Characterization of novel and large fragment deletions in exon 1 of the IL10RA gene in Chinese children with very early onset inflammatory bowel diseases

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Abstract

Background: Defects in interleukin 10 (IL10) and its receptors are particularly involved in very early onset inflammatory bowel disease (VEOIBD). However, large fragment deletions of IL10 receptor A (IL10RA) are rare.

Methods: VEOIBD patients with confirmed mutations in the IL10RA gene were enrolled from January 1, 2019 to June 30, 2020. The clinical features and endoscopic-radiological findings of the patients with large fragment deletions of the IL10RA gene were determined and followed up.

Results: Thirty-five patients with IL10RA gene mutations, namely, 28 compound heterozygous mutations and 7 homozygote mutations, were enrolled in this study. Six patients carried the reported point mutation c.301C > T (p. R101W) or c.537 G > A (p. T179T) in one locus and a large fragment deletion in exon 1 in another locus, which were novel mutations in this gene. A 333-bp deletion of exon 1 (117857034–11857366 del) was the main mutation in this locus in 85.7% of the patients with large fragment deletions. The time of disease onset ranged from birth to 4 years, and diarrhea was the main initial symptom. In total, 6/7 patients had perianal complications, including perianal abscess, fistula and skin tags. Six patients accepted thalidomide treatment, 5/7 accepted mesalamine, 3/7 accepted hematopoietic stem cell transplantation (HSCT), and 3/7 were waiting for HSCT.

Conclusions: We identified a novel large deletion of exon 1 involving the IL10RA gene for the first time and showed the characteristics of VEOIBD patients. This study expands the spectrum of Chinese VEOIBD patients with IL0RA gene mutations.

Keywords: Very early onset inflammatory bowel disease, Interleukin 10 receptor A, Large fragment deletions, Exon 1

Background

Inflammatory bowel disease (IBD) is a chronic relapsing disorder of the gastrointestinal tract with multifactorial and complex etiology. Very early onset IBD (VEOIBD) has an age of onset before 6 years old and constitutes 3–15% of pediatric IBD. VEOIBD is often associated with monogenetic disorders and is of particular interest in IBD research. Deficiencies in interleukin 10 (IL10) and its receptors (IL10RA, IL10RB) are major causes of VEOIBD [1, 2]. IL-10-related genes are most frequently associated with infantile-onset IBD (age of onset ≤ 2 years).

VEOIBD patients with mutations in the IL10 or IL10R genes present with severe gastrointestinal symptoms, such as severe colitis with hematochezia, severe perianal abscess or fistulae, repeated oral ulcer, and recurrent...
clinical and folliculitis in the first months of life [3]. These patients are refractory to immunsuppressive thera-
pies such as corticosteroids, methotrexate, and antitu-
mor necrosis factor-alpha (TNF-α) antibodies [4, 5].
For patients with severe intestinal infection or perianal lesions, enterostomy can alleviate the symptoms. Because
IL10R is expressed on most hematopoietic cells, hemat-
opoietic stem cell transplantation (HSCT) is considered
the only curative option for IL10RA-deficient VEOIBD.

Many point mutations of IL10 and IL10R have been
identified at our IBD center and by other groups [6–8].
A previous study showed that IL10RA defects were unique
mutations in East Asia compared with North America
and Europe, with the common point mutations being c.301C>T and c.537 G>A [9, 10]. The determi-
nation of the pathogenicity of candidate variants of the
IL10RA gene is critical for the diagnosis and manage-
ment of this disease. However, reports of large deletions
in the IL10RA genes and their associated clinical char-
acteristics are rare, and consequently some patients may
never receive an appropriate treatment. In this study, we
aimed to determine the characteristics of Chinese
VEOIBD patients with large fragment deletions of the
IL10RA gene.

Methods
Patient cohort
This study was approved by the Ethics Committee of the
Children’s Hospital of Fudan University. Inpatients were
recruited from a tertiary care center following a diagnosis
of IBD based on their clinical history, physical examina-
tion, endoscopic appearance, and histological findings
according to the Porto criteria. The index dates of the
IBD diagnosis were from January 1, 2019 to June 30,
2020. Fifteen VEOIBD patients with IL10RA gene point
mutations in the same period were enrolled as the con-
rol group. Patients with an infection or celiac disease
or allergic/eosinophilic gastrointestinal diseases were
excluded.

