J Neurogastroenterol Motil, Vol. 27 No. 4 October, 2021
pISSN: 2093-0879 eISSN: 2093-0887
https://doi.org/10.5056/jnm20151
Journal of Neurogastroenterology and Motility

Original Article

A Pilot Study of Clinical Evaluation and Formation Mechanism of Irritable Bowel Syndrome-like Symptoms in Inflammatory Bowel Disease Patients in Remission

Haiyang Wang,[1,2] Xiaojing Zhao,[1] Xiufang Cui,[1,3] Meifeng Wang,[1] Chunhua Jiao,[1] Jiajia Li,[1] Yan Yang,[2] Yi Li,[2]* and Hongjie Zhang[1]*

[1]Department of Gastroenterology, the First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China; [2]Department of Gastroenterology, Sir Run Run Hospital, Nanjing Medical University, Nanjing, Jiangsu, China; and [3]Department of Gastroenterology, Nanjing First Hospital, Nanjing Medical University, Nanjing, Jiangsu, China

Background/Aims
Some inflammatory bowel disease (IBD) patients in remission suffer from irritable bowel syndrome (IBS)-like symptoms (IBD-IBS). The pathogenesis has not yet been elucidated. The study aim is to evaluate relationships among quality of life (QOL), psychological status, and visceral sensitivity, and explore the formation mechanism of IBD-IBS.

Methods
Forty-seven patients with Crohn's disease in remission, 24 ulcerative colitis in remission, 26 IBS, and 20 healthy controls were included in the study. The abdominal pain, QOL, anxiety, and depression were evaluated through questionnaires. Visceral sensitivity was measured by rectal balloon distension. The serum levels of 5-hydroxytryptamine (5-HT) and nerve growth factor (NGF) were measured by enzyme-linked immunosorbent assay. The expressions of tryptase, 5-HT, NGF, and related receptors in colonic tissues were detected by immunohistochemistry and western blot.

Results
Prevalence of IBS-like symptoms in Crohn's disease and ulcerative colitis patients in clinical remission was 29.8% and 50.0%, respectively. The QOL was lower, the anxiety/depression scores were higher in IBD-IBS patients than those without IBS-like symptoms. Additionally, patients with IBD-IBS existed visceral hypersensitivity. Besides, abdominal pain was associated with poor QOL, visceral hypersensitivity, anxiety, and depression in IBD-IBS patients. The number of mast cells (MCs) and expressions of 5-HT, NGF, and related receptors were higher in IBD-IBS patients than those with no such symptoms. The serum levels of 5-HT and NGF positively correlated with abdominal pain and visceral hypersensitivity.

Conclusion
IBD-IBS patients may have low QOL and psychological abnormalities, as wells as visceral hypersensitivity which may be related to increased 5-HT and NGF levels released from activated mast cells.
(J Neurogastroenterol Motil 2021;27:612-625)

Key Words
Anxiety; Depression; Inflammatory bowel disease; Mast cells; Quality of life

Received: June 30, 2020 Revised: January 2, 2021 Accepted: February 7, 2021

*Correspondence: Hongjie Zhang and Yi Li are equally responsible for this work.
Hongjie Zhang, MD, PhD
Department of Gastroenterology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu, China
Tel: +86-25-83718836, Fax: +86-25-83674636, E-mail: hjzhang06@163.com
Yi Li, MD
Department of Gastroenterology, Sir Run Run Hospital, Nanjing Medical University, Nanjing 211100, Jiangsu, China
Tel: +86-25-87115854, Fax: +86-25-87115539, E-mail: 362008621@qq.com
Haiyang Wang, Xiaojing Zhao, and Xiufang Cui contributed equally to this work.

Introduction

Inflammatory bowel disease (IBD) mainly includes Crohn's disease (CD) and ulcerative colitis (UC), which belongs to a class of chronic recurrent gastrointestinal inflammatory diseases and immune dysfunction. Patients usually experience abdominal pain, diarrhea, bloody stool, and severe weight loss. According to the disease activity, it can be divided into the period of activity and remission.[1] Irritable bowel syndrome (IBS) is a kind of functional bowel disorder characterized by recurrent abdominal pain, disordered defecation. IBD and IBS have overlapping symptoms. Patients with IBD in clinical remission (IBDR) suffer from IBS-like symptoms such as abdominal discomfort, bellyache, diarrhea, and abnormal defecation, which are called "IBD-IBS."[2,3] According to the latest report, the prevalence of IBS-like symptoms among IBDR patients is generally between 25.0% and 60.0%[4] due to the different definition of IBD in remission, variation of inclusion criteria, and the small samples of some studies. A meta-analysis found that 35.0% of the inactive patients had IBS-like symptoms.[3] Approximately 45.0% of IBD patients with normal inflammatory markers developed IBS-like symptoms.[5]

In recent years, health-related quality of life (QOL) in chronic diseases has played a predominant role in patients' mental health, social adaptation and utilization of health resources. The QOL of IBD patients was seriously affected because of the prolonged and recurrent course.[6-9] The etiology of IBD-IBS remains unclear. It may have some similar pathogenesis as IBS, such as psychological factors, visceral hypersensitivity, impaired intestinal barrier, and so on. Hypersensitivity of viscera is a principal element underlying abdominal analgesia in IBS. Visceral hypersensitivity refers to increased visceral perception to chemical, temperature, mechanical, PH, and other stimuli, as well as decreased sensory threshold during rectal dilation stimulation. The mechanism of visceral hypersensitivity is not entirely understood. It may occur via a disturbance of the different levels of sensitization pathways: increased alertness of central system, hypersensitivity of sensory nerve endings, disturbance of autonomic nervous system, endocrine, and neurotransmitter abnormalities.[10] Mast cells (MCs) may have a critical effect on visceral hypersensitivity in IBS,[11,12] through releasing neuroendocrine substances such as 5-hydroxytryptamine (5-HT), nerve growth factor (NGF), protease, and triggering relevant receptors on nerve endings.

Currently, some studies are available on the prevalence of IBD-IBS mainly from Euro-American studies. In China, the prevalence of IBD-IBS has not yet been investigated. In addition, studies on the relationship between IBD-IBS and psychological factors, visceral hypersensitivity are not fully verified, and the formation mechanism of IBD-IBS remains incompletely clear. The purposes of this study are: (1) to evaluate the prevalence, clinical symptoms, QOL, and psychological status of Chinese IBD-IBS patients, (2) to assess the changes of visceral sensitivity in patients with IBD-IBS, and (3) to preliminarily investigate the roles of MC, 5-HT and NGF in formation of visceral hypersensitivity in IBD-IBS patients.

Materials and Methods

Patients

The patients who visited the First Affiliated Hospital of Nanjing Medical University were recruited from August 2017 to September 2018. We enrolled 71 IBDR patients (including 24 ulcerative colitis in remission [UCR] patients and 47 Crohn's disease in remission [CDR] patients), 26 IBS patients, and 20 healthy controls (HCs) for a cross-sectional study. Each IBD patient met the diagnostic criteria drafted by the European Crohn's and Colitis Organization.[13,14] The definition of IBD in remission was (1) Mayo index (UC) $\leq$ 1 with no single subitem score $>$ 1, (2) Crohn's disease activity index (CDAI) was $<$ 150, (3) C-reflection protein (CRP) was $<$ 10 mg/L, and (4) erythrocyte sedimentation rate

(ESR) was < 20 mm/hr.[8,15,16] HCs were selected from Physical Examination Center. Patients with cardio-cerebrovascular diseases, tumors, infectious diseases, use of steroids, and NSAIDs within 6 months, motility drugs with 1 month or smoking, and alcohol intake with 1 year were excluded. Peripheral blood of all patients and HCs were collected. The rectosigmoid biopsies were performed for later analysis. The Ethics Committee of the First Affiliated Hospital of Nanjing Medical University had approved this medical research and related procedures (Ethics No. 2018-SR-061).

Questionnaires

IBS-like symptom and IBS was evaluated by utilizing the Rome III diagnostic questionnaire for IBS[17]: (1) symptoms occur at least 6 months before diagnosis; (2) onset of abdominal analgesia or discomfort more than 3 days of each month within the previous 3 months; and (3) at least 2 of the following characteristics: improvement after defecation, relationship with changes in stool frequency or stool form. Abdominal pain was detected utilizing a visual analogue scale (VAS; 0 = no pain and 10 = extreme pain).[18] The general QOL was evaluated with 36-items short-form health survey (SF-36), and the disease-specific QOL (inflammatory bowel disease questionnaire [IBDQ]) were adopted as well.[19] Assessment of anxiety and depression was respectively conducted with the health 7 item generalized anxiety disorder scale (GAD-7) and the 9 item patient health questionnaire (PHQ-9).[20,21]

Endoscopic Inflammation Score of Inflammatory Bowel Disease

Two experienced gastroenterologists assessed the endoscopic scores for each IBD patient utilizing the Crohn's disease endoscopic index of severity (CDEIS)[22] and ulcerative colitis endoscopic index of severity (UCEIS).[23] The CDEIS evaluates 5 colonic segments: rectum, sigmoid and left colon, traverse colon, right colon, and ileum. The presence of ulceration, the percentage of ulcerated surface and the percentage of surface involved by CD are measured for each colonic segment. In addition, the presence of stenoses is assessed. These items are weighted and summed to a total score. UCEIS uses 3 variables: vascular pattern (normal, patchy obliteration, or obliterated); bleeding (none, mucosal, luminal mild, luminal moderate, or severe); and erosions and ulcers (none, erosions, superficial ulcer, or deep ulcer). These variables are summed to a total score.

