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*Original article*

# Profile of Iranian *GJB2* Mutations in Young Population with Novel Mutation

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## Abstract

### Objective(s)

Despite the enormous heterogeneity of genetic hearing loss, most non-syndromic hearing losses are caused by mutations in the *GJB2* gene. We aimed to characterize the mutation profiles of 100 Iranian deaf patients that were under 10 years old.

**Materials and Methods**

Patients were tested with direct sequencing of entire coding region of the *GJB2* gene.

**Results**

Eight known mutations plus one novel (358delGAG) were found in 25% of study group. The 35delG mutation (64%) constituted the majority of *GJB2* mutations.

**Conclusion**

Role of *GJB2* mutation in Iranian young deaf population is more prominent than previous study that can be a result of higher consanguine marriage in population. But our result shows that there is only 25% non-syndromic hearing loss due to high frequency of consanguine marriage in Iranian population. Identification of other genes involved in genetic deafness will help us understand the fundamental mechanisms of normal hearing, both in early diagnosis and therapy.

**Keywords:** ARNSHL, Connexin Cx26, GJB2, Hereditary hearing loss

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## Introduction

Congenital deafness is the most prevalent sensorineural disorder that affects one in 1000 neonates with 50% genetic basis (1).

Hereditary deafness is a genetically heterogeneous disorder that is classified as non-syndromic (70%) and syndromic. The majority of the non-syndromic cases (77%) show simple Mendelian autosomal recessive (ARNSHL) inheritance, while 22% are autosomal dominant and only 1% is X-linked or due to mitochondrial mutation (2).

In the field of non-syndromic hearing loss, about 130 loci have been described in previous studies; so far 47 relative genes have been mapped (3).

The first ARNSHL locus, DFNB1 (DFN: deafness; B: recessive; integer: locus in order of discovery(4)) was identified by Guilford *et al* in 1994 (5). Three years later, mutation in the gene *GJB2*, which encodes the gap junction protein connexin 26 (Cx26), was shown to be responsible for deafness at this locus (6, 7). *GJB2* is a small gene about 5500-bp length with 2 exons, of which only one contains the coding region (8).

Hearing loss can be described as mild (30-49 dB), moderate (50-69 dB), severe (70-89 dB) or profound (≥90 dB). Mutations in Cx26 are responsible for half of all moderate to profound congenital deafness in many world populations.

In the cochlea, Cx26 proteins have important roles in the recirculation of potassium ions (9).

More than 100 different mutations have been identified in association with autosomal dominant and recessive hearing loss in this gene (3). In many ethnic groups a single mutation, the 35delG mutation predominates; especially in European countries where it is established to be due to founder effect (10).

Consanguinity in the Iranian population is highly prevalent (11), so research on ARNSHL is much recommended (12).

The aim of this study was to investigate the spectrum of *GJB2* mutations among 100 patients with moderate to profound non-syndromic hearing loss in Iran.

## Materials and Methods

The study included 100 patients with moderate-to-profound, non-syndromic hearing loss with Autosomal recessive inheritance from different parts of the country. All patients were less than 10 years old. This study was approved by the Ethics Committee of Tehran University of Medical Sciences and an informed consent was signed by the patients.

The hearing loss of patients was confirmed by audiologic testing; air and bone conductions were evaluated in frequencies of 250, 500, 1000, 2000, 4000 and 8000 Hz with intensities up to120 dB.

Peripheral blood samples were taken and the genomic DNA was isolated using a Flexi Gene DNA Kit (Cat No 51204) according to the manufacturer's instructions.

An 806-bp DNA fragment containing the coding exon of the *GJB2* gene (exon 2) was amplified by PCR using the following primers: forward 5'-CTC CCT GTT CTG TCC TAG CT-3' and reverse 5'-CTC ATC CCT CTC ATG CTG TC-3' (Gene Bank NG_008358.1). PCR was performed in the following conditions: after initial denaturation at 96 ºC for 3 min, 5 cycles of denaturation at 95 ºC for 1min, annealing at 59 ºC for 1 min, extension at 72 ºC for 1 min and following with 26 cycles of denaturation at 94 ºC for 1 min, annealing at 59 ºC for 45 sec, extension at 72 ºC for 45 sec followed by 8 min of final extension at 72 ºC.

The PCR product was subjected to the direct sequencing by an ABI 3100 (Macrogen Korea), the sequencing results were analyzed by Codon Code Aligner 3.5.2.

## Results

Eight known mutations (35delG, R32H, R143W, R127H, 310del14, M163V, E101G and R184P) plus one novel variant. 358delGAG (Figure 1A) were found in 25 % of patients that enrolled in this study (Table 1).

