Author’s response to reviews

Title: Novel compound heterozygote mutations of TJP2 in a Chinese child with progressive cholestatic liver disease

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Author’s response to reviews:

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Prof. Gunadi

Editor

BMC Infectious Diseases

Dear Prof. Gunadi,

Many thanks for the letter dated November 30, 2018, regarding our manuscript entitled “Novel compound heterozygote mutations of TJP2 in a Chinese child with progressive cholestatic liver disease” (Manuscript ID MGTC-D-18-00415). We appreciate the valuable comments of the editor and reviewers. Based on the editor and reviewers’ comments and suggestions, we have revised the manuscript. Our specific point-by-point responses to the editor and reviewers’ comments and suggestions are as follows:
Editor Comments:

1. Please provide the informed consent signed by both parents.

Response: We thank for the valuable comments from the editor, and we have provided the informed consent signed by both parents of the subject (see supplementary file: Written informed consent).

2. Please provide Sanger sequencing as confirmation of NGS findings for both variants for patient, parents, and control without variant.

Response: We have provided Sanger sequencing as confirmation of NGS findings for both variants for patient, parents, and control without variant in the revised manuscript (see Figure 2).

Teguh Haryo Sasongko (Reviewer 1):

1. The conclusion offered suggest a good addition to clinical decision making. However, the current content of the manuscript does not substantiate the conclusion. I would suggest the authors to provide a review of previous PFIC cases with TJP2 mutations. The review needs to answer if there is a pattern in the patients' clinical features to lead clinicians for TJP2 mutations.

Response: We thank for the valuable comments from the reviewer 1. We have provided a review of PFIC cases with TJP2 mutations in the Discussion part (see page 8, line 161-175). It was shown that clinical variability exists in patients having mutations in TJP2, such as different age onset, and normal or high levels of GGT.

2. Description of methodologies is especially lacking. I understand that an panel of 396 hereditary liver diseases genes were used. First, it is unclear if this is something that was developed/proposed by the authors or something which is already established. If it is the latter, there is no need to put the supplementary table and a proper citation would suffice. Second, there was no description of molecular workup (eg. from which tissue was the DNA extracted, brief information on the library prep, etc). Third, a brief but sufficient detail of the bioinformatics pipeline used is needed.

Response: We have provided a description of methodologies (see page 5-6, line 99-121). The gene panel of 396 genes was developed by the authors based on OMIM (Online Mendelian
Inheritance in Man, [http://omim.org](http://omim.org), a comprehensive, authoritative and timely research resource of curated descriptions of human genes and phenotypes and the relationships between them. In addition, we have provided a description of molecular workup, as well as bioinformatics pipeline in the revised manuscript (see page 5-6, line 107-120).

3. I suggest to validate the mutation findings with Sanger sequencing.

Response: We have validated the mutation findings with Sanger sequencing (see Figure 2).

4. I wish to see the authors argue the value of the effort for doing NGS, given the cost and labor especially bioinformatics workup. Were the clinical features obtained not enough in directing the researchers to do more specific gene test? This needs to be discussed vis-a-vis clinical decision making.

Response: Given the decreasing cost of NGS, gene panel testing by NGS is available for clinical diagnosis in both hospital and company based genetic diagnostic centers in China. In addition, well-designed software is available for bioinformatics analysis. Many mutated genes important in the pathogenesis of genetic liver diseases were reported in recent years. In this study, the patient presented with a long history of jaundice, and a lot of gene mutations were reported involving in neonatal jaundice. For accurate and fast diagnosis, we used a gene panel of 396 genes related with liver diseases, including PFIC, Wilson's disease, liver failure, and metabolic liver diseases. Based on our clinical experiences, gene panel testing by NGS is good for differential diagnosis of complex genetic disorders.

Emma Andersson, PhD (Reviewer 2):

1. The authors suggest that the splice site disruption and frame shift mutation both abolish translation. It would be interesting to show from cells, or biopsies, or in overexpression experiments, that these mutations indeed abolish translation (western blot, compared to wild type TJP2 translation). Perhaps this is beyond the scope of a publication in BMC Genetics, from what I can see of other case reports, but I think it would strengthen the claims substantially.

Response: We thank for the valuable comments of reviewer 2 and fully agree with this important concern. It is very important to investigate the function of these TJP2 mutations in the future.
2. It is unclear whether informed consent was provided by one of two parents/guardians (line 169 and line 173), or whether there is only one parent/guardian that is relevant. This should be clarified and ensure that the study complies with all applicable ethics guidelines.

Response: Written informed consent was obtained from both parents of the subjects (see supplementary file: Written informed consent). Our study complied with all applicable ethics guidelines.

