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**ABOUT COVER**

Editorial Board Member of *World Journal of Gastroenterology*, King-Wah Chiu is a Distinguished Professor at the Cheng Shui University in Kaohsiung, Taiwan, Republic of China. Having received his Bachelor's degree from China Medical University College of Medicine in 1985, he rose to Chief in the Gastroenterology Division of the Kaohsiung Chang Gung Memorial Hospital Affiliated to Chang Gung University of College of Medicine in 2002. Dr. Chiu is a recognized expert in hepato-gastroenterology, having practiced for 30 years, and the pioneer of transplant hepatology in the field of liver transplantation, practicing in Kaohsiung Chang Gung Memorial Hospital since 1998. His ongoing research interests involve the application of molecular biology in transplant hepatology, particularly to study the effects of integrative basic medicine on and management of living-donor liver transplantation establishment. (L-Editor: Filipodia)

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## Retrospective Study

## Valuable clinical indicators for identifying infantile-onset inflammatory bowel disease patients with monogenic diseases

Wen Su, Yi Yu, Xu Xu, Xin-Qiong Wang, Jie-Bin Huang, Chun-Di Xu, Yuan Xiao

**ORCID number:** Wen Su 0000-0002-7853-4606; Yi Yu 0000-0002-5680-1195; Xu Xu 0000-0002-5720-945X; Xin-Qiong Wang 0000-0003-0057-3653; Jie-Bin Huang 0000-0001-9362-3511; Chun-Di Xu 0000-0002-2314-9188; Yuan Xiao 0000-0002-4927-8199.

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**Wen Su, Yi Yu, Xu Xu, Xin-Qiong Wang, Jie-Bin Huang, Chun-Di Xu, Yuan Xiao,** Department of Pediatrics, Ruijin Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai 200025, China

**Corresponding author:** Yuan Xiao, MD, PhD, Associate Chief Physician, Doctor, Department of Pediatrics, Ruijin Hospital, Shanghai Jiao Tong University, School of Medicine, No. 197 Ruijin 2 Road, Shanghai 200025, China. [yxiao.penicilinum@gmail.com](mailto:yxiao.penicilinum@gmail.com)

**Abstract****BACKGROUND**

Infantile-onset inflammatory bowel disease (IO-IBD) occurs in very young children and causes severe clinical manifestations, which has poor responses to traditional inflammatory bowel disease (IBD) treatments. At present, there are no simple and reliable laboratory indicators for early screening IO-IBD patients, especially those in whom the disease is caused by monogenic diseases.

**AIM**

To search for valuable indicators for early identifying IO-IBD patients, especially those in whom the disease is caused by monogenic diseases.

**METHODS**

A retrospective analysis was performed in 73 patients with IO-IBD admitted to our hospital in the past 5 years. Based on the next-generation sequencing results, they were divided into a monogenic IBD group (M-IBD) and a non-monogenic IBD group (NM-IBD). Forty age-matched patients with allergic proctocolitis (AP) were included in a control group. The clinical manifestations and the inflammatory factors in peripheral blood were evaluated. Logistic regression analysis and receiver operating characteristic (ROC) curve analysis were used to identify the screening factors and cut-off values of IO-IBD as well as monogenic IO-IBD, respectively.

**RESULTS**

Among the 44 M-IBD patients, 35 carried *IL-10RA* mutations, and the most common mutations were c.301C>T (p.R101W, 30/70) and the c.537G>A (p.T179T, 17/70). Patients with higher serum tumor necrosis factor (TNF)- $\alpha$  value were more likely to have IBD [odds ratio (OR) = 1.25, 95% confidence interval (CI): 1.05-1.50,  $P = 0.013$ ], while higher serum albumin level was associated with lower risk of IBD (OR = 0.86, 95%CI: 0.74-1.00,  $P = 0.048$ ). The cut-off values of TNF- $\alpha$  and

Jiaotong University School of Medicine.

**Informed consent statement:** All guardians of the enrolled pediatric patients who experienced the gene test signed an informed consent form.

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albumin were 17.40 pg/mL (sensitivity: 0.78; specificity: 0.88) and 36.50 g/L (sensitivity: 0.80; specificity: 0.90), respectively. The increased ferritin level was indicative of a genetic mutation in IO-IBD patients. Its cut-off value was 28.20 ng/mL (sensitivity: 0.93; specificity: 0.92). When interleukin (IL)-10 level was higher than 33.05 pg/mL (sensitivity: 1.00; specificity: 0.84), or the onset age was earlier than 0.21 mo (sensitivity: 0.82; specificity: 0.94), the presence of disease-causing mutations in *IL-10RA* in IO-IBD patients was strongly suggested.

## CONCLUSION

Serum TNF- $\alpha$  and albumin level could differentiate IO-IBD patients from allergic proctocolitis patients, and serum ferritin and IL-10 levels are useful indicators for early diagnosing monogenic IO-IBD.

**Key Words:** Infantile-onset inflammatory bowel disease; Immunodeficiency; Clinical indicators; Interleukin 10; Ferritin; *IL10RA*

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**Core Tip:** It is very important to identify infantile-onset inflammatory bowel disease (IO-IBD) patients, especially those in whom the disease is caused by monogenic diseases, as early as possible because these patients have poor responses to traditional inflammatory bowel disease treatments. However, there are no simple and reliable laboratory indicators for early differential diagnosis. This is the first study focusing on the laboratory indicators that could be used to distinguish O-IBD patients from allergic enteritis patients and screening IO-IBD patients with monogenic diseases. We believe that these results may be valuable in the initial investigations and diagnosis of IO-IBD.

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## INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic recurrent gastrointestinal inflammatory disease, which could be classified into Crohn's disease (CD), ulcerative colitis (UC), and IBD-unclassified (IBD-U)<sup>[1]</sup>. Common clinical manifestations include diarrhea, hematochezia, abdominal pain, growth retardation, and weight loss. Many studies have reported that the phenotypic characteristics are different in very early onset-IBD (VEO-IBD, the onset age was younger than 6 years old) children from those in adolescent-onset or adult-onset IBD patients<sup>[2]</sup>. An increasing number of studies<sup>[3]</sup> have indicated the presence of monogenic defects in VEO-IBD children, especially in infantile patients (< 2 years old). Gene sequencing, especially next generation-sequencing (NGS), could help to find the mutations to explain the cause of the disease; however, this method has high costs, and is time-consuming and not suitable for regular application at the early stage of the disease, particularly in economically underdeveloped areas. Besides, many variants of uncertain significance (VUS) after NGS was applied need laboratory indicators to verify their pathogenicity. However, there have been no reliable clinical indicators reported to identify pediatric IBD patients early, especially those who have potential gene mutations. In this retrospective study, we intended to search for early diagnostic indicators for IO-IBD children with or without the gene mutation, in order to shorten the diagnosis time, reduce medical costs, simplify the analysis of NGS, and administer targeted intervention as early as possible.

