Reviewer's report

Title: A new candidate mutation in the mitochondrial 12S rRNA, 904C >T, associated with hearing loss: systematic analysis by dHPLC

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Reviewer: Haris Kokotas

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The Japanese group, well-known in the field of genetics of deafness in their manuscript entitled 'A new candidate mutation in the mitochondrial 12S rRNA, 904C>T, associated with hearing loss: systematic analysis by dHPLC' they describe the results of a clinical and molecular evaluation of 54 prelingual and 80 postlingual sensorineural deafness Japanese subjects. They used the WAVE dHPLC technology in order to scan the mitochondrial genome of these subjects and they evaluated their results using controls and several in silico tools. Subjects previously found to harbor the A1555G or A3243G mutations were excluded from the study. I have a few minor comments:

1. In the abstract, it is stated that ‘...were subjected to mutational analysis of several mtDNA genes.’ The authors should clearly state in the abstract which genes they tested in order to help researchers access their paper when using keywords in databases (i.e. PubMed).

2. In the abstract, the statement ‘...1555A>G and 3243A>G in mtDNA frequently found in hearing loss patients’, is somewhat confusing. Can we actually refer to these mutations as ‘frequent’ ones? The A1555G mutation is indeed the most common mtDNA mutation worldwide but mtDNA deafness mutations in general are not ‘common’. The A3243G mutation is even more rare.

3. In the background section, the authors state that ‘...Mutations in mitochondrial DNA (mtDNA) are frequently responsible for hereditary hearing loss’. Again, this statement is wrong as it is known that mitochondrial mutations account for <1% of sensorineural deafness cases worldwide.

4. At the end of page 3/start of page 4: “Mutations of 7445A>C/G/T [14-16], 7472insC [17], and 7510T>C [18] in the tRNAser(UCN) are also associated with aminoglycoside-induced or nonsyndromic hearing loss.” The 7472insC mutation is also associated with syndromic deafness combined with neurological dysfunction, while the A7445G mutation has also been reported in cases with deafness and palmoplantar keratoderma.

5. At the end of page 5, the authors state that GJB2 mutations were excluded by PCR-RFLP and/or bidirectional sequencing and give references [13,42,43]. It would be helpful to mention in brief what is their strategy, i.e. they first use PCR-RFLP for frequent mutations of GJB2 in Japan (235delC) and in heterozygotes they sequence the whole coding region in order to explore the
existence of a second mutation?

6. In page 6, the authors set the age limit for prelingual deafness to be 4 years old and for postlingual to be 5 years old but they need to clarify if this is an objective classification which follows certain guidelines or it is subjective.

7. The manuscript is too wordy and could be significantly reduced in length. Especially the ‘Prediction of pathogenicity of mtDNA mutations’ and the 'Discussion' sections.

8. In page 16, ‘...the inheritance of hearing loss in the child is likely due to the transmission of an autosomal genetic mutation, not mtDNA, from the proband.’ I assume the authors mean ‘from the father’?

9. In page 17 and start of page 18, the authors discuss that the uncertainty of the role of the T7501A mutation which was found in three patients but not in controls will be enlightened by further studies. It would be essential to add that the studies needed are i) isolation of total mitochondrial RNA from lymphoblastoid cell lines derived from individuals with the T7501A mutation in order to examine the steady-state of the tRNA-Ser(UCN), and ii) measurement of the endogenous respiration rates of cell lines by determining the O2 consumption.

10. There are a few typo errors in the text (‘otoxic’ instead of ‘ototoxic’, etc).

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

**Quality of written English:** Needs some language corrections before being published

**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.