Author's response to reviews

Title: Transcript levels of Toll-like receptors 5, 8 and 9 correlate with inflammatory activity in Ulcerative Colitis

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Author's response to reviews: see over
Mexico City, September 12th, 2011

Tim Shipley, PhD
BMC Gastroenterology
Editor in Chief

Dear Editor:

Enclosed you will find the second revised version of the manuscript: MS: 1380844289464145 - Transcript levels of Toll-like receptors 5, 8 and 9 correlate with inflammatory activity in Ulcerative Colitis and the response for the reviewers, which comments were very helpful for the manuscript. All reviewer’s suggestions were incorporated in the text and marked in red. I apologize for the delay response but we incorporated new results at the protein level as suggestion from one of the reviewers.

I am looking forward hearing from you soon.

Best regards,

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**Response to reviewers.**

Reviewer: Mathias Chamaillard

TLR9 at the protein level was performed. The TLR5 and TLR8 were already done at protein level in previous studies.

Reviewer: Ingrid Arijs

Abstract:

*Results Section (page 2).*

TLR8,9 and IL6 upregulated in UC versus controls: overall UC or active UC?

It is now corrected in the text, to both quiescent and active UC.

IL6 qPCR results not mentioned in the results section of the manuscript.

IL6 and TNF qPCR results are now mentioned in the results section of the abstract.

TLR5, 8 and 9 lower in quiescent than in active UC (p<0.05)

The mention p-value (<0.05) is not correct for TLR5, it is p-value =0.06 which is not significant.

This has been taken into consideration and other significant TLRs results have been incorporated into the sentence.

What about other TLR’s, you mention a few:

TLR2, 4 is also upregulated in active UC vs controls and quiescent

TLR2, 4 is also lower in quiescent than active UC.

Results are in congruence with previous published results, but as recommended for both TLR’s results are now incorporated into the abstract results section.

*Methods*

Population and tissue samples (page 5):

Please mention table 1 in this paragraph

This is now mentioned in the first sentence of the paragraph

Sample processing RNA extraction and CDNA synthesis (page 6)

Please add ‘and’ before ‘by spectrophotometer’
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This is incorporated in the text.

Real time RT-qPCR (page 6)
Please add ‘from’ before ‘invitrogen
Please add ‘were used’ or another verb before for relative quantification’
Please add ‘was performed’ or another verb ‘using the LightCycler’
Please clarify ‘calibrator in a separate sentence

This has been corrected in page 6

Statistical analysis (page 7):
Please add a point behind program and start a new sentence with statistical significance…’

Another sentence was considered in the text.

Add a comma behind ‘differences’
Replace ‘and by a comma between TLR1 to TLR9 and TNF

Both suggestions have been addressed in the text.

*Results

TLR1 to 9 mRNA profiles in UC compared with controls (page 8)
Please add the qPCR results of TNF and IL6 also in the table 3

These results have been added in table 3 as suggested and we modified the information presented in the graphs.

Figures 1, 2 and 3 are totally not clear:
Please add in the figure what is significant
Please add in the legend what the bars representing: the median of the expression and the interquartile range??

The data previously presented in the figures 1,2 and 3 are now presented in the table 2, therefore we decided to remove it from the manuscript to avoid information redundancy.

Table 3

Please add in the legend what you are meaning with underlined numbers and numbers with star
Please add ‘UC’ behind ‘quiescent’ in the last column of the table
The transcript levels are given in means and standart deviation please mention it in the legend of the table

Suggestions have addressed into the manuscript table 3.
Please make one sentence from the second and third sentence e.g. ‘we found a significant increased expression of TLR2, TLR4, TLR8 and TLR9 mRNA levels in patients with active UC compared to patients with active UC when compared to patients with quiescent UC and healthy controls.

This has been addressed in the manuscript

Moreover the first mentioned p-value in the original sentence is not correct. It needs to be p-value <0.05 instead of p<0.02 because it is p-value 0.04 between quiescent and active UC for TLR4.

The p value was corrected as suggested

Please rewrite the sentence ‘TLR3 mRNA levels…’ to for example TLR3 mRNA showed no change in expression between the different groups.

