Original Article

Check for updates

MiR-26b-5p Predicts the Severity of Crohn's Disease and the Degree of Malnutrition

(Crohn's disease / miR-26b-5p / malnutrition / severity of the disease)

YAN-HUI SUN[1,*], XIAO-XIAO WANG[2,*], CONG WU[1]

[1]Department of Nutrition, the Seventh Medical Center of Chinese PLA General Hospital, Beijing, 100700, China
[2]Department of Obstetrics and Gynecology, Senior Department of Obstetrics and Gynecology, the Seventh Medical Center of Chinese PLA General Hospital, Beijing, 100700, China
[*]Yan-Hui Sun and Xiao-Xiao Wang contributed equally to this study.

Abstract. Crohn's disease (CD) is a chronic and recurrent inflammatory bowel disease, often accompanied by symptoms of malnutrition. MicroRNA is stably present in faeces and plays a significant role in CD. This study aimed to explore the expression of miR-26b-5p in the faeces of CD patients and its association with disease severity and nutritional status. The GEO2R online tool was used to analyse the datasets GSE144535 and GSE102134. The subjects' data were collected. The ability of miR-26b-5p to distinguish healthy individuals from CD patients was evaluated using the receiver operating characteristic curve. The subjects were grouped based on the Crohn's disease activity index, simple endoscopic score for Crohn's disease (SES-CD), malnutrition universal screening tool (MUST) and nutrition risk screening 2002 (NRS 2002) scales, and the expression levels of miR-26b-5p in the faeces of CD patients were detected. The correlation between miR-26b-5p and MUST and NRS 2002 scores was analysed by Spearman's test. The target genes were predicted by miRDB and TargetScan databases, and the biological processes and pathways involved in these genes were identified through GO/KEGG analysis. MiR-26b-5p was significantly down-regulated in the CD dataset and patient faeces, negatively correlated with the disease activity and severity of inflammation, and its expression was lower in patients with poorer nutritional status. GO/KEGG analysis revealed that the target genes of miR-26b-5p were involved in inflammation and nutrient metabolism pathways. We conclude that miR-26b-5p is a potential biomarker for CD and is negatively correlated with the severity of the disease and the degree of malnutrition.

Introduction

Crohn's disease (CD), often referred to as the "incurable cancer", is a chronic inflammatory bowel disease. It belongs to the category of inflammatory bowel disease (IBD) along with ulcerative colitis (UC) (Ungaro et al., 2014). Patients may present with a wide range of clinical manifestations, including common symptoms such as abdominal pain, diarrhoea, fever, rectal bleeding, abdominal masses and fatigue, all of which can significantly impair the daily quality of life (Xu et al. 2021; Dolinger et al. 2024). The precise pathogenesis of CD remains unclear. It is widely accepted that its onset and progression involve a complex interplay of multiple factors. Among environmental factors, changes in modern lifestyles, high-fat and high-sugar diets, and excessive use of antibiotics may all increase an individual's susceptibility to CD (Torres et al., 2017). At the genetic level, mutations in genes such as *NOD2* have been confirmed to be significantly associated with the risk of CD (Gao et al., 2022). Additionally, an imbalance in gut microbiota is considered a critical factor; alterations in the normal microbial composition can compromise mucosal barrier integrity and disrupt immune homeostasis (Caparrós et al., 2021). Ultimately, aberrant immune regulation results in a sustained or excessive inflammatory response. This cascade further aggravates intestinal

Received September 24, 2025. Accepted January 16, 2026.

Corresponding author: Cong Wu, Department of Nutrition, the Seventh Medical Center of Chinese PLA General Hospital, No. 5 Nanmengcang, Dongcheng District, Beijing, 100700, China. Tel.: (+86) 010-66721124; E-mail: congwu8520@163.com

Abbreviations: CD – Crohn's disease, CDAI – Crohn's disease activity index, GO – Gene Ontology, IBD – inflammatory bowel disease, KEGG – Kyoto Encyclopedia of Genes and Genomes, miRNA – microRNA, ROC – receiver operating characteristic, UC – ulcerative colitis.

Copyright: © 2026 Author et al. This is an open access article published under the terms of the Creative Commons Attribution 4.0 International License (CC BY 4.0).

injury and is responsible for the disease chronicity (Clough et al., 2020). The inflammatory damage to the intestine, the main target organ of CD, directly affects the digestion and absorption functions of nutrients. Meanwhile, various factors such as disease progression, medication, dietary habits and psychological conditions predispose CD patients to malnutrition, with a prevalence ranging from 65.0 % to 75.0 % (Caio et al., 2021). Malnutrition not only results in weight loss and muscle wasting but also heightens the risk of infections, delays recovery after surgery, and may lead to diminished therapeutic efficacy and increased disease recurrence (Roncoroni et al., 2022). However, the diagnosis and assessment of CD have always been challenging. Endoscopic examination combined with histological examination, as well as laboratory tests of blood and faeces are typical diagnostic methods for CD (Pasternak et al., 2023). However, due to the limitations of endoscopic surgery, the search for novel non-invasive biomarkers is of great significance for the early diagnosis and effective management of CD.

In recent years, microRNA (miRNA), as a post-transcriptional gene expression regulator, has been found in various species to be indispensable in the expression of various human diseases (Menon et al. 2022; Kimura et al. 2023; Searles, 2024). miRNA is not only stably present in peripheral blood but also widely distributed in body fluids such as urine, saliva and faeces. Among them, faecal miRNA has attracted much attention in CD research (Schönauen et al., 2018). Faecal miRNA primarily originates from the shedding and secretion of intestinal mucosal cells, as well as the metabolic activities of the gut microbiota, thereby serving as a direct reflection of localized intestinal pathological changes. Studies have shown that the miRNA expression profile in the faeces of patients with CD differs significantly from that of healthy individuals and is closely associated with the extent of intestinal mucosal inflammation and the status of intestinal barrier function. For instance, down-regulated miR-375 in the faeces of CD patients leads to increased intestinal permeability and exacerbates inflammation (Wu et al., 2019). Highly expressed miR-223 can amplify intestinal inflammation (Zhang et al., 2023). It is worth noting that miRNA plays a key regulatory role in nutritional control (Nolte-'t Hoen et al., 2015).

