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Original Article

Diarrhea-predominant Irritable Bowel Syndrome-like Symptoms in Patients With Quiescent Crohn's Disease: Comprehensive Analysis of Clinical Features and Intestinal Environment Including the Gut Microbiome, Organic Acids, and Intestinal Permeability

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Background/Aims
Diarrhea-predominant irritable bowel syndrome (IBS-D)-like symptoms frequently occur in patients with quiescent Crohn's disease (CD). To investigate the factors underlying IBS-D-like symptoms in patients with quiescent CD, we performed a comprehensive analysis of the clinical features and intestinal environment in those patients.

Methods
We performed a prospective observational study of 27 patients with quiescent CD (CD activity index [CDAI] ≤ 150; C-reactive protein ≤ 0.3 mg/dL). The presence and severity of IBS-D-like symptoms, health-related quality of life, disease-specific quality of life, and status of depression and anxiety were evaluated. The level of intestinal permeability, fecal calprotectin and organic acids and the profiles of gut microbiome were analyzed.

Results
Twelve of the 27 patients with quiescent CD (44.4%) had IBS-like symptoms, and these patients showed a significantly higher CDAI, IBS severity index and anxiety score than those without. The inflammatory bowel disease questionnaire score was significantly lower in the patients with IBS-D-like symptoms. There were no significant differences in small intestinal/colonic permeability or the levels of organic acids between the patients with and without IBS-D-like symptoms. *Fusicatenibacter* was significantly less abundant in the patients with IBS-D-like symptoms whereas their fecal calprotectin level was significantly higher (384.8 ± 310.6 mg/kg) than in patients without (161.0 ± 251.0 mg/kg). The receiver operating characteristic curve constructed to predict IBS-D-like symptoms in patients with quiescent CD using the fecal calprotectin level (cutoff, 125 mg/kg) showed a sensitivity and specificity of 73.3% and 91.7%, respectively.

Conclusion
Minimal inflammation is closely associated with the development of IBS-D-like symptoms in patients with quiescent CD.
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Key Words
Anxiety; Crohn disease; Gastrointestinal microbiome; Inflammation; Irritable bowel syndrome

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Introduction

Crohn's disease (CD) is a chronic inflammatory disease affecting the gastrointestinal tract and characterized by alternating phases of active inflammation and quiescent remission. Recently, the prevalence of CD has been increasing in not only Western but also Eastern countries.[1,2] For the treatment of CD patients, the development of biologic agents has greatly improved the situation, resulting in a high rate of quiescent remission,[3] which suggests that the number of patients with quiescent CD worldwide has increased. Although these advances in therapeutics have certainly improved the prognosis of CD patients, those with quiescent CD never attain a quality of life (QOL) equivalent to that in healthy individuals.[4] For instance, patients with quiescent CD frequently have symptoms such as abdominal pain or stool irregularities that are also characteristic of irritable bowel syndrome (IBS).[4,5] When these symptoms meet the diagnostic criteria for IBS in patients with quiescent CD, they are referred to as IBS-like symptoms.[4-6] It has been reported that IBS-like symptoms significantly impact health-related QOL,[7] and therefore it is important to clarify the underlying pathophysiology of IBS-like symptoms in patients with quiescent CD.

Approximately 30-50% of patients with quiescent CD have IBS-like symptoms.[4,5,7,8] However, the mechanism underlying such symptoms has remained unclear. Accumulating evidence has recently suggested that alteration of the gut microbiome and associated organic acids, as well as persistent mucosal microinflammation and dysfunction of the mucosal barrier, are likely common to both inflammatory bowel disease (IBD) and IBS.[6,9,10] Moreover, it is now clear that psychological stress commonly plays a pivotal role in the pathophysiology of IBS and quiescent CD.[7,11,12] In these contexts, to identify the factors crucial for the development of IBS-like symptoms in patients with quiescent CD, we comprehensively investigated the gut microbiome and associated fecal organic acids, intestinal microinflammation, and permeability in affected patients in relation to clinical data such as various aspects of QOL.

Materials and Methods

Patients

The patients with quiescent CD were enrolled into this study between September 2019 and July 2020. Quiescent CD was defined as a Crohn's disease activity index (CDAI) ≤ 150 and a C-reactive protein (CRP) level of ≤ 0.3 mg/dL.[6,13] The CDAI is a validated standard calculated using the scores for bowel habits, abdominal pain, general condition, Crohn's disease-associated findings, use of medicines for diarrhea, abdominal mass sensation, hematocrit, and body weight.[14] Prior to this study, endoscopic mucosal healing had been confirmed by colonoscopy and/or capsule endoscopy. Since we did not have a validated Japanese version of the Rome IV diagnostic questionnaire, we used a Japanese version of the Rome III diagnostic questionnaire for functional gastrointestinal disorders to evaluate IBS-like symptoms.[15] The patients classified as diarrhea-predominant IBS (IBS-D) was included in this study. The patients who completely met the conclusion criteria of both quiescent CD and IBS-D were recruited consecutively. Clinical information and symptoms were evaluated using a self-completed symptom questionnaire.

