Author's response to reviews

Title: Multi-parametric neuroimaging evaluation of cerebrotendinous xanthomatosis and their correlation with the neuropsychological presentations

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Author's response to reviews: see over
Reply to the Editor and reviewers:

Dear Professor Alam,

Thank you for considering our paper for revision and for all of the suggestions and comments. You suggested that the Introduction, Discussion and Conclusion were not well structured and required further revision. We have therefore now revised our article, especially these sections, as concisely as we can.

With regards to the editorial request for the patients’ consent forms, we will fax them to (+44 (0)20 3192 2011) and also send them by email to editorial@biomedcentral.com.

The authors declare that they have no competing interests as defined by the journal’s criteria. We have included the authors’ contributions and acknowledgements in this version. Below are our responses to the comments and we are looking forward to receiving your response.

Response to the concerns:

**Reviewer 1**

This is an interesting report on a rare neurological disorder, Cerebrotendinous xanthomatosis, in which MRI and SPECT analysis were correlated with neuropsychologic presentations. I think that the methodological aspects are correct and the results are interesting. I consider this report to be published in the present form.

Reply: We would like to thank the reviewer for the suggestion and comments.

**Reviewer 2**

1. The most important issue is that the five patients, a very small population for a VBM-TBSS study, are taken from only two families, posing a great interpretation bias in the results: are the results related to those family or are them generalized to CTX patients? Adding at least five more patients from different families would be of added value to the paper.

Reply: It is true that the study is comprised of a small sample number because of the rarity of CTX. However, we consider that the data presented in this paper are still of scientific value, since we used sophisticated and modern neuroimaging techniques to delineate the changes in CTX patients both structurally and functionally. We will include the small sample number as one
of the limitations of this work since adding five more patients is not possible from our perspective. The statements were as follows: “because the rarity of this disorder, the interpretation presented here might represent the results related to the two families. Whether it applied to the general population of CTX families required a larger population study.”

2. The authors performed a thorough imaging study, including T13D, DTI and SPECT. This is of value, but should be sustained by hypotheses that are not really clear from the introduction. What is the aim of the work? Just look everything and describe the findings?

Reply: We are sorry for the unclear statement. The reason why we used 3D-T1 MRI, DTI and SPECT was because they provide quantitative measurements of neuroimaging, which can be compared with age-matched controls and provide a better understanding of the regions being affected as compared with conventional imaging. We have included this statement in the introduction section.

3. In the introduction, the authors describe TBSS and VBM but do not really give us an understandable background of imaging in CTX.

Reply: CTX is a genetic metabolic disorder and both WM and GM are involved. Although leukoencephalopathy, signal abnormalities (low or high) of dentate nuclei of the cerebellum have been reported in brain MRI studies, the damage of WM or GM cannot be quantified by conventional imaging techniques. TBSS and VBM were used for different rationales, in that TBSS detects WM fiber tract diffusion parameter changes and provides better resolution than conventional MRI in WM tract, while VBM detects volume differences in GM and WM. We have rewritten the Introduction section, adding the physiological (or pathological) correlation related to FA, MD, axial and radial eigenvalues. We have also stated that “Diffusion MRI provides quantitative measures compared with conventional MRI in detecting WM pathology and is better suited for a more comprehensive evaluation of diffusion changes in WM”. Thank you for the suggestion.

4. In the methods section, it would be important to write the mean age of patients
(although can be calculated from the table) and the mean age of healthy controls, not given at all.

Reply: We have added the mean age of the patients in the Method sections. Since the healthy controls were selected to be age- and sex-matched, we did not repeat the information again.

5. The parameters of imaging protocol should be much more detailed as well: for FLAIR and T2, add TR TE TI, number of slices, matrix and FOV. For DTI the authors did not obtain b0 images.

Reply: For T2WI and FLAIR, we have provided the TR, TE, TI, number of slices, matrix and FOV as the reviewer suggested. We obtained one b=0 image for the DTI scanning.

6. In the VBM description the authors write that "comparisons between patients and controls were performed using the total volume of each segmented image": this is not clear, please explain.

Reply: We were sorry for the unclear statement. Therefore, we have revised the sentence as follows: The general linear model was used to assess statistical differences in GM and WM among the patient and control. Global differences in voxel intensities were used as confounding covariates in an analysis of covariance. Age and gender were considered covariates of no interest to exclude their possible effects on regional GM or WM.

