The Gut Microbiota in Collagenous Colitis Shares Characteristics With Inflammatory Bowel Disease-Associated Dysbiosis

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INTRODUCTION: **In inflammatory bowel disease (IBD), an aberrant immune response to gut microbiota is important, but the role of the microbiota in collagenous colitis (CC) is largely unknown. We aimed to characterize the microbiota of patients with CC compared with that of healthy control and patients with IBD.**

METHODS: **Fecal samples were collected from patients with CC (n = 29), age- and sex-matched healthy controls (n = 29), patients with Crohn's disease (n = 32), and patients with ulcerative colitis (n = 32). Sequence data were obtained by 454 sequencing of 16S rRNA gene amplicons, and the obtained sequences were subsequently taxonomically classified.**

RESULTS: **Analysis of similarity statistics showed a segregation between patients with CC and healthy controls with increasing taxonomic resolution, becoming significant comparing operational taxonomic unit data ($P = 0.006$). CC had a lower abundance of 10 different taxa. Taxa-specific analyses revealed a consistent lower abundance of several operational taxonomic units belonging to the Ruminococcaceae family in patients with CC, $q < 0.05$ after false discovery rate correction. Loss of these taxa was seen in patients with CC with active disease and/or corticosteroid treatment only and resembled the findings in patients with IBD.**

DISCUSSION: **CC is associated with a specific fecal microbiome seen primarily in patients with active disease or ongoing corticosteroid treatment, whereas the microbiome of CC patients in remission resembled that of healthy controls. Notably, the shift in key taxa, including the Ruminococcaceae family, was also observed in IBD. There may be common mechanisms in the pathogenesis of CC and IBD.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A61

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INTRODUCTION

Collagenous colitis (CC) is a chronic inflammatory disease of the bowel, characterized by a subepithelial collagen band of ≥10 μm plus infiltration with lymphocytes in the lamina propria. The diagnosis of CC is defined by loose stools for more than 3–4 weeks, a colonic mucosa that most often has a normal appearance, although minor changes such as edema and erythema might be present, combined with specific histopathologic criteria (1,2). Historically, CC has been considered rare, but epidemiologic studies indicate an increase in the incidence rates during the last decades. In Europe and North America, the annual incidence reaches approximately 5.2 cases per 100,000 inhabitants (2). The disease mainly affects the elderly female population, with a ratio of 3.8:1, and is a relatively common finding in the investigation of watery, non-bloody diarrhea in an elderly female patient.

The etiology of CC is largely unknown, but familial clustering and overlap with other chronic inflammatory diseases, such as celiac disease, diabetes mellitus, and rheumatoid arthritis, have been reported (3). Immunologically, there seems to be an uncontrolled mucosal inflammation, suggesting an aberrant immune

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response to luminal antigens in genetically predisposed individuals, similar to other chronic inflammatory gastrointestinal diseases such as Crohn's disease (CD) and ulcerative colitis (UC) (1). This hypothesis is supported by the fact that diversion of the fecal stream has led to recovery from inflammation in the colon among patients with CC and reconstruction of the intestinal continuity caused relapse (4,5). Several exposures that have been associated with inflammatory bowel disease (IBD), such as non-steroid anti-inflammatory drugs and smoking, have also been associated with CC (1). Interestingly, CC has been described to convert into IBD or vice versa (6–10). These observations suggest that CC and IBD might have pathophysiologic mechanisms in common.

The gut microbiome is a key factor in the pathogenesis of IBD and a dysbiosis, mainly characterized by downregulation of butyrate-producing species like *Faecalibacterium prausnitzii*, belonging to the Ruminococcaceae family, has consistently been reported in patients with IBD and especially in patients with CD (11–13). Analysis of small case series indicates that some patients with CC might display an abnormal microbial profile (14,15). However, whether CC is characterized by an altered microbiome that shares patterns with the IBD-associated dysbiosis remains unknown.

This study compares the fecal microbiota of patients with CC with that of healthy controls and identifies the possible changes, in common with CD and UC, using sequencing of 16S rRNA gene amplicons. We further aimed to associate microbial signatures with clinical variables, including disease activity and corticosteroid treatment, in patients with CC.

METHODS

Study population

Patients with established CC, CD, and UC were invited to take part in the study while attending the outpatient Gastroenterology Clinic at Örebro University Hospital, Sweden. The cohort of patients with CC has previously been described (2). The diagnosis of CC was based on the history of chronic, non-bloody diarrhea, a macroscopically normal or almost normal colonic mucosa, and typical histologic findings. The histopathologic criteria of CC included a subepithelial collagen band of $\geq$10 μm, infiltration with lymphocytes in the lamina propria, and epithelial damage. The characteristic findings had to be present in at least 2 biopsies, supported by inflammatory mucosal changes in additional biopsies and segments (1). The diagnosis of CD and UC was based on the Lennard-Jones criteria (16). All patients signed a written informed consent. Clinical disease activity in patients with CC was defined according to internationally accepted criteria, with remission defined as $<$3 stools without any watery diarrhea during the last 24 hours (1). In CD and UC, phenotype and disease activity were classified according to the Montreal classification and physician's global assessment (17). Healthy controls, matched by sex and age ($\pm$5 years) to the CC group, were identified from the population-based colonoscopy (PopCol) cohort, previously described in detail (18). All individuals were asked to provide a fecal sample and to complete a questionnaire on disease activity and use of drugs, including antibiotics. Exclusion criterion was antibiotic use within 3 months before fecal sample collection. In total, 122 individuals were included (CC: n = 29; CD: n = 32; UC: n = 32; and healthy controls: n = 29).

