ORIGINAL

Composition of Gut Microbiota and Its Relationship with Inflammatory Markers in Healthy Individuals and Patients with Ulcerative Colitis or Crohn's Disease

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Abstract : Background : The gut microbiota plays an important role in inflammatory bowel disease (IBD). However, its relationship with inflammatory markers remains unclear, especially in Asian populations. Methods : This prospective observational study examined the gut microbiota composition and its association with systemic inflammatory markers in Japanese patients with ulcerative colitis (UC) and Crohn's disease (CD) compared to healthy controls. Bacterial diversity, short-chain fatty acid-producing bacteria, and key genera were analyzed via 16S rRNA gene sequencing. Results : Patients with CD exhibited reduced bacterial diversity. *Clostridium* cluster IV was decreased in CD and showed a negative correlation with fecal calprotectin levels in UC. *Clostridium* cluster XIVa was negatively correlated with fecal calprotectin levels in both UC and CD, and with C-reactive protein (CRP) levels in CD. *Oscillibacter* positively correlated with CRP levels in CD. These findings suggest that a reduction in butyrate-producing bacteria is associated with increased inflammation in patients with IBD. Conclusion : To our knowledge, this is the first study to demonstrate a significant association between specific bacterial taxa and inflammatory markers in Japanese patients with IBD. *Clostridium* clusters IV and XIVa may serve as biomarkers, although potential confounders such as age, sex, and concurrent medication use should be recognized as study limitations. J. Med. Invest. 73 : 92-100, February, 2026

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INTRODUCTION

Inflammatory bowel disease (IBD) is a multifactorial chronic disorder that is believed to result from an abnormal immune response to intestinal antigens and is influenced by the gut microbiota, as well as genetic, environmental, and dietary factors (1, 2). Dysbiosis in IBD is characterized by a reduction in the number and diversity of bacterial species in the gut microbiota (3, 4). Although the mucosal defense mechanisms in healthy individuals prevent the attachment and invasion of gut bacteria, reports indicate that these functions are compromised in IBD, allowing extensive bacterial adherence and invasion of the mucosa (2, 5). Additionally, Westernized diets, particularly high-fat diets, have been suggested to increase mucosal permeability (leaky gut), facilitate bacterial invasion, and contribute to the development of IBD (1, 2). Liu *et al*. identified over 200 IBD-related susceptibility genes, some of which are involved in the host response to gut microbiota (6), suggesting that bacterial invasion of the mucosa triggers IBD.

Traditionally, the Mayo score has been used to assess disease activity in patients with ulcerative colitis (UC), and the Crohn's Disease Activity Index (CDAI) for patients with Crohn's disease (CD). As the infiltration of gut bacteria is a component of IBD pathogenesis, it is predicted to trigger an inflammatory response as part of the body's defense mechanism. Accordingly, inflammatory markers of IBD, such as C-reactive protein (CRP) and fecal calprotectin levels, have recently been suggested as indicators of disease activity in patients with symptomatic IBD (7). Furthermore, changes in the gut microbiota influence systemic inflammatory states (8), and reports have suggested that dysbiosis is associated with fluctuations in inflammatory markers (7). However, few studies have examined whether these relationships are found in Japanese patients. Furthermore, conventional indicators such as the Mayo score, CRP levels, and calprotectin levels have limitations in terms of sensitivity and specificity ; therefore, the identification of novel microbiota-based markers is warranted to complement the current diagnostic tools. Based on this, we examined these relationships from the perspective of variations in gut bacterial genera.

METHODS

This study investigated fecal gut microbiota in three groups : healthy individuals, patients with UC, and patients with CD. The healthy group consisted of 30 individuals (13 males and 17 females) aged 20–79 years, with a median age of 38.8 years. The UC group included 55 patients (33 males and 22 females) aged 19–77 years, with a median age of 50.4 years. The CD group comprised 40 patients (30 males and 10 females) aged 13–52 years with a median age of 27.4 years. The demographic characteristics of the study participants are summarized in Table 1.

Several patients with UC or CD received at least one therapeutic agent during the study period. A detailed list of medications used by the study participants is provided in Supplementary Table 2.

Patients with UC were categorized based on the Mayo scores as follows : 13, 19, 6, 5, 2, 8, and 2 patients scored 0, 1, 2, 3, 4,

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5, and 6, respectively. Patients with a Mayo score of ≤2 were classified as having "stable" disease (n=38), whereas those with a score of ≥3 were classified as having "active" disease (n=17).

