Author’s response to reviews

Title: Identification of gene mutations in patients with primary periodic paralysis using targeted next-generation sequencing

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Identification of gene mutations in patients with primary periodic paralysis using targeted next-generation sequencing

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Reviewer reports:

Masanori Takahashi (Reviewer 1): Luo et al. employed targeted next generation sequencing to reveal genetic cause of primary periodic paralysis in Chinese cohort. They identified several interesting variants such in OPA1 or several novel ones in SCN4A. Thus the study is potentially important but the manuscript simply describes the mutations. The manuscript would clearly benefit from some functional work.

Major

The approach of variant analysis and verification is not clear to me.

Did authors looked for mutations in 245 other genes, only if no mutation in 10 channel genes was identified? How these 10 genes were selected? Is ALG13 gene channel-related? I think there might be high possibility to find pathogenic or novel mutations in the 245 genes, if analyzed, but there is no mention. In my experience, novel mutations with unknown significance can be often encountered in large genes such as RYR1 and TTN.

Response: Thank you so much for the constructive suggestions. Apart from the 10 skeletal ion channel genes, all variants included in these 245 genes are analyzed for each participant. We analyze the variants of 245 myopathy-related genes identified in these patients and show that some giant genes like TTN, LAMA2, et al had more variants with uncertain significance (Fig.1). We are not ruling out the possibilities of the co-existence of myopathies-related causes, instead we interpret the genetic result on the basis of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. We add the interpretation process into the original Flowchart (See here Fig.2).

Fig.1 Variants with uncertain significance in 245 myopathies-related genes identified in our cohort.

Fig.2 Inclusion and diagnostic strategy for targeted NGS in patients with primary periodic paralysis

With regard to the selection criteria of these 10 ion channel genes, we included the skeletal muscle channel genes as well as central nervous system ion channel genes which are also expressed in the skeletal muscle. ALG13 gene mutations was reported in patients with Epileptic encephalopathy, early infantile, and congenital disorder of glycosylation, et al. However, we checked the omim database and found it also expressed in skeletal muscle. So we also put it into the panel.

The pathogenicity of the novel mutations are inconclusive.
The pathogenicity of the novel mutations should be investigated by co-segregation in the family, frequency of in the same ethnic cohort, and some functional analyses.

The authors mentioned segregation analysis but for which sample? If performed for unreported novel mutations, it should be clearly stated.

Response: Thanks so much for the comments and suggestions. It’s true that we should perform co-segregation analysis for patients with novel mutations. Among 8 novel variants identified in this cohort (5 SCN4A, 2 CACNA1S and 1 RYR1), we performed segregation analysis for two variants (SCN4A p.Glu36del and p.Phe1290Leu), the detailed information was listed as follows: (Tab.1). We are trying to request the parents’ biosamples for sanger sequencing, however some families refused to do so.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Co-segregation analysis</th>
<th>ACMG classification</th>
<th>Gene</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>P19</td>
<td>Yes, the father carried the same mutation, but is now paralyzed at home. He is unable to have any further EMG study or exercise test.</td>
<td>Uncertain Significance</td>
<td>SCN4A</td>
<td>c.107_109del</td>
</tr>
<tr>
<td>P20</td>
<td>Parents' biosamples are not available.</td>
<td>Likely pathogenic</td>
<td>SCN4A</td>
<td>c.121C&gt;T</td>
</tr>
<tr>
<td>P21</td>
<td>Parents' biosamples are not available. The patient responses well to the methazolamide</td>
<td>Likely pathogenic</td>
<td>SCN4A</td>
<td>c.718G&gt;A</td>
</tr>
<tr>
<td>P33/P34</td>
<td>Co-segregation analysis demonstrated the father (P33) and the son (P34) carried the same mutation and had clinical myotonia.</td>
<td>Pathogenic</td>
<td>SCN4A</td>
<td>c.3868T&gt;C</td>
</tr>
<tr>
<td>P37</td>
<td>Parents' biosamples are not available.</td>
<td>Uncertain Significance</td>
<td>SCN4A</td>
<td>c.5293G&gt;A</td>
</tr>
<tr>
<td>P39</td>
<td>Parents' biosamples are not available.</td>
<td>Uncertain Significance</td>
<td>RYR1</td>
<td>c.12428C&gt;T</td>
</tr>
<tr>
<td>P1</td>
<td>Parents' biosamples are not available.</td>
<td>Likely pathogenic</td>
<td>CACNA1S</td>
<td>c.614T&gt;A</td>
</tr>
</tbody>
</table>
Parents's biosamples are not available.

