Dear Editors

We thank the referees for the very important and helpful suggestions. We have revised the manuscript, **"Childhood-onset inflammatory bowel diseases associated with mutation of Wiskott–Aldrich syndrome protein gene" (**Manuscript NO. 36449) on the basis of the Referee's comments (shown in red font in the body of the revised draft), and we summarized these revisions by a point-by-point response to the reviewers' comments.
We hope for a positive outcome of our manuscript in *World Journal of Gastroenterology*.

Our responses to the referee's comments are as follows;

Response to the Reviewer 02440884

We wish to express our appreciation to the reviewer for his/her insightful comments, which have helped us to significantly improve the paper.

COMMENTS TO AUTHORS
The authors investigate the frequency of mutations in Wiskott Aldrich in patients suffering from IBD. The mutation WAS c.1378C>T, p.Pro460Ser is associated with a higher frequency of malignomas, which is important for the long-term follow-up. Comments 1. The study is hampered by the small number of patients included. This point should be more addressed.

Response: We thank the reviewer for this comment. We also agree the number of patients is quite small. Therefore, we stated it in limitation of our manuscript. As this is a pilot study, additional study with the greater number of patients in multicenter is now under contemplation.

Response to the Reviewer 03478404

We wish to express our appreciation to the reviewer for his/her insightful comments, which have helped us to significantly improve the paper.

COMMENTS TO AUTHORS
In this paper, the authors performed screening for Wiskott–Aldrich syndrome and chronic granulomatous disease in people with pediatric-onset of IBD. Without any doubt, the manuscript is nicely written, the structure is appropriate and references are adequate. Particularly, I've noticed attention to details. However:

1. The number of children (18) is too small and this is a real major drawback.

Response: We also agree the number of patients is quite small. Therefore, we stated it in

limitation of our manuscript. As this is a pilot study, additional study with the greater number of patients in multicenter is now under contemplation.

2. Only 3 children were found with WAS gene c.1378 C>T p.Pro460Ser mutation and they did not show neither thrombocytopenia nor increased susceptibility to infection. According to the presented data, they did not have any peculiarities, including clinical aspects, endoscopy and therapy.

Response: As the reviewer was pointed out, our three patients with WAS gene mutation has no difference in clinical course, therapy nor endoscopic findings from most of IBD patients. As we stated in the results and discussion, the patient 1 had eczema which was caused by TNF-alpha blockade therapy. WAS gene mutation might be associated with eczema caused by TNF-alpha blockade therapy, but we have no data to prove it. The patient 3 was classified into very early onset IBD (VEOIBD, 0-6 years at onset). Some VEOIBD patients showed intractable clinical course but the patient 3 was treated by 5-ASA and azathioprine with prednisolone enema and obtained clinical and endoscopic remission without TNF-alpha blockade therapy. WAS gene mutation might be associated with VEOIBD but other VEOIBD patients in our study did not have WAS gene mutation.
We added sentences and references about VEOIBD.
(page 9, line 15-16) "In 5 patients, age at the onset was 6 years or younger and they classified into very early onset IBD (VEOIBD)"
(page 10, line 11) "Patient 3 was classified into VEOIBD."
(page 12, lines 12-16) "Only one of five VEOIBD patients in our study showed low expression of WASP and *WAS* mutation. VEOIBD patients often have different symptoms from older children and adults with IBD. In general, genetics is suggested to be an important factor in VEOIBD. *WAS* mutation might be associated with pathogenesis of VEOIBD."

3. No child was found with CGD (probably, again, due to the small number of included children).

Response: As mentioned before, in additional study with the greater number of patients, we will reinvestigate about CGD.

4. The authors wrote "Despite the lack of typical clinical manifestations of WAS, low expression of WASP could be associated with the pathogenesis of a subtype of IBD patients." How do we know that this could be associated with IBD pathogenesis?

Response: We thank the reviewer for this comment. WASP dysfunction leads to impaired regulatory T cells. Such immunodeficiency affect microbiota, which may lead to IBD. Thus, we also consider that WASP plays key roles in pathogenesis of IBD in our patients. We added a sentence (page 13, line 10-11). Added sentence is as follows.
"The impaired regulatory T cells caused by WASP dysfunction also affect microbiota, which may lead to IBD/IBD-like colitis."
In our study, we aren't able to examine that whether the c.1378 C>T p.Pro460Ser mutation affects lymphocytes function. This is our study limitation and we mentioned in our manuscript.

(page14, lines 23-25)

5. What is the importance of these findings for our practice? Do they change our therapeutic and/or monitoring approach? The authors mentioned :" WAS is known to be associated with an increased risk of malignancies including lymphoma, as well as autoimmune diseases. Therefore, in any long-term follow-up, the analysis of WASP expression in children with IBD should be considered even if major symptoms of WAS are absent." But any IBD could be associated with autoimmune diseases and malignancies, including lymphoma. Therefore, we screen IBD patients for these conditions anyway. This is part (or should be part) of our daily basis practice.

