Responding letter

Major comment:

Q1: The authors are discussing on details of each diseases using about 2 pages (Line 334- Line 388), but none of these findings written here are novel. The reviewer suggest the most important result in this manuscript is that IL10 deficiency and XIAP were predominant in this cohort, in contrast to Western countries, as the authors mentioned in line 394. Emphasizing this section with more detailed citation, and summarizing description of each diseases are recommended (e.g. writing details of all the diseases in just one paragraph).

Answer: We deleted this section that discussed each disease in detail based on the advice of the reviewer and emphasized the findings of *IL10R* deficiency in our cohort.

“ In the present study, we identified monogenic IBD in nine patients, predominantly with *IL10R* mutation; five patients were diagnosed with *IL10R* mutation. Among them, one patient was the offspring of a consanguineous union with a homozygous mutation of *IL10R*, which has not been reported in the literature. All remaining patients had compound heterozygous mutations of *IL10R*. *IL10* and *IL10R* mutations have been reported previously. Patients with *IL10* signaling defects primarily present with IBD symptoms within three months of life, with severe perianal disease (abscess, fistula formation, fissure, tags) and susceptibility to infections. In addition, they are usually resistant to traditional therapies, though HSCT can induce sustained remission of intestinal inflammation [19, 26, 27]. Defects in *IL10* signaling are associated with extraintestinal inflammation, such as folliculitis and arthritis, as well as a predisposition toward B-cell lymphoma [28]. In the report of Zhiheng Huang et al [27], 42 IO-IBD patients among the Han population in China had *IL10R* mutations, 41 patients had *IL10RA* mutations, and only one patient had an *IL10RB* mutation; thus, *IL10RA* was predominant in the Han population. In the another report from China, Xiao Y et al described four patients with *IL10RA* mutation and one with *IL10RB* mutation among 13 VEO-IBD patients[19]. In the present study

and in other studies from Asia, monogenic IBD is predominately due to mutations in *IL10R* and XIAP and CGD, whereas mutations in *EPCAM, TTC37, SKIV2LLRBA,* and *TTC7A* have been reported in Western countries. These findings suggest that *IL10R* mutation is the most common cause of monogenic IBD in the Han population. Further multicenter studies are warranted.”

Q2: There are 2 limitations in this study, however the authors mentioned very little about this. The authors did not perform any functional studies for novel mutations, therefore there might be false positive for these patients, except for patients showed elevated serum IL10 levels. The authors have not described criteria for selecting patients to take genetic testing. Not testing every patients might lower detection rate of monogenic IBD among the cohort. Furthermore, the authors have used 2 methods of NGS but how these tests were chosen are not mentioned. The authors should clarify this, and the reviewer suggest using one paragraph for explaining limitations in this manuscript.

Answer: We agreed the reviewer that it is better to perform functional studies to confirm results. However, due to laboratory limitations, we did not perform functional analyses of the novel mutations. We mentioned in the manuscript that the novel mutations were all predicted to be pathogenic or likely pathogenic by SIFT (sift.bii.a-star.edu.sg/), PolyPhen-2 (genetics.bwh.harvard.edu/pph2/), and MutationTaster (www.mutationtaster.org/). This is one of the limitations in this manuscript, and we added it to the revised manuscript.

The criteria for genetic testing selection is mentioned in the manuscript: Patients with disease onset before six months of age and patients with disease onset beyond six of months but with severe perianal diseases, severe malnutrition or growth failure, or treatment resistant. We did not sequence DNA from every patient, as multiple factors might be involved. We agree that not testing every patient might lower the detection rate of monogenic IBD among the cohort. This is the second limitation in the manuscript.

WES was initially performed for all patients. Although WES is suitable for novel gene discovery, it provides less reliable gene coverage compared to TGPS in the diagnostic setting. In addition, the panel of TGPS includes all reported monogenic genes. Due to the diagnostic efficiency and cost issues, TGPS is more suitable for diagnosing monogenic IBD in the clinic.

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

Q3: The authors have written that VEO-IBD patients accounted for 34.9% of pediatric patients. However these values were not written in result section. Moreover, since how many percentages of all pediatric IBD patients in your province (or community) are referred to your hospital is unclear, it is difficult to directly compare this percentage with other cohort studies. The reviewer suggest write whole number of pediatric IBD patients and percentage of VEOIBD at the beginning of result, and delete from discussion.

Answer: We have written the number of pediatric IBD patients and the percentage of VEOIBD cases at the beginning of the Results section and deleted this information from the Discussion section.

"From October 2005 to May 2017, 137 pediatric patients were diagnosed with IBD at our hospital, among which 54 were diagnosed with VEO-IBD; thus, VEO-IBD patients accounted for 39.7 % of pediatric IBD patients in our cohort. Among the 54 VEO-IBD patients, 37 were males and 17 females, for a male to female ratio of 2.18:1."

Minor comment:

Q1: Figure 1 is missing.

