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

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Author’s response to reviews:
Dr Matteo Pasini米兰, November 15, 2017

Editor in chief
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Subject: Submission of revised manuscript MGTC-D-17-00149R1 [A donor splice site mutation in CISD2 generates multiple truncated, non-functional isoforms in Wolfram Syndrome type 2 patients]

Dear Dr Matteo Pasini
Thank you for your email enclosing the Reviewers’ comments.
Please find in attached a revised version of the above referred manuscript.
In the revised version, we addressed the issues raised by the Reviewers to further improve the significance of the data. Our responses are given in a point-by-point manner below with the relevant changes introduced in the manuscript.

We thank the Reviewers for their helpful comments and hope you will find this revised version suitable for publication.

Sincerely
Monica Cattaneo

Response to Reviewer 1:

1. As requested, we changed the sentence “biochemical assays” with “western blot analysis” (Abstract section: page 2, line 36). We eliminated the word “biochemical” (Abstract section: page 2, line 33).

2. We agree with the Reviewers that other splice variants, beyond that identified by the 5’-RACE, might exist and that they could only be detected by more efficient and deep genome-wide investigations based on unbiased methods (i.e. RNAseq). We insert the following sentence:
   “It is not excluded that other splice variants, beyond that identified by the 5’-RACE, might exist and that they could only be detected by more efficient and deep genome-wide investigations based on unbiased methods (i.e. RNAseq).” (Discussion section: page 13, lines 309-312).

   As requested, we discussed the risk for false positive. The sentence we have inserted is the following:
   “The risk of false positive splice variant identification is very low since the three isoforms were detected in both siblings (two independent samples) although at different frequency.” (Results)

3. As requested, we discussed the possibility that the splice variants can be cell-type specific. The sentence we have inserted is the following:
   “Moreover, it is not excluded that the pathogenic splice variants detected by 5’RACE might be cell type specific, therefore it is important to extend this molecular approach to some of the other relevant cell types that are most linked to WFS2 (i.e. pancreatic beta cells or neurons). The establishment of patient-derived induced pluripotent stem cells (iPSC) which can differentiate
into neurons and pancreatic beta cells will represent a suitable disease model for investigating the c.103+1G>A mutation as well as for discovering novel therapeutic targets.” (Discussion section: page 14, lane 313-319)

Two insertions have been introduced:

1. the current address of Dr Stefano Genovese and Maurizio Rondinelli in the first page, lane 18-19.
2. the symbol “#” near Monica Cattaneo in the list of the authors in order to indicate the correspondence author (page 1 lane 4)