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Microbial alteration of small bowel stoma effluents and colonic feces in infants with short bowel syndrome☆,☆☆,★

Tian Zhang [a,d,e,1], Ying Wang [b,d,e,1], Weihui Yan [b,d,e], Lina Lu [b,d,e], Yijing Tao [b,d,e], Jie Jia [c,d,e,*], Wei Cai [a,d,e,*]

[a] Department of Pediatric Surgery, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China
[b] Division of Pediatric GI and Nutrition, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China
[c] Department of Nutrition, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[d] Shanghai Institute for Pediatric Research, Shanghai, China
[e] Shanghai Key Laboratory of Pediatric Gastroenterology and Nutrition, Shanghai, China

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ABSTRACT

Background and aim: Studies about differences in microbial communities between the small intestine and colon in infants with short bowel syndrome (SBS) are rare. We aimed to characterize the bacterial diversity of small bowel stoma effluents and feces of SBS infants.
Methods: Seven SBS infants were enrolled in this study and provided two samples (one from the stoma and the other from the anus) each. Eleven age-matched healthy controls were recruited to provide one fecal sample each. 16S rRNA gene MiSeq sequencing was conducted to characterize the microbiota diversity and composition.
Results: The bacterial diversity of the stoma effluents was significantly higher than that in the feces of SBS infants. Proteobacteria dominated in both the stoma effluents and colonic. *Acinetobacter* ($P = 0.004$), *Klebsiella* ($P = 0.015$), *Citrobacter* ($P = 0.019$), and *Lactobacillus* ($P = 0.030$) were more abundant in stoma effluents compared to feces of SBS patients, while Bacteroidetes, *Bifidobacterium* and *Veillonella* were less abundant in stoma effluents. Significantly higher levels of Proteobacteria, *Enterococcus* and lower levels of *Blautia*, *Collinsella*, *Faecalibacterium*, *Veillonella* were present in the fecal samples of SBS patients than those in the healthy controls. Kyoto Encyclopedia of Genes and Genomes pathways related to metabolism and membrane function were depleted in SBS patients.
Conclusions: The predominant intestinal bacterial groups were different in SBS children before and after the fistula closure. Fecal samples of SBS patients featured overabundant Proteobacteria and less SCFA producing bacteria. Depleted functional profiles of the microbiome were found in fecal samples of SBS patients.
Level of evidence: III.

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Intestinal failure (IF) is defined as a deficiency in an intestinal function that limits the capacity to absorb water, electrolytes, and nutrients and requires parenteral nutrition (PN) support [1]. Short bowel syndrome (SBS) is the leading cause of [2]. In children, IF is often caused by small bowel resection resulting from intestinal atresia, necrotizing enterocolitis (NEC), or intestinal pseudoobstruction [3]. Children can be treated with enterostomy for symptom alleviation and to save their lives. An ileostomy or jejunostomy can cause IF even if adequate proximal intestine remains [4]. Pediatric patients who have recently undergone enterostomy, especially those with the stoma at the proximal intestine, are much more likely to suffer from nutrition-associated problems that can influence prognosis considerably [5].

Gut microbiota profoundly influences body homeostasis and is involved in physical functions, such as nutrient absorption, metabolism, immunity, and promoting intestinal mucosa growth and integrity [6]. Gut microbiota mainly matures during the first three years after birth [6,7]. During this period, the gastrointestinal microbial composition of children differs from adults and is less stable. In infants, microbial colonization and evolution are influenced by multiple factors, including delivery mode, dietary regime, antibiotic use, and environmental factors [6]. Recently, a limited number of studies have found that the gut microbiome composition in pediatric patients with SBS is characterized by an increased proportion of Proteobacteria [8,9]. Intestinal dysbiosis in children with SBS is associated with small bowel bacteria overgrowth and impaired prognosis, such as prolonged PN dependency [10], growth

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* Corresponding author:
E-mail addresses: jie.jia@shsmu.edu.cn (J. Jia), caiw204@sjtu.edu.cn (W. Cai).
[1] These authors contributed equally to this work and share the first authorship.

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retardation [11], and high risk of IF-associated liver disease [10]. However, studies about the microbial diversity of small intestine stoma output are rare. Barrett et al. found that the microbiota of the ileum/colon in infants who undergo ileostomy/colostomy exhibited large alterations at the genus level, including the absence of the strict anaerobes, *Bacteroides*, and *Clostridium* instability [12]. Host–microbe interactions in the small bowel have a great impact on development, physiology, and homeostasis of the human body [13]. Clarifying the microbial changes of stoma output may help to provide new therapies that ameliorate abdominal symptoms and promote the intestinal adaptation of patients with small intestinal stomas. Thus, this study aimed to investigate the microbial diversity of small bowel stoma effluents and identify alterations of the fecal microbiota in SBS infants compared with controls.

