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Spectrum of *GJB2* (Cx26) gene mutations in Iranian Azeri patients with nonsyndromic autosomal recessive hearing loss

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ABSTRACT

Objective: Hereditary hearing impairment is a genetically heterogeneous disorder. In spite of this, mutations in the *GJB2* gene, encoding connexin 26 (Cx26), are a major cause of nonsyndromic recessive hearing loss in many countries and are largely dependent on ethnic groups. The purpose of our study was to characterize the type and prevalence of *GJB2* mutations among Azeri population of Iran.
Methods: Fifty families presenting autosomal recessive nonsyndromic hearing loss from Ardabil province of Iran were studied for mutations in *GJB2* gene. All DNA samples were screened for c.35delG mutation by ARMS PCR. Samples from patients who were normal for c.35delG were analyzed for the other variations in *GJB2* by direct sequencing. In the absence of mutation detection, *GJB6* was screened for the del(GJB6-D13S1830) and del(GJB6-D13S1854).
Result: Thirteen families demonstrated alteration in the Cx26 (26%). The 35delG mutation was the most common one, accounting for 69.2% (9 out of 13 families). All the detected families were homozygous for this mutation. Two families were homozygous for delE120 and 299–300delAT mutations. We also identified a novel mutation: c.463–464 delTA in 2 families resulting in a frame shift mutation.
Conclusion: Our results suggest that c.35delG mutation in the *GJB2* gene is the most important cause of *GJB2* related deafness in Iranian Azeri population.

1. Introduction

Hearing loss is the most common sensorineural disorder in humans affecting about 1 in 1000 newborns. The majority of hereditary hearing loss is nonsyndromic and presents autosomal recessive pattern of inheritance (70%), the remaining are autosomal-dominant (20%), Xlinked (1%), and mitochondrial (<1%) forms [1]. So far, over 95 loci have been mapped and about 40 genes responsible for ARNSHL have been identified [2]. Although hearing loss is very heterogeneous, in some populations up to 50% of cases of prelingual non-syndromic sensorineural hearing loss (NSSHL) are due to mutations in a single gene, *GJB2* (MIM#121011), which is located at DFNB1 locus on chromosome 13q12 and encodes connexin 26 protein (Cx26). Cx26 belongs to a large family of gap junction proteins which contribute in intracellular communication. In particular, this protein has an important role in recycling potassium ions in the inner ear [3].

One of the important mutations in *GJB2* is c.35delG, which account for the majority of GJB2 related deafness in the Caucasians population [4,5]. Over 100 other mutations have been reported in the *GJB2* gene [6], of which 167delT, 235delC, V37I, W24X and R143W are the most common ones in Ashkenazi Jews, Japanese, Taiwan, Indian, and Ghanaian populations, respectively, which indicate that mutations in this gene are ethnic-specific [7–13].

Despite such diversity, the combined frequency of all *GJB2* mutations in different population is high enough to make mutation analysis of this gene a clinically useful, and therefore widely available, genetic test.

A second gap-junction gene, *GJB6*, also localizes to the DFNB1 interval. Two large deletion del(GJB6-D13S1830) and del(GJB6-D13S1854) has been reported to cause ARNSHL in homozygote or compound heterozygote individuals carrying deafness-causing allele variants of the *GJB2* [14].

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In the last eight years, numbers of studies have been conducted in Iranian population to identify the mutation spectrum and prevalence of *GJB2* gene mutations [15,16]. In this study, we attempt to determine the prevalence and spectrum of *GJB2* mutations in the Azeri hearing loss population in Ardabil province in northwest of Iran. Ardabil province is bound to the north by Azerbaijan, to the east by the Caspian Sea and to the west by east-Azerbaijan province. Consanguinity and assortative mating are very common in this population [17].

2. Materials and methods

2.1. Patients

A total of 50 families from Ardebil province were investigated. To be included in this study, families had to meet the following criteria: (1) hearing loss is documented by audiologic testing; (2) hearing loss in the absence of other clinical features; (3) a pedigree structure consistent with autosomal recessive inheritance; (4) two or more affected family members. A relatively high level of consanguinity (80%) was seen in the studied families. Puretone audiometry at 125–8000 Hz was performed for affected subjects and all have severe to profound hearing loss.

Informed consent was obtained from all family members who participated in the study and then blood samples were obtained from all family members. This study had been approved by the ethics committee of the Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran.

