Author’s response to reviews

Title: A novel missense mutation in the MYH7 gene causes an uncharacteristic phenotype of myosin storage myopathy: a case report

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Author’s response to reviews:

Dear Helena Kuivaniemi,

Thank you so much for your thorough assessment of our responses to the reviewer’s comments of your manuscript "A novel missense mutation in head region of slow/cardiac beta myosin heavy chain causes an uncharacteristic phenotype of myosin storage myopathy: a case report" (MGTC-D-18-00413R1).

Please see below our comments/answers for this round 2 of reply (in bold).

Several comments were not addressed adequately.
We’ve answered all comments/questions on this current round of reply.

Also you need to provide the CARE checklist.
CARE checklist has been provided at the initial submission. I tried to upload it back during my reply to this round 2 but there is no way to that in the sytem.
And submit the sequences to a public database.

The data has been submitted to ClinVar on September 04, 2018, with the accession number CV753034.

I am, therefore, asking you to make additional edits after which it may be possible to publish the revised version in BMC Medical Genetics.

We’ve made additional edits. Please see comments below.

Please include a cover letter with a point-by-point response to the comments, describing any additional experiments that were carried out and including a detailed rebuttal of any criticisms or requested revisions that you disagreed with. Please also ensure that all changes to the manuscript are indicated in the text by highlighting or using track changes.

All changes have been made. Please see comments below and the track changes.

Please also ensure that your revised manuscript conforms to the journal style, which can be found at the Submission Guidelines on the journal homepage.

We’ve tried to fit our style as close as possible to the journal style.

A decision will be made once we have received your revised manuscript, which we expect by 24 Apr 2019.

I look forward to receiving your revised manuscript and please do not hesitate to contact us if you have any questions.

Thank you for considering this revised manuscript in which we’ve tried to answer adequately all questions/comments raised after the first round of reply.

Additional from authors: List of cited references has been changed to follow the order within the text
Sincerely,

Jean Mamelona

Technical Comments:

Editor Comments:

Comments

A. General:

1) In your response, please give the location (page and line numbers) of the change, and copy and paste the edited text also into the response.

Agree!

2) BMC Medical Genetics guidelines state: Case reports should include an up-to-date review of all previous cases in the field. Authors should follow the CARE guidelines and the CARE checklist should be provided as an additional file. [http://www.care-statement.org/](http://www.care-statement.org/) For details, see instructions for case reports: [https://bmcmedgenet.biomedcentral.com/submission-guidelines/preparing-your-manuscript/case-report](https://bmcmedgenet.biomedcentral.com/submission-guidelines/preparing-your-manuscript/case-report)

CARE checklist has been provided at the initial submission. However, it has been removed when I submitted the documents for the first round of reply. I put it back to the system.

3) The data availability statement is wrong. The sequence data should be submitted to a public archive, such as the European Nucleotide Archive (ENA) and the accession number provided in the manuscript.
The data has been submitted to ClinVar on September 04, 2018, with the accession number CV753034. Clarification has been added within the text as follow:

Page 6, lines 4-5:

The novel variant has been submitted to ClinVar data base; with the assigned accession number CV753034.

Section Availability of data and materials: The sequence data has been submitted to ClinVar data base; with the assigned accession number CV753034.

4) All histological images need scale/size bars. Also, it would be helpful to add arrows to point to the structures that are mentioned in the figure legend.

Scale/size bars have been added for all pictures in Figure 2.

Arrows pointing to the structures mentioned in the figure legend have been added in picture D (Figure 2). This is cited in figure legend as follow:

D. Transverse view of fibres displaying dark coloured intrasarcoplasmic inclusion bodies on p62 stain (Black arrow).

5) All gene symbols should be in italics. Please also include the gene symbol in the title in parenthesis.

The title does not mention the gene. Therefore, there is no need to include the gene symbol in the title in parenthesis

B. Responses to comments by the reviewers and follow-up:

1) Page 4: Literature references for the statement (p62 immunostaining, a well-known…) on lines 19-20 is needed.

Literature reference has been added as follow:

Pag 4, lines 23-24:
…a well-known technique for revealing the presence of inclusion bodies [9].

2) One of the reviewers asked about a control for immunostaining, but I do not see a control sample in the image in Figure 2, and there was no response to that request.

Picture for control has been added in Figure 2, Picture C in replacement of the former Picture C.

