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***Retrospective Cohort Study***

**Comprehensive Screening for 10 genes in Chinese patients suffered very early onset inflammatory bowel disease**

Xiao Y *et al*. Sequencing 10 genes in Chinese VEO-IBD

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**Abstract**

**AIM:** To perform sequencing analysis in patients with very early-onset inflammatory bowel disease (VEO-IBD) to determine the genetic basis for VEO-IBD in Chinese.

**METHODS:** A total 13 Chinese pediatric patients with VEO-IBD were diagnosed from May 2012 and August 2014. The relevant clinical characteristics of these patients were analyzed. Then DNA in the peripheral blood from patients was extracted. A next generation sequencing (NGS) based on Illumina-Miseq platform was used to analyze of the exons in the coding regions of 10 candidate genes: *IL-10, IL-10RA, IL-10RB, NOD2, FUT2, IL23R, GPR35, GPR65, TNFSF15,* and *ADAM30*. The Sanger sequencing was used to verify the variations detected in NGS.

**Results:** Out of the 13 pediatric patients, 10 were diagnosed with Crohn's disease, and 3 were ulcerative colitis. Mutations in *IL-10RA* and *IL-10RB* were detected in 5 patients. There were 4 patients who had single nucleotide polymorphisms associated with IBD. Two patients had *IL-10RA* and *FUT2* polymorphisms, and 2 patients had *IL-10RB* and *FUT2* polymorphisms. Gene variations were not found in rest of the 4 patients. Children with mutations had a lower percentile body weight (1.0% *vs* 27.5%, *P =* 0.002) and lower hemoglobin (87.4 g/L *vs* 108.5 g/L, *P =* 0.040) when compared with children without mutations. Although the age of onset was earlier, height was shorter, and the response to treatment was poorer in mutation group, there was no significant difference in these factors between groups.

**Conclusion:** *IL-10RA* and *IL-10RB* mutations are common in Chinese with VEO-IBD. Patients with mutations have an earlier disease onset, lighter body weight, lower hemoglobin, and poorer prognosis.

**Key words:** Pediatric inflammatory bowel disease; Very early-onset inflammatory bowel disease; Interleukin 10 receptor; *NOD2* gene; *FUT2* gene

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**Core tip:** In this small-sample size study, we did generation sequencing for 10 candidate genes in Chinese pediatric patients with very eraly onest inflammatory bowel disease. We found that *IL-10RA* and *IL-10RB* mutations were common. There were 5 patients harbouring mutations in these 2 genes and accounted for 38.5% of all samples. Besides, there were 4 patients who had single nucleotide polymorphisms associated with inflammatory bowel disease. Pediatric patients with mutations had an earlier disease onset, lighter body weight, markedly lower hemoglobin, and poorer prognosis.

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**Introduction**

Inflammatory bowel disease (IBD) is a chronic and recurrent gastrointestinal inflammatory disease in children. Based on clinical characteristics, laboratory tests, and endoscopic and pathological presentations, IBD can be subdivided into Crohn’s disease (CD), ulcerative colitis (UC), or IBD-unclassified (IBD-U)[[1](#_ENREF_1)]. Our previous study showed the annual incidence of IBD in the 0 to 14 years age group of Shanghai residents steadily increased from 2000 to 2010[[2](#_ENREF_2)]. Although pediatric IBD mainly occurs in adolescence[[2](#_ENREF_2)], approximately 15% of IBD pediatric patients have very early-onset inflammatory bowel disease (VEO-IBD) that begins before 6 years of age, and 1% of children develop this disease before reaching 1 year of age[[3](#_ENREF_3),[4](#_ENREF_4)]. The majority of VEO-IBD cases have clinical characteristics that are distinct from classic IBD with adult and adolescent onset. VEO-IBD has more severe clinical symptoms, resistance to a variety of immunosuppressive therapies, and a poor prognosis after conventional treatments. Some scholars consider VEO-IBD to be a completely different disease from classic IBD[[5](#_ENREF_5)].

