Author’s response to reviews

Title: Identification of a novel splicing mutation in the SLC25A13 gene from a patient with NICCD: a case report

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Linlin Zhang; Yingying Li; Wenli Shi; Jinshuang Gao; Yuan Tian; Ying Li; Yaqing Guo; Shihong Cui, M.D.
BMC Pediatrics

Dear Editor:

We appreciated your interest in our manuscript, and providing us an opportunity to revise it. The reviewers' thoughtful suggestions are highly appreciated. As requested, we revised our manuscript in response to the reviewers’ comments. Our point-by-point response to the reviewers’ comments can be found below.

We wish that you will now find our revised manuscript suitable for publication in BMC Pediatrics. Thank you in advance for your kind consideration of this paper.

Sincerely yours

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Reviewer reports:

Akella Radha Rama Devi Akella (Reviewer 1):

How do you interpret the high α-fetoprotein level in the child?
Reply: In a retrospective study of liver function and islet beta cell functions for 36 patients with NICCD and 50 control individuals (Reference 10) indicates that the level of alpha-fetoprotein is significantly higher in NICCD group, as well as total bilirubin (TBIL), direct bilirubin (DBIL), alanine aminotransferase (ALT), aspartate amino transferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP). We cited this article and described these clinical features related to liver dysfunction in our revised manuscript.

Plasma amino acids and urine organic acid values are required.
Reply: We apologize that plasma amino acids and urine organic acid analysis were not performed since the HPLC test is not a routine test in our clinical laboratory. However, we believed that clinical features revealed in this patient was quite enough for differential diagnosis, which we described in a new paragraph in the revised version. Furthermore, the result of genetic test supported the diagnosis.

Duangrurdee Wattanasirichaigoon, M.D. (Reviewer 2): Dear the Editor of BMC Pediatrics

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The authors described a single case with mild jaundice. By using Next Generation Sequencing and gene panel of 137 known gene for cholestatic-typed liver diseases, the authors were able to identify a known pathogenic (c.852_855delTATG) and novel (c.1841+3_1841+4delAA) mutation of SLC25A13 gene, leading to definite diagnosis of NICCD in the patient.
Overall, the paper adds a novel mutation of SLC25A13 gene but does not provide in depth knowledge about their finding.
In addition, I had a chance to review this manuscript when it was submitted in March 2019. I found that some of my comment have not been responded appropriately as shown below.

Major comments
1) Case presentation:
   - There is not enough information about differential diagnosis based on clinical presentation. Though we know that it is impossible sometime to give a diagnosis based on clinical data, but this is a single case report so the author should provide intellectual exercise what were potential etiologic diagnosis before jumping into genetic analysis.

   - Though plasma amino acids (PAA) analysis was not performed, however PAA is not essential for confirmation of diagnosis. There are still several possible conditions that should be included in differential diagnosis.
Reply: We sincerely apologize that we didn’t respond your comments appropriately. We believe that these two comments are both correlated with the process of differential diagnosis. A new paragraph was added in the revised manuscript to describe the differential diagnosis. We hope this new paragraph could explain why we diagnose this patient as NICCD with the help of genetic test.
- The author did not explain their finding well enough, such as, Page 6, line 56: "...which is predicted to probably affect RNA splicing by HSF (Supplementary Figure 1)". I would expect the author to provide how the novel mutation affect splice site, such as, the change/loss of splice score, how the mutant RNA sequence would be like and how that affect the protein. Thought it is not practical to perform RNA and Western blot analysis to confirm the effect of the mutation at RNA and protein level for new mutation of SLC25A13 because the gene is exclusively expressed in liver tissue and there is multiple alternative splicing in the peripheral blood, at least the author should try to explain their new finding.
Reply: In this case, it seems to be impossible to confirm the effect of the mutation at RNA and protein level with experimental methods. The human splicing finder (version 3.1) is one of the most widely used bioinformatical tools of analyzing splicing-site mutations, so we predicted the effect of the mutation, and provided detailed results in Supplementary Table 2 in the revised version. However, it is beyond our knowledge as clinician and laboratorian to find other tools since the relevant case reports are limited.

- According to the GenBank database and supplementary figure 1, the novel mutation identified should be c.1841+3_1841+4delAA (though the alternate name is NC_000007.13:g.95750964delTT).
Reply: The name of mutation had been corrected in the revised manuscript.

2) Tables and Figures
- Table 2 and Figure 1 could be present as supplemental data
Reply: Table 2 and Figure 1 are removed in the revised version and provided as supplemental data.