Reviewer’s report

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

Version: 0 Date: 24 Sep 2018

Reviewer: Reviewer 2

Reviewer's report:

PEER REVIEWER COMMENTS: To view the full report from the academic peer reviewer, please see the attached file.

REVIEWER COMMENTS FROM REPORT: In this manuscript, the authors aimed to demonstrate the exosomal mRNA can be detected in CSF tissue using the next generation sequencing. While the sample size is too small to demonstrate the reliability, the authors demonstrated the technique and pipeline that might work. The paper is more like an introduction of a technique, even though one gene (CUEDC2) was highlighted as a potential biomarker. To demonstrate the reliability of a technique, additional experimental validation is typically needed, but this component lacks in the current manuscript. It may also be good to discuss or compare with other peer techniques so that the readers can understand how the technique in this work is better or more appropriate.

REQUESTED REVISIONS:

Overall, manuscript is quite straightforward to describe a technique (NGS for detecting exosomal mRNAs in human cerebrospinal fluid

As the authors corrected argued "This is, at least in part, due to insufficient detection sensitivity for exosomal mRNAs in CSF, and consequent low reproducibility." This manuscript also need to be revised to seriously address the reproducibility. The concerns included too small sample size, the biased results due to technical limitation - too many downregulated genes (see below), lack of additional validation, and the need to deposit the data for public reanalysis.

The variation of gene expression may be strong. Using four samples does not have sufficient power to address such strong variation.

In page 8, "4,580 genes were commonly detected in CSF exosomes" -> the coverage or quantitative measurements need to be provided. How abundant these genes are?
The authors need to provide technical details of calling differential gene expression in ALS patients versus normal controls. Simply mentioning DESeq2 is not enough in such a technique papers. The expression level, for example, needs to be summarized or justified in DEG analysis, especially for low expression.

Page 9: "Among these genes, 133 genes were upregulated and 410 genes were downregulated in ALS patients group." This strong difference (more down-regulated genes in ALS) is likely due to technical limitation. Since you used 4580 genes with good expression level, many of these genes in ALS samples had low expression due to technical challenges. So, the conclusion of "downregulation in ALS" would unlikely to be biologically meaningful. At least discussion on this technical issue is required, and caution should be used. In addition, as shown in Table 1, the number of short reads in ALS group is substantially lower than the controls. This may lead to very biased results. The authors need to provide such details (not general description) in the main text.

As a standard approach, to test the performance of genomic techniques, additional experiment is need to validate the results. This manuscript does not include this validation part.

"Gene ontology" or "gene ontology" -> please use "Gene Ontology".

For reproducible research, the sequence data should be deposited into GEO or other public repository database.

ADDITIONAL REQUESTS/SUGGESTIONS:

None

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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