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ABOUT COVER

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ORIGINAL ARTICLE

Observational Study

Autosomal recessive 333 base pair interleukin 10 receptor alpha subunit deletion in very early-onset inflammatory bowel disease

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Abstract

BACKGROUND

Interleukin 10 receptor alpha subunit (IL10RA) dysfunction is the main cause of very early-onset inflammatory bowel disease (VEO-IBD) in East Asians.

AIM

To identify disease-causing gene mutations in four patients with VEO-IBD and verify functional changes related to the disease-causing mutations.

METHODS

From May 2016 to September 2020, four young patients with clinically diagnosed VEO-IBD were recruited. Before hospitalization, using targeted gene panel sequencing and trio-whole-exome sequencing (WES), three patients were found to harbor a *IL10RA* mutation (c.301C>T, p.R101W in one patient; c.537G>A, p.T179T in two patients), but WES results of the fourth patient were not conclusive. We performed whole-genome sequencing (WGS) on patients A and B and reanalyzed the data from patients C and D. Peripheral blood mononuclear cells (PBMCs) from patient D were isolated and stimulated with lipopolysaccharide (LPS), interleukin 10 (IL-10), and LPS + IL-10. Serum IL-10 levels in four patients and tumor necrosis factor-α (TNF-α) in the cell supernatant were determined by enzyme-linked immunosorbent assay. Phosphorylation of signal transducer and activator of transcription 3 (STAT3) at Tyr705 and Ser727 in PBMCs was determined by western blot analysis.

RESULTS

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The four children in our study consisted of two males and two females. The age at disease onset ranged from 18 d to 9 mo. After hospitalization, a novel 333-bp deletion encompassing exon 1 of *IL10RA* was found in patients A and B using WGS and was found in patients C and D after reanalysis of their WES data. Patient D was homozygous for the 333 bp deletion. All four patients had elevated serum IL-10 levels. *In vitro*, IL-10-stimulated PBMCs from patient D failed to induce STAT3 phosphorylation at Tyr705 and only minimally suppressed TNF-α production induced by LPS. Phosphorylation at Ser727 in PBMCs was not affected by LPS or LPS + IL-10 in both healthy subjects and in patient D.

CONCLUSION
WGS revealed a novel 333-bp deletion of *IL10RA* in four patients with VEO-IBD, whereas the WES results were inconclusive.

Key Words: Interleukin 10 receptor alpha subunit mutation; Very early-onset inflammatory bowel disease; Whole-genome sequencing; Immunodeficiency; Crohn's disease; Whole-exon sequencing

Core Tip: Children less than 6 years old with very early-onset inflammatory bowel disease (VEO-IBD) exhibit severe and refractory disease phenotypes, which indicate a monogenic type disease. Here, we report four cases clinically diagnosed with VEO Crohn's disease, of which three were compound heterozygous carriers for a 333-bp deletion and an additional single-nucleotide variant, and one was homozygous for the 333-bp deletion in *IL10RA*. Based on these cases with heterozygous pathogenic variants in *IL10RA*, the possibility of another large fragment deletion that can be missed by whole-exon sequencing or gene panels should be considered, particularly when serum IL-10 is increased in patients with VEO-IBD.

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INTRODUCTION

Inflammatory bowel disease (IBD) in children < 6 years of age is known as very early-onset IBD (VEO-IBD)[1] and represents a specific disease course with a distinct phenotype that can be more severe and refractory than classic IBD[2-3]. Recent studies suggested that patients with VEO-IBD, particularly those with symptoms such as perianal disease soon after birth, suffer from failed treatment, indicating a monogenic type of disease[4-6].

By utilizing next-generation sequencing (NGS), many genetic disorders associated with epithelial defects or immunodeficiencies have been found in patients[7-9]. Notably, interleukin 10 receptor alpha subunit (IL10RA) dysfunction is the most common cause of the disease in East Asians, particularly in the Chinese, Japanese, and Korean populations[10-12].

According to our previous retrospective study, increased serum ferritin levels in VEO-IBD patients are indicative of monogenic disease, and very high serum levels of IL-10 suggest that patients with VEO-IBD are more likely to have *IL10RA* mutations [13].

We report four cases clinically diagnosed with VEO-Crohn's disease with high serum IL-10 levels, indicating *IL10RA* dysfunction. However, neither results of targeted gene panel sequencing (TGPS) nor those of whole-exome sequencing (WES) in the probands were conclusive. Whole-genome sequencing (WGS) was performed in two patients, and a novel 333-bp deletion in *IL10RA* was identified. The results of trio-WES for the other two patients were subsequently reanalyzed, and the same novel

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333-bp deletion was found.