P-Editor: Wang LL



## MATERIALS AND METHODS

### Study participants and groups

This study was approved by the ethics committee of Ruijin Hospital, Shanghai Jiaotong University School of Medicine. All guardians of the enrolled pediatric patients who experienced the gene test signed an informed consent form. This study retrospectively analyzed 73 IO-IBD patients with a disease onset before 2 years of age who were admitted to the Department of Pediatrics, Ruijin Hospital, Shanghai Jiaotong University School of Medicine from January 2014 to February 2019. The diagnostic criteria were based on the Consensus on the Diagnosis and Management of Pediatric Inflammatory Bowel Disease<sup>[4]</sup> formulated by the Chinese Society of Pediatric Gastroenterology, the Chinese Medical Association. According to the NGS results, the patients were divided into either a monogenic IBD (M-IBD) group, which comprised IO-IBD patients caused by Mendelian diseases, or a non-monogenic IBD (NM-IBD) group, which comprised IO-IBD patients without disease-causing gene mutations. Forty age-matched children who were hospitalized due to diarrhea and hematochezia during the same period and ultimately diagnosed with allergic proctocolitis (AP) were enrolled in the study as a control group.

### Clinical data analysis

The clinical data of all patients were collected, including sex, age of onset, body weight and height on admission, average daily frequency of diarrhea, hematochezia, perianal lesions, recurrent fever, and treatment outcomes (remission, non-remission, and death). The results of complete blood count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum albumin, and serum iron levels were collected and analyzed. Tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-6, and IL-10 levels were determined using chemiluminescence immunoassays with a commercial kit (Siemens). The serum ferritin concentration was detected using a double-site enzyme immunoassay with a commercial ferritin kit (ACCES).

### Next-generation sequencing and validation

Peripheral blood was collected from all IO-IBD patients for the genetic test. A FlexiGene DNA Kit (Qiagen GmbH, D-40724 Hilden) was used to extract DNA according to the manufacturer's instructions. NGS of targeted genes including primary immunodeficiency diseases and congenital diarrheal diseases (20 cases) was performed by Beijing Mygeno Gene Technology Co., Ltd and whole-exome sequencing (53 cases) was performed by Beijing Berry Genomics Co., Ltd. For the mutations found *via* NGS, Sanger sequencing was used to retest the corresponding gene sequences of the patients and their parents to verify and confirm the genetic origin. The genetic variations were identified using online databases, such as the Single Nucleotide Polymorphism Database (dbSNP), Clinvar, the Human Gene Mutation Database (HGMD), the 1000 Genomes Project, and Online Mendelian Inheritance in Man (OMIM), to verify whether they were the known pathogenic mutations. For novel mutations not included in the databases, pathogenicity was further evaluated according to the American College of Medical Genetics and Genomics (ACMG) guidelines<sup>[5]</sup>.

### Statistical analysis

Z scores for height and weight were calculated using WHO Anthro V3.2.2 software. All data were statistically analyzed using IBM SPSS Statistics 25.0. Measurement data with a normal distribution are expressed as the mean  $\pm$  standard deviation (SD), and nonnormally distributed data are expressed as the median and interquartile range (IQR). Data with a normal distribution and homogeneity of variance were analyzed using ANOVA analysis, and abnormally distributed data were analyzed by nonparametric Kruskal-Wallis analysis. Categorical data were analyzed using the chi-square test, or Fisher's exact Chi-square test. Multivariate binary logistic regression analysis was performed to identify risk factors for IO-IBD and monogenic IO-IBD. Receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic value of the identified indicators. *P* values were adjusted using the Bonferroni method for pairwise comparison. *P* < 0.05 was considered statistically significant. The statistical methods of this study were reviewed by Li J from the Clinical Research Center, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine.

## RESULTS

### Genotypes of IO-IBD patients

Among the 73 IO-IBD patients, 24 did not carry any disease-causing mutations in IBD-associated genes, and 49 had the gene mutations related to the disease. Among 49 patients with mutations, 39 carried *IL-10RA* mutations, 2 carried *CYBB* mutations, and *WAS*, *IKBKKG*, *SLC37A4*, *CD40LG*, *LIG4*, *CARD11*, *PIK3CD*, and *CXCR4* mutations were observed in one patient each (Table 1). Four of them carried the *IL-10RA* heterozygous mutation which did not meet the criteria of Mendelian disease, and one of them had a heterozygous *CXCR4* mutation which was inherited from his father with ankylosing spondylitis and recognized as a VUS according to the ACMG guidelines because of absence of typical manifestations of autosome dominant WHIM syndrome. Therefore, these five patients were included in the NM-IBD group. There were 14 different missense mutations found in *IL-10RA* among M-IBD patients. Two variations, the c.301C>T (p.R101W) and the c.537G>A (p.T179T), were considered hotspot mutations in the M-IBD patient cohort, the mutation frequencies were 42.86% (30/70) and 24.29% (17/70) respectively. We also found nine novel mutations: c.109G>T, c.302G>A, c.569T>G, and c.787C>T in *IL-10RA* gene; c.674+2T>C in *CYBB* gene; c.267delC in *CD40LG* gene; c.1144\_1145delCT in *LIG4* gene; c.155T>C in *CARD11* gene; and c.1001G>A in *CXCR4* gene. The amino acid substitutions and pathogenicity of these variations that were assessed according to the ACMG guidelines are summarized in Table 1.

