# World Journal of
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**ABOUT COVER**

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**Observational Study**

# Intestinal dysbiosis in pediatric Crohn's disease patients with *IL10RA* mutations

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## Abstract

### BACKGROUND
Several studies have employed animal models to explore the association between microbiota and interleukin (IL) 10 signaling; however, limited information is available about the human microbiome.

### AIM
To characterize the microbiome in patients with *IL10RA* mutations and to explore the association between gut dysbiosis and disease severity.

### METHODS
Fecal samples were collected from patients who were diagnosed with loss-of-function mutations in the *IL10RA* gene between January 2017 and July 2018 at the Children's Hospital of Fudan University. Age-matched volunteer children were recruited as healthy controls. Patients with Crohn's disease (CD) were used as disease controls to standardize the antibiotic exposure. Microbial DNA was extracted from the fecal samples. All analyses were based on the 16S rRNA gene sequencing data.

### RESULTS
Seventeen patients with *IL10RA* mutations (IL10RA group), 17 patients with pediatric CD, and 26 healthy children were included. Both patients with *IL10RA* mutations and those with CD exhibited a reduced diversity of gut microbiome with increased variability. The relative abundance of *Firmicutes* was substantially increased in the IL10RA group ($P = 0.02$). On further comparison of the relative abundance of taxa between patients with *IL10RA* mutations and healthy children, 13 taxa showed significant differences. The IL10RA-specific dysbiosis indices exhibited a significant positive correlation with weighted pediatric CD activity index and simple endoscopic score for CD.

### CONCLUSION

In patients with *IL10RA* mutations and early onset inflammatory bowel disease, gut dysbiosis shows a moderate association with disease severity.

**Key words:** *IL10RA* gene; Gut microbiota; Pediatric; Crohn's disease; Disease severity

**Core tip:** Understanding the role of microbes in sub-populations of inflammatory bowel disease patients is important. The focus on this relatively unique and uniform interleukin (IL)10RA group provides an excellent opportunity. In this study, clinical variables of IL10RA-deficient patients (such as disease course) were linked with changes in the stool microbiome, which implies potential clinical relevance of the changes in microbial populations.

## INTRODUCTION

Inflammatory bowel disease (IBD) includes Crohn's disease (CD), ulcerative colitis, and IBD unclassified. The pathogenetic mechanism of IBD is believed to involve inappropriate immune response to gut microbiota in genetically susceptible individuals. Recent studies have provided some insights into the role of complex host-microbiota interactions in the pathogenesis of IBD[1]. The next-generation sequencing approaches have helped unravel the genetic factors involved in the pathogenesis of infantile-onset IBD (age at diagnosis: < 2 years)[2]. The interleukin (*IL*)10 gene is one of the important genes that are known to affect the risk of IBD.

IL10 is an anti-inflammatory cytokine that inhibits intestinal inflammation. Patients with IL10 or IL10R deficiency can develop severe infantile colitis resembling CD[3]. Recent studies have revealed an association between host genetic variants and gut microbial changes; in addition, the underlying interactions were found to contribute to the onset and severity of IBD[4-7]. However, the role of microbiota in patients with infantile-onset IBD who have IL10 signaling defects is not clear. Several studies have employed animal models to explore the association between microbiota and IL10 signaling, because the link between aberrant IL10 signaling and IBD was first established in IL10$^{-/-}$ mice[8]. Colitis occurs in the presence of intestinal microbiota and changes in intestinal microbiota may also modulate the inflammatory response. For example, introduction of *Lactobacillus plantarum* 299v, *Lactobacillus salivarius* 433118, and *Bifidobacterium infantis* 35624 was shown to attenuate colitis[9-11], while introduction of *Enterococcus faecalis*, *Escherichia coli*, *Helicobacter bilis*, and *Helicobacter hepaticus* exacerbated the inflammation[12-14]. IBD (in animal models) occurs only in the presence of intestinal microbiota, as germ-free animals do not develop colitis. However, information pertaining to human microbiome is not well characterized.

Mutations in *IL10RA*, a gene that encodes one of the subunits of IL10R, have been identified as the most common causal mutations in infantile-onset IBD in China[15-17]. Thus, we conducted this study to characterize the microbiome in patients with *IL10RA* mutations and to explore the association between the disease severity and gut dysbiosis.

