Response to editor and reviewer

Dear editor,

We submit hereby a revised manuscript entitled “Serum urate is associated with an increased risk of inflammatory bowel disease: A bidirectional Mendelian randomization study” (ID: 90628) which is co-authored by Shengbing Zhao. We have carefully revised the manuscript text based on the format requirements of World Journal of Clinical Cases and the editors’ suggestions. The changes we have made are highlighted with red fonts in the marked revised manuscript. In addition, because of the contributing role of Shengbing Zhao (Funding acquisition, Writing - review & editing, Supervision and Conceptualization), we list the author as a co-corresponding author, while we did not know if it is allowed by our journal.

We would be very grateful if the manuscript could be published in World Journal of Clinical Cases. Additionally, clean revised manuscript and other supporting information are also uploaded. The following part is the point-by-point responses to the editor:

Reviewer 1

Comment: It would have really helpful to show some in vivo studies pertaining the strong correlation of urate levels with UC with IBD like analysing the cytokine level or any specific biochemistry experiments (for example- other biomolecules level other than urate level alone pertaining the urate level synthesis) in addition to the all the above mentioned experiments.

Reply: We are grateful for your valuable comments. According to your comments, we supplied results for animal studies to verify correlation between urate level and UC. Dextran sulfate sodium (DSS) was used to induce experimental colitis. Enzyme-linked immunosorbent assay (ELISA) was performed to detect cytokine levels. Our results of animal studies were as follows (Line 241-249):

Results of animal studies, HE and ELISA

To validate the positive association between serum urate levels and UC, 2% DSS

was used to induce experimental colitis. The effect of this treatment included loss of body weight (Figure 6A), increased disease activity index score (Figure 6B), shortened colon length (Figure 6C), and increased inflammatory infiltration by HE (Figure 6D). Expression levels of proinflammatory factors in the serum, including IL-6, IL-1β and TNF-α, were significantly elevated in IBD mice (Figure 6E). Additionally, serum urate level was also increased in IBD mice (Figure 6F). Together, these results provided evidence that there existed a positive association between urate levels and IBD.

Figure 6 and related legend are as follows (Line 537-544):

