Short Communication

Mutation analysis of connexin 26 gene and del(*GJB6*-D13S1830) in patients with hereditary deafness from two provinces in Iran

Abdorrahim Sadeghi[1,2], Mohammad Hossein Sanati[3]*, Fatemeh Alasti[3], Morteza Hashemzadeh Chaleshtori[4], Mitra Ataei[3]

[1]Department of Genetics, Faculty of Sciences, Tarbiat Modarres University, Tehran, I.R. Iran [2]Department of Genetics and Biochemistry, Faculty of Medicin, Arak University of Medical Sciences, Arak, I.R. Iran [3]National Institute for Genetic Engineering and Biotechnology, Tehran, I.R. Iran [4]Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, I.R. Iran

Abstract

Mutations in the connexin 26 (Cx26) gene at the DFNB1 locus on chromosome 13q12 are associated with autosomal recessive non-syndromic hearing loss (ARNSHL). There are many known mutations in this gene that cause hearing loss. A single frameshift, at position 35 (35delG) accounts for 50% of mutations in the Caucasian population with carrier frequencies of 1.5-2.5%. In this study we investigated the prevalence of Cx26 gene mutations by directly sequencing the coding exon of this gene belonging to ARNSHL individuals from 53 families in Qom and Markazi provinces of Iran. Seven different Cx26 variants were identified. Five Cx26 mutations including 35delG, 233delC, 176del16, W24X, L90P were found in 10 of 53 families (18.87%). One polymorphism V153I was also found. One variant A171T with unknown effects was also detected. Six of the 53 families were observed to have GJB2 mutations in both alleles (11.32%). The most common mutation was 35delG. Three out of 10 families (30%) with GJB2 variants contained 35delG mutation in both alleles and the frequency of 35delG allele was 0.50 among 10 out of 53 families. Also screening for the 342-kb GJB6 deletion mutant did not reveal any large deletion among families studied. Thus, in the two provinces, contribution of GJB2 (Gap Junction Protein Beta 2) mutations to familial deafness appears to be less significant. This necessitates further assessment of the other known genes regions as well as a search for new genetic factors in hereditary deafness in the Iranian population.

Keywords: *Hereditary Deafness; Iranian; Connexin 26; del(GJB6-D13S1830); GJB2*

Correspondence to:* **Mohammad Hossein Sanati, Ph.D.
Telefax: +98 21 44580346
E-mail: m-sanati@nrcgeb.ac.ir

Congenital deafness is a frequent disorder that affects 1 in 1000 neonates with approximately 50% caused by inheritance. Almost 80% of familial hearing loss is non-syndromic, with a predominant autosomal recessive mode of inheritance (Cryns *et al*., 2004; Marlin *et al*., 2005). More than 51 autosomal recessive hearing impairment loci have been reported (Hereditary Hearing Loss Homepage, http://webhost.ua.ac.be-hhh). Mutation in the connexin 26 gene (*Cx26*) is the predominant genetic cause of sensorineural hearing loss in many populations (Cryns *et al*., 2004; Marlin *et al*., 2005). Although a single mutation, known as 35delG, is a common mutation among Caucasians, it is less frequent in other populations (Shahin *et al*., 2002; Ghosh *et al*., 2004; Mustafa, 2004; Kalay *et al*., 2005). The other prevalent mutations of this gene are 235delC among the Japanese and Korean populations (Abe *et al*., 2000), the 167delT in the Ashkenazi Jews (Sobe *et al*., 1999), R143W in an African village (Brobby *et al*., 1998) and W24X in the India population (Ghosh *et al*., 2004).

Cx26 encodes a gap junction channel protein, called *GJB2*. This protein is widely expressed throughout non–sensory epithelial and connective tissue cells and is thought to be important in maintaining endocochlear potential (Kalay *et al*., 2005). A large deletion (342 kb) involving the *GJB6* gene encoding connexin 30, which is also located at the DFNB1 locus, del(*GJB6*-D13S1830), has been reported to cause ARNSHL in homozygote or compound heterozygote individuals carrying deafness-causing allele variants of the *GJB2*

on the opposite allele (Frei *et al*., 2004; Riazalhosseini *et al*., 2005). This large deletion is found in many populations, with higher frequencies observed in France and Spain where the percentage of unexpected *GJB2* heterozygotes fell to 16.0-20.9% after screening for the del(*GJB6*-D13S1830) mutation (Castillo *et al*., 2003).

