

LETTER TO THE EDITOR



Fecal microbiota transplantation as a novel approach for the treatment of atopic dermatitis

Dear Editor,

Fecal microbiota transplantation (FMT) is a kind of biological therapy that involves transplanting the intestinal microbiota from a healthy individual into a patient's gastrointestinal tract, and it has a significant curative effect on patients with digestive symptoms.¹ Atopic dermatitis (AD) is a chronic, highly pruritic skin disorder and its pathogenesis is complex, with many influencing factors, including the gut microbiota and immune modulation, which remain poorly understood. A previous study has reported that probiotic treatment in patients with AD exerted its therapeutic effects through the manipulation of gut microbiota diversity.² Moreover, Kim and colleagues have shown that the restoration of the gut microbiota via FMT significantly ameliorated AD in mice.³ Given that the gut microbiota has been shown to be associated with AD, it is important to explore FMT as a potential treatment for AD, especially in AD patients with functional gastrointestinal symptoms.

A 31-year-old female patient was admitted to our hospital in November 2019 after she was diagnosed with AD and functional constipation. Prior to hospital admission, she visited our hospital with the chief complaint of difficulty with defecation for nearly 6 months. The main purpose of this hospitalization was to solve the problem of functional constipation through FMT. Physical examination identified a diffuse rash and desquamation on the trunk of the body, and partially scabbed skin lesions (Figure 1a). The patient had a history of AD with an initial diagnosis more than 2 years previously, and she was primarily treated with topical medications (halometasone/triclosan cream, desonide cream) and oral medications (clarityne, compound glycyrrhizin tablets) at a local hospital. The patient reported seafood allergies. Fresh healthy donor feces were collected from a 28-year-old male college student, and were purified using the GenFMTer automatic purification system (FMT Medical Co. Ltd, Nanjing, Jiangsu, China), according to the manufacturer's protocol.⁴ FMT was attempted in the patient and was performed once every other day for a total of three transfers. Notably, the patient responded well to FMT treatment, reporting significant improvement in symptoms of chronic constipation (e.g. improved defecation time [<10 min] and increased Bristol stool score [4–5 points]). To our surprise, the patient's skin rash disappeared on completion of the third FMT. The follow-up examination in December 2019 showed complete recovery from functional constipation and AD (Figure 1a).

Metagenomic sequencing (whole-genome sequencing) using the fecal samples and untargeted metabolomics analysis of serum

samples were performed before and after FMT (the detailed method of metagenomic analysis is described in the Supporting Information). At the genus level (Figure 1b), the relative abundances of *Parabacteroides*, *Eisenbergiella*, *Alistipes*, *Eubacterium*, *Sutterella*, *Phascolarctobacterium*, and *Bilophila* increased, while those of *Lachnospiraceae*, *Bacteroides*, *Candidatus*, *Stoquefichus*, *Blautia*, *Lachnospiraceae*, *Tyzzelerella*, *Ruminococcus*, and *Firmicutes* decreased. At the species level (Figure 1c), the relative abundances of *Bacteroides*, *sartorii*, *Bacteroides*, *sp.*, *3_1_33FAA*, *Eubacterium*, *sp.*, *CAG:38*, *Alistipes*, *putredinis*, *Sutterella*, *sp.*, *AM11-39*, *Bacteroides*, *sp.*, *CAG:754*, *Bacteroides*, *congongensis*, *Bacteroides*, *acidifaciens*, *Bacteroides*, *sp.*, *AM16-24*, *Bacteroides*, *sp.*, *AF25-38ac*, and *Eisenbergiella*, *massiliensis* increased, while those of *Blautia*, *sp.*, *N6H1-15*, *Candidatus*, *Stoquefichus*, *sp.*, *KLE1796*, *Clostridium*, *sp.*, *CAG:299*, *Clostridium*, *sp.*, *CAG:58*, *Firmicutes*, *bacterium*, *CAG:646*, *Blautia*, *sp.*, *CAG:257*, and *Ruminococcus*, *bromii* decreased. In addition, the differences in microbiota between the healthy donor and patient were compared (Figure 1d,e). In contrast to the patient, the fecal microbiota of the healthy donor were enriched with *Oscillibacter*, *Subdoligranulum*, *Akkermansia*, *Collinsella*, *Gemmiger*, *Ruminococcus*, *Tyzzelerella*, [*Ruminococcus*], *gnavus*, and *Roseburia*, *intestinalis*. However, as this was a case study, we were unable to determine the influence of individual factors. As such, we did not conduct an in-depth analysis to assess whether the changes to the patient following FMT could be similar to a normal state. Considering the changes to the microbiota in the patient, it merits attention that the bacteria with an increased abundance following FMT (e.g. *Bacteroides*, *Alistipes*, *Bilophila*, and other biliophobic bacteria) are mainly probiotics, and are involved in bile acid metabolism, energy metabolism, anti-inflammatory pathways, and immunity.^{5–7} It was also noted that the abundance of bacteria involved in metabolic disorders and those that promote inflammation were decreased after FMT. The data concerning the metagenomic database and serum untargeted metabolomics are summarized in Supporting Information Table S1. Moreover, the Kyoto Encyclopedia of Genes and Genomes analysis was conducted to identify differentially expressed metabolites (Figure 1f), which revealed that 23 metabolites were increased, while 17 metabolites were decreased. Furthermore, glycocholic acid and methylxanthine were identified as key upregulated differentially expressed metabolites, which have been previously associated with mood disorders and gut microbiota changes in IBS.⁸ In addition, the key pathways involved were metabolic and bile

