Reviewer's report

Title: Molecular and biochemical characterisation of a novel mutation in POLG1 associated with Alpers syndrome

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Reviewer: William Copeland

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This paper adds to our collection of POLG mutations by describing a novel splice site mutation. The authors describe the molecular analysis of an Alpers patient that harbors the A467T mutation in one of the POLG alleles and a novel splice site mutation in the other POLG allele. The authors do demonstrate that this mutation does lead to excision of exon 7 in the transcripts. One strength that should be emphasized is the tissue specific differences observed in mitochondrial DNA content and OXPHOS activities between liver, muscle and in fibroblasts. Several other items need to be addressed as listed below.

Major Compulsory Revisions:

The authors state that expression of the c.1251-2A>T allele is not vulnerable to NMD, which should be true, however, the RT-PCR analysis of the transcripts shown in Figure 1D definitely shows less PCR product from Exon6,8 as compared to Exon 6,7,8 which suggests that this allele is not expressed at the same level. Visually the RT-PCR of c.1251-2A>T allele appears only at 20% the level as compared to the A467T allele. This indicates that only a small fraction of the c.1251-2A>T allele undergoes alternative splicing with skipping of exon 7. The authors need to quantitate what percent of transcripts from the c.1251-2A>T allele actually skip exon 7. This can be accomplished by sequencing individual RT-PCR clones over the exon-6-exon8 area and statistically determine the number of clones derived from each allele. Alternatively, this can be accomplished by real-time analysis with specific primers that span the new exon6-exon8 junction. Please see the following reference that describes similar analysis of a new POLG splice site (De novo mutation in POLG leads to haplotype insufficiency and Alpers syndrome. Chan SS, Naviaux RK, Basinger AA, Casas KA, Copeland WC. Mitochondrion. 2009 Sep;9(5):340-5. ).

One of the major strengths of this paper that the authors haven’t expanded on is the finding that there was little observed defects seen in the fibroblast as compared to the muscle and liver tissues. This has been observed by other investigators in the field with fibroblast and emphasizes the need to analysis primary tissue. The findings here suggest that researchers should be cautious with fibroblast as they may be misleading, and more accurate results can be obtained by analysis of primary affected tissue. This idea should be discussed and mentioned in the abstract.
Conclusion: Page 9, last paragraph. Please correct “in cis” to be “in trans” as follows: “Analysis of the patient’s parents confirmed that these mutations are present in trans in the patient.”

This paper lacks critical detail to be properly evaluated and several items are left out or mislabeled (see below for details):

In the results, please provide some statistical data for the amount of mtDNA in the fibroblasts.

Please define the control population and describe this in the methods section.

Figure 1 legend is incorrect and missing many of the panel descriptions. Panel A is genomic analysis, not the pedigree (which is panel e). Please correct this legend and supply more information to address the following: Is panel A and B derived from genomic DNA, if so, please indicate. Is panel C sequence analysis from a cDNA total population or from a gel isolated PCR fragment (such as from the gel in panel D).

Figure 2 needs labels to identify which panel is from which tissue (i.e. Panel A were from fibroblasts). Figure 2 patient data needs error bars. Please include the standard error analysis for these results. Also, please state what the internal house keeping gene control used for the real-time analysis.

Figure 3. Please show the entire gel to show the markers and loading controls. How were these activities quantitated from this gel for the results in table I.

Table 1 needs statistical analysis and standard errors reported. Please state the source of ranges for the controls.

Minor Essential Revisions
In the Abstract, please list “in trans” after “…found compound..”

Abstract conclusion: How does POLG genetic analysis prevent these mitochondrial disorders. Please reword to something more appropriate like: “…this reinforces the need to evaluate POLG before starting valproic acid treatment. “

There are several reports describing the defects of OXPHOS complexes due to POLG mutations that should be mentioned in the background (see de Vries et al., Eur J Pediatr. 2007 Mar;166(3):229-34.)

The name of the pol gamma gene is POLG, not POLG1. POLG1 is an alias.

There are numerous typographical errors throughout the paper.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interest