Rectal Balloon Distension

A high-resolution anorectal manometric electrode catheter with a balloon (MSC-2195; Medtronic, Inc, USA) was used to detect rectal perception. Each person underwent a gradual distension procedure through injecting gas into the balloon (rate: 2 mL/sec) in the lateral position. Volumes (mL) were continuously monitored. Patients were asked to rate sensations of initial perception, defecation distress, and maximum tolerance.

Immunohistochemistry

All colon tissues were stored overnight in 4% paraformaldehyde and cut into sections, then dewaxed and rehydrated. Endogenous peroxidase was blocked by 3% H_2O_2 for 15 minutes. The antigen of tryptase and NGF were retrieved by boiling in the buffer (sodium citrate, 10 mmol/L, pH = 6.0) in a microwave for 15 minutes. Antigen retrieval of 5-HT was performed by incubating in protease K liquid (20 mg/mL) for 30 minutes. Then the slides were incubated with diluted antibody at 4°C overnight (trypsin-like ab2378, Abcam, Cambridge, MA, USA,1:100; 5-HT NB120-16007, Novus, San Diego, CA, USA,1:50; NGF ab6199, Abcam, Cambridge, MA, USA, 1:200) and followed by incubating in the secondary antibody (TL-125-QPH; Thermo, Waltham, MA, USA) for 50 minutes and the DAB solution (G1211; Service bio, Wuhan, Hubei, China), then counterstained with hematoxylin. The Image Pro-Plus software (Media Cybernetics, Inc, Sarasota, FL, USA) was used to obtain a mean density of 5 random fields per slide. Tryptase-positive MCs were counted as the average of 5 random non-overlapped high-powered fields (final magnification, ×400) for each slide.

Western Blot Analysis

Protein samples obtained from human colon tissue were dissected, then transferred to the nitrocellulose membrane and sealed with 5% skim milk for 1 hour. Incubate membranes with primary antibodies (5-hydroxytryptamine 3 [5-HT_3] receptor: bs-4289R, Bioss, Beijing, China, 1:500; transient receptor potential vanilloid 1 [TRPV1]: bs-23926R, Bioss, Beijing, China, 1:500; tropomyosin receptor kinase A [TrkA]: bs-10210R, Bioss, Beijing, China, 1:500) overnight at 4℃, then incubated for 1 hour with the secondary antibody (ab6721, Abcam, Cambridge, Mass, USA, 1:10 000) at room temperature. The membrane was dropped in ECL Solution and exposure to autoradiography film (Kodak XAR film, Rochester, NY, USA). The obtained image was analyzed by Image-Pro-Plus (version 6.0).

Enzyme-linked Immunosorbent Assay

Enzyme-linked immunosorbent assay was applied to detect the

Table 1. Epidemiological and Clinical Characteristics of Patients With Inflammatory Bowel Disease in Remission

Clinical Characteristics	UCR (n = 24)		CDR (n = 47)		HCs (n = 20)	IBS (n = 26)
	UC-IBS$^+$ (n = 12)	UC-IBS$^-$ (n = 12)	CD-IBS$^+$ (n = 14)	CD-IBS$^-$ (n = 33)		
Sex (male/female)	4/8	8/4	8/6	26/7	6/14	9/17
Age (yr)	38.58 ± 8.98	39.83 ± 10.34	35.71 ± 11.48	30.61 ± 11.77	36.60 ± 11.30	37.85 ± 12.80
Duration of disease (yr)	3.88 ± 3.21	3.79 ± 2.88	3.69 ± 3.10	2.90 ± 2.64	-	3.30 ± 2.80
Age at diagnosis						
A1 (≤ 16 yr)	-	-	0	1	-	-
A2 (17-40 yr)	-	-	10	28	-	-
A3 (> 40 yr)	-	-	4	4	-	-
Disease location						
L1 (terminal ileum)	-	-	4	16	-	-
L2 (colon)	-	-	2	2	-	-
L3 (ileocolon)	-	-	6	15	-	-
L4 (upper GI)	-	-	2	0	-	-
E1 (rectum)	2	2			-	-
E2 (left-sided colon)	5	5			-	-
E3 (entire colon)	5	6			-	-
Disease behavior						
B1 (no stricture no penetration)	-	-	9	27	-	-
B2 (stricture)	-	-	4	5	-	-
B3 (penetration)	-	-	1	2	-	-
CDAI	-	-	81.42 ± 30.64[a]	46.91 ± 32.32	-	-
CDEIS	-	-	7.29 ± 3.17[b]	4.24 ± 2.33		
Mayo	0.67 ± 0.65	0.85 ± 1.21	-	-	-	-
UCEIS	1.50 ± 1.17	0.92 ± 0.80				
CRP (mg/L)	3.44 ± 1.69[d]	3.41 ± 1.73[f]	7.87 ± 5.05[c]	3.67 ± 2.94[e]	1.40 ± 0.68	2.13 ± 1.08
ESR (mm/hr)	10.67 ± 5.43	6.92 ± 3.83	12.50 ± 7.33	7.37 ± 4.65	3.32 ± 1.70	3.48 ± 1.01
Smokers	2 (16.67)	0 (0.00)	0 (0.00)	3 (10.70)	49 (25.00)	5 (19.23)
Perianal diseases	-	-	4 (28.57)	13 (39.39)	-	-
Medications						
5-ASA	12 (100.0)	9 (75.00)	5 (35.71)	6 (18.18)	-	-
Immunosuppressive agents	-	-	4 (30.77)	2 (6.06)	-	-
Anti-TNF	-	-	7 (50.00)	33 (100)	-	-
Others			1 (7.14)	2 (6.06)		
Surgery (IBD-related)	0 (0.00)	0 (0.00)	4 (28.57)	15 (45.45)	-	

[a]CDAI: CD-IBS$^+$ vs CD-IBS$^-$, $P = 0.002$.
[b]CDEIS: CD-IBS$^+$ vs CD-IBS$^-$, $P = 0.002$.
[c]CRP: CD-IBS$^+$ vs CD-IBS$^-$, $P < 0.001$.
[d]CRP: CD-IBS$^+$ vs UC-IBS$^+$, $P < 0.001$.
[e]ESR: CD-IBS$^+$ vs CD-IBS$^-$, $P = 0.001$.
[f]ESR: UC-IBS$^+$ vs UC-IBS$^-$, $P = 0.028$.

UCR, ulcerative colitis in remission; CDR, Crohn's disease in remission; HCs, healthy controls; IBS, irritable bowel syndrome; UC-IBS$^+$, ulcerative colitis in remission with IBS-like symptoms; UC-IBS$^-$, ulcerative colitis in remission without IBS-like symptoms; CD-IBS$^+$, Crohn's disease in remission with IBS-like symptoms; CD-IBS$^-$, Crohn's disease in remission without IBS-like symptoms; CDAI, CD activity index; CDEIS, Crohn's disease endoscopic index of severity; UCEIS, ulcerative colitis endoscopic index of severity; 5-ASA, 5-aminosalicylate; A1, A2, A3, age of diagnosis; L1, L2, L3, disease location; B1, B2, B3, CD disease behavior according to Montreal classification; E1, E2, E3, UC disease location with Montreal classification.
Data are expressed as n, mean ± SD, or n (%).

serum levels of 5-HT (E-EL-0033c; Elabscience, Wuhan, Hubei, China) and NGF (EK1141-24; Mutiscience, Hangzhou, Zhejiang, China) according to the manufacturer's protocol.

Statistical Methods

The data was analyzed using the IBM SPSS 21.0 software (IBM Corp, Armonk, NY, USA). The continuous data were presented as mean ± standard deviation, and the categorical data was expressed as number (percentage). Independent *t* test or ANOVA was used to compare normally distributed continuous variables, Kruskal-Wallis test for abnormal distribution parameters and χ^2 test for categorical variables. Correlations were analyzed using the Pearson's correlation coefficient. $P < 0.05$ was considered as statistical significance.

Results

Epidemiological and Clinical Characteristics

Seventy-one IBDR patients (CDR 47 and UCR 24) were included in this study (Table 1). IBS-like symptoms were found in 14 out of 47 patients with CDR (29.8%) and in 12 out of 24 patients with UCR (50.0%). The CDAI and CDEIS in CD-IBS$^+$ group were significantly higher than CD-IBS$^-$ group (CDAI $P = 0.002$; CDEIS $P = 0.002$). Compared to CD-IBS$^-$ and UC-IBS$^+$ group, the CRP level was higher in CD-IBS$^+$ group (CD-IBS$^+$ vs CD-IBS$^-$, $P < 0.001$; CD-IBS$^+$ vs UC-IBS$^+$, $P < 0.001$). Meanwhile, high ESR level was observed in IBD-IBS patients (CD-IBS$^+$ vs CD-IBS$^-$, $P = 0.001$; UC-IBS$^+$ vs UC-IBS$^-$, $P = 0.028$) (Table 1).