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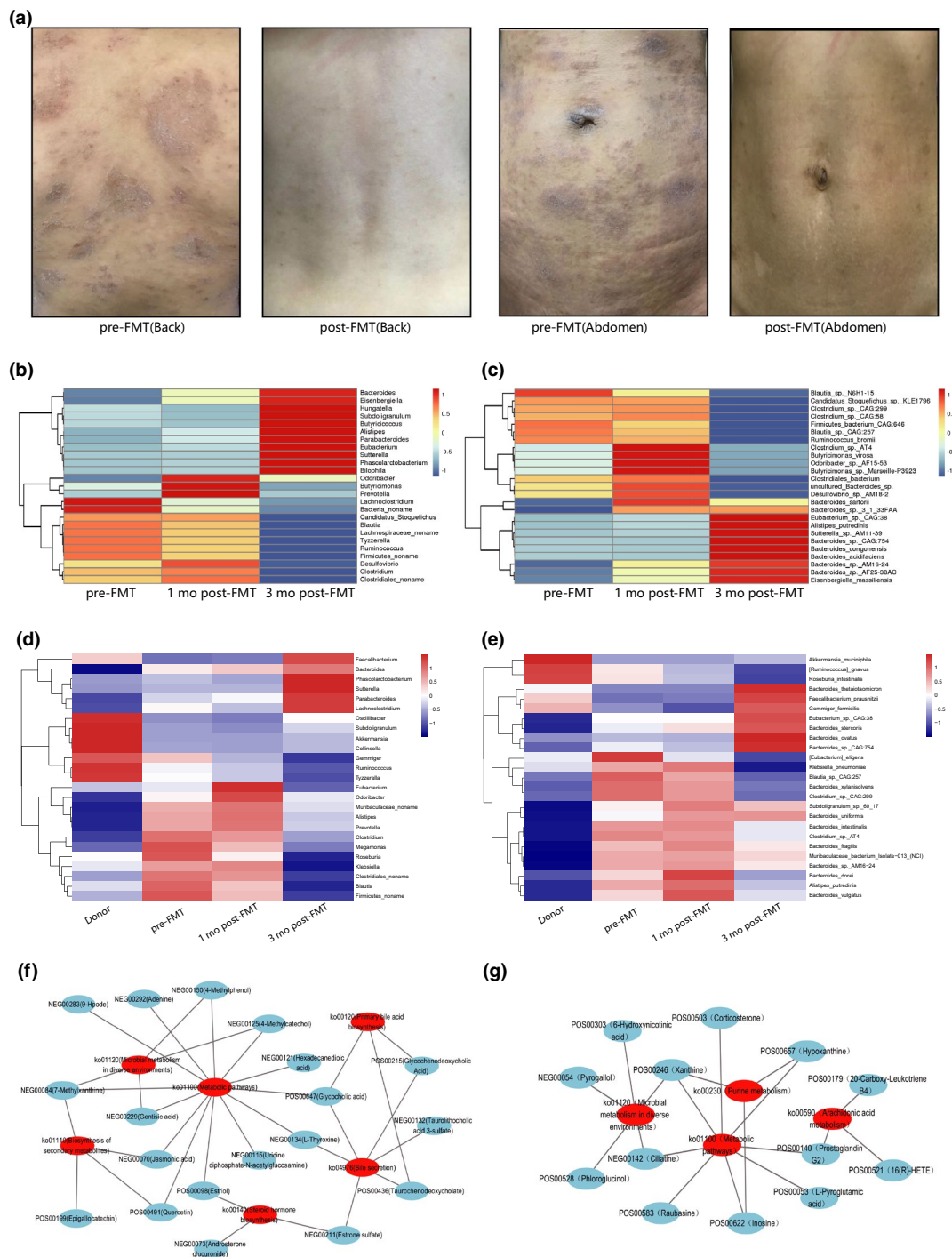


