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**Original Article**

# Original Article

# Fecal microbiota related to postoperative endoscopic recurrence in patients with Crohn's disease

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## Abstract

**Background:** Postoperative recurrence (POR) remains a major challenge for patients with Crohn's disease (CD). Gut microbial dysbiosis has been reported to be involved in the pathogenesis of POR. This study aims to investigate the relationship between fecal microbiome and endoscopic recurrence in patients with CD after ileocolonic resection.

**Methods:** This is a cross-sectional study. Fecal samples were collected from 52 patients with CD after surgical intervention from 6 to 12 months before endoscopic examination. Endoscopic recurrence was defined as Rutgeerts score $\geq$ i2. The microbiome was analyzed by sequencing the V3–V4 hypervariable regions of the 16S rRNA gene.

**Results:** A total of 52 patients were included and classified into POR ($n = 27$) and non-POR ($n = 25$) groups. Compared with the non-POR group, the POR group had a significantly lower community richness (Chao1 index: 106.5 *vs* 124, $P = 0.013$) and separated microbial community ($P = 0.007$ for Adonis, $P = 0.032$ for Anosim), combined with different distribution of 16 gut microbiotas and decrease of 11 predicted metabolic pathways ($P < 0.05$). *Lactobacillus* and *Streptococcus* were identified to closely correlate to non-POR ($P < 0.05$) after controlling for confounding factors. Kaplan-Meier analysis indicated that the patients with higher abundance of *Streptococcus* experienced longer remission periods ($P < 0.01$), but this was not for *Lactobacillus*. The predicted ethylmalonyl-coA pathway related to increased amount of succinate was positively correlated with *Streptococcus* ($r > 0.5$, $P < 0.05$).

**Conclusions:** The characteristic alterations of fecal microbiota are associated with postoperative endoscopic recurrence in patients with CD; particularly, high abundance of *Streptococcus* may be closely related to endoscopic remission.

**Keywords:** Crohn's disease; postoperative endoscopic recurrence; fecal microbiota; 16S rRNA gene sequencing

## Introduction

Crohn's disease (CD) is a chronic, relapsing inflammatory disorder of the gastrointestinal tract that mostly affects ileocecum [1]. Ileocolonic resection is often required in patients with severe complications, such as intestinal stricture, fistula or perforation [2]. However, surgery for CD is rarely curative, and nearly 65% of patients experience postoperative recurrence (POR) [3]. Ileocolonoscopy is usually performed to diagnose endoscopic recurrence according to Rutgeerts score (RS) system [3, 4]. Usually, RS $\geq$ i2 is considered to have endoscopic recurrence [5, 6]. The current guidelines indicate that postoperative prophylactic medication for patients with CD should follow the risk stratification of recurrence [7, 8]. The factors related to high risk of recurrence include active smoking, penetrating disease, history of perianal diseases, prior intestinal resection and extensive bowel lesions. Thiopurines and/or anti-tumor necrosis factor (TNF)-$\alpha$ agents should be used after surgery for these high-risk patients [9, 10]. However, the reason for POR has not been clarified yet [11].

The gut microbiota, as an important environmental factor, has been considered to involve in the pathophysiological mechanism of POR [12–14]. The previous study has reported that patients with POR of CD harbored a reduced microbial diversity and depletion of some members of Firmicutes phylum, such as *Faecalibacterium*, *Coprococcus*, *Blautia* and *Lachnospira* [15]. The decreased abundance of *Faecalibacterium prausnitzii* in CD resected ileal mucosa can predict a higher risk of endoscopic recurrence 6 months after surgery [13]. Most studies focused on the relationship between mucosa-associated microorganisms and recurrence, with less research on the role of fecal microbiota in recurrence, although fecal specimens are easier to obtain. In this study, we aimed to characterize the fecal microbial spectrum

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associated with POR in patients with CD, and to provide possible clues to underlying microbial patterns in pathophysiology of POR.

# Materials and methods

## Study population

This is a single-center cross-sectional study. A total of 84 consecutive confirmed diagnosis of patients with CD who underwent ileocolonic resection were recruited from July 2017 to December 2019 at Shanghai Tenth People's Hospital of Tongji University (Shanghai, China). All patients provided a stool sample before intestinal preparation at 6–12 months after surgery before ileocolonoscopy. The inclusion criteria for patients included (i) age >17 years, (ii) CD-related ileocolonic resection and mesenteric resection with side-to-side stapled anastomosis; (iii) ileocolonoscopy performed by experienced endoscopists from 6 to 12 months after surgery; and (d) endoscopic results included at least two images of ileocolic anastomosis, new terminal ileum and the whole colon. Endoscopic reports of patients were confirmed by agreement of another two blinded inflammatory bowel disease specialist based on these complete recorded images. The patients who received antibiotics, prebiotics or probiotics in 3 months before sample collection were excluded [16]. The other exclusion criteria included perianal or upper gastrointestinal surgery, colonic or intestinal stomy, intestinal dysplasia or cancer, active colon inflammation, and/or active perianal lesions.