### Clinical characteristics

As shown in Table 2, the proportions of male patients in the AP group, the M-IBD group, and the NM-IBD group was higher than those of female patients; however, no significant difference was observed among the three groups. The median onset age of patients in the M-IBD group was 0.51 (0.04-1.79) mo, which was much younger than that in the AP group [4.44 (1.25-11.76) mo;  $P = 0.003$ ] and the NM-IBD group [3.99 (3-9) mo;  $P = 0.001$ ]. The proportions of all IO-IBD patients who had severe diarrhea (more than 8 times/d, 86.4% in the M-IBD group and 58.6% in the NM-IBD group) and recurrent fever (63.6% in the M-IBD group and 41.4% in the NM-IBD group) were significantly higher than those in AP patients (25% of patients had severe diarrhea [ $P < 0.001$ ] and 5% of patients had a recurrent fever [ $P = 0.01$ ]). The percentages of the patients who had severe diarrhea and perianal lesions in the M-IBD group (86.4% and 79.5%, respectively) were much higher than those in the NM-IBD group (58.6% [ $P = 0.007$ ] and 24.1% [ $P = 0.005$ ]). The anthropometric results showed that the Z scores for body weight ( $-1.95 \pm 0.26$  for the M-IBD group and  $-1.15 \pm 0.29$  for the NM-IBD group) and body height ( $-1.95 \pm 0.25$  for the M-IBD group and  $-1.32 \pm 0.31$  for the NM-IBD group) of the IBD patients were all lower than those of the children in the AP group ( $-0.05 \pm 0.21$  for weight [ $P < 0.001$ ] and  $0.17 \pm 0.19$  for height [ $P < 0.001$ ]), while there was no significant difference between the M-IBD group and the NM-IBD group. Regarding the outcomes of treatment, mortality in the M-IBD group was significantly higher than that in the NM-IBD group (20.5% vs 3.4%,  $P = 0.001$ ), whereas no death occurred in the AP group.

As shown in Table 3, the blood test results showed that peripheral white blood cell count (WBC), platelet count (PLT), CRP, ESR, TNF- $\alpha$ , and IL-6 levels in the IO-IBD group were significantly higher than those in the AP group ( $P < 0.01$  for all), while serum albumin level was lower than that in the AP group ( $P < 0.001$ ). Differences in serum iron levels were not significant among the three groups. Notably, serum IL-10 [85.50 (42.75-127.25) pg/mL] and ferritin [55.90 (36.10-231.20) ng/mL] levels in the M-IBD group were significantly higher than those in the AP group [IL-10: 5.00 (5.00-5.00) pg/mL,  $P < 0.001$ ; ferritin: 22.90 (14.90-37.05) ng/mL,  $P = 0.002$ ] and in the NM-IBD group [IL-10: 6.37 (5.00-14.80) pg/mL,  $P < 0.001$ ; ferritin: 15.30 (9.40-27.60) ng/mL,  $P < 0.001$ ].

### Binary logistic regression analysis and ROC curve analysis

**Clinical indicators for risk of IO-IBD:** Binary logistic regression analysis was performed for the parameters with significant differences between the AP group and the IO-IBD group [including diarrhea (> 8 times/d), recurrent fever, Z-scores for weight, Z-scores for height, WBC, hemoglobin (Hb), PLT, CRP, ESR, TNF- $\alpha$ , IL-6, and albumin levels]. The results showed that increased peripheral WBC (odds ratio [OR] = 1.19, 95% confidence interval [CI]: 1.01-1.40,  $P = 0.040$ ), ESR (OR = 1.10, 95%CI: 1.01-1.20,  $P = 0.037$ ) and levels of TNF- $\alpha$  (OR = 1.25, 95%CI: 1.05-1.50,  $P = 0.013$ ), as well as reduced albumin levels were risk factors (OR = 0.86, 95%CI: 0.74-1.00,  $P = 0.048$ ) for

**Table 1 Genotypes of 49 infantile-onset inflammatory bowel disease patients**

Patient	Gene	Variant (allele 1)	Amino acid	ACMG (P/LP)	Variant (allele 2)	Amino acid	ACMG (P/LP)
1	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)
2	<i>IL10RA</i>	c.299T>G	V100G	P (PS1+PS3+PM2+PP3+PP4)	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)
3	<i>IL10RA</i>	c.191A>G	Y64C	LP (PM1+PM2+PM3+PP3+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
4	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
5	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)
6	<i>IL10RA</i>	c.493C>T	R165X	P (PVS1+PS1+PM2+PP4)	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)
7	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
8	<i>IL10RA</i>	c.350G>A	R117H	P (PS1+PM1+PM2+PP3+PP4)	c.493C>T	R165X	P (PVS1+PS1+PM2+PP4)
9	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.436delC	P146fs	P (PVS1+PS1+PM2+PP4)
10	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
11	<i>IL10RA</i>	c.421G>A	G141R	P (PS1+PM2+PM3+PP3+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
12	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)
13	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.350G>A	R117H	P (PS1+PM1+PM2+PP3+PP4)
14	<i>IL10RA</i>	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
15	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
16	<i>IL10RA</i>	c.99G>A	W33X	P (PVS1+PS1+PM2+PP4)	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)
17	<i>IL10RA</i>	c.493C>T	R165X	P (PVS1+PS1+PM2+PP4)	c.493C>T	R165X	P (PVS1+PS1+PM2+PP4)
18	<i>IL10RA</i>	c.109G>T	E37X	LP (PVS1+PM2+PM3+PP4)	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)
19	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
20	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
21	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
22	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.350G>A	R117H	P (PS1+PM1+PM2+PP3+PP4)
23	<i>IL10RA</i>	c.99G>A	W33X	P (PVS1+PS1+PM2+PP4)	c.299T>G	V100G	P (PS1+PS3+PM2+PP3+PP4)
24	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
25	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
26	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.493C>T	R165X	P (PVS1+PS1+PM2+PP4)
27	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
28	<i>IL10RA</i>	c.493C>T	R165X	P (PVS1+PS1+PM2+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
29	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)
30	<i>IL10RA</i>	c.302G>A	R101Q	LP (PM1+PM2+PM3+PM5+PP3+PP4)	c.349C>T	R117C	P (PS1+PM1+PM2+PP3+PP4)
31	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)
32	<i>IL10RA</i>	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)
33	<i>IL10RA</i>	c.299T>G	V100G	P (PS1+PS3+PM2+PP3+PP4)	c.569T>G	F190C	LP (PS1+PM2+PM3_VeryStrong+PP3+PP4)
34	<i>IL10RA</i>	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
35	<i>IL10RA</i>	c.299T>G	V100G	P (PS1+PS3+PM2+PP3+PP4)	c.299T>G	V100G	P (PS1+PS3+PM2+PP3+PP4)
36	<i>WAS</i>	IVS8: +3- +6 GAGT del		LP (PS1+PM2+PP3+PP4)			
37	<i>IKBK</i>	c.1217A>T	D406V	P (PS1+PM1+PM2+PP3+PP4)			
38	<i>SLC37A4</i>	c.310 ins T		P (PVS1+PM2+PP4)	c.1014-1120 del107		P (PVS1+PM2+PP4)