In this study, we investigated the *GJB2* gene mutations and the del(*GJB6*-D13S1830) mutation in 53 families with ARNSHL from Qom and Markazi provinces. 79.24% of these families had 3-10 deaf individuals and the remaining 20.76% had 2 affected children. All affected members of the families studied suffered from a prelingual hearing loss with no other associated clinical findings (non-syndromic) and no suggestive history of any other possible etiology. Informed consent was obtained from all of the participating subjects (in case of individuals under 18 years old, consent was obtained through their parents). Blood samples were taken from all individual belonging to the 53 families.

DNA was extracted from peripheral blood samples using the phenol/chloroform standard procedure (John *et al*., 1991). The coding region of *Cx26* gene (Genbank accession # M86849) was amplified using primers: forward: 5´-GTCTCCCTGTTCTGTCCTA-3´ and reverse: 5´-TCTAACAACTGGGCAATG-3´. The amplification resulted in a fragment of 743 bp containing the entire coding sequence and right splice site. After a quality check of the PCR products by electrophoresis on 1.5% agarose gel, sequencing was carried out using a capillary automated system 3700 ABI sequencer (Macrogen, South Korea). One affected sample from each family was sequenced initially. Detected mutations were confirmed by sequencing the reverse strand. Alternatively the sequencing was carried out on samples from another member of the families.

In order to analyze the 342-kb deletion in all the probands multiplex PCR was performed. The junction fragment caused by the deletion was amplified using the primer pair: forward: *GJB6*-1R, 5´-TTTAGGGCATGATTGGGGTGATT-3´ and reverse: BKR-1, 5´-CACCATGCGTAGCCTTAACCATTTT-3´. The resulting amplicon was 460 bp in length. The primer pair: forward, STS-CX636F 5´-TGCCCACCC-CCCAAGTAGAG-3´ and reverse, STS-CX636R 5´-TTTCGGTTTCATTCATTTTCCCTATT-3´ used as an internal control, generated a 360 bp fragment. The products of multiplex PCR were electrophoresed on 1% agarose gel and visualized with ethidium bromide staining under ultraviolet light to verify their size and quantity. Control samples for large deletion were a gift from the Center of Medical Genetics, at the University of Antwerp, Belgium.

In this study, 53 families were investigated for seven different genetic variantsas shown in Table 1. Six out of 53 families had *GJB2* mutations in both alleles (11.32%). These six families encompass 35delG/35delG, 35delG/W24X, 233delC/233delC and 176del16/176del16 variants. Five *GJB2* mutations including 35delG, W24X, L90P, 233delC and 176del16 were found in 10 of 53 families (18.87%). One polymorphism variant, V153I was found in 2 families. A171T variant was found in one family as heterozygote. The most common mutation was 35delG. Three out of 10 (30%) families that had *GJB2* variations containing 35delG mutation in both allele. The frequency of 35delG allele was 0.50 among 10 out of 53 families. Five out of 53 families were homozygous for one mutation in *GJB2* gene as shown in Table 1. None of the families, including those who carried one mutation in the coding region of the *Cx26* gene, revealed the 342-kb *GJB6* large deletion mutation (Fig. 1).

All families were the result of consanguineous mat-

Table 1. Cx26 genetic variants identified in Iranian ARNSHL families (compare to Genbank accession #M86849).

No.	Genotype	Effect	Frequency No. of samples
1	35delG/35delG	Frameshift	3/53(5.66%)
2	35delG/W24X	Frameshift/Stop codon	1/53(1.88%)
3	35delG/wt	Frameshift	3/53(5.66%)
4	L90P/wt	Leu at 90 into Pro	1/53(1.88%)
5	233delC/233delC	Frameshift	1/53(1.88%)
6	176del16/176del16*	Frameshift	1/53(1.88%)
7	A171T/wt	Arg at 171 into Tre	1/53(1.88%)
8	V153I/V153I	Polymorphism	1/53(1.88%)
9	V153I/wt	Polymorphism	1/53(1.88%)