POR was defined as RS i2 (>5 aphthous ulcers or larger anastomotic lesions), i3 (diffuse ileitis) or i4 (large ulcers, narrowing or diffuse inflammation). Non-POR was defined as RS i0 or i1 [4, 17]. Fifty-two patients were enrolled in the study eventually, including 27 cases in the POR group and 25 in the non-POR group (Figure 1). Azathioprine and infliximab were used from 2 to 4 weeks after surgery. Azathioprine is used as 1.0–1.5 mg/kg per day. Infliximab was regularly administered intravenously at a dose of 5 mg/kg body weight at 0, 2 and 6 weeks and then every 8 weeks until the first postoperative endoscopic examination.

The study protocol was reviewed and approved by the Institutional Ethics Committee of the Shanghai Tenth People's Hospital of Tongji University (SHSY-IEC- 4.1/20–34/02). All the patients were informed of the study purpose and provided written informed consent in accordance with the Declaration of Helsinki of 1975.

## Sample collection and DNA extraction

A total of 52 samples from each patient were collected at the first fecal discharge in the morning before intestinal preparation for ileocolonoscopy and immediately stored at −80°C until use. All DNA extraction and sequencing procedures were performed by Realbio Genomics Institute (Shanghai, China). Fecal DNA was extracted according to the 'Godon' method [18] using the QIAamp Fast DNA Stool Mini Kit (Qiagen; Hilden, Germany). DNA concentrations were quantified with a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific; Shanghai, China), and the quality was assessed by agarose gel electrophoresis.

## 16S rRNA gene sequencing

After DNA quality control, 16S ribosomal RNA (rRNA) gene sequencing was performed. The V3–V4 region of 16S rRNA gene was amplified by polymerase chain reaction (PCR) with universal primers 341F (5′-CCTACGGGRSGCAGCAG-3′) and 806R (5′-GGACTACVVGGGTATCTAATC-3′) using the KAPA HiFi Hotstart ReadyMix PCR kit. Specifically, sequencing adapters and barcodes were added to the 5' end of universal primers for PCR. Then, the qualified PCR products were quantified by Qubit and pooled in equal concentration to construct sequencing libraries, which then further sequenced on the Illumina NovaSeq platform to generate 250 bp paired-end reads according to a standard sequencing protocol.

## Bioinformatics analysis of sequencing data

Raw fastq reads were analyzed using the Quantitative Insights into Microbial Ecology 2 (QIIME2) platform [19]. Demultiplexing of sequencing reads removes the primers, barcode sequences, errors and low-quality reads according to the following criteria: (i) reads were removed when the average quality score was < 30; (ii) reads were discarded if ambiguous N bases were present; and (iii) reads not between 220 bp and 500 bp from the raw data. After trimming low-quality bases from the de-multiplexed reads, the merged overlapping paired-end reads were denoised by filtering and correcting Illumina amplicon sequencing errors using the Divisive Amplicon Denoising Algorithm 2 plugin implemented in QIIME2. Finally, amplicon sequence variants (ASVs) were obtained and quantified for each sample.

Taxonomic categories from the phylum to genus levels were assigned using a pre-trained Naive Bayes classifier (25 points) and the q2-feature-classifier function from QIIME2. The classifier was trained against version 132 of the SILVA database. After taxonomic categories of assignment, ASVs table was analyzed by Calypso software (V8.84; http://cgenome.net/calypso/) [20]. Taxa with an average relative abundance below 0.01% and over 95% zeroes were removed. Furthermore, samples with less than 3,000 sequence reads were also removed. Reads were rarefied to the minimum 8,614 sequencing reads per sample only for microbiome α diversity analysis.

## Statistical analyses

Clinical data of patients were analyzed by SPSS software (SPSS v.20.0 for Windows; SPSS; Chicago, IL, USA), and microbiota data were analyzed and visualized by R software (V3.6.3; https://www.R-project.org/). Categorical variables are presented as absolute numbers (percentage), and continuous variables are described as the median (interquartile range, IQR) or mean ± standard deviation (SD). The two groups were compared by chi square tests for categorical variables, and Mann–Whitney *U* test or Student's *t*-test was used for continuous variables. Chao1 index and Shannon index were used to calculate α diversity. β diversity was calculated based on the unweighted UniFrac distance by principal coordinate analysis (PCoA) using the PCoA function in the ape R-package [21]. The permutational multivariate analysis of variance (PERMANOVA) was used to test the difference of β diversity by the Adonis function [22]. The nonparametric analysis of similarities by Anosim function was used to evaluate homogeneity between the two groups [23]. PICRUSt analysis could be used to predict the potential functions of gut microbiotas based on MetaCyc database. The *P* values were corrected for all the multiple comparisons by controlling the false discovery rate using the Benjamini–Hochberg method with a target rate of 0.25 for q values.

The process of differential analysis at different taxonomic levels was performed by using Wilcoxon rank-sum test. A supervised comparison of the microbiota between the POR and non-POR group was conducted via linear discriminant (LDA) and effect size (LEfSe) analyses [16]. A logarithmic LDA score cutoff of 2.0 was set to identify important taxonomic differences between the POR and non-POR groups. To identify microbial taxa associated with the clinical factors, we employed linear mixed model to perform per-feature tests (https://huttenhower.sph.harvard.edu/maaslin2) [24]. The clinical characteristics were set as fixed