The Ethics Committee of Uppsala University (Dnr 2007/291) and the local Ethics Committee at Karolinska Institutet (Forskningskommitté Syd) (Dnr 394/01) approved the study.

Sampling and DNA extraction

Information on collection procedures was provided to all participants. Fecal samples were collected in plastic tubes at home by the participants, mailed by postal service, and frozen directly on arrival at the laboratory where they were stored at −70 °C. From each sample, 100 mg ($\pm$10 mg) of feces was collected in an Ultra Clean Fecal Isolation Kit Dry bead tubes (MoBio, Naxo) and eluted with 1 mL of Stool Stabilizer (NorDiag). Each sample was homogenized by 5 minutes vortexing, followed by 5 minutes of centrifugation at 6,000*g*. Five hundred microliters of each stool supernatant was transferred to a microcentrifuge tube and placed in an Arrow instrument (NorDiag) for DNA extraction using the Arrow Stool DNA cartridge to a final eluted volume of 200 μL, according to the manufacturer's instructions. DNA was concurrently extracted from all samples, according to the same extraction protocol, by the same person (A.C.).

Preparation of PCR amplicon libraries

For each sample, 3 identical polymerase chain reaction (PCR) mixes were prepared, 50 μL, containing 10 μL ×5 PCR buffer, 200 μM deoxynucleoside triphosphate (Pierce Nucleic Acid Technologies, Milwaukee), 25 μM of each primer, 0.65 μL Phusion F-530L enzyme (Finnzyme, Massachusetts), and 1 μL template DNA. The primer pairs used to amplify the hypervariable 16 rRNA regions V3-V4 were 341f (5′CCTACGGGNGGCWGCAG) complemented with adaptor B and 805r (5′GACTACHVGGGTATCTAATCC) complemented with adaptor A. In addition, each reverse primer had a unique 7 nucleotides long barcode sequence (in total 96 unique reverse primers), enabling multiplexing of samples. A PCR negative template control was also used for each primer pair. The PCR cycles used were 95 °C for 5 minutes, followed by 25 cycles of 95 °C for 40 seconds, 58 °C for 40 seconds, and 72 °C for 1 minute, followed by a final extension of 72 °C for 7 minutes.

All 3 PCR products were pooled, and 45 μL of the pooled PCR product was then purified using Agencourt AMPure beads (Beckman Coulter, CA) according to the manufacturer's protocol. The products were finally eluted in ×1 tris-ethylenediaminetetraacetic acid buffer. DNA concentration was determined by a Qubit fluorometer (Invitrogen, CA). All samples were diluted to a concentration of 3–4 ng/μL, except those where insufficient amount of DNA was obtained.

All DNA samples were pooled in 2 tubes, one for each lane on the 454 pyrosequencer. Sequencing was performed on the Roche 454-FLX GS100 using the FLX titanium kit (Roche 454 Life Sciences, Branford, CT). The obtained sequence reads were demultiplexed, filtered from low-quality reads, and denoised using the Amplicon noise pipeline. Only samples that contained $>$1,500 reads were included in the study. The sequences were then taxonomically classified using the SILVA database as previously described (19), before performing the statistical analysis.

Statistical analyses

Shannon diversity index (SDI) was calculated, and differences between the groups were compared by Kruskal-Wallis H-test and Tukey honestly signifcant difference test. Analysis of similarity (ANOSIM) was performed to test for dissimilarities between the groups (20). The Benjamini and Hochberg method was used to account for multiple comparisons, based on global *P* values of all variables compared, with a false-discovery rate of 5%. We used Wilcoxon tests to test for differences in relative abundance of specific bacterial taxa between patients with CC and healthy

controls. Only taxa prevalent in >20% of the samples were included in this analysis. To correct for multiple testing, we performed 1,000 permutations stratified for the taxonomic level of classification, i.e., genus and operational taxonomic unit (OTU) at a 97% identity threshold. For each permutation, the diseased and control individuals were randomized and tested against the relative abundance data for all OTUs. The distribution of the *P* values per OTU provided an exact test (q-value) to estimate the false-discovery rate at 5% for every OTU. We used the q-value obtained from the permutations for each OTU to estimate its significance. We repeated this analysis using the relative abundance per taxa. Comparisons between subgroups of patients with CC and healthy controls were performed by Kruskal-Wallis and Mann-Whitney *U* test. Due to the limited number of individuals in each group, patients with CC were categorized in 2 subgroups, patients in remission without corticosteroid therapy and patients with active disease or ongoing corticosteroid therapy. The prevalence of CC-associated taxa was then assessed in patients with CD and UC to explore common microbial signatures. Statistical analyses and data processing were performed in R (R Foundation for Statistical Computing, Vienna, Austria) and SPSS Statistics software (Released 2015; IBM, and IBM SPSS Statistics for Windows, Version 23.0.; IBM, Armonk, NY).

