ORIGINAL ARTICLE

Clinical Practice Study

Phenotypic and genotypic characterization of inflammatory bowel disease in children under six years of age in China

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Abstract

AIM
To analyze clinical differences between monogenic and nonmonogenic very-early-onset inflammatory bowel disease (VEO-IBD) and to characterize monogenic IBD phenotypically and genotypically *via* genetic testing.

METHODS
A retrospective analysis of children aged 0 to 6 years diagnosed with VEO-IBD in a tertiary hospital in southern China from 2005 to 2017 was performed. Clinical data for VEO-IBD patients were collected, and genetic characteristics were analyzed using whole exome sequencing or target gene panel sequencing.

RESULTS
A total of 54 VEO-IBD patients were included in this study. A diagnosis of Crohn's disease (CD) or CD-like intestinal manifestations accounted for 72.2% of the VEO-IBD cases. Nine patients (16.7%) were identified by genetic testing as having monogenic IBD. The median age of diagnosis in the monogenic group was younger than that of the nonmonogenic IBD group, at 18 mo (interquartile range (IQR): 4 to 78) and 43.5 mo (IQR: 3 to 173), respectively; the *P*-value was 0.021. The incidence of perianal disease in the monogenic group was higher than that in the

nonmonogenic group (P = 0.001). However, there were no significant differences between weight-for-age and height-for-age Z-scores between the two groups, and similar laboratory results were obtained for the two groups. Five patients were found to have *IL10* receptor mutation, two patients had chronic granulomatous disease, one patient had common variable immunodeficiency disease, and one patient had X-linked inhibitor of apoptosis protein deficiency.

CONCLUSION
A high proportion of monogenic IBD was observed in the VEO-IBD group, especially with disease onset before the age of 6 mo. Monogenic IBD and nonmonogenic IBD exhibited similar clinical features. Furthermore, next-generation sequencing played an important role in the diagnosis of monogenic IBD, and *IL10* receptor mutation was predominant in this cohort.

Key words: Monogenic; Very-early-onset inflammatory bowel disease; Primary immunodeficiency diseases; *IL10*; *IL10R*

Core tip: This study is the largest study to analyze clinical differences between monogenic and non-monogenic very-early-onset inflammatory bowel disease (VEO-IBD) in China. Moreover, we characterized monogenic IBD phenotypically and genotypically through genetic testing (whole exome sequencing and targeted gene panel sequencing). We found a high proportion of monogenic IBD in the VEO-IBD group, with the most common monogenic IBD being *IL10R* mutation. Monogenic IBD and nonmonogenic IBD showed similar clinical features. Next-generation sequencing played an important role in the diagnosis of monogenic IBD.

INTRODUCTION

Very-early-onset inflammatory bowel disease (VEO-IBD) refers to an IBD diagnosis established before the 6th year of life, including a subset of patients with disease onset before the age of 2 years, known as infantile-onset IBD (IO-IBD)[1]. VEO-IBD is rare, with an estimated incidence of 4.37 and a prevalence of 14 per 100000 children[2-4]. These patients present with abdominal pain, intestinal bleeding, diarrhea and malnutrition, similar to adults with IBD. In addition, growth failure is often observed[5,6]. However, VEO-IBD is considered to be a unique entity, and compared to adults with IBD, VEO-IBD children are more likely to present with extensive and treatment-resistant disease.

These patients may require the combined use of immunosuppressant and biologic agents and even early intestinal surgery. Although the IBD classification was recently modified to address the younger age group, this step may not be sufficient to accommodate the heterogeneous phenotypes of VEO-IBD[7]. The etiology of IBD is related to genetic and environmental factors as well as to the intestinal microbiome. Furthermore, VEO-IBD, especially IO-IBD, presents with IBD-like intestinal inflammation and shows a close association with primary immunodeficiency diseases (PIDs), defined as monogenic IBD[3].

More than 50 VEO-IBD-related genes have been reported to date, including those associated with *IL10* and *IL10* receptor mutation, immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome and X-linked inhibitor of apoptosis (XIAP) deficiency[3]. Nonetheless, the exact incidence of monogenic IBD remains unknown[8] due to variations in the genetic background of this disease. Most reports regarding monogenic IBD are based on small populations or case reports, with only a small number of genes investigated. To the best of our knowledge, this study reports the largest cohort of genetically screened patients with VEO-IBD from China.

MATERIALS AND METHODS

Study subjects
This study was approved by the Ethics Committee of the Children's Hospital of Zhejiang University School of Medicine. A total of 54 pediatric patients diagnosed with VEO-IBD in the Gastroenterology Department of Children's Hospital of Zhejiang University School of Medicine from October 2005 to May 2017 were included in this study. According to Porto criteria[9], Crohn's disease (CD) and CD-like intestinal inflammation present as noncontiguous aphthous or linear ulcers, primarily in the ileum or colon. Histologically, the disease is characterized by chronic focal inflammation with or without granulomas and perianal or fistulating disease. Ulcerative colitis (UC) or UC-like intestinal inflammation presents as continuous mucosal inflammation of the colon starting from the rectum, without small bowel involvement and without CD features. Patients with a definitive diagnosis of IBD with inflammation limited to the colon but without the typical features of UC and CD are classified as IBD of an unclassified type (IBDU).