Abdominal Pain in Patients With Inflammatory Bowel Disease in Clinical Remission

VAS scores of CD-IBS$^+$, UC-IBS$^+$, and IBS groups were 5.2 ± 2.1, 3.8 ± 1.2, and 3.8 ± 2.2, respectively. The VAS score of CD-IBS$^+$ group was increased compared to IBS ($P = 0.005$) and UC-IBS$^+$ group ($P = 0.010$).

Health-related Quality of Life in Patients With Inflammatory Bowel Disease in Clinical Remission

General QOL (total SF-36 score) of IBD-IBS patients was significantly lower than those without IBS-like symptoms (CD-IBS$^+$ vs CD-IBS$^-$, $P = 0.027$; UC-IBS$^+$ vs UC-IBS$^-$, $P < 0.001$) (Supplementary Figure A). In addition, general QOL was also decreased in IBD-IBS patients compared with IBS patients (CD-IBS$^+$ vs IBS, $P = 0.024$; UC-IBS$^+$ vs IBS, $P < 0.001$) (Supplementary Figure A). Of SF-36 dimensions, the dimension scores of physical role, general health and vitality of the UC-IBS$^+$ patients were less than those of the IBS patients (physical role, $P = 0.035$; general health, $P = 0.047$; vitality, $P = 0.018$). The dimension scores of general health were decreased in CD-IBS$^+$ patients compared with IBS patients ($P = 0.005$) (Table 2).

Disease-specific QOL (IBDQ) scores of IBD-IBS patients were obviously decreased (CD-IBS$^+$ vs CD-IBS$^-$, $P = 0.001$; UC-IBS$^+$ vs UC-IBS$^-$, $P < 0.001$) (Supplementary Figure B). CD patients with IBS-like symptoms had lower dimension scores

Table 2. Evaluation of General Quality of Life for Patients With Inflammatory Bowel Disease in Remission

SF-36 dimensions	HCs (n = 20)	IBS (n = 26)	CDR		UCR	
			CD-IBS$^+$ (n = 14)	CD-IBS$^-$ (n = 33)	UC-IBS$^+$ (n = 12)	UC-IBS$^-$ (n = 12)
Physical function	95.1 ± 5.2	83.2 ± 16.0	78.9 ± 17.9	86.2 ± 10.8	76.6 ± 15.8	90.8 ± 7.8
Physical role	93.5 ± 10.1	71.0 ± 39.0	64.3 ± 41.3	69.5 ± 35.1	47.6 ± 34.0	84.0 ± 33.9
Bodily pain	97.9 ± 5.3	77.6 ± 20.3	70.6 ± 20.6	81.4 ± 18.4	65.3 ± 19.4	91.4 ± 9.7
General health	97.7 ± 2.8	73.2 ± 22.6	51.4 ± 18.1	68.5 ± 19.5	57 ± 25.6	83.8 ± 14.5
Vitality	97.9 ± 3.5	82.7 ± 16.4	70.7 ± 26.4	78.2 ± 19.1	61.3 ± 30.6	89.9 ± 11.1
Social function	97.6 ± 4.4	74.8 ± 19.8	80.2 ± 14.6	81.4 ± 19.5	71.1 ± 17.9	85.3 ± 12.7
Emotional role	99.7 ± 0.8	73.1 ± 32.7	62.3 ± 42.4	69.2 ± 34.9	66.7 ± 34.8	91.8 ± 18.7
Mental health	98.5 ± 2.4	76.5 ± 17.4	71.7 ± 15.8	71.6 ± 21.9	65.8 ± 23.9	87.9 ± 8.3
Sum score	97.2 ± 1.2	78.9 ± 12	70.7 ± 13.8	78.4 ± 11.6	65.0 ± 13.2	88.0 ± 7.6

SF-36, 36-items short-form health survey; CDR, Crohn's disease in remission; UCR, ulcerative colitis in remission; HCs, healthy controls; IBS, irritable bowel syndrome; UC-IBS$^+$, ulcerative colitis in remission with IBS-like symptoms; UC-IBS$^-$, ulcerative colitis in remission without IBS-like symptoms; CD-IBS$^+$, Crohn's disease in remission with IBS-like symptoms; CD-IBS$^-$, Crohn's disease in remission without IBS-like symptoms.
Data represent mean ± SD.

Table 3. Disease-specific Quality of Life (Inflammatory Bowel Disease Questionnaire) Scores in Patients With Inflammatory Bowel Disease in Remission

IBDQ dimensions	CDR			UCR		
	CD-IBS$^+$ (n = 14)	CD-IBS$^-$ (n = 33)	*P*-value	UC-IBS$^+$ (n = 12)	UC-IBS$^-$ (n = 12)	*P*-value
Intestinal symptom	58.1 ± 6.5	61.9 ± 6.8	0.134	56.3 ± 13.2	64.9 ± 2.5	0.030
General symptom	24.7 ± 4.7	29.1 ± 4.1	0.004	24.6 ± 5.9	31.2 ± 1.8	0.001
Emotional function	62.1 ± 10.4	72.2 ± 8.6	< 0.001	58.0 ± 6.4	79.0 ± 3.2	< 0.001
Social function	26.0 ± 4.9	30.6 ± 4.6	0.001	27.9 ± 3.9	31.2 ± 2.3	0.065
Sum score	170.9 ± 23.2	193.8 ± 22.0	0.001	166.8 ± 25.3	206.3 ± 8.1	< 0.001

IBDQ, inflammatory bowel disease questionnaire; CDR, Crohn's disease in remission; UCR, ulcerative colitis in remission; UC-IBS$^+$, ulcerative colitis in remission with irritable bowel syndrome-like symptoms; UC-IBS$^-$, ulcerative colitis in remission without irritable bowel syndrome-like symptoms; CD-IBS$^+$, Crohn's disease in remission with -like symptoms; CD-IBS$^-$, Crohn's disease in remission without irritable bowel syndrome-like symptoms.
Data are expressed as mean ± SD.

for general symptom, social function, and emotional function than those without IBS-like symptoms (general symptom, $P = 0.004$; social function, $P = 0.001$; emotional function, $P < 0.001$). The dimension scores of general symptom, emotional function and intestinal symptom were also lower in UCR patients with IBS-like symptoms (general symptom, $P = 0.001$; emotional function, $P < 0.001$; intestinal symptom, $P = 0.030$) (Table 3).

Psychological Assessment in Patients With Inflammatory Bowel Disease in Clinical Remission

The prevalence of anxiety in CD-IBS$^+$, CD-IBS$^-$, UC-IBS$^+$, UC-IBS$^-$, and IBS groups was 64.3%, 27.3%, 75.0%, 25.0%, and 30.8%, respectively. Meanwhile, the prevalence of depression in the above 5 groups respectively was 57.1%, 42.4%, 58.7%, 16.3%, and 42.3%. IBD-IBS patients had a higher prevalence of anxiety than IBS patients and those without IBS-like symptoms (GAD-7: CD-IBS$^+$ vs CD-IBS$^-$, $P = 0.017$; CD-IBS$^+$ vs IBS, $P = 0.041$; UC-IBS$^+$ vs UC-IBS$^-$, $P = 0.014$; UC-IBS$^+$ vs IBS, $P = 0.011$). UCR patients with IBS-like symptoms presented higher depression prevalence rate than those without such symptoms ($P = 0.035$). The average scores of anxiety and depression in IBDR patients with IBS-like symptoms were much higher than the patients without such symptoms (GAD-7: CD-IBS$^+$ vs CD-IBS$^-$, $P = 0.012$; UC-IBS$^+$ vs UC-IBS$^-$, $P = 0.002$; PHQ-9: CD-IBS$^+$ vs CD-IBS$^-$, $P = 0.043$; UC-IBS$^+$ vs UC-IBS$^-$, $P = 0.044$) (Supplementary Figures C and D).

Visceral Sensitivity in Patients With Inflammatory Bowel Disease in Clinical Remission

The threshold of initial perception, defecation distress and maximum tolerance in UCR patients with IBS-like symptoms showed fewer values versus those with no such symptoms (initial perception: 35.75 ± 13.36 vs 50.00 ± 5.29, $P = 0.005$; defecation distress: 61.67 ± 17.03 vs 103.60 ± 15.16, $P < 0.001$; maximum tolerance: 89.33 ± 19.77 vs 148.20 ± 9.19, $P < 0.001$) (Fig. 1A-C). Additionally, defecation distress thresholds in CDR patients with IBS-like symptoms was also lower (57.07 ± 19.08 vs 75.21 ± 24.80, $P = 0.011$) (Fig. 1B). Compared to IBS patients, maximum tolerance threshold was reduced in UC-IBS$^+$ group (89.33 ± 19.77 vs 117.00 ± 31.59, $P < 0.001$) (Fig. 1C), the 3 sensory thresholds in CD-IBS$^+$ group were also decreased, but differences were not observed (Fig. 1A-C).

The Association Between Abdominal Pain, Psychophysiology, and Visceral Perception

There was a positive association between abdominal pain and anxiety/depression in IBD-IBS patients (GAD-7: CD-IBS$^+$, $r = 0.804$, $P < 0.001$; UC-IBS$^+$, $r = 0.713$, $P = 0.009$; PHQ-9: CD-IBS$^+$, $r = 0.833$, $P < 0.001$; UC-IBS$^+$, $r = 0.704$, $P = 0.010$). In addition, abdominal pain negatively related with rectal perception (Fig. 1D and 1E).