RESULTS

In total, 122 individuals (CC: n = 29; CD: n = 32; UC: n = 32; and healthy controls: n = 29) were included. The median (range) age of the patients with CC was 65 years (33–89 years) (Table 1). Of the 29 patients with CC, 17 were on treatment with oral corticosteroids and 10 had clinically active disease. Nine patients with CC were in clinical remission without any ongoing corticosteroid therapy. Basic demographics and clinical characteristics of the participants are shown in Table 1. Detailed information on clinical characteristics of individual patients with CC, CD, and UC is provided in Supplemental Tables 1 and 2 (see Supplementary Digital Content, http://links.lww.com/CTG/A61).

In total, 119 samples were included in the analyses, because 3 samples (CC: n = 2 and UC: n = 1) failed PCR amplification due to insufficient amount of extracted DNA. After filtering, the dataset contained a total of 349,963 reads, with a mean (range) of 2941 reads (1,643–5,271 reads) per sample. A total of 11,799 different OTUs were observed corresponding to a mean (range) of 286 OTUs (39–578 OTUs) per sample. No correlation between the number of reads and number of OTUs or the SDI was observed (see Figure 1, Supplementary Digital Content, http://links.lww.com/CTG/A61).

Microbial composition in different disease entities and healthy controls

The SDI was calculated for patients with CC, CD and UC, and also for healthy controls (Figure 1). No significant difference was found between patients with CC and healthy controls ($P = 0.25$). By contrast, CD was significantly associated with a lower diversity (mean $P < 0.0001$).

However, a principal coordinate analysis plot revealed a separation between the patients with CC and the healthy controls (Figure 2). Consistently, the ANOSIM statistics showed that the patients with CC segregated from the healthy controls with increasing taxonomic resolution (Table 2), reaching significance at the highest taxonomic resolution when OTUs were compared ($P = 0.006$).

Microbial composition in patients with CC

A principal coordinate analysis (PCoA) scatter plot indicated that samples seemed to separate according to CC vs healthy controls,

Table 1. Basic demographics and clinical characteristics of patients with CC, UC, and CD, and of healthy controls

Characteristic	Healthy controls (n = 29)	Patients with CC (n = 29)	Patients with CD (n = 32)	Patients with UC (n = 32)
Female, n (%)	19 (66)	21 (72)	15 (47)	17 (53)
Age (years), median (range)	65 (34–71)	64 (33–82)	56 (20–80)	59 (22–84)
Location, n (%)				
Terminal ileum (L1) ± upper GI (L4)			14 (44)	
Colon (L2)			15 (47)	
Ileocolon (L3) ± upper GI (L4)			3 (9)	
Behavior, n (%)				
Non-stricturing, nonpenetrating (B1)			18 (56)	
Stricturing (B2)			11 (34)	
Penetrating (B3)			3 (9)	
Extent, n (%)				
Proctitis (E1)				0
Left sided (E2)				12 (38)
Extensive (E3)				20 (63)
BMI, median (range)	24 (20–33)	25 (17–35)	26 (18–38)	27 (18–34)
Active disease, n (%)		10 (34)	5 (16)	3 (9)
Steroid treatment, n (%)		17 (59)	3 (9)	2 (6)

BMI, body mass index; CC, collagenous colitis; CD, Crohn's disease UC, ulcerative colitis.

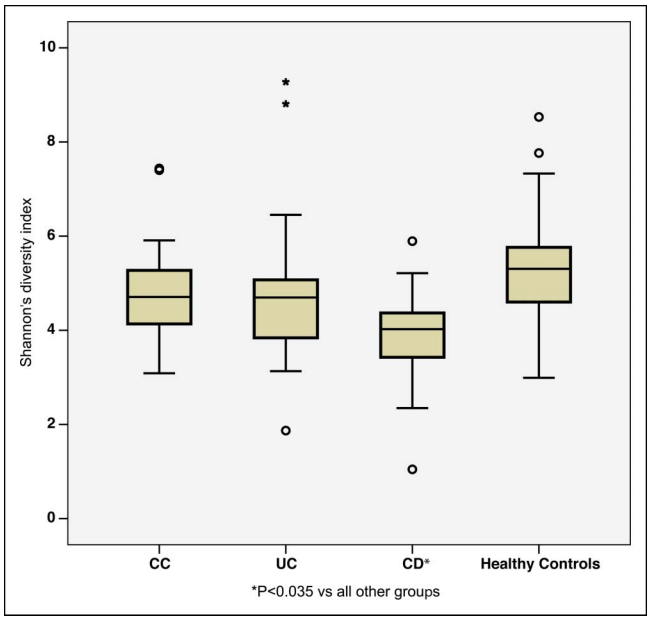

Figure 1. Shannon diversity index for patients with CC, UC, CD, and healthy controls. *Represents the highest observed significant *P* value (comparisons were based on Tukey HSD test). CC, collagenous colitis; CD, Crohn's disease; UC, ulcerative colitis.

and the ANOSIM confirmed the statistical difference between the 2 groups. To explore the impact of potential confounders, PCoA plots and ANOSIM were assessed based on sex, age, and body mass index (BMI) among patients with CC and healthy controls (see Figure 2, Supplementary Digital Content, http://links.lww.com/CTG/A61). No separation was observed with respect to sex and age, but samples separated significantly when assessed based on BMI. However, no difference in BMI was observed between patients with CC and healthy controls ($P = 0.88$).

