The Microbiome Profile In Ulcerative Colitis And Pouchitis

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Abstract

Both ulcerative colitis and pouchitis are associated with an imbalance in the intestinal microbiota, which may be related to the immune response. The objective was to determine the bacterial composition in pouchitis and ulcerative colitis in order to explore the underlying pathogenesis. Microbiome was profiled and evaluated by 16S ribosomal DNA gene sequencing in stool samples of 37 patients with ulcerative colitis, 15 patients with normal ulcerative colitis-pouch, 15 patients with ulcerative colitis-pouchitis and 18 healthy volunteers, PICRUSt and PICRUSt2 were performed to analyze the function of dominant bacteria. In our Chinese cohort, with aggravation of ulcerative colitis, intestinal microorganisms were characterized by a gradual decreased in diversity and numbers of butyrate-producing bacteria and *Bacteroides*. Besides, in addition to the decrease of probiotics, the bloom of *Escherichia-Shigella* and *Ruminococcus_gnavus* was observed in pouchitis which related to multiple infection pathways according to KEGG pathway analysis. Our results showed that pouchitis and ulcerative colitis differ in their intestinal microbial structures and metabolic pathways, but the reasons need to be further explored.

Introduction

Ulcerative colitis (UC) is an autoimmune disease that occurs in the colon and rectum, and most patients with active UC achieve remission through standardized medical treatment[1]. Surgical intervention is a better choice when medical treatment fails or when serious complications occur. Total colorectal resection with ileal pouch-anal anastomosis (IPAA) is the standard surgical treatment for UC[2–3]. However, approximately 50% of patients with pouch develop inflammation within 5 year[4], and the incidence is much higher than those with familial adenomatous polyposis(FAP) who undergo the same surgery[5–6].

Inherited early transcriptomic changes are associated with the UC-pouch, including those characteristic of enhanced immune and inflammatory responses, as well as remodeling of the extra cellular matrix[7–9]. Furthermore, similar to UC, fever, abdominal pain, diarrhea, and hematochezia are common clinical symptoms in patients with pouchitis[4]. Therefore, one hypothesis proposes that pouchitis represents the recurrence of UC in the normal small intestine[10].

However, genetic predisposition does not directly account for pouchitis, it appears to serve a prerequisite for environmental susceptibility and potential microbial instability[9]. In contrast to predominant use of antibiotics to treat pouchitis, the use of antibiotics to treat UC is controversial, even though meta-analysis has showed it helps improve clinical remission rates of patients with UC[11–12]. Intestinal microbiota may hold the key to triggering the inflammatory response[13], therefore, our aim was to determine the bacterial composition in ulcerative colitis and pouchitis and explore their potential pathogenesis.

Methods

Study subjects

We recruited patients with UC or UC-pouch who were admitted to the General Hospital of Tianjin Medical University from 2017 to 2020. Sex- and age-matched healthy volunteers served as the control group. All UC patients had a definite diagnosis, and they were divided into remission, mild, moderate, severe UC subgroup according to modified Mayo scores. All UC-pouch patients underwent ileostomy closure after at least 1 year, and inclusion criteria for patient with a normal pouch function were no inflammation and pouch dysfunction symptoms after IPAA, and their pouchitis disease activity index (PDAI < 7). While, pouchitis was defined as ≥ 2 episodes of pouchitis diagnosed according to clinical symptoms within 2 years and PDAI score ≥ 7 .

All participants and were not treated with antimicrobials for at least 4 weeks before analysis of their intestinal microbiota. Then we collected fecal samples into sterile test tubes, which were stored at -80°C . The Ethics Committee of Tianjin Medical University General Hospital approved this study (number: IRB2018-083-04), and all subjects granted their written informed consent before the study commenced.

DNA acquisition and 16S ribosomal DNA pyrosequencing

According to the OMEGA Stool DNA Kit instructions (D4015, Omega, Inc, USA), genomic DNA extraction from Stool samples will be performed. The concentration and purity of DNA samples were determined by NanoDrop2000 spectrophotometer and unqualified DNA samples will be discarded. Then the primers for the 16S ribosomalr DNA V3-V4 regions of the bacterial genome were designed in the experiment. The primer sequence of 336F was 5'-GTACTCCTACGGGAGGCAGCA-3', and the primer sequence of 806R was 5'-GTGGACTACHVGGGTWTCTAAT-3'. A PCR system was established to specifically amplify the V3-V4 region of the 16S ribosomalr DNA of the sample genome. Qualified agarose gels were sent to LC-Bio Technology (Hang Zhou, China) for Miseq library construction then performed sequencing on Illumina Miseq PE300 platform.