This study was performed with approval (No. 3158) from the Ethics Committee of Hyogo College of Medicine and registered in the University Hospital Medical Information Network (Registration No. UMIN000041577). Patients who had given fully informed consent were enrolled, and the study was conducted according to the principles governing human research stipulated by the Declaration of Helsinki. All authors had access to the study data and reviewed and approved the final manuscript.

Questionnaires

The health-related QOL (HRQOL) was scored using the 8-item short-form health survey (SF-8).[16] The disease-specific QOL was scored using the inflammatory bowel disease questionnaire (IBDQ).[17,18] Status of depression and anxiety was evaluated using the hospital anxiety and depression scale (HADS).[19,20] Fur-

thermore, we assessed the severity of IBS-like symptoms using a questionnaire based on the Japanese version of the IBS severity index.[21,22]

Measurement of Intestinal Permeability

Intestinal permeability was evaluated according to the protocol reported previously.[23] After an overnight fast, patients received orally a sugar mixture containing mannitol (1.5 g), sucralose (0.5 g), lactulose (4 g), and sucrose (15 g), and their urine for the initial 0-5 hours and for 5-24 hours was collected in separate containers, respectively. The procedures for measurement of intestinal permeability are described in Supplementary Methods.

Measurement of the Fecal Calprotectin Level

A fresh stool sample for measurement of fecal calprotectin was obtained from each patient and stored at −20℃ until analysis. The level of fecal calprotectin was quantified using a quantitative monoclonal antibody-based enzyme-linked immunosorbent assay kit (Calprotectin Mochida, Mochida, Tokyo, Japan).

Analysis of the Gut Microbiome

A separate stool sample was also collected in a tube (Sarstedt, Inc, Nümbrecht, Germany) for analysis of the gut microbiome and fecal organic acids. The collected sample was immediately frozen and stored at −80℃ until use.

Details of bacterial DNA extraction from feces, DNA sequencing and microbiome analyses are described below. Briefly, extraction of bacterial DNA was performed using the beads-phenol method.[24] Meta-analysis of fecal bacterial 16S ribosomal DNA (rDNA) sequences was performed according to the method described previously,[25] with minor modifications. The V3-V4 region of 16S rDNA was amplified on a Veriti thermal cycler (Thermo Fisher Scientific, Waltham, MA, USA), and the amplicon was purified using AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA). Sequencing was performed on the MiSeq platform using the MiSeq Reagent Kit v2 Chemistry (Illumina, Inc, San Diego, CA, USA). Comparison of each taxon in gut microbiota was conducted at the phylum and genus levels. The Shannon index, observed operational taxonomic unit, chao1, and abundance-based coverage estimator were calculated to determine the alpha diversity of microbiota in the samples. Beta diversity was estimated by calculating the Bray-Curtis distance, and the weighted and unweighted UniFrac distances between samples using QIIME, calculated using the read data obtained.[26] To compare differences in overall bacterial gut microbiota structure, principal co-ordinates analysis was applied to reduce the dimensionality of the resulting distance matrix.

Analysis of Fecal Organic Acids

Fecal organic acids were measured using a modified protocol described previously.[27,28] In brief, the ether layers containing organic acids were collected for gas chromatography–mass spectrometry analysis using GCMS-QP2010 Ultra (Shimadzu, Kyoto, Japan). The concentrations of individual organic acids were measured using external standard calibration over an appropriate concentration range.

Statistical Methods

All statistical analyses were conducted using the R statistical software package version 3.1.3.25. Data are shown as means ± SD. The Mann-Whitney *U* test and Fisher's exact test were applied for comparison of clinical data, questionnaire scores, fecal calprotectin and organic acid levels, and intestinal permeability. Statistical significance was set at $P < 0.05$. For analysis of gut microbiota, statistical significance was determined by Welch's *t* test with the Benjamini-Hochberg procedure.[29] The beta diversity was analyzed using permutational multivariate analysis of variance (PERMANOVA) for comparison of gene similarity.

Table. Characteristics of Crohn's Disease Patients With and Without Diarrhea-predominant Irritable Bowel Syndrome-like Symptoms

Characteristics	IBS-D-like symptoms		*P*-value
	Negative (n = 15)	Positive (n = 12)	
Age (yr)	47.1 ± 3.0	40.0 ± 2.6	0.124
Sex (male/female)	12/3	9/3	0.756
BMI (kg/m^2)	23.8 ± 0.8	21.6 ± 1.0	0.922
Duration of disease (yr)	11.0 ± 6.7	13.4 ± 5.6	0.086
Current medications			0.468
5-ASA	8 (53.3%)	6 (50.0%)	
Steroids	0 (0.0%)	0 (0.0%)	
Immunomodulator	2 (13.3%)	0 (0.0%)	
Biologics therapy	5 (33.3%)	3 (25.0%)	
Elemental diet	13 (86.7%)	10 (83.3%)	
Prior surgery	7 (46.7%)	9 (75.0%)	0.239
Ileectomy	3 (20.0%)	5 (41.7%)	
Colectomy	2 (13.3%)	3 (25.0%)	
Ileocolectomy	2 (13.3%)	1 (8.3%)	
C-reactive protein (mg/dL)	0.08 ± 0.04	0.14 ± 0.07	0.195

Data are expressed as mean ± SD.
CD, Crohn's disease; IBS-D, diarrhea-predominant irritable bowel syndrome; CDAI, CD activity index; 5-ASA, 5-aminosalicylate; AZA, azathioprine.