## MATERIALS AND METHODS

### Study design and sample collection

This was a single-center observational study. Pediatric patients (age: 0–18 years) who were initially diagnosed with loss-of-function mutations in the *IL10RA* gene at the Children's Hospital of Fudan University (China) between January 2017 and July 2018 were enrolled (IL10RA group). *IL10RA* gene mutations were identified by whole-exome sequencing or targeted gene panel and confirmed by Sanger sequencing, as

described elsewhere[16,17]. Patients who did not undergo endoscopy at our center or who had been diagnosed with *IL10RA* gene mutations before transfer to our hospital were excluded. Moreover, we excluded patients with a history of ostomy and those with a history of extensive bowel resection. Two groups were used as controls. Age-matched volunteer children who did not receive any medical treatment or antibiotics and had no evidence of gastrointestinal disease or symptoms were recruited as healthy controls (HC group). Patients with a confirmed diagnosis of CD based on radiological, endoscopic, and histopathological evaluations after a minimum of 6-mo follow-up were enrolled as CD controls. CD patients who developed the disease at the age of less than 6 years were screened for relevant mutations by whole exosome sequencing; the sequencing results were negative for all these patients. Fecal samples were collected prior to bowel preparation for endoscopy. Samples were transported to the laboratory and stored at -80°C prior to further processing.

### Data collection and definitions
Clinical data pertaining to the following variables were obtained from the medical records: Age, sex, weight, age at onset, medical history, diet, disease behavior, laboratory results, clinical diagnosis, treatment, and endoscopic findings. Laboratory results included hemoglobin, C-reactive protein (CRP), and IL6 levels. Clinical activity was assessed at sample collection using Simple Endoscopic Score for CD (SES-CD), weighted pediatric CD activity index (wPCDAI), and Mucosal-Inflammation Non-Invasive (MINI) index [a newly developed noninvasive index that incorporates fecal calprotectin, CRP, erythrocyte sedimentation rate (ESR), and the stool item from the PCDAI for pediatric CD[18]].

### 16S rRNA gene sequencing
Microbial DNA was extracted using the FastDNA SPIN Kit (MP Biomedicals, Santa Ana, CA, United States) according to the manufacturer's instructions. DNA concentration and purity were measured using the NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, United States), and DNA quality was checked by 1% agarose gel electrophoresis. V3-V4 hypervariable regions of the bacteria 16S rRNA gene were amplified using the thermocycler PCR system (GeneAmp 9700, ABI, United States). Purified amplicons were pooled in equimolar and paired-end sequenced on the Illumina MiSeq Platform (Illumina, San Diego, United States) according to standard protocols of the Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

### Statistical analysis
Data analyses were performed using Stata 13.1 for Windows (Stata Corp LP, TX, United States), Prism 6 version 6.02 (GraphPad Software, lnc, San Diego, CA, United States), and MedCalc Statistical Software version 19.0.4 (MedCalc Software bvba, Ostend, Belgium). Categorical variables are presented as frequencies (percentages). Continuous variables are presented as the mean (standard deviation). Fisher's exact test was used to compare categorical variables when cell sizes were less than 1. The Mann-Whitney *U* test was used to compare continuous variables between two groups, while the Kruskal-Wallis method was used to compare three or more groups. Spearman correlation analysis was performed to assess correlation between variables. The conservative Bonferroni correction was adopted for multiple tests. Two-tailed *P* values < 0.05 were considered indicative of statistical significance. For bioinformatics analyses, the α-diversity metrics and β-diversity were calculated using unweighted unifrac distances and represented in principal co-ordinates analysis (PCoA) using the open-access online Majorbio I-Sanger Cloud Platform (www.i-sanger.com).

## RESULTS

### Clinical characteristics of the patients
A total of 32 IL10RA-deficient patients were admitted to our hospital during the study reference period. Fifteen patients were excluded for the following reasons: Six patients were undergoing follow-up for disease revaluation; of these, four had undergone allo-hematopoietic stem cell transplantation while the other two were in remission after treatment with thalidomide. Four patients were excluded because of a history of ostomy. Five patients had confirmed *IL10RA* gene mutations and had undergone endoscopy prior to referral to our hospital. Our study finally included 17 IL10RA-deficient patients, defined as the IL10RA group (Supplementary Table 1). In addition, 17 patients with CD and 26 healthy children were also enrolled. The detailed clinical characteristics and medication history are shown in Table 1.