The threshold values of initial perception, defecation distress, and maximum tolerance in CD-IBS$^+$ group negatively correlated with anxiety and depression scores (Fig. 1F and 1G). Moreover, there was an inverse relationship between defecation distress/maximum volume threshold values and anxiety/depression scores in UC-IBS$^+$ group (Fig. 1H and 1I).

Activation of Mast Cells in Colonic Mucosal Tissues of Patients With Inflammatory Bowel Disease in Clinical Remission

MC tryptase is expressed in the MC cytoplasm, representing

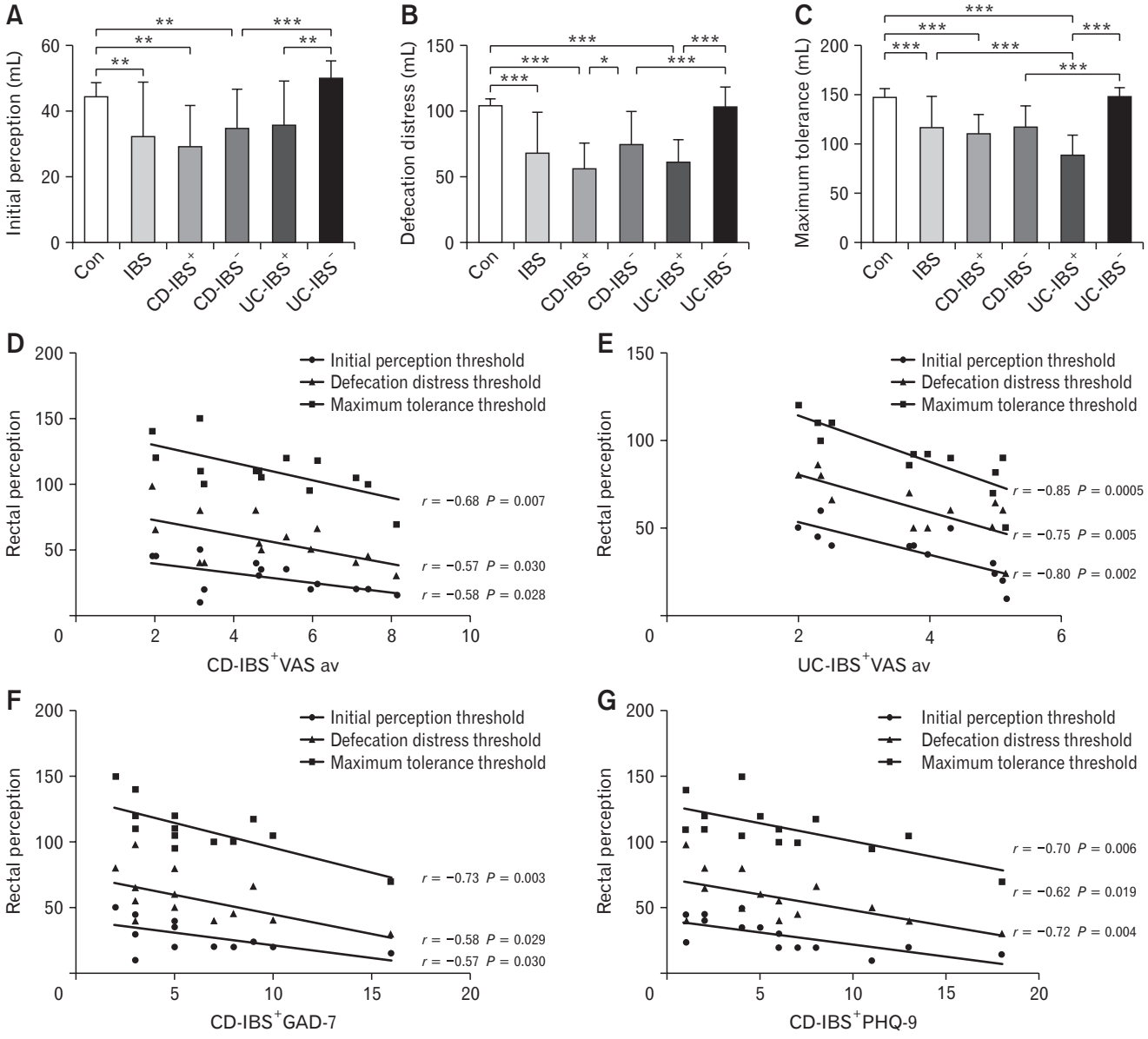

Figure 1. Rectal perception and the relationship of rectal perception with abdominal pain, anxiety and depression in patients with inflammatory bowel disease (IBD) in remission. (A) Initial perception threshold; (B) defecation distress threshold; (C) maximum tolerance threshold; (D-I) the relationship of rectal perception with abdominal pain, anxiety, and depression in patients with IBD in clinical remission suffer from irritable bowel syndrome (IBS)-like symptoms (IBD-IBS). Data are analyzed with Pearson's correlation. CD-IBS$^+$, Crohn's disease in remission with IBS-like symptoms; CD-IBS$^-$, Crohn's disease in remission without IBS-like symptoms; UC-IBS$^+$, ulcerative colitis in remission with IBS-like symptoms; UC-IBS$^-$, ulcerative colitis in remission without IBS-like symptoms; VAS, visual analogue scale; GAD-7, health 7 items generalized anxiety disorder scale; PHQ-9, nine items patients health questionnaire. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

the degranulation and activation of MCs. The results showed MCs was mainly distributed in colonic lamina propria of IBD-IBS and IBS, while MCs was not detected in HCs (Fig. 2A). The mean number for tryptase-positive MCs was higher in IBD-IBS patients than those in IBS patients (CD-IBS$^+$ vs IBS, $P < 0.001$; UC-IBS$^+$ vs IBS, $P < 0.001$) (Fig. 2B). In addition, compared with IBDR patients without IBS-like symptoms, higher mean number for tryptase-positive MCs was found in patients with such symptoms (CD-IBS$^+$ vs CD-IBS$^-$, $P = 0.001$; UC-IBS$^+$ vs UC-IBS$^-$, $P < 0.001$). The mean number of MCs was significantly higher in

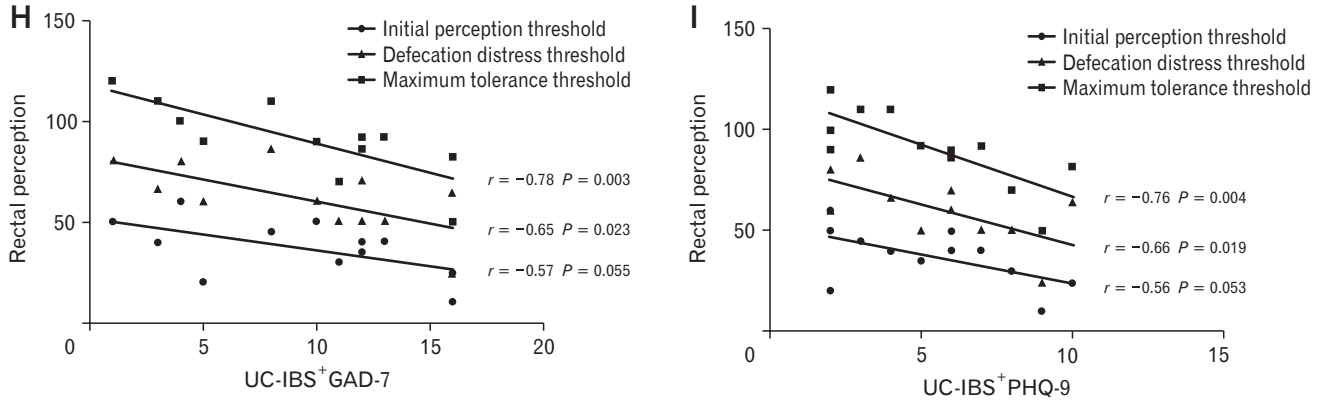

Figure 1. Continued.

