Reviewer’s report

Title: A Novel ISCA2 variant responsible for an early-onset neurodegenerative mitochondrial disorder: a case report of Multiple Mitochondrial Dysfunctions Syndrome 4

Version: 0 Date: 19 Feb 2019

Reviewer: Isabelle Thiffault

Reviewer's report:

Comments to the Author

The paper by Milad Eidi et al is an interesting and important as only three ISCA2 variants (p.L52F; p.G77S; p.R105G) were reported in Human Mutation Databases (HGMD) from five independent reports (PubMed: 25558065 ; 25539947; 27959697; 29297947; 28803783 ). In this singular condition, is rare and not well known in terms of pathophysiology and prognosis. In this case report, the authors report a putative novel pathogenic variant in a proband identified by WES (c.355G>A, p.Ala119Thr) in ISCA2 gene. As for other mitochondrial disorders, the clinical variability especially in Multiple Mitochondrial Dysfunctions Syndromes, makes the diagnosis difficult.

However, using the current guidelines for variant interpretation (https://www.acmg.net/docs/standards_guidelines_for_the_interpretation_of_sequence_variants.pdf); this missense detected in this affected proband remains a variant of unknown clinical significance without functional studies. With current guidelines: c.355G>A, p.Ala119Thr) in ISCA2

1. PM2: absent or rare in population databases (including the ethnic specific dataset)

2. PP1: homozygous in proband, heterozygous in parents. Segregation cannot be used if only one affected individual but as the boy was from an Iranian consanguineous, we could say "supporting" as no other homozygous variants were detected in genes related to patient's phenotype.

3. PP3: variant p.Ala119Thr is Moderately conserved amino acid (considering 12 species) located in the FeS cluster biogenesis domain; predicted by Sift Tolerated but Disease causing by MutationTaster.

4. To bring to Likely-pathogenic; 2 more supporting evidence are needed. At this time, the p.Ala119Thr is a variant of unknown clinical significance that is suspicious for ISCA2-deficiency / Multiple Mitochondrial Dysfunctions Syndrome. However, as this has not
been reported in other Iranian affected individuals, the current data is insufficient to confirm

1) this variant as Pathogenic (please avoid using mutation; follow ACMG & HGVS recommendation for interpretation and nomenclature [http://www.hgvs.org/]. This variant is a VUS.

2) Indeed, insufficient data to make diagnosis on this proband

3) For mitochondrial diseases, especially Multiple Mitochondrial Dysfunctions Syndromes, there is NO Clinical signs and symptoms were compatible with reported patients with bi-allelic pathogenic variants in a single gene. Very high genetic & clinical heterogeneity

There is also a need for additional details on the methods used for variant filtering. Should the author improve their ms, the interest of discussing how difficult to make a diagnosis with novel missense without functional data. Authors should contact the expert lab (Wellcome Centre for Mitochondrial Research, Institute of Neuroscience, The Medical School, Newcastle University) for functional data. They may have also seen this variants in referred cases (Hum Mutat. 2018 Apr;39(4):537-549.).

Additional points

1. Remove all use "mutation"

2. Use ACMG variant interpretation guidelines

3. Provide table with all homoz. Variants detected in proband

4. Does the ISCA2 variant located in a large regions of AOH? How many regions of AOH detected in proband? And their size…

5. Change "diagnosis" for this variant is suggestive of a ISCA2-deficiency, however, additional data / patient with same variant is required to confirm diagnosis….

6. Table 1 should be in sup data, not MS

7. Images need better resolutions

8. Figure 2-4 are not useful; sup data not MS

9. Supp Table 2: "In-sillico" is not referred as "clinical Significance".

10. Supp Table 2 should include NM_id; cDNA and protein of the variant detected
11. Supp Table should include all homoz. Variants detected; and de novo variant should not be excluded because family is consanguineous [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5515956/]

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

No

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

No

**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

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Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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None

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