This has been changed in the manuscript

Please rewrite the next sentence as followed:
- E.g. ‘Comparisons between the UC quiescent group and the control group only showed a significant up-regulation for TLR4 mRNA levels, while the TLR2, TLR8 and TLR9 mRNA levels were similar (p=0.014)
- The mentioned p-value is not correct in the original sentence, it is p=0.015 instead of p=0.04 (based on results in table 3)

This has been addressed in the manuscript

# I would delete the following sentence ‘Although the TLR2, TLR8 and TLR9 mRNA levels were similar ….’ Because you mention in the sentence before that there is ONLY one significant between controls and UC quiescent.
# Mention that the lower expression of TLR5 in quiescent UC vs active UC is borderline significant (p=0.06)

This has been changed as recommended

• You did not mention that TLR8 and TLR9 were significantly downregulated in quiescent UC vs active UC.

We mention it in the third sentence of the paragraph.

# Please delete the comma before ‘and’ between TLR4 and TLR8 in the penultimate sentence of this paragraph.

This has been changed as suggested

• Add in the table 3 the qPCR results of comparison between the overall UC
group versus controls
This has been added to table 3.

# Can you mention between brackets which other TLR’s showed no statistical significance.

It is now mentioned in the last line of the paragraph.

o Correlation of TLR5, TLR8, TLR9 and IL6 mRNA levels with endoscopic and histological activity (page 8-9):
# Can you give the reason that you only look for the correlation for TLR5, TLR8, TLR9 and IL6, and not for the other TLRs and TNF?

It is mentioned in the first line of the paragraph.

# Figure 4 and figure 5:
• The same comments that I made for the figures 1-3 (see above)

This has been corrected and as mentioned figure 4 and figure 5 are now figure 1 and 2.

# The last sentence is not clear. What are you meaning? Can you give it in a figure? There is a lower TLR expression in patients with steroid treatment?

We rewrote that sentence.

o TLRs mRNA levels correlate with IL6 and TNF mRNA levels in the colonic mucosa from UC patients
# IL6 in second sentence is not written in italic

IL6 is now written in italic.

# You mention a significant correlation between TLRs mRNA levels and IL6 and TNF, in particular with TLR5, TLR8 and TLR9. Not only TLR5, 8 and 9 but also TLR1, 2, 4 and 6. Why don’t you mention these TLRs?

The other TLRs are now mentioned in the text.

- Discussion (page 10-12):
  o First sentence:
# The authors mentioned that TLR5 is up-regulated in active UC but this is not seen in table 3, only borderline significant (p=0.06) in active UC vs quiescent UC.

This has been changed in the manuscript and only significantly involved TLRs.

# Up-regulated in active UC versus controls or versus quiescent UC or versus both controls and quiescent UC.
# Please add ‘in’ before ‘active UC patients’

This is now mentioned in the first line of the second paragraph.
o Didn’t the authors forget something behind ‘stimulated’ in the ‘TLR8 induction in human isolated epithelial cells stimulated has been reported’

We corrected this sentence.

o The correlation between TLR8 and TLR9 gene expression is only mentioned in the discussion, not in the results. Please mention it also in the results section.

After TLR9 characterization in biopsies made by us and previously reported for TLR8 we decided to do not discuss about correlation of TLR8 and TLR9 at the mRNA level.

o There is not only a trend of down-regulation for TLR5 in UC quiescent vs controls, but TLR6 shows also a trend of down-regulation in quiescent UC versus controls (it is more significant for TLR6 than for TLR5). Moreover, the mentioned p-value is not correct for TLR5, it is p=0.014 instead of p=0.011 (based on table 3).

o Why do mention only

TLR5 has been addressed in the TLR6 did not show any association with endoscopic and histological activity.

o The authors only discussed the findings of the previous studies for TLR5, TLR8 and TLR9 but not the other TLRs. E.g. The findings of TLR2 and 4 in this study are the same as in other published studies.

The TLR2 and TLR4 are now mentioned in first paragraph of the discussion.

o Please replace ‘In a paper published by Stanislawowski et al., found…’ with ‘Stanislawowski et al. found…’

This sentence has been changed in the discussion

o The sentence ‘This finding might be due to a down regulation of TLR5 in patients receiving steroids…’ is not clear.

# Please rewrite this sentence.

# Moreover, these findings are not mentioned in the results section. Can you add this to the results?

This sentence was rewritten as suggested it is also mentioned in the last sentence of the results section for correlation with endoscopic and histological activity.

o Furthermore, I cannot find the results in the results section of the upregulation of TLR5 in patients with pouchitis after total proctocolectomy.

This is result from other studies, we now clarify this in the manuscript.