Based on the ANOSIM, we explored which taxa caused the observed differences between patients with CC and healthy controls. Patients with CC had a lower abundance of 11 different taxa (see Figures 3, Supplementary Digital Content, http://links.lww.com/CTG/A61) and displayed a consistent lower abundance of several OTUs belonging to the Ruminococcaceae family (Table 3). By contrast, a diverse pattern was observed for several other families, where the mean relative abundance was increased for some OTUs but decreased for other OTUs within the same family (Table 3).

Associations between fecal microbiota and clinical data in patients with CC

We then characterized the microbiome of patients with CC further by stratifying for disease activity and corticosteroid treatment when analyzing the taxa that were identified as associated

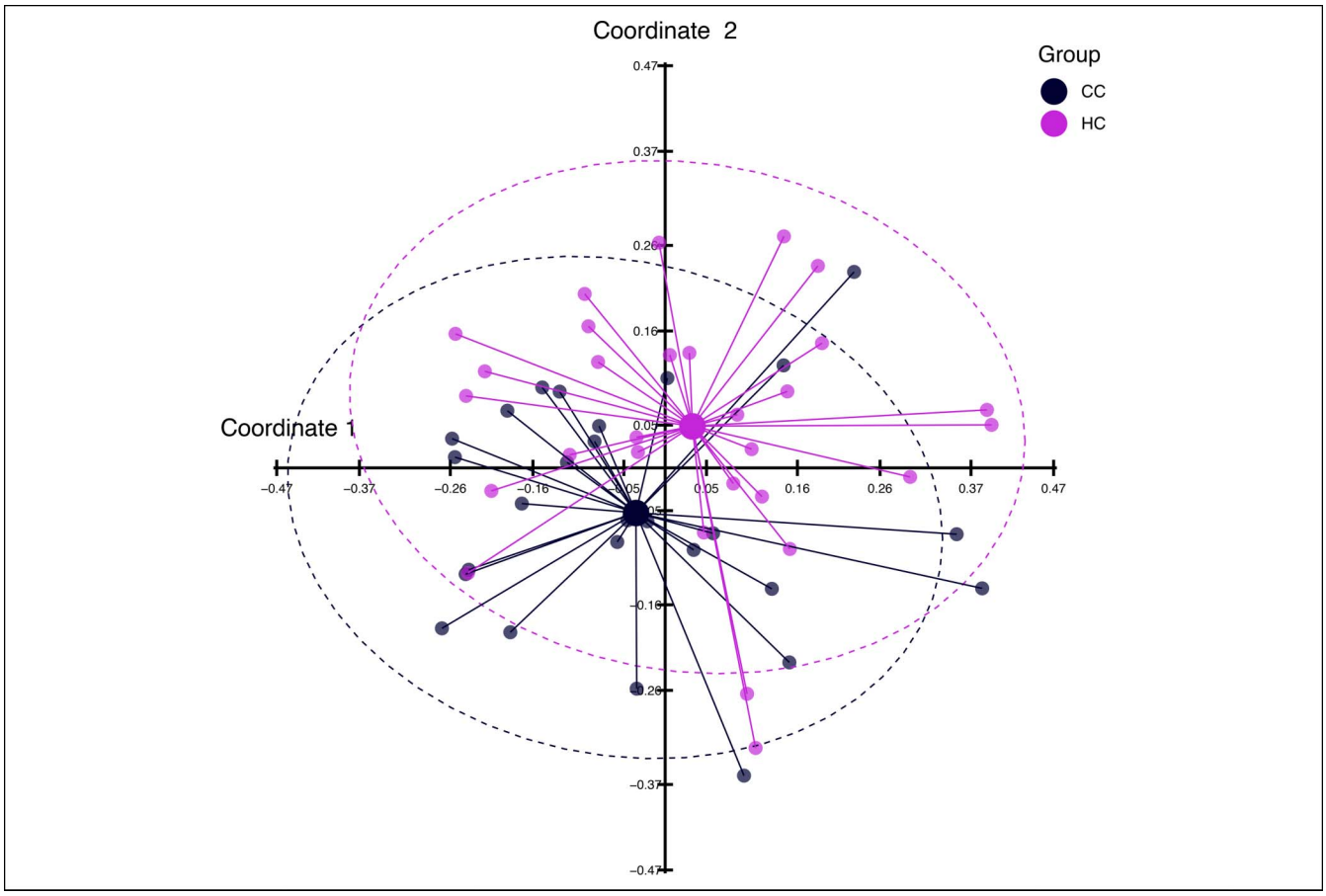

Figure 2. Principal coordinate analysis based on Bray Curtis distances of OTU data. Samples are coloured based on the groups, i.e., patients with CC and healthy controls. CC, collagenous colitis; OTU, operational taxonomic unit.

Table 2. *P* values of ANOSIM for patients with CC, UC, and CD, compared to healthy controls at different taxonomic levels based on all OTUs

	CC	UC	CD
Phylum	0.19	0.32	0.001[a]
Family	0.21	0.44	0.002[a]
Genus	0.05	0.19	<0.001[a]
OTU	0.006[a]	0.01[a]	<0.001[a]

ANOSIM, analysis of similarity; CC, collagenous colitis; CD, Crohn's disease; OTU, operational taxonomic unit; UC, ulcerative colitis.
[a]Indicates significant *P* value ($P < 0.05$) after false-discovery rate correction.

with CC. Patients were categorized into two categories: patients in remission without corticosteroid therapy and patients with active disease or ongoing corticosteroid therapy.

