Author's response to reviews

Title: LMNA mutations in Polish patients with dilated cardiomyopathy: prevalence, clinical characteristics, and in vitro studies

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Author's response to reviews: see over
Dear Editor,

First of all, we would like to thank you for your interest in this paper. We would also like to express our gratitude to both Reviewers for their valuable comments. Addressing them has considerably improved the quality of the manuscript.

Below we specify the changes introduced in the manuscript.

Regarding the comments by the first reviewer (Nicola Carboni):
- Background, page 3: The list of diseases associated to LMNA gene alterations should include more diseases: i.e. MADA, HHS (heart hand syndrome of Slovenian type et cetera) and also should be cited w references the overlapping syndromes due to LMNA gene mutations. In the aforesaid list, it is reported DCM; this is clearly wrong since the correct name is dilated cardiomyopathy with conduction defects.

Three more diseases associated with LMNA mutation were added. Also, one reference to a review that pertains to the overlapping LMNA phenotypes was added. We corrected name to dilated cardiomyopathy with conduction defects.

- page 4, line 10: Caucasian subjects with no history of cardiovascular diseases...; "Does the population include tis w muscular dystrophy or other diseases possibly linked to LMNA gene mutations?

We cannot exclude the possibility that control individuals harbored LMNA mutations. However, this is highly unlikely as these individuals were adults and did not report any symptoms of CVD. In order to clarify this we now describe this group as ‘..215 adult Caucasian subjects with no history of cardiovascular diseases as judged by an interview, randomly selected from a previously studied population cohort [11].’

- Same page: Mutations screening: where flanking intronic regions explored?

Flanking intronic regions were explored. Please, see the corrected second sentence of the first paragraph of “Mutation screening” section.

- Page 5, Expression analysis: please add a citation of manufacturer protocol (line 13).


- Clinical evaluation of the mutation carriers is really redundant. The description of index cases clinical conditions should be better summarized in a table reporting gene mutations and clinical manifestations.

The description of index cases clinical condition, as required, was summarized in Table 2 reporting gene mutations and clinical manifestations.

- Discussion: page 9, last line Synus node dysfunction at the onset of the disease is not typical....; "please support this observations w data already published or delete it.
The sentence “Synus node dysfunction at the onset of the disease is not typical...” was deleted.

- page 11, first line: His EDMD symptoms did not appear .....; Is this phenotype in keeping w EDMD? Are you sure? Any detection of elbows, TA contractures? rigid spine? More likely LGMD1B

The sentence “His EDMD symptoms did not appear...” refers to cited reference [37], not to our patient’s data. In order to make it clearer, we included an additional citation earlier, at the end of the previous sentence.

**Regarding the comments of the second reviewer (Joakim Klar):**

1. Mention the GenBank reference sequence and version number for the LMNA transcript studied.

Reference sequence numbers for the studied LMNA transcripts are mentioned in the Expression Analysis paragraph on Page 5.

2. What is known about lamin aggregate formations for the other mutations (non-sense versus missense).

We now provide this information in the 3rd paragraph of the Discussion.

3. Could the authors comment on the severity of the phenotype for different mutations (non-sense, missense, and protein domains).

The comment on the severity of the phenotype for different mutations is now given in the 4th paragraph of the Discussion and additional information is given in Table 2.

4. Write systematic names for both DNA and protein variations at the first mentioning in the methods. This will make it easier to follow how the mutations where screened for at the DNA level. Also, this could also be included in the Figure 1 text, as this describes the variants at the DNA level.

As suggested, the names for DNA variations were added to the text in the first two paragraphs of “Methods section” (Patients subsection). Figure 1 description was also modified, accordingly. At the end of the Mutation screening section, we now provide reference for the nucleotide and protein positions nomenclature.

5. Describe what the arrow indicate in Figure 4B (focal breakage) and perhaps add arrows to Figure 4A and C to draw the readers’ attention to the described changes of the nuclei.

As suggested, we now described what the arrow indicates and added the arrows to Figure 4A (abnormal distribution of chromatin) and C (halving of the nucleus).

6. The level of magnification used should be added to the text of Figure 5.

The level of magnification (63x) has been added.

In addition to changes introduced as a result of Reviewers’ comments, we also changed the sentence at the end of 1st paragraph of Results: ‘Five of the nine mutations were identified in the setting of familial disease’ into ‘Four of the nine mutations were identified in the setting
of familial disease’. This was done since we realized that the sister of the Family E proband
did not meet the criteria for DCM although she was hospitalized many times due to episodes
of severe dyspnea or fainting (variety of arrhythmia but with preserved LV systolic
function). We also amended Figure 2 accordingly. We also added one author (Agnieszka
Sioma) who by mistake was omitted from the author’ list in the first version of the paper.

Once again, we appreciate the comments and are hopeful that we were able to satisfactorily
address the issues raised by the reviewers.

Yours sincerely,

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