1. Materials and methods

1.1. Ethics

This study was approved by the Xinhua Hospital Ethics Committee (XHEC-C-2018-081). Written informed consent for sample collection was obtained from the patients' parents or guardians. This study was also registered in ClinicalTrials.gov (Identifier: NCT03590418).

1.2. Patients and controls

Seven infants with SBS were enrolled for intestinal rehabilitation at the Department of Pediatric Surgery of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine from September 2016 to December 2017. These infants all had small bowel stoma resulting from intestine resection caused by NEC, intestinal atresia, and intestinal perforation (Table 1). Inclusion criteria of children were: 1) diagnosed with SBS (a residual proximal small bowel length less than 25% of that predicted for gestational age or required parenteral nutrition for more than 42 days [14]); 2) no symptoms like abdominal distension, vomiting, diarrhea, and fever for at least one week [15]; 3) stoma output maintained less than 3 mL/kg/h [16]. All patients underwent fistula closure in our center and were discharged from the hospital when stable (no clinical symptoms, such as fever, diarrhea, abdominal distention, and vomiting, and good tolerance of EN).

Patients' demographic parameters and medical history records were collected. Serum biochemical indices were examined weekly. Patients' body weight, nutrition support information, and stoma or stool output were recorded daily.

Eleven age-matched healthy infants were recruited from the community in the Yangpu District of Shanghai by contacting their parents or guardians during the period of the present study. The inclusion criteria of healthy controls included: 1) no digestive tract disorders or congenital disabilities; 2) no treatment with probiotics, antibiotics, or other drugs within one month.

1.3. Sample collection

All samples were collected when the SBS patients had no symptoms like abdominal distension, vomiting, diarrhea, and fever; the stoma output maintained less than 3 mL/kg/h; they had no complications, such as catheter-related infection and IF-associated liver disease, that occurred in at least one week, and the proportion of PN intake was less than 20% of total caloric need. One stoma sample was collected after the enterostomy, and one fecal sample was collected after the fistula closure (Table 1). Stoma effluents were collected immediately after being excreted into the bag. One fecal sample was provided from each control child. All samples were collected immediately into a sterile tube after defecation and then stored at −20 °C. They were then transferred and stored at −80 °C within 24 h.

1.4. Stool bacterial DNA extraction and polymerase chain reaction (PCR) amplification

Microbial DNA was extracted from each fecal sample using the QIAampFast DNA Stool Mini Kit (Qiagen, Hilden, Germany). The concentration of extracted DNA was measured using a Nano-Drop 2000 spectrophotometer (Thermo Scientific, USA). PCR amplification of the 16S rRNA gene was performed according to the literature [17] using PCR primers specific for the 515–806 (V3–V4) regions. The PCR assays were carried out in triplicate as follows: 20-ml reaction solutions with 10 ng of template DNA, 4 μl of PCR reaction buffer, 0.4 mM of each primer, 2.5 mM of deoxyribonucleotide triphosphate (dNTPs), and 0.5 U of TransStartFastPfu DNA polymerase (TransGen Biotech). The conditions of PCR were as follows: 95 °C for 4 min, followed by 27 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and a final extension of 72 °C for 10 min.

1.5. Illumina MiSeq sequencing

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluo-ST (Promega, Madison, WI, USA). Purified amplicons were pooled in equimolar and paired-end sequences (2 × 250) on an Illumina MiSeq platform.

1.6. Processing of sequencing data

Raw Fastq files were demultiplexed, quality-filtered using QIIME (version 1.17) with the following criteria: (1) 300 bp reads were truncated at any site receiving an average quality score < 20 over a 50 bp sliding window, discarding the truncated reads that were shorter than

Table 1
Baseline characteristics of SBS patients.