Microbial analysis

Sequences comprising < 200 bp, ambiguous bases, and with mismatches with primer sequences or barcode labels were excluded from the analysis [14]. Qualified reads were isolated using sample-specific barcode sequences and analyzed and pruned using Illumina pipeline version 2.6. QIIME software was used to analyze the data. Sequences with 97% identity were grouped into operational taxonomic units (OTUs) [15], sparse curves were generated, and richness and diversity indexes were calculated [16]. Sequences were classified according to the tool provided by the ribosome Database Project (RDP) [17].

We used R software to evaluate the similarities between different samples, and cluster analysis and principal co-ordinates analysis (PCoA, based on unweighted Unifrac distance algorithm) were performed according to the OTU information. The distance between evolving microbial communities of each sample was calculated using the TAYC coefficient and is represented as an insignificant cluster tree between two groups. The arithmetic mean method was used to describe the difference ($= 1 - \text{similarity}$) between multiple samples [18]. Tree files in Newick format were generated. To compare the community members and community structures of different samples, the top 50 community heat maps were generated using

Mothur. The Linear discriminant analysis Effect Size (LEfSe) method was used to analyze different species among the different groups of subjects (default LDA = 3)[19]. We used PICRUST and PICRUST2 to predict functions and Kyoto Encyclopedia of Genes and Genomes pathway(KEEG) based on different intestinal microbiota[20–21].

Statistical analysis

SPSS 22.0 software was used for statistical analysis. Uparse software was used to cluster OTUs with 97% similarity, and representative OUT sequences were compared and annotated with the corresponding species according to the SILVA128 16S reference database. The confidence threshold was defined as 80%, ANOVA was used to evaluate the differences in bacterial community structures, LEfSe was used to identify different species, and Tukey’s test was used to evaluate differences in the Shannon index. PICRUST predicts the functional composition of metagenomes. P < 0.05 (corrected using false discovery rates) indicates a significant difference.

Results

We included 37 patients with UC (51% females; average age, 41.6 ± 13 years), 30 patients with UC-pouch (50% females, average age 43.3 ± 11 years), and 18 healthy individuals (50% females; average age, 38.3 ± 13 years). According to their pouchitis disease activity index, 15 patients were included in the pouchitis group.

Intestinal microorganism analysis of ulcerative colitis

The general conditions of the UC and control groups are shown in Table 1. We summarized the changes of intestinal microbiota within different UC activity levels. The abundances of *Firmicutes* decreased in patients with UC with moderate and severe activity, while that of *Proteobacteria* increased in patients with severe active UC (Fig. 1a). The compositions of the intestinal microbiota of patients with UC patients were altered at the genus level (Fig. 1b). We used Shannon’s α-diversity index to show that microbial diversities of the remission and mild groups, as well as that of the control group, were similar. In contrast, the diversities of the moderate and severe groups were significantly decreased (4.79 and 4.39 vs 5.04, P = 0.012, P = 0.002, respectively) (Fig. 1c). The results of principal coordinate analysis showed similar results, in that the microbial communities of the moderate and severe groups were significantly different compared with those of the control group (P < 0.001 and P < 0.001, respectively) (Fig. 1d).

Table 1 Demogaphic and clinical characteristics of the UC and HC group

	Healthy	UC(n=37)			
	controls	Remission	Mild	Moderate	Severe
Number of patients	18	9	10	10	8
Sex,n,M/F	9/9	4/5	3/7	5/5	3/5
Age(yr)	38.3±13.0	35.8±13.3	38.0±12.9	38.6±14.1	47.4±15.1
BMI(kg/m ²)	22.0±3.8	22.3±1.8	22.1±2.4	23.2±3.4	21.2±2.1
UC duration(yr)	NA	7.0±1.9	10.3±4.9	8.3±4.2	11.7±5.1
Mayo score	NA	≤2	4.3±0.6	8.4±1.6	11.3±0.6
Iron deficiency anemia(%)	NA	4/44.4	6/60	7/70	7/88
Faecal calprotectin[ug/g±SD]	37.3±21.7	73.2±31.5	180.7±160.8	602.1±313.5	1740.3±1215.4
Smoking(%at recruitment)	6(33.3)	1/11	2/20	1/10	1/13

UC: Ulcerative colitis; NA: Not available

When we used LEfSe analysis to further search for different species among the subgroups, we found that *Mogibacterium* species increased in patients with mildly and moderately active UC, while *Lachnoclostridium* and *Peptostreptococcus* were increased in patients with severe, active UC (Fig. 1e,f). Furthermore, *Bacteroides*, *Roseburia*, and *Blautia* gradually decreased with the development of inflammation.