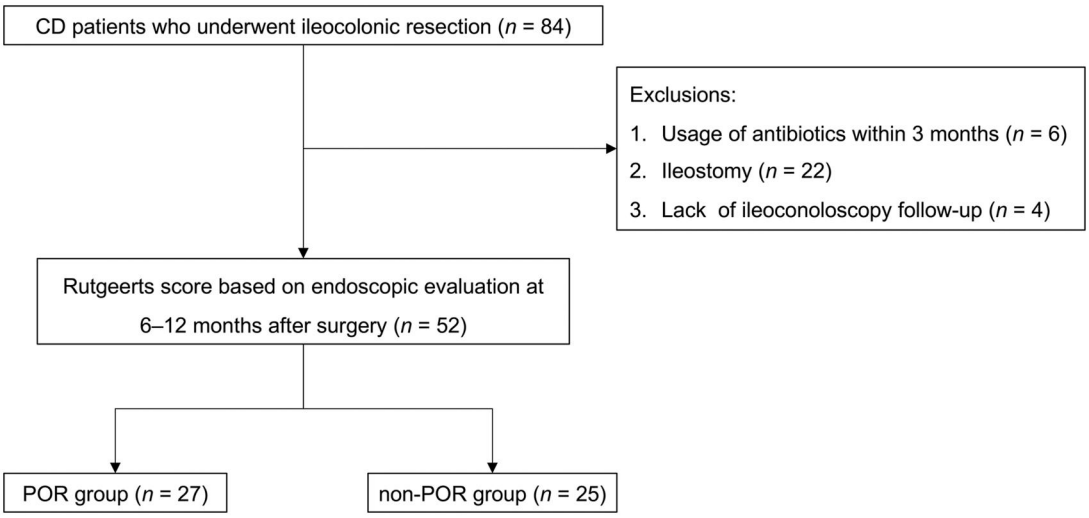


**Figure 1.** Patient flowchart of the study. Fifty-two patients with CD were divided into POR group ($n = 27$) and non-POR group ($n = 25$). CD = Crohn's disease, POR = postoperative recurrence.

effects. Cumulative rate of non-POR was estimated by the Kaplan–Meier analysis. All $P$ values $< 0.05$ were considered statistically significant.

# Results

## Clinical characteristics between patients with POR and non-POR

A total of 52 fecal samples from 52 patients with CD and ileocolonic resection were collected for microbial analysis. The clinical characteristics between the POR ($n = 27$) and non-POR ($n = 25$) groups are shown in Table 1. Factors including age, disease duration, current smoker, surgical types, surgical indications, postoperative medications and laboratory results had no significant differences between the two groups ($P > 0.05$). Male patients were more likely to be in the non-POR group than in the POR group (76.0% *vs.* 44.4%, $P = 0.020$).

## Alteration of fecal microbiotas between CD patients with POR and non-POR

The alterations of fecal microbiotas between these two groups were analyzed. Regarding phylogenetic ($\alpha$) diversity, the Chao1 index in the POR group was significantly lower than that in the non-POR group, which suggests that the patients with POR had a poor community richness (106.5 *vs.* 124, $P = 0.013$, Figure 2A). Meanwhile, the Shannon diversity in the POR patients also had the decreased tendency (2.518 *vs.* 2.910, $P = 0.165$, Figure 2B). Beta diversity showed a markedly separated distribution of fecal microbial community between the POR and non-POR groups ($P = 0.007$, Figure 2C). Multivariate analysis demonstrated a significant difference in the microbial structure between the two groups ($P = 0.032$, Figure 2D).

To further detect characteristic microbial signature in patients with POR of CD, LEfSe analysis at genus level was performed. Notably, only *Flavonifactor* genus was enriched in the POR group, while 15 genera enriched in the non-POR group, especially *Lactobacillus* and *Streptococcus* genera becoming the two top enriched taxa in the non-POR patients (LDA score $> 2$, $P < 0.05$, Figure 2E). Besides, the patients with non-POR of CD still harbored an increased abundance of *Stomatobaculum*, *Lachnoanaerobaculum*, *Oribacterium*, *Catonella*, *Streptophyta*, *Lautropia*, *Olsenella*, *Atopobium*, *Corynebacterium*, *Ezakiellla*, *Porphyromonas*, *Leptotrichia* and *Eubacterium* genera. We further performed Wilcoxon rank-sum test on the top 20 gut microbiota at the genus level and identified 12 differential genera between the two groups ($P < 0.05$, Figure 2F). Consistent with the LEfSe analysis, *Flavonifractor* is the only microbial genus with higher abundance in the patients with POR than in non-POR ($P = 0.03$, Figure 2F). Another 11 increased genera in non-POR patients were *Streptococcus*, *Lactobacillus*, *Atopobium*, *Leptotrichia*, *Oribacterium*, *Stomatobaculum*, *Eubacterium*, *Lachnoanaerobaculum*, *Corynebacterium*, *Olsenella* and *Streptophyta* ($P < 0.05$, Figure 2F). Taken together, the alliterated fecal microbial compositions may be associated with the endoscopic outcome in patients with CD after surgery.

To determine specific intestinal microbial taxa that independently contributed to POR, we adjusted age, sex and postoperative medications using MaAsLin2 to exclude the influence of confounding factors, and the results showed that *Lactobacillus* ($\beta$ coefficient $= -3.44$, $P = 0.001$) and *Streptococcus* ($\beta$ coefficient $= -2.34$, $P = 0.004$) were closely correlated to non-POR (Figure 3A and B).

