Author’s response to reviews

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

Authors:
Yiming Lin (9537237@qq.com)
Zhenzhu Zheng (smilepearl@163.com)
Wenjia Sun (senwenjia@biosan.cn)
Qingliu Fu (wrightlym@sina.com)

Version: 3 Date: 22 Nov 2017

Author’s response to reviews:

Dear Dr. Menard,

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-17-00190R2). We thank both the editor and reviewer for their positive and constructive comments and suggestions. We have revised the manuscript, according to the comments and suggestions of editor and reviewers, and responded, point by point, to the comments as listed below.

To Editor Comments:

1. Please accept all of the Track Changes in the document before responding to the comments below.

Answer: Thank you very much for your kind instruction. We have accepted all of the track changes in the revised manuscript.
2. Please clarify in the Consent for publication what the patients' guardians consented to be published, e.g. medical data, images, etc.

Answer: We have clarified this in the Consent for publication section in the revised manuscript.

3. As the sequencing data has not been included in the manuscript, the current Availability of data and materials statement cannot be used for this manuscript. Please either deposit your sequencing data to a suitable repository (https://www.biomedcentral.com/getpublished/editorial-policies#availability+of+data+and+materials), or amend the statement to one of the below:

Answer: Thank you for raising this important issue and we have amended the statement. The current Availability of data and materials statement for the manuscript has been changed to “The datasets used and/or analyzed during the present study available from the corresponding author on reasonable request”.

To Elaine B Spector-Christensen (Reviewer 3):

1. What are the approximate chromosomal locations of the beginning and end of the deletion in exon 3. I do not recall if this information is available from the MLPA kit that was utilized. Was a DNA sample with a known deletion used for a control for the MLPA?

Answer: Thanks for raising these very important questions. Because of the limitations of MLPA technique, we fail to pinpoint the exact location of this deletion. However, based on MLPA probes and subsequent qPCR experiments, we identified a loss of heterozygosity for exon 3. Firstly, GLDC exon 3 is located on chr9: 6620184-6620319, the GLDC exon 3 of MLPA probe covers chr9: 6620265-6620349 and contains a partial exon 3 sequence; Secondly, the qPCR primers were designed in the upstream and downstream of exon 3, respectively, and the coverage range of PCR amplification products was chr9: 6620328-6620414 (upstream) and chr9: 6620073-6620183 (downstream), respectively. Therefore, MLPA combined with qPCR experiment can identify the entire exon 3 heterozygous deletion, i.e. the upstream breakpoint was from the MLPA probe binding site of exon 2 to qPCR upstream primer 5’ site of exon 3, and the downstream breakpoint was from qPCR downstream primer 3’ site of exon 3 to the MLPA probe binding site of exon 4 (illustrated in Additional file 3: Figure S1). According to the HGVS nomenclature, the break point can be expressed as NG_016397.1 (NM_000170.2): c. (261_335-
95) _ (470 + 111_476) del. Accordingly, we have added this information in the Discussion section of the revised manuscript (Page 9, Lines 10-16).

Furthermore, due to NKH disease, especially heterozygous GLDC exon 3 loss is very rare, there is no corresponding positive control, so MLPA experiments only with normal samples as control group. But at the same time we conducted qPCR experiments to ensure the accuracy of the detection.

2. The ACMG/AMP 2015 Standards and Guidelines for the interpretation of sequence variants strongly discourages the use of the word mutation. It should be substituted by sequence variant or pathogenic variant etc. I will leave this up to the editors.

Answer: Thanks for your thoughtful suggestion. We have replaced the word mutation to variant in the revised manuscript as the reviewer suggested.

3. Please use the 3 letter amino acid code when describing the sequence variant. Some editing of the manuscript is still needed.

Answer: We have paid attention to this issue, and “T894A” has been corrected as “p.Thr894Ala” throughout the text in the revised manuscript.

4. What is this referring to? NKH??

Answer: Yes, we replaced the sentence as “it is possible that NKH is much more prevalent in China than it has been realized” to avoid ambiguity (Page 4, Lines 2-3).

5. Did they use a positive control. This assay is quite variable.

Answer: Due to NKH disease, especially heterozygous GLDC exon 3 loss is very rare, there is no corresponding positive control, so MLPA experiments only with normal samples as control
group. But at the same time we conducted qPCR experiments to ensure the accuracy of the detection.

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

Best regards.

Yours sincerely,

Qingliu Fu