Response to Reviewers

Name of Journal: *World Journal of Gastroenterology*

Manuscript NO: 59758

Title: Colonic vitamin D receptor expression is inversely associated with disease activity and Jmjd3 in active ulcerative colitis

Dear Editor-in-Chief Ma,
We greatly appreciate both your help and that of the reviewer concerning improvement to this paper. We hope that the revised manuscript is now suitable for publication. We have checked the manuscript, respond the comments one by one and make corresponding changes to the manuscript.

Reviewer 1
Comment: TITLE Vitamin D alleviates the disease activity of human ulcerative colitis via Jmjd3-H3K27 me3 signaling, this is an observational study showing that VDR/Serum vitamin D had an inverse correlation with disease activity in UC patients and tissue JMJD3 levels. The study is not designed to establish firmly that vitamin D alleviates the disease activity of human ulcerative colitis via jmjd3-H3K27. Hence the title is misleading. A more suitable topic would clearly state the firm results of the study without speculation.

Response: Thanks for your comment and suggestion. We have re-written the title of manuscript according to your suggestion. "Colonic vitamin D receptor expression is inversely associated with disease activity and Jmjd3 in active ulcerative colitis".

Comment: Background Authors state: the potential role of vitamin D receptor (VDR) on Jmjd3 activation remains unknown. The activation of NF-κB as an early and central event in inflammation (Pasparakis 2009). Molecular studies have revealed the reason why JMJD3 transcription could be rapidly induced by NF-κB. It is because that the promoter sequences from the first coding exon of JMJD3 contain two conserved κB sites (de Santa et al. 2007). VDR is known to downregulate NF-κB signaling and ameliorate intestinal inflammation through its interaction with NF-κB and its inhibitor inhibitory kappa B kinase beta (IKKβ). Thereby downregulating jmjd3.

Response: Thanks so much for your comment and suggestion. Indeed, the role of VDR on Jmjd3 activation has been confirmed in laboratory. However, the expression of Jmjd3 and H3K27me3 in clinical patients with UC and the correlation between VDR and Jmjd3 pathway remain unknown. We changed the background statement to "The expression of Jmjd3 and H3K27me3 in clinical patients with UC and the correlation between VDR and Jmjd3 pathway remain unknown".

Comment: Aim To explore the impact of vitamin D on disease activity and its related mechanism. The aim of the study needs to be more specific. Vitamin D is ubiquitous and has a spectrum of effects on a variety of tissues. As mentioned by the authors in the manuscript, "The purpose of this study is to study the relationship between VDR, Jmjd3 and H3K27me3. "A more descriptive aim would be to "Study the relationship between VDR, jmjd3 and H3K27me3 in patients with ulcerative colitis"

Response: Many thanks for your comment and suggestion. We have re-written the statement (see lines 50-51).

Comment: Conclusions Immunohistochemistry staining of Jmjd3 and H3K27me3 in colonic mucosa are helpful for the diagnosis of ulcerative colitis. This statement needs to be deleted or changed. This study was able to show increased levels of jmjd3 levels in colonic biopsies of UC patients in comparison to healthy controls. The study does not compare these biopsies to other inflammatory conditions of the colon. Jmjd3 is increased in a variety of tissues in a variety of conditions and is merely a marker of inflammation based on the data presented. There is no data presented here that argues in favor of jmjd3 being a specific marker for Ulcerative Colitis.

Response: Thank you very much for your comment and suggestion. As you mentioned, Jmjd3 can not be a specific diagnostic index of UC based on the data presented. Therefore, we have deleted this statement.

