Basic Study

Distinct gut microbiota profiles in patients with primary sclerosing cholangitis and ulcerative colitis

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Abstract

AIM
To characterize the gut bacterial microbiota of patients with primary sclerosing cholangitis (PSC) and ulcerative colitis (UC).
This study demonstrates specific microbial and three other genera was linked and July 7, 2017.

**RESULTS**

Microbial profiles in both PSC and UC were characterized by low bacterial diversity and significant change in global microbial composition. *Rothia, Enterococcus, Streptococcus, Veillonella*, and three other genera were markedly overrepresented in PSC regardless of concomitant inflammatory bowel disease (IBD). *Rothia, Veillonella* and *Streptococcus* were tracked to the species level to identify *Rothia mucilaginosa*, *Streptococcus infantus*, *S. alactolyticus*, and *S. equi* along with *Veillonella parvula* and *V. dispar*. PSC was further characterized by decreased abundance of *Adlercreutzia equolifaciens* and *Prevotella copri*. Decrease in genus *Phascolarctobacterium* was linked to presence of colonic inflammation regardless of IBD phenotype. *Akkermansia muciniphila*, *Butyricicoccus pullicaecorum* and *Clostridium colinum* were decreased in UC along with genus *Roseburia*. Low levels of serum albumin were significantly correlated with enrichment of order Actinomycetales.

**CONCLUSION**

PSC is associated with specific gut microbes independently of concomitant IBD and several bacterial taxa clearly distinguish IBD phenotypes (PSC-IBD and UC).

**Key words:** Dysbiosis; Inflammatory bowel disease; Ulcerative colitis; Gut microbiota; Primary sclerosing cholangitis

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Core tip: This study demonstrates specific microbial patterns associated with PSC and/or concomitant inflammatory bowel disease (PSC-IBD). Several bacterial taxa convincingly distinguish PSC-IBD from ulcerative colitis. Gut microbiota composition also differs in patients with PSC overlap with autoimmune hepatitis. Disease-specific microbial features traceable down to the species level may lead to establishing suitable biomarkers or outlining new research directions in the field of PSC and IBD pathogenesis.

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**MATERIALS AND METHODS**

**Study participants**

PSC, PSC-IBD, and UC patients for this single center, cross-sectional study were recruited at the outpatient department of the Institute for Clinical and Experimental Medicine, Prague. Healthy controls (HCs) were age and sex matched volunteers with insignificant medical history that were randomly selected from the hospital patient database. HCs were eligible if they had no history of malignant or autoimmune disease, major abdominal surgery, gastrointestinal disease or chronic gastrointestinal symptoms. Anyone, healthy or

** INTRODUCTION**

**Primary sclerosing cholangitis (PSC)** is a chronic liver disorder of unknown etiology, characterized by inflammation and stenosis of the bile ducts[2,3]. The disease may progress to severe liver fibrosis and subsequent liver cirrhosis, and may eventually lead to liver failure and death[4]. Orthotopic liver transplantation (OLT) is the only currently available effective treatment for end-stage liver disease[5]. Inflammatory bowel disease (IBD) is present in 60% to 80% of patients with PSC[6]. Concomitant colonic disease is often classified as ulcerative colitis (UC)[6], but is also considered to be a distinct phenotype and referred to as “PSC-IBD”[7].

The involvement of gut bacteria in IBD is widely accepted, but the etiology and pathogenesis of IBD is still not fully understood. It is generally assumed that the inflammation results from an aberrant immune response to antigens of gut microbiota resident in genetically susceptible individuals[7]. It has been proposed that either an imbalance of the intestinal microbiota (dysbiosis), or presence of commensal bacteria with increased virulence could promote exaggerated local and systemic immune responses by disrupting microbiota-mucosa interactions[8,9].

Evidence from animal models[11] and success of antibiotic treatment in PSC patients[12] suggest that disruption of gut microbiota may play a significant role in pathogenesis of PSC. Recently, several studies described specific changes in the gut microbiota of PSC patients that were not related to dysbiosis with concomitant IBD[13-20]. However, those studies differed in sampling, experimental methods and design, in addition to outcome.

The aim of this study was to identify microbial features specific to patients with PSC (with and without concomitant IBD), in an independent, well-characterized cohort, to compare them with healthy controls (HC) and patients with UC, and to verify previous findings.
diseased, with antibiotic use within the previous 3 mo, a history of colorectal surgery, or ongoing or recent infectious colitis were excluded. Patients who received liver transplants were excluded because we assumed that OLT and related medications, immunosuppressive therapy in particular, might significantly influence the microbiota composition, and therefore cause substantial bias. To exclude the potential impact of obesity or malnutrition, only patients and controls with a normal BMI were recruited.