3) The description of the WES is not adequate. You need to indicate e.g., the sequencer used, read depth, average length, number of reads, and QC criteria.

The description of WES has been improved. We added some description improvement as follow:

Page 5, lines 18-22:

For the sequencing, samples were loaded on an Ion HI-Q PI Chip v3 and placed onto the Ion Proton instrument (Thermo Fisher Scientific) together with an Ion PI HI-Q sequencing 200 Kit (Thermo Fisher Scientific) and sequenced for 520 cycles according to the manual (See Additional file 1: Tables S1 for parameters).

Parameters for WES are given in Additional file 1: Table S1

4) Description of the variant selection is also inadequate. The text is confusing since on page 5 it is stated that they are “suspected neuromuscular disease causing” and then on page 6, it is stated that they were excluded because they do not contribute to neurological function. Please clarify what the inclusion and exclusion criteria were.

Description of the variant selection has been highly improved. We added Figure S1 which give the filtering process. This figure has been cited in the text as follow:

Page 5; Line-23-24:

All suspected neuromuscular disease-causing candidate mutations found by WES were validated by direct Sanger sequencing (See Additional file 2: Figure S1 for filtering process).

5) List of the “suspected disease-causing variants” (page 5, line 17) should be provided as well as the primer sequences used to validate them. Provide a table that includes the prediction scores for all of these variants.
We’ve made a list of the “suspected disease-causing variants” in Table S3, which gives the prediction scores. This table is cited within the text as follow:

Page 6, Lines 4-8:

Two other candidate mutations were identified in the DNA of this patient, namely NM_003085.4:c.368C>A (p.Pro123His) in the SNCRB gene and NM_001001557.3:c.746C>A (p.Ala249Glu) in the GDF6 gene (Additional file 1: Table S3).

6) Provider of the Sanger sequencing results should be listed (page 5, lines 21-22).

Provider of the Sanger sequencing results is given within the text as follow:

Page 5: Lines 29-30: Amplicons were sent to Genewiz (https://www.genewiz.com) an external laboratory for Sanger sequencing.

C. Other:

1) Table 1: correct typo in “involvement”

Done. Thank you for bringing this up. The Table with correction has been uploaded.

2) Do not use the phrase “sequence variant mutation”. Since causality of this variant has not been proven yet, it would be best to call it a “sequence variant”.

Agree!

3) Page 5, line 25: what do you mean by “genetic baggage”?

We meant DNA of the patient. However, we’ve made a change to make it much clearer, by replacing genetic baggage by the DNA as follow:

Page 6; Lines 3-4:

… possibly linked to the clinical findings, found in the DNA of the patient as heterozygous (Figure 3).
4) Page 5, 26-30: write full sentences, not a list.

We’ve made a full list in Table S3, which is cited within the text as follow:

Page 6, Lines 4-8:

Two other candidate mutations were identified in the DNA of this patient, namely NM_003085.4:c.368C>A (p.Pro123His) in the SNCB gene and NM_001001557.3:c.746C>A (p.Ala249Glu) in the GDF6 gene (Additional file 1:Table S3).

5) Statement about limitations of the current study is needed.

Statement about limitations of the current study has been added as follow:

Page 9: Lines 1-4:

The results from this study have some limitations and data should be interpreted with caution. As we mentioned above, it was not possible to have DNA test results and clinical data for the parents of the patient. First, it is not clear if the mutation is de novo or hereditary. Second, we have not been able to demonstrate the segregation within the family.

6) I agree with the reviewers that additional editing for the text is required.

Editing has been made for the entire manuscript. Changes are tracked within the text.

Reviewer reports:

If improvements to the English language within your manuscript have been requested, you should have your manuscript reviewed by someone who is fluent in English. If you would like professional help in revising this manuscript, you can use any reputable English language editing service. We can recommend our affiliates Nature Research Editing Service (http://bit.ly/NRES_BS) and American Journal Experts (http://bit.ly/AJE_BS) for help with English usage. Please note that use of an editing service is neither a requirement nor a guarantee of publication. Free assistance is available from our English language tutorial (https://www.springer.com/gb/authors-editors/authorandreviewertutorials/writinginenglish) and our Writing resources (http://www.biomedcentral.com/getpublished/writing-resources). These cover common mistakes that occur when writing in English.

Editing has been made for the entire manuscript. Changes are tracked within the text.