Among patients in the IL10RA and CD groups, 76% were exposed to antibiotics

**Table 1  Clinical characteristics of the study population**

|  | IL10RA | CD | HC | *P* value[1] |
|---|---|---|---|---|
| Number of samples | 17 | 17 | 26 |  |
| Demographics |  |  |  |  |
| Age (mean ± SD, yr) | 1.00 ± 0.68 | 8.92 ± 4.80 | 1.61 ± 1.71 | < 0.001 |
| Sex (M/F) | 10/7 | 11/6 | 17/9 | 0.724 |
| Weight and BMI |  |  |  |  |
| Weight (mean ± SD, kg) | 7.31 ± 2.58 | 27.18 ± 13.04 | NA | < 0.001 |
| BMI (mean ± SD) | 14.53 ± 2.20 | 15.25 ± 2.44 | NA | 0.6175 |
| Disease activity |  |  |  |  |
| CRP (mean ± SD, mg/L) | 40.65 ± 28.13 | 34.06 ± 31.92 | NA | 0.4486 |
| wPCDAI (mean ± SD) | 52.21 ± 17.65 | 40.88 ± 20.02 | NA | 0.1253 |
| SES-CD (mean ± SD) | 20.56 ± 7.56 | 12.94 ± 8.60 | NA | 0.0158 |
| MINI index (mean ± SD) | 19.88 ± 4.96 | 16.47 ± 2.92 | NA | 0.0027 |
| Disease duration and anemia |  |  |  |  |
| Disease duration (mean ± SD, mo) | 9.93 ± 7.75 | 7.10 ± 9.30 | NA | 0.0706 |
| Disease location |  |  |  |  |
| Ileum only (%) | 0 (0) | 3 (18) | NA | < 0.001 |
| Colon only (%) | 17 (100) | 3 (18) | NA |  |
| Both (%) | 0 (0) | 11 (64) | NA |  |
| Disease behaviour |  |  |  |  |
| Montreal classification B1 (%) | 11 (65) | 15 (88) | NA | 0.106 |
| Montreal classification B2 (%) | 5 (29) | 2 (12) | NA | 0.203 |
| Montreal classification B3 (%) | 4 (24) | 0 (0) | NA | 0.033 |
| Montreal classification P (%) | 16 (94) | 4 (24) | NA | < 0.001 |
| Serology |  |  |  |  |
| IL6 (mean ± SD, pg/mL) | 122.47 ± 152.22 | 37.50 ± 25.08 | NA | 0.0211 |
| Birth and breast feeding |  |  |  |  |
| Cesarian section (%) | 11 (65) | 8 (47) | NA | 0.300 |
| Breast feeding (%) | 14 (82) | 13 (76) | NA | 0.671 |
| IBD medication |  |  |  |  |
| Mesalazine (%) | 11 (65) | 11 (65) | NA | 1.000 |
| Steroids (%) | 1 (6) | 2 (12) | NA | 0.542 |
| Thalidomide (%) | 4 (24) | 0 (0) | NA | 0.014 |
| Other medication |  |  |  |  |
| Antibiotics (%) | 13 (76) | 13 (76) | NA | 1.000 |
| Proton pump inhibitors (%) | 1 (6) | 9 (53) | NA | 0.001 |
| Marzulene-S (%) | 1 (6) | 1 (6) | NA | 1.000 |
| Self-reported diets |  |  |  |  |
| Amino acid formula (%) | 2 (12) | 0 (0) | NA | 0.089 |
| Extensive hydrolyzed formula (%) | 3 (18) | 1 (6) | NA | 0.277 |
| Other diet (%) | 12 (71) | 10 (59) | NA | 0.473 |

[1]*P* value for the comparison between IL10RA group and CD group. IL10RA: IL10RA group; CD: Crohn's disease group; HC: Healthy control group; NA: Not applicable; BMI: Body mass index; CRP: C-reactive protein; wPCDAI: Weighted pediatric Crohn's disease activity index; SES-CD: Simple endoscopic score for Crohn's disease; MINI: Mucosal-inflammation non-invasive; IL6: Interleukin 6.

within the last month before sample collection (Table 1). The average age at diagnosis in the IL10RA group was significantly lower than that in the CD group (*P* < 0.001). In addition, patients in the IL10RA group showed a significantly lower level of hemoglobin (*P* = 0.0035), higher level of IL6 (*P* = 0.0192), and a more severe phenotype [as reflected by a higher SES-CD score (*P* = 0.0157) and MINI index (*P* = 0.0025)] as compared to those in the CD group. Colon and the perianal region appeared to be more commonly affected in the IL10RA group (Table 1). We then performed receiver operating characteristic (ROC) curve analysis to evaluate the ability of these clinical variables in discriminating the IL10RA group from the CD

group. Of the clinical variables that showed significant differences between the IL10RA and CD groups, the age at initial admission showed the best predictive ability [area under the curve (AUC) = 0.925] with a sensitivity of 94.12% and specificity of 88.24%. Other predictive factors were MINI (AUC = 0.861), SES-CD (AUC = 0.786), hemoglobin (AUC = 0.780), and IL6 (AUC = 0.757); this showed that IL10RA-deficient patients had more severe disease than patients in the CD group (Supplementary Figure 1).

### Decreased diversity and increased variability of gut microbiome in IL10RA group

Both patients with IL10RA deficiency and patients in the CD group exhibited a reduced microbial diversity. The Shannon index values for the IL10RA, CD, and HC groups were 1.39 ± 0.85, 1.45 ± 0.87, and 2.36 ± 0.61, respectively (Figure 1A). This result was consistent with the Simpson index as an indicator of microbial diversity. The average Simpson index values were 0.47 ± 0.27, 0.44 ± 0.29, and 0.19 ± 0.11, respectively (Figure 1B). The IL10RA group showed a significantly reduced microbial diversity ($P < 0.0001$) as compared to the CD group ($P < 0.001$). As for beta diversity measured by the unweighted unifrac distance of the OTU community structure, the microbiome of the HC group clustered together and shared similar microbial profiles; however, the microbial composition in the IL10RA group was scattered and showed greater heterogeneity than that in the CD group (Figure 1C).