Sample collection, DNA extraction and amplification

Stool samples were freshly collected in standardized, sterile collection tubes by participants and brought to the clinic when they made their routine clinic visits. All samples were delivered within 6 h of collection, immediately frozen at -20 ℃ and within 2 wk transferred to a -80 ℃ freezer for long-term storage. Care was taken to prevent thawing until samples were suspended in extraction buffer^{[21,22]}. A MasterPure™ Complete DNA and RNA Purification Kit (Epicentre) was used for DNA extraction using a FastPrep®-24 Instrument homogenizer and Lysing Matrix Y zirconium oxide spheres (both MP Biomedicals). DNA concentration was equalized in each sample after quantitation with a Qubit™ 2.0 Fluorometer and a dsDNA BR Assay Kit (Life Technologies). KAPA 2G Robust Hot Start DNA Polymerase (Kapa Biosystems) was used to amplify segments of the 16S rRNA gene including the V3 and V4 regions using 341 F and 806R primers^{[23]}. The PCR protocol was initialized with 94 ℃ for 3 min, followed by 25 cycles of denaturation (94 ℃, 30 s), annealing (54.2 ℃, 1 min) and extension (72 ℃, 1 min and 15 s), with the final extension (72 ℃) for 10 min. The PCR products were purified and normalized for concentration with SequaPrep™ Normalization Plate Kit (ThermoFischer Scientific).

Library preparation and sequencing

After pooling, sample ligation was performed with a TruSeq DNA PCR-free LT Sample Preparation Kit (Illumina) following the standard protocol provided by the manufacturer. The libraries were validated by a KAPA Library Quantification Kit (Kapa Biosystems) prior to submission to The Genomics Core Facility, Central European Institute of Technology (CEITEC; Brno, Czech Republic) for sequencing on a MiSeq Platform (Illumina) using a MiSeq Reagent Kit v3 (Illumina).

Data processing and quality control

Sequencing data were processed using a QIIME package^{[24]}. Briefly, raw reads were demultiplexed and quality filtered, allowing no N characters, a maximum of three consecutive low-quality base calls, a maximum unacceptable Phred quality of Q20, and a maximum of 1.5 barcode errors. Chimeric reads were detected and discarded using USEARCH algorithms^{[25]}. In the final dataset, the median of number of reads per sample reached 42459 sequences. Community statistics and sample comparisons were done on resampled datasets at the level of 16400 sequences. This led to the exclusion of two samples, one from the PSC and one from the HC group because of an insufficient number of reads.

Statistical analysis and bioinformatics

The appropriateness of sequencing depth was checked in rarefaction plots. Alpha diversity statistics were calculated in QIIME^{[24]} using Shannon, Simpson, and observed species diversity indices, and the P value for group comparisons was determined by analysis of variance (ANOVA). Principal coordinate analysis plots were constructed to illustrate the beta diversity of samples based on phylogenetically informed weighted and unweighted UniFrac distance matrices^{[26]}. Significance of clustering patterns was tested by PERMANOVA, Adonis and ANOSIM in the R-vegan package. The multivariate homogeneity of group dispersions was assessed by Permdisp methods. Multivariate association with linear models (MaAsLin) for multivariate analysis^{[27]} applying an additive general linear model was used to assess the association between microbial abundance and patient metadata: Age, sex, gender, presence of PSC/AIH overlap, IBD activity, probiotic use (E. coli Nissle 1917), and use of anti-TNF treatment. A P value < 0.05 was considered statistically significant after adjusting for false discovery rate (FDR). Raw demultiplexed sequencing data, with sample annotations, were submitted to the Short Read Archive (http://www.ncbi.nlm.nih.gov/bioproject/368966).

Ethical approval

This study was approved by The Ethics Committee with multicenter competence of the Institute for Clinical and Experimental Medicine and Thomayer Hospital. All patients and healthy controls signed the informed consent form at the time of sample collection.

RESULTS

Characteristics of the study population

A total of 106 individuals were enrolled, including 32 patients with PSC-IBD, 11 with PSC (without IBD), 32 with UC and 31 HCs. The diagnosis of PSC or PSC overlap with autoimmune hepatitis (PSC/AIH) syndrome was established following recommended biochemical, immunological, histological, and cholangiographic evaluation^{[28-30]}. The extent of UC was categorized endoscopically^{[31]} and Mayo classification^{[32]} was used to describe disease severity. All other patient characteristics were obtained on the day of sample collection (Table 1).

Decrease of microbial diversity is tightly linked to the presence of IBD

A decrease of alpha diversity in all patients compared
Rothia and five other genera were increased in PSC regardless of concomitant IBD

We compared relative abundance corrected for FDR, and identified 12 genera that were significantly increased in patients with PSC compared with HCs (Table 2). Seven of these remained relatively more abundant regardless of concomitant IBD. These were Rothia, Enterococcus, Streptococcus, Clostridium, Veillonella and Haemophilus. On the other hand, Coprobacillus, Escherichia, Corynebacterium and Lactobacillus genera were associated with PSC-IBD, but not with isolated PSC. A distinct increase in the relative abundance of family Micrococcaceae (P < 0.001) in PSC and PSC-IBD (P < 0.05) was driven by the strong overrepresentation of Rothia. Similarly, enrichment of genus Lactobacillus and Streptococcus

with HCs was apparent from differences in the Chao1 and Shannon indices, the decrease was particularly clear in patients with IBD, especially in the UC group. No evident differences in the Simpson indices of the groups were observed (Figure 1).