Previous studies suggested that persistent intestinal immune dysfunction in a genetically susceptible population exposed to adverse environmental factors is an important mechanism for IBD development. Genome-wide linkage and association studies (GWAS) have discovered a total of 163 loci associated with the risk for IBD development[[6](#_ENREF_6)]. However, disease onset at an early stage of life suggests a leading role for rare gene variations in VEO-IBD patients, especially in children with a disease onset before the age of 1. These low frequency mutations are difficult to detect using GWAS. Next generation sequencing technology can perform the high-throughput sequencing of exons in a series of genes concurrently; therefore, rare gene variations can be discovered[[7](#_ENREF_7)]. Since Glocker *et al*[[8](#_ENREF_8)] first discovered in 2009 that mutations in genes encoding the α subunit (IL-10R1, encoding gene *IL-10RA*) and the β subunit (IL-10R2, encoding gene *IL-10RB*) of the interleukin 10 receptor could induce VEO-IBD development, a few of studies have continuously discovered mutations in genes encoding IL-10R1, IL-10R2, and interleukin 10 (IL-10)[[5](#_ENREF_5),[9-12](#_ENREF_9)]. However, current reports are limited, and the majority of studies are small-size case studies. Reports on the Han Chinese population are scarcer[[13](#_ENREF_13),[14](#_ENREF_14)].

This study used the Illumina-Miseq platform to sequence candidate genes on Han Chinese children diagnosed with VEO-IBD. The candidate genes included genes involved in the IL-10 signaling pathway, such as *IL-10*, *IL-10RA*, and *IL-10RB*, and genes highly associated with the development of CD in previous studies, including *NOD2, FUT2, IL-23R, GPR35, GPR65, TNFSF15,* and *ADAM30*. This study furthers our understanding of the genetic factors associated with VEO-IBD development in Han Chinese children.

**MaterialS and methods**

***Patient consent and ethic committee approval***

Verbal and written consent was obtained from the parents of all of patients included this study. Ethic committee approval for the study was granted by Institutional Review Boards at Ruijin Hospital affiliated to the Shanghai Jiao Tong University, School of Medicine.

***Study subjects***

A total of 13 pediatric patients with repeated diarrhea, mucus and bloody stool, or abdominal pain who were diagnosed by laboratory tests and digestive endoscopy with VEO-IBD in the Pediatric Department of Ruijin Hospital at the Shanghai Jiao Tong University, School of Medicine between May 2012 and August 2014 were included in this study. All of the patients were Han Chinese. VEO-IBD was defined as IBD onset before the age of 6, and a disease onset before 2 years of age was called infantile-onset IBD[[15](#_ENREF_15),[16](#_ENREF_16)]. The clinical characteristics of these pediatric patients, including gender, age of disease onset, body height, body weight, family history, clinical symptoms, complications, major laboratory examinations, endoscopic presentations, and therapeutic effects, were retrospectively analyzed.

***Laboratory and digestive endoscopic examinations***

Relevant laboratory examinations, including complete blood count (CBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), tumor necrosis factor α (TNF-α) level, immunoglobulin G, A, M, and E, vitamin D, HIV, human cytomegalovirus (CMV) antibody detection in serum, T lymphocyte flow cytometry sorting, stool parasite tests, stool culture, and stool *Clostridium difficile* toxin detection, were performed when the patients were admitted to the hospital. Common infectious diseases and primary immunodeficiency diseases were excluded.

All patients received a colonoscopy under general anesthesia. A biopsy of the colonic mucosa under endoscopy was performed for pathological examination.

***Illumina-Miseq platform sequencing***

**Genomic DNA extraction:** After obtaining verbal and written informed consent from the patients’ parents, genomic DNA in the peripheral blood from 13 pediatric patients was extracted using a FlexiGene DNA Kit (Qiagen Inc., Germany). Another 100 copies of DNA extracted from patients suffering from idiopathic short stature (ISS) in previous research were used to test frequency of mutant sites which were newly detected in our study.

**Multiplex PCR primer design:** Based on the stability of the Illumina-Miseq experiment and the operability of subsequent steps, the length requirement of target fragments for sequencing was < 400 bp. If the length of an exon was longer than 400 bp, an additional pair of primers was designed with overlapping bases of adjacent fragments. To avoid a high number of non-target fragment products, primers were grouped and suspended in a primer mix before the multiplex PCR performed. The concentration of each primer in the primer mix was 10 mmol/L. The basic requirement for grouping was the lack of matching sequences between two of the amplified products. Oligo 7 software was used to design primers for exons of the encoding region of the 10 candidate genes: *IL-10, IL-10RA, IL-10RB, NOD2, FUT2, IL23R, GPR35, GPR65, TNFSF15,* and *ADAM30*. A total of 86 pairs of primers were designed. The sequences are shown in supplementary Table 1.

