Author responses to Reviewers

Name of journal: *World Journal of Gastroenterology*
Manuscript NO: 86101, Case Report
Title: Inherited CHEK2 p.H371Y mutation in solitary rectal ulcer syndrome among familial patients: A case report
Authors: Chengcheng He, Shanping Wang, Peirong Zhou, Zhijun Li, Na Li, Mingsong Li

Dear Editor and Reviewers,

Thank you very much for your attention and comments on our paper "**Inherited CHEK2 p.H371Y mutation in solitary rectal ulcer syndrome among familial patients: A case report**"(**86101, Case Report**). Your valuable comments and suggestions are greatly appreciated by all co-authors. We have revised the manuscript according to your kind advices and reviewers' detailed suggestions. Enclosed please find the responses to the reviewers. We sincerely hope this manuscript will be finally acceptable to be published on **World Journal of Gastroenterology**. Thank you very much for all your help and looking forward to hearing from you soon. If you have any question about this paper, please don't hesitate to let me know.

Best regards
Sincerely yours
Prof. Mingsong Li
Please find the following Response to the comments of reviewers:
Point to point response to the reviewers' comments

Reviewers' Comments:

Referee #1 (Specific Comments to Authors):
This case report titled "Inherited CHEK2 p.H371Y mutation in solitary rectal ulcer syndrome among familial patients: A case report" presents the clinical and genetic characteristics of a Chinese mother and son diagnosed with solitary rectal ulcer syndrome (SRUS). The report highlights the presence of an inherited CHEK2 p.H371Y mutation in both patients and discusses its potential role in the development and prognosis of SRUS. Overall, the case report provides valuable information on a rare condition and highlights the importance of considering genetic factors in the etiology of SRUS. However, there are a few points to consider: Case presentation: The case presentation provides relevant details about the patients' symptoms, medical history, physical examination, laboratory tests, and imaging results. It would be beneficial to include information about any relevant lifestyle factors, such as dietary habits or previous medical conditions, which could have contributed to the development of SRUS. Genetic analysis: The identification of an inherited CHEK2 p.H371Y mutation in the mother and son suggests a potential genetic susceptibility to SRUS. However, it is important to note that this is a single case report, and the role of this specific mutation in the pathogenesis of SRUS needs further investigation. Replication studies involving larger cohorts would be necessary to establish a stronger association between the CHEK2 mutation and SRUS. Treatment and follow-up: The report briefly mentions the treatment administered to the patients, including Thalidomide, mesalazine, and biofeedback therapy. However, additional details regarding the rationale for choosing these treatments, the duration of therapy, and the specific outcomes

observed in the patients would enhance the clinical relevance of the case report. Discussion: The discussion section provides a comprehensive overview of SRUS, its clinical manifestations, diagnostic methods, and treatment options. However, further elaboration on the potential mechanisms by which the CHEK2 mutation could contribute to the development of SRUS would be beneficial. Additionally, discussing the limitations of the study, such as the small sample size and the need for further validation, would provide a more balanced interpretation of the findings.

Response: Thanks for your suggestions and comments on our paper. We have carefully revised the manuscript and provide more experimental support according to your comments.

1. Case presentation: The case presentation provides relevant details about the patients' symptoms, medical history, physical examination, laboratory tests, and imaging results. It would be beneficial to include information about any relevant lifestyle factors, such as dietary habits or previous medical conditions, which could have contributed to the development of SRUS.

Response: We sincerely appreciated the valuable suggestions. According to your comments, we added and revised the manuscript accordingly. We added the relevant lifestyle factors in the section of ***Personal and family history***. As you can see in the marked manuscript from Line 95 to Line 98, "Additionally, she and her son with SRUS like to eat mixed and coarse grains, and they have high-fiber eating habits and a sedentary lifestyle. Moreover, they are accustomed to squatting for a long time to defecate. Furthermore, they were healthy before the SRUS incidence, but are prone to anxious behaviors in life."

2. Genetic analysis: The identification of an inherited CHEK2 p.H371Y mutation in the mother and son suggests a potential genetic susceptibility to SRUS. However, it is important to note that this is a single case report, and the role of this specific mutation in the pathogenesis of SRUS needs further investigation. Replication studies involving larger cohorts would be necessary to establish a stronger association between the CHEK2 mutation and SRUS.