Patients Number	Sex	Age 1 (day)	Age 2 (month)	Age 3 (month)	Weight (kg)	Gestational age (week + day)	Diagnosis	L (cm)	Ileocecal valve	Colostomy	Antibiotics when stoma effluents collected	Duration of PN (day)
SBS1	F	12	3	7	2.65	30 + 4	NEC	80	YES	NO	Using	71
SBS2	F	3	8	13	4	28	Intestinal perforation	80	YES	NO	No use	87
SBS3	M	4	3	3.5	3.3	39 + 2	Intestinal atresia	50	YES	NO	Using	91
SBS4	F	12	11	12.5	8	29 + 5	NEC	50	YES	NO	No use	128
SBS5	M	3	5	9	3.5	33 + 1	NEC	75	NO	YES	Used	145
SBS6	M	15	2	7	2.95	30 + 1	Intestinal atresia	65	YES	NO	Used	46
SBS7	M	1	5	5.5	4.5	40	NEC	70	YES	NO	Using	150

Age 1, age of the initial surgery; Age 2, age of the stoma samples collecting; Age 3, age of the fecal samples collecting; L represents the length of proximal small bowel measured after patients accepted enterostomy; Used means patient had received antibiotics previously but was not taking them at the time of sample collection.
M, male; F, female; PN, parenteral nutrition; SBS, short bowel syndrome; NEC, necrotizing enterocolitis.

50 bp; (2) exact barcode matching, two nucleotide mismatch in primer matching, and reads containing ambiguous characters were removed; and (3) only sequences that overlapped longer than 10 bp were assembled according to their overlap sequence. Reads that could not be assembled were discarded. Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE version 7.1 (http://drive5.com/ uparse/), and chimeric sequences were identified and removed using UCHIME (http://drive5.com/index.htm). The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by RDP Classifier (http:// rdp.cme.msu.edu/) against the SILVA (SSU117/119)16S rRNA database.

1.7. Function predicting

Bacterial functions were predicted by the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) package [18]. Briefly, the procedure was carried out as follows: OTU picking against the Greengenes database (V.13.5) was performed using Mothur. At the same time, a BIOM table compatible with the PICRUSt program was generated. Metagenome prediction was made with the OTU table after normalizing for 16S copy number. The predicted functional gene was annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway [19].

1.8. Statistical analysis

Alpha-diversity parameters, including Sobs, Chao, Simpson, and Shannon, and a sequencing depth index (Good's coverage), were calculated using the Mothur software [20]. Beta diversity measurements were calculated as described [21], and partial least squares discriminant analysis (PLS-DA) based on OTU compositions were determined to evaluate the inter-group difference using the Vegan package of R software (Vienna, Austria). The Wilcoxon rank-sum test was performed to evaluate the differences in microbiota distribution and abundance of level 2 functional pathways between two groups (feces of SBS infants vs. feces of healthy controls and stoma effluents vs. colonic feces for SBS infants). Spearman's test was performed to analyze the correlation between proportions of bacteria in stoma effluents with indices of intestinal adaptation. The statistical analysis described above was performed using SPSS version 22 (Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

2. Results

2.1. Clinical characteristics of SBS infants and controls

There were no differences in age and gender between SBS infants and control infants. Average body weight and gestational age of SBS patients were significantly smaller than those of control infants ($P = 0.003$, $P = 0.022$, respectively, Table 2). Five of seven patients were using or had used antibiotics within the two months before enrollment into this study (Table 1). Patient SBS 6 was on PN with a 20% of caloric requirement, and the others had total EN tube feeding at the time the stoma sample was collected. The lipid emulsion we used in PN was Lipofundin (medium chain triglyceride [MCT]/soybean oil, B. Braun). There were no patients on PN when colonic feces were collected. All patients except patient SBS 1 (breast milk feeding) accepted extensively hydrolyzed formula feeding. The seven patients were diagnosed with SBS, which was caused by NEC ($n = 4$), intestinal atresia ($n = 2$), or intestinal perforation ($n = 1$). One patient underwent colostomy and had no ileocecal valve. No physical indices, including C-reactive protein, albumin, serum direct bilirubin, and the number of leukocytes significantly varied between the two sample collection time points.

Table 2
Comparison of baseline characteristics between the healthy controls and the SBS patients.

Parameters	SBS Patients ($n = 7$)	Healthy Controls ($n = 11$)	P value
Age (month)	5.3 ± 3.2	5.8 ± 2.4	0.694[a]
Gender (male/female)	4/3	6/5	0.916[b]
Body weight (kg)	4.1 ± 1.8	6.8 ± 1.3	0.003[a]
Gestation age (week)	33.0 ± 4.8	38.5 ± 0.9	0.022[a]

Age, body weight, and gestation age are presented as mean ± standard deviation.
SBS, short bowel syndrome.
[a] Differences between the two groups were analyzed by unpaired t-test.
[b] Differences between the two groups were analyzed by chi-squared test.

