Dear editor:

Thank you for your assessment of our manuscript. We have significantly revised the text and the figures within the manuscript. As suggested, we have prepared a point-by-point response letter that details our responses to the reviewer's comments. To assist the Reviewer, we have marked changes made to the manuscript that are directly in response to Reviewer concerns in red font.

We thank you once again for your assistance and hope that you will find our revised manuscript suitable for publication in *World Journal of Gastroenterology* .

Sincerely,

Yali Zhang/Zhipeng Tang

Point-by-point response letter

Reviewer's code: 03479673

It was great to go through your article. Before accepting, there are some suggestions to be made.

1.The two different syndromes which are described here based on TCM are not widely used in practice globally. Though authors have narrated in article about differentiating between the two, from an allopathic view point, it is still not clear to understand, how they are differentiated? It would be of great importance to enlighten the reviewers and readers with this.

Response:UC can be divided into Pi-Xu-Shi-Yun syndrome (syndrome of spleen deficiency and dampness, PXSY) and Da-Chang-Shi-Re syndrome (syndrome of dampness-heat in the large intestine, DCSR). PXSY syndrome is a deficiency syndrome in TCM theory, while the DCSR syndrome is a sthenia syndrome. Clinical manifestations of the DCSR syndrome focus on the empirical symptoms of intestinal inflammation and injury. While the clinical manifestations of PXSY syndrome focus on the deficiency symptoms of weakened digestion and absorption function. The main clinical symptoms of DCSR syndrome are as follows：abdominal pain, diarrhea, mucous purulent stool; yellowish fur and thready and slippery pulse . Some of these patients can also show the following symptoms : burning pain in anus, tenesmus, body heat, short red urine, dry or bitter mouth, ozostomia. The main clinical manifestations in patients with PXSY syndrome includes: thin sloppy stool, stool mucus is more white than red, or white frozen, pink tongue, tooth mark around tongue, white and greasy tongue coating. Some of these patients may have the following clinical symptoms: dull abdominal pain, abdominal fullness and distention, eat less and poor appetite, the body is tired, God exhausted lazy speech, thready and weak pulse or thread and slippery pulse.

We have explained these idease briefly in the Discussion as follows:

In TCM theory, PXSY syndrome is a deficiency syndrome, while DCSR syndrome is a sthenia syndrome.Clinical manifestations of the DCSR syndrome focus on the empirical symptoms of intestinal inflammation and injury. While the clinical manifestations of PXSY syndrome focus on the deficiency symptoms of weakened digestion and absorption function. Clinical manifestations of the DCSR syndrome focus on the empirical symptoms of intestinal inflammation and injury. While the clinical manifestations of PXSY syndrome focus on the deficiency symptoms of weakened digestion and absorption function. The main clinical symptoms of DCSR syndrome are as follows：abdominal pain, diarrhea, mucous purulent stool; yellowish fur and thready and slippery pulse . Some of these patients can also show the following symptoms : burning pain in anus, tenesmus, body heat, short red urine, dry or bitter mouth, ozostomia. The main clinical manifestations in patients with PXSY syndrome includes: thin sloppy stool, stool mucus is more white than red, or white frozen, pink tongue, tooth mark around tongue, white and greasy tongue coating. Some of these patients may have the following clinical symptoms: dull abdominal pain, abdominal fullness and distention, eat less and poor appetite, the body is tired,

God exhausted lazy speech, thready and weak pulse or thread and slippery pulse.

2. The weaknesses of the study, like the small sample size, non-blinding are not discussed in details, which is always important to describe to let readers know the practical applicability and authenticity of the results.

Response: As suggested, We have added the following text to the Discussion to provide more information：

The weaknesses of the study, like the small sample size, non-blinding and across-sectional study. In the following studies, the sample size needs to be further expanded to further strengthen and enrich the accuracy and stability of the research results. This study is a cross-sectional study, and it is difficult to determine the causal relationship between the results. In future studies,the intervention factors can be considered and the dynamic observation before and after the intervention can be carried out.

3.Conclusion in main manuscript body is too lengthy. It must be brief, relevant and crisp.

Response: We are very grateful for these constructive suggestions. We have modified the conclusion in main manuscript body.

The gut microbiota is different between patients with PXSY syndrome and those with DCSR syndrome. The genus Streptococcus was significantly more abundant in DCSR patients than in PXSY patients, while Lachnoclostridium was increased in PXSY patients. Dysbiosis of the gut microbiota influences the immune responses of the host, which results in inflammation, and the microbial analysis of the two TCM syndromes essentially reflected different immune activities in the human body, but they all point to promotion of inflammation in the gut.

