Disruption of the human gut microbiota affected by ulcerative colitis

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Abstract

Objective: The stability of the intestinal flora is an important guarantee for maintaining human health. Whether it will disturb the stability of the flora after ulcerative colitis (UC) is worth exploring.

Methods: An observational study was performed to evaluate the disruption of the human gut microbiota affected by UC. Chromosomal DNA was extracted, and the length of DNA fragments was examined and quantified. Gene amplification and sequencing of the V3 region of 16S rRNA were subsequently performed by Illumina’s MiSeq sequencer for high-throughput sequencing.

Results: The structure of intestinal flora in UC patients exhibited a significant increase of Proteobacteria and a remarkable decrease of Bacteroidetes compared with that of healthy controls. Among each genus in the Proteobacteria, UC patients exhibited a significant decrease of Alcaligenaceae (55.08% ± 13.90 to 0.56% ± 0.04) and a remarkable increase of Enterobacteriaceae (6.64% ± 10.22 to 98.05% ± 3.28) compared with that of healthy controls. Our results demonstrated that the disorder of the intestinal flora is associated with UC patients.

Conclusions: Therefore, UC patients exhibited a significant shift in the dominance of the gut microbiota, and restoring the homeostasis of the intestinal flora may be an important target for the treatment of UC and alleviation of symptoms.

Keywords: ulcerative colitis; intestinal flora; high-throughput 16S rRNA sequencing

Ulcerative colitis (UC) is a number of chronic non-specific inflammatory diseases of the colon and rectum (1). The incidence of UC in the United States has reached about three per thousand, and the prevalence of UC has continued to increase in recent years (2). Although there is no accurate statistics on the incidence of mainland residents in recent years, the incidence of UC is exactly increasing due to the development of the national economy and the improvement of the quality of life (3). UC usually occurs in the left colon. Although UC in some cases is limited, the ulcer of some patients can gradually expand and eventually lead to the entire colon ulceration (4). Previous studies have reported that UC patients have a long course of disease and are prone to recurrent attacks (5). UC patients have a higher risk of developing colorectal cancer through an inflammatory-dysplasia-cancer process (6, 7).

The exact cause of UC has not been clarified by pathological studies to date (8). UC has been thought to be the result of a combination of multiple factors triggered by immunity, genetics, and the environment. There are trillions of bacteria in the human intestine, of which 1 g of feces contains $10^{11}$ bacteria (9). The gut microflora is considered to be an independent organ of the human body and participates in the maintenance of colon health. The destruction of the intestinal flora affects the body’s intestinal and colorectal balance, which affects the health of the human intestine and colon.

In the present study, we aimed to study the intestinal microecology of 40 patients with UC disorders through high-throughput sequencing technology. We believed that our study provides theoretical basis for assisting UC therapy by regulating intestinal ecological balance.

Methods

Study design and patients
An observational study was performed to evaluate the disruption of the human gut microbiota affected by UC.
The study included a total of 60 volunteers, which were randomly selected. Forty of them were diagnosed as UC patients in Suzhou University. Patients were treated with medicines including steroids, mesalazine, or aminosalicylic acid. Twenty healthy subjects were randomly selected as control group. Because antibiotics have a greater impact on the intestinal flora, all participants were required to not receive any antibiotic and probiotic treatment before sampling in the last 3 months. The selected patients were not associated with recent gastrointestinal infections. Normal controls did not have digestive diseases within 3 months, and no antibiotics were used. The basic statistics of the volunteers and their disease duration were listed in Table 1. Briefly, there were 22 males and 18 females in the case group, with an average age of 56 years, and there were 7 males and 13 females in the control group, with an average age of 38 years. All participants in the experiment have signed the experimental informed consent form, and the project has been approved by Suzhou University. The samples were collected in the medical room in Suzhou University. All patient details have been deidentified. All patients have signed the consent. The reporting of this study conforms to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (10). The study was approved by Suzhou University (#lcyj-0254, approved on 11 October 2019).

**Fecal sample DNA extraction and library construction and sequencing**

DNA extraction: High-throughput sequencing has high requirements for DNA integrity. DNA from fecal was extracted using a fecal DNA extraction kit (TIANGEN BIOTECH). DNA concentration was quantified by Qubit, and DNA integrity was assessed by Agilent 2100. Library construction: Sample library construction was performed using a unique two-step PCR method. The first round of amplification was completed by PCR reaction using different sample DNA as a template. After purification of the PCR product using magnetic beads, a second round of PCR amplification was performed using primers containing different indices (the primers contain sequences paired with the linker above the sequencing flow unit to distinguish between index sequences and suspension link sequences between different samples). After purification by magnetic beads, the quality of the library was tested using Qubit and Agilent 2100. The qualified library will be sequenced using the Illumina Miseq high-throughput sequencing platform.

The project uses Illumina’s MiSeq sequencer for high-throughput sequencing. The sequencing platform can sequence one or more hypervariable regions of 16S rRNA, and the sample preparation library can start with as little as 50 ng of DNA. After that, cluster generation and sequencing are done on MiSeq.