### Key players of microbial dysbiosis in patients with IL10RA mutations

At the phylum level, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes* were the predominant phyla in all groups (Figure 2A). The relative abundance of *Firmicutes* and *Actinobacteria* showed significant differences among groups (Figure 2B). After FDR correction for multiple tests, the relative abundance of *Firmicutes* in the IL10RA group was substantially greater than that in the HC group ($P = 0.02$). On further comparison of the relative abundance between the IL10RA and HC groups, significant differences were observed with respect to 13 taxa, all of which belonged to phylum *Firmicutes* or *Actinobacteria* (Table 2). Some microbial changes which were reported to be associated with risk variants or mutations of other CD candidate genes are listed in Table 2[4,6,7,19-23].

Subsequently, we used a random forest classifier and performed linear discriminant analysis (LDA) to determine the effect size of taxa on the dysbiosis in each group (Figure 2C). Of the identified taxa in the IL10RA group, *Lactobacillales*, *Bacilli*, *Enterococcaceae*, *Enterococcus*, and *Firmicutes* were enriched in abundance with variable importance (LDA) scores of greater than 5 (Figure 2C). The LDA scores of *Clostridia*, *Clostridiales*, *Bifidobacterium*, *Bifidobacteriales*, *Bifidobacteriaceae*, and *Actinobacteria* were greater than 5 in the HC group. The top five taxa contributing to dysbiosis in the CD group were *Veillonellaceae*, *Megamonas*, *Micrococcaceae*, *Rothia*, and *Micrococcales* (Figure 2C).

### Dysbiosis index is associated with disease severity in the IL10RA group

We detected a strong correlation between SES-CD and wPCDAI ($r = 0.71$, $P = 0.0026$) within the IL10RA group. However, SES-CD did not show any correlation with wPCDAI or MINI index in the CD group ($P > 0.05$). On combining these two groups together, we found a significant correlation of SES-CD with both wPCDAI (Spearman $r = 0.58$, $P = 0.0004$) and MINI index ($r = 0.52$, $P = 0.0020$).

IL10RA-specific dysbiosis indices were calculated based on the relative abundance of five taxa at the order level (*Lactobacillales*, *Micrococcales*, *Veillonellaceae*, *Clostridiales*, and *Selenomonadales*), according to a previously defined method[24]. The dysbiosis indices were associated with the Shannon indices in both the IL10RA ($r = -0.66$, $P = 0.0052$) and CD groups ($r = -0.66$, $P = 0.0046$); this suggests that the abundance of these five taxa largely captured the dysbiosis. We found a significant correlation of wPCDAI and SES-CD scores with the dysbiosis indices (Table 3). Hemoglobin level and disease duration showed an inverse correlation with the dysbiosis indices. No significant association was found between the dysbiosis index and the MINI index and the level of IL6 or CRP. In our exploratory analysis, the dysbiosis index seemed to fit better with SES-CD score, hemoglobin, and disease duration within the IL10RA group as compared to that in the CD group (as reflected by higher values of the correlation coefficient; Table 3).

## DISCUSSION

In this observational study, we observed reduced diversity and increased variability of gut microbiome in patients with *IL10RA* mutations based on the 16S rRNA sequencing data. Patients with *IL10RA* mutations had early disease onset and
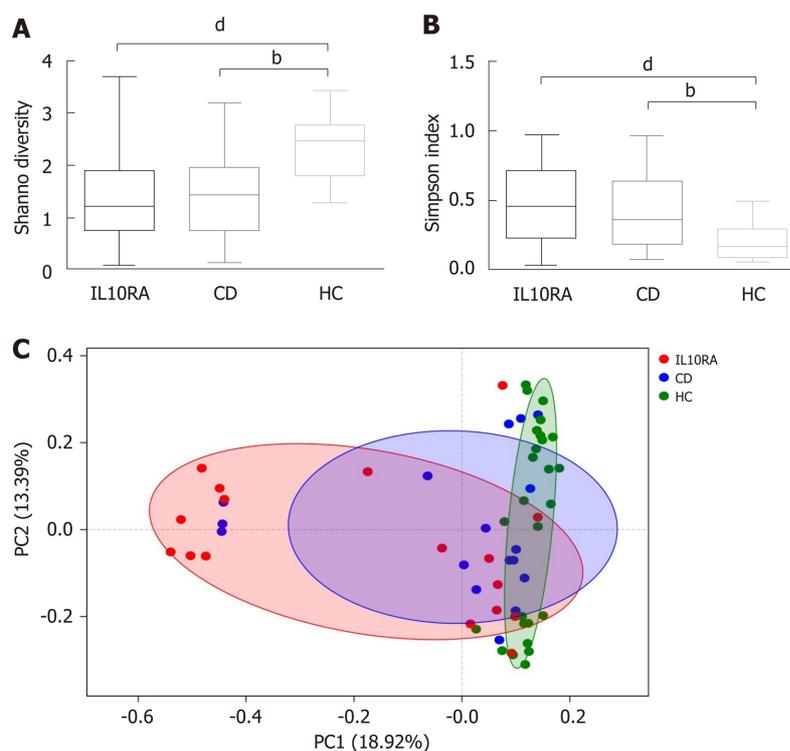
**Figure 1 Diversity of the gut microbiome at the operational taxonomic unit level.** A: Box plot of Shannon and Simpson index. For Shannon index, IL10RA *vs* healthy control (HC) group, *P* = 0.0007; Crohn's disease (CD) group *vs* HC, *P* = 0.0020. For Simpson index, IL10RA *vs* HC, *P* = 0.0008; CD *vs* HC, *P* = 0.0040. [b]*P* < 0.001, [d]*P* < 0.0001; B: PCoA using unweighted unifrac distance of operational taxonomic unit community structure. R = 0.2750, *P* = 0.0011. IL10RA: IL10RA group; CD: Crohn's disease group; HC: Healthy control group.