Results

Characteristics of Patients With Quiescent Crohn's Disease With and Without Diarrhea-predominant Irritable Bowel Syndrome-like Symptoms

Twenty-seven patients with quiescent CD were enrolled in this study. Among them, 12 patients (44.4%) had IBS-D-like symptoms (Table). None of the parameters including age, sex, body mass index, disease duration, current medications, prior surgery, or C-reactive protein level differed between the patients with and without IBS-D-like symptoms, although disease duration tended to be longer in those with IBS-D-like symptoms.

The CDAI was significantly higher in patients with IBS-D-like symptoms (104.9 ± 32.3) than in those without (60.5 ± 29.8) (Fig. 1). Furthermore, the IBS severity index was significantly higher in the patients with IBS-D-like symptoms (140.4 ± 60.8) than in those without (79.1 ± 45.9) (Fig. 1).

Disease-specific and Health-related Quality of Life in Patients With Quiescent Crohn's Disease With and Without Diarrhea-predominant Irritable Bowel Syndrome-like Symptoms

We next assessed the QOL of patients with quiescent CD. As shown in Figure 2A, the IBDQ score was significantly lower in those with IBS-D-like symptoms (167.8 ± 25.8) than in those without (188.2 ± 15.1), suggesting that disease-specific QOL is indeed worsened by IBS-D-like symptoms. We also assessed the general QOL of patients with quiescent CD using the SF-8 health survey (Fig. 2B). Neither the physical nor mental summary score differed between the patients with (physical, 53.8 ± 3.8; mental,

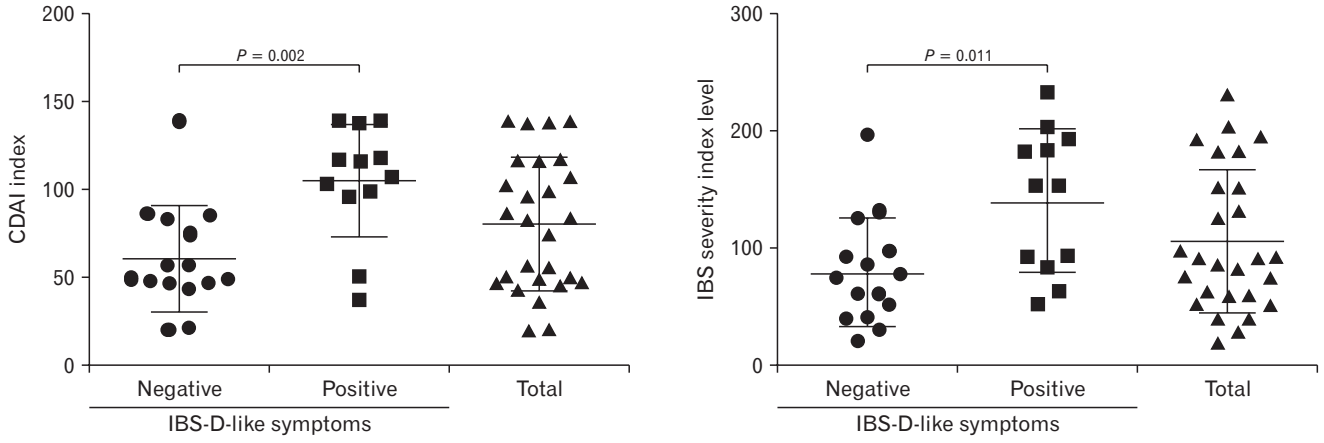


Figure 1. Relationship between diarrhea-predominant irritable bowel syndrome (IBS-D)-like symptoms and the Crohn's disease activity index (CDAI) or irritable bowel syndrome (IBS) severity index in patients with quiescent Crohn's disease. Results are expressed as mean ± SD.