MATERIALS AND METHODS

Patients

Four patients with VEO-IBD, including two boys and two girls, were enrolled in our study. The medical history and clinical characteristics of the patients are summarized in Table 1. All patients were of Chinese Han ethnicity and were born to parents who were non-consanguineous, presented disease at the age of less than 1 year (range: 11 d to 8 mo), and experienced severe diarrhea with fistulas in the perianal region; blood samples were collected from three healthy volunteers.

Written informed consent was obtained from the parents of the four patients who participated in the study. This study was approved by the Institutional Review Board of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine (No. 2019-15).

Whole-genome sequencing

Sample preparation and WGS were carried out by Beijing Berry Genomics Co., Ltd. (Beijing, China). The quality of the isolated genomic DNA was verified using the following two methods: (1) DNA degradation and contamination were monitored by electrophoresis on 1% agarose gels; and (2) DNA concentration was measured using the Qubit DNA Assay Kit and Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, United States).

A total of 1 μg DNA per sample was used as the input material for DNA library preparation. The DNA sequencing library was generated using the CLEANNGS DNA kit following the manufacturer's recommendations, and indexing codes were added to each sample. Briefly, genomic DNA samples were enzymatically disrupted to a size of 350 bp. The DNA fragments were then end-polished, a-tailed, and ligated with a full-length adapter for Illumina sequencing, followed by further polymerase chain reaction (PCR) amplification. After the PCR products were purified (AMPure XP system, Beckman, Brea, CA, United States), libraries were analyzed to determine theirsize distribution using an Agilent 2100 Bioanalyzer (Santa Clara, CA, United States) and quantified by qPCR.

Clustering of the index-coded samples was performed on a cBot Cluster Generation System using the NovaSeq 5000/6000 S4 Reagent Kit (Illumina, San Diego, CA, United States) according to the manufacturer's instructions. After cluster generation, the DNA libraries were sequenced on an Illumina NovaSeq 6000 platform, and 150-bp paired-end reads were generated.

The pathogenicity of all mutations was further evaluated according to the American College of Medical Genetics and Genomics guidelines.

Isolation and stimulation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated according to a previous study, with minor modifications[14]. Briefly, blood was drawn from patient D and healthy controls by standard venipuncture in our pediatric ward and collected into a tube containing ethylenediamine tetraacetic acid. Blood (4 mL) was diluted 1:1 with sterile RPMI 1640 medium (Hyclone, Logan, UT, United States) at room temperature (RT) and carefully dropped into a 15-mL tube (Corning, Inc., Corning, NY, United States) containing 4 mL Ficoll-Paque Plus (GE Healthcare, Little Chalfont, United Kingdom). Notably, diluted blood was present on the surface of the Ficoll gradient. The 15-mL tube was centrifuged at 800 × g at RT for 20 min (brake off), after which the buffy coat was carefully aspirated and transferred to another sterile 15-mL tube. After washing the cells with 5 mL RPMI 1640 medium three times by centrifugation at 400 × g for 15 min at RT, most of the supernatant, as much as possible, was pipetted off.

Cells were aspirated with complete RPMI 1640 (10% fetal bovine serum and 1% penicillin-streptomycin) and cultured in 6-well plates at a density of 2×10^6 cells/well. Four groups of PBMCs from patients or healthy controls were established as follows: Unstimulated phosphate buffered saline (PBS), lipopolysaccharide (LPS) (100 ng/mL), LPS (100 ng/mL) + IL-10 (20 ng/mL), and IL-10 (20 ng/mL)[15]. Cells were cultured in the indicated milieu for 12 h at 37 °C. Proteins in PBS-, LPS-, and IL-10-stimulated PBMCs were collected in radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors for western blot analysis. The supernatants of the PBS-, LPS-, and LPS + IL-10-stimulated PBMCs were collected to determine tumor necrosis factor-α (TNF-α) level.