experienced more severe colitis. We also explored the association between intestinal dysbiosis and the disease severity in these patients.

In previous studies, microbial diversity exhibited a negative correlation with severity of IBD; however, the mutation status was not factored in these studies[4,7,20,23]. In our study, patients in both the IL10RA and CD groups showed a reduced diversity compared with the healthy children. The lack of significant difference between these two groups with respect to diversity indices was likely attributable to similar antibiotic exposure (76%). Given the average age and BMI of the IL10RA-deficient patients, this might also be due to the limitation of the synthetic diversity descriptor, which potentially masks the multi-factor impact on microbiome[25]. Another interesting finding was the variability in different groups. We speculate that the resilience of the gut microbiota varied in individuals with different diseases. Patients exposed to intrinsic factors (*i.e.*, IL10RA deficiency) with an immature gut microbiome may harbor the microbiome that is most vulnerable to environmental disturbances.

Gevers *et al*[24] reported the microbial dysbiosis in new-onset pediatric CD: Increased abundance of *Enterobacteriaceae*, *Pasteurellacaea*, *Veillonellaceae*, and *Fusobacteriaceae* and decreased abundance of *Erysipelotrichales*, *Bacteroidales*, and *Clostridiales*. A systematic review showed that patients with active CD have lower abundance of *Clostridium leptum*, *Faecalibacterium prausnitzii*, and *Bifidobacterium*[26]. Consistent with previous studies, we observed increased *Veillonellaceae* in the CD group; *Clostridiales* and *Bifidobacterium* were decreased in the IL10RA group regardless of the antibiotic exposure. Gevers *et al*[24] found that exposure to antibiotics amplified the dysbiosis; however, exclusion of samples from subjects with antibiotic exposure did not change the key players. In a study by Knights *et al*[20], recent antibiotic usage was inversely associated with *Firmicutes*, *Blautia*, *Ruminococcac*, *Tenericutes*, and *Lachnospiraceae*; however, *Proteobacteria* and *Bacilli* showed a positive correlation with the medication history. Our study showed increased abundance of *Bacilli* in the IL10RA group. However, the abundance of Firmicutes was also increased in the IL10RA group.

Recent studies have shown the effects of Mendelian disorders on the intestinal microbiome, the function of the intestinal mucosa, and the immune response in the gut[4,6,7,19-23]. In parallel, studies have also identified the role of microbiota in initiating and exacerbating the disease[12-14,19]. Besides genetic defects in IL10 and its receptor, a

**Table 2 Variants/mutations in Crohn's disease candidate genes and the alteration of intestinal microbiome**

| Ref. | Gene | Human/animal | Major findings |
|------|------|--------------|----------------|
| [6,19] | *IL10* | Il10$^{-/-}$ mice | Decrease in diversity and richness |
| | | | ↑*Proteobacteria* and *Escherichia coli* (during onset of inflammation) |
| | | | ↓*Bacteroidetes* and *Firmicutes* |
| Current study | *IL10RA* | Human cohort | Decrease in diversity and increase in variability |
| | | | ↑*Firmicutes, Enterococcaceae, Enterococcus, Lactobacillales, Bacilli,* and *Micrococcales* |
| | | | ↓*Bifidobacteriales, Bifidobacteriaceae, Bifidobacterium, Veillonellaceae, Clostridiales, Clostridia, Selenomonadales,* and *Negativicutes* |
| [4,7,20] | *NOD2* | Human cohort | ↓*Roseburia, Faecalibacterium prausnitzii, Bacteroides* and *Bacteroidia* |
| | | | ↑*Eubacteriaceae* and *Enterobacteriaceae* |
| [21] | *NOD2* | Nod2$^{-/-}$ mice | Decrease in diversity and richness |
| | | | ↑*Bacteroides, Bacteroidaceae,* and *B.acidifaciens* |
| | | | ↓*Proteobacteria, Helicobacter hepaticus,* and *Desulfovibro spp* |
| [22] | *ATG16L1* | Human cohort | ↑*Fusobacteriaceae* |
| [4] | *CARD9* | Human cohort | ↓*Firmicutes* |
| [23] | *CARD9* | Card9$^{-/-}$ mice | Decreased stability |
| | | | ↓*Adlercreutzia, Actinobacteria,* and *Lactobacillus reuteri* |

