Mitochondrial DNA mutations are not a common cause of non-syndromic hearing loss in Republic of Macedonia

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INTRODUCTION

Mutations in mitochondrial DNA (mtDNA) are found to contribute to sensoryneural deafness, including both syndromic and non-syndromic forms. Hot spot regions for deafness mutations are the MTRNR1 gene, encoding the 12S rRNA and the MTTS1 gene, encoding the tRNA for Ser(UCN). Nucleotide changes are observed with a variable frequency among different populations of deaf persons. Among the known mtDNA mutations, the A1555G is the most common genetic cause of deafness, with variable frequency of 0.4 up to 5.4%, described both among non-syndromic sensoryneural hearing loss (SNHL) patients and aminoglycoside induced SNHL.

AIM OF THE STUDY

The aim of this study was to determine the presence and frequency of the most common mtDNA mutations among 130 Macedonian patients with non-syndromic hearing loss, and to determine the possible pathogenic effect of a mtDNA mutation on severity of hearing loss in patients with only one *GJB2* mutation.

MATERIAL AND METHODS

A total of 130 Macedonian patients, with profound non-syndromic sensoryneural hearing loss (NSHL), participated in this study. Five most common mitochondrial mutations associated with deafness (A827G, 961delT+Cn, T1095C, C1494T and A1555G), were analyzed using a SNaPshot method (Figure 1).

Direct sequencing using Big Dye Termination v1.1 Sequencing kit (Applied Biosystems) was performed on ABI 3130 Genetic Analyzer for analysis of other possible nucleotide changes in the MTRNR1 gene.

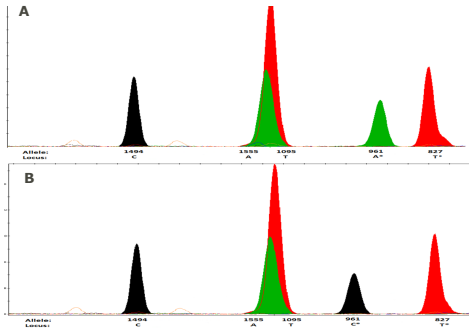

Figure 1. SNaPshot analysis of the five most common MTRNR1 gene mutations (A827G, 961delT+Cn, T1095C, C1494T and A1555G), associated with deafness. Five sets of SNaPshot extension primers were used, with 961delT+C(n) and A827G used in reverse orientation(*).

(A)Representative pattern of wild-type alleles;

(B) Capillary electrophoresis identifying a T to G nucleotide change at position 961.

RESULTS AND DISCUSSION

We have performed an cost-effective SNaPshot analysis method for simultaneous screening of the five most common mitochondrial 12S rRNA gene mutations (A827G, 961delT+Cn, T1095C, C1494T and A1555G) related with deafness. None of analyzed deafness-associated mutations were identified in the studied patients. Exeption was a G to T tranversion at position 961 detected in a patient with only one *GJB2* mutation (Figure 1). The mutation was confirmed by direct sequencing (Figure 2a). This mutation was first described by Li et al., in 2004 in five patients with distinct sets of mtDNA polymorphisms. Insertion or deletion of C at this position has also been found in association with aminoglycozide-induced deafness.

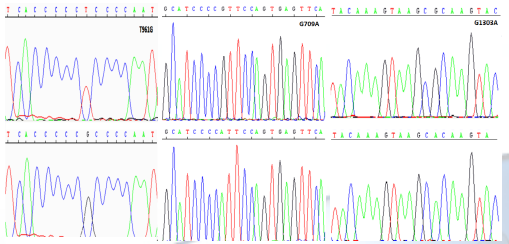

Figure 2. Sequence analysis identifying (A) T961G; (B) G709A and (C) G1303A nucleotide change in MTRNR1 gene among profoung hearing loss patients with only one *GJB2* mutation.

While screening for MTRNR mutations that could influence the pathogenic effect of a single GJB2 mutation, a G709A polymorphism was found in two unrelated patients (Figure 2B) and an unpublished variant G1303A (Figure 2C; Table 1). G1303, is not localized at conserved site, but was not found among 50 normally hearing controls. The pathogenic effect of this nucleotide change on single *GJB2* mutation should be further investigated.

Table 1. Summary of clinical and molecular characteristics among four hearing loss patients with only one *GJB2* mutation and nucleotide changes in MTRNR1 gene

12S rRNA	GJB2 mutation	Hearing impairment	Conservation *	Reported
T961G	Pro175Thr/N	Severe	T/T/A/A	Caucasians;
G709A	35delG	Severe	G/A/A/-	Wei et al, 2009
G709A	Arg127His/N	Moderate	G/A/A/-	-//-
G1303A	Thr24Stop	Severe	G/A/A/-	This study

*** (Human/Bovine/Mouse/Xenopus laevis)**

CONCLUSION

Our result suggests that mitochondrial DNA mutations do not represent a substantial risk factor for sensoryneural deafness in Macedonian population.