**Multiplex PCR amplification of candidate genes:** A Qiagen Multiple PCR Kit was used in this study. The PCR amplification reaction system had a total volume of 21 μL, including 4 μL of ddH­2O, 2 μL of Q-solution (5 x), 4 μL of 10 mM primer mix, 10 μL of buffer mix, and 1 μL of the DNA template (20 ng/μL). The reaction procedure consisted of pre-denaturation at 94 °C for 15 min, denaturation at 94 °C for 40 s, annealing at 63 °C for 1 min, and extension at 72 °C for 40 s. After each cycle, the annealing temperature was reduced by 0.5 °C for 10 cycles until the annealing temperature reached 58 °C. Next, the amplification was continued for 30 cycles with a constant annealing temperature of 58 °C. The final extension at 72 °C lasted for 10 min. The PCR products were stained with 100 x GelRed and subjected to 1% agarose electrophoresis (120 V for 60 min).

The purified multiplex PCR products were sent to Shanghai South Gene Technology Co. Ltd. for sequencing analysis with the Illumina-Miseq platform. After sequencing, the nucleotide sequence information was compared with the standard gene sequences available in GenBank. The obtained gene mutation sites were compared with information in the dbSNP, HGMD, and OMIM databases to determine if the mutations had been previously reported.

To confirm the accuracy of the results, the corresponding gene sequences for the mutations discovered using the Illumina-Miseq platform were sequenced again using the Sanger sequencing method.

The newly discovered gene variation sites were analyzed to predict their influence on protein functions using 2 online databases: SIFT ([http:// http://sift.jcvi.org/](http://provean.jcvi.org/protein_batch_submit.php?species=human)) and PolyPhen 2 (<http://genetics.bwh.harvard.edu/pph2/>).

***Statistical analysis***

According to the sequencing results, the 13 pediatric patients were divided into 2 groups. The patients who harboured pathogenic mutations were in group 1. Those without pathogenic mutations (including presence of polymorphisms only or wild type) were in group 2. The differences in diagnosis, age of disease onset, growth indicators (percentiles of body weight and height were calculated according to WHO standards), complications (perianal diseases and recurrent infection), and therapeutic effects among all groups were compared. Because the sample size was small, quantitative and ranked ordinal data were subjected to nonparametric statistics. The Mann-Whitney Test was performed, and the difference was statistically analyzed using exact probability. SPSS13.0 for Windows software was used for the statistical analysis. P<0.05 indicated a significant difference.

**Results**

***Genotyping of VEO-IBD patients***

***IL-10RA*, *IL-10RB*, and *IL-10* mutations:** *IL-10RA* mutations were detected in 4 patients, an *IL-10RB* mutation was detected in 1 patient, and an *IL-10* mutation was not detected in any of the 13 patients.

The detected *IL-10RA* mutations were all in exon 3: c.A191G (p.Y64C), c.T299G (p.V100G), c.C301T (p.R101W), and c.G350A (rs199989396) (p.R117H). The p.R101W mutation was the most common and was detected in 3 patients (patients 1-3). The other mutations were detected in only 1 patient. Patient 1 had a homozygous mutation, patients 2 and 3 had compound mutations, and patient 4 had a heterozygous mutation (Table 1 and Figure 1).

Among detected *IL-10RA* mutations, p.Y64C and p.V100G were new mutations that were predicted to be deleterious by SIFT and Polyphen 2. These novel mutant sites were not found in 100 ISS children. The other 2 mutations had been confirmed to be deleterious in several studies[[5](#_ENREF_5),[12](#_ENREF_12),[14](#_ENREF_14),[17](#_ENREF_17)].

A *IL-10RB* heterozygous mutation was detected in 1 patient (patient 5) (Table 1 and Figure 1). This c.G421A (p.E141K) (rs387907326) mutation was located in exon 4 and was also predicted as a deleterious mutation by SIFT and Polyphen 2. A nonsense mutation in same site was detected in previous study[[11](#_ENREF_11), [18](#_ENREF_18)].

