Author’s response to reviews

Title: Pure exercise intolerance and ophthalmoplegia associated with the m.12,294G>A mutation in the MT-TL2 gene: a case report

Authors:

Patrick Soldath (patricksoldath@gmail.com)
Karen Madsen (Karen.Lindhardt.Madsen@regionh.dk)
Astrid Buch (Astrid.Emilie.Buch.02@regionh.dk)
Morten Duno (Morten.Dunoe@regionh.dk)
Flemming Wibrand (Flemming.Wibrand@regionh.dk)
John Vissing (John.Vissing@regionh.dk)

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Author’s response to reviews:

Reviewer 1:

This is an interesting and well-described case report.

Thank you for the nice comment.

Were lactate levels measured after exercise or only at rest?

We see that our description of the cycle ergometry was not detailed enough. Therefore, we have rewritten the paragraph on cycle ergometry. We now begin the paragraph by mentioning that we have performed this cycle test, and then go on to describe how lactate levels as well as lactate/pyruvate ratios were measured. The changes we have made can be seen on page 5 lines 11 to 20.
Reviewer 2:

The authors describe a case with pure myopathy caused by a heteroplasmic mutation in MTTL2 in mitochondrial DNA. They present data from an exercise physiological test of the patient, and they provide confirming and supporting evidence that this mutation is pathogenic. The manuscript is easy to follow and the figures are nice and confirmative for a mitochondrial disorder.

Thank you very much.

The same mtDNA mutation has been previously reported (Ref #18 - Pulkes et al Neurology 2003) in a patient with almost identical phenotype, but this is not mentioned until in the discussion part of the present report. I think this is important information, which should be mentioned already in the introduction/background. The similarity of the two cases can be discussed later.

We agree, and have now stated in the introduction that the mutation is previously described. See page 3 line 6.

The authors' main conclusion of this study is the association between the mutation and the pure myopathy. For me this is more like circular reasoning: "The mutation is only present in muscle and therefore only muscle tissue is affected." It would be interesting if they instead could speculate WHEN the mutation arose (like in Pulkes et al). If this mutation arose early in the embryogenesis but after the differentiation of the mesoderm, I believe it is interesting that a potentially pathogenic mtDNA mutation reported only twice hitherto, arises sporadically both times, affecting the same tissue, and thus causing the same phenotype.

We agree with the reviewer’s comments. We have added a paragraph near the end of the discussion where we argue why the mutation causes a pure myopathic phenotype and state that the mutation likely arose sporadically in early embryogenesis after differentiation of the mesoderm into muscle progenitor cells. See page 7 lines 7 to 14. We have also changed both the conclusion in the abstract and conclusion in the discussion so they also take this consideration into account. See page 2 lines 19 to 20 and page 7 lines 24 to 25.
Page 2 (Abstract): In the conclusion - change "suggest" to "support"

We have done so. See page 2 line 18.

Page 2 line 27 (Background+ several more times throughout the manuscript): Mitochondrial DNA is most commonly shortened mtDNA and not Mdna

We have changed the abbreviation of mitochondrial DNA from mDNA to mtDNA throughout the entire manuscript as well as in the Abbreviations section.

Page 2 line 31 (Background): mtDNA mutations are mostly present in multiple tissues (although in varying amounts), which may be the reason why these cases are so rare …

We view this view as a statement and not a question, but we have taken note of it and changed the wording of that part of the Background section and we think the new wording is more clear and concise. See page 2 line 28 to page 3 line 1.

Page 5 line 4: Reference #9 does not describe the measurement of Lactate/Pyruvate (described in brief in ref #11)

Thank you for pointing this out. We have rewritten the paragraph on cycle ergometry, and provide a new reference (reference number 9) that refers to an original article, which conducted the cycle test in the exact same manner as we did, and where there is a very thorough description of how the cycle test was conducted, including measurement of lactate/pyruvate. See page 5 lines 11 to 20.

Page 7 (Figure 2) I assume that the sequencing electropherograms are from the Sanger sequencing

Yes, this is correct. We now state in the legend of figure 2 that they are Sanger sequencing electropherograms. See page 8 line 6.
Reviewer 3:
How was the heteroplasmy determined in patient’s skeletal muscle?

As stated in the “Molecular studies section”, the sequencing was carried out using NGS (mean coverage of >1,000), which by nature is a quantitative analysis. See page 4 line 26 to page 5 line 1.

The authors claim, that the mutation is absent in other tissues than skeletal muscle. Was this determined by Sanger sequencing? If yes, Sanger sequencing has low sensitivity for very low degrees of heteroplasmy. Authors are advised to look for low heteroplasmy using a more sensitive approach (e.g. RFLP as already described for this mutation (authors reference 18). Establishing RFLP would further allow measuring heteroplasmy of COX- vs COX+ fibres, further supporting the pathogenic nature of the mutation.

After the initial discovery, the mutation was assessed by NGS in the different tissues to a mean coverage of 10,000x. Thus, we are quite confident that the mutation is absent from urinary epithelial cells, buccal mucosa epithelial cells and leukocytes. A sentence has been introduced in the Molecular studies section to clarify this. See page 5 lines 3 to 4. We do not feel that there is a great need for measuring heteroplasmy of COX-negative vs COX-positive fibers to further support pathogenicity as this has already been carried out by Pulkes et al (reference 19) who found that the mutation segregates with COX-negative fibers.

It would be interesting to include the percentage of COX- fibres from the histology.

We agree. We have therefore changed the wording in the histology section from saying multiple COX-negative fibers to state the percentage, which is 32% COX-negative fibers. See page 4 line 14.
Please include in figure 2A control electropherogram

As the mutation is absent from buccal mucosa by NGS analysis to a coverage of 10,000, the lower sequence trace in fig 2A equals a control/normal electropherogram. Adding an additional unrelated sample to the figure does not provide further information in our opinion.

Please use mtDNA as abbreviation for mitochondrial DNA instead of mDNA

We have changed the abbreviation of mitochondrial DNA from mDNA to mtDNA throughout the entire manuscript as well as in the Abbreviations section.

Can the authors report on current treatment of the patient?

We have added a short paragraph in the end of the discussion where we briefly describe how the patient is and has been treated. See page 7 lines 15 to 19.