We further investigated the relationship of these two genera (*Lactobacillus* and *Streptococcus*) with recurrence. According to the median relative abundance of *Lactobacillus* and *Streptococcus*, the patients were classified into the low abundance group and the high abundance group. The patients in high abundance of *Streptococcus* group ($n = 26$, relative abundance: 0.899%–38.717%) had a longer period to maintain disease remission compared with those in low abundance group ($n = 26$, relative abundance: 0.0463%–0.748%, $P < 0.010$, Figure 4A). However, there was no significant difference of non-POR rate for *Lactobacillus* (high abundance group, $n = 27$, relative abundance: 0.031%–70.950%; low abundance group, $n = 25$, relative abundance: 0–0.027%, $P = 0.400$, Figure 4B). We further validated this results in two independent cohorts [5] and found that *Streptococcus* was significantly reduced in resected tissues of patients who developed POR (Table 2). This finding indicates that the depletion of *Streptococcus* in feces may play an important role in POR of patients with CD.

## Functional prediction of gut microbiotas between CD patients with POR and non-POR

The results of PICRUSt showed 11 metabolic pathways were significantly depleted in the POR group compared with the non-POR

**Table 1.** Comparison of clinical characteristics between patients with and without POR

| Characteristic | Non-POR group ($n = 25$) | POR group ($n = 27$) | *P*-value |
|---|---|---|---|
| **Clinical parameter** | | | |
| Sex, *n* (%) | | | 0.020 |
| Male | 19 (76.0) | 12 (44.4) | |
| Female | 6 (24.0) | 15 (55.6) | |
| Age at surgery (mean ± SD), years | 39.4 ± 11.6 | 39.4 ± 14.2 | 0.993 |
| Disease duration of CD (mean ± SD), months | 18.2 ± 12.7 | 19.4 ± 10.4 | 0.647 |
| Time between surgery and colonoscopy (mean ± SD), months | 9.9 ± 3.2 | 10.4 ± 2.7 | 0.441 |
| Prior bowel resection, *n* (%) | 2 (8.0) | 6 (22.2) | 0.252 |
| Previous penetrating diseases, *n* (%) | 10 (40.0) | 16 (59.3) | 0.165 |
| Active smoking at resection, *n* (%) | 11 (44.0) | 7 (25.9) | 0.171 |
| Type of operation, *n* (%) | | | 0.404 |
| Ileocecal resection | 8 (32.0) | 5 (18.5) | |
| ileocolonic resection | 17 (68.0) | 22 (81.4) | |
| History of perianal diseases, *n* (%) | 6 (24.0) | 7 (25.9) | 0.873 |
| Surgical indications, *n* (%) | | | 0.336 |
| Failure of drug therapy | 5 (20.0) | 2 (3.6) | |
| Obstruction | 14 (56.0) | 15 (55.5) | |
| Perforation | 6 (24.0) | 10 (37.0) | |
| Granulomas in the resection specimen, *n* (%) | 13 (52.0) | 17 (63.0) | 0.575 |
| Medical prophylaxis after surgery, *n* (%) | | | 0.400 |
| No | 6 (24.0) | 4 (14.8) | |
| Yes | 19 (76.0) | 23 (85.2) | |
| Postoperative medications, *n* (%) | | | 0.688 |
| AZA or IFX monotherapy | 12 (63.1) | 18(78.3) | |
| AZA + IFX | 7 (36.9) | 5 (21.7) | |
| **Laboratory examination at endoscopy, median (IQR)** | | | |
| CRP (g/L) | 3.02 (2.29–4.90) | 3.50 (3.02–19.33) | 0.120 |
| WBC ($\times 10^{12}$/L) | 4.76 (3.79–5.79) | 5.29 (4.41–7.38) | 0.096 |
| Hb (g/L) | 132.0 (114.25–142.0) | 120.0 (112.0–142.0) | 0.539 |
| PLT ($\times 10^{9}$/L) | 223.0 (167.0–271.0) | 257.0 (198.0–303.0) | 0.150 |
| Albumin (g/L) | 44.5 (39.25–48.25) | 45.0 (42.0–47.0) | 0.922 |

POR = postoperative recurrence, AZA = azathioprine, IFX = infliximab, WBC = white blood cells, Hb = hemoglobin, PLT = platelet, CRP = C-reactive protein, BMI = body mass index, IQR = interquartile ranges, SD = standard deviation.

