Peer Review File

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Reviewer A

Comment 1: the identification of the mutation in the first place needs more description. The authors indicate exome sequencing was performed in the proband and parents, but very little detail of the analysis pipeline has been provided and there is no information on the number of reads generated per sample, the mean coverage, etc. No information has been provided on the variant filtering steps either. Was the KCNC1 p.R317S variant the only potentially pathogenic variant identified? Was the variant confirmed by Sanger sequencing or another method? This section of the study should be more transparent, and should include graphical evidence of the focus variant, e.g. IGV snapshot or similar showing coverage at that site, allele balance, etc.

Reply 1: Thanks for the comment and these information are modified in "Mitochondrial and exome sequencing" section. About 500x coverage of *KCNC1* p.R317S variant provided sufficient data to determine the zygosity state. Both proband and his mother were heterozygous. And there is no homologous sequences with homology >90% in *KCNC1* p.R317S variant located region. Unfortunately, patient has deceased, and family members did not agree to perform additional test. Therefore, we were unable to carry out validation experiments.

Changes in the text: We modified "Mitochondrial and exome sequencing" section (see Page 12, line 7) and added sequencing quality scores table and *KCNC1* p.R317S variant related figure (see Page 29, line 4).

Comment 2: Was there any evidence of mosaicism in the mother? The grandparents are described as unaffected; is their mutation status known? It would be useful to have a bit more discussion of this in the manuscript.

Reply 2: Thank you for the comment. Both proband and his mother were heterozygous based on the current data. About 500x coverage of *KCNC1* variant provided sufficient data to determine the zygosity state. (proband: 489x, mother: 529x). High-depth (>1000x) amplicon next-generation sequencing can be performed on the mother to determine whether *KCNC1* variant is mosaic. However, patient has deceased, and his family refused further testing. The patient's grandparents have not been genetically tested. However, there is no clinical symptom of seizure, short stature, intellectual disability, or gait instability in his grandparents.

Changes in the text: Family history was notable for short stature and mild gait ataxia in his mother since her childhood, although there was no history of seizure. Unfortunately, patient's mother was not able to return for the Brain MRI. His maternal grandparents were not genetically tested. However, there was no clinical signs or symptoms of seizure, gait instability, intellectual disability, or short statures in his maternal grandparents. Patient had no sibling. (see Page 11, line 8)

Comment 3: The manuscript requires proofreading as there are some language errors and other inaccuracies, e.g. first sentence of the abstract "Backgrounds: Kv 3.1 is a group of voltage-gated potassium channel encoded by the KCNC1 gene."

Reply 3: Thank you for the comment. Modification is reflected in the text.

Changes in the text: *KCNC1* encodes Kv3.1, a subunit of the Kv3 voltage-gated potassium channels. It is predominantly expressed in inhibitory GABAergic interneuron and cerebellar neurons. (See Page 2, line 1)

Comment 4: Figures 3 and 4: there is insufficient description in these figure legends.

Reply 4: The legends for figures 3 and 4 are added for better clarification.

Changes in the text: We added figures 3 and 4 legends (see Page 28, line 1).

Reviewer B

Comment 1: line 115-116. I think the sentence should read as follows: "Long-range PCR was performed to acquire the whole mitochondrial genome according to methods described in a previous publication"

Reply 1: Thanks for the comment and we modify the sentence as reviewers' advice.

Changes in the text: We modify the sentence as reviewers' advice (see Page 6, line 6).

Reviewer C

Comment 1: Although the p.R317S mutation affect one of the S4 gating charges, voltage sensitivity of the channel seems no to be affected, but are other biophysical parameters such as kinetics of activation/deactivation, voltage-dependent U-type inactivation, cumulative inactivation and/or inactivation kinetics altered? This could also explain the lower current amplitudes observed for channels containing mutant subunits.

Reply 1: The reviewer rightfully noticed that more studies need to be done to fully characterize and understand the biophysical mechanism of the loss of function of R317S variant. However, the purpose of this manuscript was to show the overall functional impact of the R317S variant in the context of functional changes reported for other disease causing KCNC1 variants that did not include detailed analysis of activation/deactivation kinetics or U-type inactivation (Muona et al., 2017, Cameron et al, 2019, Park et al., 2019). While we believe such analysis would be very informative especially in understanding the channel function, it would not change the main conclusions of our study.

Changes in the text: none.

Comment 2: Another possible explanation for lower currents through the mutant channels can be a decreased trafficking to the plasma membrane. This hypothesis must be tested to clarify the main effect of the mutation.

Reply 2: We agree with the reviewer that decreased trafficking to the surface would contribute to the reduced currents but believe such analysis should not be performed in Xenopus oocytes where the cellular trafficking is different, especially compared to the neurons, and the experiments are performed at lower or room temperatures that can also affect trafficking. It will be very interesting to see such effects in mammalian cells or neurons, but that is beyond the scope of this study.

Changes in the text: none.

Comment 3: Structural analysis of the effect of p.R317S and other related KV3.1 mutation is tested using the structure of the Shaker KV1.2-KV2.1 paddle chimera channel as model. Please, show the conservation degree of the affected amino acids and the neighboring area between KV3.1 and KV1.2-KV2.1 channel. This is necessary to accept author's interpretations regarding the structural analysis of the mutations.

Reply 3: Thanks for the comment and these information are included in "Atomic structural modeling and analysis" section. Briefly, The overall sequence similarity between Kv3.1 and Kv1.2-Kv2.1) is about 40%, and neighboring area between Kv3.1 and Kv1.2-Kv2.1 channels. The selective filter regions around p.R317 position is about 80% similarity, with the complete conservation of these key Arginine (R2-R6) residues.

Changes in the text: We modified "Atomic structural modeling and analysis" section (see Page 9, line 13).

Comment 4: Please, provide explanatory legends for figures 3 and 4.

Reply 4: The legends for figures 3 and 4 are added for better clarification.

Changes in the text: We added figures 3 and 4 legends (see Page 28, line 1).

Reviewer D

Comment 1: The authors state that the first symptoms of MEAK patients were described to be myoclonus or tremor. However, some MEAK patients first presented with GTCS or ataxia.

Reply 1: Thank you for the correction. Changes were made in the text.

Change in the text: The age onset of MEAK patients was between 3-15 years. Clinical manifestations include myoclonus, ataxia, and generalized tonic-clonic seizures. (See page 16, line 21)

Comment 2: The term "mutations" should be changed to "variants".

Reply 2: Thank you for the comment. The term “mutations” has been changed to “variants”

Change in the text: “mutations” has been changed to “variants” (see page 2, line 14, line 20)

Comment 3: Was the patient diagnosed with epileptic encephalopathy or episodic encephalopathy? Define episodic encephalopathy, if this is the case. How often/rare were his episodes? Were they dependent on any specific parameters?

Reply 3: Thank you for the comment. The patient’s clinical presentation is better defined as epileptic encephalopathy as he has known epileptic seizures which contribute to the encephalopathy. Correction was made in the text.

Change in the text: “episodic encephalopathy” has been changed to “epileptic encephalopathy” (see page 12, line 4; page 20, line 15)