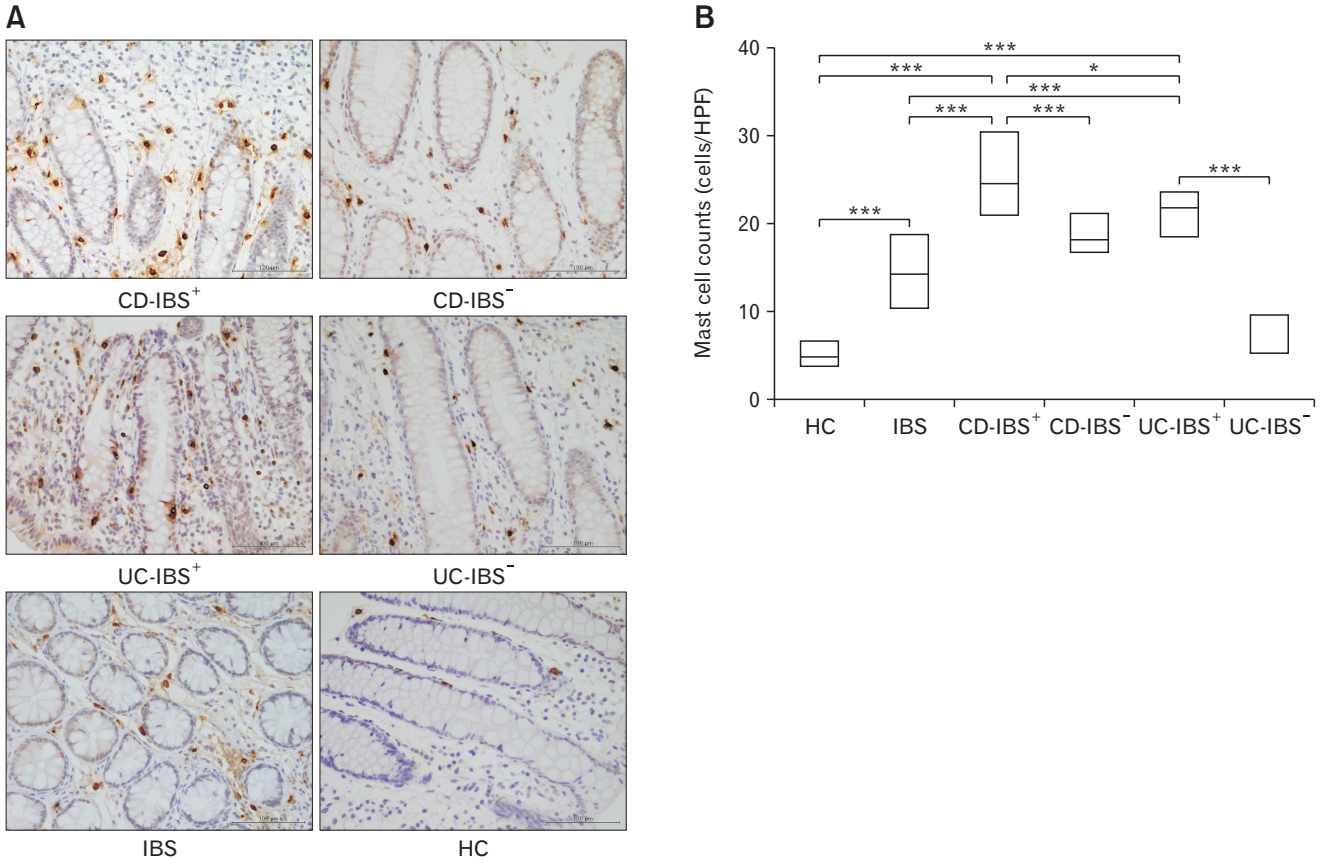

Figure 2. Mast cells in human colon tissues of patients with inflammatory bowel disease in remission. (A) Expression and distribution of mast cell (MC) tryptase among patients with Crohn's disease in remission, patients with ulcerative colitis in remission, patients with irritable bowel syndrome (IBS), and healthy controls (HCs), respectively (bar = 100 μm). (B) The mean number for tryptase-positive MCs was obtained by analyzing 5 random high-power fields (HPF) per slide. CD-IBS$^+$, Crohn's disease in remission with IBS-like symptoms; CD-IBS$^-$, Crohn's disease in remission without IBS-like symptoms; UC-IBS$^+$, ulcerative colitis in remission with IBS-like symptoms; UC-IBS$^-$, ulcerative colitis in remission without IBS-like symptoms. *$P < 0.05$, ***$P < 0.001$.

the CD-IBS$^+$ group versus UC-IBS$^+$ group (Fig. 2B).

Alteration of 5-Hydroxytryptamine and Nerve Growth Factor in Colonic Tissue and Serum of Patients With Inflammatory Bowel Disease in Clinical Remission

MCs can release 5-HT and NGF after activation. In IBD-IBS and IBS patients, 5-HT is mainly distributed in colonic glandular cells and lamina propria, and NGF is expressed in colonic submucosa. The expressions of 5-HT, as well as NGF, were not detected in normal colonic tissues (Fig. 3A and 3B). The expressions of 5-HT and NGF in colonic tissues of IBDR patients with IBS-like symptoms were upregulated compared to the patients without such symptoms (5-HT: CD-IBS$^+$ vs CD-IBS$^-$, $P = 0.007$; UC-IBS$^+$ vs UC-IBS$^-$, $P = 0.001$; NGF: CD-IBS$^+$ vs CD-IBS$^-$, $P < 0.001$; UC-IBS$^+$ vs UC-IBS$^-$, $P < 0.001$). In addition, the expression of NGF in CD-IBS$^+$ groups were higher than that in UC-IBS$^+$ groups ($P < 0.001$) (Fig. 3C and 3D).

The levels of 5-HT in serum CD-IBS$^+$, CD-IBS$^-$, UC-IBS$^+$, UC-IBS$^-$, IBS, and HCs groups were 200.2 ± 49.8, 141.5 ± 15.5, 189.7 ± 37.7, 117.8 ± 15.2, 155.7 ± 36.6, and 117.7 ± 23.4 (ng/mL), respectively. Serum levels of NGF in these groups were 44.9 ± 25.3, 14.4 ± 10.9, 33.2 ± 14.3, 6.1 ± 3.8, 22.4 ± 11.2, and 9.04 ± 6.5 (pg/mL), respectively. A significantly higher serum 5-HT and NGF levels was found in IBD-IBS patients (5-HT: CD-IBS$^+$ vs CD-IBS$^-$, $P = 0.010$; UC-IBS$^+$ vs UC-IBS$^-$, $P =$

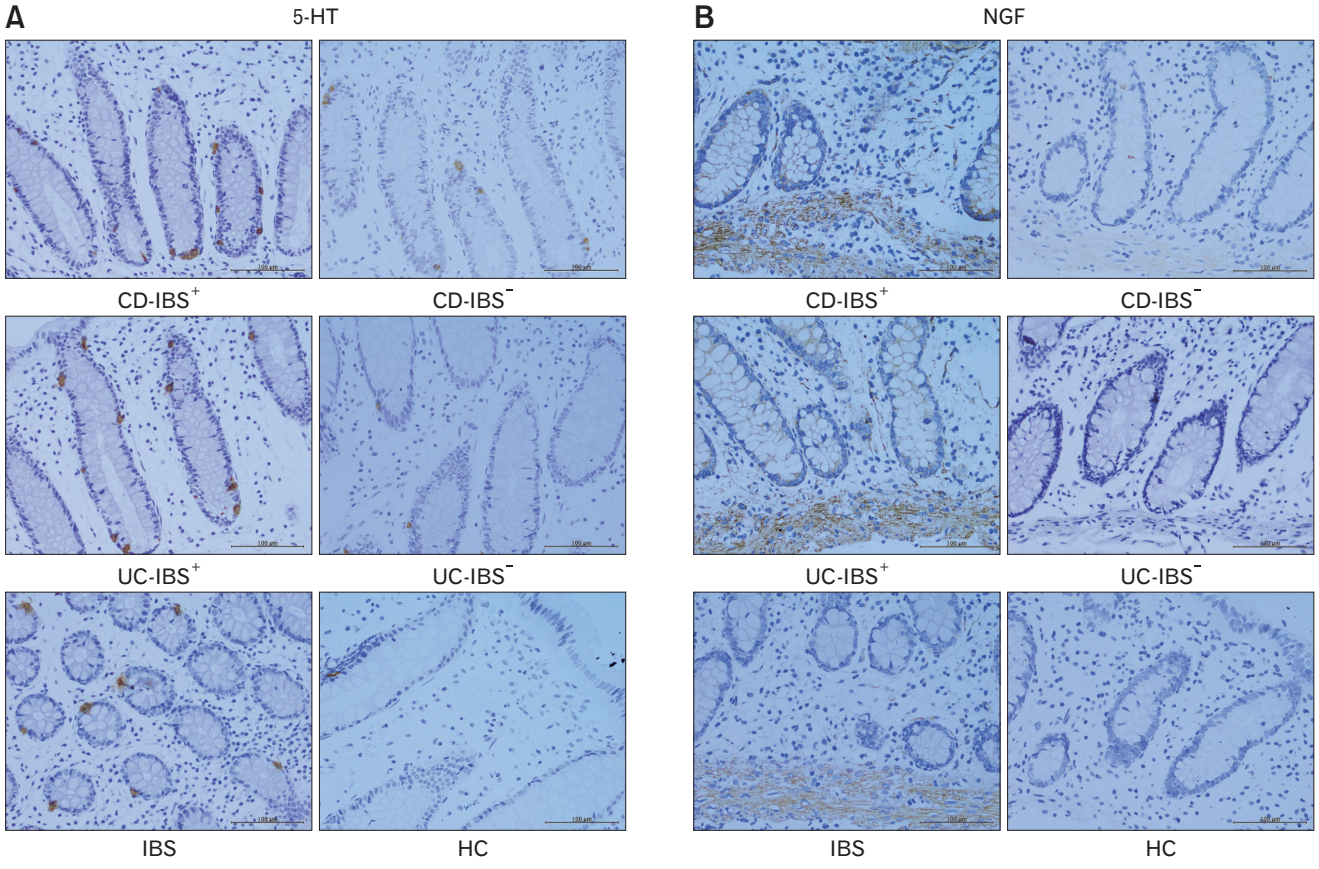

Figure 3. Alteration of 5-hydroxytrpytamine (5-HT) and nerve growth factor (NGF) in patients with inflammatory bowel disease in remission. (A, B) Expression and distribution of 5-HT/NGF in patients with Crohn's disease in remission (CDR), and patients with ulcerative colitis in remission (UCR), patients with irritable bowel syndrome (IBS), and healthy controls (HCs), respectively (bar = 100 μm). (C, D) The mean density of immunohistochemistry staining of 5-HT/NGF in human colonic biopsy specimens was obtained through analyzing 5 random fields per slide using the Image Pro-Plus software. (E, F) Serum levels of 5-HT and NGF in patients with CDR, in patients with UCR, patients with IBS, and HCs analyzed with enzyme-linked immunosorbent assay. CD-IBS$^+$, Crohn's disease in remission with IBS-like symptoms; CD-IBS$^-$, Crohn's disease in remission without IBS-like symptoms; UC-IBS$^+$, ulcerative colitis in remission with IBS-like symptoms; UC-IBS$^-$, ulcerative colitis in remission without IBS-like symptoms.*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

