Author’s response to reviews

Title: A Chinese pedigree with Brown-Vialetto-Van Laere Syndrome due to two novel mutations of SLC52A2: clinical course and response to Riboflavin

Authors:

Kaili Shi (kailihappy@126.com)
Zhen Shi (shizhen929@126.com)
Huifang Yan (yanhuifang96@163.com)
Xiaodong Wang (xdwang@ciphergene.com)
Yanling Yang (yanlingy@vip.sina.com)
Hui Xiong (xh_bjbj@163.com)
Qiang Gu (gu325q@sina.com)
Ye Wu (dryewu@263.net)
Yuwu Jiang (jiangyw@263.net)
Jingmin Wang (wang66jm@163.com)

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Author’s response to reviews:

Dear Editors,

On behalf of all the authors, I would like to say thank you for spending your time on reviewing our manuscript entitled “A Chinese pedigree with Brown-Vialetto-Van Laere Syndrome due to two novel variants of SLC52A2: clinical course and response to Riboflavin”. We followed the reviewer’s advices to revise the manuscript carefully. The revised manuscript is displayed in the form of 'Track Changes', and we will also attach the clean version. Detailed point-to-point responses are listed following the reviewer’s comments.

Editor:
1 - The manuscript requires extensive editing by a native English speaker before it can be considered further.
Answer-The manuscript was edited by a native English speaker as suggested.

2 - I suggest to use the term “variant” instead of “mutation” through the entire text, as suggested by ACMG.

Answer-We changed the term “mutation” to “variant” through the entire text.

3- Authors must describe methods used to identify the described variants. At page 6, lanes 45-48, authors say that variants were confirmed by Sanger sequencing, therefore the first screening was perhaps performed by an alternative method such as NGS (targeted panel??, exome sequencing??). Please define and at least briefly describe the method.

Answer-The content was added at page 6, lanes 9-20.
Peripheral venous blood was collected from the proband and her parents. DNA extraction was performed as previously described [10]. Whole-exome sequencing was also carried out. The sequencing libraries were prepared, and the xGen Exome Research Panel probes (IDT, USA) were used to enrich the target sequences. The enriched DNA was sequenced by Novoseq 6000 (Illumina, USA).

4- Please, within Figure 2 I suggest to add the alignment of the human protein with that of other organisms to better define the conservation of the residue 433. Is the residue involved in some particular feature/function of the protein?? Please, discuss this point.

Answer-The alignment of the human protein with that of other organisms to show the conservation of the residue 433 was added as Figure 2B, and the comment was discussed. Please see page 8, from lane 46 to the end of the paragraph:
The missense variant (c.1328G>A, p. Cys443Tyr) identified in our patient is predicted to be functionally damaged by several bioinformatics software, including SIFT, Polyphen2, and Mutation Taster. Cysteine at position 443 is evolutionarily conserved among multiple species (Figure 2B). A variant in the same amino acid of 443Cys-to-Arg was reported to be pathogenic in the study of Gahl et al. [19]. Limited information is known about the function of this region. Further studies are necessary to reveal the pathogenic mechanism of this presumably important residue.

Reviewr 1:
1. However the manuscript requires extensive editing by a native English speaker before it can be considered further.

Answer-The manuscript was edited by a native English speaker as suggested.

Reviewr 2:
1-BALASUBRAMANIEM ASHOK KUMAR, Ph.D (Reviewer 2): 1. Breath holding spells (BHS) has been recorded in the present case as one among the disease associated symptom. Authors have highlighted in the discussion part that BHS was not diagnosed in any of the BVVLS cases earlier and diagnosed first in this study. In fact, this is not correct. Nimmo et al., 2018 (Am J Med Genet. 2018; 176A:399-403) has already reported BHS in a patient, where a clinical mutation was detected in the same brain specific riboflavin transporter SLC52A2 (c.917G>A; p. Gly306Glu). Thus, authors may revise this manuscript accordingly by citing the above.

Answer-The manuscript was revised and the mentioned article was cited. Please find the revised text on page 7, lanes 39-48:
In addition, she had BHS, which was rarely reported in patients with BVVL, except in a patient with riboflavin transporter deficiency and SLC52A2 variant (c.917G>A; p. Gly306Glu); this patient had remarkable breath-holding spells since 6 months, but the evolution of the symptom and the relationship to the disease was not analyzed [12].
2-Authors are asked to check grammatical errors throughout the manuscript. The Missense variant (c.1328G>A, p.Cys443Tyr) is predicted to be functional damage by several bioinformatics software. Answer-The sentence was rewritten and other grammatical errors were checked by an English editor: The missense variant (c.1328G>A, p.Cys443Tyr) is predicted to be functionally damaged by several bioinformatics software, including SIFT, Polyphen2, and Mutation Taster.

Reviewer 3:
1-Maria Barile, Ph.D. (Reviewer 3) would have expected (preferred) some biochemical characterization of patient fluid/cell metabolic alterations. Answer-In page 4, lane 4, we wrote in previous manuscript: Serum amino acids, acylcarnitine profile, lactic acid, ammonia, hepatorenal function, microelement, and urine organic acid analysis were within normal ranges.

We described the biochemical investigations results were within normal ranges, because serum amino acids and acylcarnitine profile were basically normal, urine organic acid results only showed mildly elevated pyruvic acid and lactic acid. There were no apparent and meaningful abnormalities. In fact, biochemical investigation results varied among patients with SLC52A2 variants, the patient had slightly abnormal acylcarnitine profiles, similar to the second patient reported by Nimmo et al. (PMID: 29193829). Foley et al. reported that 10 of 17 patients with SLC52A2 variants had normal acylcarnitine profiles (PMID 24253200). We will describe the biochemical test results more objectively and accurately as following in the manuscript:

In page 5, lane 6: Ammonia, serum lactic acid, hepatorenal function, microelement, and serum amino acids were normal. Acylcarnitine profile showed mild abnormalities including mild elevation of octanoyl carnitine (C8): 0.33 μmol/L (0.01–0.30 μmol/L) and decanoyl carnitine (C10): 0.50 μmol/L(0.01–0.35 μmol/L). Other acylcarnitine species were within the normal ranges. Her urine organic acid analysis showed mildly elevated pyruvic acid and lactic acid.

Best Wishes