Reviewer's report

Title: A donor splice site mutation in CISD2 generates multiple truncated, non-functional isoforms in Wolfram Syndrome type 2 patients

Version: 1 Date: 30 Oct 2017

Reviewer: Gabriela Da Silva Xavier

Reviewer's report:

In 'A donor splice site mutation in CISD2 generates multiple truncated, non-functional isoforms in Wolfram Syndrome type 2 patients', Cattaneo and colleagues present data in support of the hypothesis that 103+1G>A mutation leads to altered splicing and results in the loss of CISD2 protein.

The authors use 5'RACE to determine the identity of splice variants and western blot analysis to demonstrate that CISD2 protein was undetectable in PBMCs of patients carrying the mutation vs carriers (parents) and healthy donor. A dose dependent effect of genotype versus protein content was demonstrated.

This is an interesting study but I have a few concerns:

1. In the abstract the authors say that they performed biochemical assays to look at protein function but in fact they only used Western blot to demonstrate presence/absence of the protein. The abstract needs to be changed to reflect this. In fact, there was no assessment of protein function in the paper because there was no protein/protein was present at levels undetectable by the antibody.

2. The 5'-RACE method used is a biased method of looking for splice variants, and arguably less efficient than newer, unbiased technologies such as e.g. RNA Seq. It should be acknowledged in the manuscript that other splice variants may exist that were not detected by the method described, and the risk for false positives should also be discussed.

3. The study is on one cell type- PBMCs- and no effort was made to assess whether the effect may be relevant to the cell types that are most linked to WFS2, e.g. pancreatic beta cells or
neurons, which the authors do mention in the discussion. This is important as splice variants can be cell-type specific, and studies in some of the other relevant cell types (modified cell lines or iPS cells) by way of comparison would have been useful. This should at least be discussed in the manuscript.

**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 am able to assess the statistics

**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 organization 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.