2.2. Bacterial diversity between patients with SBS and controls

A total of 883 OTUs were identified at 97% similarity level in all samples. The value of Good's coverage for each group was over 99%. We compared the community richness (indicated as the Sobs and Chao indexes), and community evenness (indicated as the Shannon and Simpson indexes) in stoma effluents, feces of SBS patients, and fecal samples of controls. Higher bacterial richness was demonstrated in both the stoma effluent samples obtained from SBS patients and fecal samples of controls than in the fecal samples of SBS patients (Fig. 1A). No statistical differences in the evenness parameters (Shannon and Simpson) were shown when comparing fecal samples in SBS infants to healthy controls or comparing stoma effluents to fecal samples in SBS infants (Fig. 1B). For beta diversity analysis, the microbiota compositions were analyzed and compared with the relative abundance of OTUs by using the Bray–Curtis distance matrix for each group. Results of PLS-DA revealed dissimilarities in bacterial community compositions among three groups, and most samples of each group seemed clustered together (Fig. 2A). Analysis of similarities (ANOSIM) showed that significant difference was detected in the Bray–Curtis distance ($P = 0.001$) among the three groups, suggesting that the separation among three groups was good, and intergroup variations were significantly greater than intragroup variations (Fig. 2B).

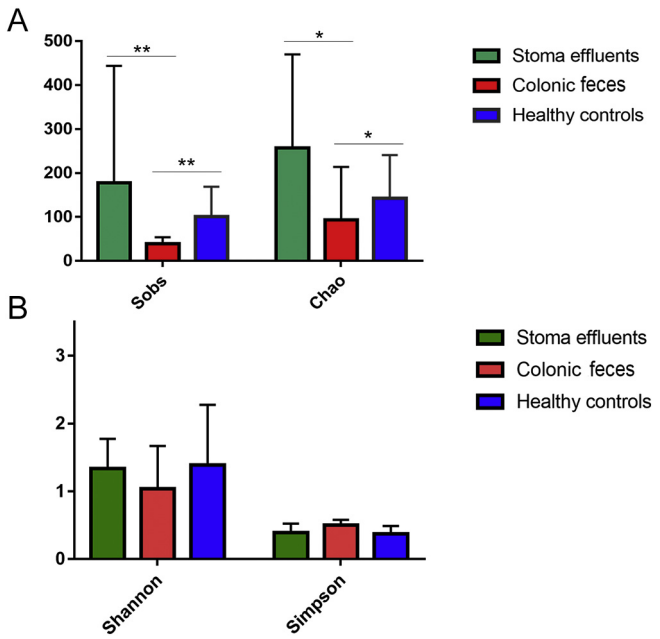

Fig. 1. Microbial alpha diversity among stoma effluents of SBS group, colonic feces of SBS group and feces of healthy controls. (A) Richness parameters Chao and Sobs. (B) Evenness indices Shannon and Simpson. Values of Sobs, Chao, Shannon, and Simpson were presented as median with interquartile range. The Wilcoxon sum-rank test analyzed differences between the two groups. *Significantly different ($P < 0.05$); **Significantly different ($P < 0.01$).

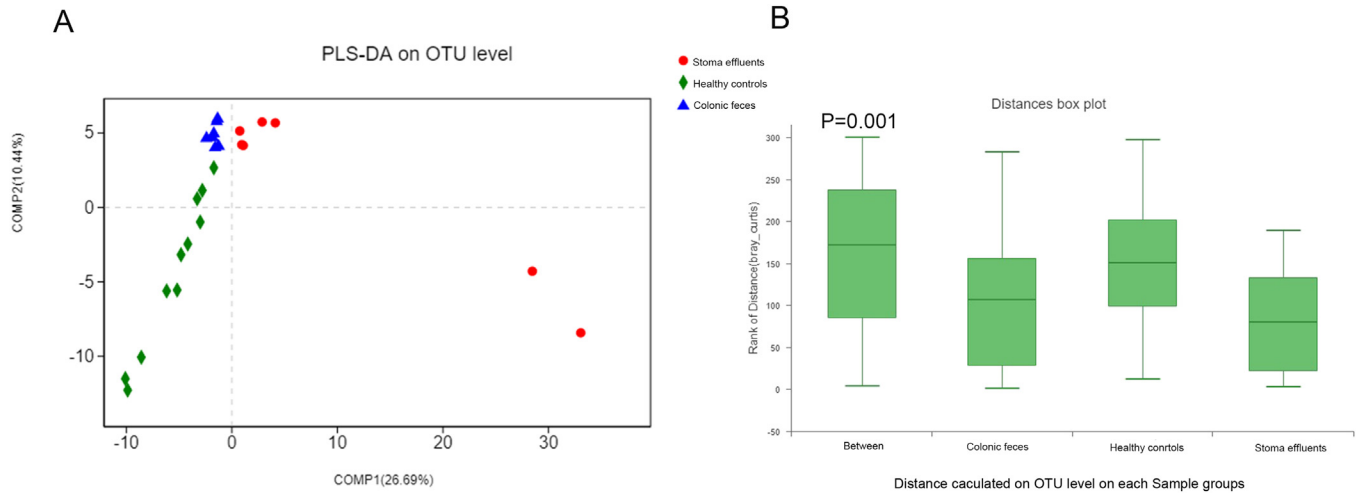

Fig. 2. Fecal bacterial beta diversity in stoma effluents of SBS group, colonic feces of SBS group, and feces of healthy controls. (A) PLS-DA with Bray–Curtis distance matrix. (B) Significant inter-group difference detected by ANOSIM analysis in Bray–Curtis distance. PLS-DA, partial least squares discriminant analysis; ANOSIM, Analysis of similarities. ANOSIM was performed with Wilcoxon sum-rank analysis.

2.3. Bacterial composition differences in different groups at the phylum level

The dominant phyla of three groups were Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes, but in different proportions (Fig. 3A). Compared with healthy controls, SBS children presented significantly higher levels of Proteobacteria in colonic feces ($P = 0.037$, Table 3). A higher level of Bacteroidetes was found in stoma effluents than in colonic feces in SBS patients ($P = 0.030$, Table 3).

2.4. Bacterial composition differences in different groups at the genus level

Bacterial compositions of three groups at the genus level were shown in Fig. 3B. Genus-level bacterial taxa distinguishing feces of the SBS group and controls included *Veillonella*, *Collinsella*, *Faecalibacterium*, *Blautia,* and *Megasphaera*, which were more abundant in controls but depleted in SBS patients, suggesting that these genera were indicative of a healthy state relative to SBS (Table 3). In contrast, genera of *Escherichia-Shigella* and *Enterococcus* were dominant in SBS patients. Stoma effluents presented higher levels of *Klebsiella*, *Lactobacillus*, *Citrobacter*, *Acinetobacter,* and *Rhodococcus* and lower levels of *Veillonella* and *Bifidobacterium* than SBS colonic feces (Table 3).

2.5. Functional analysis of gut microbiome between SBS patients and controls

Based on the 16S rRNA sequencing data, we identified distinct differences in the functional microbial pathways between the SBS group and control group. At KEGG level 2, healthy infants presented with more functions involved in energy metabolism and other cellular functions such as cellular processes and signaling compared to the SBS group (Fig. 4A). Furthermore, levels of functions responsible for amino acid metabolism, lipid metabolism, transcription, and some others were higher in stoma effluent compared with those in colonic feces in SBS patients (Fig. 4B).

3. Discussion

This study gives a new insight into the different microbial compositions of intestinal effluents and feces in SBS infants compared with the healthy controls. We found that the microbial diversity (including richness and evenness) of colonic feces in SBS infants was reduced compared with controls, consistent with previous results in SBS patients, including adults [22] and children [9]. A significantly higher level of

