MOLECULAR TESTING FOR *GJB2* MUTATIONS AMONG MACEDONIAN NONSYNDROMIC HEARING LOSS CHILDREN

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INTRODUCTION

Hearing loss the most prevalent sensory defect affecting 270 million people worldwide is influenced by both genetic and environmental factors. Inherited causes have been exposed as the most prominent etiological factor in deafness. When present in an infant it may have dramatic effects on language acquisition seriously compromising the quality of life and leading to social isolation.

The genetic basis of hearing loss is complex. There are 113 mapped loci and 51 different genes that have been shown to underlie hereditary sensorineural nonsyndromic hearing loss (NSHL) in humans. Despite the enormous genetic heterogeneity, mutations in only one gene, *GJB2*, are responsible for approximately half of all cases with NSHL.

Located at the DFNB1 locus (13q12) *GJB2* gene encodes synthesis of the transmembrane gap junction protein connexin 26.

More than 100 different mutations in this gene have been described with specific prevalence in different ethnic groups and geographic regions.

Due to the high incidence of *GJB2* mutations, molecular testing for *GJB2* mutations has rapidly become the standard of care for the diagnosis and counseling of patients with nonsyndromic hearing impairment of unknown cause.

AIM OF THE STUDY

The aim of our study was to determine the prevalence and spectrum of *GJB2* mutations among 120 Macedonian children with profound deafness as well as the presence of del(*GJB6*-D13S1830) and mitochondrial DNA mutations.

MATERIALS AND METHODS

One hundred and twenty unrelated cases of different ethnic origin [Macedonian (n=69), Albanian (n=19) and Gypsy (n=32) origin], with profound deafness were analyzed. Molecular studies were performed using direct sequencing of *GJB2* gene and specific PCR analysis for del(*GJB6*-D13S1830) mutation. Five common mtDNA mutations [A1555G, 961delT+C(n), T1095C, C1494T and A827G] were analyzed using SNaPShot method (Figure 2).

RESULTS

GJB2 mutations were found in 55 patients (45.8%), in homozygote (37), compound heterozygote (8) or heterozygote state (10). (Table 1, Figure 1). A high number of patients carrying only one mutant allele were found. The predominant *GJB2* mutation was 35delG found among Caucasians (Macedonian and Albanian patients), followed by W24X found among Gypsy patients, respectively (Table 2). Other *GJB2* mutations: R127H, V37I, V153I, Cd120delGAG and P175T were less frequent, found with allelic frequency of 2.1%, 0.8%, 0.8%, 0.4% and 0.4% respectively. None of the patients carry del(GJB6-D13S1830) or mtDNA mutation.

Due to the high prevalence of 35delG and W24X mutations found among analyzed patients (Caucasians and Gypsy, respectively), these two mutations should be tested in each routine diagnostic approach in Macedonian deaf population.

Table 1 *GJB2* genotypes determined among 120 Macedonian NSHL patients

Genotype	Patients	Ethnicity		
		Macedonian (69)	Albanian (19)	Gipsy (32)
35delG / 35delG	26	20	6	/
35delG / N	4	2	1	/
W24X / 35delG	5	/	/	5
W24X / W24X	10	/	/	10
W24X / Cd120delGAG	1	/	/	1
W24X / N	1	/	/	1
35delG / V37I	1	1	/	/
V37I / N	1	/	1	/
P175T / N	1	1	/	/
R127H / R127H	1	/	/	1
R127H / N	3	/	/	3
R127H / V153I	1	/	/	1
Total	55	24	8	22

Table 2 Allelic frequency of the *GJB2* mutations among Macedonian NSHL patients

Mutation	Prevalence		Ethnicity		
	%	No	Macedonian	Albanian	Gipsy
35delG	25.8%	(62/240)	31.9% (44/138)	34.2% (13/38)	7.8% (5/64)
W24X	11.2%	(27/240)	/	/	42.2% (27/64)
R127H	2.1%	(5/240)	/	/	7.8% (5/64)
V37I	0.8%	(2/240)	0.7% (1/138)	2.6% (1/38)	/
V153I	0.8%	(2/240)	0.7% (1/138)	/	1.6% (1/64)
Cd120delGAG	0.4%	(1/240)	/	/	1.6% (1/64)
P175T	0.4%	(1/240)	0.7% (1/138)	/	/
Total	41.7%	(100/240)	34.1% (47/138)	36.8% (14/38)	60.9% (39/64)

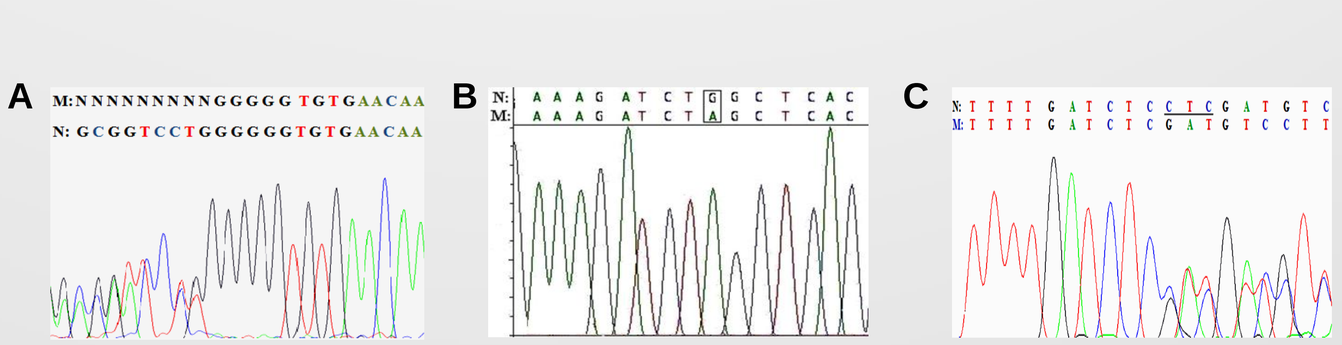

Figure 1. Representative photograph of nucleotide changes in GJB2 gene among Macedonian NSHL patients identified by sequencing reactions

A. Patient heterozygote for 35delG;
B. Homozigosity of TGG→TAG or W24X nonsense mutation in a Gypsy patient with NSHL;
C. "Inframe" trinucleotide deletion GAG at Codon 120 in heterozygote state;

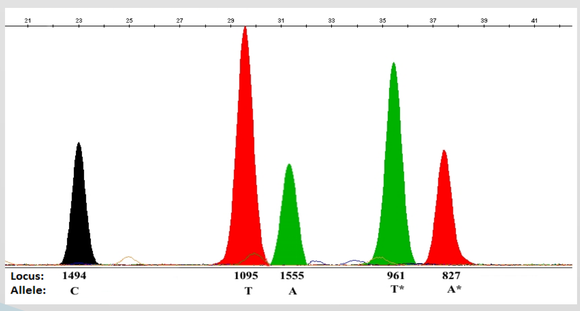

Figure 2. Representative photograph of SNaPshot analysis of the five most common mitochondrial DNA mutations* (C1494T, T1095C, A1555G, 961delT+C(n) and A827G) *causing hearing loss.

All shown fragments represent normal allele pattern.

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