Author’s response to reviews

Title: Gene expression profiling in blood from cerebral malaria patients and mild malaria patients living in Senegal

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Version: 1 Date: 30 Jul 2019

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We thank the editor and the reviewers. The constructive comments of the editor and the reviewers help us to improve the paper, and the detailed responses to these comments and the corrections are stated below. Our changes are underlined in the revised manuscript.

Reviewer 1
a) We agree that the variance can be large. The important issue is to be able to assess the variance explained by the factors of interest. That’s why we have included the information as the percentage of the variance explained by the clinical factor. Very interestingly, the mediane of the variance explained by the clinical factor was 19.2% with a maximal of 36.2% when taking into account CM, MM and
NCM whereas it was 40.3% with a maximal of 73% when taking account only CM and MM. Also, a large variance of gene expression levels can be mostly explained by the clinical factor when comparing CM to MM patients. We add the information on page 10.

b) The unit is the year. We add it in the Table 1. The reviewer is right: there were adult patients. That’s why we used the Glasgow score.

c) As requested by the reviewer, we add a text describing how the blood samples were processed on page 6.

d) I fully agree with the reviewer who wrote that a major issue is to determine what is the cause of the clinical phenotype. It is very difficult to determine it in humans because we measured the gene expression levels at the time of the clinical attack. Actually, measuring gene expression levels in the same individuals before and during the attacks would help to decipher what is the cause and what is the effect. This is a very challenging task in humans. In contrast, the experiment has been performed in mice. For instance, brain gene expression profiles associated with CM have been identified 5-7 days before the onset of CM. Thus, we state the limits of our study in humans on page 15 (discussion section), and we highlight the interest of animal models to tackle this issue. Nevertheless, we state that there is still a debate about the relevance of the mouse model.

e) I fully agree with the reviewer who wrote that studying the brain microvascular endothelium would allow to decipher crucial mechanisms involved in the pathology. Obviously, it has not been investigated in our current paper. As the reviewer said, obtaining microvascular endothelium of patients is very challenging. We discuss this aspect on page 15.

f) I understand well the comment of the reviewer concerning the power of previous GWAS. I have removed the comment on page 17 (conclusion section).

I have fixed all the minor problems that were stated by the reviewer.

Reviewer 2

We are aware of the limits of our study, and we add a paragraph on page 15 to delineate these limits, including the sample size and the bias due to cellular composition.

1) We add the labels for the cluster, as requested. We explained the change in the Figure legend and state that the list of the genes regulated in CM patients is shown in Table S1.

2) We add the hematological characteristics in Table 1 (Haemoglobin concentration, red blood cell count, and platelet count). We also add the statistical analysis on page 9. It should be stressed that we have measured the parasitaemia for 12 out of 16 patients, whereas all the patients have been tested for the presence of P falciparum infection with the immunoassay allowing to detect PfHRP2. We add this information on page 6.

3) And 4) I well understand the comment of the reviewer. However, the sample size of NCM group (n=4) is too low and did not allow us to split that group for further statistical analysis. As requested, we have performed an additional analysis after grouping CM and NCM patients. There was no significant result after correcting for multiple tests. Nevertheless, I agree with the reviewer who wrote that grouping all severe malaria patients may allow us to pick up a signature universal to severe malaria phenotypes. Also, I add this on page 17 (conclusion section).

5) The reviewer is right. The cellular composition likely influences the transcriptional profiling. Note that i) neutrophilia cannot influence the transcriptional profiles because we isolated peripheral blood mononuclear cells, from which we extracted RNA (we clarify this step in material and methods on page 6); ii) the cellular composition (lymphocytes, monocytes…) could also be reflected by the over-representation of MsigDB molecular signatures of specific immune cells. Unfortunately, we do not have the cellular composition of the individuals; we add this limit and discussed this issue on page
Minor issues:
1) We add the information on page 6.
2) We add a schematic outline of the stepwise analysis (new Figure 1), as requested by the reviewer.
3) Gene lists for each cluster from both univariate analyses and multivariate analyses are now available in Table S1. They correspond to Figures 2, 3, and 4. This information has been added in the legend of those Figures.