The differences between fecal microbiota and intestinal fluid microbiota in colon polyps

An observational study

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Abstract

Generally, intestinal microbiota can be classified into intestinal cavity microbiota and mucosal microbiota, among which, the former is the default type. This study aimed to identify the differences between fecal microbiota and intestinal fluid microbiota in colon polyps.

This study enrolled patients with colon polyps who met the Rome-III criteria to carry out 16s rDNA gene sequencing. Then, both fresh feces as well as intestinal fluid was sampled. Thereafter, α/β diversities, together with the heterogeneities with regard to microbial function and structure were assessed among those intestinal fluid and fresh feces samples collected.

According to bioinformatics analysis, difference in α-diversity was not statistically significant between intestinal fluid microbiota and fecal microbiota among patients with colorectal polyps (CPs). Non-metric multidimensional scaling analysis of β-diversity revealed that differences were of statistical significance between both groups. In addition, linear discriminant analysis effect size analysis displayed great heterogeneities in intestinal microbiota of both groups, including Firmicutes, Clostridia, and Phascolarctobacterium.

At the phylum level, difference (P = .016) in Spirochaetes was statistically significant between the intestinal fluid group and fecal group. At the family level, differences in Bacteroidaceae, Micrococcaceae, F16, Spirochaetaceae, Entero bacteriaceae, Cardiobacteriaceae, Turkish Spiribacteriaceae, Bifidobacteriaceae, and Dethiosulfovibrioaceae were statistically significant between the 2 groups. At the genus level, there were statistical differences between the 2 groups in terms of Bacteroidetes, Rothia, Actinobacillus, F16, Treponema, Oscilllospira, Turicibacter, Sharpea, Hearnophilus, Veillonella, and Cardiobacterium.

There are statistical differences in the composition between intestinal microbiota and fecal microbiota in CP patients, both of which are equally important and indispensable for analyzing the intestinal microbiota in CP patients.

Abbreviations: CP = colorectal polyp, CRC = colorectal cancer, CY group = colonic fluid group, FB group = fecal bacterial group, GI = gastrointestinal, LEfSe = linear discriminant analysis effect size, OTU = operational taxonomic unit, QIIME = quantitative insights into microbial ecology.

Keywords: colon polyps, fecal microbiota, intestinal fluid microbiota

1. Introduction

Gut microbiota has increasing influence on disease and health. At the beginning of 21st century, microbiota evolves as the “ignored organ”, however, what we have discovered in 2018 greatly alters such opinion, since microbiota is found to represent the biosystem for the intense communication with host organism.[1–3] Over the past 10 years, extensive studies have been conducted on microbiota, which allows for delineating gut microbiota functions and structures. As suggested by recent statistics, the bacterial types in gastrointestinal (GI) tract are comparable to the cells types that constitute human body.[1–3] At present, the greatly diverse human microbiota can be sufficiently...
depicted, but a majority of bacterial species in the context of disorder and health are still unclear. Gut microbial composition in human body has been substantially investigated, however, there are lots to be determined so far, which is partially because that gut microbiota in human body is complicated, and there are excessive uncertain interactions between host and microbe, between microbes and between microbe and the environment. Colorectal cancer (CRC) is a frequently seen cancer worldwide, which is associated with high morbidity and mortality globally. The CRC burden is expected to sharply elevate over the coming 20 years because of the high fat and protein consumption. Gut microbial composition may affect intestinal disorder vulnerability. As a matter of fact, research on CRC cases and animal experiments suggest that gut microbiota is related to CRC, and it helps to identify certain species of bacteria promoting tumor occurrence.

The well-known “Adenoma-Carcinoma Sequence” has been identified to exert an important part during the development of CRC. Based on molecular genetics, the accumulation of germ-like and somatic mutations will induce colonic epithelial hyperplasia and dysplasia, eventually resulting in CRC. In this regard, Tjalsma et al put forward the bacterial driver-passenger model to explain the microbial communities that were involved during CRC occurrence, and it facilitated the “Adenoma-Carcinoma Sequence” genetic paradigm. According to their results, bacterial drivers (also referred to as intestinal bacteria) had colonized in the mucosa of colon, which exhibited the protumorigenic characteristics initiating CRC occurrence.

Nonetheless, it remains unclear about how intestinal cavity microbiota affects the CRC pathogenesis in terms of “Adenoma-Carcinoma Sequence”. What’s more, a majority of existing studies only pay attention to fecal microbiota in colon with regard to intestinal cavity microbiota. This may be because that compared with fecal microbiota, intestinal fluid microbiota are difficult to access. Nevertheless, the colonic microbiota is driven by complex carbohydrates, therefore, different carriers of colonic cavity microbiota possibly result in the different microbial components, which perform diverse functions.

2. Materials and methods

2.1. Study population

The present observational study on colorectal polyps (CPs) was conducted in Jiangsu Province, China. All patients were enrolled from the Jiangsu Provincial Hospital of Chinese Medicine from January 2018 to October 2019.

The present work had gained approval from the Institutional Ethical Review Committee of Jiangsu Provincial Hospital of Traditional Chinese Medicine, China ([2017] NO. 2017NL-134-02).

The patient inclusion criteria were as follows: patients newly diagnosed with colon polyps independently by 2 expert GI pathologists, aged 18 to 85 years old, living in Jiangsu Province for a long time, displaying no abnormality in colonoscopy and conforming to Rome-III diagnostic criteria, and being able to complete the interview independently. Altogether 192 participants were excluded from this study for the following reasons: previous history of cancer (n=103); incomplete or inconsistent data (n=52); data not representative of the cohort (n=8); and familial adenomatous or hereditary non-polyposis colon cancer (n=29). The patient exclusion criteria were as follows: younger than 18 years; with no cancer or other GI tract disorders; no family history of CP in a first-degree relative; those diagnosed with disorders (parenteral or GI disorders) that might interpret GI symptoms; those with concurrent major disorders and an alcohol or drug abuse history. On the other hand, 216 controls were ineligible due to the incorrect information.

Consequently, 151 CPs were recruited into this study from the Digestive Endoscopy Center, Jiangsu Provincial Hospital of Traditional Chinese Medicine. Table 1 shows the baseline characteristics of the 151 CPs.

All subjects had provided the informed consent to participate in this study, and they completed 1 standard questionnaire respectively, which covered items with regard to demographic data as well as CPs symptomatic evaluation prior to sampling.

2.2. Sample collection

The fresh colonic feces and colonic fluid were sampled from every participant, of which, fecal sample was obtained by means of endoscopy using the disinfected catheter (2.5 mm in diameter, Olympus, Japan), while 0.5 to 1.0 mL colonic fluid was collected using the disinfected syringe after it was inserted to the junction of the transverse colon and the descending colon (spleen curvature). To obtain sufficient colonic fluid samples, sterile saline (5 mL) was added to lumen prior to sampling. Besides, endoscopes were disinfected before use. Then, sterile water was utilized to clean endoscope canal through the retrograde and antegrade endoscopy. Bowl preparation (including electrolytes and macrogol; Shenzhen Wanhe Pharmaceutical Co., Ltd., China) was completed in each patient prior to endoscopy. For each subject, fresh feces were obtained at the hospital just before sigmoidoscopy or at home on the date of sigmoidoscopy in the morning. Fresh fecal samples were extracted at once following defecation, typically, internal feces were collected to prevent contamination. After the fecal samples
were collected, they were transferred to our laboratory at once on ice. Then, each sample was stored within the 1.5 mL sterile Eppendorf cups under –80°C prior to subsequent sequencing.[19]

2.3. DNA isolation
The Metagenomic DNA was used to extract total DNA of bacterial genome from each sample by the PowerMax (stool/soil) DNA extraction kit (MoBio Laboratories, Carlsbad, CA) in accordance with protocols from manufacturer, and the extracted DNA was preserved under –20°C before subsequent assays. Later, agarose gel electrophoresis and the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) were separately used to measure DNA quality and quantity.

2.4. 16S rDNA amplification pyrosequencing
In the PCR amplification procedure for V4 region of bacterial 16S rDNA genes, the following primers were utilized, including (5’-GTGCCAGCMGCGGTAA-3’, (515F, forward) and 5’-GGACTACHVGGGTWTCTAAT-3’ (806R, reverse).

Then, the paired-end barcodes (length, 6 bp) specific to samples were added to TrueSeq adaptors to carry out multiplex sequencing. As for PCR reaction system, it consisted of the Phusion High-Fidelity PCR Master Mix (25 μL), 10 μM of each primer (3 μL), DMSO (3 μL), DNA template (10 μL), as well as ddH2O (6 μL). The PCR conditions were as follows, 30 seconds of initial denaturation under 98°C; 15 seconds of denaturation under 98°C, 15 seconds of annealing under 58°C, together with 15 seconds of extension under 72°C for 25 cycles, followed by 1 minute of final extension under 72°C. The Agencourt AMPure XP Beads (Beckman Coulter, Indianapolis, IN) were used to purify PCR amplicons, while PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA) was adopted for quantification. Following each quantification procedure, all amplicons in equivalent volumes were mixed together, and the Illumina NovoSeq6000 platform (GUHE Info technology Co., Ltd, Hangzhou, China) was used to perform pair-end 2 × 150 bp sequencing.

2.5. Analysis of sequences
All sequencing data were processed using Quantitative Insights Into Microbial Ecology (QIME, v1.9.0) pipeline according to previous description.[20] In brief, the original sequencing reads that were exactly matched with barcodes were deemed as the valid sequences and classified into different samples, respectively. The criteria below were used to filter sequences with low quality[21,22]: sequences that were < 150 bp in lengths; those with the mean Phred score < 20; those with ambiguous bases; together with those with >8 bp mononucleotide repeats. The Vsearch V2.4.4 (=fastq_mergepairs=fastq_minovel 5) was used to assemble pair-end reads, and to pick the operational taxonomic units (OTUs), including chimera screening (=uchime_ref), cluster (=cluster_fast, id=0.97), De-replication (=derep_fulllength) (Rognes 2016). For every OTU, the default parameters were used to select 1 typical sequence. The VSEARCH was used to search typical sequences based on the green database, so as to classify the OTU taxonomy.

Additionally, the OTU table was made for recording every OTU level in every sample, along with the OTU taxonomy. OTUs that contained <0.001% total sequences among the diverse samples were eliminated. For reducing the inter-sample sequencing depth heterogeneity, the 100 uniformly resampled OTU subsets were averaged to generate the rounded, average, and refine OTU table at 90% minimal sequencing depth in subsequent assays.

2.6. Bioinformatic analysis together with statistical methods
Both the R software (v3.2.0) and QIIME were employed to analyze the sequencing data. The α-diversity indexes at OTU level, including Chao1 richness estimator, Simpson index, Shannon diversity index, PD_whole_tree, and ACE metric (Abundance-based Coverage Estimator), were determined based on OTU table using QIIME. In addition, the abundance curves ranked based on the OTU level were plotted for comparing OTUs evenness and richness across diverse samples. β-diversity was also analyzed for examining the different structures among microbial communities in different samples by means of UniFrac distance metrics,[23,24] and non-metric multidimensional scaling and principal coordinate analysis were adopted for visualization.[25]

The Monte Carlo permutation test (n = 1000 iterations) and Student t test were conducted to compare the different UniFrac distances in different groups, while box-and-whiskers plots were applied in visualization. The unique and share OTUs across different samples or different groups were visualized based on the Venn diagram produced by ‘VennDiagram’ R package, according to OTUs occurrence across diverse samples/groups, and the relative levels were not considered.[26] Besides, the Kruskal test was used to compare taxa levels at species, genus, family, order, class, and phylum scales across diverse samples and different groups using the R stats package. Taxa with differential abundances among different groups were detected by the linear discriminant analysis effect size (LEfSe) by the use of default parameters.[27] In addition, phylogenetic investigation of communities by reconstruction of unobserved state was employed to predict microbial functions, according to those sequences with high quality.[28] Thereafter, the Statistical Analysis of Metagenomic Profiles (STAMP, v2.1.3) software was adopted to further analyze output file.[29] BugBase is a tool for measuring the high-level phenotypes in microbiota. A difference of P < .05 indicated statistical significance.

3. Results
A total of 151 CPs patients participated in this study. Fecal bacterial samples were available and analyzed (n = 151), which were assigned into fecal bacterial group (FB group); in addition, there were also 151 colonic fluid samples, which were referred to as colonic fluid group (CY group).

3.1. Sequencing depth and quality assessment
In the presence of small amount of sequencing, the number of OTUs gradually increases, which tends to be stable until the amount of sequencing elevates to a certain extent. As shown in Figure 1, the dilution curve tended to be flat with the increase in sequencing amount, indicating sufficient sample size. Moreover, the Shannon curve (Fig. 2) first rose and then became flattened as the depth of sequencing increased, suggesting sufficient amount of sequencing data for reflecting most bacterial biological information.
3.2. OTU composition analysis
The default was used to draw a Venn diagram, in which OTU of 97% similarity were used as the unit. The total number OTUs in the 2 samples was 8072, while that of FB group was 6522 and that of CY group was 6616. In addition, 1456 unique OTUs were identified from FB group, whereas 1550 were found from CY group. More details are presented in Figure 3.