A relatively high level (68%) of consanguineous marriages was observed in the deaf families studied, 80% of 25 mutations were found in consanguineous marriages. (Figure 2). All patients who carried a mutation in GJB2 gene had profound hearing loss. The variant mutations 35delG, R127H, M163V and E101G were identified in 8% of deaf patients in heterozygous form. Overall 15% of patients had homozygous status, with the mutations of

Table 1. Frequency of GJB2 genotypes detected in NSHHL Iranian individuals.

| Name of variants | Deaf individuals no. | Consanguineous marriage | Non consanguineous marriage |
|---|---|---|---|
| Known mutation | | | |
| 35delG/35delG | 11 | 8 | 3 |
| R32H/ R32H | 1 | 1 | 0 |
| R143W/ R143W | 2 | 2 | 0 |
| 310del14/R143W | 1 | 1 | 0 |
| 35delG/ R184P | 1 | 1 | 0 |
| 35delG/Wt | 4 | 4 | 0 |
| M163V/Wt | 2 | 1 | 1 |
| R127H/Wt | 1 | 1 | 0 |
| E101G/Wt | 1 | 0 | 1 |
| Novel mutation | | | |
| 358delGAG | 1 | 1 | 0 |
| Detected | 25 | 20 | 5 |
| Total | 100 | | |
| Polymorphism | | | |
| V153I/ V27I | 1 | 0 | 1 |
| V153I/wt | 5 | 5 | 0 |
| V27I/wt | 1 | 1 | 0 |

35delG/35delG, R32H/R32H, R143W/R143W and 358delGAG/358delGAG. Altogether 2% had 35delG/R184P and 312del14/R143W mutations in compounds with heterozygous status, respectively.

The most common mutation in this cohort study was 35delG mutation. A total of 11 homozygous and 5 heterozygous carriers of 35delG were detected (Table 2). This newly found mutation 358delGAG has not been reported previously (3). It causes a deletion at codon 120 and deletes one glutamic acid (Figure 1A). This mutation was found in a family with autosomal recessive inheritance (Figure1B).

This novel variant was analyzed further in terms of multiple sequence alignment to assess the evolutionary conservation of the amino acids caused this mutation. This human Cx26 amino acid sequence was aligned with Cx26 proteins from the human, house mouse, Norway rat, cattle, chimpanzee, common gibbon, dog, rhesus monkey, and African clawed frog and GJA3 and GJB1 protein sequences. The amino acid E120 was conserved across all aligned connexin 26 proteins and human connexin proteins GJA3 and GJB1(Figure 1C).

No mutations were found in the coding region of the *GJB2* gene in 75 out of 100 patients, but in 7 families (7%) heterozygous form, V153I, V27I polymorphisms were observed (Table 2). These polymorphisms were not encountered in patients with the *GJB2* gene mutation.

The total frequency of the *GJB2* gene mutations in patients with non-syndromic hearing loss was 21.5% among the 200 chromosomes (43/200) analyzed (Table 2). Among these nine different detected mutations, the most common was 35delG, with an allele frequency of 13.5%.

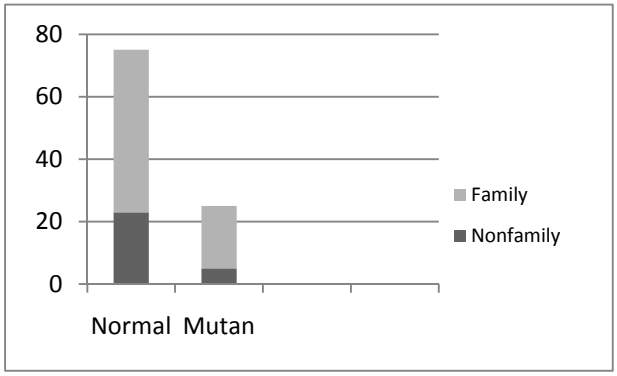
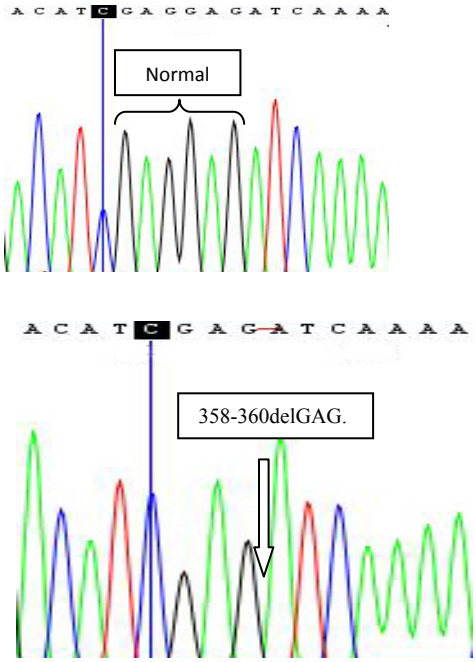
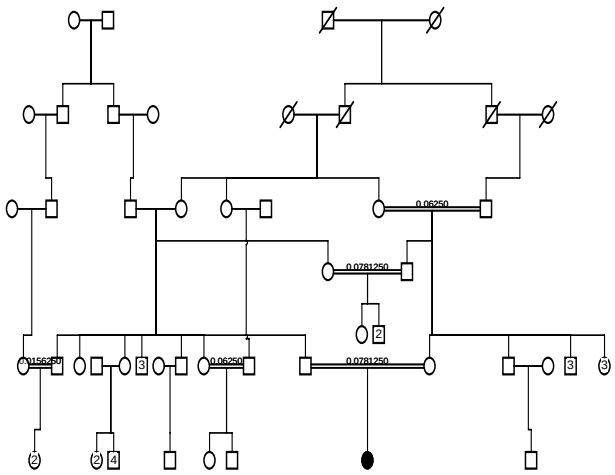



Figure 2. Comparison of consanguineous marriages and mutations.