set of causal variants and causative genes have also been identified in IBD. Variants of genes that affect the risk of IBD and have been associated with altered composition of the microbiome are listed in Table 2. These genes are involved in the intestinal immune response to microbes. For instance, impaired function of nucleotide-binding oligomerization domain-containing protein 2 (NOD2) in sensing the bacterial lipopolysaccharide may cause an increase in bacteria that produce these products (*e.g.*, *Escherichia* species and *Bacteroides vulgatus*)[4,7,20,21]. Caspase recruitment domain family member 9 (CARD9) was shown to affect the composition of the gut microbiota by altering the production of microbial metabolites[23]. Mutations in *ATG16L1* were found to decrease the secretion of antimicrobial peptides by Paneth cells and to impair the elimination of specific bacteria through phagocytosis[22]. In addition, polymorphisms in MHC class II genes affect the production of IgA in response to microbes[27]. In NHE3-deficient mice, altered electrolyte transport and mucosal pH may represent a key mechanism of reduced colonic microbial diversity[27]. We found no mutual microbial changes between patients with *IL10RA* mutations and those with other reported risk variants in IBD; this indicates that IL10 signaling defects may impact the microbiota *via* other pathways. Defects in STAT3, the signaling molecule downstream of IL10 receptors, have been recently implicated in skin microbial imbalance. In addition, failure of the MyD88-Stat3 signaling in Treg cells was shown to result in dysbiosis[28,29].

IBD is a heterogenous disease. Owing to considerable dissociation between clinical symptoms and mucosal inflammation in CD, development of therapeutic strategies targeting different sub-groups of patients based on age, disease severity, and disease location is a key challenge. Versions of PCDAI exhibited only a fair correlation with SES-CD ($r$ = 0.33-0.45)[18]. Few studies have performed parallel scoring of the dysbiosis index, SES-CD, and wPCDAI. The MINI index was also validated in our cohort, but it showed no superiority in the accordance of SES-CD. We found that the correlation between SES-CD and wPCDAI was stronger in the IL10RA group.