MiR-26b-5p was significantly down-regulated in both CD datasets of this study. Based on this, miR-26b-5p is expected to be involved in the onset and progression of CD, but this speculation currently lacks direct experimental evidence to support it. This study examined miRNA expression in faecal samples from CD patients, thoroughly investigated the expression pattern of miR-26b-5p, and elucidated its correlation with the disease severity and the nutritional status of patients, aiming to provide novel insights for the clinical diagnosis of CD.

Material and Methods

Dataset analysis

By searching for "Crohn's disease" in the GEO database (https://www.ncbi.nlm.nih.gov/gds), the GSE144535 dataset (miRNA expression profile in the faeces of CD patients) and the GSE102134 dataset (miRNA expression profile in ileal mucosa of CD patients) were obtained. Then, differentially expressed miRNAs in the datasets were screened based on $|\log_2 \text{FoldChange}| > 1$ and $P < 0.05$.

Sample size

The sample size calculation was done using the G*Power 3.1 software. The effect size (0.5), α error (0.05), power (0.80) and allocation ratio (1) were selected. The minimum sample size required for each group was determined to be 64 cases. Considering the potential risks of clinical sample dropout and data loss, a total of 85 CD patients and 80 healthy controls were finally included to ensure that the study had sufficient statistical power for testing.

Patient enrolment and sample collection

Patients diagnosed with CD at the Seventh Medical Center of Chinese PLA General Hospital were enrolled in this retrospective cohort study. The inclusion criteria were as follows: (1) meeting the diagnostic standards for CD (Inflammatory Bowel Disease Group, Chinese Society of Gastroenterology, Chinese Medical Association, 2021), including imaging, endoscopic and histopathological findings, as well as clinical manifestations; (2) age between 18 and 60 years; (3) at the time of enrolment, serum C-reactive protein (CRP) level ≥ 10 mg/l; (4) no prior treatment with biological agents or immunosuppressants, or a treatment-free interval of ≥ 3 months; (5) no intestinal surgery within the preceding 6 months; (6) absence of other severe gastrointestinal, cardiovascular, respiratory, renal, endocrine, or neurological disorders; (7) no history of allergies and not currently pregnant or lactating. Exclusion criteria included: (1) critical illness requiring emergency surgery or blood transfusion; (2) severe infections, haemorrhage, or anaemia; (3) presence of malignancy or other serious systemic diseases; (4) violation of the study protocol during the observation period, including the use of prohibited medications or significant dietary changes. Additionally, healthy control subjects with no statistically significant differences in gender, age, height, smoking, or alcohol consumption habits compared to CD patients were also enrolled. The exclusion criteria for the healthy control group were as follows: (1) no abdominal pain, diarrhoea or other intestinal symptoms in the past 6 months; (2) no chronic kidney diseases, diabetes, autoimmune diseases or other systemic diseases; (3) no use of antibiotics or probiotics in the past 3 months. Table 1 compares the demographic features between the two groups.

Table 1. Demographic characteristics of the healthy controls and CD patients in the study

Parameters	Control (N = 80)	CD (N = 85)	t/χ^2	P value
Age	32.62 ± 9.66	32.63 ± 10.99	−0.021	0.983
Gender (male), N (%)	42 (52.50 %)	45 (52.94 %)	0.003	0.955
BMI (kg/m^2)	21.24 ± 2.34	17.61 ± 1.69	11.399	< 0.001
Smoker, N (%)	49 (61.25 %)	53 (62.35 %)	0.021	0.884
Drinker, N (%)	39 (48.75 %)	43 (50.59 %)	0.056	0.813
Serum albumin (g/l)	41.42 ± 3.62	34.52 ± 2.51	14.206	< 0.001
White blood cells (×10^9/l)	6.78 ± 1.83	11.36 ± 1.55	17.349	< 0.001
Haemoglobin (g/l)	129.8 5± 11.03	105.92 ± 11.45	13.576	< 0.001
Vitamin B12 (pmol/l)	424.20 ± 147.94	192.58 ± 111.87	11.315	< 0.001

Note: CD, Crohn's disease. BMI, body mass index. Measurement data were expressed as ($\bar{X} \pm s$), and comparisons were made using Student's *t*-test. Count data were expressed as [N (%)], and comparisons were made using χ^2 tests.

Processing of faecal samples

Faecal samples of all subjects were collected on an empty stomach in the early morning and processed within 1 hour after defecation. Five g of the middle section of the faeces free of mucus, pus and blood was weighed. The faecal samples were resuspended in PBS to a final concentration of 100 mg/ml, vortexed for 15 minutes, and then centrifuged at 14,000 *g* for 10 minutes at 4 °C. The supernatant was collected, serially diluted, and an equal amount of sterile glycerol was added to the supernatant, which was stored under anaerobic conditions at −80 °C until further use.

Observational index

The Crohn's disease activity index (CDAI) was used to assess the patients' disease activity. According to this index, a score of < 150 was classified as clinical remission, whereas a score ≥ 150 denoted active disease.

The colonoscopic findings were graded for inflammatory severity using the Simple Endoscopic Score for Crohn's Disease (SES-CD; range 0–53) (Koutroumpakis and Katsanos, 2016), where a higher score denotes more severe inflammation. A score of 0 to 2 is considered an acceptable range of inflammation; 3 to 6 indicates mild inflammation; 7 to 15 indicates moderate inflammation; and ≥ 15 indicates severe inflammation.

According to whether the patients have complications such as ileitis, intestinal perforation, intestinal obstruction and anal fistula, CD patients were stratified into two groups: those without complications (CD1) and those with complications (CD2).

CD was classified by the Montreal classification (Silverberg et al., 2005). This classification includes the age of onset (A1 means ≤ 16 years, A2 means 17–40 years, A3 means > 40 years), disease behaviour (B1 means inflammatory, B2 means structuring, B3 means penetrating) and the involved sites of the disease (L1 means ileal, L2 means colonic, L3 means ileocolonic).

