Author’s response to reviews

Title: Three novel mutations in the ATP7B gene of unrelated Vietnamese patients with Wilson disease

Authors:
Nguyen Huy Hoang (nhhoang@igr.ac.vn)
Nguyen Huong (nguyenmaihuong@nch.org.vn)
Nguyen Lien (ntkimlienibt@gmail.com)
Ngo Ngoc (ngodiemngoc@gmail.com)
Nguyen Mai (nguyenphuongmai@nch.org.vn)
Nguyen Hoa (dranhhoa@gmail.com)
Le Hai (hailt@nhp.org.vn)
Phan Chi (pvchi@yahoo.com)
Ta Van (tathanhvan@hmu.edu.vn)
Tran Khanh (Khanhvan7364@gmail.com)

Version: 2 Date: 18 Apr 2018

Author’s response to reviews:

Re: Revisions for manuscript MGTC-D-18-00005R1

April 6, 2018

Dear Prof. Editors,

Editor of BMC Medical Genetics
Thank you for your comments. We have revised the manuscript as the Editor and Reviewer suggested. It is our sincere hope that this manuscript will be accepted for publication in BMC Medical Genetics.

Please find enclosed the revised version of our manuscript MGTC-D-18-00005R1. According to the highly valuable comments of the reviewer we considerably modified the manuscript:

Technical Comments:

Please provide a CARE Checklist.

This information has been added as an attachment file.

Editor Comments:

- Please use the HGVS nomenclature for describing mutations and add a transcript/protein reference used for annotating each mutation.

- Check for grammar and language errors.

- Use the correct disease name according to OMIM (#277900)

Thank you so much. We have revised the manuscript as the Editor suggested. We also checked and showed that OMIM (#277900) was used as Phenotype MIM number for Wilson disease and OMIM (#606882) was used as Gene/Locus MIM number for ATP7B gene which is the cause of Wilson disease. So we would like to use OMIM (#277900) for Wilson disease.

Reviewer reports:

Panieh Terraf (Reviewer 1): The case report describes three patients from three unrelated Vietnamese families with Wilson's disease. They report 5 mutations in the ATP7B gene, which according to authors three are novel.
Comments and Suggestions:

- Authors present His251Alafs*19 in exon 2 as a novel mutation. However, this mutation has previously been reported in a south Asian family. Please refer to gnomad.broadinstitute.org

Thank you so much for your comment. We checked this mutation in gnomad.broadinstitute.org website as suggested by the reviewer. The Genome Aggregation Database (gnomAD), is a coalition of investigators seeking to aggregate and harmonize exome and genome sequencing data from a variety of large-scale sequencing projects, and to make summary data available for the wider scientific community. However, this mutation have not been updated yet in http://www.hgmd.cf.ac.uk/ac/gene.php?gene=ATP7B. This mutation have not been published yet in any paper about Wilson disease or the patients with Wilson disease and have not been proven yet to be related to this disease. So, in this paper we reported this mutation as a novel mutation in Vietnamese patient with Wilson disease.

- I would suggest for authors to include pictures of patient's eyes pointing out the Kayser-Fleischer rings.

We have been added information as suggested by the reviewer.

- The normal range for serum ceruloplasmin is between 20-35 and not <20 as stated by authors. So please revise throughout paper and in table 1.

We have been corrected information as suggested by the reviewer.

- It would be best if authors changed the normal 24h urinary copper from <100 to <60 since this is more commonly reported. Please change throughout manuscript and in table 1.

We have been corrected information as suggested by the reviewer.

- PT stands for Prothrombin Time. Please revise throughout paper and change "prothromlin time" to "prothrombin time".

We have been corrected this word as suggested by the reviewer.
- In clinical features, authors should explain the term "insensible".

We want to describe the symptom of numbness of hands and feet in patient. However, this word maybe unknown meaning so we would like to replace “insensible” by “numbness of hands and feet”.

- The manuscript needs English proofreading. There are many grammatical errors throughout the paper.

Thank you so much. We have been corrected all of these mistake in the manuscript.

- In line 5 of page 2, please change "unrealed" to "unrelated".

We have been corrected this word as suggested by the reviewer.

- In line 3 of page 4 authors might consider revising the sentence "will be helpful in developing effective treatment" to "will be helpful in efficiently diagnosing Wilson's disease and early therapeutic intervention for patients."

This sentence have been rewritten as suggested by the reviewer.

- Line 1, page 5 "QIAamp DNA Blood Mini preparation kit" should be changed to "QIAamp DNA Blood Midi preparation kit".

We have been corrected this word as suggested by the reviewer.

- In Line 21, page 6 the sentence is not very clear. Consider changing to " The genetic analysis in his mother showed that she had a heterozygous mutation in exon 14 (Phe1026Tyr) but did not have the mutation in intron 12".

This sentence have been rewritten as suggested by the reviewer.

- The mutation in intron 12 is known to affect the correct splicing of ATP7B mRNA not the incorrect splicing. Please revise in line 14 of page 7.

This sentence have been rewritten as suggested by the reviewer.
- Line 23 and 24 of page 7 are repeated in line 14 and 15 of the same page. There is no significance for that. Please only include one or the other.

The sentence in line 23 and 24 of page 7 have been deleted as suggested by the reviewer.

- I think it would be good to change the phrase "located in the shear zone, which is affect mRNA splicing" of line 9 in page 8 to "located in the splice site, which can affect mRNA splicing".

This sentence have been rewritten as suggested by the reviewer.

- Line 16, page 9 the author's name is misspelled. Please change to Kalach et al.

We have been corrected this word as suggested by the reviewer.

- Line 17, page 9 please change to "acute liver failure".

We have been corrected this word as suggested by the reviewer.

- Line 17, page 9 the sentence is vague and unclear. Please consider changing to "Yi et al. (2012) who established neural and hepatic differentiation platforms of patient-derived induced pluripotent stem cells, showed that liver and brain are the organs most affected"

This sentence have been rewritten as suggested by the reviewer.

Evelyn Siew-ChuanKoay, Ph.D., FRCPath, F.A.A.C.B. (Reviewer 2): I have read the manuscript and found that it is not so well-written, with many grammatical errors and some typographical errors remaining in this revised 1 version. Of greater concern to me are some basic scientific issues which are not properly or appropriately addressed and need to be corrected or clarified by the authors before the article can be published. I have listed some of these below for the authors' consideration, but the list is not an exhaustive one:

- Primer sequences not provided (page 5 line 21-22).

We have been added information in Table 1 as suggested by the reviewer.
- Non-standard reference sequence of the ATP7B gene was cited (page 2 line 12; page 6 line 11).

We have been added information as suggested by the reviewer. In the original manuscript we have provided accession number of ATP7B gene in GenBank, however this information has not been specifically so we have replaced by the code number in Ensembl (ENSG00000123191).

- Non-HGVS nomenclature was used for the ATP7B protein (changes at protein level should be preceded by "p." ) (page 2 line 14-15)

We have been corrected all of these mistake in the manuscript.

- "IVS12-2" is not a standard HGVS nomenclature (page 7 line 8 & line 13).

We have been corrected all of these mistake in the manuscript.

- The term "Prothrombin time" was wrongly quoted throughout the entire text (page 4 line 13, etc).

We have been corrected this word as suggested by the reviewer.

- Authors please verify the normal range of ceruloplasmin level.

We have been corrected information as suggested by the reviewer.

- Authors please clarify the meaning of "shear zone" (page 8 line 9).

Thank you so much for your comments. This sentence has been revised as suggested by the reviewer 1.

Once again thank you very much.

Sincerely Yours,

Nguyen Huy Hoang