*This mutation is not reported for Iran.
wt is wild type

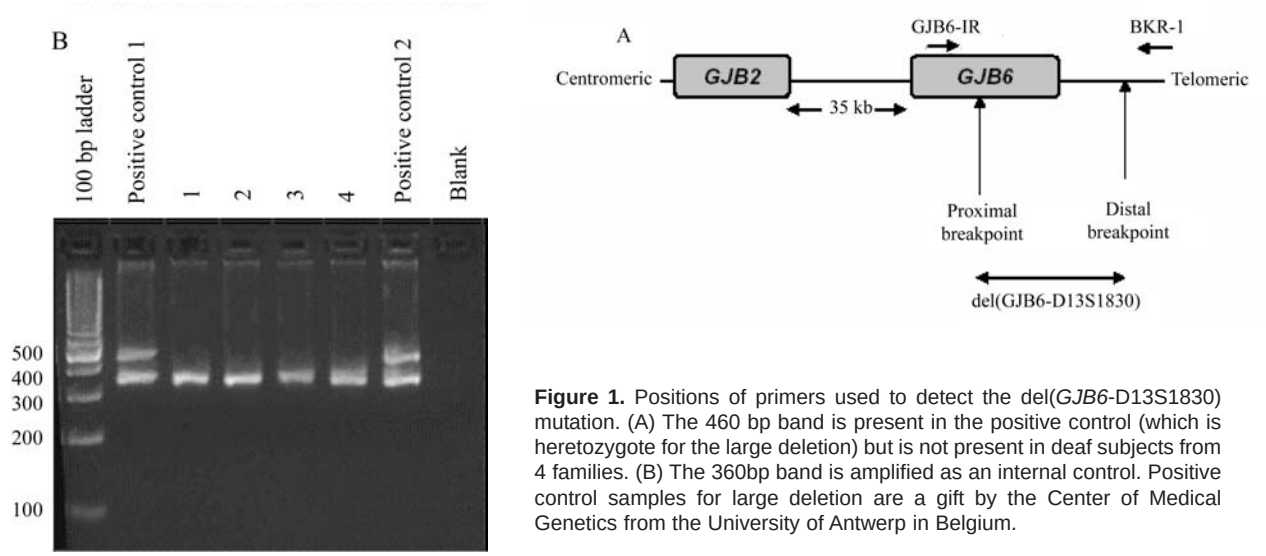

Figure 1. Positions of primers used to detect the del(*GJB6*-D13S1830) mutation. (A) The 460 bp band is present in the positive control (which is heretozygote for the large deletion) but is not present in deaf subjects from 4 families. (B) The 360bp band is amplified as an internal control. Positive control samples for large deletion are a gift by the Center of Medical Genetics from the University of Antwerp in Belgium.

ing with autosomal recessive inheritance of the disease. *GJB2*-related deafness was found in 6 of the 53 ARNSHL studied families (11.32%) and revealed that the rate of mutation in *Cx26* gene in the two populations is lower than that in the European populations (Cryns *et al*., 2004; Marlin *et al*., 2005). All of the mutations have been previously described in the Iranian population except 176del16 which was initially reported by Kudo *et al*. (2002). 35delG and L90P heterozygosity were found in 3 and 1 families, respectively and since these mutations were found in another affected member of the family therefore, they may not be due to polymorphism.

The results of this study on the frequency of *Cx26* gene mutations support the previous reports on Iranian hereditary deafness (Hashemzadeh *et al*., 2005; Najmabadi *et al*., 2005; Hosseinipour *et al*., 2005). Similar studies in Egypt (Mustafa, 2004), Turkey (Kalay *et al*., 2005), Palestine (Shahin *et al*., 2002) and India (Ghosh *et al*., 2004) demonstrated a lower rate of mutation in this gene in comparison to those in Caucasians. This study shows 35delG is the most common deafness-causing mutation in the Iranian population that confirms the previous studies in Iran (Hashemzadeh *et al*., 2005; Najmabadi *et al*., 2005; Hosseinipour *et al*., 2005). Our data and those by other researchers show that *GJB2*-related deafness in the Iranian population is due to the common mutation (35delG) similar to that in the Caucasian population, with a mutation rate resembling that in the Asian population.

Absence of the 342 kb *GJB6* variant is one of the most important findings of this study. Previous reports on the same population also presented the same finding (Riazalhosseini *et al*., 2005). The families with no mutation in the *Cx26* gene will need to be investigated further to look for the presence of mutations in other known deafness-causing genes, and new deafness-causing loci.

The Iranian population is an important resource for research on ARNSHL due to the availability of large, extended and highly consanguineous pedigrees. It should be note that the rate of familial marriage in Iran is relatively high (38.6%) Saadat *et al*. (2004), therefore an expected higher frequency of hearing loss, with more than 1 in 1000 newborn is to be expected.

In conclusion, identification of the spectrum and frequency of *GJB2* mutations and other genes in different ethnic groups of Iran can eventually help and facilitate with detection and prevention of deafness.

Acknowledgments

We would like to thank all the families, students and personnel of the schools for hearing-impairment in Qom and Arak provinces for their cooperation. Also we wish to thank other hearing impairment centers for their contribution to this study. This work was supported by Tarbiat Modarres University and the National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, I.R. Iran

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