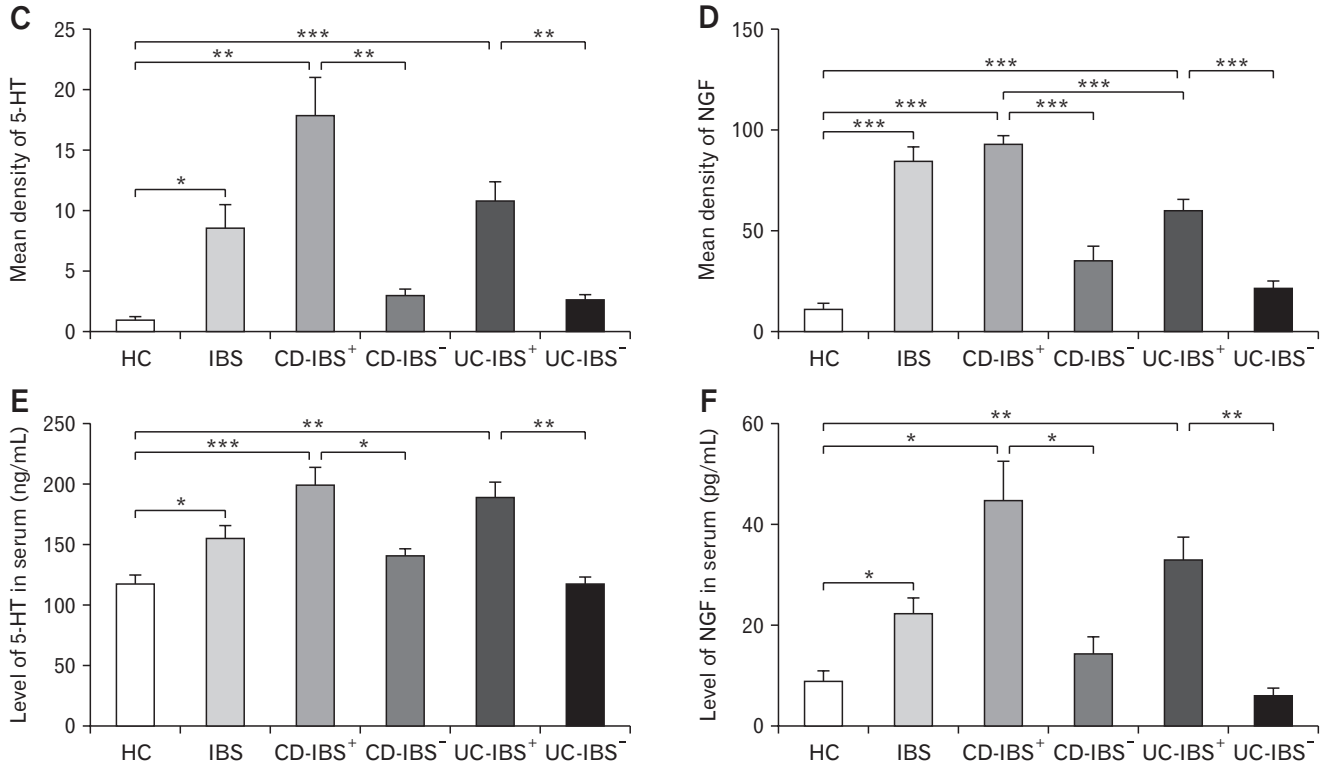

Figure 3. Continued.

Table 4. Relation Between 5-Hydroxytyptamine/Nerve Growth Factor, Abdominal Pain, Psychophysiology, and Rectal Perception

Clinical assessment	CD-IBS$^+$				UC-IBS$^+$			
	Serum 5-HT	Colonic 5-HT (MD)	Serum NGF	Colonic NGF (MD)	Serum 5-HT	Colonic 5-HT (MD)	Serum NGF	Colonic NGF (MD)
VAS average	0.9677 (< 0.001)	0.9144 (< 0.01)	0.9220 (< 0.01)	0.9278 (< 0.01)	0.9766 (< 0.001)	0.9495 (< 0.001)	0.8985 (< 0.001)	0.8859 (< 0.01)
GAD-7	0.8565 (< 0.05)	0.8962 (< 0.01)	0.9394 (< 0.01)	0.8807 (< 0.01)	0.8599 (< 0.01)	0.8325 (< 0.01)	0.9469 (< 0.001)	0.9414 (< 0.001)
PHQ-9	0.9096 (< 0.01)	0.9608 (< 0.001)	0.9823 (< 0.001)	0.9461 (< 0.01)	0.8528 (< 0.01)	0.8924 (< 0.01)	0.7592 (< 0.05)	0.9402 (< 0.001)
Initial perception threshold	−0.7859 (< 0.05)	−0.8205 (< 0.05)	−0.8135 (< 0.05)	−0.8481 (< 0.05)	−0.7645 (< 0.05)	−0.8191 (< 0.01)	−0.9048 (< 0.001)	−0.9002 (< 0.001)
Defecation distress threshold	−0.9055 (< 0.01)	−0.8748 (< 0.01)	−0.9141 (< 0.01)	−0.7884 (< 0.05)	−0.6973 (< 0.05)	−0.7255 (< 0.05)	−0.8419 (< 0.01)	−0.7958 (< 0.05)
Maximum tolerance threshold	−0.7341 (NS)	−0.8556 (< 0.05)	−0.7633 (< 0.05)	−0.8726 (< 0.05)	−0.6874 (< 0.05)	−0.6017 (NS)	−0.8035 (< 0.01)	−0.8206 (< 0.01)

UC-IBS$^+$, ulcerative colitis in remission with irritable bowel syndrome (IBS)-like symptoms; CD-IBS$^+$, Crohn's disease in remission with IBS-like symptoms; 5-HT, 5-hydroxytryptamine; MD, mean density; NGF, nerve growth factor; VAS, visual analogue scale; GAD-7, health 7 items generalized anxiety disorder scale; PHQ-9, nine items patient health questionnaire; NS, no significant difference ($P > 0.05$).
The data in the table are Pearson's coefficient.

0.001; NGF: CD-IBS$^+$ vs CD-IBS$^-$, $P = 0.034$; UC-IBS$^+$ vs UC-IBS$^-$, $P = 0.001$) (Fig. 3E and 3F).

The Relationship of 5-Hydroxytryptamine, Nerve Growth Factor With Abdominal Pain, Anxiety, Depression, and Rectal Perception

The expressions of 5-HT and NGF in IBD-IBS patients positively related with VAS scores and anxiety/depression scores, while negatively associated with rectal perception (Table 4). The results suggested that 5-HT and NGF may be correlated with abdominal pain, anxiety, depression, and visceral hypersensitivity.

Expressions of 5-Hydroxytryptamine 3 Receptor, Tropomyosin Receptor Kinase A, and Transient Receptor Potential Vanilloid 1 in the Colonic Tissues

Western blot showed that the expression of 5-HT$_3$, tropomyosin receptor kinase A (TrkA) and TRPV1 in IBD-IBS and IBS patients were upregulated compared with the HCs. Furthermore, the expressions of 5-HT$_3$, TrkA and TRPV1 in the colonic tissue of IBDR patients with IBS-like symptoms were notable higher than those of patients without such symptoms (Fig. 4).