The relative abundance of several taxa differed between the two groups (Figure 3). An association between active disease/ongoing corticosteroid therapy and a decreased relative abundance of *Collinsella*, unclassified Ruminococcaceae, unclassified Coriobacteriaceae, and unclassified Clostridiales was observed. Consistently, a decreased abundance of several OTUs corresponding to the Ruminococcaceae family was observed in patients with active disease/ongoing corticosteroid therapy but not in patients with CC in remission when taxa were compared at an OTU level (see Figures 4, Supplementary Digital Content, http://links.lww.com/CTG/A61).

Shared microbial signatures between patients with CD, UC, and CC

To explore the possible common microbial signatures, the microbial composition of patients with CC was compared with that of patients with CD and UC, restricting the analyses to taxa that separated patients with CC in remission from patients with active disease/ongoing corticosteroid treatment. Similar to the observed microbial shift in patients with CC, a decreased abundance of several OTUs corresponding to the Ruminococcaceae family was also found in patients with IBD. Of the 10 OTUs within the Ruminococcaceae family that were decreased in patients with active CC or corticosteroid treatment, 9 were also decreased in patients with CD and 4 in patients with UC (see Figure 5, Supplementary Digital Content, http://links.lww.com/CTG/A61).

DISCUSSION

By examining fecal samples from a well-characterized cohort of patients, we demonstrate that CC is associated with a specific gut microbiome and that this microbial shift is seen primarily in patients with active disease or ongoing corticosteroid treatment. By contrast, the fecal microbiome of patients with CC in remission resembled the microbiome of healthy controls. To our knowledge, this is the first detailed characterization of the microbiome of patients with CC which also identifies the possible common microbial shifts in patients with CC and IBD. Two descriptive reports on small series have been published previously (14,15). Consistent with our findings, Fischer et al. reported a marked reduction of Verrucomicrobia, *Akkermansia* spp., in 10 cases with CC. Interestingly, in our study, the patients with CC displayed a shift in some taxa, like the Ruminococcaceae family, which resembles the previously described IBD-associated dysbiosis (21,22). This could indicate that the microbiome plays a similar role in CC and IBD and that the pathogenesis of the diseases might have mechanisms related to the gut microbiome in common. The fact that we observed some of these microbial shifts in our cohort of patients with IBD strengthens this theory further.

Similar to the hypothesis in IBD, it has been proposed that microscopic colitis could result from an aberrant immune response to the commensal gut microbiome (9). This is supported by the observed clinical improvement and histologic restoration in patients with CC after fecal stream diversion due to an ileostomy. Intriguingly, clinic relapse is most often seen at subsequent restoration of intestinal continuity (4,5). An infectious etiology to CC has also been proposed. The epithelial lymphocytosis in microscopic colitis is similar to the histologic findings of "Brainerd diarrhea," i.e., outbreaks of long-standing acute watery diarrhea (23). Similarly, the reported seasonal variation in the onset of microscopic colitis and possible positive effect of antibiotics might point to the importance of an infectious agent or a microbial component (24,25). In a recent case report on the effect of fecal transplantation, a conversion from UC to CC was observed in a patient who experienced a pronounced change in the gut microbiota due to the treatment (26). In another case report, a beneficial effect of fecal transplantation was reported in a patient with steroid-resistant CC (27). Based on these observations, we aimed to characterize the fecal microbiota of patients with CC comparing with that of healthy controls and to identify possible changes, in common with patients with CD and UC, using 16S rRNA sequencing. We could not show any significant difference in the overall microbial composition, based on the SDI when patients with CC or UC were compared with healthy controls. Consistent with previous studies (11,28,29), patients with CD had a significant lower diversity compared with healthy controls. However, overall diversity might be a too simplified measure of the gut microbiota. In UC, conflicting results with both indifferent and reduced biodiversity have been shown when comparing microbiota in patients with healthy controls (28,30,31). An increasing difference in microbial composition between patients with CC and healthy controls was also observed with the degree of taxonomic resolution, becoming significant when comparing OTUs in our cohort. When specific OTUs were compared between patients with CC and healthy controls, we observed a difference in mean relative abundance of 36 OTUs. For most of these OTUs, a complex pattern was observed when analyzing the data at a family level, with an increased relative abundance for some OTUs but a decreased relative abundance for other OTUs within the same family. However, with respect to the Ruminococcaceae family, a consistent decrease in several OTUs was observed.

The Ruminococcaceae family is a member of the Firmicutes phylum and comprises a broad spectrum of species with different functional properties. An underrepresentation of species belonging to the family has previously been reported in IBD, especially in CD (22,32). Several species within the Ruminococcaceae family, like *Ruminococcus* species, are of importance for the maintenance of gut homeostasis, because they produce short-chain fatty acids and primarily butyrate. Butyrate is an important energy substrate for the intestinal mucosa and of importance for intestinal health, resistance to pathogenic microbes, and protection against colitis (11,33,34). Both *in vitro* and *in vivo* data indicate that *F. prausnitzii*, another member of the Ruminococcaceae family, also seem to have anti-inflammatory properties (11,34,35).