***Candidate gene polymorphisms***

After the sequence analysis of the coding regions of 10 candidate genes, we found that 6 patients (patient 4, 5, 6, 7, 8 and 9) had many IBD-associated single nucleotide polymorphisms (SNPs) in *IL-10RA*, *IL-10RB*, *NOD2*, and *FUT2*. The SNP loci in *IL-10RA* were rs22280554: c.G525A, p. P175P and rs22280555: c.A670G, p.I224V; the SNP locus in *IL-10RB* was rs2834167: c.A139G, p.K47E; the SNP locus in *NOD2* was rs5743277: c.C2107T, p.R703C; and the SNP locus in *FUT2* was rs1047781: c.A418T, p.I140F (Table 1).

In addition to the detected p.R117H heterozygous mutation in *IL-10RA*, patient 4 also had heterozygous SNPs in *NOD2* and *FUT2*.

Patient 5 had a heterozygous p.E141K mutation (rs387907326) in *IL-10RB* and SNPs in *IL-10RA, IL-10RB,* and *FUT2*. The SNP loci in *IL-10RA* were rs22280554 and rs22280555. The homozygous SNP loci for *IL-10RB* were rs2834167. The SNP in *FUT2* was heterozygous.

Patients 6 and 7 had SNPs in *IL-10RA* and *FUT2*. Patients 8 and 9 had SNPs in *IL-10RB* and *FUT2*.

Four patients did not show any IBD-associated variations in the coding regions of the 10 candidate genes.

There was no IBD-associated variation discovered in the coding regions of 6 genes: *IL-10, IL-23R*, *GPR35*, *GPR65*, *TNFSF15*, and *ADAM30*.

***Clinical characteristics of VEO-IBD pediatric patients***

Out of the 13 VEO-IBD pediatric patients in this study, 10 patients were diagnosed with CD (M:F = 9:1) and 3 patients had UC (M:F = 1:2). The mean age of disease onset was 5.8 ± 9.7 mo (range: birth to 3 years of age). None of the parents of the patients had a consanguineous marriage. Patient 8 had a brother that died as a neonate because of repeated diarrhea after birth. There was no clear diagnosis made at that time. The clinical symptoms of the pediatric patients included repeated abdominal pain (13/13), diarrhea (11/13), mucus and bloody stool (11/13), faliure to thrive (8/13), recurrent infection (7/13), and perianal fistulas and abscesses (5/13). The colonoscopic presentation of patients with causative mutations showed pancolitis, cobblestone-like changes in mucosa, and deep and large ulcers (Figure 2). All patients received immunosuppressive treatment with glucocorticoids, 6-mercaptopurine and/or infliximab, and thalidomide; however, varying therapeutic effects were observed. Two patient died from severe sepsis or intestinal failure, 2 patients showed no change, 4 patients showed a partial alleviation of symptoms, and 5 patients showed complete clinical remission (Table 2).

***Clinical characteristics of different genotypes***

Based on the presence of causative mutations in *IL-10RA* and *IL-10RB*, 13 patients were divided into 2 groups for analysis (Group 1: causative mutations in *IL-10RA* or *IL-10RB*; Group 2: polymorphisms and no causative mutations). The 5 patients in Group 1 were all diagnosed with CD (100%). Four of these patients had recurrent infections (80%), and 3 patients had perianal diseases (60%). In Group 2 (8 patients), 5 patients were diagnosed with CD (62.5%), and the other 3 patients were diagnosed with UC (37.5%). There were only 3 (37.5%) and 2 (25%) patients that had recurrent infections and perianal diseases, respectively. Patients in group 1 had lighter body weights percentile (1.0% *vs* 27.5%, *P =* 0.002) and lower hemoglobin concentrations (87.4 g/L *vs* 108.5 g/L, *P =* 0.040) when compared with group 2. Although patients in group 1 had a younger age of disease onset (2.7 months), lower body height percentile (5.0%), and higher CRP (60.7 mg/L), there were no significant differences when compared with group 2 (Table 3).