The global microbiota composition was clearly shifted by the presence of liver disease in PSC patients compared with HCs (Adonis R² = 0.021, P = 0.001; Figure 2A). Similarly, samples from patients with IBD clustered separately, depending significantly on the UC factor (Adonis R² = 0.021, P < 0.001) (Figure 2B). However, the homogeneity of group dispersion was significantly different when measured with Permdisp (P > 0.1). No such significant shifts were detected when comparing PSC and PSC-IBD groups. The disruption of bacterial beta diversity is shown in stack plots (Figure 3).

Table 1 Demographical characteristics of the study population categorized by disease phenotype (and healthy controls) n (%)

|                      | PSC-IBD (n = 32) | PSC (n = 11) | UC (n = 32) | Healthy controls (n = 31) |
|----------------------|------------------|-------------|-------------|--------------------------|
| Gender; male/female  | 24/8 (75%/25%)   | 10/1 (90.9%/9.1%) | 17/15 (53.1%/46.9%) | 13/18 (41.9%/58.1%)     |
| Median age (range), yr | 35 (18-60)   | 45 (18-69) | 40 (20-71) | 44 (22-72)                |
| Overlap syndrome PSC/AIH | 6 (18.8) | 2 (18.2) | N/A | N/A                     |
| Total bilirubin (μmol/L; mean ± SD) | 47 ± 56.8 | 34.4 ± 32.8 | 13.1 ± 6.7 | 12.8 ± 6.2                |
| AST (μkat/L; mean ± SD) | 1.5 ± 0.8 | 1.1 ± 0.8 | 0.4 ± 0.1 | 0.5 ± 0.3                  |
| ALT (μkat/L; mean ± SD) | 1.8 ± 0.9 | 1.3 ± 1.1 | 0.5 ± 0.2 | 0.6 ± 0.4                  |
| ALP (μkat/L; mean ± SD) | 7.4 ± 5.7 | 4.3 ± 4.9 | 1.1 ± 0.4 | 1 ± 0.3                   |
| GGT (μkat/L; mean ± SD) | 7.5 ± 7.4 | 12.2 ± 25.5 | 0.5 ± 0.5 | 0.7 ± 1.6                 |
| IBD extent:          |                 |             |             |                          |
| Pancolitis            | 28 (87.5)  | N/A | 25 (78.1) | N/A                     |
| Left-sided            | 0 (0) | N/A | 7 (21.9) | N/A                     |
| Right sided           | 4 (12.5) | N/A | 0 (0) | N/A                     |
| IBD activity:         |                 |             |             |                          |
| Mild or remission     | 24 (75)  | N/A | 20 (62.5) | N/A                     |
| Moderate              | 4 (12.5) | N/A | 3 (9.4) | N/A                     |
| Severe                | 4 (12.5) | N/A | 9 (28.1) | N/A                     |
| Medication during last month: | | | | |
| UDCA                  | 33 (100) | 11 (100) | 0 (0) | 0 (0)                   |
| 5-ASA                 | 26 (81.3) | 5 (0) | 31 (96.9) | 0 (0)                 |
| Corticosteroids       | 12 (37.5) | 2 (18.2) | 3 (9.4) | 0 (0)                   |
| Azathioprine          | 13 (40.6) | 1 (9.1) | 14 (43.8) | 0 (0)                  |
| Anti-TNFα             | 0 (0) | 0 (0) | 10 (31.3) | 0 (0)               |
| Probiotics, E. coli Nissle 1917 | 4 (12.5) | 1 (9.1) | 9 (28.1) | 0 (0)               |

PSC: Primary sclerosing cholangitis; IBD: Inflammatory bowel disease; UC: Ulcerative colitis; AIH: Autoimmune hepatitis; UDCA: Ursodeoxycholic acid; ASA: Aminosalicylic acid

Figure 1 Alpha diversity was consistently reduced in patients with ulcerative colitis as determined by Chao1 index, Shannon index and Simpson index. Groups labeled by the same letter (a, b) on the graph are not significantly different from each other (P < 0.05) as analyzed by ANOVA with Tukey's post-hoc test.
was detected only in PSC patients without concomitant IBD. *Faecalibacterium prausnitzii*, *Coprococcus catus* and *Ruminococcus gnavus* were decreased in all patients compared with HCs.