Table 3. Mean relative abundance in percentage (and % prevalence) of OTUs that differed significantly between patients with CC and healthy controls

Taxonomy	Patients with CC	Healthy controls	*P* value
A…/Coriobacteriaceae/*Collinsella*/	0.02 (26)	0.05 (52)	0.031
A…/Coriobacteriaceae/unclassified Coriobacteriaceae/	0.008 (15)	0.05 (59)	0.0004
B…/Bacteroidales/Porphyromonadaceae/*Parabacteroides*/	0.29 (63)	0.08 (41)	0.038
B…/Bacteroidales/Bacteroidaceae/*Bacteroides*/	0.88 (37)	0.32 (69)	0.042
B…/Bacteroidales/Bacteroidaceae/*Bacteroides*/	0.19 (30)	0.63 (62)	0.015
B…/Bacteroidales/Bacteroidaceae/*Bacteroides*/	0.005 (4)	0.13 (45)	0.0005
B…/Bacteroidales/Bacteroidaceae/*Bacteroides*/	0.04 (37)	0.01 (10)	0.028
B…/Bacteroidales/Bacteroidaceae/*Bacteroides*/	0.95 (37)	0.015 (7)	0.004
B…/Bacteroidales/Rikenellaceae/*Alistipes*/	0.08 (44)	0.15 (69)	0.031
B…/Bacteroidales/Rikenellaceae/*Alistipes*/	0.08 (22)	0.43 (49)	0.007
F…/Erysipelotrichaceae/Inscertae_sedis/	0.07 (11)	0.52 (38)	0.020
F…/Erysipelotrichaceae/unclassified Erysipelotrichaceae/	0.09 (52)	0.49 (76)	0.016
F…/Clostridiales/Lachnospiraceae/*Coprococcus*/	0.11 (11)	0.25 (41)	0.021
F…/Clostridiales/Lachnospiraceae/unclassified Lachnospiraceae/	0.027 (41)	0.055 (66)	0.041
F…/Clostridiales/Lachnospiraceae/unclassified Lachnospiraceae/	0.035 (44)	0.013 (21)	0.030
F…/Clostridiales/Lachnospiraceae/unclassified Lachnospiraceae/	0.18 (37)	0.003 (7)	0.005
F…/Clostridiales/Peptostreptococcaceae/*Clostridium* IX/	0.044 (48)	0.008 (10)	0.004
F…/Clostridiales/Ruminococcaceae/*Clostridium* IV/	0.017 (22)	0.049 (62)	0.002
F…/Clostridiales/Ruminococcaceae/*Clostridium* IV/	0.011 (15)	0.052 (48)	0.009
F…/Clostridiales/Ruminococcaceae/*Oscillibacter*/	0.63 (52)	1.50 (79)	0.043
F…/Clostridiales/Ruminococcaceae/unclassified Ruminococcaceae/	0.15 (37)	0.41 (66)	0.020
F…/Clostridiales/Ruminococcaceae/unclassified Ruminococcaceae/	0.11 (37)	0.12 (66)	0.047
F…/Clostridiales/Ruminococcaceae/unclassified Ruminococcaceae/	0.07 (22)	0.27 (72)	0.0004
F…/Clostridiales/Ruminococcaceae/unclassified Ruminococcaceae/	0.06 (26)	0.11 (55)	0.045
F…/Clostridiales/Ruminococcaceae/unclassified Ruminococcaceae/	0.008 (15)	0.17 (62)	<0.0001
F…/Clostridiales/Ruminococcaceae/unclassified Ruminococcaceae/	0.39 (11)	1.33 (59)	0.0003
F…/Clostridiales/Ruminococcaceae/unclassified Ruminococcaceae/	0.015 (11)	0.05 (52)	0.004
F…/Clostridiales/Ruminococcaceae/unclassified Ruminococcaceae/	0.027 (15)	0.12 (45)	0.012
F…/Clostridiales/Ruminococcaceae/unclassified Ruminococcaceae/	0.06 (15)	0.16 (41)	0.039
F…/Clostridiales/Ruminococcaceae/unclassified Ruminococcaceae/	0.006 (11)	0.021 (41)	0.016
F…/Clostridiales/unclassified Clostridiales/	0.05 (44)	0.09 (76)	0.030
F…/Clostridiales/unclassified Clostridiales/	0.05 (19)	0.23 (52)	0.012
F…/Clostridiales/unclassified Clostridiales/	0.38 (11)	0.65 (41)	0.016
V…/Verrucomicrobiaceae/*Akkermansia*	0 30 (26)	0.44 (62)	0.030
Archaea…/Methanobacteriaceae/*Methanobrevibacter*	0.11 (19)	0.10 (48)	0.030

A, Actinobacteria; B, Bacteroidetes; F, Firmicutes; CC, collagenous colitis; OTU, operational taxonomic unit; V, Verrucomicrobia.

