Reviewer’s report

Title: TLR9 polymorphism correlates with immune activation, CD4 decline and plasma IP10 levels in HIV patients.

Version: 0 Date: 07 Sep 2018

Reviewer: Mathias Tenbusch

Reviewer’s report:

Joshi et al. studied in a cohort of 50 HIV infected individuals and corresponding controls a possible relationship of two TLR9 polymorphisms (1635A/G and 1486C/T) with HIV disease progression and/or immune activation. It is stated that the presented findings provide several new insights into HIV mediated immune activation and the underlying mechanism. Although the authors demonstrate some correlations of the SNP and immune activation, mainly the IP-10 production, the overall conclusions drawn from these experiments might be overestimated which is my major concern.

Furthermore, it might be really difficult to analyze the two SNPs independently in this cohort, since 6 out of seven HIV+ patients with 1486 CC also carries the 1635AA SNP which was already described before to correlate with HIV infections, disease progression, CD4 counts and viral load. These studies were all correctly cited by the authors, but also demonstrate that most of the findings are not fundamentally new.

Main findings:

1635AA patients have reduced CD4 counts, increased frequencies of activated CD4 and CD8 cells, but no significant differences in VL (all compared to AG/GG mixed population). Using further separation into the three possible allele combinations, only significant differences were shown for CD4 counts and CD8 activation. In my opinion, the separation into these three categories would be much more meaningful and should be presented for all results.

In fig.3, it is shown that HIV+ individuals have higher levels LPS,sCD14, IP-10 in the sera compared to HIV- patients (by the way "normal" might be not best choice to indicate the controls). This also confirmed already existing data. IP-10 levels are also higher in 1635AA patients, but not the other ones. Fig 5 demonstrates that IP-10 also correlates with VL. By this finding, the authors draw the conclusion that the lower IP-10 correlates with SNP and VL and thereby the SNP with VL. But a direct correlation has not been shown in Fig 2.

In Fig. 7+8, they add the second SNP to the analyses with the above mentioned limitation and demonstrates that the AA-CC genotype seems to be the best correlate for the IP-10 levels and not AA alone, which in combination with CT is not substantially different from the other genotypes. The combination of the genotypes should be also included in the table 1.
Further major concerns:

- using a consecutive T-test as the statistical analyses for comparisons of more than two groups is not correct. ANOVA with post-test for multiple comparisons should be used. Therefore some of the significances might be questionable

- there are no mechanistic insights as it is suggested in the text. The source of IP-10 is not known and also the consequence for the T-cell activation

Therefore the study provides only very little new information to the field and mainly reproduced some previous published data in their cohort. The major contribution was the additional analyses of the 1486 SNP which has not been reported in the context of HIV disease progression. Unfortunately, the linkage disequilibrium between the two SNPs makes independent analyses very difficult. The proposed fundamental mechanistic insights are unfortunately not really analyzed and rather of theoretical nature.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

**Quality of written English**
Please indicate the quality of language in the manuscript:

Acceptable

**Declaration of competing interests**
Please complete a declaration of competing interests, considering the following questions:
1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organisation that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal