GJB2 MUTATIONS IN MACEDONIAN PATIENTS WITH NONSYNDROMIC HEARING LOSS

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INTRODUCTION

Hearing impairment is one of the most common sensory-neural disorders with the incidence of profound deafness in one per 1,000 births. At least 50% of this hearing loss is genetically determined. Mutations in the GJB2 and GJB6 genes for DFNB1 locus (13q12) are responsible for about half of all cases of autosomal recessive prelingual hearing loss. These genes encode the gap junction proteins connexin 26 and 30, respectively, implicated in the intracellular communication.

35delG mutation is the most frequent mutation in GJB2 gene accounting for approximately 70% of all GJB2 mutant alleles in most European populations. Additionally more than 100 different nucleotide substitutions are also found in GJB2 gene, of which 167delT, 235delC and R143W alleles are the most commonly hearing loss associated GJB2 alleles in Ashkenazi Jewish, Japanese and Ghanaian populations, respectively.

AIM OF THE STUDY

The aim of the study was to evaluate the frequency and type of mutations in GJB2 gene and GJB6 deletion among Macedonian patients with non-syndromic hearing loss (NSHL) and to determine the frequency of 35delG mutation among normal hearing population in Macedonia.

MATERIAL AND METHODS

The molecular analyses of GJB2 gene were been performed on DNA samples from 70 individuals with non-syndromic hearing loss belonging to 33 affected families. Twenty three families were of Macedonian ethnic origin, six Albanian, one Turkish and three Gypsy families.

SSCP analysis followed by direct sequencing of fragments with altered electrophoretic mobility was used for detection of mutations in GJB2 gene, while specific PCR using two sets of primers were used for GJB6-D13S1830del screening.

RESULTS AND DISCUSION

In 12 out of 33 patients (33.4%) mutations in GJB2 gene were found (Table 1, Figure 1C). Among 22 mutated chromosomes, 15 (68.2%) carried 35delG mutation. We have identified homozygosity for 35delG in seven unrelated patients, of whom three were Macedonian and one each of Albanian and Turkish origin, confirming that this mutation is the most prevalent one in Mediterranean countries. In addition we have determined Trp24Stop homozygosity in two unrelated patients from Gypsy origin, by SSCP method and direct sequencing with overall frequency of 6.1% (Figure 1B). The prevalence of this mutation was most frequent in India and Pakistan. Mutations Val37Ile and Arg127His, with a frequency of 3.0% and 1.6%, respectively, were also found among NSHL patients from Macedonia (Table 2, Figure 2).

GJB6-D13S1830del mutation was not found in our group of patients.

Table 1. Mutations in GJB2 gene found among NSHL patients from Macedonia

Mutation	No. of patients	Ethnicity
35delG / 35delG	7	Macedonian (5); Albanian (1); Turk (1)
35delG / Val37Ile	1	Macedonian
Val37Ile / N	1	Albanian
Trp24Stop /Trp24Stop	2	Gipsy
Arg127His / N	1	Gipsy
Total	12	/

Table 2. Frequency of the mutations in GJB2 gene found among NSHL patients from Macedonia

Mutation	Chromosomes analyzed	% of analyzed chromosomes
35delG	15 / 66	22.7%
Val37Ile	2 / 66	3.0%
Trp24Stop	4 / 66	6.1%
Arg127His	1 / 66	1.6%
Total	22 / 66	33.4%

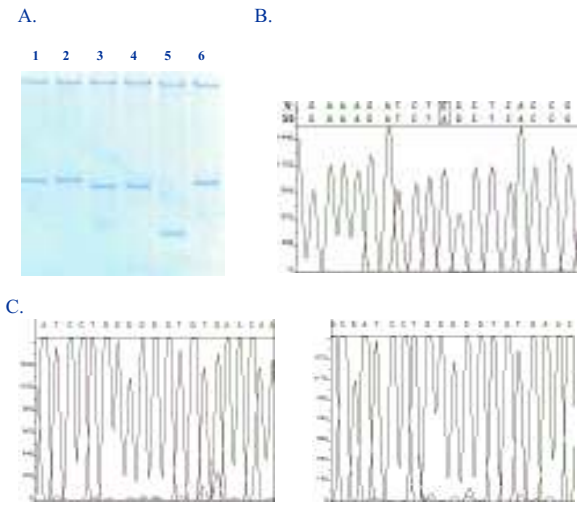

Figure 1
A. SSCP analysis identifying 35delG (lines 3 and 4) and Trp24Stop mutation (line 5) in the GJB2 gene. Lines 1 and 2 are other hearing loss individuals without mutation in GJB2; Line 6 normal control.
B. Sequence analysis determining a homozigosity of TGG→TAG or Trp→Stop nucleotide substitution at codon 24 in the GJB2 gene in a Gypsy family with non-syndromic hearing loss.
C. Normal and 35delG sequence of the GJB 2 gene

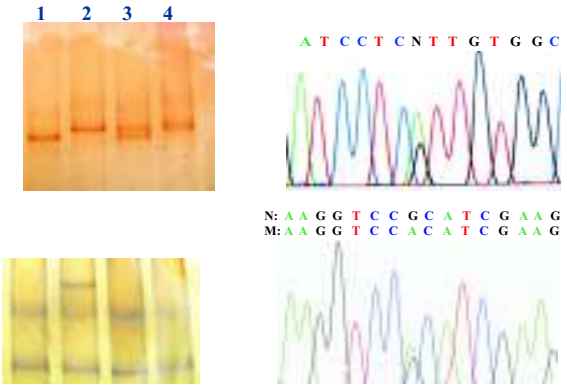

Figure 2.
Representative photograph of SSCP analysis and sequencing reactions identifying GJB2 mutations in NSHL patients from Macedonia

A) SSCP analysis identifying: homozygote for 35delG (line 1); normal control (line 2); heterozygote for 35delG (line 3); and heterozygote for Val37Ile missence mutation with sequencing reaction identifying G→A substitution at codon 37 of the GJB2 gene
B) SSCP analysis and sequencing reaction identifying G→A missence mutation at codon 127 leading to Arg→His substitution in a NSHL patient of gipsy origin

ACKNOWLEDGMENTS

This study was supported in part by project No. 09-54/1/06 from the Macedonian Academy of Sciences and Arts, R. Macedonia and FP7 project No. 229458 from the European Commission