**Several species clearly distinguish PSC-IBD from UC patients**

A distinct dysbiotic pattern characterized by the significant underrepresentation of phylum *Verrucomicrobia* (*P* < 0.001) distinguished patients in the UC group from HCs. The low signal of this group of bacteria was also evident at the family level (*Verrucomicrobiaceae*, *P* < 0.001) and genus (*Akkermansia*, *P* < 0.001) levels and with the extremely low abundance of *Akkermansia muciniphila* (*P* < 0.001), reduction of *Butyrivibrio fibrisolvens* (*P* < 0.05) and genus *Roseburia* (*P* < 0.05). The decrease of genus *Akkermansia* (*P* < 0.01) and *Akkermansia muciniphila* (*P* < 0.01) remained significant (along with the respective family and phylum signals) when compared with microbiota from PSC-IBD patients. Furthermore, *Clostridium colinum* was significantly underrepresented when compared with both HCs (*P* < 0.001) and PSC-IBD patients (*P* < 0.01). On the other hand, three genera that distinguished PSC patients from HCs (*Rothia* *P* < 0.05; *Streptococcus*, *P* < 0.05; *Veillonella*, *P* < 0.05) were increased in PSC-IBD compared to UC. The pattern continued at the species level with *R. mucilaginosa* (*P* < 0.05), *V. parvula* (*P* < 0.05), *V. dispar* (*P* < 0.05), and unclassified species of genus *Streptococcus* (*P* < 0.01) and genus *Blautia* (*P* < 0.05). Order Fusobacteriaceae (*P* < 0.05) were more abundant in UC then in PSC-IBD, but we were not able to track this tendency to lower taxonomic levels (Tables 2 and 3).

**Disturbance in order Actinomycetales correlates with serum albumin levels in the overall population and has a distinct pattern in patients with PSC/AIH overlap**

In the multivariate analysis, an OTU (operational taxonomic unit) belonging to genus *Actinomycetes*...
was significantly \((P < 0.01)\) enriched, when overlapping AIH was present in patients with PSC (including those with PSC-IBD). Multivariate analysis using MaAsLin found a significant tendency of order Actinomycetales to be increased in study subjects with low serum albumin level \((P < 0.01)\). This finding was no longer significant when adjusting for the specific patient groups and HCs (Figure 4).

**DISCUSSION**

The intestinal microbiota is involved in the pathogenesis of various diseases and is, therefore, a potential biomarker or even a therapeutic target\(^{[33]}\). We identified specific signatures of the fecal microbiota that distinguished patients with PSC from those with UC and from HCs. This is the first study of its kind from central Europe, a region characterized by specific dietary habits. Moreover, since recruitment centre may have a serious impact on microbial composition in multi-centric studies\(^{[18]}\), we decided to perform this study in a single-centre design with meticulous care for consistency of sample collection, processing and analysis.

We found disruption of gut bacteria homeostasis in patients with UC, which was characterized by decreased microbial diversity. This is in line with previous studies in which low bacterial diversity was associated with IBD\(^{[34]}\). Moreover, significant disease-specific shifts of global microbiota composition were apparent when comparing PSC patients with healthy controls and PSC-IBD with UC patients.

In complex diseases, several distinct environmental factors may influence the composition of the gut microbial community. In our series of PSC and PSC-IBD patients, it was apparent that liver disease was primary factor associated with disease-specific dysbiosis, independent of dysbiotic influences of IBD, as the changes in gut microbiota did not significantly differ in PSC patients with and without established, concomitant IBD. However, stratifying PSC patients into "with or without IBD" subphenotypes may be controversial because PSC-IBD may have a subclinical course that might be easily missed during endoscopic and histological assessment. In addition, there is no agreement on the recommended interval for endoscopic evaluation in patients with PSC and no clinical suspicion of IBD. Furthermore, previous
studies evaluated PSC-UC and PSC-CD as subgroups of their study cohorts\cite{13,16}. However, we assumed, that IBD in PSC rarely has macroscopic features typical for CD and that histological findings might be often misinterpreted and the diagnosis is often eventually changed to UC. Therefore, we included only patients with typical features of IBD associated with PSC and universally categorized the phenotype as PSC-IBD\cite{37}.

We found a close association of the relative abundance of several genera with PSC, including Rothia, Streptococcus, Enterococcus, Veillonella, Clostridium, and Haemophilus regardless of the presence or absence of concomitant IBD. The association of Rothia with PSC was the most striking, and to our knowledge, this is the first study to describe such a significant relation with PSC.

The increased abundance of genus Rothia, and R. mucilaginosa in particular, in patients with PSC suggests that oral microbiota may be overrepresented in the lower GI populations of patients with advanced liver disease, which is in line with previous reports\cite{35}. In previous studies, infections caused by Rothia species were predominantly found in immunocompromised individuals and patients with indwelling vascular devices\cite{36}. Since Rothia is sensitive to gastric fluid\cite{37}, we speculate, that our findings may reflect contamination of the intestinal microbial community by previous endoscopic retrograde cholangiopancreatography (ERCP), with repeated stenting in particular. Our data alone are insufficient to indicate a direct link to disease pathogenesis. However, recent reports of successful treatment of PSC and recurrent PSC with vancomycin\cite{12,38}, suggest that Rothia or other vancomycin-sensitive microbes\cite{39} are reasonable targets of further research in this field.

