Reviewer's report

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

Version: 1 Date: 28 July 2011

Reviewer: Malgorzata Rydzanicz

Reviewer's report:

In the article „A new candidate mutation in the mitochondrial 12S rRNA, 904C>T, associated with hearing loss: systematic analysis by dHPLC” Hideki Mutai et al., performed the mutational screening of the mitochondrial genes suggested as related to hearing impairment in 134 Japanese hearing loss patients and 137 normal hearing controls from the same population. The authors indentified 12 different sequence variants in 12S rRNA gene and one in tRNAser(UCN) suggesting three nucleotide changes, including newly identified 904C>T substitution and previously reported 1005T>C nucleotide change in 12S rRNA gene, as well as 7501T>A in tRNAser(UCN) as possible causative mutations associated with hearing loss in Japanese patients. The authors speculate about the provisional pathogenic nature of selected sequence variants based on comparison of the mutation frequency in patient and control groups, sequence conservation and secondary structure prediction.

I have found this study well performed, results properly interpreted and critically discussed. I recommend this paper for publication in the BMC Medical Genetics after the minor revision:

Please find below some concerns and suggestions to improve this article:

Discretionary Revisions

1) The general remark is that authors use the definition of mutation in huge simplification as any change in a gene sequence. I recommend to describe the sequence variants as mutations in a case of known or suggestive pathological changes, but not for all observed changes, including non-pathogenic polymorphisms and rare sequence variants with unknown function.

2) Affiliation 2 and 3, it is probably not obvious for all readers where these institutions are located, please give at least the city names.

3) Background, page 3, the authors give the frequency of 1555 A>G mutations in range 0.6-5.3% depending on the ethnic group. However, it may be even higher in the Chinese population and Hispanic patients, please verified.

4) Methods>Screening for mtDNA mutations by dHPLC, page 6 “Initially, whole mtDNA from each patient was amplified in three fragments (1351–8197; 6058–12770, and 11706–2258)…”, please add overlapping fragments.

5) Table 1. I suggest to show all sequence variants detected in the control group. Such data gives an additional valuable information about mitochondrial genome
variability in a different populations.

Minor Essential Revision

1) The title of the article is not accurate and should be rewritten.
2) Abstract should be rewritten. I have found some mistakes, including “degenerating high-performance liquid chromatography”, I suppose, instead of denaturing high-performance liquid chromatography (dHPLC). The authors states “…mutational analysis of several mtDNA genes”, which genes, the entire sequence of mtDNA was screening for deafness-related mutations/sequence variants? As abstract is one of the most viewed part of the paper, such information make it more informative for the readers and may be more valuable than “Subjects with pathogenic mutations of GJB2 or 1555A > G and 3243A > G in mtDNA frequently found in hearing loss patients were excluded from the study”, which may be or even should be given in the Materials and Methods section, where studied group is characterized. Moreover, the authors mentioned in the abstract about other mutations 961insC and 961delT+Cn in 12S rRNA gene; however, I have not found even a word about substitutions 1005 T>C and 7501 T>A which are wide discussed in the next parts of the article.
3) Methods>Subjects, page 5: “Prior to this study, the patients were confirmed not to have 1555A > G and 3243A > G mutations in mtDNA nor pathogenic mutations in GJB2 by our routine methods of RFLP-PCR or direct sequencing [13, 42, 43]”. It is not clear if the referred papers contain information about technical detail or described the results of molecular analysis, including screening for 1555 A>G, 3243 A>G and GJB2 mutations for the same group of patients. Please explain.
4) The authors need to show the audiograms of all affected subjects carrying sequence variants 904 C>T, 1005 T>C and 7501 T>A considered as putatively pathogenic in this study.
5) The authors should show the molecular data (for example: sequence chromatograms, PCR-RFLP analysis, dHPLC profiles) to improve that identified substitutions were indeed homoplasmic and/or heteroplasmic.
6) Please give the GeneBank accession number of the rCRS.
7) Discussion, page 15. The first sentence of the discussion suggests, that the authors analyzed entire sequence of mtDNA and have found sequence variants only in 2 genes; 12S rRNA and tRNAser(UCA). What about the other mitochondrial genes, no changes? Did the authors define the mitochondrial haplogroup of affected subject.
8) The manuscript text should be read by the native speaker, a number of spelling and typo errors were found throughout the paper. Some sentences are really difficult to follow.

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