3. Availability of data: "available upon reasonable request". This is unfortunately often used as a phrase to avoid sharing data (at least has been with other groups). The sequencing of the other genes should be available upon request, or deposited.

Response: We have replaced the sentence with “The sequencing of the other genes should be available upon request” (see page 10, line 214).

4. I could not find a methods section. - what sample was sequenced (blood, saliva.. other?)

More information on the targeted sequencing would be helpful (see especially comments for line 95).

Response: Peripheral blood samples were used for gene sequencing, and we have provided more information about the genetic testing (see page 5-6, line 99-121).

5. Moderate comments:

Line 79 The imaging of the patient's condition could be put into a figure, especially the biliary obstruction should be represented.

Response: We fully agree with this important concern. The patient was confirmed as biliary obstruction with radionuclide (99Tcm) hepatobiliary imaging in Nanjing Drum Tower Hospital before admitted to our center. Thus, we didn’t repeat the radionuclide hepatobiliary imaging. A scanned picture of the radionuclide hepatobiliary imaging was obtained (see supplementary image for review only). The results of radionuclide hepatobiliary imaging showed that no biliary and intestinal tract were seen from 30 minutes to 24 hours in both anterior (ANT) and posterior (POST) position of the subject.

Line 82: It could be mentioned that the biochemical profile discussed is at 23 months of age

Response: We have added the age information (see page 4, line 82).
Line 92 should the treatment not be more specifically described? (doses?)

Response: We have added doses of the drugs in the revised manuscript (see page 5, line 92-95).

Line 95: How was the selection of the 396 genes done? MYO5B is missing from the list, while it is also described as a mutation observed in low GGT cholestasis (Qiu, Hepatology 2017)

Response: The gene panel of 396 genes was developed by the authors based on OMIM (Online Mendelian Inheritance in Man, http://omim.org), a comprehensive, authoritative and timely research resource of curated descriptions of human genes and phenotypes and the relationships between them (see page 5, line 99-102). While MYO5B gene is not included in OMIM at present, our gene testing covered MYO5B gene, and no mutation was found in MYO5B gene.

Also, why is the table listing genes with mRNA accession numbers rather than gene IDs? Does this mean that genes with different transcript variants were assessed for only one transcript variant?

Response: In supplementary Table 1, mRNA accession numbers were for references only. Sequence variants were annotated using population and literature databases including 1000 Genomes, dbSNP, GnomAD, Clinvar, HGMD and OMIM. Several online softwares were used to analyze the structure of the protein, predict the conservation domain, function domain and perform the multiple sequence alignment. Variants interpretation was manipulated according to the American College of Medical Genetics (ACMG) guidelines (see page 5-6, line 107-120).

Line 100 Figure 1 is of poor quality and should be replaced by a figure of higher resolution

Response: We have replaced Figure 1 by a figure of high resolution (see Figure 1).

Line 111 of note: Byler's disease is specifically referring to PFIC1

Response: We have deleted “Byler's disease” in the first sentence of discussion.

6. Minor comments:

In general, the English language could be improved in this manuscript.
decades
which WERE inherited
neither has instead of none have
"disorders THAT are characterized"
leads RAPIDLY to end stage liver disease in untreated…
rephrase
remove of
identified AS responsible
"heterozygous mutations"
ARE described
"a special face" by particular facial features
PROTRUDING instead of FORWARD
"since the age of 6 months"
when hospitalized
"No signs were found that the lungs and heart are affected" (the phrase "no positive signs were found" is very ambiguous)
"showed a normal thoracic spine"
replace "given" by treated
"damaging", could be replaced by detrimental
"NR1H4, which linked to PFIC, were…"
"Elevated levels of serum bile acid AND…"
"when admitted at the age of 23 months."
"patients, which WERE…"
"It was shown that"
"that" by "to be"
Line 142 "causING PFIC 4"

Line 143: use "neither has" instead of "none have"

Line 145: "Animal studies have shown that loss of Zo-2 protein led to early…"

Line 147: Use treated instead of administrated

Line 150 "AS TJP2 mutations may…"

Line 151 "of HCC, patients should be monitored closely…"

Line 152 replace "firstly" by "for the first time"

Line 169, Line 173 I believe it should state "parentS" (or is there only one caretaker? In either case, it is important to ascertain whether only one parent gave consent? - If there are two parents, I imagine both should give consent)

Response: We appreciate these valuable comments, and we have improved the language writing of revised manuscript according to the comments of reviewer 2.

We hope that our revised manuscript is now suitable for publication in BMC Medical Genetics.

Sincerely Yours,

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