Table 1 Clinical and laboratory characteristics of patients

	Patient A	Patient B	Patient C	Patient D	Normal range
Sex	Male	Female	Male	Female	
Consanguinity	-	-	-	-	
Disease onset	3 mo	9 mo	11 d	18 d	
Diarrhea (times/d)	1	8	20	6	
Bloody stool	+	-	+	+	
Weight (kg) SDS	7.65 (-1.475)	6.2 (-1.4)	8.2 (-1.05)	6.3 (-1.15)	
Height (cm) SDS	76 (-1.7)	65.2 (-3.65)	78 (0.25)	67 (1)	
Perianal disease	+	+	+	+	
Extragastro-intestinal manifestations	Fever, UTI, Sepsis	Recurrent otitis media	Fever, respiratory infetion	Fever, UTI, eczama	
WBC ($\times 10^9$)	12.4	19.44	15.34	24.5	3.69-9.16
Hemoglobin (g/L)	101	77	107	96	113-151
Platelet ($\times 10^9$)	624	621	424	387	101-320
IL-10 (pg/mL)	87.3	106	43.4	80.4	< 12.9
TNF-α (pg/mL)	20.7	30.5	3.9	19.8	< 16.5
ESR (mm/h)	6	55	19	23	F: 0-20; M: 0-15
Albumin (g/L)	28	29	33	23	35-55
Ferritin	17	47.3	46.5	68.2	11-306.8
Identified mutation	*IL10RA* (c.301C>T, p.R101W): Exon 1 del	*IL10RA* (c.537G>A, p.T179T): Exon 1 del	*IL10RA* (c.537G>A, p.T179T): Exon1 del	*IL10RA* (exon.1 del): Exon 1 del	

SDS: Standard deviation score; UTI: Urinary tract infection; TNF-α: Tumor necrosis factor α; IL10RA: Interleukin-10 receptor α subunit; del: Deletion; WBC: White blood cell.

Western blot analysis

Western blotting was performed as described previously[16]. Polyvinylidene fluoride membranes were blotted with monoclonal antibodies against phos-signal transducer and activator of transcription 3 (STAT3) (Tyr705), phos-STAT3 (Ser727), STAT3 (Cell Signaling Technology, Danvers, MA, United States), and glyceraldehyde-3-phosphate dehydrogenase (Servicebio, Wuhan, China). Horseradish peroxidase-conjugated anti-mouse and anti-rabbit (Cell Signaling Technology) secondary antibodies were detected using a chemiluinescent substrate (Millipore, Billerica, MA, United States). Images were captured using an automatic chemiluminescence image analysis system (Tanon, Shanghai, China).

Enzyme-linked immunosorbent assay

The supernatant of PBMCs after stimulation with PBS, LPS or LPS + IL-10 was collected. IL-10 and TNF-α levels were determined using sandwich ELISA kits (DAKEWE, Shenzhen, China) according to the manufacturer's instructions.

Statistical analysis

Continuous variables are presented as the mean ± SEM, and the unpaired two-tailed Student's *t*-test or analysis of variance was used to compare the differences between groups as appropriate. Bonferroni correction was used for pairwise comparisons (GraphPad Prism v.5.0 software; GraphPad, Inc., La Jolla, CA, United States). Statistical significance was set at $P < 0.05$.

RESULTS

Clinical characteristics of four patients with VEO-IBD

All four patients had severe diarrhea (> 6 times/d) and hematochezia during the first year of life. Patients C and D suffered from the disease during the newborn period. In addition to gastrointestinal symptoms, all cases exhibited extraintestinal manifestations, such as perianal abscesses, skin tags (Figure 1A), rectoperineal fistula, failure to thrive, recurrent otitis, urinary tract or respiratory infection, folliculitis, and even sepsis (Table 1). Elevated numbers of peripheral white blood cells and platelets and decreased hemoglobin and albumin levels were found in each patient. Remarkably, immune-related investigations showed that all patients had high serum levels of IL-10 (Table 1). All patients underwent colonoscopy and intestinal biopsy under general anesthesia, revealing erosive lesions (Figure 1B), and were diagnosed with Crohn's disease.

Identification of a novel compound heterozygous mutation in IL10RA

Before admission, all patients underwent TGPS or trio-WES. Two heterozygous pathogenic variants of *IL10RA* were detected in three patients (patient A: c.301C>T, p.R101W; patients B and C: c.537G>A, p.T179T) (Figure 1C and Supplementary Figures 1 and 2). Mutation of c.537G>A occurred at the exon-intron boundary of exon 4, which is a variant hotspot and disrupts RNA splicing (Figure 2A). No pathogenic or likely pathogenic variants were found in patient D.

As *IL10RA* mutation causes infantile IBD in an autosomal recessive manner and serum levels of IL-10 were very high in the four infantile patients with IBD, which is a valuable clinical indicator for identifying infantile IBD as a monogenic disease as we demonstrated previously[13], we suspected that mutations had been overlooked in WES owing to the techniques' limitations. After performing WGS in patients A and B, the breakpoints of the novel deletion were identified by manual review and correction. The deletion was located at chr11:117857030 upstream of exon 1 and chr11:117857362 in intron 1, which contains the 5'-untranslated region (UTR), all of exon 1, and part of intron 1 in *IL10RA* (Figure 1D and Figure 2B). PCR revealed a paternally-inherited 333-bp deletion in addition to the point mutations mentioned above (Figure 1E). The deletion and point mutations were inherited from both parents and eventually constituted compound heterozygotes in patients B (Figure 1F) and A (Supplementary Figure 1).