Patients with CD were defined as having "stable" disease if their CDAI scores were <150 (n=29) and as having "active" disease if their CDAI scores were ≥150 (n=11). Among these patients, 37 of 40 (92.6%) patients with CD had anal involvement. For patients whose anal disease was cured or stabilized, CDAI values were calculated without considering the anal disease. One patient with UC (out of 55) and four with CD (out of 40) were classified into the "stable" and "active" phases, respectively, based on different diagnostic periods. Patients whose disease status changed during the study period were categorized according to disease activity at the time of evaluation.

Study design

This single-center, prospective observational study was conducted at Minerva Watanabe Hospital (Matsuyama, Japan) between December 25, 2018, and March 31, 2022. This study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Tokushima University (Approval No. : 3505-4). Participants were recruited between December 25, 2018, and March 31, 2022. They were provided with an informed consent form and detailed explanations were provided by their attending physicians at Minerva Watanabe Hospital. Written informed consent was obtained from all participants, and the study adhered to reporting guidelines.

Exclusion criteria

The following individuals were excluded from the study : those who did not provide consent, those for whom stool sample collection was difficult or delayed by more than two weeks from the request, those with diarrhea or bloody stools, individuals with conditions other than IBD that could affect serum CRP levels, those with limited decision-making capacity (such as dementia or psychiatric disorders), and those deemed unsuitable by the principal investigator. In addition, individuals who had taken antibiotics, prebiotics, or synbiotics within four weeks prior to stool sample collection were excluded to minimize the influence of external factors on the gut microbiota composition.

Diagnosis

UC and CD were diagnosed based on a comprehensive assessment, including clinical presentation, biochemical markers, stool tests, endoscopic findings, cross-sectional imaging, and histopathological examination, in accordance with the European Crohn's and Colitis Organisation (ECCO) guidelines. Colonoscopic examination with biopsy was routinely performed, and in some cases, histopathological assessment of the surgically resected tissues was also performed. Diagnostic procedures were outsourced to AII Pathology Imaging Laboratory Co., Ltd. (Fukuoka, Japan).

Fecal sample analysis methods

Fecal samples were collected using a fecal collection kit (Techno Suruga Lab, Shizuoka, Japan). Stool samples were subjected to 16S rRNA analysis of intestinal bacteria at Takara Bio, Inc. (Shiga, Japan) to determine the proportion of fecal bacteria.

The measurement and analysis of the alpha diversity index and the proportion of short-chain fatty acid (SCFA)-producing bacteria were sourced from Takara Bio, Inc. (Shiga, Japan). The alpha diversity index was calculated using Shannon's diversity index. Fecal calprotectin levels were measured using fluorescent enzyme immunoassay (BML Inc., Tokyo, Japan).

CRP level measurement

Blood samples were collected in BD SST tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). CRP levels were measured using AU reagent CRP (Beckman Coulter, Brea, CA, USA).

Statistical analyses

One-way analysis of variance was used for comparisons among multiple groups when the normality of distribution and homogeneity of variance assumptions were satisfied. The Kruskal–Wallis and Steel–Dwass tests were used when the assumptions were violated. The student's t-test was used to compare two groups if the normality of distribution and homogeneity of variance assumptions were satisfied, whereas the Mann–Whitney U test was used when the normality assumption was violated. The Dunnett's test was used for within-group comparisons. Correlation analyses for normally and non-normally distributed variables were performed using Pearson's and Spearman's rank correlation coefficients, respectively. The statistical methods not described here are detailed in the figure legends where applicable. Statistical analyses were performed using the EZR (version 1.55 ; Jichi Medical University, Saitama, Japan) software, which was derived from R (R Foundation for Statistical Computing, Vienna, Austria) (9). Regression analysis was performed using Microsoft Excel (Microsoft® Excel® for Microsoft 365 MSO, version 2411, build 16.0.18227.20082, 64-bit). Statistical significance was set at $p < 0.05$.

RESULTS

Patient characteristics

The detailed characteristics of patients with UC and CD are shown in Table 1. Significant differences regarding age ($p < 0.0001$) and sex distribution ($p = 0.014$) were observed between the three groups. These demographic variables may have influenced the gut microbiota composition and were considered potential confounding factors in the interpretation of the results. Our analysis was limited to an evaluation influenced by these potential confounders without statistical adjustment, which should be recognized as a limitation of this study.

Table 1. Patient characteristics

	Healthy individuals	UC patients	CD patients	Total	p-values
Number of cases	30	55	40	125	
Male to female ratio	13:17	33:22	31:9	77:48	0.014
Age (Mean +/- SD [Median])	38.8 +/- 14.1 [37]	50.4 +/- 13.2 [51]	27.4 +/- 9.0 [24]	40.1 +/- 15.6 [38]	<0.0001

P-values were obtained using the chi-square test for sex ratio and the Kruskal–Wallis test for age.
UC, ulcerative colitis ; CD, Crohn's disease ; SD, standard deviation.