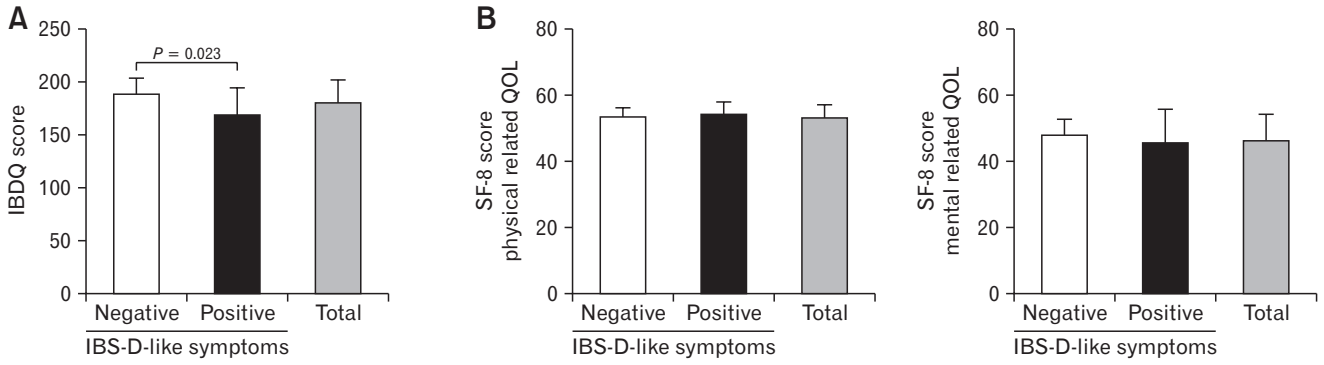


Figure 2. Relationship between diarrhea-predominant irritable bowel syndrome (IBS-D)-like symptoms and (A) the inflammatory bowel disease questionnaire (IBDQ) score or (B) 8-item short-form health survey (SF-8) scores in patients with quiescent Crohn's disease. Results are expressed as mean ± SD. QOL, quality of life.

45.2 ± 10.6) and without (physical, 52.8 ± 3.5; mental, 47.8 ± 4.9) IBS-D-like symptoms, although the mental summary scores for all of the patients were basically lower than 50 (the Japanese standard value for healthy subjects).[30]

Status of Depression and Anxiety in Patients With Quiescent Crohn's Disease With and Without Diarrhea-predominant Irritable Bowel Syndrome-like Symptoms

Using the HADS questionnaire, we assessed more precisely the status of depression and anxiety in patients with quiescent CD (Fig. 3). Although the depression score did not differ between

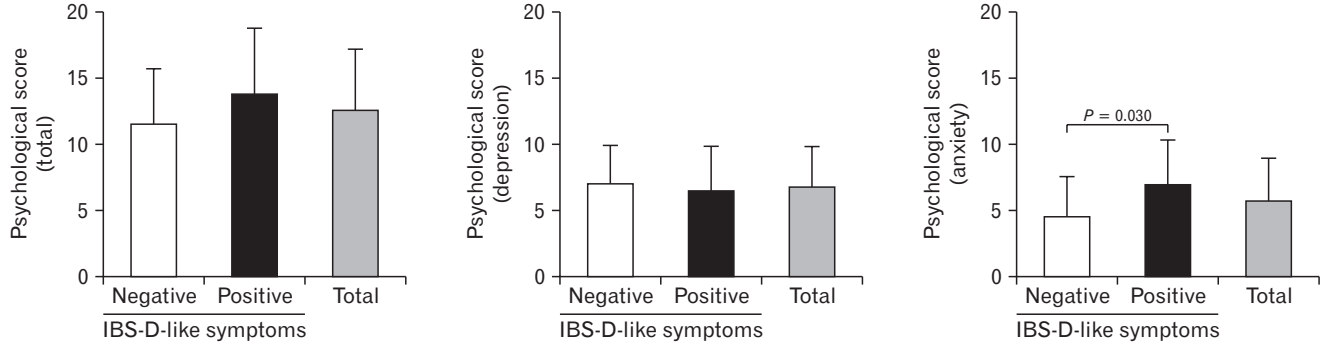


Figure 3. Relationship between diarrhea-predominant irritable bowel syndrome (IBS-D)-like symptoms and psychological scores in patients with quiescent Crohn's disease. Results are expressed as mean ± SD.

