Author’s response to reviews

Title: A novel compound heterozygous variant identified in GLDC gene in a Chinese family with non-ketotic hyperglycinemia

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Version: 2 Date: 22 Sep 2017

Author’s response to reviews:

Dear Dr. Dalal,

Thank you for arranging a timely review for our manuscript entitled “A novel compound heterozygous mutation identified in GLDC gene in a Chinese family with non-ketotic hyperglycinemia” (ID: MGTC-D-17-00190R1). We have carefully evaluated the reviewers’ critical comments and thoughtful suggestions, responded to these suggestions point-by-point, and revised the manuscript accordingly. The main corrections in the paper and the responds to the reviewers’ comments are as flowing:

To Reviewer 1

1. Comment #1: Authors reported compound heterozygous variants in GLDC gene which includes a novel missense variant c.2680A>G and a previously reported exon 3 deletion, in a Chinese family affected with Non-ketotic hyperglycinemia (NKH). Importantly, the phenotype is consistent with mutations in GLDC gene and further delineates the mutation spectrum of this gene in NKH. The findings of the paper are well described however text (method and result sections) should be shortened and reorganized to make it more compact. The database and in silico analytical tools information and outcomes can be summarized in tabular forms and provided as a supplementary file. Figures 1, 2 and 3 should be clubbed up into one figure.
Answer: Considering the reviews’ suggestion, we have rewritten the method and the result sections to make it more compact and read more smoothly (the main corrections include: page 5, line 1-3, line 8, line 18-20; page 6, line 5-7, line 18-21; page 7, line 13). The database and in silico analytical tools information has been summarized in tabular forms and provided as additional file 2 (Table S2. Pathogenicity prediction analysis of GLDC c.2680A>G alteration). Outcomes of the siblings are illustrated in Table 1 and we believe kept a brief description in the result section will contribute to a better understanding of NKH. Meanwhile, figures 1, 2 and 3 has been integrated into one figure and shown in the figure 1.

2. Comment #2: Page 4, 2nd line statement "However, few studies refer to the genetic profile for NKH patients in China have been reported." should be supported with appropriate references.

Answer: We revised the sentence to make it more informative (Background section, page 4, line 2-6) and supplemented two corresponding references as the reviewer suggested in our revised manuscript.


3. Comment #3: Page 5, Quantitative PCR (Q-PCR) section: The methods should be described elaborately for Q-PCR (SYBR green and other reagent details if any). What is the reference gene used for normalization in 2-ΔΔCT calculation?

Answer: We thank the reviewer to raise this important issue, SYBR Green Dye was used for Q-PCR and TERT gene was chosen as endogenous control in this study, which has been further clarified in the revised manuscript (page 5, line 18-20).
4. Comment #4: If MRI pictures are available for the patients authors should provide that in the manuscript by clubbing up with figures 1, 2 and 3. If not available that should be clearly mentioned in the 3.1 Clinical data and auxiliary examination" subsection under "Results".

Answer: We have added MRI picture of the proband’s younger sister in the revised manuscript (Fig. 1b).

To Reviewer 2

1. Grammatical errors need to be corrected in multiple places preferably by a native English speaker.

Answer: This has been done by us as well as the native English professionals.

2. Some information can be presented in a more concise manner eg. mutation pathogenicity prediction can be summarised in a table.

Answer: Mutation pathogenicity prediction information has been summarized in tabular forms and provided as additional file 2.

3. Did the authors perform sequencing of all three genes followed by MLPA of GLDC? Logically, since GLDC mutations are predominant, it would make sense to do Sanger for GLDC, and as one mutation was identified followed by MLPA of GLDC to look for CNVs.

The authors need to clarify this in methods section, and suggest the best approach to molecular testing of NKH.

Answer: We would like to thank the reviewer for this important and instructive comment. Directly sequencing GLDC gene is theoretically possible, but in the currently available research works, to our knowledge, the three genes are directly and simultaneously sequenced and rarely a single GLDC gene had been sequenced. Moreover, if no mutation is found in GLDC, sequencing the other two genes would be much more troublesome. In addition, due to (1) the rapid development of the patient's condition, directly sequencing all three genes can help identify the mutant gene fastly, (2) the GCSH and AMT genes are relatively small with a limited number of
exons, thus rendering the sequencing not expensive and time-consuming, so I personally think that the ideal strategy would be to use the sanger sequencing technology to identify possible pathogenic mutations in GLDC, AMT and GCSH genes, if necessary, MLPA can be performed at the same time to detect CNV. All these statements have been addressed in the discussion section of the revised manuscript (page 9, line 5-8).

In our work, we have completed the tests for GLDC, AMT and GCSH genes, including CNV analysis which are essential for evaluating NKH. Specifically, we have performed sequencing of GLDC, AMT and GCSH genes and only a single heterozygous mutation of GLDC gene was identified. In addition, MLPA was applied to detect potential mutations in all three genes, because this SALSA® MLPA® probemix is designed to detect deletions/duplications of all three genes in a DNA sample.

We have tried our best to improve the manuscript and made some changes in the manuscript. We appreciate for your comments and suggestions, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.

Thank you and best regards.

Yours sincerely,

Qingliu Fu