Intestinal microorganism analysis of pouchitis

Certain baseline characteristics of patients with pouch is shown in Table 2. At the level of the Bacteriophyta, compared with the normal pouch, *Bacteroidetes* decreased while *Proteobacteria* significantly increased in the pouchitis group (Fig. 2a). The pouchitis group had a lower Shannon α-diversity level (4.09 vs 5.07, respectively, P = 0.049) (Fig. 2b) and a significantly different community structure compared with the normal pouch group (P < 0.001) (Fig. 2c). The relative abundances of *Escherichia-Shigella* and *Bacteroides* were respectively over-represented and significantly lower compared with controls (Fig. 2d,e). Furthermore, the results of LEfSe analysis were similar in that *Escherichia-Shigella* increased while *Bacteroides* and *Faecalibacterium* decreased in the pouchitis group (Fig. 2f).

Table 2 Demographic and clinical characteristics of the pouch group

	Pouch (n=30)	
	Pouchitis	NP
Number of patients	15	15
Sex,n,M/F	7/8	8/7
Age(yr)	39.0±8.7	46.4±13.1
Pouch duration(yr)	2.7±1.8	3.9±2.0
BMI(kg/m ²)	24.5±2.1	25.1±1.8
PDAI score	≥7	≥7
Age at colectomy	38.4±12.1	42.5±10.9
Smoking(%at recruitment)	0/0	0/0
iron deficiency anemia(%)	10/67	7/47
Number of patients with chronic pouchitis(%)	6/40	0/0
Crohn's-like disease of the pouch(%)	4(26)	1(7)
Primary sclerosing cholangitis	1(7)	0(0)
Faecal calprotectin[ug/g±SD]	351±207	103±79
Pouchitis: Inflammation of ileal pouch;NP:Patients without pouchitis		

The structure of intestinal microbiota differ between active UC and UC-pouchitis

Colon metaplasia in pouches provides the basis for comparison between UC and pouchitis. We next integrated the intestinal microbiota data of patients with active UC and patients with pouchitis. Though the Shannon α -diversity index of pouchitis was close to that of moderate-activity UC, there was no significant difference between the pouchitis group and the other two subgroups (Fig. 3a). The microbial community of the pouchitis group was significantly different compared with that of the active UC group ($P = 0.001$) (Fig. 3b).

Functional analysis of intestinal microbiota in ulcerative colitis and pouchitis

The findings described in Sect. 3.1 and 3.3 prompt us to use the PICRUSt2 algorithm to analyze the combined data for the moderate and severe UC subgroups (MSUC). This analysis revealed 77 and 132 functional differences in patients with MSUC and those with pouchitis, respectively (Supplementary Table S1). For example, the *Bifidobacterium* shunt, inosine – 5'-phosphate biosynthesis, and heterolactic fermentation were enhanced in patients with UC, while the L – alanine and demethylmenaquinol pathways were increased in those with pouchitis. Furthermore, the functional prediction indicates overproduction of Enterobactin in patients with pouchitis (Fig. 4a,b). Further Spearman correlation

analysis of different microbiota based on PICRUSt showed that *Escherichia-Shigella*, as well as other bacteria such as *Ruminococcus_gnavus*, may be associated with the production of Enterobactin (Fig. 4c).

According to KEGG analysis, (all data was displayed in Supplementary Table S2), the enrichment of pathways associated with the immune system and infectious diseases varied among the bacteria included in the heat map (Fig. 4d). These results show that these pathogens, mainly *Escherichia-Shigella*, were involved in diverse infection pathways.

Discussion

IBD comprises two main inflammatory pathways involving the intestinal microbiota. In one pathway, pathogens secrete toxins, and in the other, the host's immune system is overactive because of the instability of the compositions of their microbiota[22–23]. Clinical remission is achieved using Metronidazole/ ciprofloxacin for patients with acute pouchitis(Whether or not they are relapse), while 5aminosalicylic acid and biological agents should be considered to chronic antibiotic-refractory pouchitis which share more autoimmune features[24–25]. These effects of treatment indicate similarities in microbial pathogenesis between specific types of pouchitis and UC, but most cases of pouchitis differ from UC.

We show here that the diversity of the intestinal microbiota decreased in patients with UC or pouchitis as previously reported and that UC and pouchitis were associated with different microbial structures compared with the control group[26]. When it comes to active UC, though evidence indicates that *Lachnoclostridium* contributes to produce sulfide that inhibits butyrate utilization[27], and *Peptostreptococcuse* triggers a pro-inflammatory response based on activating nuclear factor kappa-B(NF-κB) activation, the increased abundance of these bacteria suggests that their role in promoting the occurrence of UC is limited(Fig. 1b)[28]. Therefore, we proposed that the mechanism of pathogenesis of UC caused by microorganisms may involve the contributions of numerous genera (e.g., *Bacteroides* and butyrate-producing bacteria)[29].