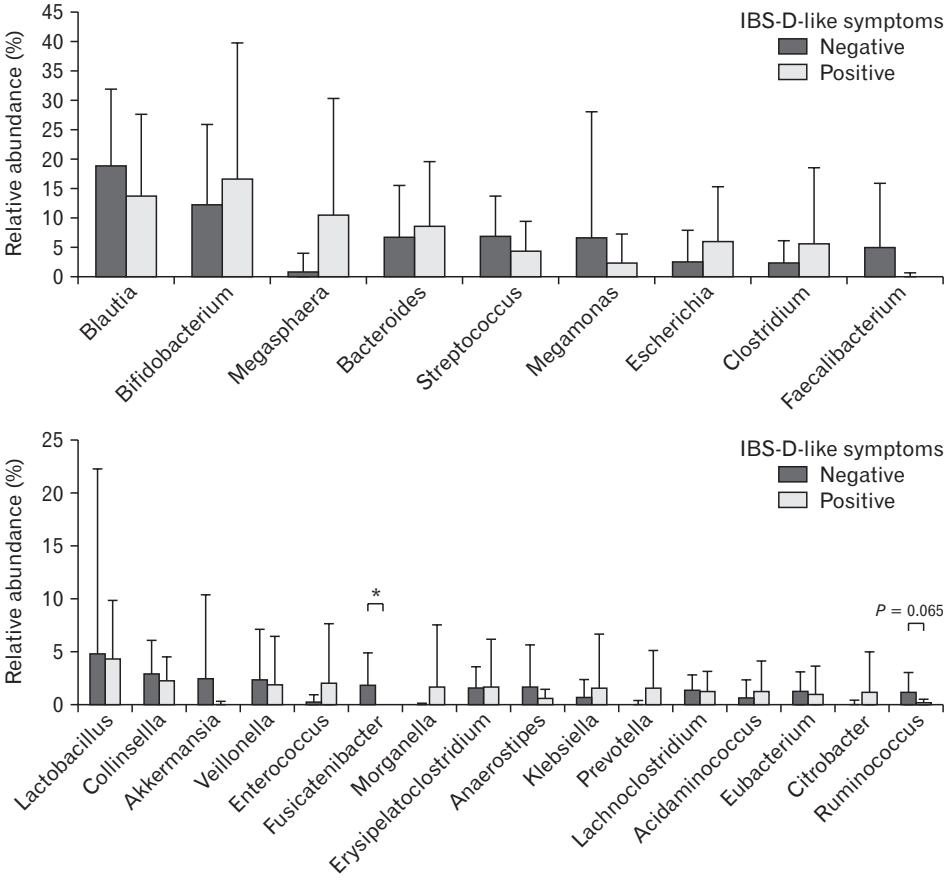


Figure 4. Relationship between diarrhea-predominant irritable bowel syndrome (IBS-D)-like symptoms and the relative abundance of intestinal bacteria at the genus level in patients with quiescent Crohn's disease. Results are expressed as mean ± SD. *$P < 0.05$.

the patients with (6.4 ± 3.3) and without (6.9 ± 2.9) IBS-D-like symptoms, the anxiety score was significantly higher in the patients with such symptoms (6.8 ± 3.4) than in those without (4.5 ± 3.0).

Intestinal Environment Including Fecal Microbiome, Organic Acids, Calprotectin, and Intestinal Permeability

Principal coordinate analysis revealed no significant difference in gut microbiome structure between the patients with quiescent CD with and without IBS-D-like symptoms (Supplementary Figure A). In addition, the diversity of the gut microbiome and the abundance of the gut microbiome at the phylum level did not differ between the 2 groups (Supplementary Figure B and C). We also analyzed the gut microbiome profile at the genus level. This revealed that *Fusicatenibacter* was significantly less abundant in the patients with IBS-D-like symptoms (Fig. 4), and that *Ruminococcus* also tended to be less abundant in the same group ($P = 0.065$).

The fecal organic acid profiles in patients with quiescent CD are shown in Figure 5. We found no significant differences in the levels of any of the 6 organic acids examined between the patients with and without IBS-D-like symptoms (Fig. 5A).

To evaluate the inflammatory condition of the intestinal environment, we measured the level of fecal calprotectin. This was found to be significantly higher in patients with IBS-D-like symptoms (384.8 ± 310.6 mg/kg) than in those without (161.0 ± 251.0 mg/kg) (Fig. 5B).

There was no difference in the degree of small-intestinal permeability (in terms of the lactulose/mannitol ratio) between the patients with (0.011 ± 0.008) and without (0.012 ± 0.007) IBS-D-like symptoms (Fig. 5C). There was also no significant difference of colonic permeability (in terms of sucralose excretion) between the patients with (0.594 ± 0.463%) and without (0.536 ± 0.256%) IBS-D-like symptoms (Fig. 5C). As a reference, the degree of permeability of the small intestine and colon in healthy controls determined using a similar protocol[31,32] were similar to those in patients with quiescent CD.

