Author’s response to reviews

Title: Isolated and repeated stroke-like episodes in a middle-aged man with a mitochondrial ND3 T10158C mutation: a case report.

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30th August, 2017

Dr. Marc Alain Babi

Editor

BMC Neurology
Dear Dr. Babi,

We would like to sincerely thank the editor and the reviewers for their careful review and constructive comments that helped us to improve this manuscript. In this revision, we have responded to the reviewers’ comments point by point and have shown the changes in red text in the revised manuscript. We hope that the editor and the reviewers will be satisfied by our responses and that our manuscript is now suitable for publication in BMC Neurology.

Thank you for your consideration of this manuscript.

Sincerely yours,

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Reviewer 1 (Francisco Alvarez):

The Authors report an interesting case of mitochondrial disease related to ND3 mutation.

I have 2 questions:

- were 14.3.3 protein and adenosine deaminase in CSF determined?

We had measured 14-3-3 protein and adenosine deaminase in CSF and had confirmed that concentrations of these molecules in CSF were within normal limits. We have now described this in the revised manuscript on page 6, lines 96-97.

- is available ADC sequence in MRI study?

According to the suggestion, we have added ADC images in Fig. 1 and have mentioned the findings on page 6, lines 101-104 in case presentation and page 15-16, lines 299-302 in the Figure legends.

Reviewer 2 (Yasmin Ali O'Keefe):

Overall well written paper, interesting case. I enjoyed the discussion the most as it was a good refresher of the pathophysiology and causative factors of this disease process. You may do well
to a little more prominently highlight the preferential predilection of mitochondrial disease to present in pediatric demographics (perhaps in the abstract) to catch the reader's attention as to why this is a pertinent case report, but otherwise overall well done.

Thank you for your valuable comment. Accordingly, we have revised the abstract as follows on page 3, lines 47-51: “Genetic analysis of biopsied biceps brachii muscle, but not of peripheral white blood cells, revealed a T10158C mutation in the mtDNA-encoded gene of NADH dehydrogenase subunit 3 (ND3), which has previously been thought to be associated with severe or fatal mitochondrial disorders that develop during the neonatal period or in infancy.

Reviewer 3 (Salman Al Jerdi):

#1. I think you did a good job stating that the mutation MAY cause the disease, as the burden of proof for this is difficult even with a much higher number of cases.

Thank you for your valuable comment. We agree that we cannot completely exclude the possibility that the T10158C mutation may not directly cause the disease state of the present case. Thus, in regard to the diagnosis, we have avoided the definite statement that the mutation directly caused the disease state of the present case. We have described in the revised manuscript that “We therefore suggest that a possible diagnosis for the patient could be isolated mitochondrial encephalopathy associated with the mutation.” on page 8, lines 136-137.

#2. The pattern you describe with occipital lobe involvement may be seen with conditions such as PRES, CNS vasculitis, and some other rare conditions. I do not see in the paper a mention or discussion of these possibilities and how they were ruled out. For example, although your patient was very thin, his systolic BP was 158. This is presumably high due to anxiety or symptoms, but a discussion about hypertensive encephalopathy is due. Also, CNS vasculitis, RCVS, and other conditions are usually addressed with vessel imaging initially. I do not see a discussion about vessel imaging. Did you guys perform invasive or non-invasive angiography? If so, please include it.

Thank you for your valuable comments. We had raised ischemic stroke, sinus thrombosis, PRES, CNS vasculitis, encephalopathy, malignant lymphoma, and CJD, as differential diagnoses. We think that we could exclude these disorders by blood and CSF tests, MR imaging, including ADC, MRA, and MRV, and EEG. We have now described this issue on page 8, lines 127-132. Furthermore, according to the reviewers’ suggestions, we have included images of ADC, MRA, and MRV in Fig.1 of the revised manuscript and updated the legend for Fig. 1 accordingly.

#3. Why do you think this mutation only presented in adult-life, with such high burden of mutation (80%)? This may be difficult to answer, but should be addressed if possible, since such mutations are usually quite devastating. Also, what do you think is the significance to the mutation being found in the muscles and not the WBCs? How does this speak to the time/stage at which the mutation occurs?
Thank you for your valuable comments. Based on several findings in the literature and our case, the T10158C mutation may occur de novo and attain high-level heteroplasmy, presenting encephalopathy very rapidly in some cases or slowly over 40 years in others. The present case belongs to the latter. We speculate that there may be additional factors that slow the onset of dysfunction and/or accumulation of the abnormal mtDNA in the slowly progressing cases, in contrast to the rapidly progressing cases. We have discussed this point on page 11, lines 181-186.

Due to the heteroplasmic characteristics of mitochondrial disorders, white blood cells in the present case may be spared from the distribution of abnormal mitochondria during the developmental stage. We speculate that the biopsied muscles did not show phenotypic changes due to “threshold effects” despite harboring 76% of the mutated mtDNA: it is reported that threshold values of mutational load presenting phenotypic changes may be around 60% for mtDNA deletions, while around 90% for mtDNA point mutations. We therefore speculate that the mutational load in affected CNS lesions in the present case may be over 90%. Alternatively, the mutation may disrupt the functions of the CNS more easily than those of other organs, such as muscles, even with a similar mutational load of around 80%. However, we would need to perform a brain biopsy and genetic analysis to determine this. We have discussed this point on page 10, lines 168-176.