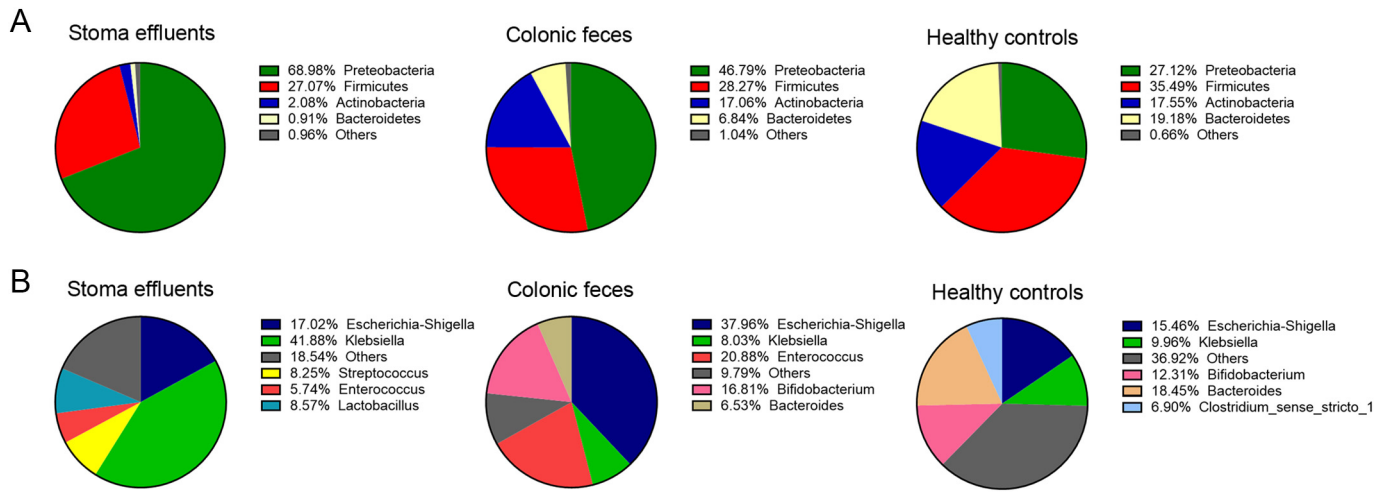

Fig. 3. Pie charts representing the overall microbial composition of the stoma effluent samples obtained from the SBS group, colonic feces of SBS infants and feces of healthy controls. (A) At the phylum level. (B) At the genus level. Proportions of bacteria and means are shown in these charts.

Table 3
Comparison of predominant bacterial compositions in stoma effluents of SBS group, feces of SBS group and feces of healthy controls.

Bacterial Taxa	Stoma effluents of SBS patients (%)	Colonic feces of SBS patients (%)	Healthy controls (%)	P1 value	P2 value
Phylum level					
Proteobacteria	68.98 ± 24.25	46.79 ± 25.71	27.12 ± 33.71	0.037	0.125
Bacteroidetes	0.91 ± 1.50	6.84 ± 18.07	19.18 ± 29.84	0.124	0.030
Firmicutes	27.07 ± 23.99	28.27 ± 22.74	35.49 ± 26.14	0.469	0.898
Actinobacteria	2.08 ± 2.36	17.06 ± 16.70	17.55 ± 19.96	0.856	0.125
Genus level					
Collinsella	0.00 ± 0.00	NA	5.05 ± 8.69	0.027	0.173
Faecalibacterium	0.05 ± 0.09	NA	3.01 ± 7.30	0.027	0.075
Blautia	0.03 ± 0.05	0.00 ± 0.00	1.23 ± 2.39	0.004	0.262
Megasphaera	0.02 ± 0.02	0.00 ± 0.00	0.72 ± 1.51	0.029	0.101
Bifidobacterium	0.37 ± 0.77	16.81 ± 16.51	12.31 ± 12.93	0.587	0.021
Citrobacter	1.87 ± 4.68	0.30 ± 0.79	0.02 ± 0.02	0.076	0.019
Klebsiella	41.88 ± 32.89	8.03 ± 21.21	9.96 ± 20.36	0.147	0.015
Lactobacillus	8.57 ± 17.66	0.09 ± 0.15	5.78 ± 16.38	0.174	0.030
Rhodococcus	1.13 ± 1.19	0.01 ± 0.01	0.04 ± 0.06	0.011	0.021
Acinetobacter	1.50 ± 2.67	NA	0.21 ± 0.59	0.027	0.004
Enterococcus	5.74 ± 10.25	20.88 ± 23.93	0.20 ± 0.29	0.002	0.125
Veillonella	1.72 ± 1.66	2.04 ± 5.36	5.45 ± 13.44	0.018	0.040
Escherichia-Shigella	17.02 ± 26.02	37.96 ± 30.27	15.46 ± 23.01	0.057	0.097
Bacteroides	0.05 ± 0.07	6.53 ± 17.26	18.45 ± 29.86	0.102	0.433
Streptococcus	8.25 ± 18.41	1.12 ± 1.67	0.45 ± 0.57	0.786	0.250

Proportions of bacteria are presented as mean ± standard deviation.
The Wilcoxon rank-sum test analyzed microbial differences among the three groups.
P1, the P value of colonic feces of SBS patients vs. healthy controls; P2, the P value of stoma effluents vs. colonic feces of SBS patients; SBS, short bowel syndrome; NA, not available.