The clinical characteristics of the pediatric patients, including sex, first symptom(s), time of onset of symptom(s), clinical features, surgical history, medication

history and family history, were collected from medical records.

The following were criteria for genetic testing: (1) patients with disease onset before 6 mo of age; and (2) patients with disease onset beyond 6 mo accompanied with severe perianal disease, severe malnutrition or growth failure, or resistance to treatment.

Whole exome sequencing and targeted gene panel sequencing

Genomic DNA was isolated from peripheral blood of the patients. For whole exome sequencing (WES), whole exome library preparation was based on modifications of a protocol using the Agilent SureSelect XT Human All Exon Kit. Sequencing was performed using the Illumina HiSeq 2500 platform.

For targeted gene panel sequencing (TGPS), target sequences were enriched using customized capture probe chips (SureSelect Inherited Disease Panel; Agilent Technologies), which included 4,503 genes known to cause inherited disorders. DNA probes were designed for exons and flanking 10 bp intronic sequences. Briefly, 1 μg of genomic DNA was fragmented into 200-300 bp lengths by the Covaris Acoustics system. The DNA fragments were subsequently processed by end-repairing, A-tailing and adaptor ligation, 4-cycle pre-capture polymerase chain reaction (PCR) amplification, and targeted sequence capture. The captured DNA fragments were eluted and amplified by 15-cycle postcapture PCR. The final products were sequenced with 150-bp paired-end reads using the Illumina HiSeq X Ten platform according to the standard manual.

Raw data were processed by the Illumina pipeline (version 1.3.4) for image analysis, error estimation, base calling and generating the primary sequence data. For quality control, Cutadapt (https://pypi.python.org/pypi/cutadapt) and FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) were used to remove 3'-/5'- adapters and low-quality reads, respectively. Clean short reads were mapped to the human genome (hg19) using Burrows Wheeler Aligner software (http://sourceforge.net/projects/bio-bwa/). SOAPsnp software (http: //soap.genomics.org.cn/) and SAM tools Pileup software (http://sourceforge.net/projects/samtools/) were used to detect single-nucleotide polymorphisms and small insertions and deletions.

Variants were annotated using ANNOVAR software (http://www.openbioinformatics.org/annovar/), which includes function implications (gene region, functional effect, mRNA GenBank accession number, amino acid change, cytoband) and allele frequencies in dbSNP, 1000 genomes (http://www.1000genomes.org), ESP6500 (http://evs.gs.washington.edu/EVS/) and ExAc (http://exac.broadinstitute.org/). Damaging missense mutations were predicted by SIFT (http://sift.bii.a-star.edu.sg/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and MutationTaster (http://www.mutationtaster.org/). Interpretation of variants was based on recommended standards from the American College of Medical Genetics and Genomics[1,2], and all variants were categorized as pathogenic, likely pathogenic, variants of unknown significance, likely benign or benign.

To validate the variants based on next-generation sequencing (NGS) and WES, PCR amplification and Sanger sequencing were performed for the sequence centers on variant genes.

Statistical analysis

The categorical variables were presented as number (percentage). The continuous variables of normal distribution were presented as mean ± SD, otherwise as median ± interquartile range (IQR). The normality test was performed by Shapiro-Wilk test. The difference in variables with normal/abnormal distribution between nonmonogenic and monogenic IBD were estimated by Student's *t*-test and Mann-Whitney test, respectively. The differences in categorical variables between non-monogenic and monogenic IBD were assessed using the chi-square test; however, if total sample size was less than 40 or the number in one cell was less than 5, Fisher's exact test was used for testing the difference. $P < 0.05$ was considered to be a statistically significant difference. All statistical analyses were conducted with SPSS 22.0 statistical software (SPSS Inc., IBM Corp., Armonk, NY, United States).

RESULTS

General characteristics of VEO-IBD patients

From October 2005 to May 2017, 136 pediatric patients were diagnosed with IBD at our hospital, among which 54 were diagnosed with VEO-IBD; thus, VEO-IBD patients accounted for 39.7% of pediatric IBD patients in our cohort. Among the 54 VEO-IBD patients, 37 were males and 17 females, for a male to female ratio of 2.18:1. Thirty-one VEO-IBD patients (57.4%) had disease onset before the age of 2 years.

Of the 54 patients with VEO-IBD, 72.2% had a diagnosis of CD or CD-like intestinal manifestations, 7.4% had a diagnosis of UC or UC-like intestinal manifestations, and 20.4% had a diagnosis of IBDU. Only one patient was the offspring of a consanguineous union, and his older sister also had similar symptoms during the neonatal period, though without a definitive diagnosis. None of the remaining patients had a positive family history of IBD or PID.