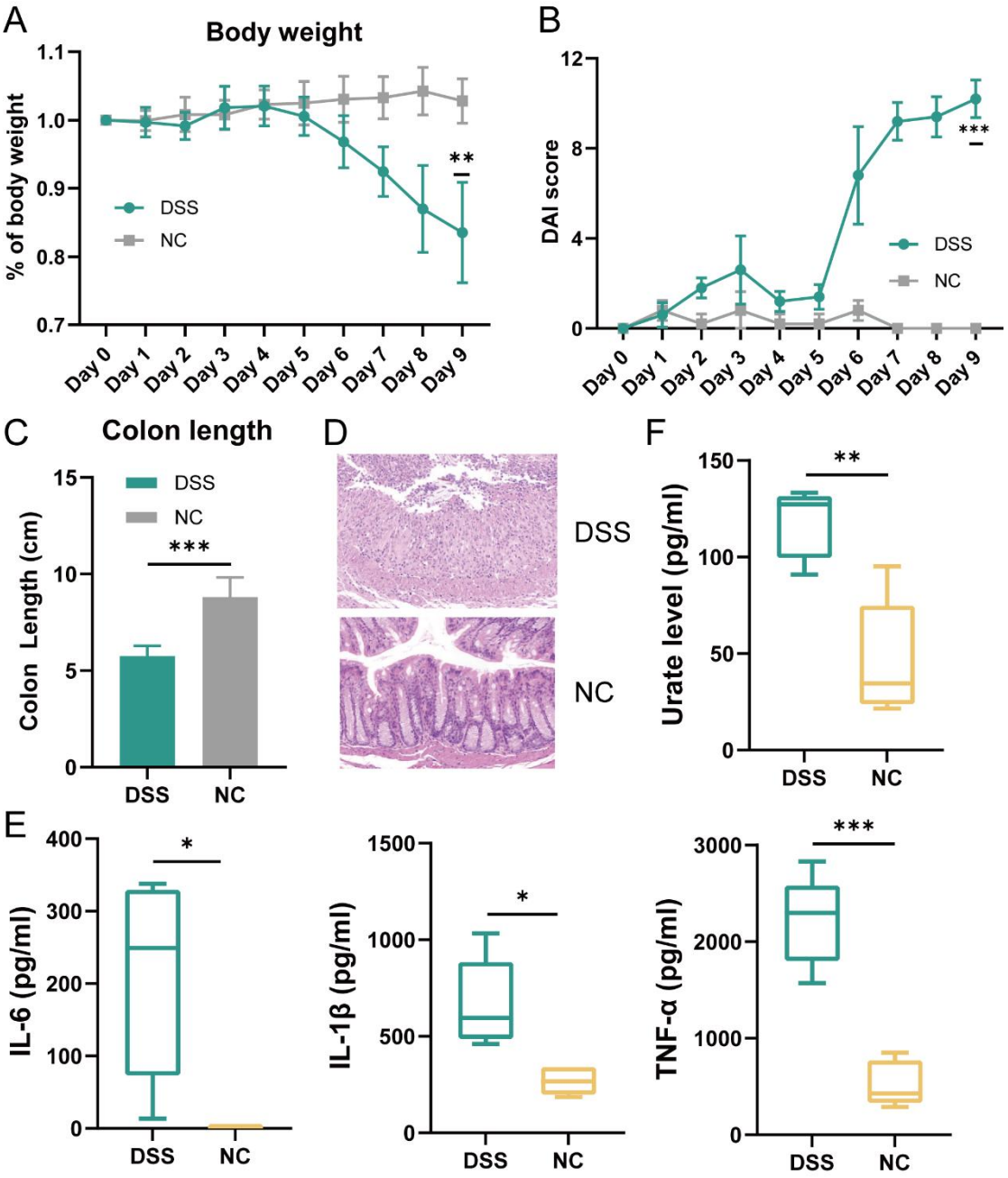

Figure 6. *DSS contributed to increase of inflammation in IBD mice.*

*C57BL/6 mice were administered DSS (2%) for 7 days (and a control group was provided with water only for comparison) and 2 days for water. DSS group (n = 5) exhibited a significant aggravation of IBD-associated changes in of body weight (**A**), disease activity index (**B**), colon length (**C**), inflammatory infiltration (**D**) and increased levels of IL-6, IL-1β and TNF-α (**E**). Compared with control group (n = 5), DSS group demonstrated increased levels of urate levels (**F**).*

Other changes:

Line 4, “, #” was added.

Line 23-25, “Shengbing Zhao, M.D., Department of Gastroenterology, Changhai Hospital, Naval Medical University, 168 Changhai Road, Shanghai, China. Tel: +86-21-31162770, E-mail: zhaoshengbing@hotmail.com” was added.

Line 48-49, “Animal studies confirmed the positive association between urate levels and UC.” was added.

Line 101, “In addition, we conducted in vivo animal studies to verify the association between urate levels and IBD.” was added.

Line 170-191, animal-related experiments were added:

Animal studies

All animal experimental procedures were approved and conducted in accordance with the guidelines of the Animal Care Committee of Navy Medical University. Co-housed, seven-week-old male C57BL/6 mice were administrated 2% dextran sulfate sodium (DSS) (36–50 kDa; MP Biomedicals) in their drinking water ad libitum for 7 consecutive days, following by 2 days of normal water.

Disease activity score and histological analysis in mice

Body weight, the presence of occult per rectum, stool consistency, and colon length were documented. A scoring system was used to assess diarrhea and the presence of occult or overt blood in the stool. Changes in body weight are reported as percentage loss of baseline body weight[26]. The ring of the rectum was harvested postmortem, fixed in 4% buffered formalin, and embedded in paraffin for following H&E staining procedure.

Enzyme-linked immunosorbent assay (ELISA)

IL-6, IL-1β, TNF-α and urate levels in serum were quantified using commercial ELISA kits in accordance to the manufacturer’s instructions (Multi Sciences LTD, Hangzhou, China).

MR results were presented as odds ratios (OR) with 95% confidence intervals (CIs) of the outcome risk of a unit change in exposure. A two-sided p value <

0.05 was considered to indicate statistical significance. Statistical analyses were performed mainly in R software (version 4.2.0, The R Foundation for Statistical Computing; TwosampleMR and MR-PRESSO package) and SPSS 26.0.

Line 255, “Animal studies confirmed the association between high urate levels and IBD.” was added.

Line 267, “Animal studies further demonstrated a positive association between urate levels and colitis.” was added.

Line 273, “dextran sulfate sodium” was deleted.

Line 280, “agreed with” was replaced as “supported”.