3.3. Analysis of microbial diversity
For estimating microbial diversities in CY group and FB group, the α-diversity indexes, including Chao index,
Shannon index, and Simpson index, were analyzed (Fig. 4) \( (P > .05) \).

### 3.4. Differential analysis of microbial composition

Based on microbial community compositions across diverse samples, this study additionally examined the sample clustering patterns through principal coordinate analysis according to Bray Curtis distance metrics. As observed from our results, the structures of bacterial communities were distinctly separated, and CY group samples were clustered together (Fig. 5). Using non-metric multidimensional scaling analysis, CY group again differed from FB group (Fig. 6). Moreover, LEfSe analysis on genus scale suggested that, CY group samples were different from those in FB group (Fig. 7) in terms of more than 50 microbial genera including Firmicutes, Clostridia, and Phascolarctobacterium.
3.5. Characterization of microbiota

The percentages for diverse phyla that comprised microbiota were alike between CY and FB groups. Among them, the 3 major bacterial phyla were Firmicutes, Bacteroidetes, and Proteobacteria. However, significantly more Spirochaetes were observed from CY group than from FB group, with the difference being statistically significant ($P = .016$) (Fig. 8A). Based on the family classification criteria, the top 3 most dominant bacteria in CY group and FB group were the same, which were Lachnospiraceae, Bacteroidaceae, and Ruminococcaceae. In addition, it was found after comparing CY group with FB group that, the statistically different mycobacterial families were Bacteroidaceae, Micrococcaceae, F16, Spirochaetaceae, Enterobacteriaceae, Cardiobacteriaceae, Turicibacteriaceae, Bifidobacteriaceae, and Dethiosulfovibrionaceae (Fig. 8B). According to the genus classification criteria, the top 3 most dominant bacterial genera in CY group were Roseburia, Bacteroidetes, and Megamonas, while those in FB group were

**Figure 5.** PCoA of CY and FB groups. CY group = colonic fluid group, FB group = fecal bacterial group, PCoA = principal coordinate analysis.

**Figure 6.** NMDS of CY and FB groups. CY group = colonic fluid group, FB group = fecal bacterial group, NMDS = non-metric multidimensional scaling.
Bacteroidetes, Enterobactiaceae, and Roseburia, respectively. As shown in Figure 8C, bacterial genera that had statistical differences between CY group and FB group were Bacteroidetes, Rothia, Actinobacillus, F16, Treponema, Treponema Genus (Oscillospira), Turicibacter, Sharpea, Heamophilus, Veillonella, and Cardiobacterium.

3.6. Analysis of functional genes

For revealing the potential differences in metabolism between the 2 groups, phylogenetic investigation of communities by reconstruction of unobserved state\(^{29}\) was employed for predicting the functional contents based on 16S rDNA. Nonetheless, differences between fecal and colonic fluid samples from CPs cases were detected. When comparing the phenotype classification based on the BugBase between CY group and FB group, differences were of statistical significance of both 2 groups (KS\(_P\) value < .05) (Fig. 9), including Anaerobic (9A), Aerobic (9B), Potentially Pathogenic (9C), Stress Tolerant (9D), Gram Positive (9E), and Gram Negative (9F).

4. Discussion

Intestinal flora is related to most systematic diseases in human body, such as autism, hypertension, inflammatory bowel disease, type 2 diabetes mellitus, immune diseases, cardiovascular disease, and polycystic ovary syndrome. There are over 99% anaerobic bacteria and small amounts of aerobic and combined anaerobic bacteria among the microorganisms in human intestines.

Research on gut microbiota among CPs cases is mainly based on muscoal microbiota or fecal microbiota. This study described the differences between fecal microbiota and colonic fluid microbiota in the same individuals. Generally, intestinal microbiota in human body can be divided into 3 types. Physiological bacteria (anaerobic bacteria), including Lactobacillus, digestive cocci, Bacteroides, and bifidobacteria, which are all the dominant intestinal microbiotas that play a role in nutrient metabolism and immunity regulation. Conditional pathogenic bacteria (facultative aerobic bacteria), such as Enterobacter and Enterococcus, which are harmful to the host health once the intestinal microecological balance is destroyed, but they are harmless to the host in normal microecological environment. Pathogens, such as Proteus, Clostridium wechnerii, and Pseudomonas, which may cause disease when the number increases to break the microecological balance.