This observational study was a pilot effort to characterize IL10RA-specific microbial alterations and has several limitations. First, the descriptive nature of the study does not permit any causal inferences. Prospective trials enrolling larger treatment-naive populations at high risk with longitudinal follow-up would provide insights into the role of microbes in the onset of inflammation. Second, 16S rRNA sequencing has its limitations; shotgun metagenomic sequencing with a higher
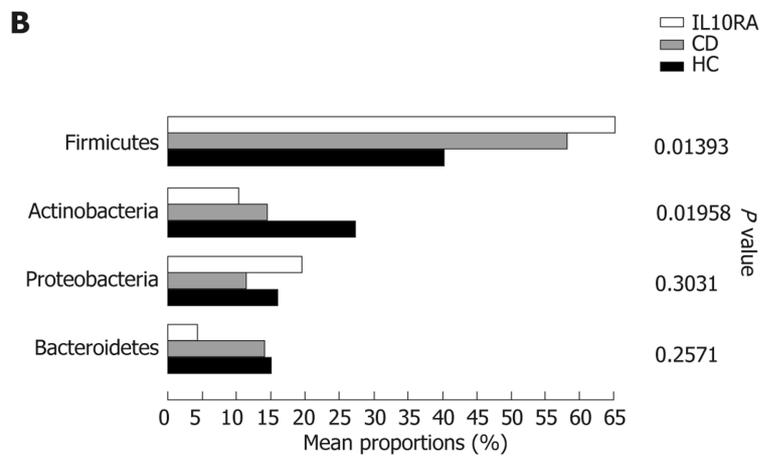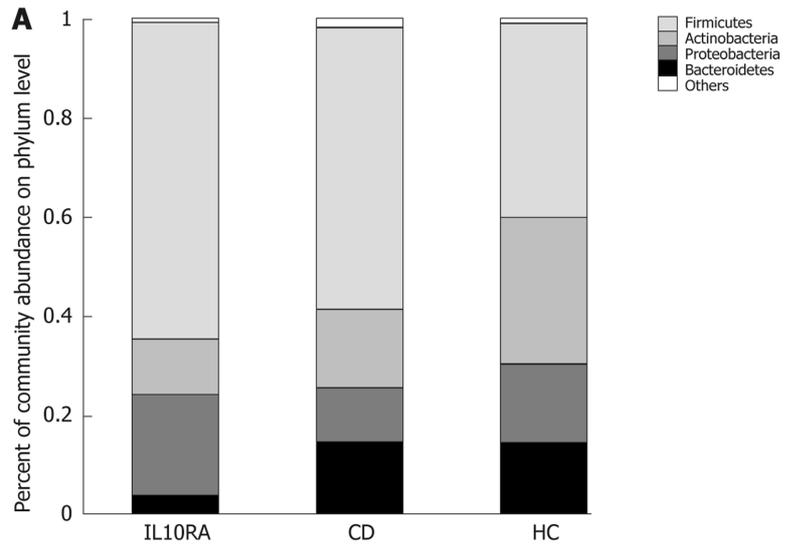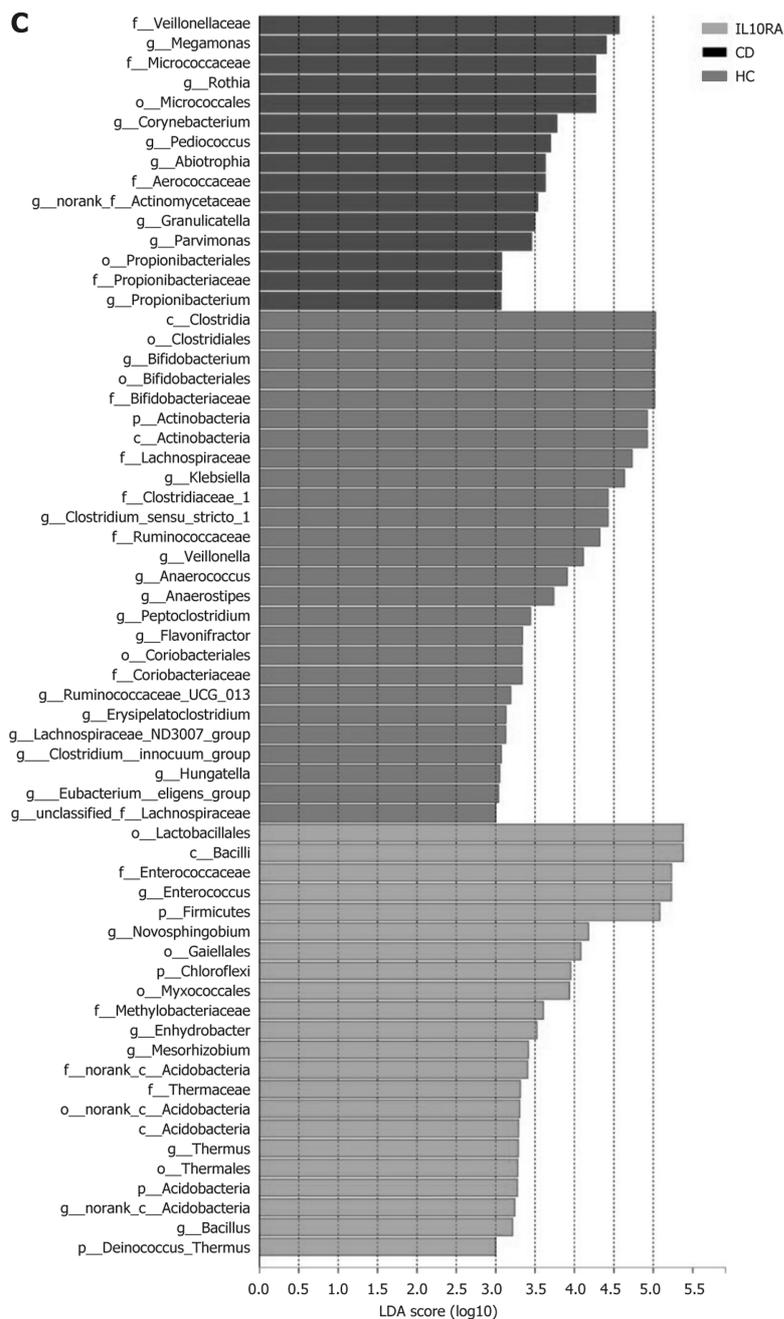
**Figure 2 Community barplot and Kruskal-Wallis *H* test bar plot of the relative abundance of microbiome at phylum level (A, B) and LEfSe bar of the different taxa between groups using the linear discriminant analysis (C).** Taxa with higher linear discriminant analysis scores had a greater effect on the dysbiosis in each group. IL10RA: IL10RA group; CD: Crohn's disease group; HC: Healthy control group; LDA: Linear discriminant analysis.

taxonomic resolution may capture microbial shifts in full complexity. Further investigations may be warranted to identify the shifts in functional or metabolic capabilities of the microbiome. Third, comparison between CD patients and those with *IL10RA* mutations was not corrected for other factors; patients with *IL10RA* mutations are young and typically have a greater propensity for colonic disease.