To explore the role of the microbiome in CC further, we stratified patients with CC based on clinical variables, i.e., disease activity and corticosteroid treatment. Budesonide, a corticosteroid, is the drug of choice for treating CC, because it is effective in both inducing and maintaining remission (36). However, the relapse rate is high at dose reduction and after discontinuation (37). Therefore, many patients are kept at a minimal dose of corticosteroid to maintain remission. The patients with CC were divided into 2 categories; patients in remission without corticosteroid therapy and patients with active disease or ongoing corticosteroid therapy. For several taxa, a difference in relative abundance was observed in patients with active disease or ongoing corticosteroid treatment only, when compared with healthy controls. Thus, some taxa seem to be associated with disease activity, although our study design does not allow us to confirm causality. Several of the taxa that showed an association with

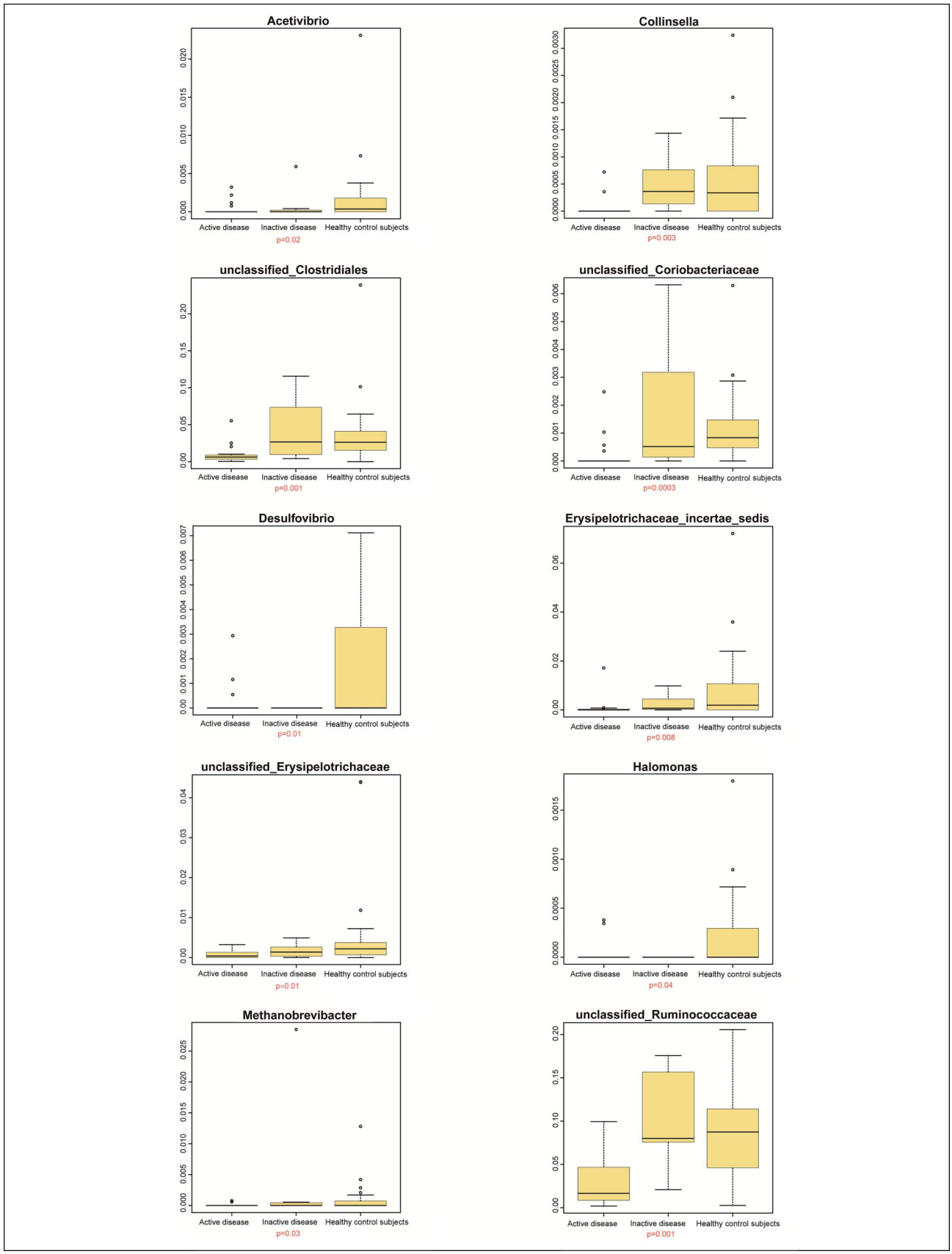

Figure 3. Relative abundance of different taxa in patients with active (defined as clinical active disease or ongoing corticosteroid treatment) and inactive CC compared with those in healthy controls (comparisons were based on Kruskal-Wallis test). CC, collagenous colitis.

disease activity or ongoing corticosteroid treatment belonged to the Ruminococcaceae family. However, a decreased relative abundance of *Collinsella*, Clostridiales, Coriobacteriaceae, and Erysipelotrichaceae was also observed in this subgroup of patients with CC.

Similar to the findings in patients with CC, a decreased abundance of several OTUs corresponding to the Ruminococcaceae family was also observed in patients with IBD. The shared microbial shift between CC and IBD is of great interest, because a shift from CC to IBD and vice versa has been reported in some patients (6,7). Thus, further characterization of virulence factors and gene expression profiles of taxa within the Ruminococcaceae family might be of importance to clarify the possible common microbial pathways of CC and IBD.

