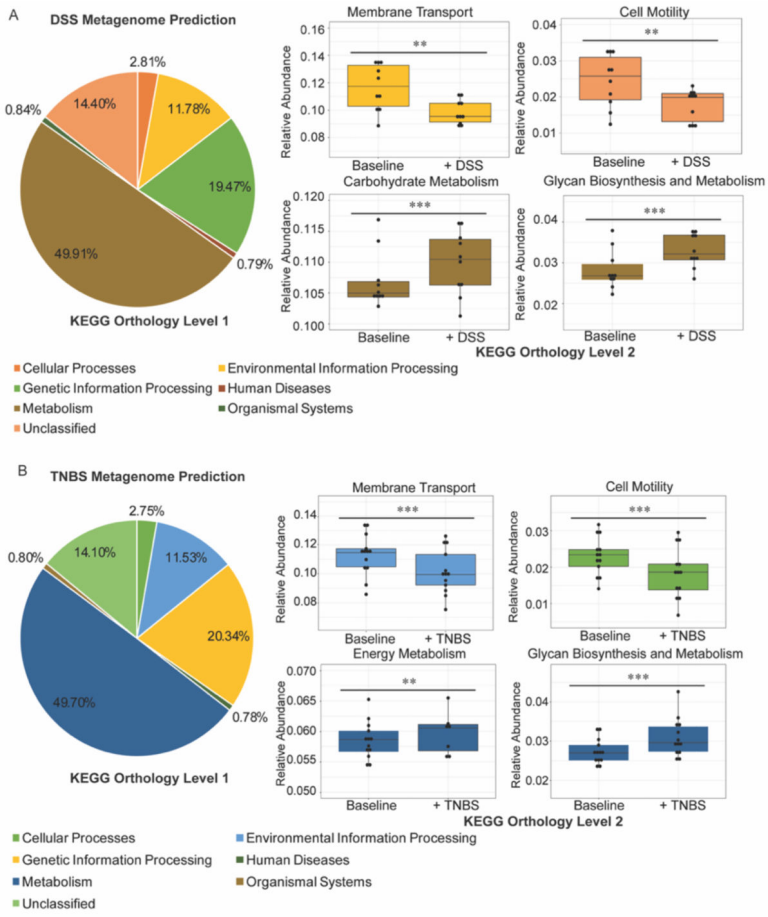

Figure 5.

Metagenome of DSS and TNBS models were predicted by KO functions at level 1 and 2. The pie charts stand for the composition of KO level 1 functions at inflammation status. The right panels of boxplots indicate the most 2 different level 2 functions under health or IBD-induced condition. The colors filled in boxes are corresponding to the colors of level 1 function slides in pie charts.