Author’s response to reviews

Title: Identification and characterization of a novel 43-bp deletion mutation of the ATP7B gene in a Chinese patient with Wilson's disease: a case report

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Response to Reviewers’ Comments

Prahlad Balakrishnan (Reviewer 1):

The manuscript entitled 'Identification and characterization of a novel 43-bp deletion mutation of the ATP7B gene in a Chinese patient with Wilson's disease: a case report' is written moderately well, but can be improved little more. The molecular genetics of Wilson's disease has been reported quite often, but the characterization and mechanism of deletions / duplications or complex rearrangements are rarely found in literature. This is an interesting article describing the molecular genetics part as well as the possible mechanism behind the novel deletion in ATP7B with adequate pictures. The article follows a satisfactory way of presentation, but can be modified well before the acceptance.

Response:
Thanks for your comments and suggestions. We have carefully addressed, point-by-point, the issues raised in the comments. Details of our responses to these comments were listed below.

Specific comments:

Comment 1: Gene name to be changed to italics. e.g., ATP7B gene to be *ATP7B* gene or ATP7B.

Response: Revised as suggested.

Comment 2: ATP7B protein to be changed to ATP7B.

Response: Revised as suggested.

Comment 3: In multiple places it has been mention, Pathogenic mutations / deletion mutation etc... it can be changed to either mutations or pathogenic variants.

Response: In our manuscript, “Pathogenic mutations” was revised as “mutations”. We kept using “deletion mutation” for clear presentation.

Comment 4: In para 1 of Background, ‘ATP7B gene mutations lead to ATP7B protein dysfunction, which in turn causes a massive accumulation of copper in the liver, brain, kidneys and corneas, with a wide range of clinical symptoms, including hepatic disorders, neuronal degeneration of the brain, and Kayser-Fleischer rings at the corneal limbus [5, 6]’. (‘a massive' can be removed)

Response: We corrected it in the revised manuscript. Thank you. (line 71, Page 4)

Comment 5: In para 1 of Background, ‘However, mutations are identified in only one allele or none in a substantial number of WD patients’. (reference to be included if possible)

Response: The reference has been added to support the statement. (line 83, Page 4)


Comment 6: In Para 1 of Case presentation, ‘The proband was the first child of healthy non-consanguineous Chinese parents form Jiangsu Province.’ (It is not 'form', it is 'from').
Response: Corrected correspondingly. Thank you. (line 95, Page 5)

Comment 7: In Para 2 of Case presentation, ‘Sanger direct sequencing of the 21 exons of the ATP7B gene revealed two heterozygous mutations in the proband, including c.3517G>A and c.532_574del. The c.3517G>A mutation in exon 16 resulted in the conversion of glutamate to lysine at amino acid position 1173 (p.Glu1173Lys), and is responsible for Wilson's disease [17]. The c.532_574del deletion covered a 43-bp region starting from nucleotide 481 to 523 in exon 2; it included CTCAGCAACCAAGAGGCCGTCATCACTTATCAGCCTTATCTCA, and resulted in a frame shift mutation (p.Leu178PhefsX10). His parents were screened for mutations, and his mother was heterozygous for the c.3517G>A mutation, while the father was heterozygous for c.532_574del (Figure 1). Accordingly, the proband was compound heterozygous for c.3517G>A and c.532_574del mutations in both alleles inherited from his mother and father, respectively. According to previous reports and the Wilson Disease Mutation Database (http://www.wilsondisease.med.ualberta.ca/databa...

Response: We made our efforts to improve the writing of this paragraph, we hope that the improvement will meet the requirements. We revised the sentences as “Sanger direct sequencing of the 21 exons of the ATP7B gene revealed two heterozygous mutations in the proband: one missense variant, c.3517G>A (p.Glu1173Lys) and one deletion variant of 43 base pairs, c.532_574del. His mother was heterozygous for the c.3517G>A mutation, while the father was heterozygous for c.532_574del (Figure 1). Accordingly, the proband was compound heterozygous for c.3517G>A and c.532_574del mutations in both alleles inherited from his mother and father, respectively. The missense mutation p.Glu1173Lys has been reported three times in other cases[18]. The c.532_574del mutation was predicted to cause a premature termination codon (p.Leu178PhefsX10) in the N-terminal region. According to previous reports and the WD Mutation Database, the c.532_574del mutation in our WD patient was unknown so far. Owing to definite genetic cause of WD in our patient, the parents was offered prenatal diagnosis during a second pregnancy (testing the amniotic fluid) in the family was carried out, and the unborn sibling of the proband harbored only the heterozygous c.532_574del mutation and not c.3517G>A.” (The above paragraph is to be re-written in more scientific way)

Comment 8: In the discussion and conclusions part, ‘the deletion mutation c.532_574del (p.Leu178PhefsX10) in the ATP7B gene firstly reported in this study existed in the proband's parent, was therefore not a de novo mutation.’ (can be made in like, the deletion mutation c.532_574del (p.Leu178PhefsX10) in the ATP7B gene was reported for the first time in present study, and was observed in proband's parent, was therefore not a de novo mutation).

Response: Revised as suggested. Thank you. (line 119-130, Page 6-7)

Comment 8: In the discussion and conclusions part, ‘the deletion mutation c.532_574del (p.Leu178PhefsX10) in the ATP7B gene firstly reported in this study existed in the proband's parent, was therefore not a de novo mutation.’ (can be made in like, the deletion mutation c.532_574del (p.Leu178PhefsX10) in the ATP7B gene was reported for the first time in present study, and was observed in proband's parent, was therefore not a de novo mutation).

Response: Revised as suggested. Thank you. (line 146-147, Page 7)
Comment 9: In the same paragraph, unable to understand the meaning of 'the severe mutation'. What is it? Please change to more attractive way like, "The variant would be considered as a pathogenic variant with more severe phenotype than the other missense pathogenic variants".

Response: Revised as suggested for clear description. (line 154-156, Page 8)

Comment 10: MMEJ is currently used to explain the possible formation of large deletions belonging to structural variations (SVs), commonly referred to as copy number variants (CNVs) which are generally defined as DNA regions of approximately 50 bp and larger in size, and not small deletions belonging to indels which are small insertions or deletions generally between 1 and 50 bp in size. (any reference).

Response: The reference has been added to support the corresponding description. (line 174, Page 9)


Comment 11: The presence of DNA sequence motifs, including 2 "Deletion hotspot consensus", 2 "DNA polymerase arrest site", 1 "Ig heavy chain class switch repeat 1", 1 "Ig heavy chain class switch repeat 2", 1 "Vaccinia topoisomerase I consensus", and 1 "oligo(G)n tracts" in adjacent regions of the c.532_574del mutation, is......(repeated in the same paragraph).

Response: The sentence was revised as “The DNA motifs around the deletion breakpoints would likely cause DNA strand breaks”. (line 175-176, Page 9)

Comment 12: None of the supplementary files have a connection with the main article.

Response: Revised as commented. There were two additional files in our manuscript. Additional file 1 was a detailed description of materials and methods related to this manuscript. Additional file 2 summarized previously reported ATP7B deletions due to FoSTeS/MMBIR mechanism. The two files have been cited in the main text (line 117-118, Page 6; line 187, Page 9).

Ahmet Koç (Reviewer 2):

It is a case study about WD and involves deletion of a segment of DNA on exon2. This deletion mutation has not been previously described and thus I believe this work has scientific merits. The authors also proposed a mechanism about how this deletion might have happen in the parents. The paper is organized and written well and I believe it could be published in BMC Medical Genetics.
Response: Thank you so much for the positive comments on our manuscript.