Author’s response to reviews

Title: VARS2-LINKED MITOCHONDRIAL ENCEPHALOPATHY. TWO CASE REPORTS ENLARGING THE CLINICAL PHENOTYPE

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Author’s response to reviews:

Dear Editor,

We are very grateful for the careful evaluation and the constructive suggestions from both review-ers and yourself, which have significantly improved the paper.
We now submit a revised version of the manuscript for further consideration on your journal.

Please note that changes of the first version are either highlighted for added sentences or strikethrough for deleted sentences in the revised (clean) version.

For your convenience, below please find our point-by-point response explaining how we have addressed each of the reviewers’ comments.

We hope to have satisfied your requirements and that the paper is now suitable for publication on your Journal.

Best regards

Yours sincerely,

On behalf of the co-authors
Chiara Begliuomini, MD

Editor’s Comment:

Your manuscript "VARS2-LINKED ...(omissis)… by our reviewers.
Our reply: thanks for your appreciation and offer. We kindly ask, if it is possible, to modify the affiliation of Filippo M. Santorelli, Denise Cassandrini and Claudia Nesti as follows “Molecular Medicine for Neurodegenerative and Neuromuscular Diseases Unit, IRCCS Fondazione Stella Maris, Viale del Tirreno, 331 56018 Calambrone, Pisa, Italy”. (Page 1, line 18).

Moreover we would like to inform you that we also added some minor changes while revising the manuscript. In particular we added in page 3, line 10 “cardiomyoencephalopathies” as related to VARS2 mutations. We also changed the word count as follows: Abstract: 266; Text: 1717.

Reviewer(s)’ Comments to Author:
Marie Sissler (Reviewer 1): In the present manuscript, C. Begliuomini and co-workers ...(omissis).. exemplified the complexity of AARS2 related disorders. As a very minor comments:

- Reference Kemp et al. 2011, may not ...(omissis)... highly e-specific reaction.
- Could it be considered that the cardiomyopathy is not yet visible in the patients, but that it may occur over time since the two of them suffer from hypotonia
Our reply: thank you for this comment. In the Conclusions section (page 9, line 6) we added a sentence to point out this issue: “As for clinical phenotype almost all patients with VARS2 mutations present with severe early-onset encephalopathy with hypotonia. Albeit hypertrophic cardiomyopathy has never been observed in patients carrying the biallelic c.1100C>T (p.Thr367Ile) variant, this is a common feature of other known VARS2 gene mutations, including those who are compound heterozygous for the p.Thr367Ile variant (Bruni et al. 2018). The early onset and the hypertrophic features suggest that cardiomyopathy could be related to a genetic etiology, rather than being a consequence of global hypotonia.”

Sandra Jackson, Ph.D. (Reviewer 2): Summary:
The authors present 2 further patients ...(omissis)... North Sardinians are an inbred population.

General comments.
- In the background section, please provide more information ...(omissis)... with this disease.
Our reply: thank you for pointing this out. We reformulated the sentence as follows (page 5, lines 7-13):” Rare bi-allelic variants in VARS2 have been associated with mitochondrial encephalopathies or cardiomyoencephalopathies in 13 families with 17 affected individuals worldwide (Baertling et al. 2017, Diodato et al. 2014, Ma et al. 2018, Pereira et al. 2018, Taylor et al. 2014). To date the p.Thr367Ile variant is the most common. The homozygous c.1100C>T (p.Thr367Ile) mutation has been described in six patients presenting with encephalopathy (Bruni et al. 2018, Diodato et al. 2014, Pereira et al. 2018) and the correlation between genotype and phenotype appears loose.”

- In the section Conclusions, the authors should discuss ...(omissis)... variants in VARS2.
Our reply: In the Conclusions section (page 9, line 6) we added a sentence to point out this important issue raised by the reviewer: “As for clinical phenotype almost all patients with VARS2 mutations present with severe early-onset encephalopathy with hypotonia. Albeit hypertrophic cardiomyopathy has never been observed in patients carrying the biallelic c.1100C>T (p.Thr367Ile) variant, this is a common feature of other known VARS2 gene mutations, including those who are compound heterozygous for the p.Thr367Ile variant (Bruni et al. 2018).”

