To dr. Y. Qi
Science editor, World Journal of Gastroenterology

Leiden, the Netherlands, 14-11-2016

Dear dr. Qi,

We are very grateful for your decision to grant us the opportunity to revise our manuscript entitled 'Cerebral magnetic resonance imaging in quiescent Crohn's disease patients with fatigue – a pilot study' (Ms. Ref. No.: 29758). Additionally, we would like to thank the reviewers for his/her helpful comments. We have addressed the issues and concerns that were raised by the reviewers and believe these changes have improved the manuscript. Please find enclosed the revised version of the manuscript, we have used a red font to indicate the changes made.

We look forward to receiving your editorial response to the revised version of the manuscript.

Yours sincerely,

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Reviewer 1:

1. What is the hypothesis? The conclusion first raises a hypothesis with regards to systemic inflammation influencing the brain. This study is however not designed to study this proposed hypothesis.
   We fully agree with the reviewer the introduction can be more clear on this point. The aim was to study the effects of systemic inflammation on the brain in CD patients in remission. Previous performed studies indicates that although CD is in remission, pro-inflammatory cytokines may be present in these patients (Refs: Kader HA et al. Protein microarray analysis of disease activity in paediatric inflammatory bowel disease demonstrates elevated serum PLGF,IL-7, TGF-1 and IL-12p40 levels in crohn's disease and ulcerative colitis patients in remission versus active disease. Am J Gastroenterology 2005;100:414-423 and Hagel AF et al. Plasma histamine and tumour necrosis factor-alpha levels in Crohn's disease and ulcerative colitis at various stage of disease. J Physiol Pharmacol 2015;66(4):549-56). Therefore, although CD is in remission in the present study based on the clinical symptoms and the Harvey Bradshaw Index (HBI) score, some systemic inflammation may be present and may affect brain and cognitive functioning. We have clarified this in the introduction part (page 7 "Although quiescent … are present").
2. Abstract: "CD patients encountered significantly more depressive symptoms" and Page 17, line 12: "No correlations were found between mental status and MRI findings." A major weakness in this study is an attempt to define fatigue, since this symptom is the basis for this clinical research. It is presently unclear whether the authors are defining fatigue as a pathopsychologic disorder, in which case the study confirms a multitude of studies of underlying depression in individuals with chronic disease and the present MRI findings are not helpful, or whether the authors are defining fatigue as a pathophysiologic disorder, in which case they have not mentioned allied disorders in Crohn's disease including malnutrition, the potential side-effects of chronic pharmacologic therapy, etc.
   In the present study, we have not defined fatigue as underlying symptom of depression, but as an independent variable measured with a different questionnaire mentioned in the methods part (page 10 and 14). Fatigue was assessed with the Multidimensional Fatigue Index (MFI) and the Visual Analogue Scale (VAS) and depression was measured with the HADS (Hospital Anxiety Depression Scale) as a part of the mental status together with anxiety. On page 17, line 12 we refer to the mental status, including depression and anxiety. We have clarified this in the revised manuscript (page 14). Although more depressive symptoms were present in CD patients, no correlation was found between depression and MRI changes.
   Based on previous research, we have mentioned in the introduction (pages 7-8) that fatigue is related with bot active and quiescent CD and is associated with structural changes in the brain. We agree with the reviewer that more allied disorders and differences between CD patients and healthy controls can be explored, but since this is a pilot study we have decided to focus on the two most important differences between the two study groups. Further research is required to investigate more allied disorders including malnutrition, side-effects of therapy etc.

3. Core tip: "the effects of systemic inflammation on the brain". This study was not designed to study this question e.g. the title "quiescent Crohn's Disease".
   We agree that this sentence mentioned in the Core tip seems to be in contrast with the title of this manuscript. As mentioned before, although quiescent CD patients are included, systemic inflammation may be present but is not expressed as an active CD due to the low HBI score and few clinical symptoms.
4. Introduction: "The pathogenesis of CD is ...and eventually CD". If the authors have evidence of the pathogenesis of this chronic idiopathic disorder please either provide a reference supporting that pathogenesis or provide the evidence in their present manuscript. Otherwise, it is "proposed" pathogenesis or "prior pathophysiological studies support the notion that".
   We agree with the reviewer and we have inserted an additional reference supporting the multifactorial pathogenesis of CD (page 7). Now, these two references provide a clear evidence of the pathogenesis based on previous performed studies in both human and mice.
5. Introduction, paragraph 2: "cerebral infiltration of monocytes ... inflammation in the brain". Authors please provide reference.
   We agree with the reviewer a reference should be provided. We have added three references (page 7).
6. Page 8, Paragraph 2: "systemic inflammation contributes to cognitive decline". I am assuming that the authors therefore have a reference to provide us to back up their claim by showing that control of inflammation does prevent cognitive decline. In the same paragraph, "previous studies found an association between structural changes in the brain and fatigue". Authors, please list those structural changes.
   On page 8, paragraph 2, after the sentence "systemic inflammation contributes to cognitive decline" we have explained in more detail to what kind of cognitive decline activation of the immune system, and thus systemic inflammation, may lead to including Alzheimer disease, multiple sclerosis (MS) and Parkinsons' disease all provided with a reference. We agree that in this same paragraph the structural changes referred to in patients with fatigue needs to be more clarified. We have add this information in the manuscript (page 8 "In CFS … in CFS").
7. Page 8, third paragraph: "The aim ... what extent systemic inflammation affects the brain of CD patients". This study is not designed to study this issue and so a new aim needs to be provided.
   We understand the confusion, but hopefully we have clarified this matter now, by describing systemic inflammation may be present in CD patients in remission due to the presence of pro-inflammatory cytokines. We have clarified this matter in the introduction (page 7 "Although quiescent … are present").
8. MATERIALS: "patients had endoscopic proven CD for at least 3 months before inclusion". This definition does not support chronicity and it is unclear whether subacute enteric infections have been excluded. Unfortunately, the definition of chronicity in CD is not universally accepted but many prior studies settled on 6 months with evidence by repeated studies for chronicity during that 6 month period.
   We fully agree with the reviewer that the definition of chronicity in CD is mostly defined as CD for at least 6 months, but it was not our aim to focus on the chronicity