Nutritional risk in patients with CD was screened using the Malnutrition Universal Screening Tool (MUST). A score of < 2 indicates low to medium risk and a score of ≥ 2 indicates high risk.

Nutritional screening for CD patients was conducted using the Nutrition Risk Screening 2002 (NRS 2002) score sheet (Kondrup et al., 2003). A total score of ≥ 3 indicates the presence of nutritional risk; a total score of < 3 suggests a relatively low current nutritional risk. The total score was positively correlated with the degree of nutritional risk.

RT-qPCR analysis

The mirVana isolation kit (Thermo Fisher, Waltham, MA) was used to extract and collect miRNA from faeces. The concentration and purity of the isolated miRNA were measured and assessed using a nanoDrop 2000 spectrophotometer (Thermo Fisher). After synthesizing cDNA using the miRCURY LNA RT kit (Qiagen, Hilden, Germany), it was mixed with 2× miRCURY SYBR Green Master Mix (Qiagen) for RT-qPCR amplification. U6 snRNA was used as an internal reference, and the change in miR-26b-5p expression was determined by calculating the relative expression fold.

Gene function enrichment analysis

The downstream target genes of miR-26b-5p were predicted using the TargetScan (https://www.targetscan.org/vert_80/) and miRDB (https://mirdb.org/) databases. Subsequently, the predicted genes underwent Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis via the Shanghai OE Biotech. Co., Ltd. cloud platform (https://cloud.oebiotech.com/).

Statistical analysis

For data analysis, SPSS 23 (SPSS Inc., Chicago, IL) and GraphPad Prism 10.1.2 (GraphPad Software Inc., La Jolla, CA) were employed. Each set of experimental data came from at least three independent repeated trials. The diagnostic capacity of miR-26b-5p for differentiating the two subject groups was evaluated via the receiver operating characteristic (ROC) curve. For intergroup statistical

comparisons, Student's *t*-test was used for two groups and one-way ANOVA was adopted for multiple groups. $P < 0.05$ was considered statistically significant.

Results

MiR-26b-5p is expressed at a low level in CD

Two datasets related to CD were retrieved from the GEO database. The GSE144535 dataset contains miRNA expression profiles from faecal samples of CD patients and healthy controls, while the GSE102134 dataset includes miRNA expression profiles from ileal mucosal samples of CD patients. After analysis using the GEO2R online tool and screening with $\log_2FC > 1$ and $P < 0.05$, miR-26b-5p was identified as down-regulated in both datasets (Fig. 1A). From the clinical characteristics of the subjects (Table 1), it can be seen that there were no significant differences in age (the age of the control group was 32.62 ± 9.66 years, while that of the CD group was