Response: Thanks for the reviewer's good evaluation and kind suggestion. Indeed, as you commented, this is a single case report and the limitations of the study showed the small sample size, so the role of this specific mutation in the pathogenesis of SRUS needs further investigation. According to your comments, we added and revised the manuscript accordingly. First of all, SRUS is uncommon, large cohorts of SRUS are very difficult to collect. Then we did our best to obtain three sporadic SRUS specimens in a short period of time for experimental verification. We hope you can understand the difficulty and complexity of this experiment.

We added the relevant experimental results in the section of ***FURTHER DIAGNOSTIC WORK-UP.*** As you can see in the marked manuscript from Line 140 to Line 145, "The SRUS groups contain familial patients in our case and non-familial cases (sporadic cases). The IHC results revealed that the CHEK2 mutation did not affect the expression of CHEK2 protein whether in familial SRUS cases or sporadic SRUS cases, but it would affect CHEK2 functions to different degrees. CDC25A expression level variations are more significant in familial SRUS cases, while p-p53 expression level changes are more pronounced in sporadic SRUS cases." And we have supplemented the contents of Figure 2 accordingly and discussed the result accordingly. As you can see in the marked manuscript from Line 217 to Line 222, "Both familial and sporadic SRUS cases showed weakened function in CHEK2 protein, and familial SRUS cases are mainly characterized by changes in CDC25A of CHEK2 downstream, while sporadic SRUS cases are mainly characterized by

variations in p-p53 of CHEK2 downstream. We speculate similarities and differences in the pathogenesis and prognosis between familial SRUS and non-familial SRUS. ”

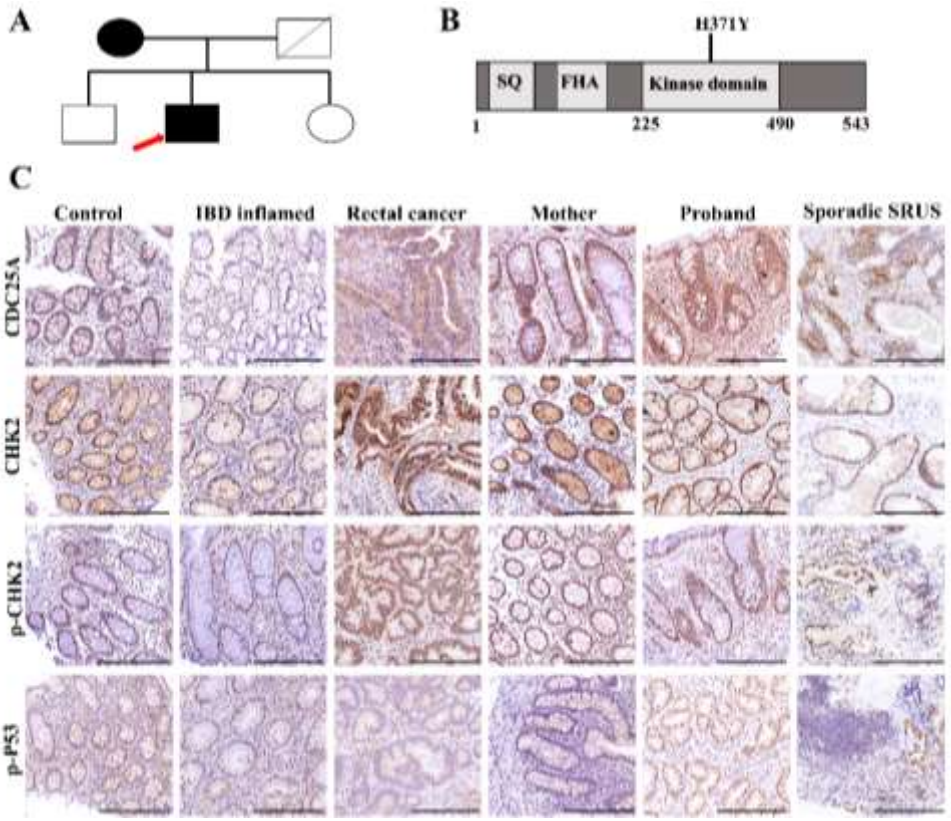


Figure 2. Genetic background of the patients with solitary rectal ulcer syndrome patients in a mother-son relationship. A. Family tree of the familial patients with solitary rectal ulcer syndrome. Squares indicate male family members; circles indicate female family members; black indicate affected patients; white indicate healthy family members; slashes indicate deceased family members; arrow indicate the first diagnosed patient of the family. **B**. Schematic diagram of the CHEK2 protein. **C**. The differential expressions of CHEK2 and the downstream genes among the healthy control, and patients with inflammatory bowel diseases, rectal cancer, and solitary rectal ulcer syndrome, using the immunohistochemical staining; scale bars, 100μm. IBD, inflammatory bowel diseases; SRUS, solitary rectal ulcer syndrome.

