Reviewer’s report

Title: Identification of biomarkers for amyotrophic lateral sclerosis by comprehensive analysis of exosomal mRNAs in human cerebrospinal fluid.

Version: 1  Date: 31 Oct 2018

Reviewer: Reviewer 2

Reviewer's report:

"REVISION ASSESSMENT FROM THE ACADEMIC PEER REVIEWER:

Has the author addressed your concerns sufficiently for you to now recommend the work as a technically sound contribution?

No

Reviewer comments: The authors made good response and added additional materials (some more details of the data, deposit of the data to GEO, RT-PCR, among others). however, the main concern of large variation of gene expression among four samples remains. As you can see Figure 3D, the variation is quite strong. That says, it is always easy to find some genes showing strong difference among the disease versus control samples. This is a big scientific issue - reproducibility of the results. The statistical power is not sufficient in this study.

The authors replied "5,006 genes passed threshold for calculation (line 180-182, 592-593)." This itself has a big concern. What is the hypothesis here and statistical test? If this observation is reliable, then the whole transcriptome has been changed in the ALS disease form.

Line 180-182, it is not clear how the FDR was calculated. Given that so many genes were compared and sample size was very small, it would be important to disclose with more details, instead of stating "(data not shown, see Additional file 3)."

The authors stated "However, we think it should be noted that representative DEGs were also confirmed by qRT-PCR." This may help, but again the authors did not even much understand the scientific design or result interpretation. It only confirms the expression, but it is risky to conclude this gene is statistically significant. Would the expression represent the same trend if many more samples were selected? This is a fundamental statistical issue - the authors need to consult with biostatistician.

They added QC and make the data available to the readers via GEO GSE121519. This dataset is not currently searchable in GEO - this is likely the authors have embargo data and it is understandable. The authors must assure to have it available as academic standard.
As above, while this reviewer still has main concern, the authors need to at least state more clearly this paper is technique based. "

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

No

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

No

**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.

Not relevant to this manuscript

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

Acceptable

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