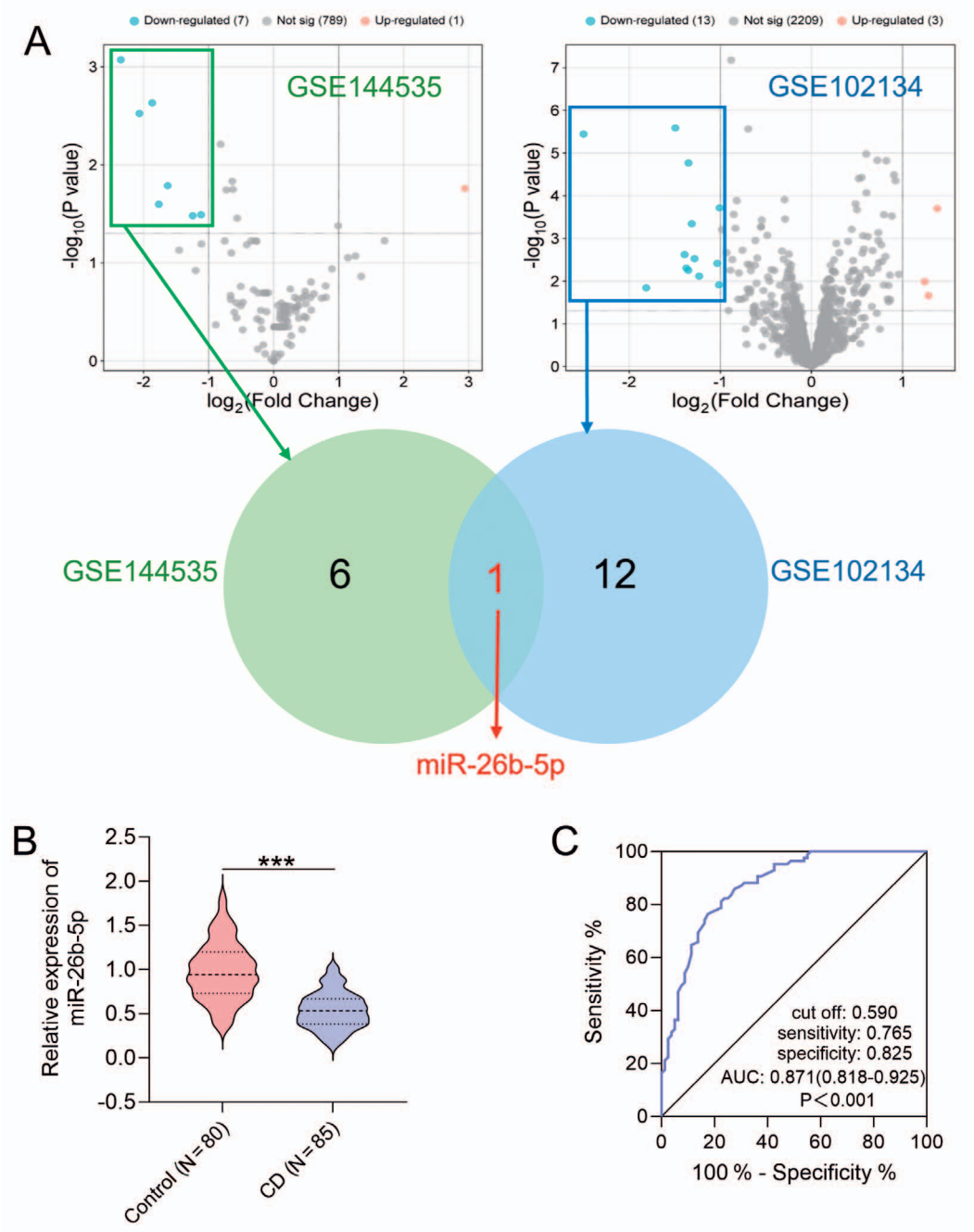

Fig. 1. MiR-26b-5p was significantly down-regulated in CD. **(A)** MiR-26b-5p was identified as a down-regulated miRNA by screening the GSE144535 and GSE102134 datasets using the GEO2R tool with the criteria of $|\log_2FoldChange| > 1$ and $P < 0.05$. **(B)** Compared with healthy subjects (N = 80), miR-26b-5p was expressed at a low level in the faeces of CD patients (N = 85). **(C)** The ROC curve of miR-26b-5p in diagnosing CD patients ($P < 0.001^{***}$).

32.63 ± 10.99 years, t = −0.021, P = 0.983), gender (the proportion of males in the control group was 52.50 % and that in the CD group was 52.94 %, $\chi^2 = 0.003$, P = 0.955), smoking history (61.25 % in the control group, 62.35 % in the CD group, $\chi^2 = 0.021$, P = 0.884), and drinking history (48.75 % in the control group, 50.59 % in the CD group, $\chi^2 = 0.056$, P = 0.813) between CD patients (N = 85) and healthy individuals (N = 80), but there were significant differences in BMI (21.24 ± 2.34 kg/m² in the control group, 17.61 ± 1.69 kg/m² in the CD group, t = 11.399, P < 0.001), serum albumin (41.42 ± 3.62 g/l in the control group, 34.52 ± 2.51 g/l in the CD group, t = 14.206, P < 0.001), white blood cell count (6.78 ± 1.83×10⁹/l in the control group, 11.36 ± 1.55 × 10⁹/l in the CD group, t = 17.349, P < 0.001), haemoglobin (129.85 ± 11.03 g/l in the control group, 105.92 ± 11.45 g/l, t = 13.576 in the CD group, P < 0.001) and vitamin B12 (424.20 ± 147.94 pmol/l in the control group, 92.58 ± 111.87 pmol/l in the CD group, t = 11.315, P < 0.001). The RT-qPCR results showed that miR-26b-5p was significantly down-regulated in faecal samples of CD patients compared to healthy individuals (Fig. 1B). The ROC curve indicated that miR-26b-5p had a good ability to distinguish healthy volunteers from CD patients (Fig. 1C, AUC = 0.871, cut off = 0.590, sensitivity = 0.765, specificity = 0.825, P < 0.001).

MiR-26b-5p is associated with the severity of disease in CD patients

MiR-26b-5p was significantly down-regulated in the faeces of patients with CD. Following the classification of CD patients into active (N =74) and inactive groups (N = 11) based on the CDAI, miR-26b-5p was found to be significantly down-regulated in patients with active disease (Fig. 2A, P < 0.01). When patients were categorized according to the SES-CD, miR-26b-5p expression showed a progressive decrease with increasing SES-CD scores (Fig. 2B, P < 0.05). Furthermore, miR-26b-5p expression was significantly lower in the CD2 group (N = 56) compared to the CD1 group (N = 29) (Fig. 2C, P < 0.01). Additionally, CD patients were classified into a low-miR-26b-5p group and a high-miR-26b-5p group based on the median faecal expression level of miR-26b-5p, followed by Montreal classification analysis of the two groups (Table 2). The results in Table 2 show that there was a statistically significant difference in the disease behaviour between high-miR-26b-5p and low-miR-26b-5p groups (P = 0.014), while there was no difference in the disease location and age of onset.

MiR-26b-5p is associated with malnutrition in patients with CD

MUST and NRS 2002 were used to screen and evaluate the overall nutritional status of patients. The MUST results showed that miR-26b-5p was lowly expressed in the group with a score ≥ 2 (N =74) compared with the group with a score < 2 (N =11) (Fig. 3A, P < 0.001), and the results of its ROC curve indicated that miR-26b-5p could distinguish CD patients with a MUST score < 2 from those with a score ≥ 2 (Fig. 3B, AUC = 0.839, cut off = 0.486, sensitivity = 0.581, specificity = 0.905, P < 0.001). The results of Spearman correlation analysis showed that the expression of miR-26b-5p was negatively correlated with the MUST score (Fig. 3C, r = −0.781, P < 0.001). The NRS 2002 results indicated that miR-26b-5p was significantly down-regulated in patients with a score ≥ 3 (N =73) compared to those with a score < 3 (N =12) (Fig. 3D, P < 0.001). Similarly, the ROC