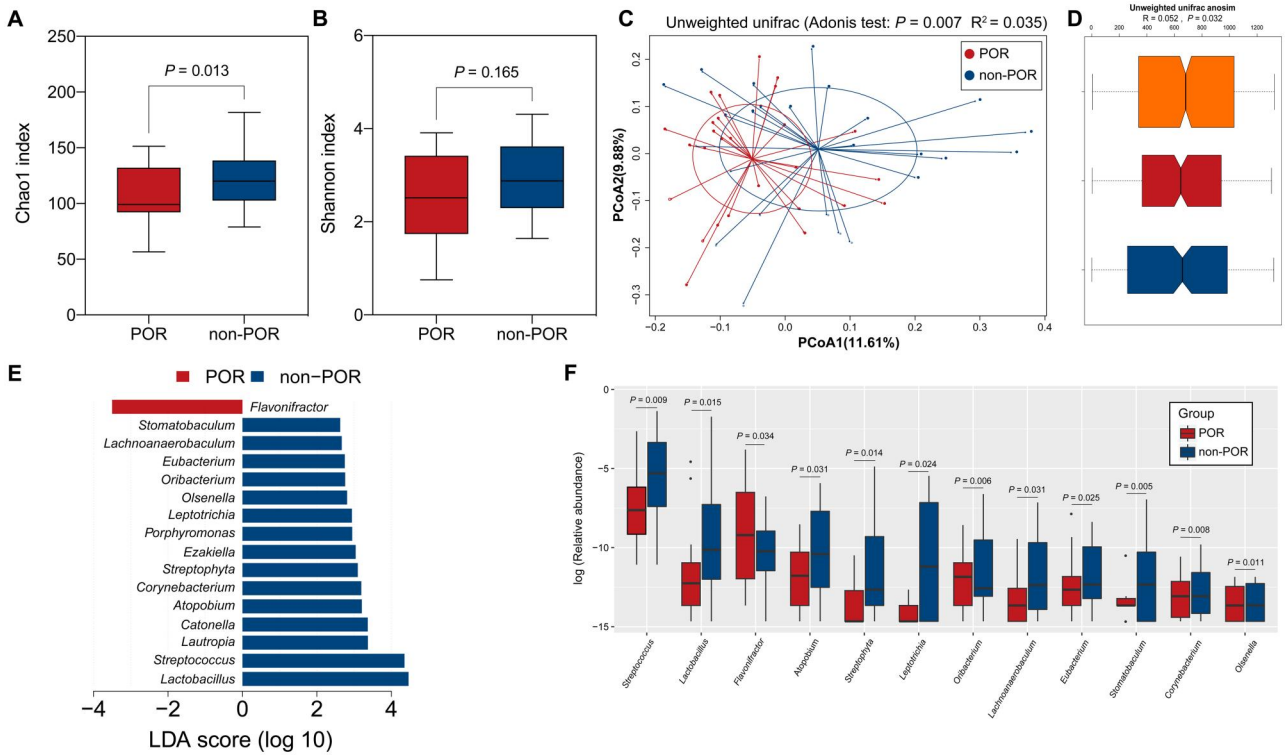


**Figure 2.** Alterations of fecal microbiotas between patients with POR and non-POR. Alpha diversity was calculated by (A) Chao1 ($P = 0.013$) and (B) Shannon indexes ($P = 0.165$). (C) Multivariate analysis of variance (Adonis) and (D) nonparametric analysis of similarities (Anosim) based on unweighted UniFrac distances demonstrated different microbial structure between patients with and without POR ($P = 0.007$ for Adonis, $P = 0.032$ for Anosim). (E) LEfSe analysis revealed significant microbial signatures at the genus level in the patients with POR (LDA score >2, $P < 0.05$). (F) Significant differences at the genus level between the patients with POR and non-POR assessed by Wilcoxon rank-sum test ($P < 0.05$). POR = postoperative recurrence, LEfSe = linear discriminant analysis effect size.

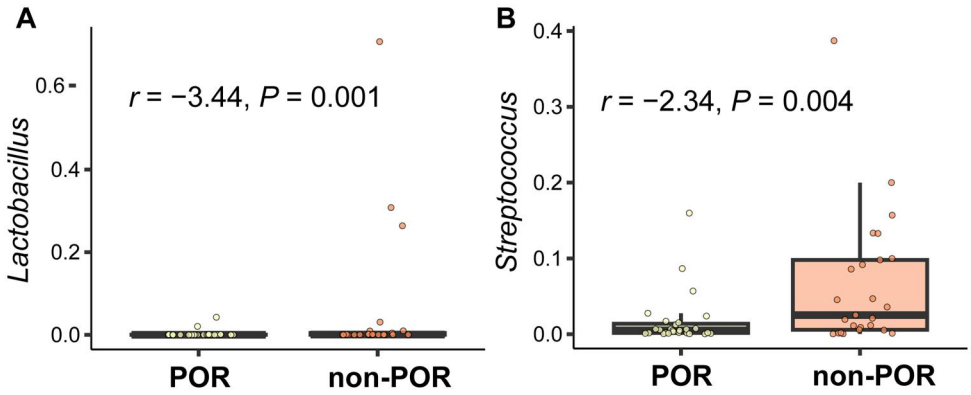


**Figure 3.** *Lactobacillus* (A) and *Streptococcus* (B) were closely correlated with non-POR by microbiome multivariable association with linear models after adjusting the confounding factors ($P < 0.01$). POR = postoperative recurrence.

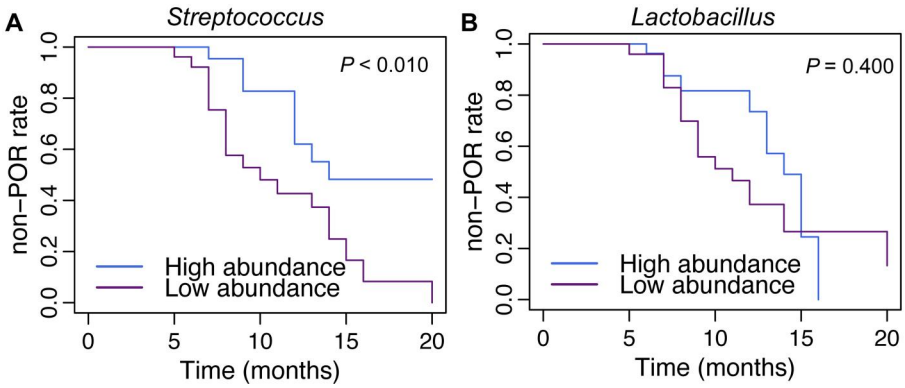


**Figure 4.** Kaplan–Meier's curve showed the proportion of patients with non-POR of CD in different abundance of (A) *Streptococcus* and (B) *Lactobacillus*. CD = Crohn's disease, POR = postoperative recurrence.

