Author's response to reviews

Title: Tryptophan degradation in Irritable Bowel Syndrome: Evidence of indoleamine 2,3-dioxygenase activation in a male cohort.

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Version: 2 Date: 6 November 2008

Author's response to reviews: see over
Dr Hans Zauner, PhD.
Senior Assistant Editor
BMC Gastroenterology

November 06th 2008

Dear Dr Zauner,

Please find attached our revised manuscript (MS:6111190812085863) entitled “Tryptophan degradation in Irritable Bowel Syndrome: Evidence of indoleamine 2,3-dioxygenase activation in a male cohort” which we are resubmitting to BMC Gastroenterology. We would like to thank the editor and the reviewers for their important, positive and constructive comments. We have addressed all the points of the referees in the accompanying letter. We hope that our revised manuscript is now deemed suitable for publication.

Reviewer 1: Sylvie Bradesi

MAJOR COMPULSORY REVISIONS:

Comment 1: In the discussion it is stated that plasma kynurenine levels and kynurenine/tryptophan ratio are higher in IBS patients ‘providing compelling evidence of increased tryptophan breakdown’. This statement is in contrast with the actual data showing that tryptophan levels are not altered in IBS patients. Although it is briefly discussed later in the discussion, conclusions should be consistent throughout the manuscript.

This statement is now altered to more accurately say that the evidence shows increased activity of the enzymes responsible for tryptophan degradation (Discussion, Paragraph 1, page 9)

MINOR ESSENTIAL REVISIONS:

Comment: The nature of the samples should be indicated in the abstract

The ‘Methods’ section of the abstract now clearly states that we used plasma samples in the study.
DISCRETIONARY REVISIONS

Comment: The authors indicate that interferon gamma levels could not be reported because they were below the limit of quantitation of the analysis method. Could a different analysis method be used to assess the current samples for interferon gamma?

We have considered this point, but unfortunately there is insufficient plasma sample remaining to carry out any additional analysis.

Reviewer 2: Dietmar Fuchs

Comment 1: The rather lengthy discussion of the data is not really justified

We have taken this comment into consideration and shortened the discussion by removing discussion items on pages 10, 11 and 12 of the original manuscript. The discussion in our first manuscript was 1228 words long, it is now 1008 words.

Comment 2: The significance to distinguish patients and controls is much greater than that from tryptophan or the ratio. Authors should comment on that finding.

We assume that the reviewer was referring to the greater degree of difference in neopterin levels between IBS patients and control subjects data compared to that seen with the tryptophan measures. We have commented on this finding at the end of page 10/start of page 11 in the discussion section of the revised manuscript.

Comment 3: Authors should comment on the rather huge overlap of tryptophan and kynurenine between groups

We have now noted on this interesting overlap at the start of the discussion on page 9 of the revised manuscript.

Comment 4: Neopterin has been described as a potential marker of disease activity in IBD patients, however not cited.
Indeed we are aware of these interesting findings in colitis, we have now included an appropriate citation at the end of the introduction on page 4 of the revised manuscript.

Comment 5: Authors measured tryptophan metabolic abnormalities + neopterin. To substantiate the association between neopterin and tryptophan degradation, the correlation should be calculated and shown on a graph.

This correlation (p<0.05, r = 0.7055) has now been included in both the results section on page 9 and as figure 3B. The figure legends (page 16) have also been updated as has the data analysis section of the methods on page 8.

Comment 6: Discussion, line 8:...decreased availability of tryptophan...has not been found in patients. So to discuss the potential consequences of subnormal tryptophan seems rather obsolete

The actions we have taken to account for comment 1 by this reviewer takes on board this suggestion. We have also addressed this point in the alterations made to the manuscript on the basis of the comments made by reviewer 1.

Comment 7: Tables 1 and 2 are not really necessary, content can be easily included in the text. All marker concentrations were independent of age so the details for all individual compounds are not necessary. Group comparisons are given anyhow.

Table 1 is removed from the revised manuscript. Data from table 1 not previously included in the text of the results section is now added to that section. Table 2 is also removed from the revised manuscript.

Comment 7: Concentrations of tryptophan and catabolites should be given in µmol/L instead of ng/ml).

All relevant concentrations are now given in µmol/L.

MINOR

Comment 1: Should read L-kynurenine instead of l-kynurenine throughout
Reviewer 3: John Potokar

Comment 1: Was the study powered for just 10 patients and what was this sample size based on?

The study was powered to detect differences at the p=0.05 level with n=10 in both groups. We felt that the inclusion of additional controls was necessary to adequately show the variation that can occur for these measures in a normal population and to get a superior baseline measure. Comparisons between a more restricted control group do not alter the findings of the study.

Comment 2: The IBS group is much older and neither group are adequately described- this needs to be addressed before publication. Were there any age associated confounders that might be relevant?

Our ANCOVA analysis shows the age is not a factor. However we now state clearly in the paper that “Due to the possibility of their being an influence of age on the results, we used age as a covariate assessed by analysis of co-variance (ANCOVA)”.

Moreover, additional information is now included in the ‘Subjects’ section on page 5 of the revised manuscript.

Comment 3: Were any of either group on serotonergic medication?

As is now indicated in the ‘Subjects’ section on page 5 of the revised manuscript, all individuals from both groups were free of serotonergic medications.

Comment 4: Were there differences in smoking between the two groups?

Two of the patients (20%) and four of the controls (15.4%) were classified as smokers. This data is also now included in the ‘Subjects’ section on page 5 of the revised manuscript.

Comment 5: Where were the controls recruited from?
As is now indicated in the ‘Subjects’ section on page 5 of the revised manuscript, the controls were recruited from the complement of staff affiliated to the University College Cork and its teaching hospitals.

Comment 6: p9, last sentence remove ‘there’
Correction made

Comment 7: I am not clear how this study clarifies that if the increased tryptophan degradation is IDO dependant, the knock on consequences for serotonergic signalling are greater than if it is TDO-mediated
We wanted to address this point because IDO can also metabolise serotonin and this was referenced in the discussion. However this is now reformatted and made clearer on page 11 of the revised manuscript.

We look forward to hearing from you in due course,

With best regards,

Gerard Clarke
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