Comparison of number of bacterial species, diversity index, and proportion of SCFA-producing bacteria among healthy individuals, patients with UC, and patients with CD

The number of bacterial species was 110.9±27.1 in healthy individuals, 97.8±28.4 in patients with UC, and 65.6±23.5 in patients with CD. There was a significant decrease in the number of bacterial species in patients with CD compared to healthy controls (Fig. 1A). The alpha diversity index was 4.69±0.46 in healthy individuals, 4.26±0.80 in patients with UC, and 3.94±0.78 in patients with CD. Patients with UC and CD showed a significant decrease in the alpha diversity index compared with healthy individuals (Fig. 1B). The proportions of SCFA-producing bacteria were 0.21±0.10 in healthy individuals, 0.20±0.12 in patients with UC, and 0.18±0.14 in patients with CD, which were comparable (Fig. 1C).

Correlation analyses between variations in the number of bacterial species and the diversity index revealed a strong positive correlation in healthy individuals. Both the UC and CD groups showed significant correlations during the overall period (combined stable and active phases) and the stable phase. Notably, strong positive correlations were observed in the overall UC group, the active phase of UC, and the stable phase of CD. In contrast, no significant correlations were observed during the active phase in the CD group (Table 2).

Variations in CRP and fecal calprotectin levels in the UC and CD groups

The relationship between inflammatory markers (CRP and fecal calprotectin) and the disease state of IBD was also investigated. In the UC group, there was no significant difference in the CRP levels between the stable and active phases. In contrast, the CD group showed a significant increase in CRP levels during the active phase compared with the stable phase (Fig. 2A). Fecal calprotectin levels were significantly higher in the active phase than in the stable phase in both the UC and CD groups (Fig. 2B).

The correlations between CRP and fecal calprotectin levels and microbial parameters, including bacterial counts, diversity indices, and SCFA-producing bacteria, are summarized in Table 3. In the CD group, CRP levels showed a significantly weak negative correlation with the number of bacterial species and a significantly moderate negative correlation with the diversity index. Specifically, during the active phase of CD, a significantly strong negative correlation was observed between CRP levels and diversity indices.

Similarly, fecal calprotectin levels in the CD group showed a significantly weak negative correlation with the diversity index.

Correlation between IBD activity scores and inflammatory markers

As shown in Table 4, inflammatory marker levels (CRP and fecal calprotectin) were positively correlated with disease

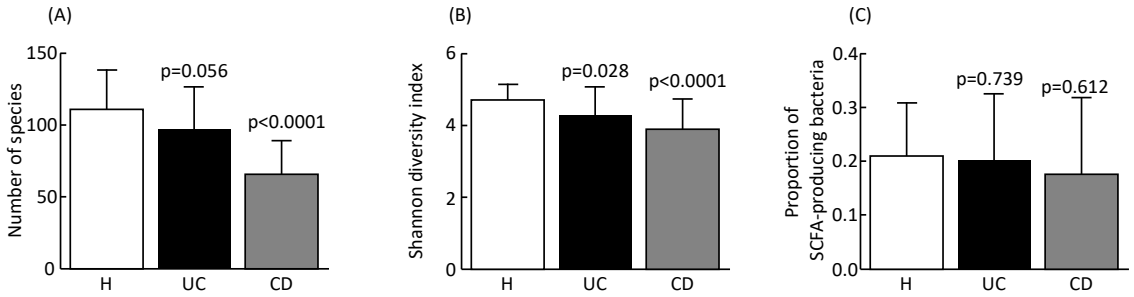

Fig 1. Number of bacterial species (A), the diversity index (B), and the proportion of short-chain fatty acid (SCFA)-producing bacteria (C) in healthy controls, the UC group, and the CD group.
UC, ulcerative colitis ; CD, Crohn's disease ; SCFA, short-chain fatty acid.

Table 2. Gut microbial counts and diversity index in healthy individuals, patients with UC, and patients with CD

Group		Correlation coefficient	p-values
Healthy individuals (30)		0.8004	<0.0001
UC patients	Total (55)	0.6908	<0.0001
	Stable phase (38)	0.6852	<0.0001
	Active phase (17)	0.7564	0.0004
CD patients	Total (40)	0.6555	<0.0001
	Stable phase (28)	0.7496	<0.0001
	Active phase (12)	0.5264	0.0791

The correlation coefficients range from -1 to 1, with positive values indicating a direct relationship and negative values indicating an inverse relationship. The numbers in parentheses indicate the number of cases evaluated. UC, ulcerative colitis ; CD, Crohn's disease.

