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Rostock, Jan. 29, 2018

Dear Editor,

Thank you and the referees for the constructive review of our paper entitled

"NOD2- and disease-specific gene expression profiles of peripheral blood mononuclear cells from Crohn's disease patients" (Manuscript No: wjg/37608). To meet the reviewer's concerns, we have included the requested data, and thoroughly revised the manuscript according to the suggestions. Furthermore, we have corrected some errors, such as the age of the patients (Table 4), which now refers to the time of blood sampling. The changes are highlighted in yellow. Please find enclosed the edited manuscript in Word format. Subsequently, we provide specific point-to-point replies to each reviewer's comments.

We hope that our manuscript, in its revised form, is now acceptable for publication in the *World Journal of Gastroenterology*.

Sincerely yours,

Robert Jaster

(corresponding author)

Point-to-point reply to the referee's comments

We thank the referees for their constructive suggestions and helpful advices.

Reviewer 1:

The study characterized different gene expression profiles of peripheral blood mononuclear cells in CD and healthy controls correlating to the NOD2 mutation status.The research design is reasonable and the conclusion is credible. It helps us to understand the role of NOD2 mutation in the Pathophysiology in CD.

We thank the reviewer for the encouraging comments.

Reviewer 2:

Authors analyzed disease-specific gene expression profiles of peripheral blood mononuclear cells (PBMCs) of Crohn's disease (CD) patients in clinical remission. They identified CLEC5A and LYZ as CD- and NOD2-associated genes of PBMCs. I think this may be an interesting finding for future investigations but due to the small number of patients enrolled no conclusions can be made. Furthermore, this study issue is basic science thus I think it would better fit in a journal dedicated to that.

We thank the referee for the constructive criticism. Indeed, the number of patients was rather small, which is however not unusal for a basic study like this. Using appropriate statistical analysis methods, we were nevertheless able to obtain statistically significant results. As a matter of course, the findings need to be substantiated in follow-up studies. Furthermore, we did our best to interpret the data as cautious as possible.

Since WJG is also dedicated to the publication of basic studies with clinical implications, we believe that our work would fit in this journal if the manuscript should be accepted.

Reviewer 3:

We thank the referee for the detailed and constructive comments.

1. *There are a number of errors of English language word usage/grammar that all need correction.*
   To overcome these shortcomings, we have involved Nature Publishing Group Language Editing service and provide a language certificate.

2. *The authors should revise the term "CD patients" to read "patients with CD" as per various international guidelines.*
   We apologize for this inconsistency, which has been corrected throughout the manuscript.

3. *In the INTRODUCTION, the authors refer to NOD2 and CD. This sentence should be revised to be more clear and precise. NOD2 is but one of the at risk genes/NOD2 is a risk factor in Caucasian populations predominantly.*
   We have revised the sentence as suggested; thank you for the comment.

4. *The word regime is used incorrectly: this should be correct to the word "regimen".*
   The error has been corrected.

5. *The manuscript includes a combined Results/Discussion section, which is not usual for the journal.*
   Both sections are now presented separately.

6. *If the criteria for inclusion was clinical remission (CDAI <150) why was one patient included who did not fit that specific criterion?*
   The patient mentioned by the reviewer displayed an only modestly increased CDAI of 166 and a normal CRP. The latter value is compatible with a state of very low disease activity. We therefore considered the inclusion of the patient as justified.

7. *Whilst the CDAI score may include a presence/absence of symptoms, it may not indicate disease activity. Did the group of patients with CD who had low CDAI scores also have normal CRP (or calprotectin or other inflammatory markers)?*
   As requested by the reviewer, we have now included the CRP values of the patients. As shown in the revised TABLE 4, all patients except of three had a normal CRP (below 5 mg/l). The CRP values of the other three patients were slightly increased (all below 14 mg/l), and corresponded only partially to CDAIs in the upper range of the group. This is now also mentioned in the text (page 15). Taken together, the findings suggest a low disease activity in all of our patients.

8. *Can the authors be sure that the drug exposures did not affect the gene profiles of interest?*
   Thank you for raising this important question, which we have addressed in the course of our studies. For *TREM1*, *lysozyme* and *CLEC5A* (the three genes with CD-associated expression patterns), no significant association between treatment with prednisolon, azathioprine or anti-TNF-α and expression could be found. This is stated on page 16 of the revised manuscript.

9. *As stated vitamin D is important in the context of IBD, and vitamin D was used in the ex vivo work. What was the vitamin D status of the subjects prior to collection of PBMC?*
   Thank you for this important question. Out of the 16 patients, only two displayed normal vitamin D levels (> 75 nmol/L). The other patients presented with vitamin D values of 50-75 nmol/L (n=8) or even below 50 nmol/L, indicating a deficiency (n=6). Since all samples were collected during the winter season, these findings were not unexpected. In cases of a vitamin D deficiency, a substitution therapy was initiated.
   The vitamin D levels of all patients have been included into TABLE 4, and some implications are now discussed in the text (page 15).

10. *In TABLE 2 we are told of the maximum upregulation. One assumes that this fold difference relates to just one of the genes of interest (the one with maximal change). The other genes may have or may not have substantially different fold difference. Hence this is not that helpful.*
    The idea of TABLE2 was to give a first overview of the number of differentially expressed genes in the individual groups, as well as of the magnitudes of the effects. While the lower border was the same everywhere (by definition, ≥ 2-fold with a

$p < 0.05$), the maximum effects that could be observed were quite different. Although these maxima refer to just one gene per group, we would therefore prefer to include this information.

11. *TABLE 4 lists the disease and other defining characteristics for the 16 subjects with CD. A number of the subjects are listed to have multiple disease locations (e.g. L1 L2 L3).*
    The corrections have been performed as suggested.

12. *How long were cells exposed to vitamin D? Figure 1 says 22 hours. Table S2 says 26 hours. This is not clear.*
    Unfortunately, Fig. 1 contained an error – 26 h is correct. Thank you very much.