The increased abundance of genera Enterococcus and Lactobacillus confirms the results of a recent complex study from Belgium\cite{40}. Even though we did not find a significant increase of genus Fusobacterium (one of three significantly increased genera in the Belgian study), we observed the clear enrichment of family Fusobacteriaceae in PSC-IBD patients ($P < 0.001$).

We could replicate previous results of enrichment of Veillonella, notably V. parvula and V. dispar, as a key feature of PSC-associated dysbiosis\cite{13}. Previously, this genus was associated with several other progressive fibrotic disorders\cite{40}. As in our study, Kummen et al\cite{13} did not evaluate the influence of liver cirrhosis, which might be the condition that predisposes towards Veillonella increase, not necessarily PSC itself. This is further supported by Sabino et al\cite{16} who found that Veillonella abundance in PSC was no longer significantly changed when patients with confirmed liver cirrhosis were excluded from the analysis. Sabino et al\cite{16} diagnosed liver cirrhosis diagnosis biopsy and/or MRI imaging and/or elastography. Confirming cirrhosis in advanced cholestatic liver disease by elastography may be challenging because of imaging distortion, and biopsy can miss focally expressed morphological features\cite{41}. Therefore, it is not certain, whether the alteration in microbiota composition was a PSC-specific characteristic, or more likely reflects homeostatic disruption in advanced chronic liver disease in general.

In our dataset, genus Adlercreutzia was significantly decreased in PSC compared with HCs. Adlercreutzia is a genus that includes only one species (A. equolifaciens) capable of converting ingested isoflavones, which are abundant in legumes and soya beans, into equol\cite{42}. Equol has a high affinity for estrogen receptors (ERs) and may be a selective estrogen receptor modulator\cite{43}. ER expression on cholangiocytes is increased in cholestatic liver diseases but is absent in healthy individuals\cite{44}. Taking into account the fact that the prevalence of PSC is much decreased in women and Asian populations\cite{45} (and presumably in populations with higher than average consumption of isoflavones), we could speculate upon potential pathogenetic pathways with significant impact on disease development that are influenced by
dietary habits and endocrine signaling.

We observed a significant decrease of *Ruminococcus gnavus* in all diseased individuals, Png et al. described a manifold increase of this mucin-degrading species in the colonic mucosa of both CD and UC patients. This discrepancy could be, however, caused by the fact that the luminal and mucosal microbiota are significantly different. The low abundance of another mucolytic bacteria, *Akkermansia muciniphila* (and therefore genus *Akkermansia*), in UC is consistent with Png et al. The absence of this bacterium from the microbial community could affect the use of mucins as a carbon source by other bacteria that are important members of microbial community in healthy individuals. In our study, the UC phenotype was characterized by a substantial decrease of *Butyricicoccus pullicaecorum* sp., which is in line with Eeckhaut et al. who found that *B. pullicaecorum* attenuated trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats, and that supernatants of cultures of *B. pullicaecorum* increased transepithelial resistance. Decreased abundance of *Faecalibacterium prausnitzii* has recently been reported in IBD-associated dysbiosis, which also occurred in this study in both UC and with PSC. The decrease of *F. prausnitzii* was particularly significant in patients with an inflamed colon, regardless of IBD phenotype. The decreased abundance of *B. pullicaecorum*, *F. prausnitzii*, and genus *Roseburia* demonstrates disruption in butyrate-producing bacteria, which probably have anti-inflammatory activity. Furthermore, it has been proposed recently that the anti-inflammatory properties of *F. prausnitzii* is associated with the production of 15 kDa protein capable of inhibiting the NF-κB pathway in intestinal epithelial cells.

Bile acids and changes in production, circulation, and conversion may be associated with changes of microbiota composition. Microbiota might thus be influenced by long-term use of ursodeoxycholic acid (UDCA) leading to substantial changes in bile composition. Sabino et al. reported that only 66.7% of a series of 147 patients with PSC-UC used UDCA. In our series, all patients with an established diagnosis of PSC received UDCA as routine clinical practice at our center follows the recommendations of EASL and ECCO, which are contradictory to the AASLD guidelines advocating against routine use of UDCA in PSC. We were thus not able to evaluate the influence of UDCA on microbiota composition. However, recent reports have not reported substantial differences in the microbiota of PSC patients treated or not treated with UDCA.

The impact of antibiotics on gut microbiota composition is clear, but there is no consensus on how to adjust for the antibiotic use, we included only subjects who had not used antibiotics within the previous 3 mo. Sabino et al. enforced only 1 mo cutoff, and Kummen et al. observed no substantial differences related to antibiotic use within the previous 12 mo.

The role of biologics for PSC treatment is currently not sufficiently clear. α4β7 integrin antagonists have demonstrated a positive effect on PSC course and the effect of anti-TNF treatment is dubious with potential risk of serious adverse events. In our series, no PSC patients had been treated with biologics. Nearly one-third of the UC patients had received infliximab, adalimumab, or golimumab, but these agents did not result in microbial shifts that distinguished the UC group.