**Table 2.** *Sterptococcus* is significantly enriched in the non-POR group by two independent cohorts

| Cohort | Order | Family | Genus | Non-POR | POR | log2 Fold change | P.adjust |
|---|---|---|---|---|---|---|---|
| UNC | Lactobacillales | Streptococcaceae | *Streptococcus* | 0.00487 ($n = 11$) | 0.00033 ($n = 9$) | −2.31217 | 0.000061 |
| WashU | Lactobacillales | Streptococcaceae | *Streptococcus* | 0.000533 ($n = 43$) | 0 ($n = 21$) | −2.10746 | 1.96E-11 |

POR = postoperative recurrence.

group ($P < 0.05$, Figure 5A), including P122-PWY heterolactic fermentation, P124-PWY Bifidobacterium shunt, P164-PWY purine nucleobases degradation I (anaerobic), PWY-3661 glycine betaine degradation I, PWY-5507 adenosylcobalamin biosynthesis I (early cobalt insertion), PWY-5647 2-nitrobenzoate degradation I, PWY-5741 ethylmalonyl-CoA pathway, PWY-5971 palmitate biosynthesis II (bacteria and plants), PWY-6505 L-tryptophan degradation XII (Geobacillus), PWY-7255 ergothioneine biosynthesis I (bacteria) and PWY0-41 allantoin degradation IV (anaerobic). Further Spearman correlation analysis revealed that *Streptococcus* was positively correlated with three carbon metabolisms, including P122-PWY heterolactic fermentation, P124-PWY Bifidobacterium shunt and PWY-5741 ethylmalonyl-CoA pathway ($r > 0.5$, $P < 0.05$, Figure 5B). Among them, ethylmalonyl-CoA pathway has been reported to increase amount of succinate, further produces butyrate and ameliorates DSS-induced colitis [25]. Consequently, these results indicate that the composition and function of the gut microbiota can affect the state of the disease. The increased abundance of *Streptococcus* helps to maintain a non-recurrence state after surgery in patients with CD.

## Discussion

In this study, we found the characteristic fecal microbiota related to POR of patients with CD. Moreover, the enrichment of *Streptococcus* and *Lactobacillus* were positively associated with non-POR status. The high abundance of *Streptococcus* has close relationship with longer disease remission during the postoperative period, and its predicted metabolic pathway ethylmalonyl-CoA may reduce intestinal inflammation as previous studies. Our findings may clarify a newly potential pathophysiology of POR and indicate the specific fecal microbiota as diagnostic biomarker for POR.

Until now, only few studies have investigated the associations of fecal microbial communities and disease status in patients with CD after surgery [15, 26]. Recently, Kathleen *et al.* [15] found an enrichment of *Fusobacterium* but a depletion of *Bifidobacterium* in feces of POR patients through 16S rRNA gene sequencing-based approach. Using genetic analysis GA-map technology, another study found a higher abundance of *Actinobacteria* and a reduced abundance of *Alistipes* in the patients with POR [26].

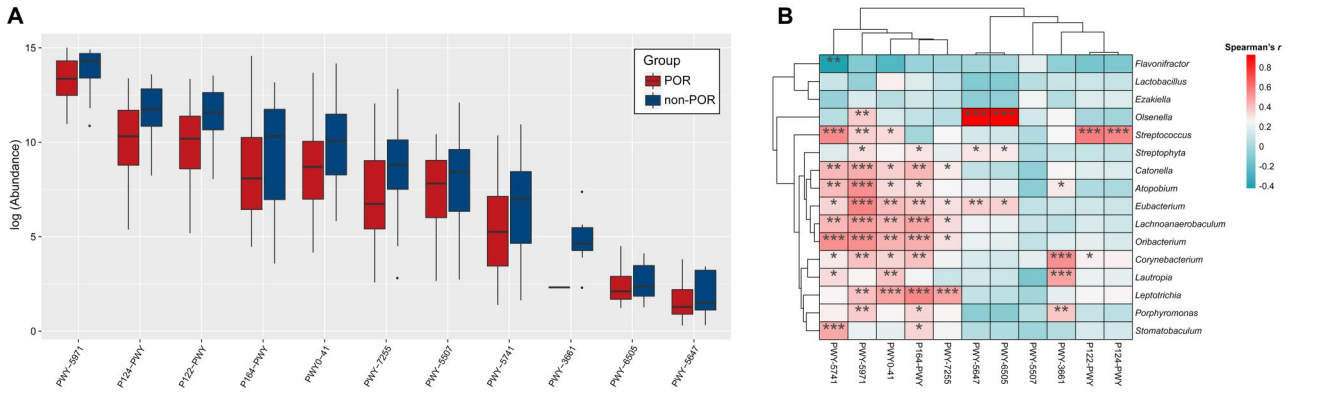


**Figure 5.** The metabolic pathways predicted by PICRUSt dataset in *Lactobacillus* and *Streptococcus*. (A) Mann–Whitney *U* test revealed the differentially enriched bacterial functions between POR and non-POR groups ($P < 0.05$). (B) Heatmap of the Spearman analysis showed the microbial functions and compositions with postoperative recurrence. *< 0.05, **$P < 0.01$, ***$P < 0.001$. POR, postoperative recurrence.