39	CYBB	c.674+2T>C		P (PVS1+PM2+PP4)
40	CYBB	c.1272G>A	W424X	P (PVS1+PS1+PM2+PP4)
41	CD40LG	c.267delC	Q90Sfs*6	LP (PVS1+PM2+PP4)
42	LIG4	c.833G>T	R278L	LP (PM1+PM2+PM3+PM5+PP3+PP4)
				c.1144_1145delCT L382Efs*5 LP (PVS1+PS1+PM2+PP4)
43	CARD11	c.155T>C	I52T	LP (PS2+PM2+PP4)
44	PIK3CD	c.3061G>A	E1021K	P (PS1+PS3+PM2+PP3+PP4)
45	IL10RA	c.350G>A	R117H	P (PS1+PM1+PM2+PP3+PP4)
46	IL10RA	c.537G>A	T179T	P (PS1+PS3+PM2+PP3+PP4)
47	IL10RA	c.787C>T	R263X	LP (PVS1+PM2+PP4)
48	IL10RA	c.301C>T	R101W	P (PS1+PS3+PM1+PM2+PP3+PP4)
49	CXCR4	c.1001G>A	R334Q	VUS (PM2+PP3+PP4:BS4)

ACMG: American College of Medical Genetics and Genomics; P/LP: Pathogenic/likely pathogenic; VUS: Variants of uncertain significance.

**Table 2 Clinical features and growth parameters among three groups of patients**

	AP	M-IBD	NM-IBD	P value
Total number	40	44	29	
Male (%)	25 (62.50)	27 (61.36)	21 (72.41)	0.591
Median onset age (mo, IQR)	4.44 (1.25,11.76)	0.51 (0.04,1.79) <sup>1</sup>	3.99 (3.00,9.00)	< 0.001
Diarrhea (≥ 8 times/d, %)	10 (25.00) <sup>1</sup>	38 (86.40) <sup>1</sup>	17 (58.60)	< 0.001
Diarrhea with blood (%)	30 (75.00)	29 (65.90)	24 (82.80)	0.320
Perianal disease (%)	0 (0)	35 (79.54) <sup>1</sup>	7 (24.14)	0.005
Fever (%)	2 (5.00) <sup>1</sup>	28 (63.63)	12 (41.38)	< 0.001
Death (%)	0 (0.00)	9 (20.45)	1 (3.45) <sup>2</sup>	0.001
Z scores for weight (mean ± SD)	-0.05 ± 0.21 <sup>1</sup>	-1.95 ± 0.26	-1.15 ± 0.29	< 0.001
Z scores for height (mean ± SD)	0.19 ± 0.19 <sup>1</sup>	-1.95 ± 0.25	-1.32 ± 0.31	< 0.001
Z scores for BMI (mean ± SD)	-0.21 ± 0.24 <sup>2</sup>	-1.30 ± 0.28	-0.52 ± 0.27	0.026

<sup>1</sup>Compared with the other two groups, adjusted *P* value < 0.05.

<sup>2</sup>Compared with the monogenic inflammatory bowel disease (M-IBD) group, adjusted *P* value < 0.05. AP: Allergic proctocolitis; M-IBD: Monogenic inflammatory bowel disease; NM-IBD: Non-monogenic inflammatory bowel disease; IQR: Interquartile range; SD: Standard deviation; BMI: Body mass index.

the diagnosis of IO-IBD (Table 4). We further investigated these four indicators using ROC curve analysis to explore their diagnostic value. The areas under the curves (AUCs) and the cut-off values are shown in Figure 1A and B. For WBC, the AUC was 0.78 (95%CI: 0.69-0.86, *P* < 0.001) and the cut-off value was  $11.96 \times 10^9/L$  (sensitivity: 0.66; specificity: 0.88). For ESR, the AUC was 0.81 (95%CI: 0.73-0.89, *P* < 0.001), and the cut-off value was 12.50 mm/h (sensitivity: 0.70; specificity: 0.90). For TNF- $\alpha$ , the AUC was 0.80 (95%CI: 0.72-0.89, *P* < 0.001) and the cut-off value was 17.40 pg/mL (sensitivity: 0.78; specificity: 0.88). For albumin, the AUC was 0.88 (95%CI: 0.81-0.94, *P* < 0.001), and the cut-off value was 36.50 g/L (sensitivity: 0.80; specificity: 0.90). Based on the results, WBC and ESR had a low sensitivity for recognizing IO-IBD. While TNF- $\alpha$  and albumin level had a higher sensitivity and specificity, and we speculated they may be used for early identifying IO-IBD patients.

**Clinical indicators for risk of M-IBD:** Binary logistic regression analysis was performed using the parameters with significant differences between the M-IBD group and the NM-IBD group [including median onset age, diarrhea (> 8 times/d), perianal disease, CRP, IL-10, and ferritin levels]. The results showed that very early onset age

**Table 3 Results of blood test and inflammatory factors among three groups of patients (median, interquartile range)**

	AP	M-IBD	NM-IBD	P value
WBC ( $\times 10^9$ )	8.77 (6.78, 10.75) <sup>1</sup>	14.88 (9.84, 19.37)	13.77 (8.87, 18.51)	< 0.001
Hemoglobin (mg/L)	119 (115, 126) <sup>1</sup>	98 (90, 113)	100 (80.50, 116)	< 0.001
PLT ( $\times 10^9$ )	304 (254, 383) <sup>1</sup>	447 (306.75, 591.50)	477 (323.50, 601.50)	0.002
CRP (mg/L)	1 (0.20, 1.00) <sup>1</sup>	48 (7.98, 75.23) <sup>1</sup>	21 (1.00, 44.35)	< 0.001
ESR (mm/h)	6 (4, 8) <sup>1</sup>	18.50 (8, 39)	21 (6, 42)	< 0.001
TNF- $\alpha$ (pg/mL)	14.60 (10.68, 17.03) <sup>1</sup>	21.50 (17.58, 27.88)	22.90 (14.48, 61.28)	< 0.001
IL-6 (pg/mL)	2.85 (2.00, 3.35) <sup>1</sup>	21.00 (10.20, 50.30)	14.55 (6.81, 30.00)	< 0.001
IL-10 (pg/mL)	500 (5, 5)	85.50 (42.75, 127.25) <sup>1</sup>	6.37 (5.00, 14.80)	< 0.001
Ferritin (ng/mL)	22.90 (14.90, 37.05)	55.90 (36.10, 231.20) <sup>1</sup>	15.50 (10.60, 27.72)	< 0.001
Albumin (g/L)	41 (38, 43) <sup>1</sup>	31 (27, 35)	33 (27.00, 36.50)	< 0.001
Serum iron ( $\mu$ mol/L)	8.00 (5.90, 10.20)	3.90 (2.60, 6.88)	5.70 (4.35, 11.38)	0.069

<sup>1</sup>Compared with the other two groups, adjusted *P* value < 0.05. AP: Allergic proctocolitis; M-IBD: Monogenic inflammatory bowel disease; NM-IBD: Non-monogenic inflammatory bowel disease; WBC: White blood cells; PLT: Platelets; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; TNF: Tumor necrosis factor; IL: Interleukin.