Comment: Core Tip This knowledge unveiled a novel mechanism by which vitamin D involves in the alleviation of UC pathology via anti-inflammation. Additionally, detection of signaling molecules of Jmjd3 and H3K27me3 in colonic mucosa may help for development of the diagnostic markers of UC. Please correct this statement. Surely VDR has been shown to downregulate NFKB and mitigate the downstream inflammation. Additionally NFKB manifests its inflammatory effects by recruitment of jmjd3 in some tissues. However jmjd3 is known to have both pro and anti inflammatory effects depending on the type of cells being studied. Similarly end results of VDR upregulation vary widely. For instance, Fabio Periera et al have shown through their work that 1,25(OH)(2)D(3) activates the JMJD3 gene promoter and increases the level of JMJD3 RNA in human cancer cells. JMJD3 upregulation was strictly dependent on vitamin D receptor (VDR) expression and was abolished by cycloheximide. In colon cancer cells, VDR promotes the transcription of jmjd3 RNA Therefore, It would be more accurate to say that our findings show that VDR expression is inversely related to jmjd3 expression and disease activity in colonic mucosa of patients with UC. Further studies are warranted investigate this relationship.

Response: Thanks for your comment and suggestion. We have corrected this statement in core tip. In addition, we have downplayed the statement relating to causality and used the term correlation (see lines 83-90).

Comment: Discussion Second paragraph: A multiyear cohort study showed that low serum vitamin D level increased the frequence Change to "frequency"

Response: Many thanks for pointing this out. We have meticulously revised the whole manuscript and corrected the mistakes.

Comment: Vitamin D level and VDR expression decreased and were inversely correlated with the disease activity, and simultaneously, increased Jmjd3 and decreased H3K27me3 expression was noted in active UC patients, suggesting that the alleviated affect of Vitamin D and VDR on the disease activity of human ulcerative colitis is associated with activation of Jmjd3 and inhibition of H3K27me3 presumedly via NF-κB and STAT 1/STAT 3 signaling. Change to "effect"

Response: Thanks for your comment. We have corrected the word.

Reviewer 2

Comment: Vitamin D alleviates the disease activity of human ulcerative colitis via Jmjd3-H3K27 me3 signaling. Most major single point. How can the authors say this?

Response: Thanks for your question. As you mentioned, this study only verified the correlation among vitamin D, VDR, Jmjd3 and H3K27me3. Besides, VDR, Jmjd3 and H3K27me3 expressions were detected only by immunohistochemical method due to the limitation of intestinal mucosal specimens in patients with UC. Therefore, we have corrected the title in our manuscript. "Colonic vitamin D receptor expression is inversely associated with disease activity and Jmjd3 in active ulcerative colitis".

Comment: "This knowledge unveiled a novel mechanism by which vitamin D involves in the alleviation of UC pathology via anti-inflammation." Where are the inflammatory measurements?

Response: Many thanks for your question. The inappropriate expression has been changed to "These findings indicate that VDR expression was inversely related to Jmjd3 expression and disease activity in the colonic mucosa of patients with UC".

Comment: Where are the silencing/over-expression expts of said mechanisms

in cell systems to back up these claims. Unless such expts exist these claims can NOT be made and we are simply looking at a series of physiological correlations. Please change this language to reflect your data.

Response: Thanks for your comment and suggestion. Previous studies (*Hum Mol Genet. 2011; 20 (23):4655-4665; Cell Cycle. 2012; 11(6):1081-1089*) revealed that vitamin D regulates the RNA expression of the genes encoding Jmjd3 in human colon cancer cells. Jmjd3 is a target gene of vitamin D which has been demonstrated in these articles. Therefore, we have changed the language to reflect our data (see lines 132-136).

Comment: This is all simply correlation? Major points: 1. The VDR correlation with Mayo score is frankly startling? Fig 5B? and definitely the most provocative bit of data presented.

Response: Thanks for your thoughtful question. We only conducted correlation analysis because of lacking sufficient samples. Further quantitative detection and mechanism study will be carried out in future. However, the relationship between VDR and disease activity in patients with UC is controversial among different studies. Previous studies (*Oncol Rep. 2009; 22(5):1021-1025; J Natl Med Assoc. 2018; 110(3):276-280*) revealed that VDR expression in colonic mucosa of patients with UC was significantly decreased, but was not inversely correlated with disease activity. However, a significantly inverse correlation between VDR and histological scores in UC was observed in this study (T*herap Adv Gastroenterol. 2019; 12:1756284818822566*). In our study, UC disease activity was defined based on the Mayo score. An inverse correlation was observed between VDR and Mayo score. The difference of the inverse correlation between our research and other studies may be due to the diversity of judgment indicators and population difference.