**Discussion**

The currently recognized pathogenetic mechanism of IBD is the involvement of many environmental triggers and a genetic susceptibility that causes intestinal immune dysfunction. However, the influence of genes are likely more important than environmental factors for VEO-IBD patients with a disease onset prior to 6 years of age, especially patients with an infantile onset prior to 1 year of age[[19](#_ENREF_19)]. GWAS studies suggested that SNPs of *IL-10* and *STAT3* wereassociated with IBD[[20-23](#_ENREF_20)]. Previous studies confirmed that IL-10 or IL-10 receptor gene knockout mice had severe chronic inflammation of the intestinal tract[[24](#_ENREF_24)]. IL-10 forms a complex with 2 molecules of IL-10R1 and 2 molecules of IL-10R2 to activate Janus kinase 1 (Jak1) and tyrosine kinase 2 (Tyk2). This activation results in the phosphorylation of signal transducer and activator of transcription 3 (STAT3), which regulates the transcription of specific genes. Studies suggested that IL-10-mediated signals effectively reduced the number of Th17 cells and relieved intestinal inflammation in CD[[25](#_ENREF_25)]. These data indicated that the anti-inflammatory IL-10 signaling pathway played a critical role in the regulation of intestinal immune homeostasis.

Since Glocker *et al*[[8](#_ENREF_8)] first reported in 2009 that gene mutations in *IL-10RA* and *IL-10RB* caused infantile onset IBD[[8](#_ENREF_8)], studies have continuously reported mutations in *IL-10*, *IL-10RA*, and *IL-10RB* in patients with infantile onset IBD[[9-12](#_ENREF_9),[18](#_ENREF_18),[26](#_ENREF_26)]. In these limited data, the majority of patients were Arabian or Caucasian and the offspring of a consanguineous marriage. There are few reports on the Han Chinese population, including only 3 pediatric patients to date[[13](#_ENREF_13),[14](#_ENREF_14)].

In this study, we used high-throughput next generation sequencing technology to sequence 10 IBD-associated genes, *IL-10*, *IL-10RA*, *IL-10RB*, *NOD2*, *FUT2*, *IL-23R*, *GPR35*, *GPR65*, *TNFSF15*, and *ADAM30*, in 13 Han Chinese children diagnosed with VEO-IBD. A total of 4 mutations were discovered in *IL-10RA*, including 2 novel mutations. There was 1 mutation in *IL-10RB*. These pathogenic mutations were found in 5 patients, which accounted for 38.5% of all VEO-IBD. Among these patients, 1 patient had an *IL-10RA* homozygous mutation, 2 patients had *IL-10RA* compound heterozygous mutations*,* 1 patient had an *IL-10RA* heterozygous mutation, and 1 patient had an *IL-10RB* heterozygous mutation. All *IL-10RA* mutations were in exon 3, and c.C301T (p.R101W) showed the highest frequency. The c.C301T (p.R101W) and c.G350A (p.R117H) mutations in *IL-10RA* were previously reported in similar pediatric patients. These mutations may disrupt signal transduction after activation of the IL-10 receptor; therefore, STAT3 is not phosphorylated and intractable inflammatory reactions in the intestinal tract of pediatric patients develop[[5](#_ENREF_5),[12](#_ENREF_12)]. The 2 novel mutations in *IL-10RA* discovered in this study were c.A191G (p.Y64C) and c.T299G (p.V100G). Because of condition limitations, we did not perform functional studies on these mutations. However, the SIFT prediction results for these 2 mutations were deleterious (scores of 0 and 0.002, respectively), and the Polyphen 2 prediction results were probably damaging (both scores were 1.000). These predictions suggest that these 2 mutations are pathogenic. According to the recommendation of the American College of Medical Genetics (ACMG) and Genomics and the Association for Molecular Pathology (AMP)[[27](#_ENREF_27)], these 2 mutations were defined as pathological supporting.Therefore, we speculate that these 2 novel mutations individually formed compound heterozygotes with the c.C301T (p.R101W) mutation to cause the disease symptoms observed in patients 2 and 3.