Likely pathogenic CACNA1S c.2965G>A

Tab.1 Co-segregation analysis and ACMG classification for all novel 8 variants

The authors should also provide data showing that the novel mutations identified are not present in their normal ethnic population. I have not fully checked but found existence of several variants in Asian population in ExAC database. In case of Ala1765Thr in SCN4A, the variant is present 64 in 16490 South Asian alleles.

Response: These novel mutations have been checked in 1000Genome, ExAC, ESP6500 and GnomAD database (Frequency sheet in “Novel Mutation Summary.xlsx”). According to the frequency of Ala1765Thr in SCN4A in South Asian, this variant probably is a polymorphism, but the frequency of this variant is very low in East Asian population (Please find the attached file: Novel Mutation Summary.xlsx).

I think mutation in OPA1 is of interest but some functional study is necessary to establish its pathogenicity.

Response: Yes, thanks for the comment and functional study is necessary to establish its pathogenicity. To investigate the relation between the mitochondrial defect and the ions imbalance would be of interest. We first conducted the prediction using Interpro Software, this mutation is confirmed that it is located in OPA1 Dynamin-type guanine nucleotide-binding (G) domain. The p.Ala357Thr is located in the GTPase domain adjacent to its active site, which is critical for OPA function. However, further in vitro functional analysis should be conducted.

Minor

Abstracts

Many will read only the abstract and simply believe the listed mutations as pathogenic. Thus I think the detailed information of the novel mutations should not be listed in the abstract, unless their pathogenicity was evident.


Methods
Authors stated about the analysis of CNV and RNA in muscle but there is no mention in the results.

Response: Thanks for the suggestions. We added the CNV screening results in the results section.

References

There are many references which are miss-numbered. Authors should cite original reference for the reported mutations, especially for minor mutation such as Arg1451Leu in SCN4A.

Response: We rearranged the references and added original references for SCN4A Arg1451Leu.

Discussion

Discussion should be shortened. Repetition of the description already appeared in the results should be avoided.

Response: Thanks for the suggestions. We shortened the discussion and removed the repetitive description, especially for the first paragraph in discussion section and also, page 20 last paragraph recapturing the benefits of targeted next generation sequencing.

Saïd Bendahhou (Reviewer 2): The manuscript describes a panel of 60 patients with primary PP using the NGS technique for the identification of polymorphisms in 10 chosen ion channel genes and 245 muscular dystrophy- and myopathy-related genes. This strategy led to the identification of novel variants in the SCN4A, CACNA1S, and RYR1 genes. These variants were confirmed by the classical Sanger sequencing.

The major comment:

The authors did conduct any functional study on the novel identified mutations may turn out to be benign polymorphisms. The authors may have to be more cautious describing these mutations as disease-causing mutations unless they provide clear functional data correlating the genotype to the phenotype.

Response: Thanks so much for the suggestions. To avoid misleading and misinterpretation, we removed the description of the novel variants in the abstract section.

Minors:
Figure 3A: Mutation T704M is mislocated on the cartoon. It is usually shown on the segment S5 close to the intracellular face.

Response: It’s true that SCN4A T704M is mislocated in the cartoon, we revised it.

The cartoons (Figures 3 and 4) are of poor quality and could be improved.

Response: Sorry for the low quality figures, we improved the cartoon quality accordingly. Please use "download" button to see the clear figures, if necessary I can re-upload or send them directly to the editors.

Additional files: Table 1 is hard to read (Column titles). Please modify the layout accordingly.

Response: We rearranged the format to make it more suitable for reading. Thanks for pointing out that.