**Table 4 Binary logistic analysis for identifying risk factors for infantile-onset inflammatory bowel disease**

	AP (median, IQR)	IO-IBD (median, IQR)	Adjusted OR (95%CI)	P value
WBC ( $\times 10^9$ )	8.77 (6.78, 10.75)	14.12 (8.85, 18.27)	1.19 (1.01, 1.40)	0.040
ESR (mm/h)	6 (4, 8)	19.00 (8.00, 39.00)	1.10 (1.01, 1.20)	0.037
TNF- $\alpha$ (pg/mL)	14.60 (10.68, 17.03)	21.65 (17.33, 29.45)	1.25 (1.05, 1.50)	0.013
Albumin (g/L)	41 (38, 43)	32 (27, 36)	0.86 (0.74, 1.00)	0.048

Binary logistic analysis showed that white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), tumor necrosis factor (TNF)- $\alpha$ , and albumin in peripheral blood could be used to identify the infantile-onset inflammatory bowel disease from AP if a child had diarrhea and hematochezia in early childhood. Higher WBC, ESR, and TNF- $\alpha$ , as well as lower level of albumin suggested a higher probability of inflammatory bowel disease. AP: Allergic proctocolitis; IO-IBD: Infantile-onset inflammatory bowel disease; IQR: Interquartile range; OR: Odds ratio; CI: Confidence interval; WBC: White blood cells; ESR: Erythrocyte sedimentation rate; TNF: Tumor necrosis factor.

(OR = 3.47, 95%CI: 1.29-9.29, *P* = 0.013], presence of perianal diseases (OR = 11.42, 95%CI: 1.71-76.23, *P* = 0.012), and high levels of serum ferritin (OR = 1.14, 95%CI: 1.06-1.24, *P* = 0.001) and IL-10 (OR = 1.04, 95%CI: 1.01-1.08, *P* = 0.005) were the main risk factors for M-IBD diagnosis (Table 5). Because the *IL-10RA* mutations in this study accounted for a dominant proportion (79.6%) in the M-IBD group, to avoid bias caused by sample selection, we further compared the above high-risk factors among patients with *IL-10RA* mutations or non-*IL-10RA* mutations in the M-IBD group, and those without any disease-causing mutations in the NM-IBD group. The results showed that only serum ferritin could discriminate M-IBD and NM-IBD regardless of genotypes. The level of serum ferritin was significantly lower in the NM-IBD group than in the other two groups (Table 6) and could be used as an indicator for recognizing IO-IBD children with genetic mutations. The ROC curve analysis was also performed to explore the indicators for the diagnosis of M-IBD. Using gene sequencing results as the gold standard, all IO-IBD patients were included in the analysis. The AUC of ferritin for diagnosing M-IBD was 0.94 (95%CI: 0.87-1.00, *P* < 0.001), with a cut-off value of 28.20 ng/mL (sensitivity: 0.93; specificity: 0.92; Figure 1C), which showed high diagnostic value. Besides, we further analyzed the value of serum IL-10 level and onset age for early noticing *IL-10RA* mutation in M-IBD patients. The AUC for IL-10 was 0.91 (95%CI: 0.86-0.99, *P* < 0.001), and the cut-off value was 33.05 pg/mL (sensitivity: 1.00; specificity: 0.84); the AUC for onset age was 0.87 (95%CI: 0.78-0.96, *P* < 0.001), and the cut-off value was 0.21 mo (sensitivity: 0.82; specificity: 0.94; Figure 1D and E). These results indicated that the two indicators had high diagnostic value and may be

**Table 5 Binary logistic analysis for identifying risk factors for monogenic inflammatory bowel disease**

	M-IBD (median, IQR)	NM-IBD (median, IQR)	Adjusted OR (95%CI)	P value
Ferritin (ng/mL)	55.90 (36.10, 231.20)	15.30 (9.40, 27.60)	1.14 (1.06, 1.24)	0.001
IL-10 (pg/mL)	85.20 (43.30, 126.50)	5.00 (5.00, 9.11)	1.04 (1.01, 1.08)	0.005
Median onset age (mo)	0.51 (0.04, 1.79)	3.99 (3.00, 9.00)	3.47 (1.29, 9.29)	0.013
Perianal disease (%)	35 (79.50)	7 (24.10)	11.42 (1.71, 76.23)	0.012

Binary logistic analysis showed that higher levels of ferritin and interleukin (IL)-10 in peripheral blood, earlier onset age, and presence of perianal disease suggested that inflammatory bowel disease children may have the gene mutation. However, most of the mutations (79.6%) in this study occurred in *IL-10RA*, and in order to avoid the bias, we did further analyses as showed in Table 6. M-IBD: Monogenic inflammatory bowel disease; NM-IBD: Non-monogenic inflammatory bowel disease; IL: Interleukin; IQR: Interquartile range; OR: Odds ratio; CI: Confidence interval.

**Table 6 Comparison of risk factors among infantile-onset inflammatory bowel disease patients with or without *IL-10RA* mutation and non-monogenic inflammatory bowel disease (median, interquartile range)**

	<i>IL-10RA</i> M-IBD	Non- <i>IL-10RA</i> M-IBD	NM-IBD	P value
Ferritin (ng/mL)	55.90 (53.12, 74.73)	45.25 (16.58, 68.70)	15.30 (14.55, 17.95) <sup>1</sup>	< 0.001
IL-10 (pg/mL)	85.20 (66.20, 125.00) <sup>1</sup>	8.90 (5.00, 85.20)	5.00 (5.00, 9.11)	< 0.001
Median onset age (mo)	0.04 (0.00, 0.08) <sup>1</sup>	2 (0.41, 4.08)	3.99 (3, 9)	< 0.001
Perianal disease (%)	32 (91.43) <sup>1</sup>	3 (33.33)	7 (24.14)	0.002