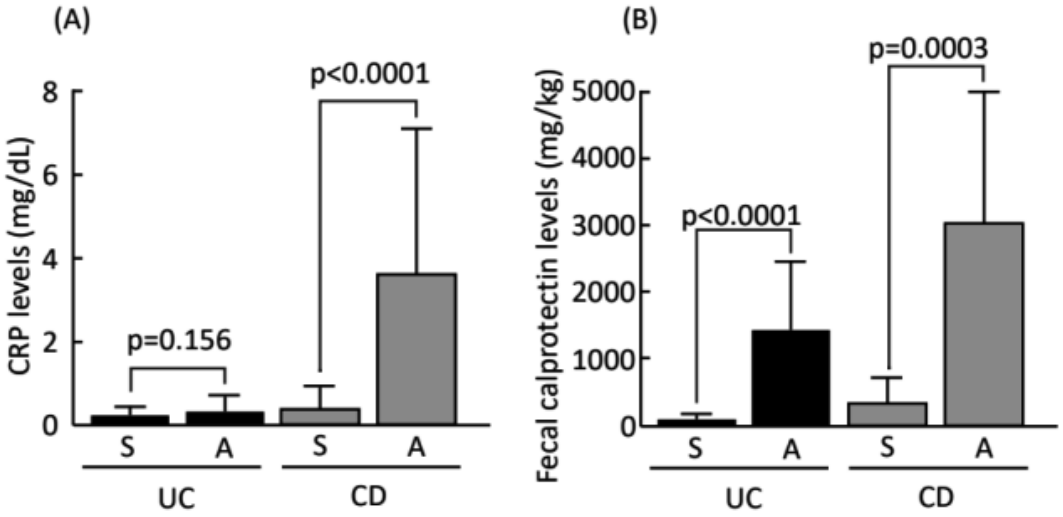

Fig 2. CRP (A) and fecal calprotectin levels (B) according to the disease stage in the UC and CD groups.
The UC group was categorized as follows : a Mayo score of ≤2 was defined as the stable stage (n=38, denoted as S), and a score of ≥3 was defined as the active stage (n=17, denoted as A). In the CD group, a CDAI score of <150 was defined as the stable stage (n=29, S), and a score of ≥150 was defined as the active stage (n=11, A).
UC, ulcerative colitis ; CD, Crohn's disease ; CRP, C-reactive protein ; CDAI, Crohn's Disease Activity Index.

Table 3. Correlation between gut microbiota and inflammatory markers

Group	Disease phase	Marker	Number of species (r / p)	Shannon diversity index (r / p)	SCFA-producing bacteria (r / p)
UC patients	Total	CRP	0.069 / 0.617 (55)	0.035 / 0.802 (55)	-0.238 / 0.103 (48)
		FC	-0.050 / 0.716 (55)	-0.159 / 0.247 (55)	0.131 / 0.372 (48)
	Stable phase	CRP	-0.018 / 0.915 (38)	0.053 / 0.754 (38)	-0.290 / 0.113 (31)
		FC	0.029 / 0.864 (38)	-0.105 / 0.530 (38)	-0.209 / 0.260 (31)
	Active phase	CRP	0.308 / 0.229 (17)	0.155 / 0.552 (17)	-0.281 / 0.274 (17)
		FC	0.218 / 0.402 (17)	0.193 / 0.458 (17)	0.038 / 0.886 (17)
CD patients	Total	CRP	-0.335 / 0.035 (40)	-0.698 / <0.001 (40)	-0.188 / 0.288 (34)
		FC	-0.255 / 0.118 (39)	-0.435 / 0.006 (39)	0.130 / 0.470 (33)
	Stable phase	CRP	0.032 / 0.872 (28)	0.162 / 0.410 (28)	0.085 / 0.687 (25)
		FC	-0.284 / 0.151 (27)	0.265 / 0.181 (27)	0.014 / 0.595 (24)
	Active phase	CRP	-0.403 / 0.194 (12)	-0.809 / 0.001 (12)	-0.501 / 0.170 (9)
		FC	0.110 / 0.733 (12)	-0.162 / 0.616 (12)	0.250 / 0.517 (9)

A Mayo score of ≤ 2 was defined as the stable phase, a score of ≥ 3 as the active phase, a CDAI score of < 150 as the stable phase, and a score of ≥150 as the active phase. Numbers in parentheses indicate the number of cases evaluated. UC, ulcerative colitis ; CD, Crohn's disease ; SCFA, short-chain fatty acid ; CRP, C-reactive protein ; FC, fecal calprotectin ; r, correlation coefficient ; p, p-value.