Multivariate analysis revealed a significant negative correlation of serum albumin level and the relative contribution of order Actinomycetales in the total study population. This might reflect a decrease in this microbial subgroup in patients with advanced liver disease and subsequent alteration of proteosynthesis. As the relation could not be assigned to any specific subgroup of patients, the relevance of this observation is not clear.

Due to the advances in sequencing and bioinformatics, we were able to found some of the most striking changes at the low taxonomic level (species). These disease-specific bacteria could be the cornerstone in search for suitable biomarkers for PSC development or in non-invasive distinguishing of different IBD phenotypes. The fact that most of our findings were consistent with those reported in previous studies validates the study design and methodology. The current evidence supports a key role of microbiota in PSC pathogenesis which, however, needs to be further elucidated by future mechanistic studies.

In conclusion, PSC- and UC-associated dysbiosis was characterized by reduced bacterial diversity, significant changes in global bacterial composition and relative abundance of distinct taxa, primarily at the genus and species levels. The most prominent changes related to PSC were in the genera *Rotthia*, *Streptococcus*, *Veillonella*, *Enterococcus*, *Clostridium*, *Haemophilus*, and *Adlercreutzia*. Several microbes including *Akkermansia muciniphila*, *Butyricicoccus pullicaecorum* and *Clostridium colinum* clearly distinguished the UC and PSC-IBD phenotypes. Specific changes in occurring with PSC/AIH overlap involved enrichment of *Actinomyces spp.*

**COMMENTS**

**Background**

Primary sclerosing cholangitis (PSC) is a chronic liver disease with particularly high incidence in northern Europe and North America. Concomitant inflammatory bowel disease (IBD) is present in majority of patients with PSC. This IBD phenotype is distinct from Crohn’s disease and ulcerative colitis and is often referred to as PSC-IBD. Gut microbiota composition is most likely involved in pathogenesis of this complex and often unfavorable clinical entity that involves the liver and intestine.

**Research frontiers**

Disruption of microbial ecology has previously been described in several clinical conditions including IBD. The few reports on gut microbiota composition in PSC and PSC-IBD have used diverse methods and yielded inconsistent results.
Innovations and breakthroughs
This study describes specific gut microbial patterns associated with PSC and differs in individual IBD phenotypes (UC and PSC-IBD).

Applications
Identifying disease-specific microbial features could be the cornerstone of further research in the field of PSC and IBD pathogenesis as well as the search for suitable biomarkers.

Terminology
PSC is a severe liver disease characterized by fibrosis and stenosis of both intra- and extrahepatic bile ducts; PSC-IBD is a specific phenotype of inflammatory bowel disease associated with PSC; gut microbiota comprises the ecological community of microorganisms residing in the (human) intestine.

Peer-review
Interesting and complex article, a very good work and a beautiful writing. It was difficult to read, because includes so many data, but also very useful in the end.