4.It would also be appreciable to provide table/algorithm showing normal or prevalent taxa in otherwise normal population.

Response: Thank you very much for reviewers' suggestions. We will supplement these contents in future research.

5.Will it be possible or practical to screen high risk / symptomatic population and prevent or modify natural history of disease- would like to know comments of authors under the heading of 'future possibilities'.

Response:

We are very grateful for these constructive suggestions. As suggested, We have added the following contents:

The clinical manifestations of UC are related to disease activity, lesion location and range. Based on the clinical manifestations, TCM can be divided into different syndromes and treated according to syndrome differentiation. The relationship between TCM syndromes and intestinal flora is an interesting and important research. We compared the differences of intestinal flora between UC patients and healthy patients, and between patients with PXSY Syndrome and DCSR Syndrome, to explore

the relationship between intestinal flora and UC, as well as intestinal flora characteristics related to TCM deficiency and positive syndrome. TCM syndrome differentiation is the premise and basis for the establishment of treatment methods. This study preliminarily discussed the characteristics and differences of intestinal flora of patients with two different TCM syndrome.

6.*Inclusion of subjects is based on 'diarrhea – diagnosis standard of ulcerative colitis', it is always give details of the 'diagnosis standard of ulcerative colitis' in methods section.*

Response: We are very grateful for these constructive suggestions. We have supplemented the diagnostic criteria of method section.

The main clinical manifestations of UC are diarrhea, mucous pus and blood accompanied by abdominal pain, tenesmus and back weight, and systemic symptoms of varying degrees. The course of disease is more than 4 ~ 6 weeks. Mucous purulent blood is the most common symptom of UC. UC lacks the gold standard for diagnosis, which is mainly combined with clinical manifestations, endoscopy and pathological histology for comprehensive analysis, and the diagnosis is made on the basis of excluding infectious and non-infectious colitis. We can distinguish these two syndromes according to patients' clinical manifestations, tongue coating and pulse condition. Clinical manifestations of DCSR syndrome were abdominal pain, tenesmus, stools containing blood and white mucus, a burning sensation around the auns and scanty, dark-yellow urine. The tongue coating was greasy and slightly yellow. Pulse was slippery and rapid . While clinical manifestations of PXSY syndrome were recurrent loose stools and increased frequency of bowel movements, especially after eating greasy food, poor appetite, gastric or abdominal distension, a sallow complexion, and lassitude.The tongue coating was pale with a thin, white coating.The pulse was weak and thready.

7.*It seems to be the first article of its kind, if there are other published articles similar or relevant to this study, please provide the details.*

Response: No other articles were published.

Reviewer's code:03538272

The authors have performed an observational study comparing the microbiata between two traditional chinese medicine syndromes of UC and controls using 16S ribosomal RNA sequencing of stool. They found increased abundance of Streptococcus in DCSR patients while Lachnoclostridium was increased in PXSY patients.

The major issue with this paper is controlling for confounders that may impact on the microbial diversity and several points need to be discussed to clarify this. Were any further analyses performed to evaluate this?

Response: We tried to exclude the possible factors affecting the intestinal flora

examination, for example: (1)No microecological preparations or antibiotics were used within the previous two weeks before sampling. (2)With the increase of age, the number of beneficial bacteria in human body will decrease, while the number of harmful bacteria will continue to rise. The patients included are between 18 and 60 years old, and there is no difference in the baseline level between the three groups.

Additionally, more details are needed to on the methodology of the study and overall study aims. - the authors need to state their initial hypothesis - was it assumed that you would find certain species to be different amongst the two groups initially?

Response: We are very grateful for these constructive suggestions. We have supplemented the details of method and aim sections. Our initial hypothesis are: There were more intestinal bacteria in healthy people than in UC patients. There were differences in intestinal flora between patients with UC with two different syndromes.

How was the required sample size calculated?

Response: It is not easy to collect a large number of cases that meet the requirements of clinical trials. We did our best to collect samples from 93 patients.

What was the criteria for diagnosing UC?

Response: The main clinical manifestations of UC are diarrhea, mucous pus and blood accompanied by abdominal pain, tenesmus and back weight, and systemic symptoms of varying degrees. The course of disease is more than 4 ~ 6 weeks. Mucous purulent blood is the most common symptom of UC. UC lacks the gold standard for diagnosis, which is mainly combined with clinical manifestations, endoscopy and pathological histology for comprehensive analysis, and the diagnosis is made on the basis of excluding infectious and non-infectious colitis.

Could patients with symptoms of the sydromes and no endoscopic disease activity be counted as DCSR or PXCY?