Genetic analysis
Whole-exome sequencing (WES) was performed as
described previously [11]. Target gene capture and next-
generation sequencing (NGS) were also used to ana-
lyze the patients. The gene panel (MyGenostics, Beijing,
China) contained 347 genes related to diarrhea. The
library was prepared using the Library Preparation Kit
developed by MyGenostics Co., Ltd. according to the Illu-
mina platform requirements. High-throughput sequenc-
ing was performed using an Illumina ×10 sequencer.
Exon 1 deletion was found by CapCNV analysis, followed
by a CNVkit protocol (https://cnvkit.readthedocs.io/en/
stable/pipeline.html).

PCR and sanger sequencing
To verify the mutation sites detected by WES or NGS,
the following procedure was used. The 200-bp DNA
fragment including this site was amplified by polymer-
ase chain reaction (PCR) and sequenced using an ABI
3730 Genetic Analyzer (Applied Biosystems, CA). To
verify the exon 1 deletion, the DNA fragment including
exon 1 was amplified by PCR using special primers, and
two bands were obtained by agarose gel electrophore-
sis for the patients and their parents. The mutated DNA
sequences were analyzed by BLAST in UCSC to confirm
the deleted region. When identifying the novel sequence
variant, pathogenicity was determined based on the
American College of Medical Genetics and Genomics
(ACMG) Standards and Guidelines [12].

Laboratory indices
The erythrocyte sedimentation rate, C-reactive pro-
tein level, complete blood cell count, hemoglobin, total
protein and albumin levels, and prealbumin level were
recorded at the time of diagnosis. The available height for
age (HFA), weight for age (WFA), and body mass index
(BMI) Z score at the time of the first diagnosis for each
patient were calculated using the World Health Organiza-
tion (WHO) Anthro (version 3.2.2) software. All the
patients with large fragment deletions of the IL10RA
gene in this study had undergone follow-up for treatment
and prognostic information.

The data were analyzed using SPSS 24.0 for Windows
(SPSS Inc., Chicago, IL, USA). Continuous data are pre-
sented as the mean and standard deviation (SD) or the
median and interquartile range (IQR). Categorical vari-
ables are reported as frequencies and percentages. A
two-tailed value for $P<0.05$ was considered statistically
significant.

Results
Demographics of the patients
From January 1, 2019 to June 30, 2020, 35 patients with
IL10RA gene mutations, including 33 compound het-
erozygous mutations and 2 homozygote mutations, were
enrolled in the study. Seven patients with compound
heterozygous mutations were found to carry point muta-
tions c.301C>T (p. R101RW), c.537 G>A (p. T179T) or
c.106G>A (p. A36T) in one locus and a large fragment
deletion of the IL10RA gene in another locus (Table 1).
The deletions in these 7 patients were all located in exon
1 of the IL10RA gene. Six patients (cases 1, 3, 4, 5, 6 and
7) (85.7%) had the same 333-bp deletion. The pathogenic
evidence of this 333-bp deletion and untested mutation
was PVS1+PM2. To determine the impact of the 333-
bp deletion on mRNA production, we performed PCR
A representative figure of the exon 1 deletion of patient 1 is shown in Fig. 1a, b.

More detailed clinical characteristics of the 7 patients with identified mutations in \( \text{IL10RA} \) are shown in Table 2. For patient 4, although the disease onset was at 4 years of age, he still had refractory diarrhea and a bloody stool. We compared the characteristic features of patients with a large fragment deletion in exon 1 of the \( \text{IL10RA} \) gene with 15 point mutations of the \( \text{IL10RA} \) gene. Both the patients with a large fragment deletion and the control patients had complete clinical data and were diagnosed with Crohn's disease by endoscopy.

The laboratory data of these patients at the first outpatient visit to the hospital were collected. Although no significant difference was found, both groups with \( \text{IL10RA} \) gene mutations displayed increased white blood cell counts, C-reactive protein levels, platelet counts, erythrocyte sedimentation rates, and fecal calprotectin levels, while the expression levels of Hb, serum total protein, albumin and prealbumin were decreased. The patients with \( \text{IL10RA} \) mutations showed decreases in the average HFA, WFA and BMI (Table 3).

The patients with large deletions in exon 1 were compared with other patients with the \( \text{IL10RA} \) gene point mutation. We could not find a significant difference between the two groups except on the day of diarrhea and the percentage of eczema.

