Reviewer's report

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

Version: 0 Date: 12 Dec 2018

Reviewer: Sandra Jackson

Reviewer's report:

Summary:

The authors present 2 further patients from two independent families who are homozygous for the c.1100C >T p.(Thr367Ile) pathogenic sequence variant in VARS2, expanding the number of individuals homozygous for this sequence change reported to date to eight (from 6 independent families). Although these individuals originate from Poland (one individual), Portugal (one individual) and Afghanistan (three individuals from one family), three of the six affected families, including the two described in this manuscript, originate from Northern Sardinia, which the authors partly attribute to the fact that North Sardinians are an inbred population.

Comments to the authors.

General comments.
In the background section, please provide more information about the total number of patients who have been described to date with pathogenic sequence variants in VARS2 (13 families with 17 affected individuals). The authors should mention that to date, the p.(Thr367Ile) sequence variation is the most common mutation associated with this disease.

In the section Conclusions, the authors should discuss whether the clinical features shown by the patients homozygous for the p.(Thr367Ile) variant are broadly similar or different to the clinical features of patients who harbor different pathogenic sequence variants in VARS2.

Methods
The methods are not described in any detail at all. The authors should provide more details of the sequencing protocol or should provide a reference for the protocol used.

Further points:
1) In the abstract, it is stated in the background section that only six cases harbouring such a mutation have been described worldwide. The use of the term "such a mutation" renders it unclear as to whether the authors are referring to the p.Thr367Ile sequence variant or to all pathogenic sequence variants so far described in VARS2. If the former is true, the authors should refer to the variant as "this" mutation rather than "such" a mutation. Additionally, this statement is not accurate: six individuals homozygous
for the p.(Thr367Ile) sequence variant have been described to date, but a further 4 individuals have been described who harbour this sequence variant together with a second pathogenic sequence variant (Baertling et al., 2017; Bruni et al., 2018: families 2, 4, and 6). The authors should more correctly state that only 6 patients homozygous for this sequence variant have been described worldwide.

2) In the same section, the authors state that the rare homozygous p.(Thr367Ile) mutation presents with progressive developmental delay, axial hypotonia, ataxia, limb spasticity, drug resistant epilepsy leading to premature death. This gives the impression that these are homogeneous, invariant symptoms found in all affected individuals, yet ataxia was only reported in one patient homozygous for the p.(Thr367Ile) sequence change (Bruni et al., 2018, patient 1), and indeed has only been described in one other patient who harbours two different pathogenic sequence changes in VARS2 (Taylor et al., 2014). Further, two of the individuals who have presented with this sequence variant in homozygous form were alive at the time of reporting (Bruni et al., 2018, patient 1 and patient 13), so the authors should amend this sentence accordingly.

3) In the Conclusion section of the Abstract, the authors state that three out of eight cases so far described are of Sardinian heritage. In fact, three out of these eight cases belong to the same family, which is of Afghan heritage, so the authors should more accurately state that three out of the five pedigrees in which the sequence variant has been identified in homozygous form are of Northern Sardinian extraction.

4) In the Background section (page 4, line 18) the authors should include the reference of Ma et al (Ma K et al. A novel compound heterozygous mutation in VARS2 in a newborn with mitochondrial cardiomyopathy: a case report of a Chinese family. BMC Med Genet. 2018 Nov 20;19(1):202 . doi: 10.1186/s12881-018-0689-3) which was published after submission of this article).

5) Page 5, line 50: The authors should supply the NM number of the VARS2 transcript variant, and use the correct nomenclature for the sequence variant - c.1100C>T p.(Thr367Ile) - according to HGVS nomenclature.

6) Conclusions, Page 8, line 1: The authors state that: As for clinical phenotype, and in contrast to other VARS2 gene mutations, the biallelic c.1100C>T (p.Thr367Ile) variant is not associated with cardiomyopathy, which may suggest a protective effect to the heart. The authors should state more clearly that cardiomyopathy has been reported in all other patients described to date who harbor other pathogenic sequence changes in VARS2, including those who are compound heterozygous for the p.Thr367Ile variant together with a second sequence change. It is highly unlikely that the pathogenic sequence change has a "protective effect" on the heart, as stated by the authors. It would seem more likely that homozygosity for this sequence change is less deleterious to the heart than the other sequence variant combinations described, and the term "protective effect on the heart" should be amended accordingly.

7) Page 8, line 18. Complications related to epilepsy, hypotonia and global debilitation is responsible for exitus, which occurs between 2 and 8 years (P1, P3, P4 and P6). As stated above not all of the patients described with this mutation are reported to have died. The sentence should be amended to reflect this. The term exitus should be replaced by death.

8) Table 1. Ideally, more information should be provided in the table. A column in which the age at the
time of reporting or age at death is provided would be useful. A further column in which laboratory findings such as plasma or csf lactate levels are provided would be informative. Also, the fact that patients P3-P5 are siblings should be indicated.

9) Table 1. Some clinical details are missing from the Table. Patient 1 had facial dysmorphia, in common with the patients reported in the current manuscript. Patient 2 was reported to have ataxia and pathological visual evoked potentials. The fact that Patient A has nystagmus is also not recorded in the table. These symptoms should be added 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.

11) Legend to Table 1, line 16. The authors write "Clinical update on P1, posthumous to 2014 publication, was obtained from his medical records at the Unit of Child Neuropsychiatry, University Hospital of Sassari, where he has been assisted. This sentence is unclear. Do the authors mean "subsequent" to the 2014 publication rather than posthumous to the 2014 publication? Do the authors mean "where he was treated" rather than "assisted"?

English

Although the quality of the written English is generally acceptable, some very minor corrections should be made:

Page 5, line 25: "The patient could barely speak into sentences" should read "The patient could barely speak in sentences"

Page 6, line 1: "She is attending school and daily global rehabilitation with normal social interaction ability". This sentence is unclear and should be restructured.

Line 38. The meaning of the sentence "At age 3, the patient underwent a global psychomotor rehabilitation" is unclear. Do the authors mean that the patient underwent an improvement in her psychomotor skills? Please make the sentence clearer.

Page 7, line 42 the authors refer to the uselessness of routine testing. This would be better described as routine testing is of limited use or is uninformative

Line 49, the same page. The authors write: "When clinical and imaging findings were read upon multigene sequencing, we could reach a formal diagnosis". This sentence is unclear, and probably should read something like: When the clinical and imaging findings were interpreted together with the findings obtained from multigene sequencing, we were able to reach a formal diagnosis.

**Are the methods appropriate and well described?**

If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**

If not, please specify which controls are required in your comments to the authors.

Yes
Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

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