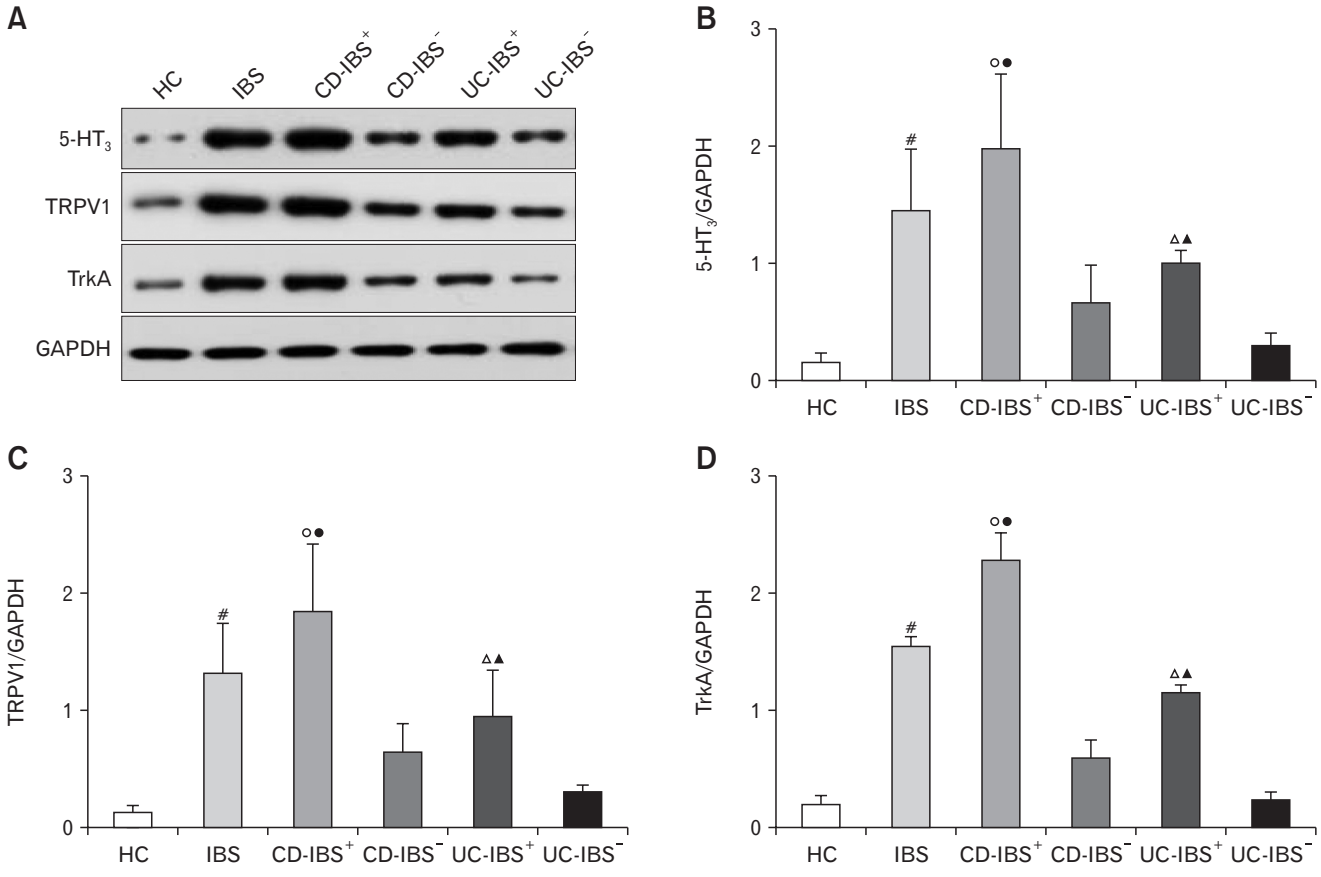

Figure 4. Expression of 5-hydroxytryptamine 3 [5-HT$_3$] receptor, transient receptor potential vanilloid 1 (TRPV1), and tropomyosin receptor kinase A (TrkA) in the colonic tissues of in inflammatory bowel disease (IBD) patients in remission. (A) Western-blot detection of expression of 5-HT$_3$, TRPV1, and TrkA in human colonic biopsy specimens, all normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for every 5 samples in each group. (B-D) The integrated optical density of gray scanning of 5-HT$_3$, TRPV1, and TrkA was analyzed using the Image Pro-Plus software. HC, healthy control; IBS, irritable bowel syndrome; CD-IBS$^+$, Crohn's disease in remission with IBS-like symptoms; CD-IBS$^-$, Crohn's disease in remission without IBS-like symptoms; UC-IBS$^+$, ulcerative colitis in remission with IBS-like symptoms; UC-IBS$^-$, ulcerative colitis in remission without IBS-like symptoms. $^{\#}P < 0.01$, IBS vs HC; $^{\circ}P < 0.05$, CD-IBS$^+$ vs HC; $^{\bullet}P < 0.05$, CD-IBS$^+$ vs CD-IBS$^-$; $^{\triangle}P < 0.05$, UC-IBS$^+$ vs HC; $^{\blacktriangle}P < 0.05$, UC-IBS$^+$ vs UC-IBS$^-$.

Discussion

In this investigation, the prevalence of IBS-like symptoms in CDR patients was 29.8% and 50.0% in UCR patients with remission, similar to the previous studies.[5,7,8,24] The present study confirmed that IBDR patients with IBS-like symptoms had higher VAS scores than those without such symptoms, especially for CD-IBS $^{+}$ patients, which may be due to the long course, a wide range of lesions, and transmural inflammation. Additionally, we found that IBDR patients with IBS-like symptoms had worse QOL than those without such symptoms, which were consistent with previous studies.[8,25-27] UCR patients with IBS-like symptoms were predisposed to suffer from worsened physical role, general health, and vitality. The general health of CDR patients with IBS-like symptoms was impacted as well. Through a questionnaire, we confirmed that patients with IBS-like symptoms had important differences in the dimensions of general symptom and emotional function compared to patients without such symptoms. These results indicated that IBD-IBS patients are not only affected by physical symptoms, but also by emotional, social function, and mental state. We showed that the prevalence of anxiety and depression in IBD-IBS patients was 57.1-75.0%, which was consistent with the results previously reported.[28,29] IBD-IBS patients were more likely to have anxiety and depression than those who had no IBS-like symptoms. Further analysis indicated that there was a tight relationship between anxiety, depression, and abdominal pain. Therefore, detection of anxiety and depression in IBDR patients and the early psychological intervention for patients with IBS-like symptoms may be conducive to improving the physical condition and quality of life.

Visceral hypersensitivity is one of the important pathophysiological changes in IBS, which is related to abdominal pain. The present study revealed that the values of initial sensory threshold, defecation distress threshold, and the maximum tolerance threshold were decreased, which showed the visceral hypersensitivity in IBD-IBS patients. In addition, visceral hypersensitivity was associated with abdominal pain and anxiety/depression in IBD-IBS patients. In murine colitis models, depression may lead to recurrence of colitis by damaging cholinergic nerve function and antidepressant treatment may improve colitis.[30] Therefore, we speculate that psychological abnormalities may increase visceral sensitivity and thus participate in the occurrence of IBS-like symptoms in IBDR patients.

This study found that CDAI, CDEIS, CRP, and ESR was higher in CD-IBS^{+} patients, and low-grade inflammation such as inflammatory cells infiltration was suspected to be related with the development of IBS-like symptoms. Previous studies confirmed that MCs were involved in IBS patients.[31-33] Vivinus et al[34] found that the number of intraepithelial lymphocytes and MCs were significantly increased in IBD patients with IBS-like symptoms, the number of eosinophils was significantly higher in UC patients compared with HCs, but was not significantly different between patients with or without IBS-like symptoms. Another study determined that the total number of MCs in colonic mucosa of UCR patients as well as the percentage of MCs close to nerve endings increased.[18] Visceral hypersensitivity could be reduced by blocking MC activation in IBS-like rat models.[35] In this study, we focused on the relationship between MCs and IBS-like symptoms in IBD patients. The present study found that colonic MCs in IBD-IBS patients were significantly more active than those patients without IBS-like symptoms, especially in CD-IBS^{+} patients. 5-HT and NGF, 2 bioactive substances released from MCs, were reported to be linked to visceral hypersensitivity and intestinal motility dysfunction in IBS patients. The expression of the 5-HT catalytic enzyme, and NGF significantly increased in CDR patients with IBS-like symptoms.[36,37] In this study, the expressions of 5-HT and NGF and receptor protein 5-HT_3 and TrkA were increased in IBD-IBS patients, expression of NGF in CD-IBS^{+} patients was obviously upregulated compared to UC-IBS^{+} patients. Furthermore, serum levels of 5-HT and NGF in IBD-IBS patients positively correlated with abdominal pain, anxiety, depression, and visceral hypersensitivity, which suggest that 5-HT and NGF may be involved in visceral hypersensitivity. On the other hand, anxiety and depression may further stimulate activated MCs to release 5-HT and NGF.

TRPV1 is expressed in primary sensory afferent nerve fibers and can produce pain by activation with capsaicin, NGF, and other substances. Akbar et al[38] reported that the number of TRPV1 positive nerve fibers in the colonic mucosal tissues in IBD-IBS patients had a strong correlation with abdominal pain. Lower visceral sensitivity was observed in TRPV1 knockout mice as opposed to wild-type mice during inflammatory recovery.[39] In this investigation, we revealed that the expressions of TRPV1 in IBD-IBS patients and IBS patients was obviously upregulated versus those in the healthy controls. Compared with IBDR patients without IBS-like symptoms, TRPV1 expressed highly in those patients with IBS-like symptoms. Generally, TRPV1 may play a vital role in the process of abdominal pain in IBD-IBS.

The present study has several limitations. First, we defined inactive IBD as clinical remission, and the prevalence of IBS-like symptoms in IBD patients with deep remission needs to be as-

sessed. Second, the limited sample size of CD, especially UC, may affect the results. Third, we did not subgroup the IBS-like symptoms. Future studies will have to focus on the correlation between certain IBS subtypes and IBD.

In conclusion, our findings indicated: IBD-IBS patients may have low QOL and psychological abnormalities, as well as visceral hypersensitivity which may be related to increased 5-HT and NGF levels released from activated MCs and upregulated 5-HT_3, TrkA, and TRPV1 expressions in the colonic tissue.

Supplementary Material

Note: To access the supplementary figure 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/jnm20151.