In this study, we found that the abundance of *Flavonifactor* was markedly enriched in the patients with POR, while the *Streptococcus* and *Lactobacillus* strongly related to non-POR. *Flavonifractor* is a genus which potentially influences oxidative stress and inflammatory reaction [27]. Previous results have shown the association of *Flavonifractor* with increased oxidative stress and low-grade inflammation in patients with active CD [28], which is consistent with our result.

In addition, we found the enrichment of *Streptococcus* and *Lactobacillus* was positively associated with non-POR. *Lactobacillus* has been confirmed to have anti-inflammatory function in patients with CD after induced remission [29, 30]. Administration of *Lactobacillus* is beneficial for alleviating inflammation in post-infectious irritable bowel syndrome mouse model [31]. *Streptococcus* has been reported to improve glucose metabolism, producing lactic acid and acetic acid, as well as relieving chronic inflammation [32]. The high abundance of fecal *Streptococcus* from patients with ileal CD was associated with small intestinal mucosal healing [16]. Shahir NM *et al.* [5] recently also noted a common lower abundance of *Streptococcus* in resected bowel of patients with CD who developed POR in two independent cohorts. Moreover, we indicated that *Streptococcus* was positively correlated with the disease remission during the postoperative period, indicating the anti-inflammation role of *Streptococcus*.

Furthermore, we found that *Streptococcus* was related to the functions of three carbon metabolic pathways, i.e. heterolactic fermentation, Bifidobacterium shunt and ethylmalonyl-CoA pathways. Among them, Bifidobacterium shunt is a unique, effective central fermentative pathway of bifidobacterial, and can product acetate and lactate [33]. Bifidobacterium contributes to antibacterial and viral properties, anti-inflammatory properties, and immune enhancement, exhibiting antioxidant activity [33]. The ethylmalonyl-CoA pathway is an anaplerotic reaction sequence in the central carbon metabolism of some gut microbiota. Increased ethylmalonyl-CoA pathway may suggest increased amount of succinate which can be used to produce butyrate to ameliorate the DSS-induced colitis inflammation [25]. These findings support the complex impact of gut environment on gut microbiota function and interactions between bacterial communities, as changes in gut microbiota composition and function may lead to different outcomes in postoperative CD.

There are some limitations in our study. Firstly, this is a single-center study with limited fecal samples, and the baseline characteristics had some unbalanced distribution of sex. We used multivariate correlation analysis to adjust sex and postoperative medication to avoid these potential confounding effects. Secondly, all patients included were hospitalized patients with relatively severe conditions. There is a certain bias in patient selection. Thirdly, this study lacks baseline fecal microbiota data before treatment to investigate the relationship between pre-treatment characteristics of fecal microbiotas and postoperative outcome. And we will investigate the alterations of fecal microbiota before and after operation or treatment in the future prospective study.

## Conclusion

In conclusion, the abundance of *Streptococcus* is closely related to maintain endoscopic remission after surgery in patients with CD. The characteristic changes of fecal microbial communities may become the potential biomarker for diagnosis of POR.

## Data availability

The raw reads of 16S rRNA sequencing can be obtained from the NCBI sequence read archive (SRA) under the accession code PRJNA800594.

## Authors' contributions

H.W., G.Y. and X. W. designed the study. X. W. and X. G. supervised the study. Y. W. and D. Z. collected fecal samples and clinical information. H. W., G. Y. and X. G. performed data analysis and interpretation, created figures and wrote the manuscript. X. W., X. G. and Z. L. performed critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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## Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## References