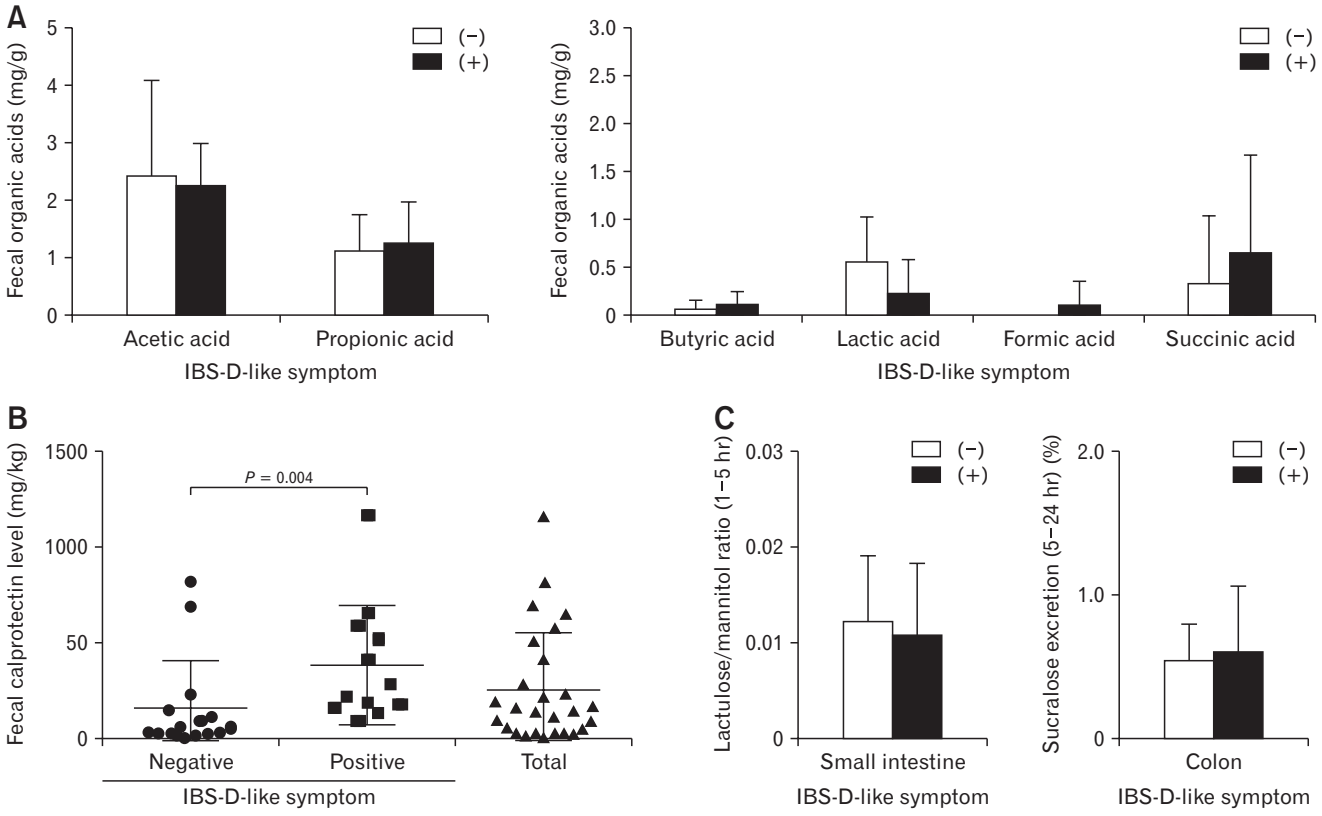
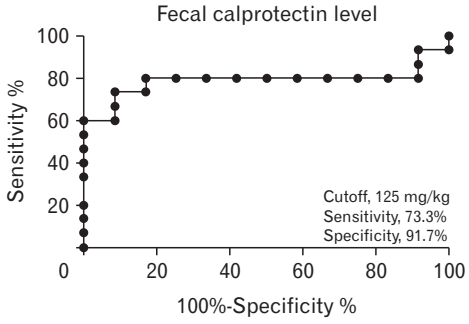
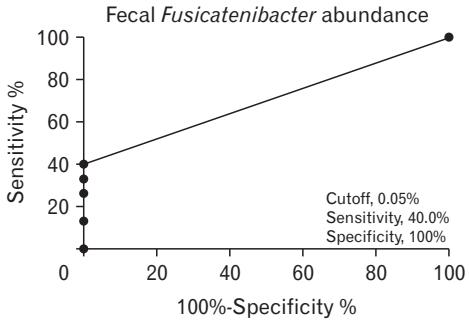


Figure 5. Relationship between diarrhea-predominant irritable bowel syndrome (IBS-D)-like symptoms and (A) fecal organic acids, (B) fecal calprotectin, and (C) intestinal permeability in patients with quiescent Crohn's disease. Results are expressed as mean ± SD.



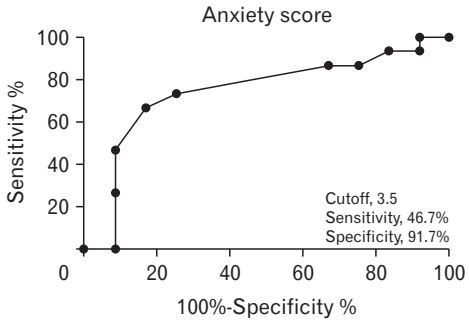
Calprotectin	*Fusicatenibacter* High	*Fusicatenibacter* Low
High	2	13
Low	5	7

$P = 0.185$



Calprotectin	Anxiety score High	Anxiety score Low
High	9	4
Low	4	8

$P = 0.252$



Anxiety score	*Fusicatenibacter* High	*Fusicatenibacter* Low
High	1	12
Low	6	8

$P = 0.077$

Figure 6. Receiver-operating characteristic curves for prediction of diarrhea-predominant irritable bowel syndrome (IBS-D)-like symptoms in patients with quiescent Crohn's disease. The cutoff level, sensitivity, and specificity for each factor are shown in the panels. Relationships among the fecal calprotectin level, *Fusicatenibacter* abundance and anxiety score were analyzed by Fisher's exact test.