Response: Yes, patients with symptoms of the sydromes and no endoscopic disease activity could be counted as DCSR or PXCY.

How was the diagnosis of PXCY and DCSR made - was a set lot of diagnostic criteria

applied?

Response: The standard of TCM syndrome differentiation and classification was referred to the "dysentery" standard in the consensus on TCM diagnosis and treatment of ulcerative colitis (2009). We can distinguish these two syndromes according to patients' clinical manifestations, tongue coating and pulse condition. Clinical manifestations of DCSR syndrome were abdominal pain, tenesmus, stools containing blood and white mucus, a burning sensation around the auns and scanty, dark-yellow urine. The tongue coating was greasy and slightly yellow. Pulse was slippery and rapid . While clinical manifestations of PXSY syndrome were recurrent loose stools and increased frequency of bowel movements, especially after eating greasy food, poor appetite, gastric or abdominal distension, a sallow complexion, and lassitude.The tongue coating was pale with a thin, white coating.The pulse was weak and thready.

Did disease severity affect which group people were placed in.

Response: No，disease severity didn't affect which group people were placed in. There was no difference in baseline comparison of patients.

Given treatment may impact on microbiata, what therapies were people on?

Response: All the included patients were UC patients who went to longhua hospital for the first time. Patients take (or do not take) medications for UC in accordance with the guidelines. There was no history of antibiotic or microecological preparation in the 2 weeks before sampling. In this study, the effects of microecological agents and antibiotics were basically excluded, and the effects of other drugs on the results of UC treatment were excluded as far as possible.

What was the endoscopic severity for patients? - how was clinical activity determined? Was a score like the partial Mayo or SCCAI applied?

Response: The Sutherland Index was used to evaluate the disease activity. A score ≤2 indicated remission, 3-5 indicated mildly active, 6-10 indicated moderately active, and 11-12 indicated severely active. Based on disease severity, UC patients were divided into the clinical alleviate ,mild, moderate, and severe groups.

Reference:

D'Haens G, Sandborn WJ, Feagan BG, et al. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. Gastroenterology 2007; 132:763-86.

Were people evaluating the microbiata analysis blinded to the diagnosis of patients?

Response: According to the experimental procedure, the person who detects 16s-RNA is only responsible for the test results, and will not be responsible for the samples from which the test results come (normal, DCSR or PXCY)

Was there any difference in diets between the groups?

Response: I'm sorry we didn't analyze the effects of dietary factors. And we also didn't do the baseline analysis of calprotectin.

Were baseline calprotectin or bloods performed?

Response: Routine blood tests are used to determine the severity of the disease.

Were any patients on treatment for UC?

Response: Yes,there were some patients on treatment for UC. However, for those who have not received traditional Chinese medicine treatment, the purpose is to exclude the influence of traditional Chinese medicine on test results.

Reviewer's code:00035982

This MS focuses on the microbial patterns seen in adults with UC (two groupings) and healthy controls SPECIFIC COMMENTS

1.The title includes an abbreviation

Response: Thanks for your comments, we have corrected the error in the text.

2. The methods of the ABSTRACT includes results - these should be moved

Response: As suggested, we have re-written this part according to the Reviewer's suggestion.

Methods: From May 2015 to February 2016, UC patients presenting to Longhua Hospital who met the established inclusion and exclusion criteria

were enrolled in this retrospective study. Fresh stool specimens of UC patients with PXSY or DCSR were collected .The feces of the control group came from the health examination population of Longhua Hospital.The composition of gut bacterial communities in stool samples was determined by the pyrosequencing of 16S ribosomal RNA. The high-throughput sequencing reads were processed by QIIME, and biological functions were predicted using PICRUSt.

3. The conclusion of the ABSTRACT indicates that this work has described a mechanism, which is not the case. this is a descriptive study that proposes a theory. Further how does TCM directly lead to the development of UC (as suggested)? TCM is a system of interpretation is it not - it does not itself lead to the development of this or any other disease.

Response: According to the reviewers' comments, we have re-written this part ：

Results: The composition of gut bacterial communities in 93 stool samples (30 healthy controls, 32 patients with PXSY syndrome, and 31 patients with DCSR syndrome) was determined by the pyrosequencing of 16S ribosomal RNA. Beta diversity showed that the composition of the microbiota was different among the three groups. At the family level, Porphyromonadaceae, Rikeneliaceae, and Lachnospiraceae significantly decreased while Enterococcus, Streptococcus and other potential pathogens significantly increased in UC patients compared to healthy subjects. At the genus level, Parabacteroides, Dorea and Ruminococcus were decreased while Faecalibacterium showed increased abundance in UC compared to healthy controls. Five differential taxa were identified between PXSY and DCSR syndromes. On the genus level, a significantly increased abundance of Streptococcus was observed in DCSR patients, while Lachnoclostridium was increased in PXSY patients. The differential functional pathways of the gut microbiome between the PXSY and DCSR groups mainly included lipid metabolism, immunity, and the metabolism of polypeptides.