Proteobacteria was found in SBS infants than in healthy controls, similar to previous studies in SBS patients [11,22]. Proteobacteria normally accounts for a small proportion of the total gut microbiota in healthy people, and most members of Proteobacteria are facultative or obligate anaerobic. The overgrowth of Proteobacteria in SBS patients may result from gastrointestinal environmental changes, such as pH value and intestinal structure [23]. Ralls et al. also proved that the use of PN and deprival of EN could result in the overabundance of Proteobacteria in adults and pediatric patients with small bowel resection [24]. Overgrowth of Proteobacteria increased intestinal permeability and has been strongly associated with liver steatosis and portal and intestinal inflammation in patients receiving PN[9]. This may be a reason that an ileostomy sited distally enough can cause functional SBS, even if adequate intestine remains. *Enterococcus* can always produce lots of harmful and proinflammation chemicals [25,26] and was found significantly increased in the colonic feces of SBS patients. Short chain fatty acids (SCFAs) were predominant metabolites of gut microbiota and exerted an anti-inflammatory effect in the gut by regulating the function of regulator T cells [27]. However, lower levels of SCFA producing genera, such as *Veillonella*, *Blautia,* and *Faecalibacterium,* were depleted in SBS patients in the present study, further suggesting the dysbiosis of gut microbiota, although the continuity of intestine has been reconstructed in the SBS patients.

Next, we compared the gut microbiota before and after fistula closure and found that the microbial richness of the small intestinal stoma effluents was significantly higher than that of fecal samples in SBS infants. The previous study found that elevated oxygen concentrations near patients' ileostomies could increase the relative abundance of Enterobacteriaceae, while the microbial community returns to its normal composition after fistula closure [28]. At the same time, antibiotic treatment could generate a micro-aerobic niche for Enterobacteriaceae [29]. In our study, we also observed a higher abundance of Enterobacteriaceae in stoma effluents than that in the SBS fecal samples (data not shown). The potential mechanisms of high microbial richness and overgrowth of Proteobacteria in the stoma effluents still need to be explored in the future. Additionally, stoma effluents presented higher levels of *Acinetobacter, Citrobacter,* and *Klebsiella*, which were all members of Proteobacteria. Expansion of the small intestinal Proteobacteria in patients with SBS is rarely reported. *Citrobacter* can be found in the human intestine and is rarely the source of illnesses but also could result in infant meningitis and sepsis [30]. As the commensal gut bacteria, *Klebsiella* species can also behave as opportunistic pathogens [31]. SBS patients have a high risk of infections and often require oral or intravenous antibiotic treatments. There has been evidence that antibiotic exposure in children increased the level of *Klebsiella* in the gastrointestinal tract [32].

In the current study, a depleted carbohydrate metabolism pathway module with increased Enterobacteriaceae and decreased Firmicutes was shown in the fecal samples of SBS patients compared with healthy controls, consistent with the previous study in SBS patients [11]. Piper et al. found that glycolysis, gluconeogenesis, and pentose-phosphate pathway modules were depleted in children with SBS, which were explained by Enterobacteriaceae enriched gut microbial composition [11], as many members of Enterobacteriaceae use the Entner–Doudoroff pathway for glucose metabolism and are unable to catabolize through glycolysis [33]. Interestingly, pathways responsible for amino acid metabolism, lipid metabolism, membrane transport, and gene transcription were dominant in stoma effluents, compared with those in feces of SBS patients in our study, suggesting that the small bowel microbiota was more involved in the nutrient digestion and metabolism than colonic bacteria. There has been evidence that microbiota in the small bowel interacts with the host's metabolism through a variety of physiological processes [34,35]. Small intestine microbiota could regulate digestive and absorptive adaptive responses to lipid intake [13]. The change of microbiota in the proximal small intestine played a vital role in metabolic diseases like type II diabetes [36]. The function of the small intestinal microbiota of SBS patients with small bowel stoma needs further investigation.

There were a few limitations to this study. Stoma effluents were collected from the stoma bag. We attempted to collect samples directly from the lumen, which, however, was more invasive for patients with poor compliance. The stoma and fecal samples were collected when SBS patients had no complications with receiving the total EN, or minimum PN support, and there was a relatively long time between the two sample collection points. Including more sampling time like the sample from the stoma just prior to stoma closure and the fecal sample just after the stoma closure may provide more information on microbial changes in relation to location and health status. Also, five of seven patients were using or had used antibiotics intravenously to reduce the risk of infections when the stoma samples were collected. Then, several days of protective antibiotic treatment (cefmetazole) were administered to the patients after they underwent fistula closure. Antibiotics are known to