The thorough clinical characterization of the patients with CC is a major strength of our study, although clinical activity at acquisition of fecal samples was not confirmed by histologic assessment. Comparisons with patients with CD and UC and with healthy controls from the general population strengthen the results further. Even though our study included 122 individuals, the number of patients with CC in each subgroup was limited when stratifying for disease activity and corticosteroid medication. The comparisons of patients with CD and UC with healthy controls were hampered by differences in age and sex, because the matching was performed on the basis of age and sex of the patients with CC. A limitation with the present study was that, in common with many previous studies of the gut microbiome in relation to various diagnoses, samples were obtained at a single point in time. Analyses of sequential samples from patients with CC, including those which cover periods of remission, relapse, and changes in treatment, as well as matched samples obtained over time from healthy controls would be a more powerful design to reveal the influence of the microbiome in CC. The use of fecal samples is another possible limitation, because it can be anticipated that it is the mucosal microbiome that is most important in CC. Further research on mucosal biopsies should be undertaken to investigate host-microbiota interactions in patients with CC. Alterations in the gut microbiota secondary to induced diarrhea and functional diarrhea have previously been described. Some of the shifts in the gut microbiota composition that have been associated with bowel cleansing resemble the alterations that have been seen in IBD (22,38,39). By contrast, the gut microbiota in patients with irritable bowel syndrome with diarrhea has shown a different pattern. This indicates that diarrhea per se might have an impact on microbial composition and may represent a limitation for this study, even though the previous literature is inconsistent (40,41). Effects of retarded growth of certain species and overgrowth of other species due to environmental factors could have been minimized using a DNA-stabilizing agent. In general, samples are expected to have been shipped within 24 hours, but the fact that the duration of individual shipments was not specifically recorded limits the study further. Intersample differences in the number of obtained reads represent another potential source of uncertainty because we did not normalize the data by randomly picking a fixed number of reads. However, no correlation between the number of reads and number of OTUs or SDI was observed. The use of 16S amplicon sequencing may have negatively affected our possibilities to identify rare taxa, because the technique has limited the ability to detect low abundant taxa. Thus, the absence of CC-specific taxa must be interpreted with caution. Enrichment of antigens prevalent in different species of bacteria may also be of importance, with respect to the pathogenesis of CC. However, such enrichment cannot be detected by the method used, and further shotgun metagenomic sequencing-based studies of individuals with CC are warranted. Other clinical factors such as diet, blood trait, age, sex, BMI, and host genotype have also been associated with gut microbiota composition (42). Especially age, sex, and BMI have been identified as important potential confounders. To rule out that the results were confounded by any of these variables, the impact of age, sex, and BMI was examined by a principal coordinate analysis and an ANOSIM (see Figures 2, Supplementary Digital Content, http://links.lww.com/CTG/A61). However, no differences were found, except for BMI, where a difference was observed between lean (BMI < 25) and obese (BMI > 30) participants. However, because obese participants were equally distributed in CC group and controls, we do not expect any impact on the observed differences between CC and healthy controls.

To our knowledge, this is the first study to demonstrate that CC is associated with a specific gut microbiome and that the altered microbiota is seen primarily in patients with active disease and/or corticosteroid treatment. Interestingly, the shift in some taxa, like the Ruminococcaceae family, resembles an IBD-associated dysbiosis and was also observed in patients with IBD in our study. This may indicate that CC and IBD are underpinned by similar microbial mechanisms.

CONFLICTS OF INTEREST

Guarantor of the article: Jonas Halfvarson, MD, PhD.

Specific author contributions: A.C.: planned and conducted the study; collected, analyzed, and interpreted the data; drafted the manuscript; and approved the final version submitted. J.D.: planned the study, analyzed and interpreted the data, drafted the manuscript, and approved the final draft submitted. R.N., M.L.: analyzed and interpreted the data and approved the final draft submitted. A.A.: collected data, revised the manuscript, and approved the final draft submitted. J.B.: collected material and data, revised the manuscript, and approved the final draft submitted. C.T.: collected material, interpreted the data, planned, revised the manuscript, and approved the final draft submitted. N.T., L.A.: collected data, revised the manuscript, and approved the final draft submitted. L.E.: planned and conducted the study, collected and interpreted the data, revised the manuscript, and approved the final draft submitted. J.H.: planned and conducted the study, interpreted the data, drafted the manuscript, and approved the final draft submitted.

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Ethics approval and consent to participate: The Ethics Committee of Uppsala University (Dnr 2007/291) and the local Ethics Committee at Karolinska Institutet (Forskningskommitté Syd) (Dnr 394/01) approved the study. All participants submitted written consent to participate in the study.

Availability of data and materials: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Study Highlights

WHAT IS KNOWN

- ✓ Gut microbiota is of importance for the maintenance of health.
- ✓ Similar to IBD, CC is characterized by an aberrant immune response to luminal factors.
- ✓ Gut microbiota plays an important role in the pathogenesis of IBD, but its potential role in CC is largely unknown.

WHAT IS NEW HERE

- ✓ Analysis of fecal samples revealed an altered microbial composition, with decreased abundance of several members of the Ruminococcaceae family in patients with CC.
- ✓ Alterations in the gut microbiota composition is associated with active CC or ongoing corticosteroid treatment, whereas the microbiome of patients with CC in remission resembled that of healthy controls.
- ✓ Common mechanisms related to the pathogenesis of CC and IBD may exist, because similar alterations were observed in both groups.

TRANSLATIONAL IMPACT

- ✓ Future microbiota-directed therapies may be of interest in patients with active CC.

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