Author's response to reviews

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

Authors:

Tuomas Komulainen (mikakomu@mail.student.oulu.fi)
Reetta Hinttala (reetta.hinttala@oulu.fi)
Mikko Kärppä (mikko.karppa@oulu.fi)
Leila Pajunen (leila.pajunen@ppshp.fi)
Saara Finnilä (saara.finnila@hotmail.com)
Hannu Tuominen (hannu.tuominen@ppshp.fi)
Heikki Rantala (heikki.rantala@oulu.fi)
Ilmo Hassinen (ilmo.hassinen@oulu.fi)
Kari Majamaa (kari.majamaa@oulu.fi)
Johanna Uusimaa (johanna.uusimaa@oulu.fi)

Version: 3 Date: 31 January 2010

Author's response to reviews: see over
RESPONSE TO THE COMMENTS BY THE REVIEWERS

Reviewer 1:

Comment 1. I think, that for providing the pathogenic role of the p.R722H mutation it would be important to exclude mutation in other possible nuclear genes causing multiple mtDNA deletions (PEO1, ANT1, POLG2).

Response 1: We have sequenced the PEO1 (Twinkle), ANT1 and POLG2 genes from blood DNA of patients A1 and A2 and no mutations in these genes were found.

Location of changes: 1) Abstract. 2) Methods and results.

Comment 2. The patients with homozygous p.R722H had a late onset mild phenotype for an autosomal recessive POLG defect. Memory impairment, dementia and psychiatric symptoms were the main presenting features in these patients. I think the authors should make a point for the discussion about this as well, since this is not typical for other POLG mutations.

Response 2: This matter is taken account in the discussion.

Location of changes: Discussion.

Reviewer 2:

Comment 1. 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.

Response 1: Unfortunately, muscle DNA of the siblings was not available, therefore we were not able to perform long PCR assay. Southern blot with biotin-labeled probe was performed on muscle DNA of patient A1. Long PCR was performed again on patient A1 and four healthy controls, age of which ranged from 75 years to 89 years.

Location of changes: 1) Methods, results and discussion. 2) Former image presenting long PCR assay is replaced with image of the new long PCR assay.

Comment 2. 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.

Response 2: A family tree of family A is included and medical history and mutation status with respect to p.R722H are presented in Table 2.
Comment 3. MtDNA multiple deletions have been associated to mutations in several genes. Apart from POLG1, other genes should be eventually investigated (PEO1, ANT1, POLG2).

Response 3: See Response 1 to Reviewer 1.

Comment 4. 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?

Response 4: Aside to long PCR assay performed on available buccal and blood DNA samples, mitochondrial DNA was not analyzed.

Comment 5. 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.

Response 5: Role of arginines is commented and 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.

Response: A family tree of the first family is included.

Location of changes: A family tree included as Figure 2.

- A panel showing histological and histochemical findings of the muscle biopsy of Patient 1 should be included.

Response: The histological and histochemical findings of the muscle biopsy is presented in Table 1.

Location of changes: Table 1 included.

- Page 10: please include sequence of reverse primer used to detect R722H

Response: The sequence of reverse primer is included as suggested.

- Page 11: reference 26 is inappropriate for the sentence.

Response: Corrected as suggested.
Response: The sequence of LNA primers are included as suggested.

Response: As the family tree of the first family (family A) is included, patients are now coded as A1, A2, A3 and so on to avoid confusion with the members of the second family (family B). Therefore, patient 1 is patient A1 after this addition.

Response: Corrected as suggested.

Comment 1. Evidence of pathogenicity of the p.R722H POLG1 polymorphism is not entirely convincing. Luoma et al. found this polymorphism in 1% of controls. The authors claim that the mitochondrial abnormalities detected in muscle of patient 1 support pathogenicity; however, muscle of healthy people over age 60 typically shows a few ragged-red fibers and multiple deletions of mtDNA by PCR. What proportion of muscle fibers were ragged-red or cytochrome oxidase deficient? Southern blot analysis should be performed to better assess the levels of the mtDNA deletions. Also, if possible, biochemical activities of mitochondrial respiratory chain enzymes should be measured.

Response 1: The muscle biopsy sample was studied by a pathologist, who noticed technical difficulties concerning preservation of the sample. Ragged-red fibers and cytochrome c oxidase negative fibers were present, but the margin of the biopsy was technically defective and the proportion of ragged-red fibers and cytochrome c oxidase negative fibers could not be evaluated with certainty. Southern blot analysis was performed on two healthy controls (aged 80 and 89 years). We were not able to measure biochemical activities of mitochondrial respiratory chain enzymes.

Comment 2. The fact that patients 1, 2, and 3 are siblings must be clearly stated in the Abstract or in the descriptions of the patients.

Response 2: Corrected as suggested.

Comment 3. The left basal ganglia infarct in patient 1 is not visible in Figure 1. Is the lesion suggestive of a lacunar infarct?

Response 3: Figure 1 does not show the lacunar infarct as the image slice is above basal ganglia. The lacunar infarct would be seen in lower slices. The main finding in Figure 1 is cortical and central atrophy.

Comment 4. Additional family history of patients 1, 2, and 3 should be reported. Were there any unaffected siblings? Were parents consanguineous? Did any relative have Parkinson disease?

Response 4: A family tree and medical history are included.

Location of changes: 1) A family tree included as Figure 2. 2) Additional family history is presented in Table 2.
Comment 5. The p.W748S mutation has been frequently identified in cis with the p.E1143G polymorphism. Did patients 4 and 5 have this second polymorphism?

Response 5: Sequencing revealed p.E1143G polymorphism in patient 4, but blood and buccal DNA samples of patient 5 (patient B2) were exhausted during p.R722H and p.W748S detections and long PCR. Thus, POLG1 gene was not sequenced from patient 5 and therefore we were not able to confirm this polymorphism in patient 5.

Location of changes: This has now been clarified in the Results section.

Comment 6. The manuscript requires editing to correct grammatical errors.

Response 6: The original version of the manuscript has been revised by a native English speaker.