Financial support: This work was supported by the National Natural Science Foundation of China (No. 81770553) and Science and Technology Development Fund of Nanjing Medical University (No. NMUB2018291).

Conflicts of interest: None.

Author contributions: Haiyang Wang and Hongjie Zhang designed the study; Yi Li, Chunhua Jiao, Xiaojing Zhao, and Yan Yang collected specimens; Haiyang Wang, Xiaojing Zhao, and Meifeng Wang performed the research; Haiyang Wang, Jiajia Li, and Xiufang Cui analyzed the data; Haiyang Wang wrote this manuscript; and Hongjie Zhang and Yi Li supervised the report.

References

1. Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med 2009;361:2066-2078.
2. Burgell RE, Asthana AK, Gibson PR. Irritable bowel syndrome in quiescent inflammatory bowel disease: a review. Minerva Gastroenterol Dietol 2015;61:201-213.
3. Halpin SJ, Ford AC. Prevalence of symptoms meeting criteria for irritable bowel syndrome in inflammatory bowel disease: systematic review and meta-analysis. Am J Gastroenterol 2012;107:1474-1482.
4. Kruis W. Commentary: symptoms of irritable bowel syndrome in patients with inflammatory bowel disease--organic disease or diseased organ? Aliment Pharmacol Ther 2013;38:440.
5. Hoekman DR, Zeevenhooven J, D'Haens GR, Benninga MA. The prevalence of irritable bowel syndrome-type symptoms in inflammatory bowel disease patients in remission. Eur J Gastroenterol Hepatol 2017;29:1086-1090.
6. Piche T, Ducrotté P, Sabate JM, et al. Impact of functional bowel symptoms on quality of life and fatigue in quiescent Crohn's disease and irritable bowel syndrome. Neurogastroenterol Motil 2010;22:626-e174.
7. Keohane J, O'Mahony C, O'Mahony L, O'Mahony S, Quigley EM, Shanahan F. Irritable bowel syndrome-type symptoms in patients with inflammatory bowel disease: a real association or reflection of occult inflammation? Am J Gastroenterol 2010;105:1788-1794.
8. Tomita T, Kato Y, Takimoto M, et al. Prevalence of irritable bowel syndrome-like symptoms in Japanese patients with inactive inflammatory bowel disease. J Neurogastroenterol Motil 2016;22:661-669.
9. Gracie DJ, Williams CJ, Sood R, et al. Negative effects on psychological health and quality of life of genuine irritable bowel syndrome-type symptoms in patients with onflammatory bowel disease. Clin Gastroenterol Hepatol 2017;15:376-384, e5.
10. Stasi C, Rosselli M, Bellini M, Laffi G, Milani S. Altered neuro-endocrine-immune pathways in the irritable bowel syndrome: the top-down and the bottom-up model. J Gastroenterol 2012;47:1177-1185.
11. Schemann M, Camilleri M. Functions and imaging of mast cell and neural axis of the gut. Gastroenterol 2013;144:698-704, e4.
12. Feng B, La JH, Schwartz ES, Gebhart GF. Irritable bowel syndrome: methods, mechanisms, and pathophysiology. Neural and neuro-immune mechanisms of visceral hypersensitivity in irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 2012;302:G1085-G1098.
13. Magro F, Gionchetti P, Eliakim R, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. J Crohns Colitis 2017;11:649-670.
14. Gomollón F, Dignass A, Annese V, et al. 3rd European evidence-based consensus on the diagnosis and management of crohn's disease 2016: part 1: diagnosis and medical management. J Crohns Colitis 2017;11:3-25.
15. Weimers P, Burisch J. The importance of detecting irritable bowel-like symptoms in inflammatory bowel disease patients. J Crohns Colitis 2018;12:385-386.
16. Ishihara S, Kawashima K, Fukuba N, et al. Irritable bowel syndrome-like symptoms in ulcerative colitis patients in clinical remission: association with residual colonic inflammation. Digestion 2019;99:46-51.
17. Quigley EM, Fried M, Gwee KA, et al. World gastroenterology organisation global guidelines irritable bowel syndrome: a global perspective update september 2015. J Clin Gastroenterol 2016;50:704-713.
18. van Hoboken EA, Thijssen AY, Verhaaren R, et al. Symptoms in patients with ulcerative colitis in remission are associated with visceral hypersensitivity and mast cell activity. Scand J Gastroenterol 2011;46:981-987.
19. Parra RS, Chebli JMF, Amarante HMBS, et al. Quality of life, work productivity impairment and healthcare resources in inflammatory bowel diseases in Brazil. World J Gastroenterol 2019;25:5862-5882.
20. Quon BS, Bentham WD, Unutzer J, Chan YF, Goss CH, Aitken ML. Prevalence of symptoms of depression and anxiety in adults with cystic fibrosis based on the PHQ-9 and GAD-7 screening questionnaires. Psychosomatics 2015;56:345-353.
21. Moriarty AS, Gilbody S, McMillan D, Manea L. Screening and case finding for major depressive disorder using the patient health question-

naire (PHQ-9): a meta-analysis. Gen Hosp Psychiatry 2015;37:567-576.
22. Travis SP, Schnell D, Krzeski P, et al. Developing an instrument to assess the endoscopic severity of ulcerative colitis: the ulcerative colitis endoscopic index of severity (UCEIS). Gut 2012;61:535-542.
23. Mary JY, Modigliani R. Development and validation of an endoscopic index of the severity for Crohn's disease: a prospective multicentre study. Groupe d'etudes thérapeutiques des affections inflammatoires du tube digestif (GETAID). Gut 1989;30:983-989.
24. Berrill JW, Green JT, Hood K, Campbell AK. Symptoms of irritable bowel syndrome in patients with inflammatory bowel disease: examining the role of sub-clinical inflammation and the impact on clinical assessment of disease activity. Aliment Pharmacol Ther 2013;38:44-51.
25. Jelsness-Jørgensen LP, Bernklev T, Moum B. Coexisting irritable bowel-like symptoms in inflammatory bowel disease in remission is associated with impaired social functioning and increased bodily pain. Gastroenterol Nurs 2014;37:280-287.
26. Gracie DJ, Ford AC. Irritable bowel syndrome-type symptoms are associated with psychological comorbidity, reduced quality of life, and health care use in patients with inflammatory bowel disease. Gastroenterology 2017;153:324-325.
27. Minderhoud IM, Oldenburg B, Wismeijer JA, van Berge Henegouwen GP, Smout AJ. IBS-like symptoms in patients with inflammatory bowel disease in remission; relationships with quality of life and coping behavior. Dig Dis Sci 2004;49:469-474.
28. Mikocka-Walus AA, Turnbull DA, Moulding NT, Wilson IG, Andrews JM, Holtmann GJ. Controversies surrounding the comorbidity of depression and anxiety in inflammatory bowel disease patients: a literature review. Inflamm Bowel Dis 2007;13:225-234.
29. Mittermaier C, Dejaco C, Waldhoer T, et al. Impact of depressive mood on relapse in patients with inflammatory bowel disease: a prospective 18-month follow-up study. Psychosom Med 2004;66:79-84.
30. Mikocka-Walus AA, Gordon AL, Stewart BJ, Andrews JM. A magic pill? A qualitative analysis of patients' views on the role of antidepressant therapy in inflammatory bowel disease (IBD). BMC Gastroenterol 2012;12:93.
31. Robles A, Perez Ingles D, Myneedu K, et al. Mast cells are increased in the small intestinal mucosa of patients with irritable bowel syndrome: a systematic review and meta-analysis. Neurogastroenterol Motil 2019;31:e13718.
32. Zhang L, Song J, Hou X. Mast cells and irritable bowel syndrome: from the bench to the bedside. J Neurogastroenterol Motil 2016;22:181-192.
33. Lee H, Park JH, Park DI, et al. Mucosal mast cell count is associated with intestinal permeability in patients with diarrhea predominant irritable bowel syndrome. J Neurogastroenterol Motil 2013;19:244-250.
34. Vivinus-Nébot M, Frin-Mathy G, Bzioueche H, et al. Functional bowel symptoms in quiescent inflammatory bowel diseases: role of epithelial barrier disruption and low-grade inflammation. Gut 2014;63:744-752.
35. Li Y, Yang T, Yao Q, et al. Metformin prevents colonic barrier dysfunction by inhibiting mast cell activation in maternal separation-induced IBS-like rats. Neurogastroenterol Motil 2019;31:e13556.
36. Minderhoud IM, Oldenburg B, Schipper ME, ter Linde JJ, Samsom M. Serotonin synthesis and uptake in symptomatic patients with Crohn's disease in remission. Clin Gastroenterol Hepatol 2007;5:714-720.
37. di Mola FF, Friess H, Zhu ZW, et al. Nerve growth factor and Trk high affinity receptor (TrkA) gene expression in inflammatory bowel disease. Gut 2000;46:670-679.
38. Akbar A, Yiangou Y, Facer P, et al. Expression of the TRPV1 receptor differs in quiescent inflammatory bowel disease with or without abdominal pain. Gut 2010;59:767-774.
39. Lapointe TK, Basso L, Iftinca MC, et al. TRPV1 sensitization mediates postinflammatory visceral pain following acute colitis. Am J Physiol Gastrointest Liver Physiol 2015;309:G87-G99.