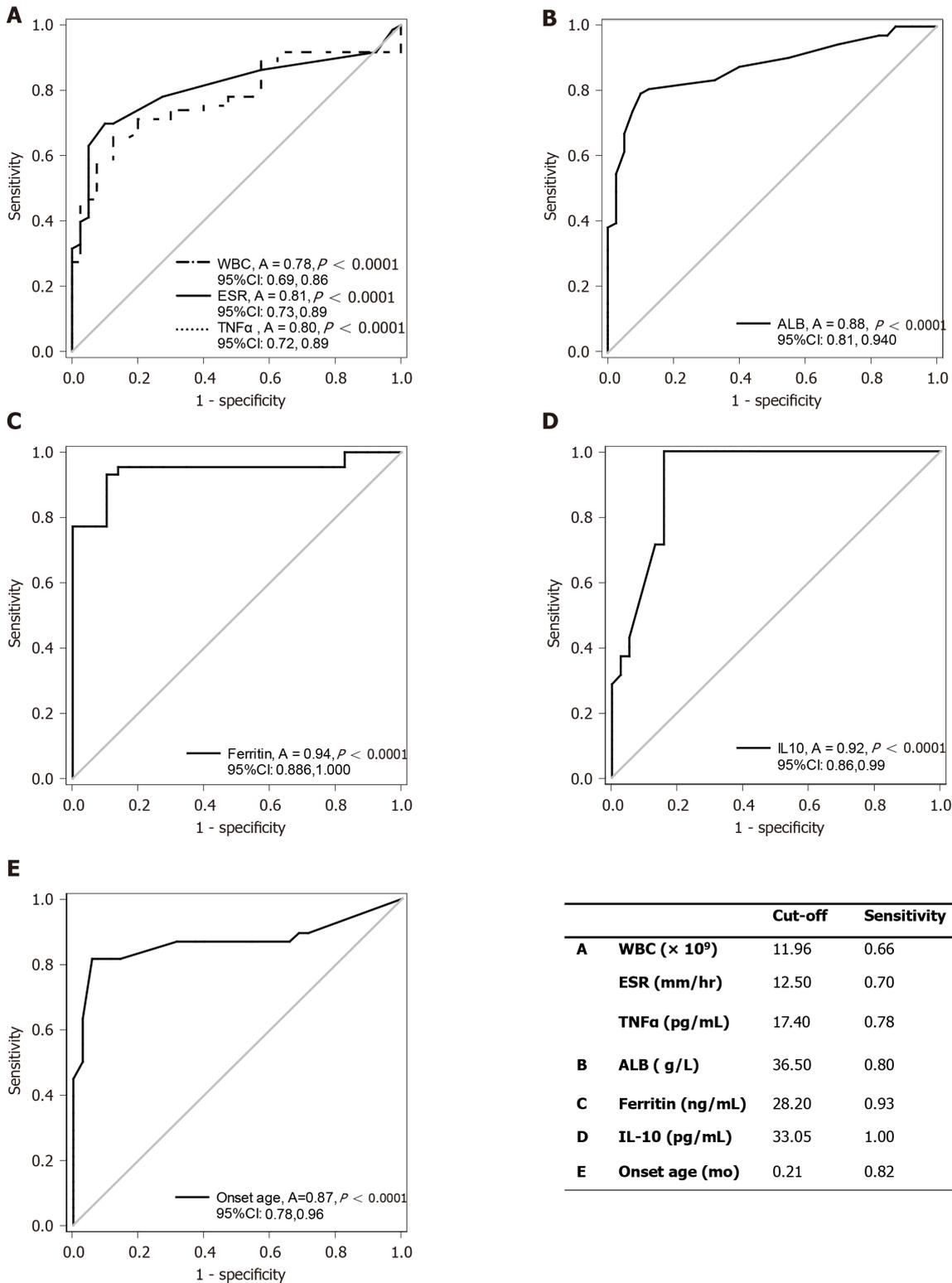
<sup>1</sup>Compared with the other two groups, adjusted P value < 0.01. M-IBD: Monogenic inflammatory bowel disease; NM-IBD: Non-monogenic inflammatory bowel disease; IL: Interleukin.

used in clinical practice. All above results suggested that, when serum ferritin levels were higher than 28.20 ng/mL, it might indicate the existence of pathogenic gene mutations in IO-IBD patients. If the infant got IO-IBD within 1 wk after the birth or their serum IL-10 levels were higher than 33.05 pg/mL, it might strongly suggest the presence of *IL-10RA* gene mutations.

## DISCUSSION

Approximately 15% of pediatric patients were categorized as having VEO-IBD<sup>[2]</sup>, and approximately 1% of pediatric patients developed symptoms during infancy (IO-IBD). Over the past decade, the prevalence of VEO-IBD had increased by more than 50%<sup>[6,7]</sup>. Increasing studies believed that genetic factors, such as monogenic diseases, play important roles in the development of VEO-IBD, especially IO-IBD. Currently, there is no epidemiological data for IO-IBD patients in China. However, in the past 5 years, more than 200 cases of VEO-IBD have been reported by our hospital and other hospitals in China<sup>[8-13]</sup>, and more than half of the patients presented genetic defects. Among the 73 pediatric patients in this study, 49 (67.12%) carried disease-related gene mutations, which was much higher than the reported proportions in European and American countries<sup>[14,15]</sup> but similar to the proportion reported by Huang *et al*<sup>[10]</sup>, suggesting that the presence of monogenic diseases is more common in Chinese VEO-IBD patients.

A total of ten mutated genes were identified in this study, among which the *IL-10RA* mutation was the most common (39/49). This finding was similar to those reported by other researchers in China<sup>[8]</sup>, while *IL-10RB* and *IL-10* mutations were not observed. Among the 14 *IL-10RA* variants, ten have been reported previously, among which c.537G>A, c.493C>T, c.436delC, c.299T>G, and c.191A>G mutations were observed in children only in China or other Asian countries<sup>[11,16,17]</sup> but have not been reported in Western countries, suggesting a genetic founder effect in *IL-10RA*. The four identified novel variants were c.109G>T, c.302G>A, c.569T>G, and c.787C>T, which were likely pathogenic mutations according to the ACMG guidelines. In this study, the most common *IL-10RA* mutation locus was c.301C>T, followed by c.537G>A, which was



