Author's response to reviews

Title: Prenatal Diagnosis Using Genetic Sequencing and Identification of a Novel Mutation in MMACHC

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Title: Identification of a Novel Mutation in MMACHC and Development of a New Prenatal Diagnostic Technique Using Genetic Sequencing

Response to Reviewer’s comments
Dear Dr. Tim Sands,

We thank you and the Journal for considering our work for publication. We answered to all issues raised by the Reviewer. We provide a point-by-point response below.

Major compulsory revisions
• Please revise the title to something like: “Prenatal diagnosis using genetic sequencing and identification of a novel mutation in MMACHC” instead of “….Development of a New Prenatal Diagnostic Technique Using Genetic Sequencing”.

Answer: I have revised the title to “Prenatal diagnosis using genetic sequencing and identification of a novel mutation in MMACHC”, as the reviewer recommended to make it more accurate and concise. As the reviewer pointed out, the technique used in our study was standard methodology for chorionic villi sample-based prenatal genetic diagnosis. It is not a new prenatal diagnostic technique either in China or in other countries. Although there is no report of prenatal diagnosis for cblC deficiency through a simple genetic diagnosis in China.

• Clinical information is limited. Unless further clarified the description “some patients also had acidosis, urine ketone positive, elevated lactate and ammonia” would better be removed, as it raises questions to the readers of the manuscript, unless more information can be added on table 2 or else in the paper.

Answer: I have removed the description “some patients also had acidosis, urine ketone positive, elevated lactate and ammonia”. Although we made a follow-up through the telephone, some details were still inexact. [Page.4, Paragraphs2, Line 3]

• Given the number of normal controls screened was small, additional information on the frequency of the mutations from whole exome sequencing databases, such as the Exome Variant Server or ExAC browser should be provided.

Answer: We have searched the HGMD database, Exome Variant Server and ExAC browser. The G155R mutation of MMACHC gene has not been reported before in any of the databases, thus it is considered to be a novel mutation. Besides the frequency of some known mutations were as follows, c.394C>T, f=0.00009951, c.609G>A, f=0.00003312, c.658~660delAAG, f=0.00004143 etc. and c.321G>A (V107V) was a synonymous mutation with the frequency of 0.4664 on the ExAC browser. [Page.7, Paragraphs3, Line 1-2]
Minor Essential revisions:

• Subtypes of combined methylmalonic acidemia and homocystinuria include: MMACHC (cblC), MMADHC (cblD combined, cblD-MMA and cblD-HCY), LMBRD1 (cblF), and ABCD4 (cblJ) – please describe appropriately.

  Answer: I have rewritten the classification of methylmalonic acidemia and combined methylmalonic acidemia and homocystinuria. MMA can be classified as isolated methylmalonic acidemia and combined methylmalonic acidemia and hyperhomocysteinemia based on biochemical features. Combined methylmalonic aciduria and homocystinuria consists of four subtypes, MMACHC(cblC), MMADHC (cblD combined, cblD-MMA and cblD-HCY), LMBRD1(cblF) and ABCD4(cblJ).

• Italicize gene names as well as cobalamin subtype (cblC, D, J, F) names throughout the manuscript.

  Answer: I have revised the written form of gene names and cobalamin subtypes throughout the manuscript.

• cblC deficiency should not be referred to as MMA in the document (see conclusion in the abstract etc). Please replace with combined methylmalonic acidemia and homocystinemia or cblC deficiency.

  Answer: I have replaced “MMA” in the conclusion of the abstract and other parts of the document with cblC deficiency to make it more concise.

• Rephrase the sentence: “Although partial MMA is a treatable genetic disease, both the high mortality during…”. It is unclear what the authors refer to by “partial MMA”, may want to re-phrase to “MMA is a partially treatable disorder”.

  Answer: I have revised the sentence to “Although MMA is a partially treatable disorder, both the high mortality during the acute phase and the chronic damage to the nervous system, will lower the quality of patient’s life and increase the family economic burden.” to make it more clear and read more smoothly.

• Indicate amino acid change and numbers in Figure 2.

  Answer: I have added information of amino acid change and numbers in Figure 2. Thank you very much for your suggestion.

• Patient 6 is reported as having normal HCY and MMA at follow-up – this would be
particularly unusual for patients with cblC disease, please clarify.

**Answer:** I have clarified the follow-up outcome of patient 6 in the revised manuscript,[ Page11,Paragraphs2], as follows.

In general, late-onset patients have better survival and response to treatment compared with early-onset patients. As an early-onset patient, patient 6 in this study had been given treatment with hydroxycobalamin, carnitine, betanine and folinic acid, as well as rehabilitation programs promptly after diagnosis. During follow-up at 3 years old, we found that his urine levels of MMA decreased to normal and plasma levels of homocystenuria decreased to 10.6$\mu$mol/l. The result confirmed the importance of early intervention of the disease.

- **Add age at follow-up before the clinical details**
  
  **Answer:** I have added information of the patients’ age at follow-up in table 2 before the clinical details.

- **On patient 8 in table 2, “Recovered well after rehabilitation and taking medicine” – please rephrase or clarify.**
  
  **Answer:** I have revised the sentence to “taking medicine promptly and properly for a period of time, the patient has being in good recovery.” in table 2

- **Comment on prenatal treatment of the mothers with B12 in the confirmed MMACHC affected fetuses.**
  
  **Answer:** I have added the sentence “Prenatal OHCbl administration may reduce the maternal metabolites, however the complications of cbl deficiency such as retinopathy could not be ameliorated.” [Page11,Paragraphs3,Line 8-9]

Best regards,
Dr.Kong XD