Response: As you indicated, both IBD and WAS are associated with an increased risk of malignancies and their long-term follow-up is same. Some patients with WAS develop brain tumors. There may be some differences of malignancy between IBD and WAS. Recently, in XIAP deficiency patient complicated by enterocolitis, hematopoietic stem cell transplantation is reported to be curative therapy (J Clin Immunol. 2017 Jan;37(1):85-91). Similarly, in WAS patient complicated by enterocolitis, hematopoietic stem cell transplantation may be one of the treatment options. In this point, we consider that it is useful to diagnose WAS, although we must be verified. Fundamental treatment strategy differs between two etiologies.

6. The authors wrote "IBD is caused by both genetic and environmental factors". Please do not forget about epigenetics, microbiota and immune responses.

Response: We thank the reviewer for this comment. We revised sentences. (page 6 lines 2-3). Revised sentence is as follows.
"IBD is caused by multiple factors; genetics, epigenetics, environment, microbiota and immune responses."

7. I thought a control group would have been very important. And, indeed, later in their paper, the authors wrote "Blood samples were also collected from healthy young adults as a control." What were the findings in this control group? I did not find any mention. This could be of crucial importance.

Responses: In our analysis, we used heathy controls as a comparison with patients. WASP expressions of healthy controls were not low. We added a sentence (page 9 line 21). Added sentence is as follows.
"WASP expressions of healthy controls were normal"

8. The authors wrote: "Screening for underlying immunodeficiencies may contribute to improving patient management and outcome." I fully agree with this statement, but this is not the conclusion after analysing the patients mentioned in this study.

Response: We thank the reviewer for this comment. We revised this sentence (page 14, line 6-9). Revised sentence is as follows.

"Screening for underlying immunodeficiencies including WAS and CGD may contribute to improving IBD patient management and outcome. Especially, physicians can pay more attention to the increased future risk of malignancy and autoimmune disease in IBD patients with WAS mutation."

9. Legend of the endoscopic images should be re-written to correspond to the pictures.

Response: In accordance with the Reviewer's comment, we have changed the following text in the legend of figure 3:

"(A) Patient 1: aphthae and diffuse longitudinal ulcers in the ileum (B, C) Patient 2: bloody ulcerated mucosa in the descending colon and sigmoid colon (D) Patient 3: ulcerated and edematous mucosa in the rectum"

to

"(A) Patient 1: long linear ulcerations and cobble stone appearance in the ileum. (B, C) Patient 2: edematous and friable mucosa with superficial bleeding in the descending colon and sigmoid colon. (D) Patient 3: edematous mucosa with granularity and erythema in the rectum."

Response to the Reviewer 02446483

We wish to express our appreciation to the reviewer for his/her insightful comments, which have helped us to significantly improve the paper.

COMMENTS TO AUTHORS

Inflammatory bowel disease (IBD) is chronic in nature with a relapsing course and both comprises Crohn's disease (CD) and ulcerative colitis (UC) as well as indeterminate colitis with overlapping features of CD and UC. Although affecting people of the 2nd and 3rd decades of life, IBD may also affect infants and children. IBD is suggested to result from disturbed interactions between the immune system and commensal bacteria of the gut, but theories may involve the environmental factors as well. The immune theory is substantially backed by murine models showing that colitis does not develop in gnotobiotic mice, but emerges on reconstitution of the gut flora. The authors present interesting data with involvement of the Wiskott -Aldrich gene. The manuscript is very interesting, although terminology needs to be addressed (there are some inconsistencies) and in the discussion the role of the environment and epigenomics should be emphasized.

Response: We thank the reviewer for this comment. We regret to inform you that we were not able to find the terminology to be addressed. Please tell us specifically. As you indicated, the environment and epigenomics are important in IBD pathogenesis.
We revised a sentence (page 6 line 2-3). Revised sentence is as follows.
"IBD is caused by multiple factors; genetics, epigenetics, environment, microbiota and immune responses."

WASP dysfunction leads to impaired regulatory T cells. Such immunodeficiency affect microbiota, which may lead to IBD. Thus, we also consider that WASP plays key roles in pathogenesis of IBD in our patients. We added a sentence (page 13, line 10-11). Added sentence is as follows.

"The impaired regulatory T cells caused by WASP dysfunction also affect microbiota, which may lead to IBD/IBD-like colitis."

Response to the Reviewer 01557050

We wish to express our appreciation to the reviewer for his/her insightful comments, which have helped us to significantly improve the paper.

COMMENTS TO AUTHORS

1) General comments Dr. Ohya and Yanagimachi, et al. investigated 'Childhood-onset inflammatory bowel diseases associated with mutation of Wiskott-Aldrich syndrome protein gene. The article is informative and well-presented. The reviewer has some comments. Comments 1) Please describe in Discussion whether WAS mutation would be the prediction for cutaneous complication with TNF-α or not.

We thank the reviewer for this comment. We added a sentence (page 12 line 9-11). Added sentence is as follows.

"There may be possibility that *WAS* mutation is associated with TNFα blockade-cutaneous complication and prediction for the complication."