**Endoscopic and imaging findings**

Endoscopy revealed that patient 1 had irregular intestinal ulcers in the rectum, sigmoid colon and ileocecal junction and longitudinal ulcers in the transverse colon and descending colon. Nodules and erythema were present in the sigmoid colon, descending colon and rectum of patient 2. Patient 3 had an intestinal ulcer in the colon and cobblestone-like polyposis. A patched mucosal ulcer was observed from the rectum to the ascending colon in patient 4. Patients 5, 6 and 7 had colon and rectal ulcers, inflammatory polyps and rectal stenosis (Fig. 2a–d).

The typical imaging findings of these patients were evaluated. Radiological examination showed colon and rectal disease in enhanced CT and MRI examinations (Fig. 3a–c).

### Table 1 The baseline information of the VEOIBD patients with \( \text{IL10RA} \) gene mutations

| No | Sex | Consanguinity | Onset age, initial symptom | Main symptom | Perianal complications | Paris classification |
|----|-----|---------------|----------------------------|--------------|------------------------|---------------------|
| 1  | M   | NO            | 4 m, diarrhea              | Diarrhea, bloody stool | Skin tag               | A1aL3B1G1           |
| 2  | F   | NO            | 10 d, oral ulcer, eczema   | Diarrhea      | Abscess                | A1aL2B1G0           |
| 3  | M   | NO            | 18 d, fever                | Diarrhea, eczema | Abscess                | A1aL3B1G1           |
| 4  | M   | NO            | 4 y, bloody stool          | Diarrhea, bloody stool | –                     | A1aL3B1G0           |
| 5  | M   | NO            | 2 m, diarrhea              | Diarrhea      | Abscess, fistula       | A1aL2B1G1           |
| 6  | M   | NO            | 14d, fever                 | Diarrhea, oral ulcer, eczema | Abscess, fistula | A1aL2B1G0           |
| 7  | F   | NO            | 0 d, fever                 | Diarrhea, eczema | Abscess, fistula       | A1aL2B2G1           |

BMI body mass index, d day, F female, HFA height for age, M male, m month, WFA weight for age, y year
Treatment and follow-up

Six of the patients agreed to thalidomide treatment. Two patients agreed to mesalazine treatment. Two patients agreed to infliximab infusion and stopped because of several allergies. Two patients underwent HSCT. Patient 7 underwent enterostomy because of severe infection and perianal lesions. Patients 1 and 3 also had cytomegalovirus (CMV)-related inflammation and recovered after antiviral treatment.
After follow-up, patients 5 and 6 were in remission after HSCT, and Sanger sequencing confirmed IL10RA gene repair. Patient 2 died of sepsis after HSCT (Fig. 3D). Patients 2, 4 and 7 were waiting for HSCT, while patient 1 had no intention of undergoing HSCT because of the stability of the disease.

Literature review

We searched the literature from January 2009 to June 2020 in the PubMed and HGMD databases, and only one paper on large deletions in the IL10RA gene was reported by Engelhardt et al. [13]. An Arabic patient was found to have deletions in exons 1, 2, and 3 of IL10RA (Ex1_3del; homozygous). The onset age for this child was 2 months. No transplantation was performed until 1 year of age at the time of manuscript drafting. The main clinical findings were severe colitis and severe otitis media/urinary tract infections. This infant received therapy with prednisone and azathioprine and showed Candida species-induced septicemia. Follow-up showed that the infant had ongoing colitis with no remission. To date, no additional article has reported large deletions of the IL10RA genes, particularly in the East Asian population, where the point mutations c.301C>T (p. R101RW) and c.537 G>A (p. T179T) of IL10RA are common.

Discussion

VEOIBD patients with IL10/IL10R signaling pathway deficiency are characterized by early onset refractory diarrhea and severe infectious diseases, oral ulcers, and perianal diseases (abscess, fistula formation, fissure, and skin tags) early in life with severe evolution in some cases [14, 15].

Glocker et al. first reported patients with IL10R signaling defects [16]. Since then, other defects in IL10 and its receptors IL10RA and IL10RB have been reported.
Our group also reported the phenotype and genotype of IL10R-mutated VEOIBD patients in our collaborative study. We identified c.301C>T (p. R101RW) and c.537 G>A (p. T179T) as common mutations of IL10RA in the East Asian population [9].