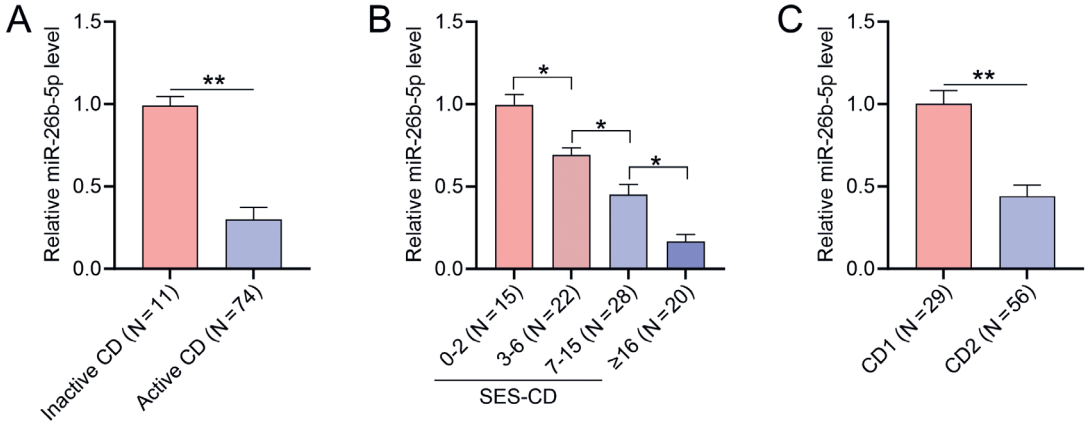

Fig. 2. The expression level of miR-26b-5p was related to the severity of CD. **(A)** After grouping CD patients according to the CDAI score, compared with the inactive group (N = 11), miR-26b-5p was expressed at a lower level in the active group (N = 74). **(B)** According to the SES-CD score, CD patients were divided into 0–2 points group (N = 15), 3–6 points group (N = 22), 7–15 points group (N = 28) and ≥ 16 points group (N = 20), and the expression of miR-26b-5p decreased as the SES-CD score increased. **(C)** The expression level of miR-26b-5p was lower in the group with CD complications (CD2, N = 56) than in the group without complications (CD1, N = 29). Experiments were repeated three times (N = 3) (P < 0.05*, P < 0.01**).

Table 2. Clinical characteristics of CD patients

Parameters	Low-miR-26b-5p (N = 42)	High-miR-26b-5p (N = 43)	χ^2	P value
Disease location (%)			1.159	0.799
L1 (ileal)	6	7		
L2 (colonic)	8	5		
L3 (ileocolonic)	17	17		
L4 (upper gastrointestinal tract)	11	14		
Disease behaviour (%)			8.571	0.014
B1 (inflammatory)	6	18		
B2 (stricturing)	24	19		
B3 (penetrating)	12	6		
Age of onset (%)			1.189	0.616
A1 (≤16 years)	11	12		
A2 (17–40 years)	28	25		
A3 (>40 years)	3	6		

Note: CD, Crohn's disease. Count data were expressed as [N (%)], and comparisons were made using χ^2 tests.

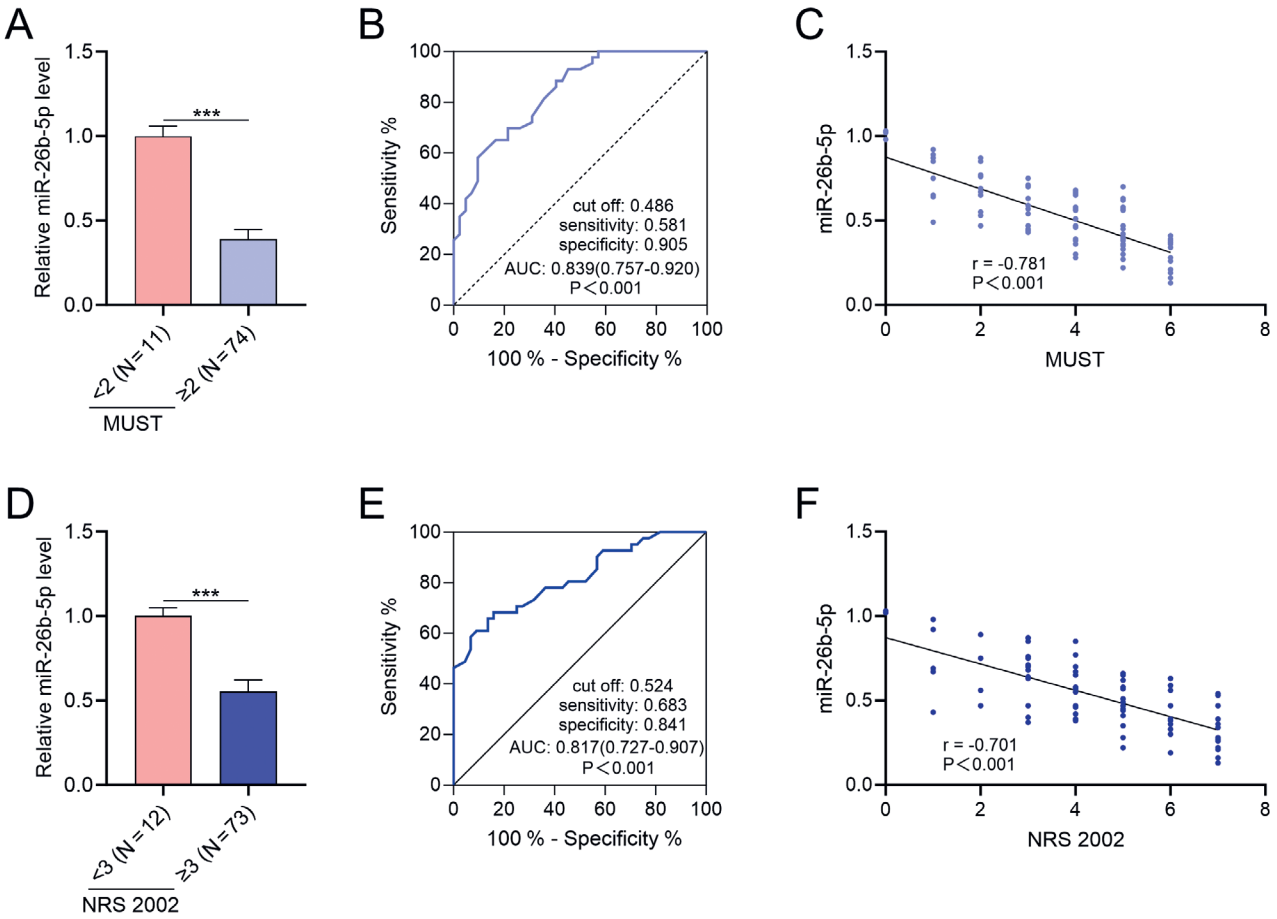

Fig. 3. The expression level of miR-26b-5p was associated with malnutrition in CD. The expression levels of miR-26b-5p in patients with MUST ≥ 2 (N = 74) and MUST < 2 (N = 11) **(A)**, the ROC curve **(B)**, and the correlation between miR-26b-5p and MUST **(C)**. The expression levels of miR-26b-5p in patients with NRS 2002 ≥ 3 (N = 73) and NRS 2002 < 3 (N = 12) **(D)**, the ROC curve **(E)**, and the correlation between miR-26b-5p and NRS 2002 **(F)**. Experiments were repeated three times (N = 3) ($P < 0.001^{***}$).

curve results indicated that miR-26b-5p had the ability to distinguish CD patients with a score < 3 from those with a score ≥ 3 on the NRS 2002 scale (Fig. 3E, AUC = 0.817, cut off = 0.524, sensitivity = 0.683, specificity = 0.841, $P < 0.001$). Meanwhile, the results of Spearman correlation analysis showed that miR-26b-5p was negatively correlated with the NRS 2002 score (Fig. 3F, $r = -0.701$, $P < 0.001$).