- Methods. The methods are not ...(omissis)... the protocol used.
Our reply: thank you for raising this point. We added in page 6, line 24 “Blood DNA from the proband was analysed using a customized targeted resequencing panel (MitoChip) able to investigate the coding regions of 1172 nuclear genes encoding the “MitoExome” (De Michele et al. 2018). Using this strategy we identified the homozygous c.1100C>T (p.Thr367Ile) in VARS2 (NM_001167734)”, and in page 8, line 7 “Exome sequencing using reported methodologies (Diodato D et al. 2014) revealed the homozygous c.1100C>T (p.Thr367Ile) mutation in VARS2.”

Further points:
1) In the abstract, it is stated ...(omissis)... have been described worldwide.
Our reply: We reformulated the sentence as “Yet only six cases (of which three are siblings from the same parents) harbouring this homozygous mutation have been described worldwide” (Abstract, background section. Page 3, line 10).

2) In the same section, ...(omissis)... amend this sentence accordingly.
Our reply: We definitely agree with your meaningful comment and changed the sentence, as suggested, as follows: “Among these, the rare homozygous c.1100C>T, p. Thr367Ile c.1100C>T (p.Thr367Ile) mutation variably presents with progressive developmental delay, axial hypotonia, ataxia, limbs spasticity, drug-resistant epilepsy leading, in some cases, to premature death”. (Abstract, background section, page 3, line 7-10).
3) In the Conclusion section …(omissis)… form are of Northern Sardinian extraction. Our reply: thanks to have raised the issue. In the conclusion section of the Abstract we replaced the sentence “Moreover, noteworthy, three out of eight cases so far described belong to the Northern Sardinia ethnicity” with “Moreover, noteworthy, three out of five pedigrees so far described belong to the Northern Sardinia ethnicity.” (Page 4, line 3).

4) In the Background section (page 4, line 18) …(omissis)… after submission of this article). Our comment: thank you for pointing this out. We included the reference as suggested.

5) Page 5, line 50: The authors should supply t …(omissis)… according to HGVS nomenclature. Our reply: we replaced “c.1100C>T, p. Thr367Ile “ with the correct nomenclature“ c.1100C>T (p.Thr367Ile) mutation”, as you kindly suggested.

6) Conclusions, Page 8, line 1: The authors …(omissis)… should be amended accordingly. Our reply: thank your for stressing this point, we welcome the comment and we reformulated the sentence as “The early onset and the hypertrophic features suggest that cardiomyopathy could be related to a genetic etiology, rather than being a consequence of global hypotonia” (Conclusions section, page 9, line 10).

7) Page 8, line 18. Complications related to epilepsy, …(omissis)… exitus should be replaced by death. Our reply: as for this appropriate suggestion we replaced the term “exitus” with “death” and we also reformulated the sentence as follows : “Complications related to epilepsy, hypotonia and global debilitation are responsible for exitus death, which occurs between 2 and 8 years (P1, P3, P4 and P6) occurred for P1, P3, P4 and P6 between 2 and 8 years”. (Page 9, line 19-21).

8) Table 1. Ideally, …(omissis)… should be indicated. Our reply: we welcome this suggestion. We added a column indicating “age at time of report-ing/death and another indicating serum and CSF lactate levels.

9) Table 1. Some clinical details …(omissis)… added to Table 1. Our reply: thank you for noticing. We added the missing symptoms to Table 1.

10) Table 1. What do the superscript 5 and 6 in the second column (Origin) refer to? This is not indicated in the legend to the table. Our reply: thank you for bringing this inconsistency to our attention. It was a typing error and we removed the numbers.

11) Legend to Table 1, line 16. The authors …(omissis)… rather than "assisted”? Our reply: we welcome the comment and changed the sentence as suggested. “Clinical update on P1, subsequent to 2014 publication, was obtained from his medical records at the Unit of Child Neuropsychiatry, University Hospital of Sassari, where he has been treated.”

- English. Although the quality of the written English…(omissis)… to reach a formal diagnosis.

Our reply: thank you very much for the suggested corrections; accordingly, we have modified the text as follows:
- page 6, line 20 “The patient could barely speak in sentences”;
- page 7, line 8 “She is attending school with good social skills. Moreover she is undergoing daily
global psychomotor training”;  
- page 7, line 24 “At age 3, the patient underwent a global psychomotor training”;  
- page 8 line 14 “Owing to genetic and clinical heterogeneity, to nonspecific early signs and routine testing that are often uninformative, diagnosing mitochondrial diseases remains challenging”;  
- page 9, line 1 “When clinical and imaging findings were interpreted together with multigene sequencing, a formal diagnosis was finally formulated”.