WES and targeted gene panel sequencing have emerged as powerful tools to screen genes of interest [22, 23]. Point mutations, small fragment deletions and insertions can be identified by WES and targeted gene panel sequencing. Although point mutations and small region deletions are easily detected with WES, large fragment deletions may be missed. The failure to identify associations between IL10 variants and more common forms of Crohn’s disease may be explained by the current limitations of NGS bioinformatics analysis. Small region deletions and point mutations such as deletions and insertions can be found by Sanger sequencing; unfortunately, large fragment deletions or copy number variations are easily missed [24]. Large fragment deletions of genes cause the loss of protein function. With the development of bioinformatics analysis, not only point mutations but also large fragment deletions can be identified. Large fragment deletions of genes can be further confirmed by multiplex ligation-dependent probe amplification or QT-PCR.

For large fragment deletions of the IL10RA gene, Engelhardt et al. first reported an exon 1_3 large deletion in an Arabic patient. In the current study, we found a novel large deletion of exon 1 of IL10RA in 7 patients for the first time. This 333-bp deletion of exon 1:117857034–11857366 was the main mutation form in these patients. We further confirmed a mutation at a possible hotspot of the IL10RA gene large fragment deletions. Regarding VEOIBD patients, particularly those with perianal diseases, IL10/IL10R-related monogenic diseases are often suspected [25]. In addition to point mutations, small fragment deletions and insertions and large fragment deletions should be considered. Other methods, such as multiplex ligation-dependent probe amplification or QT-PCR, should also be used [20, 26]. Identification of the large fragment deletions of the IL10RA gene has an important effect on clinical practice. For those patients, identifying large fragment deletions of the IL10RA gene helps the genetic diagnosis of VEOIBD. Furthermore, after confirming the compound mutation of IL10RA with a point mutation and large fragment deletions, these children can undergo HSCT to cure this disease. In our study, among the 7 patients with large fragment deletions, 3 accepted HSCT, 2 were in remission, and 3 were awaiting HSCT. Furthermore, the confirmation of
a compound mutation of IL10RA with a point mutation and large fragment deletions helps the prenatal diagnosis and consultation for parents and perinatal gastricians.

Children with VEOIBD and IL10/IL10R signaling pathway deficiency have more extensive, severe, and refractory disease than older children and adults with IBD. The treatment for these patients is challenging [27]. In this study, large fragment deletions of the IL10RA gene caused severe intestinal complications. To determine the differences and similarities in these patients, we compared patients with large deletions in exon 1 with the control group. There was no significant difference except in the time of diarrhea and the percentage of eczema. These two kinds of mutations all cause deleterious effects. The patients with large fragment deletions of the IL10RA gene received mesalazine, thalidomide, infliximab infusion, HSCT, and enterostomy. Thalidomide and enterostomy have shown to be efficient treatments for remission in previous studies. HSCT has been proven to be a radical cure. Furthermore, two patients had CMV infection because of the high expression of CMV DNA. Therefore, for IL10RA-mutated VEOIBD, opportunistic infection should be considered because of immunodeficiency disorders. Our study has several limitations. First, comprehensive functional studies are lacking concerning these patients. Additionally, the comparison with other mutations of IL10RA may have a bias because of the small number of VEOIBD patients with large fragment deletions in the IL10RA gene.

Conclusions
Our study is the first to report large fragment deletions in the IL10RA gene in Chinese VEOIBD patients. This study expands the spectrum of Chinese VEOIBD patients with ILORA gene mutations.

Abbreviations
ACMG: American College of Medical Genetics and Genomics; BMI: Body mass index; HFA: Height for age; HSCT: Hematopoietic stem cell transplantation; IL10: Interleukin 10; IQR: Interquartile range; SD: Standard deviation; NGS: Next-generation sequencing; PCR: Polymerase chain reaction; VEOIBD: Very early onset inflammatory bowel disease; WFA: Weight for age; WHO: World Health Organization.

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Authors’ contributions
ZT and ZH contributed to the conception of the study and drafted the manuscript. MJ, PZ, CY, and RZ contributed to the data collection and analysis. YH and ZH designed the study. ZT and PZ contributed equally to this work. All the authors read and approved the final manuscript.

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Availability of data and materials
The datasets generated and/or analyzed during the current study are available in the ClinVar repository [Accession Nos. SCV001547199, SCV001519656].

Declarations
Ethics approval and consent to participate
The study was approved by the ethics committee of the Children's Hospital of Fudan University. Administrative permissions and licenses were granted by the corresponding author to access the data used in our research. Written consent to participate was obtained from the parents/guardians of the minors included in this study.

Consent for publication
Written consent to write and publish this manuscript was obtained from the patients. The parents/guardians gave their written consent for their child's personal or clinical details along with any identifying images to be published in this study.

Competing interests
The authors declare that they have no competing interests.

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