Due to the convenience of sample collection, it is not harmful and invasive to the human body. Many studies on intestinal cavity bacteria have adopted feces as the samples to investigate microbiota. However, the fecal microbiota itself is mixed with food residues, passing bacteria, and shedding intestinal mucosa. Therefore, the author believes that the content of fecal microbiota is complex, which involves numerous variable factors. In this regard, this experimental study was designed, in which 2 types of samples, including colonic fluid and feces, were collected to examine the differences between them. As suggested by our final experimental results, there were statistical differences between colonic fluid microbiota and fecal microbiota, and the fecal microbiota did not completely represent the microbiological environment of intestinal cavity. In particular, within intestines in CPs patients, the intestinal mucosal barrier was broken and in the states of stress and inflammation. Thus, the fecal microbiota and intestinal fluid microbiota might be more convincing.

The present work applied high-throughput sequencing technology in performing 16S amplicon sequencing and biological analysis on DNA extracted from both colonic fluid samples and

Figure 7. LEfSe of CY and FB groups. CY group = colonic fluid group, FB group = fecal bacterial group, LEfSe = linear discriminant analysis effect size.
fecal samples. Notably, this technology allows to comprehensively and accurately analyze the bacterial flora information from the aspects of microbial diversity, spatial distribution, and morphological determination.

Microbial diversity has been employed as a precise means to assess the overall microecological structure. Under normal health, the more proximal intestinal diversity is lower than that in fecal microbiota. In this study on patients with intestinal polyps, no statistical difference is detected in the diversities between intestinal fluid and fecal flora near the spleen. It can be inferred that, the intestinal barrier function in patients with intestinal polyps may be destroyed, the intestinal microenvironment is imbalanced, a large number of pathogens and toxins invade the intestine, and local inflammation reactions may occur, which thereby triggers the proliferation of intestinal epithelial cells. The microbial diversity of corresponding proximal intestine also increases by the addition of these foreign and pathogenic bacteria. In this study, LEfSe analysis on intestinal microbiota and fecal microbiota was also conducted to analyze those markedly different microbiotas. It was discovered that there were several anaerobic bacteria associated with periodontal disease, including Synergistales, the newly discovered anaerobic bacteria in recent years. In addition, Clostridium nucleas, the anaerobic bacterium not normally present in the intestine but only exists in the oral cavity, was also detected. These 2 microbiotas are frequently enriched in the GI tract of CRC patients, and it is reported in related literature that periodontal disease-related bacteria are tightly associated with CRC genesis and progression.\textsuperscript{30,31} Intestinal polyps themselves may be the precancerous lesions of CRC. Exogenous colonization of pathogenic bacteria, such as Synergistales and Clostridium spp., has strengthened the relationship between intestinal polyps and intestinal cancer.

After analyzing the differences between colonic fluid and fecal samples on the levels of phylum, family, as well as genera, the following thoughts were obtained. At the phylum level, Fusobacteria, Firmicutes, and Bacteroidetes were identified as the predominant bacteria in both CY group and fecal group. Pachyphytes and Bacteroides accounted for more than 90% of all human gut microbiotas. In terms of disease prevention and treatment, both groups of bacteria inhibited the inflammatory response, regulated bacterial toxins, removed free radicals, and boosted the immune function, thereby preventing the occurrence of GI diseases. Typically, the top 3 dominant bacteria within the