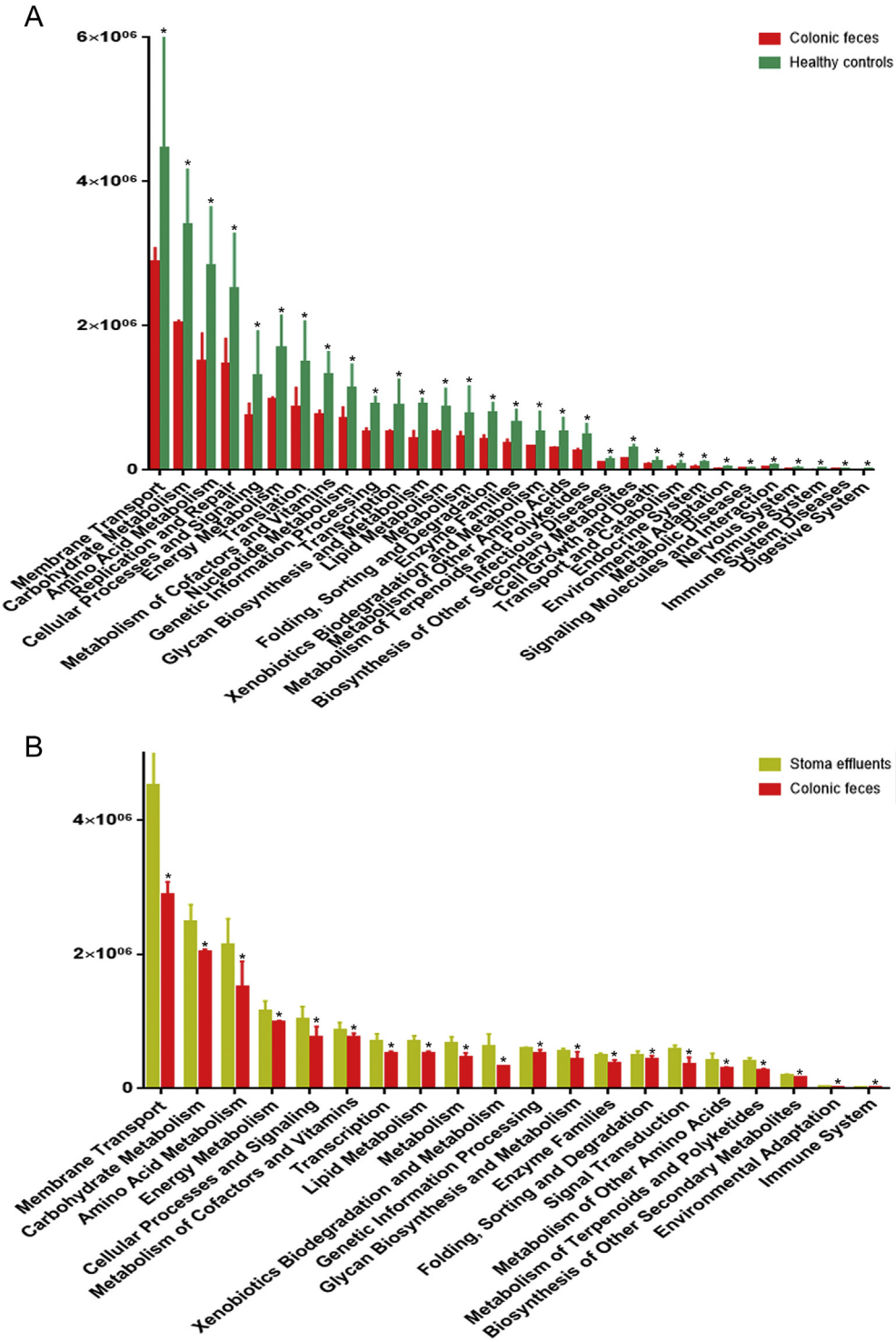

Fig. 4. KEGG pathways exhibit significant between-group differences in relative abundance. (A) SBS feces vs. healthy controls; (B) Stoma effluents vs. SBS feces. Values were presented with medians with interquartile range; Comparisons were performed with Wilcoxon sum-rank analysis. *Significantly different ($P < 0.05$).

have a profound influence on gut microbiota. Therefore, expanding the sample size to include a larger cohort with minimum antibiotic use at multiple times may offer a comprehensive picture of microbial alterations of the intestine in SBS children.

In summary, marked alterations in the microbial compositions of intestinal stoma effluents and colonic feces were demonstrated in SBS pediatric patients in this study. An overabundance of Proteobacteria and reduced levels of SCFA-producing bacteria (*Veillonella*, *Blautia,* and *Faecalibacterium*) were found in the SBS fecal samples compared with the healthy controls. Stoma effluents presented higher levels of *Acinetobacter*, *Citrobacter, Klebsiella,* and *Lactobacillus* than those in feces. Metabolic and cellular membrane function of the fecal microbiome was depleted in SBS patients. Through clarifying the composition and function of gut microbiota in the patients at different stages of the disease, relevant microbial diagnosis or modulation strategies might be precisely determined during early disease stages, promoting intestinal homeostasis in SBS patients.

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