Previous analyses showed that the colitis caused by gene mutations in IL-10 and its receptor exhibited an autosomal recessive inheritance pattern. In the current study, patients 4 and 5 were carriers of heterozygous mutations in *IL-10RA* and *IL-10RB*, respectively. The c.G350A (p.R117H) mutation in *IL-10RA* carried by patient 4 was a pathogenic mutation[[5](#_ENREF_5),[12](#_ENREF_12),[17](#_ENREF_17)]. The c.G421A (p.E141K) mutation in *IL-10RB* carried by patient 5 may affect protein function as predicted by SIFT (score= 0.026) and Polyphen 2 (score = 0.946). However, the clinical presentation of these 2 patients was similar to the symptoms of patients with other IL-10 receptor mutations: disease onset was within 1 year of age, the presence of perianal diseases and recurrent infection, and resistance to conventional medication treatment. Based on currently available knowledge, there are at least 50 single-gene genetic conditions that induce IBD-like diseases, and the majority of conditions are related to immunodeficiency[[4](#_ENREF_4),[6](#_ENREF_6)]. Therefore, the 2 patients that did not conform to a Mendelian genetic pattern might also carry abnormal sites on other genes that cause the disease symptoms. In addition to carrying a pathogenic mutation in *IL-10RA*, patient 4 also had a non-synonymous SNP (nsSNP): rs5743277 in *NOD2*. SIFT prediction results suggest the nsSNP is deleterious (score = 0), and the Polyphen 2 prediction results suggest the nsSNP is probably damaging (score = 0.999). This polymorphism was already present in the HGMD database and has been considered to cause susceptibility to CD[[28](#_ENREF_28)]. Patient 5 had a similar condition. In addition to carrying an *IL-10RB* mutation, patient 5 also had multiple polymorphisms: rs22280554 (homozygous) and rs22280555 (homozygous) in *IL-10RA*, rs2834167 (homozygous) in *IL-10RB*, and rs1047781 (heterozygous) in *FUT2*. There are previous reports on the pathogenicity of these SNPs. For example, Galatola *et al*[[29](#_ENREF_29)] reported that the heterozygous rs2834167 in *IL-10RB* and the heterozygous mutation in the promoter region of *IL-10RA* caused the development of UC in an 18-month-old patient. Although rs22280554 did not cause a change in the amino acid sequence of IL-10R1, a study by Moran *et al*[[30](#_ENREF_30)] showed that rs22280554 and rs2228055 in *IL-10RA* may increase the risk for VEO-IBD, especially VEO-UC. Furthermore, in the Han Chinese population, the rs1047781 polymorphism in *FUT2* may increase the risk for CD development[[31](#_ENREF_31)]. The above SNPs were also detected in 4 patients in this study. Therefore, their disease development may be due to “trans-heterzygous”: the collective effects of a variety of detected mutations. Another possible cause is that the pathogenic genes were not detected in this study.

When genotypes and phenotypes were combined for analyses, the results showed that the disease phenotype in patients with mutations were more severe. The age of disease onset was earlier, the patients were more likely to have combined recurrent infections and perianal diseases, their body weight and height were low, anemia was more severe, inflammatory indicators were high, and the prognosis was much poorer. These results are in accordance with previous studies[[5](#_ENREF_5),[8-14](#_ENREF_8),[18](#_ENREF_18),[32](#_ENREF_32)]. However, the sample size of this study was small, and significant differences were only found with body weight and hemoglobin parameters. Because of the influence of cultural ideas, family members find difficulty in accepting an ileostomy as a disease treatment. Past literature reported that pediatric patients with *IL-10RA* and *IL-10RB* mutations could be cured through hematopoietic stem cell transplantation[[4](#_ENREF_4),[6](#_ENREF_6),[8](#_ENREF_8),[17](#_ENREF_17)]; therefore, some patients are waiting for a donor match.

In this study, we found that mutations in *IL-10RA* and *IL-10RB* were more common in Han Chinese VEO-IBD patients and accounted for 38.5% of all VEO-IBD. The high percentage is probably due to the small number of patient in the cohort as most of our patients who were referred by other clinical IBD centers were very ill. There was a selection bias. Because VEO-IBD is relatively rare, multi-center studies on the relationship between genotypes and phenotypes in VEO-IBD patients in China are necessary. And the implementation of hematopoietic stem cell transplantation therapy are the focus in research agenda.

**comments**

***Background***

Very early onset inflammatory bowel disease (VEO-IBD) may have stronger genetic contribution. Recently, a few of studies on genetic defects in the IL-10 signaling pathway have provided new insights into IBD, especially in VEO-IBD. Furthermore, a lot of genes associated with IBD were identified, such as *NOD2*, *FUT2, IL-23R, GPR35, GPR65, TNFSF15,* and *ADAM30*. Becaue of different genetic background, this study was set to disclose whether mutations in these genes contributed to VEO-IBD in Chinese childred.

***Research frontiers***

In addition to the polygenic variants associate with IBD, there are rare monogenic disorders, including many immunodeficiencies that can present with IBD-like intestinal inflammation, especially in early life.