In contrast, KEGG analysis showed that much pathogens enriched in pouchitis were related to numerous pathways associated with infectious diseases. That means infectious bacteria play an important role in the occurrence of pouchitis. What's more, we found that 'Enterobactin, which is a siderophore secreted by many pathogens, will confer a survival advantage upon E. coli in the inflammatory bowel was associated with many pathogens enriched in pouchitis[30]. Therefore, we conclude that the blooms of these pathogenic bacteria are the direct cause of UC-pouchitis, which likely explains why the use of antibiotic monotherapy achieves clinical remission in most patients with pouchitis.

One important question is that whether it is logical to compare pouch microbiome to UC microbiome as we are dealing with two different anatomical reservoirs. We think this is doable because the pouch itself is designed to slow stool removal, just like a removed colon. In fact, colon metaplasia is a very common occurrence in pouches. Therefore, we compare pouch microbiome to UC microbiome. The similar

microbial characteristics of UC and pouchitis include the decrease of the population of butyrate-producing bacteria and *Bacteroides*. Butyrate is an energy source required for intestinal epithelial cells to maintain the function of intestinal barrier[31–33]. *Bacteroides* produces vitamin K and has anti-inflammatory potential[34–35]. The depletion of these bacteria weakens the intestinal barrier and lead to more severe inflammation. For pouchitis, antibiotic therapy is effective, but relapse is really common, and the bigger trouble is development pouchitis with antibiotic-dependence. Recent study has shown that the recurrence of antibiotic-dependent pouchitis is related to blooms of oral and disease-associated bacteria rather than antibiotic-resistant intestinal microbiome[36]. This suggests that long-term antibiotic therapy will exert a severely destructive effect on the intestinal barrier, making it easier to colonize by exogenous pathogens, which leads to the recurrence of pouchitis. Therefore, we should avoid infecting normal bacteria when we use antibiotics. According to our result, Enterobactin maybe the best candidate. Some research had generated “Trojan horse” strategy, they employing an Enterobactin-ciprofloxacin conjugate to targeted elimination of Enterocrocin, and that could be a future treatment for chronic pouchitis[37–38].

Some patients present Crohn's-like (CD) disease of the pouch. Interestingly, the bloom of *E. coli*, *Ruminococcus_gnavus*, and the consumption of *Faecalibacterium* are prominent microbiological features of CD[39–40]. Pouchitis and CD share certain similarities such as predisposition to perianal infection and anal fistula, which may be associated with specific pathogens. Moreover, patients with CD with perianal abscesses or anal fistulas undergo the same treatment as patients with pouchitis (first-line metronidazole/ciprofloxacin)[41]. We should therefore carefully consider the contribution to IBD of infectious agents and their products.

Together, our present data strongly support the conclusion that different microbial mechanisms operate in pouchitis compared with those of UC. We believe therefore that UC appears to be associated with the instability of intestinal microbiota (especially the decrease of beneficial bacteria) while pathogenic bacteria such as *Escherichia-Shigella* and *Ruminococcus_gnavus* contribute to the pathogenesis of pouchitis. Incidents of infection in IBD have been noted, further research will help to clarify the causes of infection in the UC-pouch, as well as deepen the understanding of the relationship between gut microbes, autoinflammation response, and infection.

Conclusions

Pouchitis and UC differ in their intestinal microbial structures and metabolic pathways. They were all associated with the significant decrease in probiotics, while blooms of infectious bacteria is present in pouchitis which support the antibiotic strategy.

Declarations

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Conflict of Interest

The authors of this manuscript report no conflict of interest.

Availability of data and material

Sequencing data for intestinal microbiota is available on request.

Code availability

Not applicable

Authors' contributions

Gang Liu designed the study. Li-Sheng Yang and Di Huang recruited patients for this study. Xin Gao and An-Qi He collected patients' data. Xin Gao and Li-Sheng Yang drafted the manuscript and all authors reviewed it. Xin Gao and Kai-Yu Li approved final version. Xin Gao and Tong Liu performed the statistical analysis.

Ethics approval

The Ethics Committee of Tianjin Medical University General Hospital approved this study (number: IRB2018-083-04), and all subjects granted their written informed consent before the study commenced.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent for publication

Patients signed informed consent regarding publishing their intestinal flora sequencing data.

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Supplemental Data

Supplemental tables are not available with this version.

Figures

Figure 1

Figure 3

Comparison of intestinal microbiota between active UC and pouchitis.(a) The α -diversity of pouchitis and active UC, (b) the microbial structure between active UC and pouchitis were different

Figure 4

Microbial Function and KEEG pathway analysis. The 30 most significant functional differences among MSUC, pouchitis, and control group, (c) positive correlation between Enterobactin levels and Escherichia-Shigella, (d) Escherichia-Shigella and other bacteria enriched in pouchitis were associated with numerous infection pathways compared with MSUC.