FIGURE 1 Clinical findings, gut microbiota profiles, and metabolite profiles before and after fecal microbiota transplantation (FMT). (a) Clinical findings of atopic dermatitis (AD) before and after FMT. Relative abundance of the gut microbiota (b,d) at the genus level, and (c,e) at the species level. (f) Comparative analysis of metabolites before and after FMT. (g) Molecular pathway analysis

secretion pathways (Figure 1g). Among the differentially expressed metabolites that were downregulated was santhine, which has been positively associated with type-2 diabetes and insulin resistance.⁹

To the best of our knowledge, this is the first report of AD being successfully treated with FMT. Taken together, FMT may exert its therapeutic role in AD through restoration of the gut microbiota, mainly by enhancing the abundance of bacteria related to bile

secretion, energy metabolism, and anti-inflammatory pathways. However, it is still unclear whether FMT is able to directly affect dermatitis. There is the possibility that recovery from constipation might be the important factor for the improvement of dermatitis in this case. Therefore, FMT may not be generally effective for AD, but may be effective for AD patients also suffering from constipation. As such, the effect of FMT in AD may provide a novel therapeutic

option for physicians, but more clinical studies are required for validation.

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CONFLICT OF INTEREST

None declared.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of the article at the publisher's website.

Table S1. Changes of metabolites after FMT and related signal pathways in this case

Increase		Decrease	
POS & NEG	KEGG	POS & NEG	KEGG
POS00098 (Estriol)	ko01100; ko00140	POS00053 (L-Pyroglutamic acid)	ko01100; ko00480
POS00199 (Epigallocatechin)	ko01110	POS00140 (Prostaglandin G2)	ko01100; ko04726; ko00590; ko04611
POS00215 (Glycochenodeoxycholic Acid)	ko04976; ko00120	POS00149 (Bialaphos)	ko01130
POS00370 (Ergothioneine)	ko00340	POS00179 (20-Carboxy-Leukotriene B4)	ko00590
POS00436 (Taurochenodeoxycholate)	ko04976; ko00120	POS00211 (Panthenol)	ko00770
POS00450 (D-Raffinose)	ko02010; ko00052	POS00246 (Xanthine)	ko01100; ko01120; ko01110; ko00230; ko00232
POS00491 (Quercetin)	ko01100; ko01110; ko04152	POS00284 (Xanthurenic Acid)	ko00380
POS00507 (Hippuric acid)	ko00360	POS00303 (6-Hydroxynicotinic acid)	ko01120; ko00760