Table 4. Correlation between disease activity and inflammatory markers

Group	Disease phase	CRP vs Activity (r/p)	FC vs Activity (r/p)	CRP vs FC (r/p)
UC patients	Total	0.192 / 0.160 (55)	0.703 / <0.001 (55)	0.462 / <0.001 (55)
	Stable phase	-0.053 / 0.751 (38)	-0.121 / 0.470 (38)	0.024 / 0.886 (38)
	Active phase	0.143 / 0.584 (17)	0.281 / 0.274 (17)	0.708 / 0.001 (17)
CD patients	Total	0.595 / <0.001 (40)	0.741 / <0.001 (39)	0.708 / <0.001 (39)
	Stable phase	0.090 / 0.648 (28)	0.015 / 0.942 (27)	0.275 / 0.164 (27)
	Active phase	0.145 / 0.652 (12)	0.281 / 0.376 (12)	0.494 / 0.103 (12)

A Mayo score of ≤2 was defined as the stable phase, a score of ≥3 as the active phase, a CDAI score of <150 as the stable phase, and a score of ≥150 as the active phase. Numbers in parentheses indicate the number of cases evaluated. UC, ulcerative colitis ; CD, Crohn's disease ; CRP, C-reactive protein ; FC, fecal calprotectin ; r, correlation coefficient ; p, p-value.

activity status in the complete groups of both UC and CD, particularly in patients with CD. Additionally, CRP and calprotectin levels were strongly correlated in patients with UC and CD.

Changes in the proportion of butyrate-producing bacteria and other genera in IBD

Butyrate-producing bacteria, such as those that produce SCFAs, are decreased in patients with IBD (10). In this study, the proportion of butyrate-producing bacteria and other genera in the total bacterial population was examined in healthy individuals and patients with IBD. The analysis revealed a significant decrease in the proportion of bacteria belonging to *Clostridium* cluster IV in patients with CD compared to healthy individuals (Fig. 3).

Correlation between butyrate-producing bacterial genera and IBD inflammatory markers

In this study, we examined the correlation between 181 bacterial genera and levels of inflammatory markers in patients with IBD. Specifically, the 14 genera with the highest relative abundances (*Alistipes*, *Bacteroides*, *Bifidobacterium*, *Blautia*, *Clostridium* IV, *Clostridium* XIVa, *Clostridium* XVIII, *Flavonifractor*, *Lachnospiracea incertae sedis*, *Oscillibacter*, *Parabacteroides*, *Ruminococcus*, *Streptococcus*, and *Veillonella*) were analyzed for their correlation with inflammatory marker levels in IBD using multiple regression analysis.

Detailed genus-level correlations between bacterial abundance and inflammatory markers are presented in Supplementary Table 1. Specifically, the genus *Clostridium* IV was significantly negatively correlated with fecal calprotectin levels in patients with UC. The genus *Clostridium* XIVa was significantly negatively correlated with fecal calprotectin levels in patients with UC and with levels of inflammatory markers of IBD in patients with CD. Additionally, the genus *Oscillibacter* significantly and positively correlated with CRP levels in patients with CD.

DISCUSSION

This study examined the relationship between IBD activity, inflammatory marker levels, and gut microbiota. Reduced bacterial diversity was found in patients with UC and CD, and CRP and fecal calprotectin levels correlated with disease activity. Patients with CD showed a significant decrease in *Clostridium* cluster IV levels, and several bacterial genera were recently identified to be associated with IBD inflammation.

The gut contains a diverse array of cells that comprise the intestinal immune system and maintain homeostasis (11, 12). IBD is a multifactorial chronic disease, with genetic factors (gene mutations and family history), immune system abnormalities (abnormal immune responses and autoimmune reactions), environmental factors (diet and smoking), psychological stress, and gut microbiota dysbiosis contributing to its etiology (10, 13). In healthy individuals, the mucosal defense mechanisms prevent the attachment and invasion of gut bacteria. However, in IBD, this function is compromised, allowing extensive bacterial adherence and mucosal invasion, which are implicated in disease onset (14).