After identifying the 333-bp deletion in the gene in patients A and B, we reanalyzed the trio-WES data for patients C and D, specifically in the region from 117857030 and 117857362 on chromosome 11 and detected the same deletion. Patient C was a compound heterozygous carrier for c.537G>A, p.T179T (maternal), and the 333-bp deletion (paternal) (Supplementary Figure 2), and patient D was homozygous for the 333-bp deletion.

Histological and functional analysis of patient DIL10RA deletion

Histological findings in a specimen from the colon obtained during colonoscopy revealed oval-shaped intramural abscesses in the submucosa (Figure 3A). Figure 3B shows a higher magnification of inset 1 in Figure 3A, depicting intramural microabscesses.

To determine whether the novel 333-bp deletion in *IL10RA* caused IL-10R dysfunction and subsequently inhibited TNF-α production, supernatants of cultured PBMCs were collected and used to determine TNF-α levels. In healthy controls, LPS stimulation caused a remarkable increase in TNF-α production, whereas addition of IL-10 significantly decreased its abundance. Although LPS led to increased TNF-α production in patient D, this phenomenon was not reversed by the addition of IL-10, as observed in the healthy controls (Figure 3C).

To clarify the exact mechanisms involved, PBMCs were isolated from patient D because the patient was homozygous for the *IL10RA* deletion. PBMCs were stimulated with LPS in the presence or absence of IL-10. The results of western blot analysis showed that in PBMCs from healthy controls, both LPS and IL-10 stimulation caused an increase in the phosphorylation of STAT3 at Tyr705 but not at Ser727 (Figure 3D). In PBMCs from patient D, LPS stimulation also induced increased phosphorylation of STAT3 at Tyr705 but not at Ser727. However, IL-10 stimulation failed to significantly increase phosphorylation of STAT3 at Tyr705 in PBMCs of patient D compared with that in PBS-stimulated PBMCs. No significant differences in STAT3 phosphorylation at Ser727 were observed among the PBS-, LPS-, and IL-10-stimulated PBMCs from patient D (Figure 3D).