Comment: It would have been nice to see some quantitative measure used, i.e western blot or Elisa but I understand these were sampled during endoscopy and there would be limited tissue available.

Response: Thanks for your suggestion and understanding. As you mentioned, quantitative measures were not performed for detecting VDR, Jmjd3 and H3K27me3 because of lacking sufficient tissue specimens. More samples will be collected for quantitative measurements using WB and ELISA in future.

Comment: Statements such as "VDR may down-regulate the expression of Jmjd3," really need to at least have some in silico support. i. e. are there VDRES n promoters of any of the genes correlated?

Response: Thanks for your critical question. Jmjd3 is the target gene of vitamin D, which has been studied in previous studies (*Hum Mol Genet. 2011; 20 (23):4655-4665; Cell Cycle. 2012; 11(6):1081-1089*). In any case, this mechanism has not been confirmed in colonic epithelial cells. Therefore, we focus on the physiological relationship between VDR and Jmjd3 pathway in the revised manuscript in clinical patients. The description has been revised.

Comment: As expected patients were taking anti-inflammatory or steroid agents. This would have a long term effect on inflammatory outcomes of course but how can the authors rule out effects on the measures taken here?

Response: This is really a good question. Indeed, the effect of anti-inflammatory treatments on UC pathology can not be ruled out in UC patients, as the animal model in laboratory. However, we did not consider the effect of anti-inflammatory treatment on the pathological alleviation of the colonic mucosa. Here we just observed the association of vitamin D, VDR, Jmjd3 and H3K27me3 in UC patients, rather than the effect of the outcomes of the tissue pathology on the change of the molecules examined.

Comment: 25OHD status appears unaffected by steroids? Fig 2C but what about the other anti-inflammatory treatments.

Response: This is really a very instructive question. Our study showed that there was no significant difference in vitamin D level between the steroids group and the non-steroids group. In any case, we cannot completely rule out the effect of anti-inflammatory treatments on vitamin D level or VDR, Jmjd3 and H3K27me3 expression. We will further explore this topic in future research. Thank you very much for your constructive question.

Comment: Healthy levels of 25OHD are in dispute and many American studies would say levels below 20ng/ml (i.e. the mean of both groups) would be vitamin D deficient? So all patients were deficient.

Response: Thank you for your valuable comment and suggestion. As you mentioned, we noted that serum vitamin D level in patients with UC and controls are lower than those in many American studies. However, there is no uniform criteria for vitamin D level in healthy people and patients with UC due to the lack of relevant research in China. The possible reason is the limitation of population and region.

Comment: On ta related point what does a 2.5ng/ml i.e 6.25nM decrease in 25OHD really mean?

Response: Thanks for your question. Although there was no remarkable

decrease in vitamin D level in patients with UC, statistical analysis showed that there was a significant difference in vitamin D level between UC and controls, suggesting that vitamin D deficiency may affect the disease activity of UC.

Comment: Minor points Typos I can't give page position because it was NOT included. There are numerous small mistakes that need fixing. Shown to be inversely {associated} with disease activity [8-10].

Response: Thank you for pointing out the mistake. We have carefully corrected the grammatical structure and errors of the manuscript.

Comment: Loss of VDR in intestinal were measured in the all UC patients?

Response: Thanks for your question. Immunohistochemical examination for VDR were measured in 56 UC patients and 22 healthy controls.

Comment: In UC patients of smokers not presented a significantly low vitamin D level?

Response: Many thanks for your question. Smokers had lower levels of vitamin D than non-smokers was not observed in our study. A relative research has showed that CD patients who smoke have lower vitamin D level than patients who are non-smokers (*J Crohns Colitis. 2013; 7(10):e407-e413*). Furthermore, current smokers have an increased risk of CD, and decreased risk of UC compared to never smokers, which was observed in several studies (*Aliment Pharmacol Ther. 2005; 21(8):921-931; Gastroenterology. 2019; 157(3):647-659*). Therefore, serum vitamin D level may not be affected by smoking in UC patients. This point has been added to the discussion section (see lines 340-342). However, multicenter large sample population studies are still required for further confirmation.