Author's response to reviews

Title: Polg1 mutations and stroke like episodes: A distinct clinical entity rather by an atypical MELAS syndrome

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Author's response to reviews: see over
Polg1 mutations and stroke like episodes: A distinct clinical entity rather by an atypical MELAS syndrome

Dear Editor,

Please find enclosed the revised versions of the manuscript that we resubmit to Editors, after careful revision and editing the manuscript (using colored text) according to Editor and Reviewers suggestion. Detailed answers to reviewers are reported below. English language was edited too by native speaker, as requested.

We hope that our paper could now be suitable for publication in your Journal.

Your Sincerely,

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Reviewer 1: Rita Horwath

The manuscript by Cheldi et al. reports on a patient with a MELAS-like clinical presentation, who carries mutations in the POLG gene. The clinical presentation of the patient is late onset and relatively mild. The authors discuss MELAS-like presentation as a clinical phenotype of POLG mutations.

Comments:

1. The abstract should summarize the findings of the reported patient, and should not focus on discussing a previous paper (not very recent, from 2007).

   We agree with reviewer comment and modified abstract as suggested.

2. Patient’s history: The focal hyperintensities are described as older ischaemic lesions. Do the authors think that these lesions are stroke-like lesions related to the POLG mutations, or signs of a real cerebrovascular problem? The later would not be unusual in a 74-79 years old patient.

   We are not completely sure about a common origin of all found lesions. Since some lesions were not confined to a specific vascular territory, a common pathogenetic origin can be supposed, although an overlapping cerebrovascular disease cannot be excluded. However we modified MRI description and added a comment in the discussion part.

3. The detailed listing of the facial muscles, but not the muscles of the extremities for electrophysiological examination is very unusual. Did the patient have peripheral neuropathy? This would be very common in POLG deficiency.

   As suggested by reviewer we listed examined muscles and changed electrophysiological examination description.

4. Were there any family members tested for the detected POLG mutations? The p.T251I and p.P587L mutations were repeatedly reported in cis on the same allele. I think it would be good to show that the third mutation, p.D1186H is on the
other allele, which could be shown by analysis of family members.

We did not test any other family member for POLG mutations since parents are not alive and siblings are not available. Unfortunately, proband’s muscle is not available to obtain RNA in order to check, after cloning of RT-PCR products, if the detected mutations are in cis or in trans. The hypothesis of a compound heterozygous asset of [p.T251I and p.P587L] and p. D1186H mutations is highly probable, although it cannot be definitely demonstrated.

5. Conclusions: POLG has 23 exons not 22, however exon 1 is not coding. I don’t understand the final conclusion of the paper.

We apologize for mistake and made the change.

6. Do the authors believe that there is a MELAS-like presentation of POLG deficiency? I think it is not possible to contradict it based on the clinical presentation of this single patient.

We discussed this point in discussion part

Minor comments:

There are also some small grammatical mistakes and the English would require corrections.

English language and grammatical mistakes were edited by native speaker, as requested.

“Oculistic” examination – ophthalmological examination

Should not use any abbreviations, such as 79 y.o. in the abstract.

We made the changes as suggested

Reviewer 2: Marcus Deschauer

The authors present an interesting patient with mitochondrial disease due to POLG1 mutations who suffered from stroke symptoms at age 74 and 78 years. A very late onset of stroke-like episodes is unusual for patients with POLG1 defects.

There are two major questions that should be answered by the authors:

1. Could the episodes be “normal” strokes in an older patient in co-incidence with
mitochondrial disease? This is not discussed in the paper. Did the patient have vascular risk factors for cerebral ischemia?

**We added this comment in the discussion part as suggested by reviewer**

In MELAS syndrome due to the 3243A>G mutation as well as in POLG1-associated encephalopathy headache and seizures during the episodes are frequent. Missing of these symptoms argues against stroke-like episodes but for “normal” strokes in the patient. This has to be discussed. Was an EEG performed that frequently shows epileptic activity in POLG1 defects?

**EEG resulted normal and we added this aspect to case report description. We added also data about headache and seizures.**

If we believe that there were stroke-like episodes due to mitochondrial disease the next question is how to classify them (MELAS or POLG1-associated encephalopathy). This question is addressed in the paper highlighting the complexity of the genotype–phenotype relationship in mitochondrial disorders. However, the authors should emphasize that MELAS was defined as a clinical syndrome in 1984 and POLG1 associated encephalopathy is a molecular genetically defined term thus it is difficult to compare them?

**We emphasized this aspect in the discussion modifying some points of this part**

2. The authors have to highlight the novel findings in their case. Is it just a novel POLG1 mutation p.D1186H (with uncertain pathogenicity)?

