Peer Review File

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Reviewer A: The article entitled “Osteoarticular manifestations as initial symptoms of WD with novel compound 2 heterozygote mutations in the ATP7B gene: a case report” describes a 13 year old female individual having prominent symptoms of WD like low plasma ceruloplasmin level, Kayser Fleischer ring and T2 weighted MRI pattern suggestive of Wilson disease.

The article documents the osteoarticular changes as a rare symptom of WD patient. I appreciate the authors for properly documenting all the clinical parameters for the patient.

However, the way the manuscript has been written, may be improved.

Comment 1: In the entire manuscript, the authors have never mentioned the ethnicity of the subject.

Reply 1: Thank you for reminding. We have added this information to the manuscript (See Page 4, line 31).

Changes in the text: A 13-year-old girl of Chinese Han ethnicity presented to the Department of Rheumatology at Renji Hospital with a 6-month history of joint pain, and limb tremors, clumsy gait, and speech difficulties lasting 3 weeks.

Comment 2: The authors also mentioned, they have performed genetic screening of ATP7B, but it is not clear whether they have performed targeted bi-directional Sanger sequencing or anything else!

Reply 2: Thank you for your suggestion. We apologized for this omission to have left this point unclear. We detected the mutation using SureSelect clinical research exome V2 and then verified this mutation via targeted bi-directional Sanger sequencing. We have added this information to the manuscript. (See Page 6, line 78-82)

Changes in the text: Subsequently, we performed genetic testing for ATP7B gene mutations，which are responsible for the pathogenesis of WD (2)，using SureSelect Clinical Research Exome v2 （Agilent）. A heterozygous mutation of NM_000053.4 (ATP7B): c.3884C>T p. Ala1295Val in exon 18 was detected and then verified using

bi-directional Sanger sequencing. The mutation was found to be inherited from the patient's father (Figure 3).

Comment 3: The authors must provide a pedigree and the chromatogram of the variant identified.

Reply 3: We agreed with the reviewer's comments. We have added a pedigree and the chromatogram of the variant identified of the whole family to the manuscript. (See Page 11, line 204-205, and Figure 3)

Changes in the text:

Figure 3. Family pedigree of the patient and a heterozygote along the sequence exon 18 of the ATPase copper transporting beta (ATP7B) genes using Sanger analysis.

Comment 4: Previously, some studies have reported bone dysfunction among WD patients from different parts of the world. The authors should mention some of their findings.

Reply 4: Thanks for your suggestion. According to your suggestion, a relevant literature review has been added to the revised manuscript. (See Page 7, line 102-109)

Changes in the text: Quemeneur et al. summarized musculoskeletal conditions associated with WD. They observed spinal radiological abnormalities, including scoliosis, diffuse bone demineralization, and osteochondritis (6,7). Several groups have reported that decreased BMD and a high prevalence of osteoporosis, especially in the lumbar spine, in patients with WD (8-10). In adults patient with normal BMD, Quemeneur et al. also reported a prevalence of vertebral and peripheral fracture (11).

Comment 5: A report of hypoparathyroidism has been reported previously in a WD patient. The authors must check the PTH and VitD3 levels in order to investigate bone demineralization as a possible cause of Osteo-skeletal dysfunction.

Reply 5: We totally agree with the reviewer. PTH plays a role in bone metabolism. The patient's serum level of PTH was 25.4 pg/ml (normal range 12-88), and 25-OH-D3 was 19.14 ng/ml (normal range >20). The bone mineral density (BMD) of the patient's hip and lumber spine, as measured by dual-energy X-ray

absorptiometry, was normal. We have added these parameters to the manuscript. (See Page 5, line 64-68; Page 7, line 111-113)
Changes in the text: Her serum calcium was 2.16 mmol/L (normal value: 2.08–2.6), phosphorus 1.62 mmol/L (normal value: 0.96–1.62), 25-OH-vitamin D3 19.14 ng/ml (normal value: >20) ,and parathyroid hormone 25.4 pg/mL (normal value: 12–88). The bone mineral density (BMD) of the patient's hip and lumber spine, as measured by dual-energy X-ray absorptiometry, was normal.
The patient did not have osteoporosis, and her serum levels of calcium, phosphorus and 25-OH-Vitamin D3 were within normal limits.

Comment 6: In the discussion section, the authors must explain a possible involvement of ATP7B or copper in bone mineralization or calcium metabolism.
Reply 6: We appreciated the reviewer's suggestion. We have added the description of the role of ATP7B and copper in bone mineralization in the discussion section, based on the review's comment. (See Page 7, line 113-119; Page 8,line 130-133)
Changes in the text: Copper deposition might play a role in the etiology of arthropathy and fracture. However, the mechanisms of osseous abnormality are still unclear. Renal tubular dysfunction and abnormal calcium and phosphorus metabolism have been linked to bone conditions (2,10). Copper is an essential component of proteins and enzymes and is involved in multiple metabolic pathways. At excess levels, copper induces both the production of reactive oxygen species (ROS) and mitochondrial dysfunction, leading to osteoarticular disorders (10,12).
ATP7B mutations caused a defect in hepatocellular copper transportation, resulting in the accumulation of copper in various tissues and organs. Xie et al. reported that skeletal copper content increased 4 times in patients with WD, which might lead to bone and joint changes (15).

Comment 7: Line 33; they have mentioned 'heterozygosis' please change it to compound heterozygote or compound heterozygous mutation.
Reply 7: Thank you very much. We have changed "heterozygosis "to"heterozygous mutation". (See Page 3, line 11)
Changes in the text: Genetic analysis found that the patient carried a novel compound heterozygous mutation in ATP7B on both chromosomes to be inherited

from her asymptomatic parents.

Comment 8: Line 44; include the word ‘variable’ otherwise next line will have no meaning.

Reply 8: Thank you for your excellent suggestion. We have modified the corresponding part of the manuscript in line with this comment. (See Page 3, line 21-22)

Changes in the text: Patients present with hepatic, neurologic and psychiatric manifestations, and presentations vary considerably among patients (1). Due to this variability, making an early diagnosis is difficult, which affects therapeutic outcome and prognosis.

Comment 9: Expand the acronym: CARE

Reply 9: Thank you. We have expanded the acronym. (See Page 4,line 28)

Changes in the text: We present the following case in accordance with the CARE reporting checklist.

Reviewer B: Nice case presentation

Reply: Thank you for your comments.