2.2. Mutation detection

Genomic DNA was extracted from peripheral blood of the family members following the standard salting out procedure [18]. At first step, *GJB2* was screened for c.35delG mutation using allele-specific PCR amplification (ARMS PCR) with primers as described by Scott et al. [19]. No further testing was done on homozygote individuals for the 35delG allele variant of *GJB2*, and in this group, the diagnosis of DFNB1 deafness was made. In normal ones for the 35delG allele, the entire exons 1 and 2 were later sequenced for other allele variants using Big Dye Terminators (Applied Biosystems 3130 Genetic Analyzer). Probands who were negative or only have one *GJB2* mutated allele were analyzed for the presence of the del(GJB6-D13S1830) and del(GJB6-D13S1854) of the *GJB6* gene as described by del Castillo et al. [14].

Table 1
Genetic variants in the *GJB2* gene identified in this study with percentage of consanguinity.

Genotype	Frequency	Consanguinity
35delG/35delG	9/50	6/9[a]
463–464delTA/463–464delTA	2/50	Consanguineous
delE120/delE120	1/50	Consanguineous
299–300delAT/299–300delAT	1/50	Non consanguineous
A171T/WT	1/50	Consanguineous
V27I+E114G/WT	1/50	Consanguineous

[a] 6 out of nine mutated families have consanguineous marriage.

3. Results

13 out of 50 Iranian families presenting ARNSHL from Ardabil province in Northwestern of Iran, showed mutations in the *GJB2* gene (26%) (Table 1). Homozygous 35delG mutation was observed in 9 families, accounting for 69.2% of the *GJB2* mutations in this ethnic group. Other detected mutations were delE120 and 299–300delAT, each in one family. A novel c.463–464delTA mutation was also observed in two families (Fig. 1). This mutation causes a frame shift mutation at codon 155 followed by a stop codon at 208 which resulting to a truncated protein.

We also could identify A171T and V27I+E114G variants in heterozygote form. No mutation was detected in exon 1.

Screening for the two *GJB6* deletions did not reveal a positive sample among *GJB2* negative or heterozygote samples.

4. Discussion

Deafness related to DFNB1 locus is the most common cause of ARNSD in many countries throughout the world [4,20]. Based on extreme heterogeneity of autosomal recessive non syndromic hearing Loss, study of diverse ethnic groups are necessary to establish the frequency of different genes involved in HL and specially *GJB2* mutations as a cause of hearing impairment. The frequency of mutations may vary between countries or even within different ethnic groups of a particular country. For example, the prevalence of *GJB2* mutations in Slovakia is 45.6 [21], France 39.8 [22], Turkey 25% [23], Pakistan 6.1% [24] and 0% in Oman [25].

The Iranian population is composed of many different ethnic groups, so it is important to generate ethnic specific data.

The study performed by Najmabadi et al. [14] indicated that *GJB2* related deafness account for 16.7% in Iranian population and 35delG mutation was the most common deafness-causing allele, occurring in 71.6% of persons with DFNB1 deafness. They also found the highest percentage of GJB2-related deafness in the north

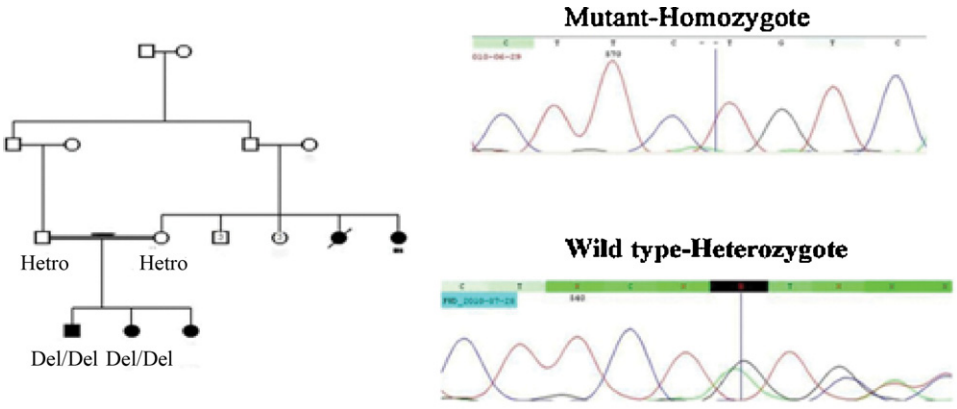

Fig. 1. One family's pedigree with novel identified mutation and segregation of the c.463–464delTA in this family.