REFERENCES
1. Gow PJ, Chapman RW. Liver transplantation for primary sclerosing cholangitis. Liver 2000; 20: 97-103 [PMID: 10847476]
2. Chapman RW, Arbogath BA, Rhodes JM, Summerfield JA, Dick R, Scheuer PJ, Sherlock S. Primary sclerosing cholangitis: a review of its clinical features, cholangiography, and hepatic histology. Gut 1980; 21: 870-877 [PMID: 749807]
3. Levy C, Lindor KD. Primary sclerosing cholangitis: epidemiology, natural history, and prognosis. Semin Liver Dis 2006; 26: 22-39 [PMID: 16469230 DOI: 10.1055/s-2006-933560]
4. Karlsen TH, Schrumpf E, Boberg KM. Update on primary sclerosing cholangitis. Dig Liver Dis 2010; 42: 390-400 [PMID: 21072772 DOI: 10.1016/j.dld.2010.01.011]
5. Chapman R, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, Gores GJ. Diagnosis and management of primary sclerosing cholangitis. Hepatology 2010; 51: 660-678 [PMID: 20101749 DOI: 10.1002/hep.23294]
6. Hirschfeld GM, Karlsen TH, Lindor KD, Adams DH. Primary sclerosing cholangitis. Lancet 2013; 382: 1587-1599 [PMID: 23810223 DOI: 10.1016/S0140-6736(13)60096-3]
7. Loftus EV, Harewood GC, Kirsner JB, Tremaine WJ, Harmsen WS, Zinsmeister AR, Jewell DA, Sandborn WJ. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. Gastroenterology 2005; 128: 822-835 [PMID: 16082901 DOI: 10.1053/gastro.2004.21059]
8. Sartor RB. Microbial influences in inflammatory bowel diseases. Gastroenterology 2008; 134: 577-594 [PMID: 18242222 DOI: 10.1053/j.gastro.2007.11.059]
9. Bentley RW, Keenan JI, Geary RB, Kennedy MA, Barclay ML, Roberts RL. Incidence of Mycobacterium avium subspecies paratuberculosis in a population-based cohort of patients with Crohn’s disease and control subjects. Am J Gastroenterol 2008; 103: 1168-1172 [PMID: 18371139 DOI: 10.1111/j.1572-0241.2007.01742.x]
10. Tannock GW. Molecular analysis of the intestinal microflora in IBD. Microbial Immunol 2008; 1 Suppl 1: S15-S18 [PMID: 19079221 DOI: 10.1038/mi.2008.54]
11. Lichtman SN, Koka J, Clark RL, Schwab JH, Sartor RB. Biliary tract disease in rats with experimental small bowel bacterial overgrowth. Hepatology 1991; 13: 766-772 [PMID: 2010172]
12. Tabibian JH, Weeding E, Jorgensen RA, Petz JL, Keach JC, Talwalkar JA, Lindor KD. Randomised clinical trial: vancomycin or metronidazole in patients with primary sclerosing cholangitis - a pilot study. Aliment Pharmacol Ther 2013; 37: 604-612 [PMID: 23384404 DOI: 10.1111/apt.12232]
13. Kummer M, Holm K, Anmarkrud JA, Nygård S, Vesterhus M, Hov JR. The gut microbial profile in patients with primary sclerosing cholangitis is distinct from patients with ulcerative colitis without biliary disease and healthy controls. Gut 2017; 66: 611-619 [PMID: 26887816 DOI: 10.1136/gutjnl-2015-310500]
14. Kummer M, Holm K, Anmarkrud JA, Nygård S, Vesterhus M, Hov JR. Response to ‘Faecal microbiota profiles as diagnostic biomarkers in primary sclerosing cholangitis’ by Rühlemann et al. Gut 2017; 66: 755-756 [PMID: 27340491 DOI: 10.1136/gutjnl-2016-312347]
15. Torres J, Bao X, Goel A, Colombel JF, Pekow J, Jabri B, Williams KM, Castillo A, Odin JA, Meckel F, Kasiibidden F, Peter I, Itzkowitz S, Hsu J. The features of mucosa-associated microbiota in primary sclerosing cholangitis. Aliment Pharmacol Ther 2016; 43: 790-801 [PMID: 26857696 DOI: 10.1111/apt.13552]
16. Sabino J, Vieira-Silva S, Machiels K, Joossens M, Falony G, Ballet V, Ferrante M, Van Assche G, Van der Meers S, Vermeiren S, Raes J. Primary sclerosing cholangitis is characterised by intestinal dysbiosis independent from IBD. Gut 2016; 65: 1681-1689 [PMID: 27207975 DOI: 10.1136/gutjnl-2015-311004]
17. Quraishi MN, Sergeant M, Kay G, Ishai T, Chan J, Constantinidou C, Trivedi P, Ferguson J, Adams DH, Pallen M, Hirschfeld GM. The gut-adherent microbiota of PSC-IBD is distinct to that of IBD. Gut 2017; 66: 386-388 [PMID: 27196590 DOI: 10.1136/gutjnl-2016-319191]
18. Kevas D, Tyler AD, Holm K, Jorgensen KK, Vann MH, Karlsen TH, Kaplan GG, Eksteen B, Gevers D, Hov JR, Silverberg MS. Characterization of Intestinal Microbiota in Ulcerative Colitis Patients with and without Primary Sclerosing Cholangitis. J Crohns Colitis 2016; 10: 330-337 [PMID: 26526357 DOI: 10.1093/jcc/jjv204]
19. Rossen NG, Fuentes S, Boonstra K, D’Haens GR, Heilig HG, Zoetendal EG, de Vos WM, Ponsioen CY. The mucosa-associated microbiota of PSC patients is characterized by low diversity and low abundance of uncultured Clostridiales II. J Crohns Colitis 2015; 9: 342-348 [PMID: 25547975 DOI: 10.1093/jcc/jju023]
20. Rühlemann MC, Heinzen FA, Zonouzi R, Lieb W, Franke A, Schramm C. Faecal microbiota profiles as diagnostic biomarkers in primary sclerosing cholangitis. Gut 2016; 65: 753-754 [PMID: 27216937 DOI: 10.1136/gutjnl-2016-312180]
21. Cardona S, Eck A, Cassellas M, Gallart M, Ablaee C, Lore D, Azpíroz F, Roca J, Guarnier F, Manchichan C. Storage conditions of intestinal microbiota matter in metagenomic analysis. BMC Microbiol 2012; 12: 158 [PMID: 22846661 DOI: 10.1186/1471-2179-12-158]
22. Tedjo DI, Jonkers DM, Savelkoul PH, Maslee AA, van Best N, Pierik MJ, Penders J. The effect of sampling and storage on the fecal microbiota composition in healthy and diseased subjects. PLoS One 2013; 10: e0126685 [PMID: 26024217 DOI: 10.1371/journal.pone.0126685]
23. Yu Y, Lee C, Kim J, Hwang S. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol Bioeng 2005; 89: 670-679 [PMID: 15696537 DOI: 10.1002/bit.20347]
24. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttenhower C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Applied and Environmental Microbiology 2013; 79: 869-874 [PMID: 23490135 DOI: 10.1128/AEM.01762-12]
25. Edgar RC, Flyvbjerg H. Error filtering, pair assembly and error correction for next-generation sequencing reads. Bioinformatics 2015; 31: 3476-3482 [PMID: 26139637 DOI: 10.1093/bioinformatics/btv401]
26. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 2005; 71: 8228-8235 [PMID: 16323807 DOI: 10.1128/AEM.71.12.8228-
Bajer L et al. Microbiota profiles in PSC and UC