Some IBD susceptibility genes are related to the host response to the gut microbiota, and many factors are reportedly involved in the immune system and mucosal defense functions (6). Dysbiosis, characterized by decreased bacterial diversity and altered gut microbiota composition, occurs in IBD (3, 4). This study demonstrated a decrease in the number of bacterial species in patients with UC and a significant reduction in those with CD compared with healthy individuals, with a significant reduction in the diversity index in both patients with UC and CD, consistent with previous reports of dysbiosis (Fig. 1).

Butyrate-producing bacteria, such as those that produce SCFAs, are decreased in patients with IBD (10). These bacteria not only help mitigate IBD symptoms but also absorb the SCFAs they produce into the bloodstream and reportedly exhibit a wide range of physiological activities, including anti-inflammatory, anti-obesity, metabolic regulatory, anti-angiogenic, and antioxidant effects (15, 16). Recent studies have also reported that SCFA-producing bacteria induce M2 macrophage polarization in vivo and in vitro and that a decrease in these bacteria is negatively correlated with the inflammatory marker CRP (17). This suggests that the loss of SCFA-producing bacteria may impair anti-inflammatory immune regulation, thereby contributing to the systemic inflammation and elevated CRP levels observed in patients with IBD.

Immunological homeostasis in the gut mucosa is maintained by the balance between Treg and Th17 cells, and SCFAs play a crucial role in inducing Treg cell differentiation and expansion in the colon (18, 19). SCFAs also regulate Treg cell function (20).

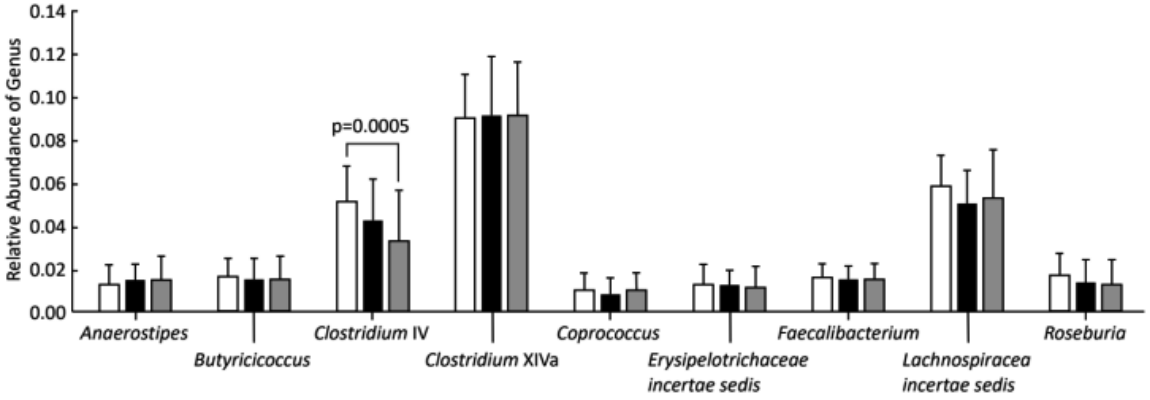

Fig 3. Comparison of the presence ratio of bacteria in the "genera," including butyrate-producing bacteria. Black, white, and gray bars represent healthy controls, patients with UC, and those with CD, respectively. For *Clostridium* cluster IV, no significant decrease in the bacterial presence ratio was observed in patients with UC (p=0.0963), whereas a significant decrease was observed in patients with CD (p=0.0005).
UC, ulcerative colitis ; CD, Crohn's diseases.

This study examined the bacterial species whose abundance was reduced during dysbiosis. Although no significant changes were observed in the overall proportion of SCFA-producing bacteria in patients with IBD (Table 3), butyrate is particularly important for gut immune regulation (10, 21). A comparison of the proportions of bacteria, including butyrate-producing genera, revealed a significant decrease in *Clostridium* cluster IV levels in patients with CD compared with controls. This cluster includes *Faecalibacterium prausnitzii*, a notable butyrate-producing bacterium (15), suggesting a potential decrease in butyrate production in patients with CD.

The *Clostridium* XIVa cluster, like the *Clostridium* IV cluster, includes prominent butyrate-producing bacteria such as *Butyrivibrio crossotus* and *Roseburia intestinalis* (15). Previous reports indicated that a decrease in butyrate-producing bacteria can lead to impaired Treg cell induction, and increased butyrate levels have been shown to induce Treg cells and alleviate the symptoms of experimental colitis (22, 23). Additionally, Clostridium species have been reported to continuously induce Tregs in the colon (18), highlighting the significance of these findings in the context of IBD, particularly CD, in which Treg cell induction and the involvement of *Clostridium* clusters IV and XIVa are of interest.

