Reviewer's report

Title: POLG1 R722H mutation associated with multiple mtDNA deletions and a neurological phenotype

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Reviewer: Nereo Bresolin

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In this paper, Komulainen and colleagues describe two families presenting a neurological phenotype associated to R722H variant in POLG1 gene. In the first family the proband is an 83-year-old man with sensorineural hearing impairment, type 2 diabetes mellitus, dysphagia and external ophthalmoplegia. He and his two affected sisters carry R722H in homozygous state.

In the second pedigree, R722H is described in compound heterozygosity with W748S substitution in two siblings with mental retardation, psychiatric symptoms and mild bilateral ptosis.

R722H has been previously reported in two patients with sporadic Parkinson’s disease and in one control but its pathogenic role is not well-established yet. Here, for the first time, this variant is associated with mitochondrial presentations and multiple mtDNA deletions.

This work contributes to increase clinical heterogeneity of POLG1-mutated patients but it is not conclusive about the pathogenic role of R722H variant in POLG1 gene.

Several important issues must be addressed before final acceptance.

- Molecular analysis by long-PCR disclosed the presence of multiple mitochondrial deletions in Patient 1 muscle. Unfortunately, this assay has not been performed in muscle tissues derived from his two siblings.

  Southern blot analysis is still a straightforward technique to detect large scale mtDNA rearrangements. Can the authors confirm mtDNA deletions by Southern blot analysis? In Figure 4 is the control healthy subject age-related to Patient 1? Accumulation of mtDNA multiple deletions in muscle from elderly people is not uncommon.

- Authors state that dementia and sensorineural hearing impairment segregated consistently with the R722H mutation in Family 1. However, clinical features of other family members are lacking. No anamnesis are provided for ascendants and descendants of the affected members. The identification of healthy R722H carriers would be useful to define the recessive inheritance of this mutation and its pathogenic role.

- mtDNA multiple deletions have been associated to mutations in several genes. Apart from POLG1, other genes should be eventually investigated (PEO1, ANT1,
POLG2).

- In Family 2, Patient 4 and 5 present a similar phenotype. According to authors, their mother shows cardiac symptoms, cognitive impairment and impaired hearing. These findings are not uncommon in several mitochondrial syndromes. Maternal inheritance due to a mutation in mitochondrial DNA could explain the worsening presentation of the offspring. Did authors analyze mitochondrial DNA? Moreover, as observed by the authors, W748S mutation alone has been shown to cause a catalytic defect involving poor DNA synthesis and primer extension. Carrier status for this mutation could account for maternal milder phenotype?

- Poor conservation of R722 residue is not suggestive of a recessive pattern of inheritance. Can the authors better comment the putative importance of this amino acid in POLG1 linker region? The specific role of arginines should be better discussed.

Minor revisions

- Parental relationships are not fully clear. We learn that Patients 2 and 3 are sisters of Patient 1 only in the discussion. A better comprehension would be achieved including family trees.
- A panel showing histological and histochemical findings of the muscle biopsy of Patient 1 should be included.
- Page 10: please include sequence of reverse primer used to detect R722H
- Page 11: reference 26 is inappropriate for the sentence.
- Page 11: reference 28 could be omitted (please include LNA-primer sequences)
- Page 11 (line 13): Patient A1 should be replaced with Patient 1
- Page 15 (line 10): “is” must be removed

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

'I declare that I have no competing interests'