Relationships Among Fecal Calprotectin, *Fusicatenibacter* Abundance and Anxiety Score in Patients With Quiescent Crohn's Disease

In the present study, we found significant correlations between the presence of IBS-D-like symptoms and fecal calprotectin, *Fusicatenibacter* abundance or anxiety score in patients with quiescent CD. We further constructed a receiver operating characteristic curve using the fecal calprotectin level, *Fusicatenibacter* abundance or anxiety score to predict IBS-D-like symptoms in these patients (Fig. 6). The cutoff level for fecal calprotectin was determined as 125 mg/kg, and the sensitivity and specificity were 73.3% and 91.7%, respectively. The cutoff level for fecal *Fusicatenibacter* abundance was 0.05%, and the sensitivity and specificity were 40.0% and 100%, respectively. The cutoff anxiety score was 3.5, and the sensitivity and specificity were 46.7% and 91.7%, respectively. When analyzed the associations among these 3 factors, patients with a high fecal calprotectin level tended to have a lower abundance of fecal *Fusicatenibacter* and a higher anxiety score. In addition, those with a higher anxiety score tended to have a lower abundance of fecal *Fusicatenibacter*.

Discussion

As it is anticipated that the population of patients with quiescent CD will continuously increase as a result of improved medication, the management of such patients is becoming a focus of attention. The QOL of patients with quiescent CD is apparently lower than that of healthy subjects,[4] and indeed IBS-D-like symptoms significantly impact the QOL of such patients.[33] In the present study, IBS-D-like symptoms were confirmed in 44.4% of patients with quiescent CD, similar to findings reported previously.[4,5,7,8] Here we investigated not only IBS-D-like symptoms but also physical/mental symptoms, and this revealed that mental, but not physi-

cal, QOL was significantly lower in the patients with IBS-D-like symptoms than in those without. Among mental-related factors, the anxiety score was significantly lower in the patients with IBS-D-like symptoms, consistent with previous reports.[5,7] It is clearly debatable whether IBS-D-like symptoms induce anxiety in patients with quiescent CD or whether such patients are more sensitive to mental stress and thus more likely to complain of IBS-D-like symptoms. However, it was interesting that the disease duration tended to be longer in the patients with IBS-D-like symptoms than in those without. Subsequent analysis revealed no significant correlation between disease duration and anxiety score, suggesting that the development of IBS-D-like symptoms is not simply attributable to exacerbation of anxiety in the setting of long-standing disease.

In the present study, we investigated the gut microbiome profile of patients with quiescent CD as it is a factor known to play a pivotal role in the pathophysiology of not only IBD but also IBS-D.[34] However, one limitation of the study was that we were unable to enroll healthy subjects, so consequently could not compare the data obtained with those for healthy individuals. However, our data did indicate a non-significant tendency for gut microbiome profiles to differ between the patients with and without IBS-D-like symptoms, and also for its diversity to be lower in those with IBS-D-like symptoms. In addition, analysis of the gut microbiome at the genus level revealed that the abundance of *Fusicatenibacter* was significantly reduced in the patients with IBS-D-like symptoms. Interestingly, a lower abundance of *Fusicatenibacter* has been reported in not only CD patients[35] but also an animal model of IBS-D.[36] *Fusicatenibacter* is a recently isolated genus[37] considered likely to ameliorate colitis by enhancing IL-10 production in the colonic mucosa.[38] These findings suggest that a decreased abundance of *Fusicatenibacter* may underlie susceptibility to microinflammation in patients with quiescent CD who suffer IBS-D-like symptoms.