The downstream target genes of miR-26b-5p are mainly involved in inflammatory responses and nutrient supply

The target genes of miR-26b-5p were predicted using miRDB and TargetScan (the predicted target genes and overlapping genes are shown in Supplemental Table 1). The overlapping genes were selected for subsequent GO and KEGG pathway analyses. As illustrated in Figure 4A, in terms of molecular function, the target genes were predominantly enriched in oxalate transport, inorganic anion transport, bicarbonate transport, L-serine transport, and other ions or nutrients. It is suggested that the overlapping genes are involved in the transport of oxalate, bicarbonate, L-serine, ions or nutrients. Regarding cellular components, these genes were primarily associated with the cell body, cytoplasm, growth cone and cytoplasmic stress granules. In the context of biological processes, the differentially expressed genes were mainly involved in mRNA 3'-UTR binding, L-serine transmembrane transport activity, dehydroascorbic acid transmembrane transport activity and glucose transmembrane transporter activity. KEGG pathway analysis (Fig. 4B) revealed that the target genes were significantly enriched in pathways related to the phosphatidylinositol signalling system, the Wnt signalling pathway, the glucagon signalling pathway and other associated pathways. This indicates that these genes play an irreplaceable role in the inflammatory response and nutrient supply pathways.

Discussion

CD can accumulate throughout the entire digestive tract from the mouth to the anus (Dolinger et al., 2024). Clinical diagnosis mainly relies on endoscopic examination at present, but its invasiveness leads to poor patient tolerance (Catt et al. 2019). In comparison, miRNAs in faeces have outstanding advantages such as non-invasiveness and convenience, which leads to high patient compliance. This study found that miR-26b-5p was expressed at a low level in patients with CD, and it was able to effectively distinguish healthy individuals from CD patients, demonstrating its potential as a non-invasive diagnostic marker. CDAI and SES-CD have an important reference value in clinical disease management. The expression of miR-26b-5p was lower in active CD and gradually decreased with the increase of SES-CD score, suggesting that it can reflect the severity of CD inflammation and provide a reference for dynamic detection of the disease. Meanwhile, miR-26b-5p was lowly expressed in CD patients with complications, indicating that it may be involved in the key mechanism of complication occurrence and become an early warning molecular marker for predicting the risk of complications. However, this discovery awaits further verification. In addition, this study also found, based on the Montreal classification, that miR-26b-5p showed differences in the disease behaviour in CD patients. Patients with low expression of miR-26b-5p were more numerous in the B2/B3 category, indicating that miR-26b-5p was related to fibrosis and epithelial-mesenchymal transition in CD. This was consistent with the regulatory roles of molecules such as miR-200 and miR-21 in regulating intestinal fibrosis and epithelial-mesenchymal transition (EMT) in CD found in previous studies (Mehta et al. 2018; Wang et al. 2022), further confirming the association between miR-26b-5p and the severity of the disease and the risk of complications. However, the study results showed that miR-26b-5p displayed no significant differences in the disease sites in the Montreal classification. The possible reason for this result lies in the fact that miR-26b-5p is mainly involved in regulating the common pathways of disease progression such as post-inflammatory tissue repair and fibrosis. These processes can occur in different sites, so these expression changes are more related to the disease behaviour. In addition, the detection of miRNA in faeces is easily affected by factors such as intestinal flora (Zhang et al., 2025), which may to some extent weaken its ability to reflect site-specific lesions.

It is well-documented that malnutrition frequently occurs in individuals with CD, with significant reductions in serum albumin, haemoglobin, vitamin B12 and other indicators (Jabłońska and Mrowiec, 2023). This study also confirmed this. What's more, this study found that in patients with high nutritional risk scores, miR-26b-5p showed significantly low expression, and miR-26b-5p was negatively correlated with MUST and NRS 2002 scores, proving that it was closely related to the malnutrition status of CD patients. Furthermore, following the GO and KEGG enrichment analysis of the predicted target genes of miR-26b-5p, it was observed that these genes exhibited multi-dimensional functional overlaps within the nutritional metabolism and inflammatory regulation networks associated with CD. According to the GO analysis, the biological processes such as "regulation of endothelial barrier formation" and "negative regulation of cell migration" are directly linked to a key pathological feature of intestinal epithelial barrier damage. A compromised barrier allows translocation of gut microbiota and their metabolites into the mucosal layer, thereby initiating an immune-inflammatory cascade (Wallace et al., 2014). Additionally, the enrichment of molecular functions, including "glucose and L-serine transmembrane transport activity," highlights the molecular basis underlying nutritional absorption impairments in CD patients. KEGG enrichment analysis further confirmed the cross-talk among multiple signalling pathways. Adherens junctions and the Notch signalling pathway serve as key regulatory axes in maintaining the in-