Answer: We added Figure 1 to the revised manuscript.

Q2: Write every gene names in Italic letters.

Answer: Gene names are italicized.

Q3: Use term 'monogenic' but not 'monogenitic'.

Answer: We changed 'monogenetic' to 'monogenic' throughout the manuscript.

Q4: What is assembly of chromosome locations described in this manuscript? GRCh37 or CRCh38?

Answer: GRCh37.

Q5: Add SNP IDs (start with rs….) for all known variants (Table 5).

Answer: SNP IDs for all known variants have been added to Table 5.

Q6: Results are divided to 7 sections, and only some of these titles are written in Bold letters. Check the manuscript again and use bold letters for all the titles, or use numbers instead.

Answer: We have checked the manuscript again and use bold letters for all titles.

Q7: Some results are written in percentages, however considering sample size, all percentage values should be rounded off to the first decimal (e.g. 57.41% to 57.4%).

Answer: We checked the results again and corrected the percentage values.

Q8： Line 121: The term 'incidence' is used as number of patients who developed disease per 'general population' in a year. When the overall cohort only includes patients, this term cannot be used. In this manuscript, the reviewer suggest using the term 'the percentage of VEO-IBD among all pediatric IBD patients'.

Answer: Thank you for pointing out the improper use the term of "incidence". We have corrected the description in the manuscript.

Q9: Line 125: Add non-abbreviated term for 'M' (months).

Answer: The abbreviation for months (M) has been defined.

Q10: Line 129: Add non-abbreviated term for BMI.

Answer: The abbreviation for body mass index (BMI) has been defined.

Q11: Line 145: The term 'Time to diagnosis' is unclear. Did you want to mean 'Median time from the disease onset to diagnosis'?

Answer: Yes, "time to diagnosis" means "the median time from disease onset to diagnosis"; this is corrected in the manuscript.

Q12: Line 164: Add non-abbreviated term for CVID (and note mistyped as CIVD here).

Answer: The abbreviation for common variable immune deficiency (CVID) has been defined.

Q13: Line 173: 'Level of IL10' should be 'serum IL10 level'

Answer: The phrase 'level of IL10' has been replaced with 'serum IL10 level'.

Q14: Line187: Is ‘amino acid formula’ same as ‘elemental formula’?

Answer: Yes, the term has been corrected.

Q15: Line 207: In the sentence ‘His older sister had one mutation’, did you want to say the sister is a hereditary carrier?

Answer: Yes, his sister is a hereditary carrier.

Q16: Line 213: Please add adult normal range of serum IL10.

Answer: The normal range of serum IL10 has been added to the manuscript.

Q17: Line 236: CD4/CD >CD4/CD8?

Answer: Yes, it is CD4/CD8.

Q18: Line 243: Add non-abbreviated term for Ig (Immunoglobulin)

Answer: The abbreviation for immunoglobulin (Ig) has been defined.

Q19: Line 244: Add non-abbreviated term for IVIG

Answer: The abbreviation for intravenous immunoglobulin (IVIG) has been defined.

Q20: Line 251: Please specify that these mutations are on TNFRSF13B gene.

Answer: This is specified in the manuscript.

Q21: Line274: Most of the studies on clinical features of VEOIBD include monogenic IBD patients. Do the authors suggest monogenic IBD patients should be excluded from these studies, or just want to say the geographic, ethnic difference among studies affected difference in genetic background and therefore caused conflicting results? If the latter is what the authors want to say, describe in that way.

Answer: We changed the description in the manuscript.

Q22: Line 281: Please change description of citation as follows; Kammermeirer et al.

Answer: The description has been changed.

Q23: Line 291; When using the term ‘prevalence’ solely, usually it means ‘number of patients per general population. If you want to describe ‘the percentage of VEOIBD among all the pediatric IBD patients in your hospital’, the reviewer suggest writing in that way. Alternatively you can write ‘prevalence of VEOIBD among all the pediatric IBD patients in our hospital’.

Answer: The description has been changed.

Q24: Line 310: The reviewer suggest the author should mention if clinical findings mentioned in the citation are similar to this cohort or not.

Answer: I am not sure what the reviewer refers to. Because the lines may have changed in the different versions of the manuscript.

Q25: Line 332: The sentence ‘However…’ should be deleted from here and write a paragraph about limitation of this manuscript instead.

Answer: We added a paragraph to present the limitations.

Q26: Line 334-388: Nothing novel is written here. The reviewer suggest this section should be completely deleted or summarized.

Answer: We agree, and we deleted this section.

Q27: Line 400: The importance of this manuscript and what is new in this manuscript should be described in conclusion.

Answer:

The conclusion has been revised as follows:

“Using WES and TGPS, we identified underlying PID gene mutations in pediatric patients with VEO-IBD in a Chinese population. There was a high proportion of monogenic IBD in the VEO-IBD group, especially with disease onset before six months of age. IL10 R mutation was predominant in our cohort.”