**Figure 1 Receiver operating characteristic curve analysis for diagnostic indicators.** A and B: Parameters that may be used to distinguish allergic proctocolitis from infantile-onset inflammatory bowel disease (IO-IBD). The increased peripheral white blood cell count, erythrocyte sedimentation, rate and tumor necrosis factor (TNF)- $\alpha$ , as well as the reduced albumin levels were risk factors for the diagnosis of IO-IBD, and TNF- $\alpha$  and albumin levels had higher sensitivity and specificity; C: Serum ferritin level has high diagnostic value in discriminating IO-IBD patients with gene mutation (M-IBD) or without (NM-IBD) when serum ferritin levels are above 28.20 ng/mL. It might indicate the existence of pathogenic gene mutations in IO-IBD patients; D and E: Serum interleukin (IL)-10 level and onset age can be used to screen *IL-10RA* mutation in M-IBD patients. If an infant got IO-IBD within a week in life or a patient's serum IL-10 levels were higher than 33.05 pg/mL, the presence of *IL-10RA* gene mutations was strongly suggested. WBC: White blood cell; ESR: Erythrocyte sedimentation rate; TNF: Tumor necrosis factor; ALB: Albumin; IL: Interleukin; CI: Confidence interval.

consistent with the previous reports in Asian children<sup>[8]</sup>. Notably, c.537G>A mutation was located in exon 4, which was always missed in the reports as it was mistaken for a synonymous mutation; however, it had been confirmed as a splicing mutation that has a deleterious effect on IL-10 receptor<sup>[41]</sup>. For IBD children who are highly suspected of having abnormal IL-10 signaling pathways, especially those who have a heterozygous *IL-10RA* mutation after gene sequencing, clinicians should be aware of the sequencing report and not to miss the c.537G>A mutation.

In addition to *IL-10RA*, the other eight genetic mutations that could cause immunodeficiency diseases were also observed in this study. These included *CYBB* mutation, which caused X-linked chronic granulomatous disease<sup>[18]</sup>, *IKBK* mutation, which caused ectodermal dysplasia and immunodeficiency<sup>[19,20]</sup>, *PIK3CD* mutation, which caused activated phosphoinositide 3-kinase  $\delta$  syndrome<sup>[21]</sup>, *CD40LG* mutation, which caused X-linked hyper-IgM syndrome<sup>[22]</sup>, *SLC37A4* mutation, which caused glycogen storage disease type 1B<sup>[23]</sup>, *LIG4* mutation, which caused LIG4 syndrome<sup>[24]</sup>, *WAS* mutation, which caused Wiskott-Aldrich syndrome<sup>[25]</sup>, and *CARD11* mutation, which caused immunodeficiency type 11B with atopic dermatitis<sup>[26]</sup>. These diseases have been confirmed to cause IBD-like colitis in infants, such as recurrent diarrhea, hematochezia, infections, perianal abscess, and growth retardation.

Because of an insufficient understanding of IO-IBD, especially that caused by monogenic diseases, most patients could not be diagnosed as early as possible. It has been reported that the median time from the occurrence of symptoms to disease diagnosis was approximately 6 mo<sup>[15]</sup>. The most common misdiagnosis was allergic diseases, therefore, this study included a group of age-matched patients who were admitted to the hospital due to diarrhea and hematochezia and were eventually diagnosed as allergic proctocolitis. Although the AP children had diarrhea, hematochezia, or other similar symptoms with the IBD children, there was less onset of failure to thrive, perianal lesions, and death in AP patients; meanwhile, the daily frequency of diarrhea was significantly higher in IO-IBD children than in AP children. Inflammatory markers in peripheral blood such as WBC, CRP, ESR, PLT, TNF- $\alpha$ , and IL-6 in IO-IBD children were significantly higher than those in AP children, while the nutritional indicators such as albumin and hemoglobin were lower in IBD patients than in AP patients. Logistic regression analysis further confirmed that increased levels of WBC, ESR, and TNF- $\alpha$ , as well as lower serum albumin level were associated with a higher likelihood of IBD. Although it has been reported that hematological examinations had little value in the differential diagnosis of food allergy and IBD<sup>[15]</sup>, the ROC curve analysis in this study showed that TNF- $\alpha$  and serum albumin level had moderate value for differentiating IBD from allergic disease. Moreover, the high sensitivities and specificities of cut-off values for TNF- $\alpha$  and serum albumin may help clinicians, especially those in areas with limited medical resources, to identify IO-IBD patients earlier.

Genetic diagnosis is currently the gold standard for the diagnosis of IO-IBD caused by gene defects. However, the economic and time costs of this detection are relatively high in China. Most parents are not willing to choose this method at initial visits, and this would delay the diagnosis and treatment. The binary logistic regression analysis showed the serum ferritin (OR = 1.14) and IL-10 (OR = 1.04) may indicate the presence of gene defects in IO-IBD children; however, in view of the high proportion of *IL-10RA* mutations in our cohort, we further compared these factors among patients with *IL-10RA* mutations, those with non-*IL-10RA* mutations, and those without any disease-causing mutations. The final results showed that serum ferritin was abnormally elevated in IO-IBD patients with gene mutations and was not affected by the genotypes. Further ROC curve analysis showed that serum ferritin might be a valuable diagnostic indicator for monogenic IBD. Serum ferritin was acknowledged as an inflammatory marker, which would reflect the acute and chronic inflammatory state in infectious diseases, immunological diseases, hematological diseases, and malignant diseases<sup>[27]</sup>. A variety of cells could synthesize and secrete ferritin, of which macrophages were the main source and could induce diseases<sup>[27]</sup>. The role of ferritin in inflammatory responses is still unclear. It was hypothesized that in the inflammatory state, the Toll-like receptor (TLR) 9 signaling pathway is activated, IL-1 $\beta$  and IL-18 levels increased, then macrophages were activated, and synthesized more ferritin, which could further amplify the inflammatory response through the TLR9 signaling pathway, thereby forming a positive feedback loop and resulting in continuous aggravation of inflammation<sup>[28-32]</sup>. Some researchers believed that serum ferritin could bind to T and B lymphocytes directly<sup>[32,33]</sup>, then inhibit T cell proliferation, B cell maturation, and immunoglobulin synthesis<sup>[33-35]</sup>. In addition, serum ferritin could also promote regulatory T cell differentiation through the IL-10 signaling pathway and play an immunoregulatory role<sup>[36]</sup>. In our study, the serum ferritin level was

significantly elevated in M-IBD children, but whether it plays a pro-inflammatory or immunomodulatory role needs further study.