The median age of disease onset was 14 mo (IQR: 0 to 72 mo), and the median age of diagnosis was 35.5 mo (IQR: 3 to 173 mo). The median time to diagnosis was 9 mo (IQR: 0.4 to 104 mo). The Z-scores for median weight-for-age, height-for-age and body mass index -for-age were -1.69 (IQR: -4.69 to 2.2), -1.41 (IQR: -6.43 to 2.51) (*n* = 51) and 14 (IQR: 0 to 72) respectively.

Mortality

The overall mortality rate was 11.1% (6/54). The

Table 1 Clinical features of dead very-early-onset inflammatory bowel disease patients

Patient	Sex	Age of disease onset	Chief complaints	Perianal diseases	EIMs	Colonoscopy features	Disease cause death	Genetic diagnosis
1	M	Neonate	Diarrhea	Perianal abscess and fistula	Arthritis, oral ulcers	Pancolitis, ulcers and hyperplasia	Lymphoma	NA
2	M	2 mo	Diarrhea, bloody stools	None	None	Proctitis, linear and aphthous ulcers	Suspension of treatment	NA
3	M	1 yr 5 mo	Diarrhea, bloody stools	Perianal abscess	None	Pancolitis and terminal ilium ulcers and hyperplasia	Suspension of treatment	NA
4	F	3 yr 10 mo	Abdominal pain	None	None	Pancolitis, ulcers and cobble stone appearance of colon	Sepsis, multiple organ failure	NA
5	M	4 yr 11 mo	Diarrhea, bloody stools, fever, malnutrition	None	Rash, oral erosion	Pancolitis, erosion, ulcers and multiple hyperplasia lesions	Intestinal bleeding	NA
6	M	2 mo 13 d	Diarrhea, fever	Perianal abscess	None	Proctitis, longitudinal ulcer	Sepsis and shock	CGD

EIMs: Extraintestinal manifestations.

Table 2 General data of very-early-onset inflammatory bowel disease patients

	Monogenic IBD	Nonmonogenic IBD	*P* value
Patients	9	45	
Sex, M/F	8/1	28/17	
Median age of disease onset, in mo	1 (0, 72)	19.5 (0, 72)	0.008[a]
Median age of disease diagnosis, in mo	18 (4, 78)	43.5 (3, 173)	0.021[a]
Duration before diagnosis, in mo	6 (2, 29)	9 (0, 104)	0.668

[a]$P < 0.05$. IBD: Inflammatory bowel disease.

clinical conditions of these 6 patients are listed in Table 1. Among the 6 patients, 4 had a diagnosis of CD and 2 a diagnosis of IBDU. Moreover, 2 of the patients had disease onset before 2 years of age.

Clinical characteristics

Nine patients (16.7%) were diagnosed with monogenic IBD; among them, 6 were diagnosed with CD and 3 with IBDU. We also compared the clinical manifestations of monogenic and nonmonogenic IBD patients. The monogenic group was predominately male, with a male to female ratio of 8:1. Four patients had disease onset during the neonatal period, and two patients presented with symptoms before 1 year of age. The median age of disease onset for the monogenic patients was earlier than that for the nonmonogenic patients at 1 mo (IQR: 0 to 72) and 19.5 mo (IQR: 0 to 72) respectively (P = 0.008). Similarly, the median age of diagnosis for the monogenic group was earlier than that for the nonmonogenic group, at 18 mo (IQR: 4 to 78) and 43.5 mo (IQR: 3 to 17) respectively (P = 0.021). However, there was no significant difference in the median time from disease onset to diagnosis between the two groups (P = 0.668; Table 2).

The incidence of perianal disease in the monogenic group was higher than that in the nonmonogenic group (P = 0.001); when comparing weight-for-age and height-for-age Z-scores, no significant differences between the two groups were found. Moreover, laboratory findings were similar between the two groups. Mesalazine was more commonly used in the monogenic than in the nonmonogenic group (P = 0.009). A greater number of

patients underwent intestinal surgery in the monogenic group than in the nonmonogenic group; however, this difference was not statistically significant at P = 0.050. To date, more patients in the nonmonogenic group have died compared to the monogenic group (P = 0.000; Table 3).

Genetics

A total of 16 patients underwent WES or TGPS, 6 and 12 respectively (2 patients were tested by both WES and TGPS). Two patients underwent genetic testing (*IL10RA*) at another hospital. Nine patients were diagnosed with monogenic IBD. Seven of these patients were diagnosed by TGPS, and two were diagnosed by both methods. Five patients were diagnosed with *IL10R* mutation, two patients were diagnosed with chronic granulomatous disease (CGD) with *CYBB* mutation, and one patient was diagnosed with XIAP deficiency. One patient was diagnosed with common variable immune deficiency (CVID) with *TNFRSF13B* mutation. The clinical information for the 9 monogenic patients are listed in Table 4, and the genetic phenotypes of the 18 patients are provided in Table 5.