The 16S rRNA V3 region gene amplification and sequencing were performed on the extracted DNA samples. The amplification primers are 5’-NNNNNNTTACGGGAGGCAGCAG-3’ and 5’-NNNNNNTTATTACCAGCTGTAGTGG-3’. ‘NNNNNNNN’ is a unique tag sequence set for the amplification of each sample. PCR amplification and overall sequencing analysis of each sample were performed.

**Bioinformatics analysis of sequencing results**

For the sequencing results, different sample-related sequences are distinguished according to different tag sequences, and corresponding libraries are established. The CD-HIT software was used for cluster analysis, and the analysis of operational taxonomic units in each library was further completed. Relying on a new generation of DNA sequencer and computing platform, the genome distribution of intestinal flora is obtained by macrogenome analysis.

**Results**

**Classification analysis at phylum-level**

The basic statistics of the volunteers were listed in Table 1. The structure of intestinal flora in UC patients was studied and compared with the structure of healthy human intestinal flora to find out the correlation between intestinal flora changes and UC. As shown in Fig. 1, the structure of intestinal flora in UC patients exhibited a significant decrease of Bacteroidetes and an increase of Proteobacteria compared with that of healthy controls. In healthy individuals as controls, Bacteroidetes dominated, and in UC patients, sequencing found that the abundance of Proteobacteria was clearly dominant.

**Classification analysis at genus-level**

We next analyzed the components of each genus in the Proteobacteria. As shown in Fig. 2, the structure of intestinal...
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Fig. 1. Patients with ulcerative colitis show differential disruption in gut microbiota at the phylum-level.

Fig. 2. Comparison of patients with high proteobacteria phylum.
flora in UC patients exhibited a significant decrease of Alcaligenaceae and a remarkable increase of Enterobacteriaceae compared with that of healthy controls. In addition, the proportion of Pasteurellaceae was decreased in UC patients. Our data revealed that, among healthy individuals, the genus Alcaligenaceae is dominant, while in UC patients, the dominant genus is Enterobacteriaceae.

Discussion
Intestinal flora and microbiota alterations play an important role in the development of UC (11, 12). However, the research on UC intestinal flora is not enough to clearly and completely describe its composition. It has been reported that the commensal bacterial community is implicated in the pathogenesis of inflammatory bowel disease (IBD), including UC (13).

In the present study, 60 cases of intestinal flora were sequenced to describe the characteristics of intestinal flora in UC patients. Fecal samples from 40 UC patients and 20 healthy control volunteers were selected as subjects. The diversity of the V3 region of the bacterial 16S rRNA gene was detected by the sequencing technique combined with statistical methods. The results indicated that the intestinal flora structure of patients with UC was different from that of the healthy control group, mainly represented by a decrease in Bacteroidetes and Firmicutes, and an increase in Proteobacteria. Therefore, we next analyzed the components of each genus in the Proteobacteria. Our results showed that the proportion of Alcaligenaceae and Pasteurellaceae was decreased in UC patients compared with that of healthy controls. In addition, an increase of Enterobacteriaceae in UC patients was observed. This study mainly uses high-throughput technology to explore the characteristics of the intestinal flora of UC patients, which might provide a reference for the development of drugs targeting the flora.

In fact, there are still certain limitations of this study. First, the average age of patients in the healthy control group and the UC group is not exactly the same. Different ages may affect the distribution of intestinal flora, which, in turn, affects the accuracy of our research results. Second, the patient’s dietary habits are a factor that affects the distribution of intestinal flora. Due to time and resource constraints, we are unable to record and analyze the dietary habits and history of all study participants. We have simplified the experimental process under the condition of ensuring the accuracy of the research results as much as possible. Third, we have limited understanding of the pathogenesis of UC, and our research focuses on the comparative effects of UC on the colonic bacterial microbiota. We are now conducting more detailed study of larger sample size to further validate our results.

In conclusion, we used high-throughput sequencing technology to detect the distribution of intestinal flora in UC and normal populations and further explored the bacterial structure that is significantly associated with the formation and development of UC. We demonstrated that UC patients exhibited a significant shift in the dominance of the gut microbiota. Our research may provide a reference for the development of drugs targeting microorganisms, which possess certain clinical value.

Conflicts of interest and funding
The authors declare that they have no competing interests. This study was supported by the General Support Project for Outstanding Youth in Universities in Anhui Province (gxyq2017091); R & D Projects Entrusted by Enterprises (2020XHX026); Priority Projects on Natural Science of Suzhou University (2016yzd04).

Authors’ contributions
Haichao Wang, Changhao Wu, and Dehui Kong conceived the idea. Haichao Wang, Changhao Wu, and Dehui Kong designed the study, performed research, and analyzed data. Haichao Wang wrote the paper.

Consent to publish
All of the authors have consented to publication of this research.

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