Author's response to reviews

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

Authors:

Xiang Dong Kong (kongxd@263.net)  
Ning Liu (crystalningxin@163.com)  
Qinghua Wu (yangyx85@126.com)  
Zhenhua Zhao (princesszzh@163.com)

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Author's response to reviews: see over
Dear editors and reviewers,

We greatly appreciate the time and effort you’ve spent in reviewing our manuscript entitled “Identification of a Novel Mutation in MMACHC and Development of a New Prenatal Diagnostic Technique Using Genetic Sequencing”. (MS: 4336040571443951) We have studied comments carefully and have made revisions. The main revisions in the new manuscript are:

EDITORIAL REQUIREMENTS:

- Please include the email address of all authors in the title page.

We have added the email address of all authors in the title page. It must be pointed out that we revised the authors after reevaluated the contribution of each author to this paper. Zong Yanan is submitted as the first author because she participated in the design and coordination of the study, followed up of the patients and helped to draft the manuscript. Wu Qinghua was removed considering that her efforts to draft this manuscript was limited. Wu Qinghua has agreed with this modification. We are really sorry for this mistake.

The answers to the questions of reviewer #1:

1. Change HC to HCY in all the document
   We have changed HC to HCY in all the document.

2. Include cblJ (MIM 603214) in line 50 and change to 9 subtypes.
   I searched the literatures and revised that there are 9 subtypes of defects in cellular cobalamin metabolism (complementation groups cblA-cblJ). [Page.3, Paragraphs 3, Line 7-8]
   Reference article: [2] Nuria CC, David A, Charles PV. Disorders of intracellular cobalamin metabolism. Gene Reviews [Internet].

3. Change GC-detection to organic acid levels or MMA levels and define the kind of specimen used (urine or plasma), in all the document.
   I have rewritten the sentences in [Page.5, Paragraphs 3, Line 2-4] and [Page.10, Paragraphs 1, Line 1-2] as follows:
   Urinary organic acid analysed by gas chromatography/mass spectrometry (GC-MS, QP2010, Schimadzu, Japan) showed high levels of methylmalonic acid.
   Organic acid levels of urine and serum total homocysteine of the two newborns were normal at 1
month old.

4. Line 146. Change serum homocysteine detection to serum total homocysteine quantification. Correct this matter also in line 188.
I have rewritten the sentences as follows:
Organic acid levels of urine and serum total homocysteine were detected for two newborns at 1 month old. [Page.8,Paragraphs 4,Line 2]
Organic acid levels of urine and serum total homocysteine of the two newborns were normal at 1 month old. [Page.10,Paragraphs 1,Line 1-2]

5. Line 169. Provide more information of the results of these analysis if possible.
Besides the missense variants p.G155R was considered putative pathogenic mutations because PolyPhen2 predicted that the functional consequences is probably damaging(score of 1.000). In addition, the PROVEAN scores were −7.167(cutoff: −2.5). [Page.9,Paragraphs 1,Line 1-4]

6. Lines 189-190. The last sentence is not necessary.
The sentence “One year later, telephone follow-up confirmed that the babies were healthy.” is removed. [Page.10,Paragraphs 1]

7. Line 198. Change "to have a risk of disease" to "to have the disease"
I have revised this English mistake. [Page.10,Paragraphs 2,Line 6]

8. Line 208. Change delayed to late-onset type.
I have revised this word. [Page.10,Paragraphs 3,Line 9-10]

9. Line 171. Change the title to "Prenatal diagnosis of MMACHC gene and follow-up"
The sub-heading of this section is changed into "Prenatal diagnosis of MMACHC gene and follow-up". [Page.8, the sub-heading]

10. Lines 134-142. Specify the time in which genetic-sequencing-based prenatal diagnosis was carried out.
The genetic-sequence-based prenatal diagnosis will be carried out within 1 week. [Page.7, Paragraphs 3, Line 9-10]

11. Line 192. Discuss the advantages of this technique vs the biochemical prenatal diagnosis.
The published articles concerning the prenatal diagnosis of MMA are not many[17]. There seems to be a lack of consensus on the most reliable method, and preferred sample on which to conduct these investigations. Mutation analysis is a useful method for prenatal diagnosis. If molecular genetic testing is not possible, prenatal testing for pregnancies of intracellular cobalamin metabolism is possible by a combination of complementation analysis of cultured amniocytes and measurement of MMA and HCY concentration in amniotic fluid using mass spectrometric techniques. But it is reported that the results could be false negative/positive[2,17]. So it is recommended to perform prenatal diagnosis by two independent methods. Our success with this
Technique of \textit{MMACHC} genetic diagnosis shows that genetic-sequence-based prenatal diagnosis is accurate and economical. It could also be an effective genetic screening method for affected families. But the information of the proband or the parents must be available, and maternal contamination of the DNA sample should be excluded by marker analysis. Preimplantation genetic diagnosis could be considered for the families whose pathogenic mutation have been identified. The result will be available within 10 workdays. Although the metabolic analysis of amniotic fluid will be carried out simply, the molecular genetic testing is the best method to make prenatal diagnose in the first trimester, since amniocentesis shouldn’t be performed before the 16th gestational week to avoid complications.