IL10 and IL10R mutations

Patient 1 was a male offspring from a consanguineous union, with disease onset during the neonatal period. The first manifestation was a perianal abscess followed by recurrent fever, bloody stools, anal fistula and anal abscess formation, and skin infections. Colonoscopic findings included strictures of the colon near the splenic flexure, extensive polypoid hyperplasia, irregular ulcers,

Table 3 Comparison of clinical features of monogenic and nonmonogenic inflammatory bowel disease groups

	Nonmonogenic IBD	Monogenic IBD	*P* value
Diagnosis			
CD, %	75.0	50.0	
IBD-U, %	18.2	40.0	
UC, %	6.8	10.0	
Abdominal pain, *n*	25	2	0.142
Diarrhea, *n*	36	8	1.000
Bloody stools, *n*	26	8	0.131
Malnutrition, *n*	19	5	0.489
Growth failure, *n*	14	4	0.461
Oral ulcers, *n*	3	1	0.529
Persistent fever, *n*	3	0	1.000
Perianal diseases, *n*	9	7	0.001[a]
Body weight Z-score	-1.54 (-4.69, 2.20)	-2.04 (-3.66, 0.96)	0.303
Height Z-score	-1.38 (-6.43, 2.51)	-1.76 (-4.92, 0.46)	0.597
WBC, as [a]10^9	15.04 ± 1.72	12.53 ± 1.72	0.382
HGB, in g/L	103.02 ± 2.57	106.67 ± 5.00	0.496
PLT, as [a]10^{12}	454.60 ± 28.40	421.89 ± 54.97	0.632
CRP, in mg/L	51.16 ± 8.43	20.22 ± 2.43	0.668
ESR, in mm/h	29.22 ± 4.70	21.00 ± 4.56	0.923
ALB, in g/L	33.80 ± 1.10	36.60 ± 1.77	0.283
Steroid, *n*	36	6	0.399
Antibiotics, *n*	29	7	0.484
Mesalazine, *n*	32	2	0.009[a]
Immunosuppressants, *n*	25	8	0.075
Nutrition, *n*	33	5	0.425
Infliximab, *n*	14	4	0.461
Surgery, *n*	6	4	0.050
Death, *n*	5	1	0.000[a]

[a]$P < 0.05$. IBD: Inflammatory bowel disease.

and inflammatory tags. Esophagogastroduodenoscopy was normal. Preliminary immune tests and the serum IL10 level were normal. The patient underwent a colostomy at the age of 1 year and 1 mo due to recurrent diarrhea and perianal abscesses. At 2 years and 5 mo of age, he underwent transverse partial colectomy, small bowel internal fistula resection and anastomosis. The pathological evaluation revealed transmural inflammatory infiltrate and linear serpentine ulcerations. The patient also underwent incision, drainage and seton placement for multiple abscesses. He was resistant to treatment with antibiotics, steroids, total parenteral nutrition (TPN), enteral nutrition (EN) and infliximab. At the time of writing this manuscript, he is in clinical remission on a combination treatment of azathioprine and thalidomide. Genetic testing by both TGS and WES revealed the following homozygous *IL10RB* mutation: Chr21:34660499: c.737G>A, p.W246X. This mutation has not been reported in HGMDpro.

Patient 2 was a female with VEO-IBD of neonatal onset, with first symptoms of recurrent watery diarrhea and blood and mucous in stools followed by a perianal abscess, fistula formation and recurrent fever. The patient was initially treated with elemental formula due to suspected cow's milk allergy. She had her first colostomy at 1 year and 11 mo of age. Colonoscopy showed a cobble stone appearance and linear ulcerations in the colon. The pathological evaluation revealed classic CD features (strictures, transmural inflammatory infiltrate, linear serpentine ulcerations and noncaseating granulomas). Preliminary immune tests were normal, and the serum IL10 level was slightly elevated. She was resistant to treatment with steroids, mesalazine, antibiotics, immunosuppressants and infliximab. At 3 years and 1 mo of age, she underwent repair for a small bowel perforation, pancolectomy and ileal pouch-anal anastomosis. TGPS revealed the following compound heterozygous *IL10RA* mutations: Chr11:117864125: c.537G>A, p.T179T and Chr11:117860269: c.301C>T, p.R101W. The two mutations were derived from the mother and father, respectively. Both mutations are reported in HGMDpro[10,11].

Patient 3 was a male with disease onset at 1 mo of age. The patient had symptoms of recurrent diarrhea with mucous and blood, oral ulcers, anal fistula, obvious malnutrition and growth failure. Initial immune tests were normal, and the IL10 level was elevated at 85.5 pg/mL (normal range from 2.6 to 4.9 pg/mL). Colonoscopy showed deep ulcers in the rectum. The pathology evaluation showed nonspecific inflammation of the colon. The traditional treatments of antibiotics, TPN, EN and steroids were unsuccessful. Genetic testing at an outside hospital detected the following compound heterozygous *IL10RA* mutations: Chr11:117860269, c.301C>T, p.R101W, Chr11:117993268: c.470A>G, p.Y157C. The two mutations were from his mother and father, respectively. His older sister is a hereditary carrier.