of CD. The most important aim of this study is the comparison between brain changes in CD patients and healthy controls. An inclusion criteria for CD patients was being diagnosed with CD for at least 3 months, proven by endoscopy. Since all patients prior to study enrolment underwent endoscopic assessment it was excluded that patients had subacute enteric infections since CD has a typical inflammation pattern in the intestine.

9. The most significant MRI findings are: a. obtained by MRS analysis, but only 9 CD and 9 age/gender matched controls are included. Do the authors have any references that support low variability in MRS studies of small numbers of patients? Was the nutritional status of these 9 CD patients closely examined? and b. obtained by blood flow analysis. Table 1 shows that 55% of CD patients are smokers: are these the same individuals with altered blood flow.

   We thank the reviewer for this comment. Indeed, in the MRS analysis only 9 CD and 9 age and gender matched controls were included since data was excluded due to patients' motion or bad shimming (page 20). MRS studies may show different degree of variation depending on the field strength (1.5 Tesla, 3 Tesla or 7 Tesla), the region of the brain that is studied and the different MRS acquisition parameters that are used (such as voxel size, acquisition method, number of averages, echo time). To our knowledge, only a few single volume MRS variability studies were performed with a 3 Tesla human MRI scanner (Mullins et al. MRM 2008, Wellard et al. AJNR 2005, Bednařík et al. NMR in Biomedicine 2015, Terpstra et al MRM 2015) and only one measured physiologic variability of the MRS results obtained from white matter (Wellard et al. AJNR 2005). This study reported an absolute variability of 5%, 7%, 7%, 19%, 12% and 9% for NAA, NAA+NAAG, Cr, Ins, Choline and Glu+Gln respectively based on two separate scans of 2 weeks apart. The different acquisition parameters that are used in this study (PRESS sequence, echo time of 30 ms, 20x20x20 mm^3 voxel size) makes it difficult to have a direct comparison with our results. Nonetheless, the variability measurement reported by Wellard et al. for Glu+Gln (9%) being half of the percentage difference in Glu+Gln that we have measured in between the CD patients and healthy controls (18%), indicate that our results are above a possible physiological variability.

   As a response to the question of the reviewer about the altered blood flow and the percentage of the CD patients who smoke: no significant association was in found the CD patients between smoking and the altered blood flow (p=0.74). Although more CD patients were smokers compared with the healthy controls, smoking did not influence the blood flow.

10. Page 13, final paragraph: "the cognitive functioning of adolescents with IBD has not been fully previously investigated"; Why is this mentioned: according to Table 1, the mean age of CD patients was 30.1 years old.

    We agree that the use of the word "adolescent" in this manuscript is completely misused and we have changed this in the text (page 13).

11. Page 12, Mood: "Cognitive performance depends upon the psychiatric status of the patient". What about cognitive performance with regards to their sleep schedule, social environment, and nutritional status?

First of all we have changed "Mood" into "Mental status" due to the fact it was not clear to the reviewer we mean the same with depression and mental status (comment 2). "Cognitive performance depends upon the psychiatric status of the patient": the psychiatric status was assessed in the same way as the mental status with the HADS questionnaire. The HADS questionnaire concerns questions about having pleasure in life and enjoying daily things. We agree with the reviewer that all mentioned variables (sleep schedule, social environment and nutritional status) may influence cognitive performance as well as mental status, but this is beyond the scope of this pilot study.

12. Page 13: Statistics: "A p-value </= 0.05 was considered statistically significant"; Then the authors need to go back through this manuscript and not refer to p values that are > 0.05 as being significant.
    We agree with the reviewer and we need to stick to the p-value ≤ 0.05 as statistically significant. In the results section (page 16) we have mentioned "just missed significance" which is not considered significant.

Reviewer 2:

1. This manuscript does not contain enough number of patients in the study. I suggest the authors should accumulate more patients in this work to improve the statistics.
   We agree with the reviewer statistics will be improved by accumulating more patients. Since this is a pilot study, the population size was limited to examine the differences between the most extreme cases; quiescent CD patients with fatigue and healthy controls without fatigue. Significant differences have been found between both study groups and now further research with more patients is required. We have addressed this matter in the discussion part.

Reviewer 3:

1. An interesting pilot study that opens the door to further explore the connect between the gut and brain using advanced MRI techniques.
   We thank the reviewer for this comment. This pilot study is a first step in understanding brain involvement in CD patients and implies that it is important to focus not only on symptoms in CD related to the gastrointestinal tract, but also on the effects of systemic inflammation on the brain. Understanding these effects may help gastroenterologists to set up interventions to maintain CD remission and improve mood status and quality of life.