1. Torres J, Mehandru S, Colombel JF *et al.* Crohn's disease. *Lancet* 2017;**389**:1741–55.
2. Bemelman WA, Warusavitarne J, Sampietro GM *et al.* ECCO-ESCP consensus on surgery for Crohn's disease. *J Crohns Colitis* 2018;**12**:1–16.
3. Pittet V, Rogler G, Michetti P *et al.*; Swiss Inflammatory Bowel Disease Cohort Study Group. Penetrating or stricturing diseases are the major determinants of time to first and repeat resection surgery in Crohn's disease. *Digestion* 2013;**87**:212–21.
4. Rutgeerts P, Geboes K, Vantrappen G *et al.* Predictability of the postoperative course of Crohn's disease. *Gastroenterology* 1990; **99**:956–63.
5. Shahir NM, Wang JR, Wolber EA *et al.* Crohn's disease differentially affects region-specific composition and aerotolerance profiles of mucosally adherent bacteria. *Inflamm Bowel Dis* 2020; **26**:1843–55.
6. Sokol H, Brot L, Stefanescu C *et al.*; REMIND Study Group Investigators. Prominence of ileal mucosa-associated microbiota to predict postoperative endoscopic recurrence in Crohn's disease. *Gut* 2020;**69**:462–72.
7. Gionchetti P, Dignass A, Danese S *et al.*; ECCO. 3rd European evidence-based consensus on the diagnosis and management of Crohn's disease 2016: Part 2: surgical management and special situations. *J Crohns Colitis* 2017;**11**:135–49.
8. Nguyen GC, Loftus EV Jr, Hirano I *et al.*; AGA Institute Clinical Guidelines Committee. American Gastroenterological Association Institute guideline on the management of Crohn's disease after surgical resection. *Gastroenterology* 2017;**152**:271–5.
9. Oh TG, Kim SM, Caussy C *et al.* A universal gut-microbiome-derived signature predicts cirrhosis. Cell Metab 2020;**32**:878–88.e6.
10. Liu Y, Geng R, Liu L *et al.* Gut microbiota-based algorithms in the prediction of metachronous adenoma in colorectal cancer patients following surgery. *Front Microbiol* 2020;**11**:1106.
11. De Cruz P, Kamm MA, Hamilton AL *et al.* Crohn's disease management after intestinal resection: a randomised trial. *Lancet* 2015;**385**:1406–17.
12. Neut C, Bulois P, Desreumaux P *et al.* Changes in the bacterial flora of the neoterminal ileum after ileocolonic resection for Crohn's disease. *Am J Gastroenterol* 2002;**97**:939–46.
13. Dey N, Soergel DA, Repo S *et al.* Association of gut microbiota with post-operative clinical course in Crohn's disease. *BMC Gastroenterol* 2013;**13**:131.
14. De Cruz P, Kang S, Wagner J *et al.* Association between specific mucosa-associated microbiota in Crohn's disease at the time of resection and subsequent disease recurrence: a pilot study. *J Gastroenterol Hepatol* 2015;**30**:268–78.
15. Machiels K, Pozuelo Del Río M, Martinez-De La Torre A *et al.* Early postoperative endoscopic recurrence in Crohn's disease is characterised by distinct microbiota recolonisation. J Crohns Colitis 2020;**14**:1535–46.
16. Hattori S, Nakamura M, Yamamura T *et al.* The microbiome can predict mucosal healing in small intestine in patients with Crohn's disease. *J Gastroenterol* 2020;**55**:1138–49.
17. Rutgeerts P, Geboes K, Vantrappen G *et al.* Natural history of recurrent Crohn's disease at the ileocolonic anastomosis after curative surgery. *Gut* 1984;**25**:665–72.
18. Santiago A, Panda S, Mengels G *et al.* Processing faecal samples: a step forward for standards in microbial community analysis. *BMC Microbiol* 2014;**14**:112.
19. Bolyen E, Rideout JR, Dillon MR *et al.* Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 2019;**37**:852–7.
20. Zakrzewski M, Proietti C, Ellis JJ *et al.* Calypso: a user-friendly web-server for mining and visualizing microbiome-environment interactions. *Bioinformatics* 2017;**33**:782–3.
21. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol* 2005; **71**:8228–35.
22. Anderson MJ. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 2001;**26**:32–46.
23. Anderson MJ. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* 2006;**62**:245–53.
24. Thingholm LB, Rühlemann MC, Koch M *et al.* Obese individuals with and without type 2 diabetes show different gut microbial functional capacity and composition. *Cell Host Microbe* 2019;**26**: 252–64.e10.
25. Kim J, Choi JH, Ko G *et al.* Anti-inflammatory properties and gut microbiota modulation of porphyra tenera extracts in dextran sodium sulfate-induced colitis in mice. *Antioxidants (Basel)* 2020; **9**:988.
26. Strombeck A, Lasson A, Strid H *et al.* Fecal microbiota composition is linked to the postoperative disease course in patients with Crohn's disease. *BMC Gastroenterol* 2020;**20**:130.
27. Coello K, Hansen TH, Sorensen N *et al.* Affective disorders impact prevalence of Flavonifractor and abundance of Christensenellaceae in gut microbiota. *Prog Neuropsychopharmacol Biol Psychiatry* 2021; **110**:110300.
28. Luceri C, Bigagli E, Agostiniani S *et al.* Analysis of oxidative stress-related markers in Crohn's disease patients at surgery and correlations with clinical findings. *Antioxidants (Basel)* 2019; **8**:378.
29. Wang Y, Gao X, Ghozlane A *et al.* Characteristics of faecal microbiota in paediatric Crohn's disease and their dynamic changes during infliximab therapy. *J Crohns Colitis* 2018;**12**:337–46.
30. He C, Wang H, Liao WD *et al.* Characteristics of mucosa-associated gut microbiota during treatment in Crohn's disease. *World J Gastroenterol* 2019;**25**:2204–16.
31. Wang H, Gong J, Wang W *et al.* Are there any different effects of Bifidobacterium, Lactobacillus and Streptococcus on intestinal sensation, barrier function and intestinal immunity in PI-IBS mouse model? *PLoS One* 2014;**9**:e90153.
32. Hwang JE, Kim KT, Paik HD. Improved antioxidant, anti-inflammatory, and anti-adipogenic properties of hydroponic ginseng fermented by leuconostoc mesenteroides KCCM 12010P. *Molecules* 2019;**24**:3359.
33. Killinger BJ, Whidbey C, Sadler NC *et al.* Activity-based protein profiling identifies alternating activation of enzymes involved in the bifidobacterium shunt pathway or mucin degradation in the gut microbiome response to soluble dietary fiber. *NPJ Biofilms Microbiomes* 2022;**8**:60.