**While the pathogenetic role of p.T251I and p.P587L mutations is undisputable, the missense mutation c.3556G>C, leading to protein change p.D1186H, has never been investigated by functional studies. Nevertheless several elements support its pathogenetic significance: i) it has never been detected in more than 200 ethnic-matched controls; ii) is affects an evolutionary conserved residue; iii) three software programs (Polyphen2, SIFT, PMut) predicted a highly
deleterious effect of p.D1186H on POLG structure or stability; iv) D1186 residues is located in the DNA binding channel, a fundamental domain of POLG enzyme.

Some other minor details should be addressed:

Background:

Our paper (Deschauer et al. 2007) has reported a young man with a single stroke-like-episode (not episodes as mentioned in the manuscript) fulfilling clinical criteria of MELAS.

Case presentation:

1. Hemiparesis should be graduated using MRC score. MRC can be quantified as 4 in proximal left arm muscles. However we did not report this scale in the paper since it is not the best scale to evaluate strength in stroke patients or patients with central nervous system lesion. We added indeed NIHSS score.

2. The authors should specify neurophysiological examination. I guess they performed needle EMG. What do they mean by “mouth muscle”? Why did the authors examine several facial muscles? No facies myopathica is mentioned. If the authors believe that this is important they should define EMG findings in detail as EMG of facial muscles shows small and shorts potentials in normal individuals. Which other muscles were examined in legs and arms?

   We detailed neurophysiological examination as requested.

3. What was the course of aphasia after the second episode.

   We added the patient’s follow-up as requested.

4. Sensory neuronopathy and cerebellar ataxia are frequent finding in patients with POLG1 mutations. Thus the authors should state if there was sensory or cerebellar ataxia?
As suggested by reviewers, we added this information in the case presentation.

5. What was the time interval between onset of symptoms and MRI scans? This information is important for interpretation of diffusion weighted images.

We changed the MRI description according to reviewer suggestion.

5. It is unnecessary to mention the results of histological investigation and genetic testing in the patient’s history as they are presented in detail later.

We deleted these mentions and changed this part, as suggested by reviewer.

6. At the end of the patient’s history the authors state that other mtDNA mutations were searched. What is meant by “other”? Are other mtDNA point mutations (as mentioned in the introduction) excluded?

We deleted the incorrect sentence.

7. It is not important to mention the restriction enzyme Hae III for RFLP as the method is not shown in detail. In contrast the authors should explain briefly (or give a reference for) the PCR assay showing multiple mtDNA deletions as this is demonstrated in figure 2B.

PCR assay for detecting multiple deletions used two primers (forward 7440–7465 and reverse complement 16135–16110) and the following amplification protocol: an initial denaturation at 94°C for 2 min, followed by 25 cycles (94°C for 30 s, 55°C for 30 s, and 68°C for 90 s) and a final extension for 2 min at 72°C (Platinum HiFi Taq Polymerase by Invitrogen, Carlsbad, CA).

8. The authors should quantify the amount of ragged-red fibers and COX-negative fibers.

We changed the sentence adding the available data

9. The authors should clearly state in the section "Genetic analysis" if the POLG1 mutations were detected in heterozygous state.

All the POLG1 variants were detected in heterozygous state.

Discussion
1. In the case presentation the authors show a normal lactate level but in the discussion they have mentioned lactic acidosis.

We apologise for the mistake and deleted it in the discussion part

2. The authors state that the p.T251I and p.P587L mutations are reported already but give no references. Is p.D1186H a novel mutation? Are the reported mutations known as recessive mutations? The authors should examine family members to find out if the mutations are present in cis or trans.

The variants p.T251I and p.P587L were reported several times in subjects showing multiple clinical phenotypes, according to the Human DNA Polymerase Gamma Mutation Database (http://tools.niehs.nih.gov/polg/). The change p.D1186H has not been previously described. We did not test any other family member for POLG mutations since parents are not alive and siblings are not available. Unfortunately, proband’s muscle is not available to obtain RNA in order to check, after cloning of RT-PCR products, if the detected mutations are in cis or in trans. The hypothesis of a compound heterozygous asset of [p.T251I and p.P587L] and p. D1186H mutations is highly probable, although it cannot be definitely demonstrated.

3. The author should discuss the results of diffusion MRI regarding normal stroke or mitochondrial disease. How do they explain that MRI was unchanged after the second episode despite global aphasia and right-sided hemiparesis.

We apologise for the mistake but we examined carefully MRI images and DWI sequences and found a DWI restricted parieto-temporo-occipital ischemic lesion in the last MRI explaining aphasia and right hemiparesis. We also added this findings in the case presentation.

Finally there are several typos or unusual terms such as brachial bicep muscle, sensorimotor bilateral hypoacusia, oculistic examination, POLG-encefalopathy, mithochondrial disease, mithochondrial POLG1, mithochondrial signs.....

We apologise for typos and unusual terms and changed incorrect terms.