4. *The INTRODUCTION could be shortened and focused more. The description of the two TCM patterns includes comments such as the quality of the fur - yet these were humans.*

Response: We are very grateful for these constructive suggestions. We have revised the introduction.

5. Further the TCM patterns includes much overlap. the features are subjective in part. The distinction between the two types does not include a defined biological marker (a specific objective marker). How were the patients classified then?

Response: TCM syndrome is a unique TCM clinical classification method based on symptom group characteristics, which is used to guide clinical TCM treatment. Our TCM syndrome differentiation was based on the "dysentery" standard in the consensus on TCM diagnosis and treatment of ulcerative colitis (2009).We can distinguish these two syndromes according to patients' clinical manifestations, tongue coating and pulse condition. Clinical manifestations of DCSR syndrome were abdominal pain, tenesmus, stools containing blood and white mucus, a burning sensation around the auns and scanty, dark-yellow urine. The tongue coating was greasy and slightly yellow. And also the pulse was slippery and rapid . While clinical manifestations of PXSY syndrome were recurrent loose stools and increased frequency of bowel movements, especially after eating greasy food, poor appetite, gastric or abdominal distension, a sallow complexion, and lassitude.The tongue coating was pale with a thin, white coating.The pulse was weak and thready.

6. The study evaluated the intestinal microbiota only. It did not include any metabolomic features or any host features - these limit the ability to confirm any underlying molecular mechanism

Response: Thank you very much for reviewers' suggestions. We will improve our research plan in future studies, including further evaluation of metabolomics characteristics.

7. The METHODS section includes the details of the number of subjects included 0- these are results and should be moved accordingly

Response: Thanks for the reviewers' suggestions, we have revised them.

8.It is not clear why pregnancy, lactation and uncooperative nature and included together?

Response: Thanks for your comments, we have corrected the error in the text.

Pregnancy, lactation or planned pregnancy; Uncooperative patients;

9. The MS has numbered sections. However, the discussion returns to 1. If these do not suit the journal style requirements, then they should all be removed

Response: We are very grateful for these constructive suggestions. We have revised them all.

10.The DISUSSION could be shortened and more focused. More discussion on the similarity or differences of the observed patterns compared to other such reports would enhance

Response: We are very grateful for these constructive suggestions. We have revised the discussion section.

11. Table 1 would be enhanced with a better title. the column of p values could be removed as all are above 0.05 and replaced with a simple sentence in the legend

Response: Thanks for the reviewers' suggestions, we have revised it.

12. Table 1 does not include information about medication use, smoking history, and other important disease or environmental factors.

Response: We are very grateful for these constructive suggestions. In future studies, our baseline analysis will be more comprehensive.

13. Figure three is titled to feature differences between the two groups of patients with UC, but also contains control patterns as well

Response: Thanks for the reviewers' suggestions, we have corrected the error in the text.

Reviewer's code:00044509

Although IBD is still an unexplained refractory disease, it is generally accepted to divide it into UC and CD.

1.The biggest point in this paper is to divide UC into two different syndromes such and PXSY and DCSR based on TCM. The idea of dividing UC into PXSY and DCSR is unfortunately not considered to be accepted internationally. The authors should clearly show, for example, a table showing what differs between PXSY and DCSR, eg, clinical symptoms or laboratory data, or endoscopic findings.

Response: We are very grateful for these constructive suggestions. We have supplemented this section:

We can distinguish these two syndromes according to patients' clinical manifestations, tongue coating and pulse condition. Clinical manifestations of DCSR syndrome were abdominal pain, tenesmus, stools containing blood and white mucus, a burning sensation around the auns and scanty, dark-yellow urine. The tongue coating was greasy and slightly yellow. And also the pulse was slippery and rapid . While clinical manifestations of PXSY syndrome were recurrent loose stools and increased frequency of bowel movements, especially after eating greasy food, poor appetite, gastric or abdominal distension, a sallow complexion, and lassitude.The tongue coating was pale with a thin, white coating.The pulse was weak and thready.

2. The gut microbiota have been shown to be affected by therapeutic agents such as antibiotics and pro and/or prebiotics. The author should describe and discuss the patient's treatment.