It has been challenging to clarify the role of the gut microbiome in the development of symptoms including abdominal pain and/or diarrhea in patients with quiescent CD. Accumulating evidence has revealed that the gut microbiome interacts with epithelial cells, endocrine cells, immune cells, or neural cells through the organic acids produced by the gut flora.[39] In this context, we investigated the levels of fecal organic acids in patients with quiescent CD but found no significant differences between the patients with IBS-D-like symptoms and those without. For example, several studies have reported that fecal acetic acid is decreased in both CD[40] and IBS patients;[28] however, data for fecal organic acids in CD[41,42] or IBS[43,44] patients have been conflicting, and are still scarce, especially for patients with quiescent CD. As it is known that gut microbiome profiles and/or the organic acids produced are greatly affected by various factors such as species, food, or medication,[45,46] it is not surprising that the data on organic acids in CD or IBS patients have tended to be conflicting. As alteration of mucosal permeability is responsible for the passage of bacteria and/or antigens into the lamina propria, thus affecting mucosal immune conditions and the enteric nervous system,[47] we also investigated the permeability of the intestinal mucosa. Although we were unable to compare intestinal permeability between healthy individuals and patients with quiescent CD, we found that the overall values for patients with quiescent CD were not very different from those for healthy subjects reported previously.[31,32] We then expected that intestinal permeability might differ between patients with and without IBS-D-like symptoms, but again found no significant difference. As the level of intestinal permeability must reflect the quality of mucosal barrier function, these findings at least suggest that among patients with quiescent CD the damage to mucosal integrity does not differ markedly between those who have IBS-D-like symptoms and those who do not. On the other hand, the average fecal calprotectin level (317 mg/kg) in the patients with quiescent CD overall was understandably over the normal range (< 50 mg/kg). This suggests that intestinal mucosa appears to be healed endoscopically but invisible inflammation must be persisted in the mucosa of patients with quiescent CD. Of note, our comprehensive survey revealed that the level of fecal calprotectin in patients with IBS-D-like symptoms was slightly but significantly higher than that in the patients without. Thus, it appears that some degree of intestinal inflammation not sufficiently severe to affect intestinal permeability might persist in patients with quiescent CD with IBS-D-like symptoms, and we speculate that slight exacerbation beyond a certain threshold level might be associated with the development of IBS-D-like symptoms. It has been suggested that the severity of IBS-D symptoms correlates with the levels of inflammation in the intestine[48] and that the inflammatory state in the intestinal tract is possible to sensitize the signaling from peripheral nerves to central nerve system in the gut-brain axis, causing emotional and depressive disorders in patients with IBS-D.[48,49] Furthermore, it has been suggested that pro-inflammatory cytokines including IL-1β, IL-6, IL-8, IL-12, and TNF-α may play a pivotal role to sensitize the signaling in the gut-brain axis.[50-52] In this regard, we are regrettable that we could not assess gut immune dysfunction including the alterations of pro-inflammatory cytokines profile in the intestinal tissues, indicating the limitation of this study.

It has been suggested that in post-infectious IBS, minimal intestinal inflammation is a key to the development of IBS symptoms.[53] Indeed, an increase of macrophage/mast cells and the

cytokines they produce is evident in the intestinal mucosa of IBS patients.[54,55] In this context, we have also found that the macrophage population is increased in not only the lamina propria but also the muscular layer in mice with remission after dextran sulfate sodium-induced colitis.[56] Thus, it is currently hypothesized that minimal inflammation accompanied by activated immune cells may act on the enteric nervous system, resulting in visceral hypersensitivity and gastrointestinal tract dysmotility.[57] As it is impossible to examine the enteric nervous system in biopsy samples from IBD and/or IBS patients, any mechanistic analysis of the interaction between immune cells and the enteric nervous system would require an adequate animal model. To overcome this experimental limitation, we were able to investigate the correlation between the fecal calprotectin level and data for various clinical parameters. We found that the fecal calprotectin level tended to have a positive association with the anxiety score in patients with quiescent CD. It is recognized that gastrointestinal inflammation may affect the brain-gut axis, resulting in psychological disturbance.[58,59] In contrast, psychological stress is likely to cause inflammation in the intestinal tract.[59,60] This invites speculation as to whether intestinal minimal inflammation or psychological stress is the crucial factor triggering IBS-D-like symptoms in patients with quiescent CD. Are these 2 factors mutually casual, or are they independent of each other? Although we have no definitive answer, it is tempting to speculate that intestinal minimal inflammation affects the brain-gut axis and is closely associated with the development of IBS-D-like symptoms in patients with quiescent CD.

In summary, we have comprehensively investigated clinical data sets and intestinal environmental factors in patients with quiescent CD suffering IBS-D-like symptoms. Among many factors examined, we found that intestinal minimal inflammation and anxiety were closely associated with the development of IBS-D-like symptoms in these patients. On the other hand, we have to know a limitation that the number of patients examined was small to confirm those findings. In the present study, the definition of quiescent CD was very strict and furthermore, we designed to do comprehensively several difficult tests for intestinal environment including gut microbiome, organic acids, or intestinal permeability, leading to a difficult recruitment of target patients. However, we believe that this work has some novelty because this is a first report investigating clinical features and various aspects of intestinal environment in quiescent CD patients with IBS-D-like symptoms. Another limitation of this study is a lack of a healthy control group. Thus, we could not compare the quiescent CD patients without IBS-D-like symptoms with healthy subjects in terms of microinflammation. In this context, the level of fecal calprotectin in patients without IBS-D-like symptoms is relatively higher than that in healthy control (normal range, $<$ 50 mg/kg), and therefore, we are tempted to speculated that the patients with quiescent CD have a threshold level of microinflammation for the development of IBS-D-like symptoms. Although we were unable to clarify the mechanism of interaction between intestinal minimal inflammation and anxiety in terms of the brain-gut axis, we demonstrated that reduction of *Fusicatenibacter* strains may be involved in intestinal minimal inflammation in these patients. This suggests that probiotics might offer a promising avenue of therapy for correcting the gut microbiota profile in patients with quiescent CD who suffer IBS-D-like symptoms, and we are now initiating a clinical trial to examine this possibility.

Supplementary Materials

Note: To access the supplementary figure and methods mentioned in this article, visit the online version of *Journal of Neurogastroenterology and Motility* at http://www.jnmjournal.org/, and at https://doi.org/10.5056/jnm22027.

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