Louis *et al*. (24) reported that most butyrate-producing bacteria in the human colon belong to *Clostridium* IV and XIVa clusters, with a reduction in these clusters observed in patients with CD (25). No significant correlation was found between the number of bacterial species and inflammatory markers in either the UC or CD groups (Table 3). However, a significant negative correlation was observed between the diversity index and fecal calprotectin levels in patients with CD. A significant positive correlation was observed between the number of bacterial species and diversity index in patients with both UC and CD (Table 2), suggesting that a reduction in the diversity index, rather than the number of bacterial species, is associated with inflammation in CD. However, this correlation was not observed during the active CD phase. Xu *et al*. reported that richness (Chao1) and diversity (Shannon) both decreased during the active phase of CD ; however, the reduction in richness was more pronounced, and the difference in Shannon diversity was significant only when compared with healthy controls (26). Both microbial counts and the Chao1 index indicate species richness, with Chao1 accounting for rare taxa. These findings indicate that under active inflammation, species richness and diversity may not change in parallel, which could explain the discrepancy observed in our study.

Although there have been few reports on the correlation between IBD inflammatory marker levels (CRP and fecal calprotectin) and IBD activity, this study found significant positive correlations between the Mayo score and fecal calprotectin levels in patients with UC and between the CDAI and both CRP and fecal calprotectin levels in patients with CD (Table 4). Additionally, examination of the correlation between the relative abundance of specific bacterial genera and inflammatory markers revealed significant negative correlations between the *Clostridium* XIVa cluster and both CRP ($R=-0.425$, $p=0.006$) and fecal calprotectin ($R=-0.6081$, $p<0.001$) levels in patients with CD, as well as between *Clostridium* XIVa and fecal calprotectin levels in patients with UC ($R=-0.357$, $p=0.007$) (Supplementary Table 1). This highlights the correlation between inflammatory markers and the relative abundance of specific bacterial genera.

This study had several limitations. First, there were notable differences in age and sex distributions among the healthy, UC, and CD groups (Table 1). We believe that this reflects the clinical epidemiology of CD, which is known to occur predominantly in adolescents and young adults, particularly in male patients in Japan (27). Therefore, the observed imbalance in our cohort likely mirrors this established disease tendency rather than representing a methodological bias. Age and sex are known to influence the gut microbiota composition, and these demographic imbalances may have acted as potential confounding factors in the comparison of microbiota profiles. Although our analyses focused on disease-related differences in microbial diversity and inflammatory markers, some of the observed microbial patterns may have been partially influenced by these demographic factors. Second, this study was conducted at a single regional center, which may have limited the generalizability of the findings. Further validation using a larger multicenter cohort with better age- and sex-matched participants or statistical adjustments is warranted to confirm the observed associations.

Furthermore, we identified multiple bacterial genera with potential associations with CRP and fecal calprotectin levels. Detailed correlation data for the 14 representative genera are presented in Supplementary Table 1. In the main text, we highlight key genera, such as *Clostridium* clusters IV and XIVa, that showed the most notable associations.

Importantly, while this study provides valuable insights into the relationship between the gut microbiota and inflammation in Japanese patients with IBD, it also has limitations that should be acknowledged. In addition to the demographic imbalances noted earlier, the use of various medications that are known or suspected to influence microbiota composition is a potential confounding factor.

A potential limitation of this study is that many patients with UC and CD were taking medications that may have affected their gut microbiota composition. Several biological agents used in this study, including adalimumab, infliximab, ustekinumab, and vedolizumab, have been reported to influence gut microbial profile (28, 29). Among corticosteroids, prednisolone has shown similar effects (30). Both the 5-aminosalicylic acid (5-ASA) agents, mesalazine and sulfasalazine, are known to affect the gut microbiota (31) ; however, the influence of azathioprine remains uncertain (32). Moreover, butyrate-producing bacterial preparations were also administered, which were expected to alter the gut microbiota. Although this reflects real-world clinical settings, the possibility that these treatments influenced the observed microbial profiles cannot be excluded. The specific medications used by the participants are summarized in Supplementary Table 2.

Future studies involving stratified analyses and medication-naïve cohorts are required to validate and extend these findings.

In conclusion, this study showed that CRP and fecal calprotectin levels effectively assessed IBD activity at our hospital, consistent with overseas findings. Dysbiosis with reduced gut microbial diversity, including a significant decrease in *Clostridium* clusters IV and XIVa in patients with CD, has been linked to systemic inflammation and worsening of IBD symptoms. Importantly, this study is, to the best of our knowledge, the first to report significant correlations between *Clostridium* clusters IV and XIVa and both CRP and fecal calprotectin levels, specifically in a Japanese cohort with IBD. This finding provides new insights into population-specific microbiota–host interactions and suggests a possible microbial signature relevant to inflammatory activity in Japanese patients with IBD.