POS00647 (Glycocholic acid)	ko01100; ko04976; ko00120	POS00503 (Corticosterone)	ko01100; ko00140; ko04925; ko04923; ko05020
NEG00070 (Jasmonic acid)	ko01100; ko01110; ko00592	POS00521 (16(R)-HETE)	ko00590
NEG00073 (Androsterone glucuronide)	ko00140	POS00528 (Phloroglucinol)	ko01120
NEG00084 (7-Methylxanthine)	ko01100; ko01120; ko01110; ko00232	POS00583 (Raubasine)	ko01100; ko01110
NEG00115 (Uridine diphosphate-N-acetylglucosamine)	ko01100; ko01130; ko04931; ko00520; ko00524	POS00622 (Inosine)	ko01100; ko00230
NEG00118 (Hippuric Acid)	ko00360	POS00657 (Hypoxanthine)	ko01100; ko00230
NEG00121 (Hexadecanedioic acid)	ko01100	POS00672 ((R)-(+)-2-Pyrrolidone-5-carboxyli c acid)	ko00471
NEG00125 (4-Methylcatechol)	ko01100; ko01120; ko01220	NEG00054 (Pyrogallol)	ko01120
NEG00132 (Tauroolithocholic acid 3-sulfate)	ko04976	NEG00142 (Ciliatine)	ko01100; ko0112; ko02010
NEG00134 (L-Thyroxine)	ko01100; ko04976; ko04080; ko00350; ko04918; ko04919; ko05320		
NEG00150 (4-Methylphenol)	ko01100; ko01120; ko01220; ko04974		

NEG00211 (Estrone sulfate)	ko04976; ko00140
NEG00229 (Gentisic acid)	ko01100; ko01120; ko00350
NEG00283 (9-Hpode)	ko01100; ko00591
NEG00292 (Adenine)	ko01100; ko00230

Supplementary Methods

Metagenomic analysis

Sample quality control

DNA quality control was performed with several steps, including: 1) DNA degradation degree and potential contamination was monitored on 1% agarose gels; 2) DNA purity (OD₂₆₀/OD₂₈₀, OD₂₆₀/OD₂₃₀) was verified using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA); and 3) DNA concentration was measured using the Qubit® dsDNA Assay Kit with the Qubit® 2.0 Fluorometer (Life Technologies, CA, USA). The OD values were between 1.8-2.0, and DNA contents above 1 µg were used to construct the library.

Library construction

DNA (1 µg per sample) was used as the input material for DNA sample preparations. Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's recommendations and index codes were added to attribute sequences to each sample. Briefly, the DNA sample was fragmented by sonication to a size of 350 bp, then DNA fragments were end-polished, A-tailed, and ligated with the full-length adaptor for Illumina sequencing with further PCR amplification. Finally, PCR products were purified (AMPure XP system) and libraries were analyzed for size distribution using the Agilent2100 Bioanalyzer and quantified using real-time PCR.

Sequencing

The clustering of the index-coded samples was performed on a cBot Cluster Generation System according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina HiSeq platform and paired-end reads were generated.

Clean reads filtering

Quality trimming is an essential step to generate a high confidence of variant calling. As such, raw reads were processed to obtain high quality clean reads according to three stringent filtering standards: 1) removing reads with $\geq 10\%$ unidentified nucleotides (N); 2) removing reads with $> 50\%$ bases having a Phred quality score ≤ 20 ; and 3) removing reads aligned to the barcode adapter.

Assembly

Illumina sequence data of each sample were assembled individually using MEGAHIT^[1] stepping over a k-mer range of 21 to 99 to generate the sample-derived assembly. Overall *de novo* assembly statistics were evaluated as a combination of percent paired or singleton reads realigning to the assembly using BWA^[2]. The unmapped reads of each sample were pooled to the re-assembly using MEGAHIT to generate the mixed assembly. The sample-derived assembly and mixed assembly were combined to obtain the final assembly for further analysis. Gene prediction and gene catalogue Open Reading Frame (ORF) were predicted based on the final assembly contigs (> 500 bp) using MetaGeneMark^[3]. The predicted ORF ≥ 300 bp in length from all samples were pooled and combined based on $\geq 95\%$ identity and 90%

read coverage using CD-HIT^[4] to reduce the number of redundant genes for the downstream assembly step. The reads were re-aligned to predicted genes using BWA to count read numbers. A final gene catalogue was obtained from non-redundant genes with gene read counts > 2.

Taxonomic profiling

After read filtering, clean reads were used to generate a taxonomic profile using a k-mer-based taxonomic classifier, MetaOthello^[5]. MetaOthello employs a novel data structure, called l-Othello, to support efficient querying of taxonomic information from NCBI using read k-mer signatures 31 bp in length.

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