Response: All the included patients were UC patients who went to longhua hospital for the first time. Patients take (or do not take) medications for UC in accordance with the guidelines. There was no history of antibiotic or microecological preparation in the 2 weeks before sampling. In this study, the effects of microecological agents and antibiotics were basically excluded, and the effects of other drugs on the results of UC treatment were excluded as far as possible.

Reviewer's code:03478404

This study appears to be the first of its kind, investigating differences between gut microbiota dysbiosis in PXSY and DCSR syndromes (and versus healthy controls) and originality is always appreciated. Structure of the manuscript is overall respected (maybe discussion and conclusion could be shortened and made clearer). I consider, for a pilot study, the number of included patients to be enough. Figures and tables are of good quality. References are pertinent. However, I have some comments:

A. Major: 1.Traditional Chinese Medicine (TCM) is very different from allopathic medicine. The two syndromes (PXSY and DCSR) do not exist in evidence-based medicine and these syndromes are based on clinical symptoms/signs that are not usually included in the diagnosis of UC across the world. Please clarify in what way this paper would benefit doctors and their patients, across the globe.

Response: We are very grateful for these constructive suggestions. TCM syndrome is a unique TCM clinical classification method based on symptom group characteristics, which is used to guide clinical TCM treatment. Our TCM syndrome differentiation was based on the "dysentery" standard in the consensus on TCM diagnosis and treatment of ulcerative colitis (2009). We know UC from another perspective, which has enriched people's understanding of this refractory disease.

2. Patient recruitment: The authors wrote "Two types of TCM syndromes of UC, DCSR syndrome and PXSY syndrome, were identified with the "diarrhoea" diagnosis standard of ulcerative colitis.24" Please define how the diagnosis of UC was made. Only diarrhea? Later on, the authors wrote: "Exclusion criteria of UC patients are as follows: ....Have diarrhea or other gastrointestinal diseases". This is confusing. Moreover, in the "Results" paragraph, we learn that diagnosis was based on colonoscopy. What criteria were used for diagnosis at colonoscopy? Please correct / clarify and detail in the Methods section. What are the endoscopic lesions in the two syndromes? Correct diagnosis is crucial!

Response: As suggested, we have re-written this part according to the Reviewer's suggestion.

The main clinical manifestations of UC are diarrhea, mucous pus and blood accompanied by abdominal pain, tenesmus and back weight, and systemic symptoms of varying degrees. The course of disease is more than 4 ~ 6 weeks. Mucous purulent blood is the most common symptom of UC. UC lacks the gold standard for diagnosis, which is mainly combined with clinical manifestations, endoscopy and pathological histology for comprehensive analysis, and the diagnosis is made on the basis of excluding infectious and non-infectious colitis.

3. It is not clear whether patients with UC were recruited at diagnosis or not. The reason I am asking this is that in Table 1, we learn that at recruitment, some patients with UC were in clinical remission. But, it was mentioned that patients had symptoms. Therefore, were they under some sort of therapy to induce clinical remission? If so, in what way this paper shows the role of gut microbiota in the etiopathogenesis of UC? Please clarify.

Response: We are very grateful for these constructive suggestions. All the included patients were UC patients who went to longhua hospital for the first time. Patients take (or do not take) medications for UC in accordance with the guidelines. There was no history of antibiotic or microecological preparation in the 2 weeks before sampling. In this study, the effects of microecological agents and antibiotics were basically excluded, and the effects of other drugs on the results of UC treatment were excluded as far as possible.

4. The authors wrote "According to TCM theory, the same disease can be divided into different syndromes, and different treatments would be used clinically depending on the different syndromes". Could the authors present in what way the findings of their study could contribute to different therapies and what would these therapies include? Or what are the future perspectives?

Response: The treatment principle of DCSR syndrome was clear heat, resolve dampness and regulate qi and blood.While the treatment principle of PXSY syndrome were strengthen the spleen ,supplement qi and transform dampness. Our treatments include Chinese medicine, massage, acupuncture and so on. These therapies may play a role in regulating intestinal flora. There are similarities between

the same treatment of different diseases in traditional Chinese medicine and precision treatment in western medicine.

B. Minor: 1. Please clarify the following sentence: "Based on the microbiome analysis, the fact that TCM has an essential role in the development of UC precision medicine could be highlighted" 2. Please clarify the following sentence: "The molecular mechanism of TCM was elucidated to explore precision medicine for UC." Since both sentences are part of the Abstract, they should be clearer. 3. The following sentence: "All volunteers signed the informed consent before participating in the experiment." should be moved from Results to Patient recruitment. 4. Conflict-of-Interest Disclosure Form is not appropriate.

Response: We are very grateful for these constructive suggestions. We have corrected these contents.