In summary, the advent of new methodologies can facilitate a better understanding of the interactions between genetic factors and the gut microbiome. To the best of our knowledge, this is the first report of microbial dysbiosis in this sub-population of IBD patients with *IL10RA* mutations; our findings may facilitate further attempts to develop microbial therapeutics. Gut dysbiosis in patients with *IL10RA* mutations showed a moderate association with disease severity in this study. Further studies should focus on the precise role of the microbiota in the etiology of IBD in terms of host genetic susceptibility; this constitutes an attractive target for a given host genome.

**Table 3 Correlation of dysbiosis index with clinical variables**

| Variable | Combined, r[1] (*P* value) | IL10RA, *r* (*P* value) | CD, *r* (*P* value) |
|---|---|---|---|
| wPCDAI | 0.52 (0.002) | 0.51 (0.040) | 0.54 (0.027) |
| SES-CD | 0.48 (0.005) | 0.51 (0.044) | 0.35 (0.174) |
| MINI index | 0.26 (0.131) | 0.32 (0.213) | 0.17 (0.510) |
| CRP, mg/L | 0.11 (0.532) | 0.16 (0.526) | 0.18 (0.493) |
| IL6, pg/mL | 0.17 (0.346) | 0.22 (0.417) | 0.01 (0.961) |
| Hb, g/L | -0.54 (0.0009) | -0.56 (0.022) | -0.51 (0.037) |
| Disease duration (mo) | -0.35 (0.045) | -0.50 (0.043) | -0.40 (0.109) |

[1]Spearman's rank order correlation coefficient. wPCDAI: Weighted pediatric Crohn's disease activity index; SES-CD: Simple endoscopic score for Crohn's disease; MINI: Mucosal-inflammation non-invasive; CRP: C-reactive protein; IL6: Interleukin 6; Hb: Hemoglobin.

## ARTICLE HIGHLIGHTS

### Research background

Several studies have employed animal models to explore the association between microbiota and interleukin (IL)10 signaling; however, limited information is available about the human microbiome. To the best of our knowledge, this is the first report of microbial dysbiosis in this sub-population of inflammatory bowel diseases (IBD) patients with *IL10RA* mutations.

### Research motivation

Patients with *IL10RA* mutations had early disease onset and experienced more severe colitis. Recent studies have revealed an association between host genetic variants and gut microbial changes; in addition, the underlying interactions were found to contribute to the onset and severity of IBD. However, the role of microbiota in patients with infantile-onset IBD who have IL10 signaling defects is not clear. Our findings may facilitate further attempts to develop microbial therapeutics in these patients.

### Research objectives

We aimed to characterize the microbiome in patients with *IL10RA* mutations and to explore the association between gut dysbiosis and disease severity. We observed a reduced diversity and increased variability of gut microbiome in patients with *IL10RA* mutations. We also explored the association between intestinal dysbiosis and the disease severity in these patients. Further studies should focus on the precise role of the microbiota in the etiology of IBD in terms of host genetic susceptibility; this constitutes an attractive target for a given host genome.

### Research methods

Fecal samples were collected from patients who were diagnosed with loss-of-function mutations in the *IL10RA* gene. Age-matched volunteer children were recruited as healthy controls. Patients with Crohn's disease (CD) were used as disease controls to standardize the antibiotic exposure. Microbial DNA was extracted from the fecal samples. All analyses were based on the 16S rRNA gene sequencing data.

### Research results

Seventeen patients with *IL10RA* mutations, 17 patients with pediatric CD, and 26 healthy children were included. Both patients with *IL10RA* mutations and those with CD exhibited a reduced diversity of gut microbiome with increased variability. The relative abundance of Firmicutes was substantially increased in the IL10RA group. On further comparison of the relative abundance of taxa between patients with *IL10RA* mutations and healthy children, 13 taxa showed significant differences. The IL10RA-specific dysbiosis indices exhibited a significant positive correlation with weighted pediatric CD activity index and simple endoscopic score for CD. This observational study was a pilot effort to characterize IL10RA-specific microbial alterations and does not permit any causal inferences.

### Research conclusions

In patients with *IL10RA* mutations and early onset IBD, gut dysbiosis showed a moderate association with disease severity. In this study, clinical variables of *IL10RA*-deficient patients (such as disease course) were linked with changes in the stool microbiome, which implies potential clinical relevance of the changes in microbial populations.

### Research perspectives

16S rRNA sequencing has its limitations; shotgun metagenomic sequencing with a higher taxonomic resolution may capture microbial shifts in full complexity. Further investigations may be warranted to identify the shifts in functional or metabolic capabilities of the microbiome. Prospective trials enrolling larger treatment-naive populations at high risk with longitudinal follow-up would provide insights into the role of microbes in the onset of inflammation.

## ACKNOWLEDGEMENTS

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