Additionally, we also emphasized this limitations of small cohorts in the Discussion section. As you can see in the marked manuscript from Line 238 to Line 243, “Limitations related to a small-cohort study and the patient heterogeneity exist. The following possible drawbacks may occur in our study. Firstly, this is a single case report and a retrospective study, and the role of CHEK2 mutation in SRUS pathogenesis needs further investigation. Secondly, this study did not involve the detailed mechanism of CHEK2 mutation causing SRUS. Therefore, subsequent verification of large samples and more detailed experimental verification should be prepared.”

3. Treatment and follow-up: The report briefly mentions the treatment administered to the patients, including Thalidomide, mesalazine, and biofeedback therapy. However, additional details regarding the rationale for choosing these treatments, the duration of therapy, and the specific outcomes observed in the patients would enhance the clinical relevance of the case report.

Response: Thanks for your kind suggestion. According to your suggestion, we added and revised the manuscript accordingly. We added the detailed treatment plan in the section of ***Treatment and follow-up***. As you can see in the marked manuscript from Line 147 to Line 171, "SRUS treatment should be comprehensive and aimed at restoring the patient's normal bowel pattern, including behavior modification, medication, biofeedback, and surgery. Initially, we guided patient's lifestyles and eating habits and advised them to change their sedentary habits and appropriately reduce the amount of dietary fiber in their food. Simultaneously, we guided them to develop good defecation habits, avoid forceful defecation, set defecation time and body position, and artificially limit defecation frequency. Then, we provide psychological care to patients and encourage them to appropriately participate in social activities to vent their bad emotions. Concurrently, biofeedback, which can limit the change of toilet frequency in patients with frequent bowel movements, was recommended as an effective treatment. Biofeedback training can help resolve symptoms, especially in patients who remain symptomatic postoperatively. Finally, we advise patients to use thalidomide and mesalazine for rapid improvement of inflammation, considering the long medical history of the patient, especially frequent diarrhea in the male patients. Mesalazine is a commonly used drug for SRUS and thalidomide is used for anti-inflammation and its side effects of constipation and improved sleeping happen to help patients relieve diarrhea and help them sleep.

Changes in eating and living habits need to be maintained for a long time. Patients are advised to undergo re-examination after 3 months drug treatment and biofeedback adjuvant therapy. A significant improvement can be maintained for a long time under the condition of monitoring drug side effects. Additionally, the medication regimen was adjusted following the patient's symptoms during the follow-up. Patients' symptoms were significantly improved under the comprehensive treatment. The reexamination results after 3 months indicated a significantly relieved mucosa (Figure 1F). Patients remain under regular follow-up and treatment annually. "

4. Discussion: The discussion section provides a comprehensive overview of SRUS, its clinical manifestations, diagnostic methods, and treatment options. However, further elaboration on the potential mechanisms by which the CHEK2 mutation could contribute to the development of SRUS would be beneficial.

Response: Thanks for your kind suggestion. According to your suggestion, we added and revised the manuscript accordingly. We added the potential mechanisms in the section of ***Discussion***. As you can see in the marked manuscript from Line 223 to Line 230, "Previous studies have revealed that CHEK2 is associated with inflammation and functions through the kinase mechanism to down-regulate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway in macrophages to alleviate *Staphylococcus aureus*-induced pneumonia in mice. Additionally, phospho-CHEK2 was associated with high macrophage infiltration in UC[9]. Moreover, SRUS is a manifestation of inflammation and CHEK2 mutation may contribute to the development of SRUS via effects on inflammatory pathways, such as the NF-κB pathway[10]."