2835-2005

Tickle T, Waldron L, Yiren Lu HC, Multivariate association of microbial communities with rich metadata in high-dimensional study

Beuers U, Hepatic overlap syndromes. J Hepatol 2005; 42 Suppl: S93-S99 [PMID: 15777577 DOI: 10.1016/j.jhep.2004.11.009]

Beuers U, Rust C. Overlap syndromes. Semin Liver Dis 2005; 25: 311-320 [PMID: 16143946 DOI: 10.1055/s-2005-916322]

Lindor KD, Kovlevy K, Harrison ME. ACG Clinical Guideline: Primary Sclerosing Cholangitis. Am J Gastroenterol 2015; 110: 646-659, quiz 660 [PMID: 25869391 DOI: 10.1038/ajg.2015.112]

Langan RC, Gotsch PB, Krafczyk MA, Skillinger DD. Ulcerative colitis: diagnosis and treatment. Am Fam Physician 2007; 76: 1323-1330 [PMID: 18019875]

Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N Engl J Med 1987; 317: 1625-1629 [PMID: 33170057 DOI: 10.1096/NEJM198712243172603]

Kverka M, Tiskalova-Hogenova H. Intestinal Microbiota: Facts and Fiction. Dig Dis 2017; 35: 139-147 [PMID: 28147364 DOI: 10.1159/000449095]

Alipour M, Zaidi D, Valcheva R, JoveI J, Martinez I, Sergi C, Walter J, Mason AL, Wong GK, Dieleman LA, Carroll MW, Huynh HQ, Wine E. Mucosal Barrier Depletion and Loss of Bacterial Diversity are Primary Abnormalities in Paediatric Ulcerative Colitis. J Crohns Colitis 2016; 10: 462-471 [PMID: 26669040 DOI: 10.1093/ecco-jcc/jjv223]

Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, Guo J, Le Chatelier E, Yao J, Wu L, Zhou J, Ni S, Liu L, Pone N, Batto JM, Kennedy SP, Leonard P, Yuan C, Ding W, Chen Y, Hu X, Zhou B, Qian G, Xu W, Ehrlich SD, Zheng S, Li L. Alterations of the gut microbiome in liver cirrhosis. Nature 2014; 513: 59-64 [PMID: 25079328 DOI: 10.1038/nature13568]

Ramanan P, Barreto JN, Osmon DR, Tosh PK. Rothia bacteremia: a 10-year experience at Mayo Clinic, Rochester, Minnesota. J Clin Microbiol 2014; 52: 3184-3189 [PMID: 24951810 DOI: 10.1128/JCM.01270-14]

Rosen R, Amirault J, Liu H, Mitchell P, Hu L, Khatwa U, Waldron L, Yiren Lu HC. Multivariate association of microbiota profiles in PSC and UC with IBD and UC. Gut 2014; 63: 59-64 [PMID: 25079328 DOI: 10.1038/nature13568]

Bajaj J, Hylemon PB, Ridlon JM, Heuman DM, Daita K, Tsay CJ, Caccamo DV, Cox KM, Castillo RO, Lackner K, Oldenburg B, Panes J, Rogler G, Stein J, Tilg H, Travis S, Lindsay JO. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 3: special considerations. J Crohns Colitis 2013; 7: 1-33 [PMID: 23040453 DOI: 10.1016/j.crohns.2012.09.005]

Hallibasie E, Fuchs C, Hofer H, Paungartner G, Trauner M. Therapy of Primary Sclerosing Cholangitis--Today and Tomorrow. Dig Dis 2015; 33 Suppl: 2: 149-163 [PMID: 26641422 DOI: 10.1159/000440827]

Hommes DW, Erkelens W, Ponsioen C, Stokkers P, Raauw E, van der Spek M, ten Kate F, van Deventer SJ. A double-blind, placebo-controlled, randomized study of infliximab in primary sclerosing cholangitis. J Clin Gastroenterol 2008; 42: 522-526 [PMID: 18344886 DOI: 10.1097/MCG.0b013e3181662426]
Franceschet I, Cazzagon N, Del Ross T, D’Incà R, Buja A, Floreani A. Primary sclerosing cholangitis associated with inflammatory bowel disease: an observational study in a Southern Europe population focusing on new therapeutic options. *Eur J Gastroenterol Hepatol* 2016; 28: 508-513 [PMID: 26872110 DOI: 10.1097/MEG.0000000000000596]

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