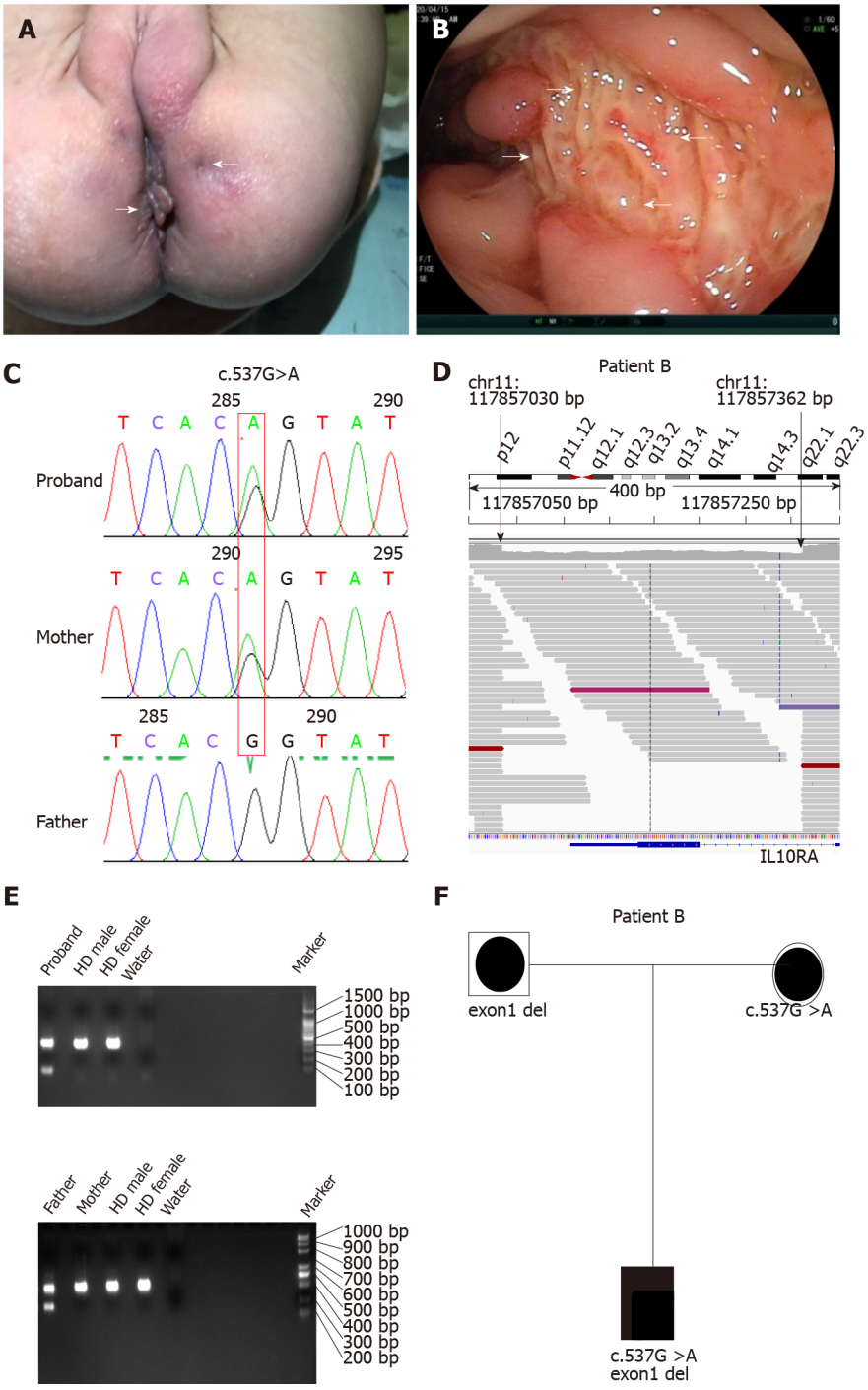

Figure 1 Identification of a novel 333-bp deletion spanning interleukin 10 receptor alpha subunit exon1. A: Perianal skin tag; B: Endoscopic image of ulcerations; C: Sanger DNA sequencing verified a compound heterozygous variant (c.537G>A) inherited from the mother in patient B; D: Whole-genome sequencing (WGS) data showing sequencing read pairs at breakpoints chr:117857030 and chr:117857362 of interleukin 10 receptor alpha subunit (*IL10RA*); E: Polymerase chain reaction validated the heterozygous deletion of 333 bp spanning exon1 inherited from the father; F: WGS revealed compound heterozygous variants of *IL10RA* in patient B with very early-onset inflammatory bowel disease. bp: Base pair; HD: Healthy donor.

DISCUSSION

VEO-IBD is challenging to diagnose and treat because the patients are critically ill and exhibit numerous potential mono-genic defects. Approximately 56 Mendelian genetic defects that can lead to IBD-like colitis have been identified, some of which show almost 100% penetrance, such as defects in *IL-10*, *IL10RA*, *IL10RB*, *FoxP3*, and *XIAP*[1]. NGS has led to breakthroughs in the diagnosis of genetic diseases, including monogenic VEO-IBD. According to a recent single-center study performed by Crowley *et al*[17], 7.8% of VEO-IBD (141 patients) and 13.8% of infantile-onset IBD (29 patients) cases had rare variations associated with monogenic genes. This prevalence was lower