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Figure 8. Different level difference analysis of CY and FB groups. (A) Phylum level difference analysis of CY and FB groups. (B) Family level difference analysis of CY and FB groups. (C) Genus level difference analysis of CY and FB groups. CY group = colonic fluid group, FB group = fecal bacterial group.
The intestinal tract of normal healthy human are Firmicutes, Bacteroidetes, and Proteobacteria. According to our results in this study, the proportion of Clostridia in both enterobacterial and fecal microbiotas of patients with intestinal polyps exceeded that of Proteobacteria, finally rising to the third place. Clostridium itself is a type of normal microbiota within human intestine. In the process of substance metabolism, Clostridium fermentation may produce butyrate, which will affect the development of intestinal epithelial cells and energy metabolism. Gaines et al. found that, the intestinal diversity and proportion of Clostridium in CRC cases significantly increased compared with those in normal subjects. At the same time, similar results were also discovered in the intestine of patients with ulcerative colitis. So far, Clostridium has been recognized as the fungus related to CRC in the world. As demonstrated in 2 related intestinal tumor experiments, the high expression of Clostridium causes microsatellite instability and MLH1 methylation. In addition, Clostridium is associated with tumor recurrence and metastasis. Moreover, it is reported that, the Clostridium-immunized mice are more likely to develop tumors than those colonized with other strains, and the toxicity of Clostridium to the host not only increases by its own toxicity, but may also elevate through gene transfer toxicity. Currently, many experiments have been carried out on the relationship between intestinal polyps and Clostridium. Nonetheless, our findings also indicated that the Clostridium proportion and diversity increased in intestine of patients with CRC characteristics. This may be related to the anti-intestinal inflammatory stress responses of Clostridium and its products. Meanwhile, intestinal spirochadium was detected in intestinal fluid group, which was significantly different from that in the stool. On the other hand, intestinal Borrelia is a pathogen that can damage the intestinal mucosal cells and invade the intestinal epithelial cells in some severe cases.

At the family level, the Enterobacter proportion of FB group statistically increased relative to that of CY group. On the one hand, this may be because that the feces are closer to the end of the colon than the intestinal fluid collected and the enterobacteria proportion increases with the different intestinal microbial characteristics. On the other hand, Bacillus is involved in digestion and absorption, and protects the intestinal function in human body. Besides, it can regulate the transcriptional response of genes in the case of impaired mucosal defense function. It is speculated that there may be an increase in the enterobacteria proportion in the intestinal mucosa of CPs patients. Feces pass through the entire intestinal cavity, and the mixed mucosal microbiota may reflect inflammation in the intestinal cavity compared with colonic fluid samples. In addition, the increase in Bifidobacteriaceae also has certain known research significance. The “Driver-Passenger” model is proposed to illustrate the mechanism of CRC occurrence. Some strains that induce the damages to intestinal mucosal epithelial cells and/or chronic intestinal inflammation are called “drivers”, while those that follow the cancerous process and proliferate due to the changed microenvironment (beneficial or pathogenic bacteria) are referred to as “passengers”. Bifidobacteria are the beneficial bacteria in the “passengers” position in the context of chronic intestinal inflammation, which are highly expressed in the intestine under conditions of epithelial cell damage and microenvironment changes.

From the genus level, Veillonella was the representative genus for both CY and FB groups. Weyococcus is a pathogen that often suggests the presence of an inflammatory response. The results of this study may be explained by the fact that, the microecological environment of patients with intestinal polyps is imbalanced, which leads to clustering of some pathogenic bacteria and the relative reduction in beneficial bacteria. By contrast, stool
samples farther away from the colon did not show an increase in the proportion such pathogens. It remained unknown that whether it was related to the growth site of polyps in the intestine. There were significant differences in Treponema and Rothia between 2 groups of samples, which were also related to the intestinal epithelial damage. However, the evidence for intestinal polyps-related etiology is insufficient, which should be further explored.

The present study recruited patients from hospital to eliminate the confounding factors. However, some limitations should still be noted in the study. First of all, the differences in fecal microbiota and intestinal fluid microbiota might result in residual or unmeasured confounding factors, such as age, exercise, and smoking, even though the identified factors were adjusted in the inclusion and exclusion criteria. Another limitation was the number of cases enrolled. Due to the limited number of colonoscopy to healthy person, we did not add the health group and only the CP patients were studied. Also the colonoscopy preparation with fluids could influence the results, we still need to find a new method to collect the intestinal fluid. Therefore, these results should be validated with additional studies.

5. Conclusions
In our study, it is discovered the relationship between intestinal polyps and intestinal microbiota that, the intestinal fluid microbiota and fecal microbiota are equally important and indispensable.

This experiment investigates the changes in intestinal fluid microbiota and fecal microbiota among patients with intestinal polyps. The results suggest that, patients with intestinal polyps show typical alterations of microbial composition, proportion, and diversity. Besides, any disorder in intestinal flora may probably cause intestinal polyps, which continues to participate in disease development as the starting factor.

In addition, the different variations of intestinal fluid flora and fecal flora in patients suggest that disease research should focus on investigating the relationship between gut microbes.

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