Table 4 Clinical characteristics of monogenic very-early-onset inflammatory bowel disease patients

Patient	Sex	Disease onset	Z-score of height	Z-score of body weight	Chief complaints	Others	Disease locations and behavior	Clinical and genetic diagnosis	Intestinal surgery	Treatment	Prognosis
1	M	< 1 mo	0.22	-1.18	Diarrhea, bloody stools	Pyoderma	L2, P, B2B3	CD, IL10RB mutation	Colostomy	Steroid, IFX, thalidomide, 6-MP/MTX/AZA	Remission
2	F	4 mo	-1.76	-0.96	Diarrhea, bloody stools	Pyoderma	L2,L4b, P, B2B3	CD, IL10RA mutation	Intestinal perforation repair, colostomy and J-POUCH	Steroid, mesalazine, IFX, MTX/CsA	Remission
3	M	1 mo+	-0.79	-2.21	Diarrhea, bloody stools	Elevation of ALT	L2, P, B2	CD, IL10RA mutation	None	None	Waiting HSCT
4	M	1 mo+	-3.11	-3.66	Diarrhea, bloody stools	None	L2, P, B1	IBDU, IL10RA mutation	None	Antibiotics	Give up
5	M	11 d	-4.92	-3.38	Diarrhea, bloody stools	NEC	L2,L4b, P, B1	CD, IL10RA mutation	None	Steroids, AZA	Give up
6	M	< 1 mo	0.46	-1.08	Diarrhea, bloody stools	Epilepsy	L2, P, B1	IBDU, CGD	None	None	Remission
7	M	< 1 mo	-0.84	-2.04	Persistent fever	Intestinal malrotation, elevation of ALT	L2, P, B1	IBDU, CGD	None	None	Dead
8	M	9 mo	-4.63	-2.95	Diarrhea, bloody stools	Hepatosplenomegaly	L2, B1	CD, CVID	Intestinal resection and anastomosis	Steroid, 6MP, mesalazine, thalidomide	Partial remission
9	M	5 yr 11 mo	-2.2	-1.57	Abdominal pain, diarrhea, bloody stools	None	L2, L4b	CD, XIAP deficiency	None	DXM, IFX, 6MP/AZA	Remission

6-MP: 6-mercaptopurine; ALT: Alanine aminotransferase; AZA: Azathioprine; CGD: Chronic granulomatous disease; CsA: Cyclosporin A; CVID: Common variable immunodeficiency; HSCT: Hematopoietic stem cell transplantation; IFX: Infliximab; IL10R: Interleukin 10 receptor; MTX: Methotrexate; XIAP: X-linked inhibitor of apoptosis protein.

At the time of this writing, he is awaiting hematopoietic stem cell transplantation (HSCT). The pathogenic mutation c.301C>T is reported in HGMDpro[10], and c.470A>G was predicted by software mentioned in the methods section to be the likely pathogenic gene.

Patient 4 was a male with first symptoms of recurrent perianal abscess followed by watery diarrhea with scant fresh blood, rash and severe malnutrition. Immune tests were normal, and the IL10 level was 47.2 pg/mL. Colonoscopy showed multiple irregular ulcers at the sigmoid colon and rectum with polypoid hyperplasia and ulcers of the anus. Pathological examination revealed nonspecific findings. TGPS revealed the following homozygous mutation of IL10RA: Chr11:117860269: c.301C>T, p.R101W. The mutation was verified in his mother. The mutation is reported in HGMDpro to be pathogenic[10].

Patient 5 was a male with disease onset during the neonatal period. He initially presented with bloody diarrhea and was diagnosed with necrotizing enterocolitis; however, his symptoms persisted after surgery. This patient subsequently presented with a perianal fistula and severe malnutrition. Colonoscopy showed polypoid hyperplasia at the colon and ulcers at the rectum. This patient was treated with mesalazine and enemas. Genetic testing at an outside hospital revealed the following compound heterozygous IL10RA mutations: Chr11:117860269: c.301C>T, p.R101W, c.350G>A, p.R117H. The two mutations are reported in the literature to be pathogenic[10,12].

CGD

Patient 6 was a male with disease onset during the neonatal period. He presented with recurrent diarrhea, bloody stools and a perianal abscess. Preliminary immune tests were normal. Colonoscopy showed pancolitis and a perianal abscess. TGPS revealed the following hemizygotic mutation of CYBB: ChrX:37658209: c.676C>T, p.R226X. The mutation originates from his mother and is reported in HGMDpro to be pathogenic. The mutation is a termination mutation, which can alter the function of the encoded protein[13].