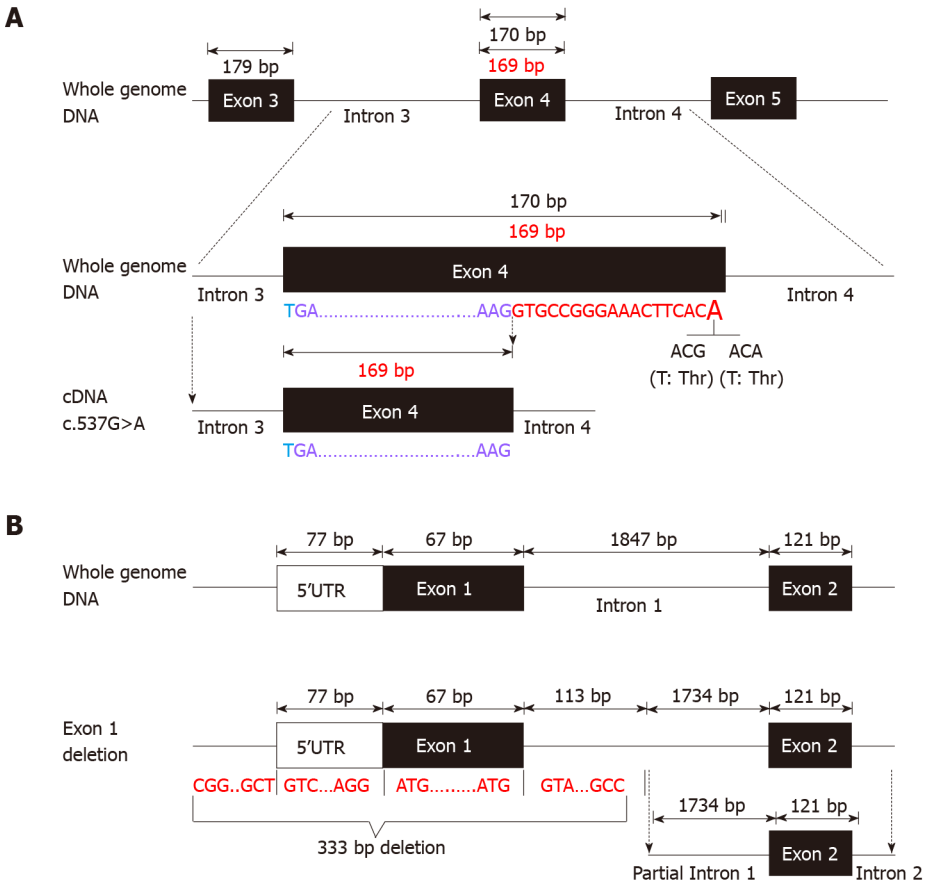

Figure 2 Schematic diagram of disrupted RNA splicing and 333-bp deletion in interleukin 10 receptor alpha subunit. A: Schematic diagram of disrupted splicing caused by c.537G>A in interleukin 10 receptor alpha subunit (*IL10RA)* between the boundary of exon4 and intron4; B: Schematic diagram of *IL10RA* 333-bp deletion. bp: Base pair.

than that reported in Chinese or European studies, which was approximately 31.9%-45.2%[12,18]. In our center, we found that 60.3% of patients with infant-onset IBD had monogenic disease, with mutations in *IL10RA* identified as the most common defect [13].

We evaluated four patients who had Crohn's disease from early infancy and exhibited failure to thrive, severe perianal disease, and resistance to medication. These characteristics indicate the presence of underlying genetic conditions[7]. Although the patients underwent NGS in a local hospital, neither TGPS nor WES revealed conclusive results. However, all patients showed very high serum IL-10 levels, and three patients had a disease-causing heterozygous mutation in *IL10RA*. According to our previous research, serum IL-10 levels > 33.05 pg/mL in patients with VEO-IBD strongly indicates the presence of IL10RA dysfunction[13]. Thus, we predicted that additional *IL10RA* mutations were missed during TGPS or WES. We did not detect the 333-bp deletion in *IL10RA* in two patients until WGS was performed. We then analyzed the same deletion in the other two patients by reanalyzing the trio-WES data. Finally, all patients were precisely diagnosed with VEO-IBD owing to compound heterozygous mutations in *IL10RA* in three patients and homozygous deletion involving *IL10RA* in one patient.

NGS, including TGPS and WES, is a powerful tool for identifying Mendelian genetic diseases in patients with VEO-IBD. The position paper on VEO-IBD by NASPGHAN/ESPGHAN suggests that NGS combined with the patient clinical history represents a vital component of the diagnostic approach[1]. A previous multicenter study showed that molecular diagnosis was achieved in 32% of patients with VEO-IBD when NGS was employed[18]. However, clinical NGS applications have limitations such as short read lengths, relatively high error rates, and incomplete coverage. Non-coding, yet potentially functional regions, and approximately 5% of exons are poorly covered in WES[19]. It is difficult to detect variants involving extensive deletions/insertions or short tandem repeats[20]. Charbit-Henrion *et al*[18] reported three WES-negative cases harboring large deletions in *LRBA* and *NCF1*. Compared to WES, WGS can detect all single-nucleotide variants, small indels, large