7. In the TBSS section, although in the title is written that you performed TBSS for the three eigenvalues as well, in the description it looks like you only performed the FA and MD voxelwise analysis. It is not clear also which version of TBSS was used (1.0 1.1 or 1.2?) Moreover, while in data preprocessing section you say you used FSL version 4.0, in the TBSS section you state to have used FSL version 3.3.

Reply: For TBSS, we performed three eigenvalues as well. The results have now been added to this revision (Result-WM tract and Figure 5). We used FSL 4.0 and TBSS 1.2 for DTI study preprocessing and analysis. We are sorry for the mistyping of the version in FSL.
8. Why did you only correlate CASI scores with FA? What is the purpose of reporting all the other neuropsychological tests that you name before?

Reply: We did not extensively correlate the cognitive results with FA since the small case number might have caused statistical bias for multiple comparisons. Therefore, we used CASI total score because they represent a general assessment in cognitive performances as compared with the subdomains. We reported the CASI subdomains in Table 1 for a better understanding of the subdomains affected in CTX. We did not use MMSE for correlation since the domains in CASI already cover most of the test items in MMSE. The WAIS-R score presented here was to delineate the changes of intelligence quotient at onset and at follow-up. Since the interpretation of WAIS-R was normalized to the norm rather than raw score, we did not use the normalized data to correlate with FA since the CTX group might have shown a floor effect in WAIS-R as compared with CASI.

9. It is not clear how the authors performed this correlation of CASI with FA, with which software and which methodology.

Reply: In the statistical analyses section, we wrote “Correlations between CASI total scores and FA were carried out using Spearman’s rank order correlation. The statistical analysis was conducted using the Statistical Package for Social Sciences software package (version 13 for Windows®, SPSS Inc, Chicago, IL).” Thank you for the reminder.

10. In the results, you should give the p values with FWE of the VBM findings.

Reply: For GM and WM, we both accepted p<0.05 FWE for multiple comparison.

11. In the discussion there are many non justified assumptions; the authors should review the discussion almost completely. They should start from the pathological findings in CTX and discuss the correlation of their findings with pathology in a more clear way.

Reply: Thank you for the suggestion. We have arranged the discussion as follows:
we first summarize the main findings in the first two paragraphs and elaborate the possible linkage of the findings with pathological studies thereafter. An important notion in our discussion is the combined axonopathy in CTX in addition to the conventionally considered demyelinating process. With the combined interpretation of DTI with VBM of WM atrophy, we found that WM regions such as peri-dentate nucleus or midbrain were severely affected despite the treatment with CDCA.

12. The discussion and conclusions are not well balanced and are not adequately supported by the data.

Reply: Thank you for the comment. We have revised the Conclusion to balance the evidence we provided. We considered that DTI and VBM can provide complementary information regarding the GM or WM involvement in CTX and are more sensitive than conventional neuroimaging studies. From imaging study results, WM involvement including demyelination, axonal changes and tissue loss suggested irreversibility especially in the peridentate areas. Major associative fiber involvement as well as cortical atrophy accounted for the broad spectrum of cognitive deficits in these patients. The cognitive decline and image changes in conventional MRI indicate neuro-toxicity and possible irreversibility of this disease despite using CDCA.

13. The limitations of the work are not clearly stated

Reply: We have added one paragraph at the end of the article regarding the limitation of the study as follows: There are several limitations to this study. First, this study enrolled a small number of patients. Because the rarity of this disorder, the interpretation presented here might represented the results related to the two families. Whether it applied to the general population of CTX families required a larger population study. Second, we cannot provide histology proof in this study because this is a cross sectional study on human subjects. Therefore, the interpretation of the pathological changes through neuroimaging findings (as reflected in changes in axial and radial eigenvalues) cannot be proved. Nonetheless, the results are consistent with previous pathology studies where myelin damage is constant with or without axonal
destruction. It is worth pointing out that a direct correlation between axial and radial diffusivity parameters and the microscopic pathology of white matter injury has not been conclusive. As such, interpretation of changes in axial/radial eigenvalues as demyelination or axonopathy should be treated with great care especially in regions where neuron fibers cross.

14. The writing is acceptable although sometimes the authors use non proper words.

Reply: We have revised the paper as concisely as we can. We also sent the paper for English editing in the first and this revised version.