***Innovations and breakthroughs***

To our knowledge, this is the first cohort study to apply NGS in 13 Chinese pediatric patients with VEO-IBD to discover gene variations in this children. The result revealed that IL-10RA and IL-10RB mutations were common in Chinese VEO-IBD, especially in infantile IBD. And these monogenic IBD patients had a more severe clinical features.

***Applications***

According to the results of this study and previous studies of VEO-IBD, we suggest that screening for gene mutations in IL-10 signaling pathway is necessary.

***Peer-review***

The clinical study is focused on gene expression analysis in VEO-IBD by NGS. The authors conclude that mutations in the IL-10 pathway are commonly in VEO-IBD.

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**Figure 1 Causative mutations in *IL-10RA* (A-D) or *IL-10RB* (E).** A: Patient 1, c.C301T, p.R101W, homozygote; B: Patient 2, c.T299G, p.V100G and c. C301T, p.R101W, compound heterozygote; C: Patient 3, c.A191G, p.Y64C and c. C301T, p.R101W, compound heterozygote; D: Patient 4, c.G35A, p.R117H (rs199989396), heterozygote; E: Patient 5, c.G421A, p.E141K (rs387907326), heterozygote).



**Figure 2 Colonoscopic presentation of patients with causative mutations showed pancolitis, cobblestone-like changes in mucosa, and deep and large ulcers.** A to E presents patient 1 to patient 5 respectively).

**Table 1 Genotypes of 13 patients diagnosed as very early onset inflammatory bowel disease**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Patient** | **Gene** | **Variation** | **Homo/Heterozygotie** | **Function defect** |
| 1 | *IL-10RA* | p.R101W | Homozygote | Yes |
| 2 | *IL-10RA* | p.R101W | Compound heterozygote | Yes |
| p.V100G (novel mutation) | Pathogenic supporting by Polyphen2 and SIFT |
| 3 | *IL-10RA* | p.R101W | Compound heterozygote | Yes |
| p.Y64C (novel mutation) | Pathogenic supporting by Polyphen2 and SIFT |
| 4 | *IL-10RA* | p.R117H (rs199989396) | Heterozygote | Yes |
| *NOD2* | p.R703C (rs5743277) | Heterozygote | Susceptibility to CD recorded in HGMD |
| *FUT2* | p.I140F (rs1047781) | Heterozygote | Susceptibility to CD in Chinese population reported by Hu *et al*[31]. |
| 5 | *IL-10RB* | p.K47E (rs2834167) | Homozygote | SNP in a VEO-UC child reported by Galatola *et al*[29]. |
| p.E141K (rs387907326) | Heterozygote | Pathogenic supporting by Polyphen2 and SIFT |
| *IL-10RA* | p.P115P (rs22280554) | Homozygote | Susceptibility to VEO-IBD reported by Moran *et al*[30]. |
| p.I224V (rs22280555) | Homozygote |
| *FUT2* | p.I140F (rs1047781) | Heterozygote | Susceptibility to CD in Chinese population reported by Hu *et al*[31]. |
| 6 | *IL-10RA* | p.P115P (rs22280554) | Homozygote | Susceptibility to VEO-IBD reported by Moran *et al*[30]. |
| p.I224V (rs22280555) | Homozygote |
| *FUT2* | p.I140F (rs1047781) | Homozygote | Susceptibility to CD in Chinese population reported by Hu *et al*[31]. |
| 7 | *IL-10RA* | p.P115P (rs22280554) | Homozygote | Susceptibility to VEO-IBD reported by Moran *et al*[30]. |
| p.I224V (rs22280555) | Homozygote |
| *FUT2* | p.I140F (rs1047781) | Heterozygote | Susceptibility to CD in Chinese population reported by Hu *et al*[31]. |
| 8 | *IL-10RB* | p.K47E (rs2834167) | Homozygote | SNP in a VEO-UC child reported by Galatola *et al*[29]. |
| *FUT2* | p.I140F (rs1047781) | Heterozygote | Susceptibility to CD in Chinese population reported by Hu *et al*[31]. |
| 9 | *IL-10RB* | p.K47E (rs2834167) | Heterozygote | SNP in a VEO-UC child reported by Galatola *et al*[29]. |
| *FUT2* | p.I140F (rs1047781) | Heterozygote | Susceptibility to CD in Chinese population reported by Hu *et al*[31]. |

Patient 10, 11, 12 and 13 were wild types in all these genes. CD: Crohn's disease; UC: Ulcerative colitis; VEO-IBD: Very early onset inflammatory bowel disease; VEO-UC: Very early onset ulcerative colitis; SNP: Single nucleotide polymorphism; HGMD: The Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php).