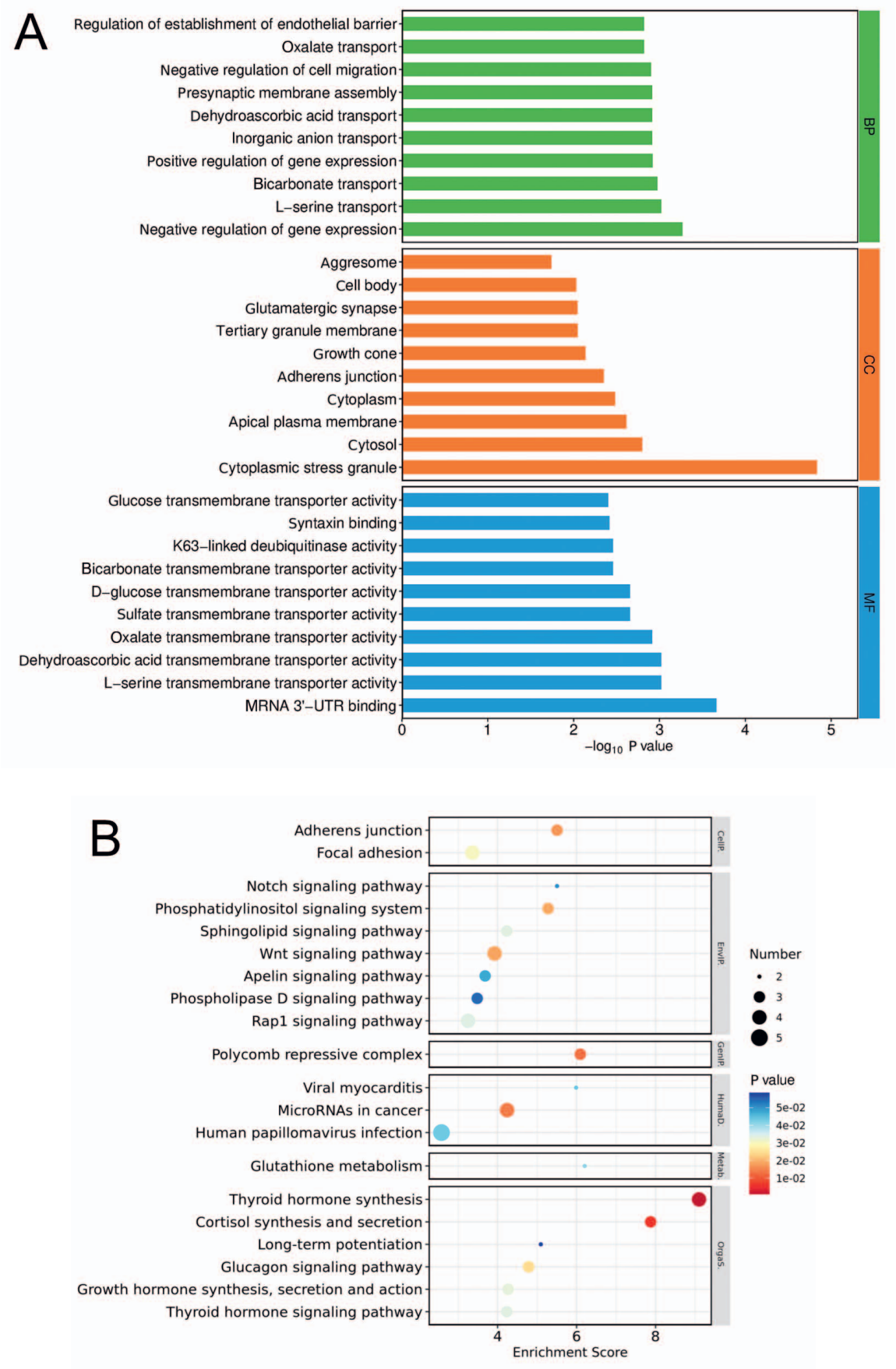

Fig. 4. The downstream target genes of miR-26b-5p were mainly involved in nutrient metabolism and inflammatory responses. **(A)** GO functional analysis of the targets of miR-26b-5p. **(B)** KEGG pathway enrichment analysis of the miR-26b-5p target genes.

testinal physical barrier and promoting EMT. Functional impairments in these pathways directly compromise the "first line of defence" in CD (Ortiz-Masiá et al., 2016). The glutathione metabolism pathway is involved in oxidative stress (Muro et al., 2024), thereby amplifying inflammatory responses and impeding mucosal repair. Moreover, the enrichment of metabolism-related pathways, such as the thyroid hormone signalling pathway and glucagon signalling pathway, indicates a bidirectional regulatory mechanism between nutritional metabolism and immune inflammation. These results link miR-26b-5p to the key aspects of CD clinical progression, namely disease severity and nutritional status.

Admittedly, this study has some limitations. Firstly, this study is a single-centre retrospective study, and all samples are from a single medical centre. Secondly, the inclusion criteria limited serum CRP ≥ 10 mg/l, which made the inclusion of CD patients insufficiently comprehensive. Finally, no experiments were conducted on the specific molecular mechanisms by which miR-26b-5p regulates inflammation and nutritional metabolism in CD. Therefore, subsequently, multi-centre and large-sample studies will be carried out to verify the universality of this research, and the patient inclusion scope will be expanded to comprehensively evaluate the value of miR-26b-5p in CD patients with different disease phenotypes and inflammatory levels. Meanwhile, the related molecular mechanisms of miR-26b-5p regulating inflammation and nutritional metabolism in CD will be deeply explored through cell models such as intestinal epithelial cells.

Conclusion

In summary, miR-26b-5p shows promising diagnostic value, positioning it as a potential biomarker for CD. Additionally, the expression level of miR-26b-5p is negatively correlated with the severity of the disease and the degree of malnutrition in CD patients.

Authors' contributions

Conceptualization: Y.S., X.W. and C.W.; Data curation: Y.S., X.W. and C.W.; Formal analysis: Y.S., X.W. and C.W.; Funding acquisition: C.W.; Investigation: Y.S. and X.W.; Methodology: Y.S., X.W. and C.W.; Project administration: C.W.; Resources: Y.S. and X.W.; Software: Y.S. and X.W.; Supervision: C.W.; Validation: Y.S. and X.W.; Visualization: Y.S. and X.W.; Roles/Writing – original draft: Y.S. and X.W.; Writing – review and editing: C.W.