Patient 7 was a male diagnosed with intestinal malrotation during the neonatal period. He developed diarrhea and fever during hospitalization. The immunoglobulin level and CD3 (46.81%), CD4 (29.86%), natural killer cell (7.79%), CD4/CD8 (1.86) levels were normal. Colonoscopy showed a longitudinal ulcer at the rectum. Soon thereafter, the patient developed multiple organ failure due to severe infection. TGPS

He presented with pneumonia, perianal abscess and liver dysfunction; he developed diarrhea and fever during hospitalization. The immunoglobulin level and CD3 (46.81%), CD4 (29.86%), natural killer cell (7.79%), CD4/CD8 (1.86) levels were normal. Colonoscopy showed a longitudinal ulcer at the rectum. Soon thereafter, the patient developed multiple organ failure due to severe infection. TGPS

Table 5 Genetic testing of very-early-onset inflammatory bowel disease patients

Patient	Sex	Age of disease onset	WES/TGPS	Genetic mutation	Location of mutation	Mutation of parents	Homo/Heterozygote	SIFT score/ prediction	Polyphen2 score/ prediction	MutationTaster score/ prediction
1	M	Neonate	Both	IL10RB	Chr21:34660499 c.737G>A p.W246X	Heterozygotic mutation of parents c.737G>A	Homozygote	-	-	6/D
2	F	4 mo	TGPS	IL10RA	Chr11:117860269 c.301C>T p.R101W	Heterozygotic mutation of father c.301C>T mother c.537G>A	Compound heterozygote	0/D	1/D	101/D
					Chr11:117864125 c.537G>A p.T179T			1/T	-	-
3	M	1 mo+	TGPS	IL10RA	Chr11:117860269 c.301C>T p.R101W	Heterozygotic mutation of father c.301C>T mother c.470A>G	Compound heterozygote	0/D	1/D	101/D
					Chr11:117864058 c.470A>G p.Y157C (rs102750306)			0.001/D	1/D	194/N
4	M	1 mo+	TGPS	IL10RA	Chr11:117860269 c.301C>T p.R101W	Heterozygotic mutation of parents c.301C>T	Homozygote	0/D	1/D	101/D
5	M	Neonate	TGPS	IL10RA	Chr11:117860269 c.301C>T p.R101W	Heterozygotic mutation of father c.301C>T mother c.350G>A	Compound heterozygote	0/D	1/D	101/D
					c.350G>A p.R117H (rs199989396)			0.011/D	1/D	29/D
6	M	Neonate	TGPS	CYBB	ChrX:37658209 c.676C>T p.R226X (rs137854592)	Heterozygotic mutation of mother c.676C>T	Hemizygotic mutation	-	-	6/D
7	M	Neonate	TGPS	CYBB	ChrX:37642741 c.142-2A>G splicing	Heterozygotic mutation of mother c.142-2A>G	Hemizygotic mutation	-	-	-
8	M	9 mo	Both	TNFRSF13B	Chr17:16843819 c.452C>T p.P151L (rs200037919)	Heterozygotic mutation of father c.452C>T	Compound heterozygote	0.568/T	0.005/B	98/N
					Chr17:16852132 c.365G>A p.R122Q (rs755343222)	mother c.365G>A		0.485/T	0.136/B	43/N
9	M	5 yr 11 mo	TGPS	XIAP	ChrX:123022501 c.910G>T p.G304X	No mutation in parents	Hemizygotic mutation	-	-	6/D
10	F	5 yr 9 mo	TGPS	IL10RB	Chr21:34652146 c.421G>A p.E141K (rs387907326)	Heterozygotic mutation in father c.421G>A	Heterozygote	0.026/D	0.946/D	56/D
11	F	4 mo	WES	-						
12	M	8 mo	WES	-						
13	F	3 yr 3 mo	TGPS	-						
14	F	4 yr	TGPS	-						
15	M	1 yr 10 mo	WES	-						
16	F	4 yr 8 mo	WES	-						

TGPS: Targeted gene panel sequencing; WES: Whole exome sequencing.

revealed the following hemizygotic mutation of *CYBB*: ChrX:37642741: c.142-2A>G splicing. The mutation is reported as pathogenic in HGMDpro[14].

CVID

Patient 8 was a male who presented with jaundice and recurrent respiratory infections during the neonatal period. Immunoglobulin (Ig)G and IgM levels were below normal. He was treated with antibiotics and intravenous immunoglobulin. He gradually developed splenomegaly. At the age of 9 mo, this patient presented with recurrent bloody stools. Colonoscopy showed ulcers and polypoid hyperplasia, which eventually spread to involve the entire colon. Pathological examination of the intestinal biopsy showed nonspecific inflammatory changes. The patient underwent laparotomy, which revealed intestinal swelling and with fissure ulcers, and ulcers involving the tunica muscularis. Treatments with steroids, TPN, EN, mesalazine, infliximab and multiple immunosuppressants were unsuccessful. Both WES and TGPS showed the following compound heterozygous mutations in *TNFRSF13B*: Chr17:16843819: c.452C>T, p.P151L and Chr17:16852132:c.365G>A, p.R122Q. These mutations are reported to be associated with CVID, though they have not been reported in HGMDpro. Further tests are required to determine the effects of these mutations.

