

Peer Review File

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Reviewer A

Zhang et al. report on 6 patients with cerebrotendinous xanthomatosis (CTX) followed for 2 years. They performed nerve biopsy in 3 patients that showed a predominant demyelinating neuropathy.

Overall, the paper does not provide much novel data related to CTX and there has been already several publications of Chinese patients, including 19 patients recently reported - Clinical and genetic characteristics of Chinese patients with cerebrotendinous xanthomatosis. Tao QQ, Zhang Y, Lin HX, Dong HL, Ni W, Wu ZY. Orphanet J Rare Dis. 2019 Dec 3;14(1):282.

Major comments otherwise:

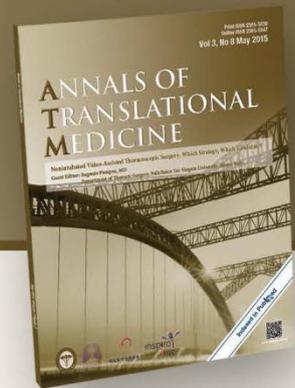
Comment 1. English needs to be edited

Reply 1: We polished the language in this manuscript by a native speaker. We corrected the grammar mistakes and made the flow of text more concise and coherent.

Changes in the text: Words with a different color of text showed the changes.

Comment 2. Cholestanol levels are not available: this was already mentioned in previous articles as a pitfall. Given the importance of that simple metabolic test for functional validation of pathogenic variants in CYP27A1 and response to treatment, why not having a least one lab in China that can perform such analysis? Instead of reporting more patients without new findings.

Reply 2: Cholestanol levels are potent methods to confirm the diagnosis, verify the pathogenicity of novel mutations and evaluate the treatment response. We've collected blood sample of our patients but no lab in China can provide such a test at present. Detecting cholestanol level is a intricate and technical process that we can't perform in our own lab. Building at least one lab will definitely promote the diagnosis and screening of is curable disease. With more CTX cases emerged from population and more attention to rare disease, we will try our best to promote the laboratory examination of the disease. We believe this



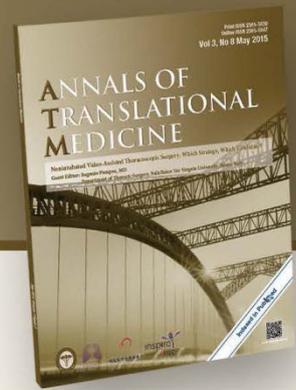
test will soon be established in China. We have evaluated clinical symptoms of our patients and considered to perform functional verification through animal model next.

Changes in the test: Response to treatment had been reevaluated and rewrote in the follow-up part.(See Page 9) Still, we could not perform such a test in this submission.

Comment 3. About the 6 patients detailed here: the section about treatment needs to be re-written. It is not possible to assess the efficacy of CDCA (or UDCA) with only 6 to 12 months of follow-up. Furthermore, none of the assessment is standardized and each report with treatment is anecdotal. The statement about UDCA (UDCA and lipid-lowering therapy also contributed to clinical stabilization) is misleading as only 2 patients were treated with UDCA, one who reportedly worsened and the other who improved. And the dramatic improvement of gait after 6 months is very suspicious given the slow rate of improvement observed over years in all other major patient series.

Reply 3: We just performed a recent follow-up after receiving this review comments. All the description of follow-up data was based on facts. CDCA was only produced by one pharmaceutical company at home and can't be provided in hospital, so patients had to buy this medicine themselves. This decreased the adherence of regular administration of CDCA. As a result, the duration of treatment among patients varies, with the least taking only 6 months. At 1-year or 2-year follow-up, patients were evaluated by face-to-face interviews or telephone. Symptoms relieved or aggravated were collected and neurological examination was performed. mRS scores were used to quantified general status. We made a mistake in the first submission that Patient 6 took CDCA after diagnosis, not UDCA. After treating for 6 months, his cerebral ataxia, slow response and cognitive impairment improved. So his improvement was the effect of CDCA. Only patient 2 took UDCA and progressed chronically. Overall, CDCA could only alleviated some of the symptoms partly and could not cease the progression of the disease.

Changes in the test: We have re-written the follow-up part, updated clinical data and added more details.(See page 9 Follow-up part). To delineate data clearly, we added Table 4.Treatment response evaluation in 6 patients during a 2-year follow-up.(Page 27 Table 4)



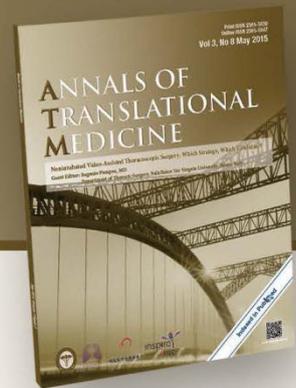
Comment 4. The neuropathological description is interesting but the predominance of demyelinating features has already been reported in CTX.

Reply 4: Literature focused on CTX-related neuropathy was relatively rare. Although the demyelinating features have been reported, larger series and literature review from abroad seemed to consider axon dominant neuropathy as the most common type, confirmed by NCV/EMG. Demyelinating features mainly came from sporadic cases or small series. In China, CTX was considered extremely rare and might be under-diagnosed. Neuropathy in Chinese CTX cases had never be summarized. We found the predominate demyelinating type in our own cases and then found other reported cases from China seemed to present a similar type of neuropathy. When the peripheral neuropathy lesion was slight, sensorimotor demyelinating is the prominent feature, with motor nerve more vulnerable. In severe neuropathy, demyelinating and axonal degeneration tend to coexist. So we focused on this part and made a more detailed discussion. CTX-related peripheral neuropathy turned to be a highlight of this assay.