Both in our study and other studies in China, *IL-10RA* is the most common mutated gene in Chinese VEO-IBD patients. We also found that compared to the patients with other gene mutations, patients with the *IL-10RA* deficiency had unique features. First, the age of onset was particularly early. The median onset age was 0.04-mo-old, while it was 2-mo-old in patients with other gene mutations, indicating that diarrhea and hematochezia existed from birth in *IL-10RA* mutated children. Second, serum IL-10 level in these patients was particularly high. IL-10 is an important anti-inflammatory factor secreted by a variety of immune cells. After binding to IL-10R, IL-10 can activate a series of cascade reactions to maintain immune homeostasis by inhibiting inflammatory factors such as TNF- $\alpha$ <sup>[37]</sup>. IL-10R is composed of two subunits, IL-10RA and IL-10RB, and IL-10RA only binds to IL-10<sup>[38]</sup>. It was previously believed that the increased expression of IL-10 in IBD patients indicated a good steroid response and prognosis<sup>[39]</sup>. However, when IL-10R is deficient, the anti-inflammatory function of IL-10 could not be achieved. Glocker *et al*<sup>[40]</sup> confirmed that human peripheral blood mononuclear cells (PBMCs) could secrete large amounts of TNF- $\alpha$  after stimulation with lipopolysaccharide (LPS) *in vitro*, but its level decreased significantly when exogenous IL-10 was added into the culture medium, while this phenomenon could not be observed in the PBMC culture supernatant from *IL-10RA* deficient children, indicating the important role of the IL-10 signaling pathway in anti-inflammatory processes. In our study, both high IL-10 and TNF- $\alpha$  levels were observed in patients with *IL-10RA* mutation, which indicated that even such a high level of IL-10 could not inhibit TNF- $\alpha$  release and alleviate the inflammation in these children. These findings were similar to the *in vitro* research mentioned above. Therefore, we speculated that IL-10 level could be used as an *in vivo* detective indicator to verify the function of novel mutations of the *IL-10RA* gene. In this study, ROC curve analysis also confirmed that serum IL-10 level had a high sensitivity and specificity for identifying the presence of *IL-10RA* mutations, which suggested that if IO-IBD patients have a remarkably elevated IL-10 level, the gene sequencing is strongly recommended to identify the existence of *IL-10RA* deficiency. In fact, we did further investigation by whole-genome sequencing (WGS) in two patients of this cohort, whose clinical manifestations were similar to those of *IL-10RA* mutated patients, especially the increased significantly IL-10 level but whole-exome sequencing only found heterozygous mutations in *IL-10RA*. However, the results of WGS showed that there was another heterozygous deletion of 333bp in *IL-10RA* which led to exon 1 absence. This result further supported that serum IL-10 level may help distinguish *IL-10RA* mutated patients.

IO-IBD patients, especially those caused by gene mutations, are rare. Although the cohort included in this study was larger than many published studies, the absolute number was not large, which may lead to statistical bias. Besides, we needed further prospective studies to evaluate the diagnostic value of the indicators identified in this study.

## CONCLUSION

Children with IO-IBD have a higher proportion of Mendelian diseases. For Chinese Han pediatric patients, *IL-10RA* mutation is the most common pathogenic gene. A complete medical history and clinical evaluation are necessary to differentiate IBD and allergic proctocolitis earlier. Diarrhea frequency, body weight, height, serum albumin, and TNF- $\alpha$  level could help distinguish IO-IBD and allergy. Elevated serum ferritin level could be used for identifying IO-IBD caused by gene mutations, and the remarkably increased serum IL-10 level strongly suggests the presence of *IL-10RA* gene mutation. These clinical characteristics and hematological parameters would help to shorten the time of diagnosis so that the patients are able to receive timely and correct treatment and then reduce social and financial burdens.

## ARTICLE HIGHLIGHTS

### Research background

Infantile-onset inflammatory bowel disease (IO-IBD) causes severe clinical manifestations and responds poorly to traditional inflammatory bowel disease (IBD)

treatments. At present, there are no simple and reliable laboratory indicators for early screening IO-IBD patients, especially those in whom the disease is caused by monogenic diseases.

### Research motivation

Because of an insufficient understanding of IO-IBD, especially that caused by monogenic diseases, most patients could not be diagnosed as early as possible. It is hard to persuade the parents to accept endoscopic examination or genetic diagnosis at initial visits, especially those in areas with limited medical resources.

### Research objectives

We intended to search for early diagnostic indicators for IO-IBD children with or without gene mutations, in order to shorten the diagnosis time, reduce medical costs, simplify the analysis of next generation-sequencing, and administer targeted intervention as early as possible.

### Research methods

A retrospective analysis was performed in 73 patients with IO-IBD admitted to our hospital in the past 5 years. Based on the next-generation sequencing results, they were divided into either a monogenic IBD group (M-IBD) or non-monogenic IBD group (NM-IBD). Forty age-matched patients with allergic proctocolitis (AP) were included as a control group. The clinical manifestations and the inflammatory factors in peripheral blood were evaluated. Logistic regression analysis and receiver operating characteristic (ROC) curve analysis were performed to find the screening factors and cut-off values of IO-IBD as well as monogenic IO-IBD.

### Research results

Among the 44 M-IBD patients, 35 carried *IL-10RA* mutations, and the most common mutations were c.301C>T (p.R101W, 30/70) and c.537G>A (p.T179T, 17/70). Patients with higher serum tumor necrosis factor (TNF)- $\alpha$  value were more likely have IBD [odds ratio (OR) = 1.25, 95% confidence interval (CI): 1.05-1.50,  $P = 0.013$ ], while higher serum albumin level was associated with lower risk of IBD (OR = 0.86, 95% CI: 0.74-1.00,  $P = 0.048$ ). The cut-off values of TNF- $\alpha$  and albumin were 17.40 pg/mL (sensitivity: 0.78; specificity: 0.88) and 36.50 g/L (sensitivity: 0.80; specificity: 0.90), respectively. The increased ferritin level was indicative of a genetic mutation in IO-IBD patients. Its cut-off value was 28.20 ng/mL (sensitivity: 0.93; specificity: 0.92). When interleukin (IL)-10 level was higher than 33.05 pg/mL (sensitivity: 1.00; specificity: 0.84), or the onset age was earlier than 0.21 mo (sensitivity: 0.82; specificity: 0.94), the presence of disease-causing mutations in *IL-10RA* in IO-IBD patients was strongly suggested.

### Research conclusions

Serum TNF- $\alpha$  and albumin level could differentiate IO-IBD patients from allergic proctocolitis patients, and serum ferritin and IL-10 level are useful indicators for early diagnosing monogenic IO-IBD.

### Research perspectives

Using serum TNF- $\alpha$  and albumin level may contribute to distinguishing IO-IBD which needs further investigations, such as gastrointestinal endoscopy. High levels of serum ferritin and IL-10 can infer a monogenic disease in IO-IBD patients, especially the mutations in the *IL-10RA* gene. The diagnostic value of the indicators identified in this study should be evaluated by prospective studies.

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