

Peer Review File

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Review Comments (Round 1)

Reviewer A

The author reported the individual with PIGS variants, the predicted splicing variant from mother and the frameshift variant from father. The affected boy was suffered from early onset epilepsy and severe developmental delay, and pyridoxine treatment was effective to control his seizures. It is important to accumulate the various clinical symptoms of this rare disease.

Reply: Thank you very much for your valuable comments and professional advice. Our responses to your comments as followings:

Major comments

Comment 1: I think that c.935-6C>G is 3' splice site of the intron 8. How does this variant cause skipping exon10? This variant localized at pyrimidine repeat, it is possible that it causes several kinds of abnormal splicing, such as intron 8 retention (?) etc. It is also possible that c.935-6C>G makes new AG, a splicing donor site, leading to 5 base addition which causes frameshift.

Reply: Thank you very much for your valuable comments and professional advice.

We used the rMATS software for computational analysis. Two modes were set for analysis, including the difference between the proband and the parents and the difference between the proband, the mother who carried the c.935-6C>G variant and the father who did not. Computational splice analysis of the c. 935-6C>G variant predicted two possible effects that prompted RNA studies: (1) skipping of exon 10; (2) mutually exclusive exons between exon 6 and exon 8(Supplementary data 1). In vitro RNA analysis of the boy and the parents revealed all had only one same amplicon from exon 5 to exon 9 (Supplementary data 2). Finally, the boy had two amplicons which were further verified by sanger sequence (Supplementary data 3).

The mechanism exon skipping in the proband is still unclear and we will do some work on it.

Comment 2: The author said that he used computational analysis prediction tool, what tool did you use? Please show the details of the prediction results in case of this variant.

Reply: It is rMATS software.

Changes in the text: We add the name of software in the manuscript (Page 3, L39) and submitted the prediction results (Supplementary data 1) as the supplementary data.

Comment 3: In Figure 2A, the sample from proband showed three bands, however there was a single band from his mother who had the same variant. Why? In Figure 2B, are these models based on the sequence of the bands of Figure 2A? As the forward primer localizes on the exon 9, I think the abnormal splicing variants, which I mentioned above could not be detected. The primer should be put on exon 8.

Reply: The sample from the proband had two bands and another interference band. A same interference band was very indistinct both in samples from the parents.

There was only a single band in the sample from the mother. We supposed there might be some coordination in the two variants, and it is needed to be interpreted later.

In Figure 2B, these models were based on the sequence of the bands of Figure 2A.

Thanks for your valuable advice. Based on the results of computational analysis, the primer was put on exon 9.

Comment 4: I also think that both variants would be pathogenic but the author should re-analyze the cDNA sequence of the proband together with his parents, showing details of abnormal splicing as well as decreased expression of normal transcript.

Reply: Thanks a lot for your very constructive advice. The boy had two amplicons which were further verified by sanger sequence (Supplementary data 3). The fresh blood from the family is unavailable now because the family lives in a remote city that is far away from us and the condition of COVID-19. We will do the expression study.

Minor comments

Figure 2B, as the sequence showing c.935-6C site is reverse, it is difficult to understand. Showing GGATC being the start of the exon9 would help.

Reply: Thanks a lot for your very constructive advice.

Changes in the text: We added some details in the figure 2B.

Reviewer B

The authors described a patient with a novel variant in the PIGS gene. The feature of this patient seems typical for inherited GPI deficiency syndrome. And I recognize their method confirming the pathogenicity of the novel splice variant as appropriate.

Comment 1: As a case presentation, I agree with the content of this article. I found some mistakes in scientific terms and English grammar. The authors wrote a marker of flow cytometry of FLAER as FLEAR. It is not correct and necessary to replace.

Reply: We apologize for the mistake.

Changes in the text: We have revised it in the manuscript (Page 5, L5 and L9).

Comment 2: As for grammatical mistakes, I found many lacks of space after the period and improper capitalization in the middle of the sentence. As I am not an English native, it is impossible to point out all the grammatical mistakes, but there are several sentences that I felt comfortable. I would recommend proofreading this article.

Reply: Thanks for your advice. The revised manuscript has been partly edited by the editorial. There will be the extensive language editing later.

Review Comments (Round 2)

The authors revised their manuscript. However, I found several grammatical mistakes and collocation.

Comment 1: In page 5 and line 6 to 11, it is unavoidable that they could not perform the flow cytometry, because they have no resource in their environment. Then, they have no material or information to discuss the meaning of flow cytometry from the result of their patient. Therefore, it is not suitable to discuss the meaning of flow cytometry from their patient, and it would be appropriate to remove the sentences of discussion.

Reply: Thank you for your valuable advices. We removed that paragraph.

Comment 2: In page 2 and line 41, the first sentence is interrupted with “and.” It seems that the next sentence would follow this sentence.

Reply: Sorry for that mistake. We deleted it.

Comment 3: In page 3 and line 5, interictal electroencephalographic (EEG) is adjective. It should be replaced by interictal electroencephalograph (EEG) or interictal electroencephalogram (EEG).

Reply: Thank you for your valuable advices. We revised it " electroencephalograph ".

Comment 4: In page 3 and line 17, smile usually means the facial expression of happiness silently. The authors wrote loudly. If he presented some sound or voice, it should be replaced by laughed.

Reply: Thank you for your valuable advices. We revised it as "laughed".

Comment 5: In page 3 and line 23, frontal blossing might be misspelling of frontal bossing.

Reply: Sorry for that mistake. We revised it as " frontal bossing ".

Comment 6: In page 3 and line 35, it seems the capitalization is not necessary in Junction analysis.

Reply: Sorry for that mistake. We revised it as " junction ".

Comment 7: In page 4 and line 33, aggressive is not usual adjective for seizure. I recommend it to another word.

Reply: Sorry for that mistake. We revised it as " severe ".