5. **Additionally, discussing the limitations of the study, such as the small sample size and the need for further validation, would provide a more balanced interpretation of the findings.**

Response: Thanks for your kind suggestion. According to your suggestion, we added and revised the manuscript accordingly. We added the limitations in the section of ***Discussion***. As you can see

in the marked manuscript from Line 238 to Line 243, “Limitations related to a small-cohort study and the patient heterogeneity exist. The following possible drawbacks may occur in our study. Firstly, this is a single case report and a retrospective study, and the role of CHEK2 mutation in SRUS pathogenesis needs further investigation. Secondly, this study did not involve the detailed mechanism of CHEK2 mutation causing SRUS. Therefore, subsequent verification of large samples and more detailed experimental verification should be prepared.”

Moreover, we tried our best to improve the manuscript and also, we sent our paper to Enago for further polishing. We believe the revised manuscript has been significantly improved in terms of language and flow. Once again, thank you very much for your comments and suggestions.

Referee #2 (Specific Comments to Authors):

The topic of this study is very interesting and innovative. In real clinical practice, this condition presents numerous challenges. Firstly, it is extremely difficult to diagnose. There are no precise diagnostic criteria, making it challenging to differentiate between inflammatory bowel diseases, mechanical mucosal damage caused by rectal prolapse or injury. Contrary to its name, this condition can involve multiple ulcers, and they may not be limited to the rectum alone; they can also appear in the sigmoid colon or descending colon. Some researchers have suggested a potential association with Helicobacter pylori. Therefore, there are doubts about whether these various spectrums of the disease truly represent a single condition. Secondly, the prognosis varies greatly. Some cases are managed without treatment, while others may require the use of infliximab, and in extreme cases, such as the patient described in this paper, surgery may be necessary. As stated in the paper, if certain genes can help predict the diagnosis and prognosis of SRUS, it would be a groundbreaking test. I believe that the efforts of the researchers have significant value and deserve publication. However, there are several assumptions that need to be considered. Firstly, there is insufficient information regarding whether male patients were truly diagnosed with SRUS. Since surgery is not common and persistent diarrhea even after surgery raises doubts about the possibility of inflammatory bowel disease, further investigation is needed. Secondly, based on my experience, the frequency of a family history is not clearly established. As SRUS is not a common condition and has diverse causes, additional information on the frequency of family history is necessary before analyzing genes. Thirdly, it would be helpful to provide detailed information on the patient's treatment progress. I am interested in knowing if biofeedback, mesalamine, antibiotics, steroids, or other treatments were used. Lastly, in the supplementary Figure, the A photograph appears to be a postoperative image, but it should be presented as a preoperative image. Thank you.

Response: Thanks for the reviewer’s good evaluation and kind suggestion. The manuscript has been revised according to your suggestions. The detailed amendments are listed as follows:

1. **Firstly, there is insufficient information regarding whether male patients were truly diagnosed with SRUS. Since surgery is not common and persistent diarrhea even after surgery raises doubts about the possibility of inflammatory bowel disease, further investigation is needed.**

Response: Thanks for your kind suggestion. According to your suggestion, we added and revised the manuscript accordingly. First of all, we provided pictures of the patient's surgical specimen and surgical pathology in Supplementary Figure 1. The postoperative pathology confirmed that the

diagnosis of SRUS was indeed in line with the preoperative pathological diagnosis of SRUS.

2. **Secondly, based on my experience, the frequency of a family history is not clearly established. As SRUS is not a common condition and has diverse causes, additional information on the frequency of family history is necessary before analyzing genes.**

Response: Thanks for your kind suggestion. First of all, we reviewed and counted the reports of SRUS around the world, and did not find any family-related reports. This is also the biggest highlight of our study. It is the first report of a mother and child diagnosed with SRUS. And as you mentioned, SRUS is uncommon, it is difficult to diagnose, and clinicians may have insufficient awareness, therefore the diagnosis rate is low. There have been no reports of familial illness, but it may be related to the low diagnostic rate. Therefore, it is also hoped that our case will provide clinicians with new insights into family screening of populations with SRUS.

