Author's response to reviews

Title: Novel loss-of-function PRRT2 mutation causes paroxysmal kinesigenic dyskinesia in a Han Chinese family

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Author's response to reviews: see over
Dear editor:

We greatly appreciate this opportunity to revise our manuscript (6077534951256325) and thank you very much for your reconsideration with our manuscript to publish as Original Article. We would like to thank the reviewers for their kind comments regarding our work and for their efforts in reviewing this paper. We have carefully read the editor’s and the reviewer’s comments regarding our submission and have responded point by point as listed below. We feel that the reviewer’s comments have greatly helped us to strengthen our manuscript and we hope that the revised version will be acceptable for publication in *BMC Neurology*.

Sincerely yours,

Fu Xiong

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<th>Comments</th>
<th>Our answers or modification</th>
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<td>(1). The minigene assay is not used to check the NMD and the design for the minigene assay is not very clear and may be somehow wrong (at least one primer should be in the vector). I suggest that its data should be removed from discussion, and instead, considering use the words &quot;the NMD mechanism of this mutation needs further investigation.&quot; (Reviewer #1)</td>
<td>We would like to thank reviewer for this good suggestion and we have revised this discussion accordingly as follow: “Based on the experiments performed in vitro, we think that haploinsufficiency is not disputed. And nonsense mediated decay is one of possible mechanism to haploinsufficiency, the mechanism of this mutation needs further investigation.”</td>
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<td>(2) Previously they mentioned and now they confirmed by QRT-PCR that mutant allele has twice amount of RNA expression level than the wild type allele in transfected cells, but giving no explanation, readers may want to know the reasons, moreover, does it have any impact on the design of co-transfection when they perform the 4:4, 4:3, 4:2, and 4:1 ratios? (Reviewer #1)</td>
<td>We think the mutant allele has twice amount of RNA expression level than the wild type allele which may be caused by the mutation leading to the mRNA premature termination. The truncating mutation strengthened the abnormal mRNA expression and resulted in the mutant type allele have twice amount of RNA expression level than the wild type allele in transfected cells. The primer used in our experiment cannot distinguish the mutation type from the wild type when they co-transfection performed the 4:4, 4:3, 4:2, and 4:1 ratios, so we can’t provide the result with the co-transfection different ratios with the wild and mutant type alleles. But we think that the QRT-PCR result should be similar with previous works.</td>
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<td>(3) Minor Essential Revisions 1. As they said that HEK293 has no endogenous PRRT2, the first sentence in result para. in the abstract need to be modified (add &quot;transfected&quot; ?) 2. 2nd para. of discussion “398bp” should be changed to &quot;codon 398&quot; 3. The last sentence before the conclusion need rewrite (the novel mutant protein...), as they already said the different localization of mutant protein is the result of loss of function, not the cause of gain of function. 4. Fig1, use +/- or +/- for the genotypes of the family members instead of the words in the legend. Table 1, in the columns of PRRT2 mutation and Protein alternation for II-1 and II-4, they should be “NA” instead of &quot;No&quot; since they don't have the sanger sequencing data as they mentioned in the legend (Reviewer #1)</td>
<td>We very appreciate reviewer for this comment and have checked our manuscript accordingly. We have revised the points 1, 2 in revised manuscript directly. The point 3 has been revised as follow: “The novel mutant protein was located in the nucleus and might influence on the ion channels loss of function.” The point 4 revised in Figure1 and Table 1. (See the Figure1 and Table 1 in the revised manuscript);</td>
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