STATEMENTS

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STATEMENT OF ETHICS

Study approval statement : The study protocol was reviewed and approved by the Ethics Committee of Tokushima University (Approval No. : 3505-4).

Consent to participate statement : Participants were recruited between December 25, 2018, and March 31, 2022. They were provided with an informed consent form, and detailed explanations were provided by their attending physicians at the Minerva Watanabe Hospital. Written informed consent was obtained from all the participants.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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AUTHOR CONTRIBUTIONS

H. W. conceived the study ; K. T. and H. W. designed and supervised the study ; Y. O. and K. T. contributed to the experiments ; K. T., T. O., and H. W. contributed to the discussion ; K. T. prepared the tables and figures ; K. T. wrote the manuscript.

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Supplementary Table 1. The proportion of each bacterium within the "genus" and its correlation with inflammatory response markers in patients with IBD

Genus	Correlation coefficient (R) and p-values(p)			
	UC patients		CD patients	
	CRP (55)	Fecal calprotectin levels (55)	CRP (40)	Fecal calprotectin levels (39)
Alistipes	R=-0.043, p=0.752	R=-0.166, p=0.226	R=0.092, p=0.571	R=0.281, p=0.083
Bacteroides	R=-0.030, p=0.834	R=0.033, p=0.810	R=0.058, p=0.722	R=-0.094, p=0.568
Bifidobacterium	R=-0.093, p=0.499	R=-0.216, p=0.875	R=0.041, p=0.803	R=0.086, p=0.603
Blautia	R=-0.053, p=0.700	R=0.101, p=0.464	R=-0.193, p=0.232	R=-0.224, p=0.171
Clostridium IV	R=-0.071, p=0.608	R=-0.312, p=0.019	R=0.016, p=0.924	R=-0.008, p=0.960
Clostridium XIVa	R=-0.203, p=0.137	R=-0.357, p=0.007	R=-0.425, p=0.006	R=-0.608, p<0.001
Clostridium XVIII	R=0.121, p=0.380	R=0.098, p=0.477	R=-0.259, p=0.106	R=-0.212, p=0.195
Flavonifractor	R=0.063, p=0.644	R=-0.007, p=0. 960	R=-0.250, p=0.120	R=-0.135, p=0.412
Lachnospiracea incertae sedis	R=0.002, p=0.986	R=0.144, p=0.295	R=-0.246, p=0.125	R=-0.158, p=0.336
Oscillibacter	R=0.152, p=0.269	R=-0.193, p=0.158	R=0.347, p=0.028	R=0.213, p=0.193
Parabacteroides	R=-0.122, p=0.373	R=-0.043, p=0.753	R=-0.162, p=0.317	R=-0.197, p=0.231
Ruminococcus	R=-0.051, p=0.709	R=-0.132, p=0.338	R=-0.287, p=0.073	R=-0.188, p=0.253
Streptococcus	R=0.138, p=0.312	R=0.157, p=0.251	R=0.105, p=0.521	R=0.077, p=0.641
Veillonella	R=0.079, p=0.568	R=-0.053, p=0.700	R=-0.036, p=0.824	R=0.077, p=0.643

The numbers in parentheses indicate the number of cases evaluated. IBD, inflammatory bowel disease ; UC, ulcerative colitis ; CD, Crohn's disease ; CRP, C-reactive protein. These data were evaluated using multiple regression analysis.
The correlation coefficient was determined based on the slope of the regression line, and Excel provided the absolute value of multiple R.

Supplementary Table 2. Medication use in healthy controls and patients with ulcerative colitis (UC) and Crohn's disease (CD)

Group		Medications (cases)												
		Azathioprine	Adalimumab	Infliximab	Ustekinumab	Sulfasalazine	Magnesium oxide	Budesonide	Predonisolone	Betamethasone	Vedolizumab	Mesalazine	Elemental diet	Butylate -producing bacteria
Healthy Individuals (30)		0	0	0	0	0	0	0	0	0	0	0	0	0
UC patients (55)	Stable phase (38)	2	0	1	0	0	1	0	0	0	0	9	0	3
	Active phase (17)	0	1	0	0	1	0	0	2	1	2	4	0	3
CD patients (40)	Stable phase (28)	2	2	9	5	0	0	0	0	0	0	0	0	0
	Active phase (12)	0	3	0	3	0	0	2	0	0	0	1	1	0

Numbers in parenthesis indicate the number of cases evaluated.