Changes in the test: We laid our stress on neuropathy in CTX and rewrote the literature review part, see page 10, line 8.”Literature review of CTX patients with peripheral neuropathy in China”. Also, we gave a more detailed discussion about the type and possible underlying mechanism of CTX-related neuropathy.(See page 13, line 8) Furthermore, we modified table 2 and deleted the description of 2 reported cases, because message was extracted from literature and may have bias when compared to ours. So we just provided our own neuropathological description and analyzed in the discussion part.(See Table 2)

Comment 5. The authors also report on 31 other Chinese patients. However, these data are confusing. Is this a literature review from Pubmed? Or did the authors go back to patient’s charts to get the actual data? To which extent this literature review is distinct from the Tao et al paper cited above?

Reply 5: In the first admission, we reviewed literature focused on clinical symptoms and genetic features. Those data from 31 reported cases were reviewed from PubMed. After collecting our cases, we tried to summarize the clinical, pathophysiological and genetic features of Chinese CTX cases to promote diagnosis. However, Tao et al had done the



analysis and reported their cases before us. They didn't evaluate nerve biopsy and no follow-up data were included. So we just reported our 6 cases and given a similar analysis by pooling their cases and other reported cases together. Even though, our data wasn't novel enough because conclusions were just similar. So in this submission, we just cut out the literature review of 31 cases about clinical and genetic features to avoid repetition. Pathological and electrophysiological analysis about peripheral neuropathy were interesting and rarely detailed studied in China, so we just turn our attention to this aspect. Literature review was focused on CTX cases with descriptive NCV/EMG findings or neuromuscular abnormalities. In this new submission, we include 21 cases from PubMed and also mentioned in method part. In that case, we reported our cases, identified novel mutations and given features of peripheral neuropathy in Chinese population, which was distinct from Tao et al's paper.

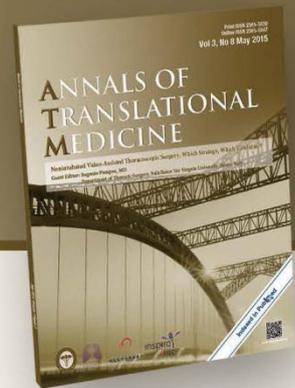
Changes in the test: We delete the previous literature review about clinical symptoms and genetic features and substitute with a new review about CTX-related peripheral neuropathy. (See page 13, line 8) Those 21 Chinese cases (including ours) were collected from Pubmed, which was mentioned in the Method part.(See page 5, line 10)

Comment 6. Along the same line, Figure 3 shall be redone. The data shall be presented instead as a Kaplan-Meier analysis showing a which age each main symptom occurred for each patient (as in Degos et al, Natural history of cerebrotendinous xanthomatosis: a paediatric disease diagnosed in adulthood. Orphanet J Rare Dis. 2016 Apr 16;11:41), or as a detailed table (as in Tao et al).

Reply 6: Since we deleted the part of clinical features analysis based on literature review, the Figure 3 was cut off correspondingly. In fact, previous data presentation profile was indeed not very appropriate. A Kaplan-Meier analysis or precise table would be fine. However, we cut that content to avoid repetition.

Changes in the test: We deleted figure 3 and 4 as well as corresponding analysis in the text.

Comment 7. In the abstract and result sections, there is mention of mental retardation occurring



at a median age of 13 years of age – this is not possible. Mental retardation is a developmental feature with very early onset in infancy.

Reply 7: Mental retardation developed early from infancy and a feature of CTX. We should use the word "cognitive impairment" or "school difficulties" or "bad school performance" instead of "mental retardation". Even though mental retardation really existed early in CTX patients, Chinese parents usually ignored or denied in a subjective manner until their kid had much "school difficulties" or "bad school performance". In the literature, authors usually mentioned at which age patients emerged school difficulties or bad performance, indicating an evident sign of cognitive impairment. So that's why we use the wrong word. School difficulties at an average 13 years old would be reasonable. However, we decided to delete that kind of age-symptom analysis because of small size, inappropriate method and kind of repetition.

Changes in the test: We deleted mental retardation occurring at a median age of 13 year old in the abstract and result sections.(See the new abstract section--page 2; and the new result section--page 5 Result)

Comment 8. The numbering of references is incorrect: "CYP27A1 had recently been identified as a possible candidate gene for ALS in a large and comprehensive genome-wide screening" this is reference 40 and not 39 (I have not checked all other references) and a reference from 2012 is not recent.

Reply 8: All number of references has been corrected and checked before re-submission. Though not recent, this reference was meaningful. CTX was a rare metabolic disease to some extent quite mimicking ALS with its pyramidal signs, muscle weakness, advance bulbar palsy and progressively deterioration. Misdiagnosis was not uncommon clinically. It was inspiring to find that these to degenerating disease do had some genetic relationship. Despite not recent, this reference was an important millstone in exploring the underlying mechanism of CTX. Also, this reference gave us some clue that specific cholic acid intermediate might act as motor neuron-protectors and might shed light on the potential treatment of ALS. So we still cited this literature.

Changes in the test: This reference had been corrected.