3. **Thirdly, it would be helpful to provide detailed information on the patient's treatment progress. I am interested in knowing if biofeedback, mesalamine, antibiotics, steroids, or other treatments were used.**

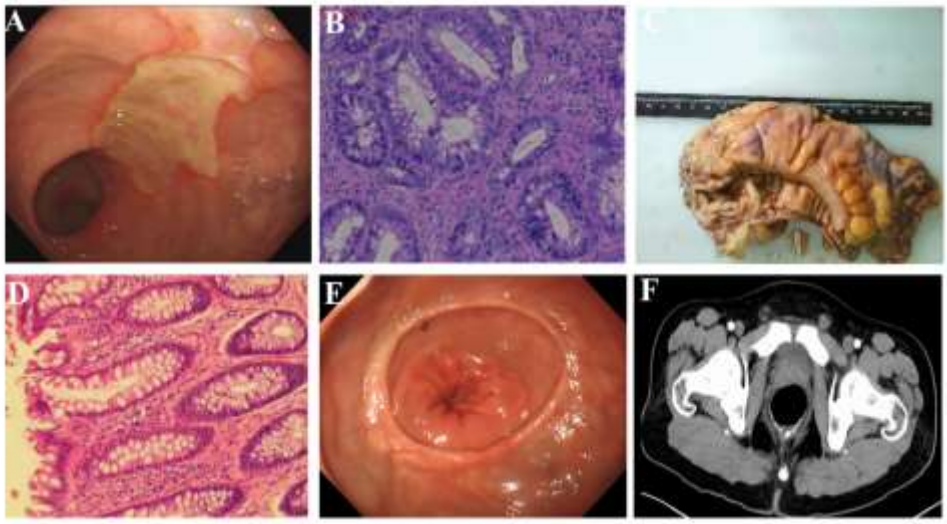
Response: Thanks for your kind suggestion. According to your comments, we added and revised the manuscript accordingly. We added the detailed treatment plan in the section of ***Treatment and follow-up***. As you can see in the marked manuscript from Line 147 to Line 171, "SRUS treatment should be comprehensive and aimed at restoring the patient's normal bowel pattern, including behavior modification, medication, biofeedback, and surgery. Initially, we guided patient's lifestyles and eating habits and advised them to change their sedentary habits and appropriately reduce the amount of dietary fiber in their food. Simultaneously, we guided them to develop good defecation habits, avoid forceful defecation, set defecation time and body position, and artificially limit defecation frequency. Then, we provide psychological care to patients and encourage them to appropriately participate in social activities to vent their bad emotions. Concurrently, biofeedback, which can limit the change of toilet frequency in patients with frequent bowel movements, was recommended as an effective treatment. Biofeedback training can help resolve symptoms, especially in patients who remain symptomatic postoperatively. Finally, we advise patients to use thalidomide and mesalazine for rapid improvement of inflammation, considering the long medical history of the patient, especially frequent diarrhea in the male patients. Mesalazine is a commonly used drug for SRUS and thalidomide is used for anti-inflammation and its side effects of constipation and improved sleeping happen to help patients relieve diarrhea and help them sleep.

Changes in eating and living habits need to be maintained for a long time. Patients are advised to undergo re-examination after 3 months drug treatment and biofeedback adjuvant therapy. A significant improvement can be maintained for a long time under the condition of monitoring drug side effects. Additionally, the medication regimen was adjusted following the patient's symptoms during the follow-up. Patients' symptoms were significantly improved under the comprehensive treatment. The reexamination results after 3 months indicated a significantly relieved mucosa (Figure 1F). Patients remain under regular follow-up and treatment annually. "

4. **Lastly, in the supplementary Figure, the A photograph appears to be a postoperative image, but it should be presented as a preoperative image.**

Response: Thanks for your kind suggestion. According to your comments, we added and revised

the manuscript accordingly. We added the preoperative images and surgical pathology images in Supplementary Figure 1.



Supplementary Figure 1. Clinical features of the male patient with solitary rectal ulcer syndrome. A. The colonoscopy image of the male patient preoperatively. **B**. Pathological results of the ulcer indicated solitary rectal ulcer syndrome. **C**. The surgical specimen image of the male patient. **D**. The surgical pathology of the male patient confirms the diagnosis of solitary rectal ulcer syndrome. **E**. Colonoscopy image upon his reexamination postoperatively. **F**. The intestinal computed tomography enhancement results upon his reexamination postoperatively.

Again, we are very grateful for your valuable and kind advice. All of these comments are of great importance to our article and have contributed a lot to improve the quality of our article.