XIAP deficiency

Patient 9 was a male, aged 5 years and 11 mo, who presented with recurrent bloody stools, fever and abdominal pain. He had been diagnosed with hemophagocytic syndrome at 4 years of age. Colonoscopy showed blunting and flattening of villi at the terminal ileum. The entire colon showed irregular deep ulcers and aphthous ulcers. Capsule endoscopy revealed sporadic jejunal ulcers. Immune tests were normal. He did not respond to exclusive enteral nutrition therapy and oral steroids. He was treated with infliximab and 6-Mercaptopurine and is currently in clinical remission. His colonoscopy findings have also improved. TGPS revealed the following mutation associated with XIAP deficiency: ChrX:123022501: c.910G>T, p.G304X. The mutation was not acquired from his parents. This mutation has not been reported in HGMDpro.

DISCUSSION

VEO-IBD children are generally recognized as having more severe clinical symptoms, more extensive intestinal inflammation, higher rates of treatment resistances and rapid disease progression compared to patients with adolescence- or adult-onset IBD[15-17]. In contrast, several studies have reported that VEO-IBD patients are not prone to a more severe clinical course compared to those with adolescence-onset IBD[18]. However, geographic and/or ethnic differences can affect genetic background and therefore cause conflicting results among studies. Monogenic IBD typically presents early, with severe clinical features and is resistant to traditional therapy; a portion of VEO-IBD patients even require HSCT[19].

In the study by Jochen Kammermeier *et al*[16], a high rate of IBDU (71%) in patients younger than 2-years-old was observed, among whom 29% were offspring from consanguineous unions, 18% had a positive family history of IBD in a first-degree relative, and 31% were diagnosed with monogenic IBD. In that study, high rates of consanguineous unions and positive family history of IBD resulted in high rates of monogenic IBD, exhibiting a more severe clinical course than traditional IBD[16]. Abdulrahman Al-Hussaini *et al*[20] analyzed 352 IBD patients in Saudi Arabia and found that 21.6% were diagnosed with IBD before the age of 6 years and 9% before the age of 3 years, and the consanguinity rate was significantly higher in the infantile or toddler-onset CD subgroup (57.1%).

In our study, the percentage of VEO-IBD patients among cases of pediatric IBD was similar to that reported by Oren Ledder *et al*[17]. Monogenic IBD patients showed earlier disease onset than nonmonogenic IBD patients, and CD was the predominant diagnosis in both groups. Nonetheless, IBDU was more common in the monogenic group, which suggests that most of the monogenic IBD patients had disease limited to the colon. The results of laboratory tests were similar between the two groups. Moreover, patients in the monogenic group did not show a propensity to be prescribed infliximab and immunosuppressants earlier than those in the nonmonogenic group.

According to the diagnostic guidelines for pediatric IBD by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition[9], CD is likely to be the diagnosis when colonoscopy shows classic or nonclassic CD features with small bowel involvement or the presence of fistula/perianal disease. IBDU classification is reserved for patients with inflammation limited to the colon, in the absence of features suggestive of either UC or CD. If the initial immune work-up is normal, further investigation may be considered by physicians to be unnecessary. However, monogenic IBD, including IL10/R and XIAP deficiencies, CGD, and IPEX syndrome, presents with such features as perianal/fistulating disease, linear serpentine ulcerations, and even non-caseating granulomas[21-23], and these patients are likely to be diagnosed with IBDU or CD. It is recognized that onset of disease before 6 mo of age, growth stunting (height-for-age Z-score < 3), extensive disease and epithelial abnormalities are significantly more prevalent in monogenic IBD[16], which may indicate the need for immune tests and even genetic screening in this group of patients[3].

PID can present as IBD-like symptoms with intestinal manifestations and can be diagnosed by immune tests. CGD is characterized by genetic defects in components of the phagocyte reduced nicotinamide

adenine dinucleotide phosphate (NADPH) oxidase (phox) complex, which can be detected by the neutrophil oxidative burst assay. High levels of IgG may indicate FOXP3 deficiency[16,24]. However, Wiskott-Aldrich syndrome, hyper-IgE syndrome and *IL10* or *IL10R* mutations are not detected by basic immunodeficiency screening tests and require specific functional analyses. Defects in gene *IL10RA* and *IL10RB* can be detected by assays that determine whether exogenous IL10 will suppress lipopolysaccharide-induced peripheral blood mononuclear cell cytokine secretion or IL10-induced STAT3 phosphorylation[22]. Flow cytometry can detect functional defects in muramyl dipeptide signaling in patients with XIAP deficiency[25].

Given the large number of potential candidate genes and overlapping phenotypes, single-gene sequencing and immune tests are becoming less appropriate in children with suspected monogenic IBD. NGS techniques have significantly improved in recent years, with lower cost and diagnosis time. Although WES is suitable for novel gene discovery, it offers less reliable gene coverage in the diagnostic setting compared to TGPS[8]. TGPS and WES were both used in our study, with 16 VEO-IBD patients with suspected immune deficiency undergoing WES and/or TGPS, which revealed 9 cases of monogenic IBD (*IL10RA, IL10RB, XIAP* and *CYBB*). Among the 9 patients, 4 had disease onset during the neonatal period, and 4 had symptoms within 1 year of age. The phenotypes of the patients were in accordance with the genotypes. SIFT, Polyphen2 and MutationTaster were used to predict the innocuousness of the mutated genes.