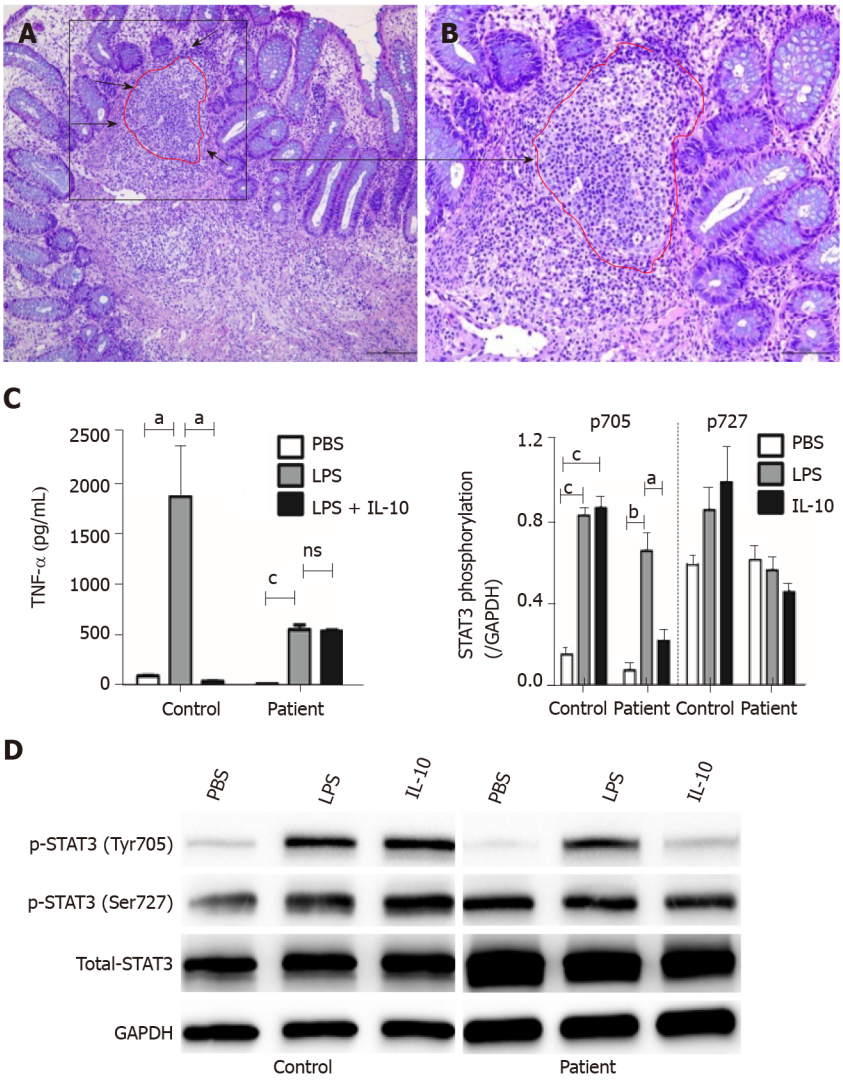

Figure 3 Histopathochemistry and functional results of homozygous interleukin 10 receptor alpha subunit mutation. A: Histological findings in a colonic specimen obtained during colonoscopy showing oval-shaped intramural abscesses; B: Higher magnification of inset A; C: Determination of TNF-α levels in the supernatant of cultured PBMCs from patient D after stimulation with PBS, LPS, or LPS + IL-10 *in vitro*; D: Western blot results of PBMCs isolated from patient D after stimulation with LPS or IL-10. Phosphorylation of STAT3 at Tyr705 and Ser727 and total STAT3 protein were detected (n = 3). $^{a}P < 0.05$, $^{b}P < 0.01$, and $^{c}P < 0.001$ were considered as statistically different.

indels, and copy number variants. In our cases, the 333-bp *IL10RA* deletion contained a 5′-UTR, exon 1, and part of intron 1. This large deletion was easy to overlook when WES was used because of its technical limitations and insufficient bioinformatics analysis. After detecting this deletion, we requested re-analysis of the WES data for patients C and D, specifically for the 333-bp region spanning *IL10RA* exon 1. As expected, these two patients harbored the deletion. These results indicate that WES can detect the 333-bp deletion, which was easily overlooked in bioinformatics analysis because of algorithm defects and insufficient experience. WGS compensates for the limitations of WES. Our results indicated that WGS should be performed in VEO-IBD cases with negative WES results, particularly in those with infantile-onset IBD and treatment failure.

A comprehensive range of defects may be associated with VEO-IBD. It is difficult to differentiate every patient based on these defects and their underlying genetic disorders. A small number of patients show distinct phenotypes associated with specific functions, such as IL-10/IL-10R defects, IPEX, CGD, and XIAP[1]. *IL10RA* mutation-induced VEO-IBD shows specific characteristics such as refractory pan-colitis, perianal defects, fistulas, and growth failure, which occur during the neonatal period. An assay that can detect the lack of IL-10 inhibition in LPS can confirm receptor mutations[1]. Nevertheless, this assay is complicated and not routinely available in most hospitals in China. In a retrospective study, we found that the assay was useful for diagnosing IL10RA defects when the serum level of IL-10 was > 33.05

pg/mL. The assay sensitivity was very close to 100%, and the specificity was approximately 84%[13]. Elevated serum levels of IL-10 in patients with VEO-IBD indicated that even such high level of IL-10 could not inhibit TNF-α release and alleviate inflammation. Thus, serum IL-10 level may be a substitute for determining IL-10 inhibition in LPS functional testing. Therefore, when classic symptoms and laboratory results indicate that patients may have IL-10/IL-10R dysfunction but WES is inconclusive, *IL-10/IL10RA/IL-10RB* must be investigated and analyzed. It is recommended to use WGS or specific PCR to detect whether a large deletion involving these genes has occurred.