References:

The answers to the questions of reviewer #2:
Thank you for all your suggestions.

Major Revisions:
1. A. the methodology and conclusions lack novelty
   To our knowledge, there is no report of prenatal diagnosis through a simple genetic diagnosis in China. In our study, \textit{MMACHC} mutations were analyzed in 10 families with a birth history of children diagnosed with combined MMA and HCY (cblC type). A novel mutation, c.463G>C (G155R), was found. We also performed genetic-sequencing–based prenatal diagnosis in 3 families. Our success with this technique of \textit{MMACHC} genetic diagnosis shows that genetic-sequence-based prenatal diagnosis is accurate and economical. It could also be an effective genetic screening method for affected families.

   B. failed to mention the previously described c.464G>A, p.G155E mutation
   Another type of mutation has been previously reported as pathogenic at the same codon, c.464G>A, p.G155E[14]. Comparative analysis of the amino acid sequence of MMACHC from human, chimpanzee, zebrafish, rodents, and lizards was performed, and we found that the site G155 is highly conserved between various species (Fig. 2) indicating it might be essential for the normal function of MMACHC. [Page.8, Paragraphs 4, Line 2-3]


   C. The application of sanger sequencing and CVS-chronic villus sampling
   The technology application in the prenatal setting is not novel in the center of prenatal diagnosis in
the first affiliated hospital of Zhengzhou University. We have performed invasive prenatal
diagnosis for pregnant women since 2011 in our center, mainly for those women who were
high-risk for single gene diseases. The use of Sanger sequencing for clinical diagnosis has already
become an mature technology in the field of genetic diseases. We carried out every test
conscientiously according to the standard protocol.

2. A. I have revised the letter “translocase” to “mutase”.
   B. The number of mutations in the MMACHC gene has been retrieved on HGMD(human
genetic mutation database). I revised “about 50 mutations” to “77 mutations” in [Page.4,
Paragraphs 1,Line 2] and [Page.10,Paragraphs 3,Line 4]
   C. About MMA is treatable
   The sentence “Although MMA is a treatable genetic disease, both the high mortality during the
acute phase and the chronic damage to the nervous system, which causes a serious reduction in
children's quality of life and increased mortality rate, place an enormous burden on families. ” is
replaced by the sentence “Although partial MMA is a treatable genetic disease, both the high
mortality during the acute phase and the chronic damage to the nervous system, which causes a
serious reduction in children's quality of life and increased mortality rate, place an enormous
burden on families. ” [Page.4, Paragraphs 2,Line 3-5]

3. The phenotype information provided is very limited.
   The clinical information of our study is complemented after collection and analysis of the
patients’ materials. We also make a follow-up through the telephone. However in pedigree 4, 5 and
9, the probands were diagnosed at other hospitals, and we could not get their medical records. And
all of these 3 probands died under 1 years old, at least 1.5 years ago, so it’s hard for their parents
to provide any detail information about the disease progression and eye involvement. There are 4
probands suffered with ocular impairment in different levels. In pedigree 2 and 8 the parents said
that there were not any ocular abnormalities with the probands without any test by the doctor. The
revised Table 2 is provided at the end of the letter.

Minor Revisions:
The term odinopoeia is replaced by termination of pregnancy (TOP).
Figure 1 legend is added an arrow in red color.

We hope the Reviewers and the Editors will be satisfied with the revisions for the original
manuscript.
Thanks and Best regards!
Yours Sincerely,
Kong Xiangdong & Zong Yanan
2015-02-15
<table>
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<th>No.</th>
<th>Proband</th>
<th>Mutation Maternal</th>
<th>Mutation Paternal</th>
<th>Onset age</th>
<th>Clinical data</th>
<th>Follow-up</th>
<th>Fetus Genotype</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HC (µM)</td>
<td>MMA (µM)</td>
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<tr>
<td>1*#</td>
<td>/</td>
<td>220delK</td>
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<td>W203X</td>
<td>R132X</td>
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<td>G155R</td>
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<td>c.567dupT</td>
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<td>/</td>
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<td>220del K</td>
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<td></td>
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<tr>
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<td>/</td>
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<td>R206W</td>
<td>W203X</td>
<td>40 days</td>
<td>158</td>
<td>45.1</td>
<td>nystagmus developmental delay. HC, MMAare normal</td>
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<tr>
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<td>220delK</td>
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<td>8*</td>
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<td>W203X</td>
<td>W203X</td>
<td>2 weeks</td>
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<td>215.2</td>
<td>Normal^ Recovered well after rehabilitation and taking medicine</td>
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<td>W203X</td>
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<td>W203X</td>
<td>W203X</td>
<td>3 mon -ths</td>
<td>108</td>
<td>29.4</td>
<td>Can’t raise his head now(6months old), developmental delay</td>
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