There were limitations in this study. First, we did not perform functional analyses of novel mutations, and such studies should be performed to confirm the results. However, the novel mutations were all predicted to be pathogenic and likely pathogenic by SIFT, PolyPhen-2 and MutationTaster. Second, not all of the VEO-IBD patients were assessed by genetic testing. In cases in which immune deficiency disease was strongly suspected but could not be diagnosed by initial immune tests, genetic testing was recommended. At our hospital, the identification of monogenic IBD and genetic testing in VEO-IBD patients have only been performed during the last 5 years. Several IO-IBD patients diagnosed in early years who met the criteria for genetic testing had died, and their blood samples were not collected. For this reason, more cases of monogenetic IBD might have been present in our cohort.

In the present study, we identified monogenic IBD in 9 patients, predominantly with *IL10R* mutation; five patients were diagnosed with *IL10R* mutation. Among them, 1 patient was the offspring of a consanguineous union, with a homozygous mutation of *IL10R* which has not been reported in the literature. All remaining patients had compound heterozygous mutations of *IL10R*. *IL10* and *IL10R* mutations have been reported

previously. Patients with *IL10* signaling defects primarily present with IBD symptoms within 3 mo of life, with severe perianal disease (abscess, fistula formation, fissure, tags) and susceptibility to infections. In addition, they are usually resistant to traditional therapies, though HSCT can induce sustained remission of intestinal inflammation[19,26,27]. Defects in *IL10* signaling are associated with extraintestinal inflammation, such as folliculitis and arthritis, as well as a predisposition toward B-cell lymphoma[28].

In the report of Zhiheng Huang *et al*[27], 42 IO-IBD patients among the Han population in China had *IL10R* mutations, 41 patients had *IL10RA* mutations, and only 1 patient had an *IL10RB* mutation; thus, *IL10RA* was predominant in the Han population. In another report from China, Xiao *et al*[19] described 4 patients with *IL10RA* mutation and 1 with *IL10RB* mutation among 13 VEO-IBD patients. In the present study and in other studies from Asia[26], monogenic IBD is predominately due to mutations in *IL10R* and *XIAP* and CGD, whereas mutations in *EPCAM, TTC37, SKIV2LLRBA* and *TTC7A* have been reported in Western countries[16]. These findings suggest that *IL10R* mutation is the most common cause of monogenic IBD in the Han population. Further multicenter studies are warranted.

The genetic background of VEO-IBD patients is not clear, especially among different ethnicities. Due to the heterogeneity of VEO-IBD with overlapping phenotypes between different genotypes, TGSP is appropriate for rapid recognition of monogenic IBD in VEO-IBD patients with the signs and features of monogenic IBD. This approach may help guide a more appropriate treatment strategy. For example, sustained remission after HSCT can be achieved with *IL10R/IL10* mutation[12], XIAP deficiency[29] and FOXP3 deficiency[30].

In conclusion, using WES and TGPS, we identified underlying PID gene mutations in pediatric patients with VEO-IBD in a Chinese population. There was a high proportion of monogenic IBD in the VEO-IBD group, especially with disease onset before 6 mo of age. *IL10R* mutation was predominant in our cohort.

ARTICLE HIGHLIGHTS

Research background
Very-early-onset inflammatory bowel disease (VEO-IBD) patients show a close association with primary immunodeficiency diseases, defined as monogenic IBD. More than 50 VEO-IBD related genes have been reported to date. Nonetheless, the incidence of monogenic IBD in Chinese population remains unknown.

Research motivation
Most reports regarding monogenic IBD were based on small population or case report, with only a small number of genes investigated. This study reports the largest cohort of genetically screened patients with VEO-IBD from China.

Research objectives
The objective of this research is to characterize monogenic IBD phenotypically and genotypically *via* genetic testing and to analyze clinical differences between

monogenic and nonmonogenic VEO-IBD patients.

Research methods

A retrospective analysis of children aged 0 to 6 years diagnosed with VEO-IBD in a tertiary hospital in southern China from 2005 to 2017 was performed. Clinical data for VEO-IBD patients were collected, and their genetic characteristics were analyzed using whole exome sequencing or target gene panel sequencing.

Research results

Nine patients (16.7%) were identified to have monogenic IBD by genetic testing. Five patients were shown to have *IL10R* mutation, two patients had chronic granulomatous disease, one patient had common variable immunodeficiency disease, and one patient had X-linked inhibitor of apoptosis deficiency.

Research conclusions

A high proportion of monogenic IBD was observed among the VEO-IBD group, especially with disease onset before the age of 6 mo. *IL10RA* was the predominant mutation in this cohort. Monogenic IBD and nonmonogenic IBD demonstrated similar clinical features. Next-generation sequencing played an important role in the diagnosis of monogenic IBD.

Research perspectives

Next-generation sequencing revealed a high proportion of monogenic IBD in our VEO-IBD cohort. Multicenter prospective studies are expected to determine the incidence of monogenic IBD in the Chinese VEO-IBD population and to investigate the genetic characteristics of monogenic IBD in China.

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