**Table 2 Clinical manifestation of very early onset inflammatory bowel disease**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Patient 1** | **Patient 2** | **Patient 3** | **Patient 4** | **Patient 5** | **Patient 6** | **Patient 7** | **Patient 8** | **Patient 9** | **Patient 10** | **Patient 11** | **Patient 12** | **Patient 13** |
| **Gender** | F | M | M | M | M | M | M | M | M | M | M | F | F |
| **Age of onset (month)** | 8  | 1 | 0.3 | 0.3 | 4 | 0.2 | 9 | 2 | 0.5 | 3 | 0.7 | 10 | 36 |
| **Height percentile** | 19% | 1% | 3% | 1% | 1% | 1% | 52% | 1% | 15% | 1% | 19% | 16% | 20% |
| **Weight percentile** | 1% | 1% | 1% | 1% | 1% | 20% | 55% | 13% | 8% | 15% | 16% | 60% | 33% |
| **Diahrrea (times/day)** | > 10 | 7-8 | > 10 | 10 | 5-10 | 5-6 | 7-8 | 2-4 | 7-8 | No diarrhea | 7-8 | No diarrhea | 2-3 |
| **Bloody stool** | + | + | + | + | + | - | + | + | + | - | + | + | + |
| **Infection** | Sepsis | Pneumonia | No | Pneumonia, *clostridium difficile* infection | Sepsis,oral candidiasis,fungemia,*clostridium difficile* infection | Recurrent respiratory infection | No | No | No | Repeated fever of unknow origin | Oral candidiasis，gingivitis | No | No |
| **Perianal lesion** | Fistulae | No | No | Eexcrescence | Fistulae,abscess,excrescence | Fistulae,ulcer | No | No | No | No | Fistulae,abscess,excrescence | No | No |
| **Clinical diagnosis** | CD | CD | CD | CD | CD | CD | CD | CD | UC | CD | CD | UC | UC |
| **Medication** | GC, 6-MP | IFX,THD | GC,THD | GC,IFX1,THD | GC,IFX,THD | GC,IFX1 | GC,IFX,MES | GC,IFX1,THD,6-MP | GC,MES | GC,6-MP,THD | GC,IFX,THD,6-MP | MES | GC,MES |
| **Clinical status** | NR | PR | Died at 2 yr because of severe sepsis | PR | Died at 3yr because of intestinal failure | NR | CR | PR | CR | CR | PR | CR | CR |

1allergic to IFX

1allergic to IFX. CD: Crohn's disease; UC: Ulcerative colitis; GC: Glucocorticoid; 6-MP: 6-mercaptopurine; IFX: Infliximab; THD: Thalidomide; MES: Mesalazine; NR: Non-remission; PR: Partial remission; CR: Complete remission.

**Table 3 Comparison of features between patients with mutations and polymorphisms**

|  |  |  |  |
| --- | --- | --- | --- |
| 　 | 　 | **Group 1** | **Group 2** |
| **Size of sample** | 5 | 8 |
| **Age of onset (mo)** | 2.7 | 7.7 |
| **Height percentile** | 5.0% | 15.6% |
| **Weight percentile1** | 1.0% | 27.5% |
| **WBC (× 10**-9) | 15.2 | 16.3 |
| **Hemoglobin (g/L)1** | 87.4 | 108.5 |
| **Platelet (× 10**-9) | 538.4 | 424 |
| **C reactive protein (mg/L)** | 60.7 | 35.9 |
| **ESR (mm/H)** | 32.2 | 16.6 |
| **TNFα (pg/ml)** | 44.5 | 51.6 |
| **Diagnosis of CD** | 100.0% | 62.5% |
| **Recurrent infection** | 80.0% | 25.0% |
| **Perianal disease** | 60.0% | 25.0% |

1The *P* value < 0.05. All measurement data are expressed as mean. Group 1: mutations in IL-10RA or IL-10RB; Group 2: polymorphisms. Height and weight percentile was calculated according to WHO charts. WBC: White blood cell; ESR: Erythrocyte sedimentation rate; TNFα: Tumor necrosis factor alpha; CD: Crohn's disease.