Interestingly, TNF-α production in LPS-stimulated PBMCs was not as robust in patient D as in control subjects; in our study, patient D was administered with anti-TNF-α antibody (Infliximab) before blood collection, which may have led to relatively low TNF-α production in the supernatant of LPS-stimulated PBMCs compared to that in healthy controls. Another possible reason is that increased TNF-α in the blood of patient D led to activation of the TNF-α receptor, resulting in JNK signaling-dependent inhibition of Bcl-2 expression, which acts as a major anti-apoptosis protein[21].The lack of a comprehensive functional test for *IL10RA* mutation is the major limitation of our study, although we conducted western blotting to determine the possible mechanism of *IL10RA* mutation-induced dysfunction of IL10RA signaling. Further studies are needed to explore other potential differences in IL10RA signaling.

CONCLUSION

Using WGS, we identified a novel 333-bp deletion in *IL10RA* that contributed to four cases of clinically diagnosed VEO-IBD with inconclusive *IL10RA* mutations. Most importantly, we confirmed that typical clinical manifestations and increased serum levels of IL-10 strongly indicate the existence of IL-10R dysfunction even when WES results are negative.

ARTICLE HIGHLIGHTS

Research background

Interleukin 10 receptor alpha (*IL10RA*) gene mutations constitute the most common monogenic disease in East Asia, affecting the health of children. However, identifying disease-causing mutant sites or copy number variants remains challenging in the clinic.

Research motivation

According to the results of our previous study, severe clinical symptoms as well as significantly increased serum IL-10 indicate IL10RA dysfunction, a monogenic phenotype of very early-onset inflammatory bowel disease (VEO-IBD). In addition, such very early-onset IBD showed a heterozygous *IL10RA* gene mutation by whole exon sequencing, leading to the employment of WGS and subsequent identification of 333-bp deletions in *IL10RA*.

Research objectives

We investigated the potential disease-causing gene mutations missed by WES and target gene panel sequencing (TGPS). Our results may contribute to monogenic disease diagnosis.

Research methods

Four patients clinically diagnosed with VEO-IBD during the past 5 years were recruited for this study. Based on their severe clinical phenotypes and the fact that before hospitalization, three patients harbored an *IL10RA* mutation (c.301C>T, p.R101W in one patient; c.537G>A, p.T179T in two patients), as detected by TGPS and trio-WES, and because WES did not show conclusive results in the fourth patient, we performed whole-genome sequencing (WGS) on patients A and B and reanalyzed the trio-WES data from patients C and D. To verify the functional change caused by the novel mutation, peripheral blood mononuclear cells (PBMCs) from patient D were isolated and stimulated *in vitro* with lipopolysaccharide (LPS), IL-10, and LPS + IL-10. Serum IL-10 levels in four patients and tumor necrosis factor-α (TNF-α) in the cell

supernatant were determined by ELISA. Phosphorylation of signal transducer and activator of transcription 3 (STAT3) at Tyr705 and Ser727 in PBMCs was determined by western blot analysis.

Research results

Results of WGS revealed a novel 333-bp deletion encompassing exon 1 of *IL10RA* in patients A and B, which was also found in patients C and D after reanalyzing their WES data. Patient D was homozygous for the 333-bp deletion. All four patients showed elevated serum IL-10 levels. *In vitro*, IL-10-stimulated PBMCs from patient D failed to induce STAT3 phosphorylation at Tyr705 and minimally suppressed TNF-α production induced by LPS. Phosphorylation at Ser727 in PBMCs was not affected by LPS or LPS + IL-10 in both healthy subjects and patient D.

Research conclusions

Genome-wide uniformity of coverage of WGS identified a novel 333-bp deletion in *IL10RA* in four patients with VEO-IBD, whereas the results of initially performed WES were inconclusive. WGS, which was more informative than WES, is the most important comprehensive second-tier genomic test for monogenic diseases in the clinic.

Research perspectives

We will customize a multiplex ligation-dependent amplification probe of the 333-bp deletion in *IL10RA* to help diagnose *IL10RA* mutation-related monogenic diseases.

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