Conflicts of Interest

There is no conflict of interest in this study.

References

Caio, G., Lungaro, L., Caputo, F. et al. (2021) Nutritional treatment in Crohn's disease. *Nutrients* **13**, 1628.

Caparrós, E., Wiest, R., Scharl, M. et al. (2021) Dysbiotic microbiota interactions in Crohn's disease. *Gut Microbes* **13**, 1949096.

Catt, H., Hughes, D., Kirkham, J. J. et al. (2019) Systematic review: outcomes and adverse events from randomised trials in Crohn's disease. *Aliment. Pharmacol. Ther.* **49**, 978-996.

Clough, J. N., Omer, O. S., Tasker, S. et al. (2020) Regulatory T-cell therapy in Crohn's disease: challenges and advances. *Gut* **69**, 942-952.

Dolinger, M., Torres, J., Vermeire, S. (2024) Crohn's disease. *Lancet* **403**, 1177-1191.

Gao, J., Zhao, X., Hu, S. et al. (2022) Gut microbial DL-endopeptidase alleviates Crohn's disease via the NOD2 pathway. *Cell Host Microbe* **30**, 1435-1449.e9.

Inflammatory Bowel Disease Group, Chinese Society of Gastroenterology, Chinese Medical Association (2021) Chinese consensus on diagnosis and treatment in inflammatory bowel disease (2018, Beijing). *J. Dig. Dis.* **22**, 298-317.

Jabłońska, B., Mrowiec, S. (2023) Nutritional status and its detection in patients with inflammatory bowel diseases. *Nutrients* **15**, 1991.

Kimura, M., Kothari, S., Gohir, W. et al. (2023) MicroRNAs in infectious diseases: potential diagnostic biomarkers and therapeutic targets. *Clin. Microbiol. Rev.* **36**, e0001523.

Kondrup, J., Rasmussen, H. H., Hamberg, O. et al. (2003) Nutritional risk screening (NRS 2002): a new method based on an analysis of controlled clinical trials. *Clin. Nutr.* **22**, 321-336.

Koutroumpakis, E., Katsanos, K. H. (2016) Implementation of the simple endoscopic activity score in Crohn's disease. *Saudi J. Gastroenterol.* **22**, 183-191.

Mehta, S. J., Lewis, A., Nijhuis, A., et al. (2018) Epithelial down-regulation of the miR-200 family in fibrostenosing Crohn's disease is associated with features of epithelial to mesenchymal transition. *J. Cell. Mol. Med.* **22**, 5617-5628.

Menon, A., Abd-Aziz, N., Khalid, K. et al. (2022) miRNA: a promising therapeutic target in cancer. *Int. J. Mol. Sci*. **23**, 11502.

Muro, P., Zhang, L., Li, S. et al. (2024) The emerging role of oxidative stress in inflammatory bowel disease. *Front. Endocrinol. (Lausanne)* **15**, 1390351.

Nolte-'t Hoen, E. N., Van Rooij, E., Bushell, M. et al. (2015) The role of microRNA in nutritional control. *J. Intern. Med.* **278**, 99-109.

Ortiz-Masiá, D., Cosín-Roger, J., Calatayud, S. et al. (2016) M1 macrophages activate Notch signalling in epithelial cells: relevance in Crohn's disease. *J. Crohns Colitis* **10**, 582-592.

Pasternak, G., Chrzanowski, G., Aebisher, D. et al. (2023) Crohn's disease: basic characteristics of the disease, diagnostic methods, the role of biomarkers, and analysis of metalloproteinases: a review. *Life (Basel)* **13**, 2062.

Roncoroni, L., Gori, R., Elli, L. et al. (2022) Nutrition in patients with inflammatory bowel diseases: a narrative review. *Nutrients* **14**, 751.

Schönauen, K., Le, N., von Arnim, U. et al. (2018) Circulating and fecal microRNAs as biomarkers for inflammatory bowel diseases. *Inflamm. Bowel Dis.* **24**, 1547-1557.

Searles, C. D. (2024) MicroRNAs and cardiovascular disease risk. *Curr. Cardiol. Rep.* **26**, 51-60.

Silverberg, M. S., Satsangi, J., Ahmad, T. et al. (2005) Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can. J. Gastroenterol.* **19 (Suppl. A)**, 5A-36A.

Torres, J., Mehandru, S., Colombel, J. F. et al. (2017) Crohn's disease. *Lancet* **389**, 1741-1755.

Ungaro, R., Bernstein, C. N., Gearry, R. et al. (2014) Antibiotics associated with increased risk of new-onset Crohn's disease but not ulcerative colitis: a meta-analysis. *Am. J. Gastroenterol.* **109**, 1728-1738.

Wallace, K. L., Zheng, L. B., Kanazawa, Y. et al. (2014) Immunopathology of inflammatory bowel disease. *World J. Gastroenterol.* **20**, 6-21.

Wang, Z., Zhou, H., Cheng, F. et al. (2022) MiR-21 regulates epithelial-mesenchymal transition in intestinal fibrosis of Crohn's disease by targeting PTEN/mTOR. *Dig. Liver Dis.* **54**, 1358-1366.

Wu, C. P., Bi, Y. J., Liu, D. M. et al. (2019) Hsa-miR-375 promotes the progression of inflammatory bowel disease by upregulating TLR4. *Eur. Rev. Med. Pharmacol. Sci.* **23**, 7543-7549.

Xu, F., Carlson, S. A., Liu, Y. et al. (2021) Prevalence of inflammatory bowel disease among medicare fee-for-service beneficiaries – United States, 2001-2018. *MMWR Morb. Mortal. Wkly Rep.* **70**, 698-701.

Zhang, J., Guo, Z., Wang, Z. et al. (2023) Fecal miR-223 is a noninvasive biomarker for estimating Crohn's disease activity. *Immun. Inflamm. Dis.* **11**, e1131.

Zhang, Q., Liu, Y., Li, Y. et al. (2025) Implications of gut microbiota-mediated epigenetic modifications in intestinal diseases. *Gut Microbes* **17**, 2508426.