**A)**




**B)**



**C)**

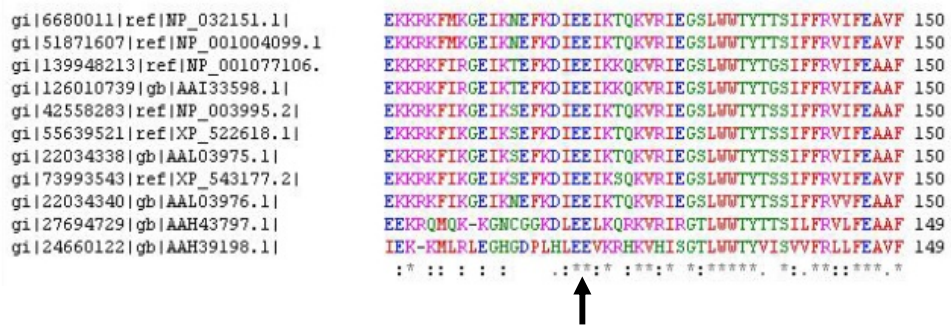



Figure 1. **A)** Nucleotide sequence of the novel variant 358delGAG compare with normal control. **B)** The pedigree of family with new mutation. **C)** Multiple sequence alignment of connexin 26 amino acid sequences. Arrow indicates position of the amino acids affected by novel mutation 358delGAG.

Table 2. Frequency of GJB2 variations detected NSHHL Iranian individuals.

| Name of variants | Nucleotide change | Number of alleles (% of 200) | Proportion of 41mutations (%) |
|---|---|---|---|
| Mutations | | | |
| 35delG | Deletion of G at 35 | 27 (13.5%) | 64% |
| R143W | C to T at 427 | 5 (2.5%) | 12% |
| R32H | G to A at 95 | 2 (1%) | 4.7% |
| M163V | A to G at 487 | 2 (1%) | 4.7% |
| 310DEL14 | Del 14 nucleotide at 310 | 1 (0.5%) | 2.3% |
| R184W | G to C at 551 | 1 (0.5%) | 2.3% |
| R127H | G to A at 380 | 1 (0.5%) | 2.3% |
| E101G | G to A at 302 | 1 (0.5%) | 2.3% |
| Novel mutation | | | |
| 358-360delGAG | Del GAG at 358 | 2 (1%) | 4.7% |
| Detected | | 42 | |
| Total | | 200 | |
| Polymorphism | | | |
| V153I | G to A at 457 | 6 | |
| V27I | G to A at 79 | 2 | |

## Discussion

In a previous study from Iran, Hashemzade *et al* (2007) showed that the *GJB2* gene mutations are present in approximately 14.6% of deaf families (12). In addition, Najmabadi *et al* (2005) revealed that 16.7% of deaf families had *GJB2* mutations in Iran (13).

Our results showed that *GJB2* mutations were present in about 25% of patients with non-syndromic hearing loss (Table 1).

The mutation detection rate in connexin 26 in our study was higher than the previous studies (12, 13). This discrepancy may be due to the facts that our study groups were less than 10 years old; whereas, in the previous studies, samples were collected from different ages. This result showed an increase of *GJB2* mutation in young generation of Iranian population. This increase of *GJB2* in young patients can be due to:

Firstly, an increase of consanguine marriage in the Iranian population. The frequency of consanguine marriage in study group was 68%, whereas, in the previous study it was 37.5% (11). Secondly, other genes or factors which can cause late hearing loss do not appear in our study group, so the result was higher prevalence of *GJB2* mutation.

The 35delG mutation constituted the majority of *GJB2* mutations (64%) in our study group (Table 2), which is in agreement with the previous reports (12, 13). The novel variant 358delGAG could be a pathologic variation because this mutation removed three nucleotides; therefore, one glutamic acid of CL (cytoplasmic linking) domain was deleted. This domain and C-terminal domain are involved in pH gating of the channel (14).

Also, there are 2 glutamic acids consecutively in sequence of *GJB2* gene. These 2 glutamic acids at residue 119 (15) and 120 of Cx26 are almost conserved among the different species and among human connexin proteins (Figure 1C) and finally this variation does not exist in normal Iranian population (15). These results show that mutation must be examined for functional effects.

In heterozygous carriers of the 35delG, R32H, R143W, R127H, M163V and E101G mutations, no other mutations had been detected in the coding region of *GJB2* (exon 2). A second mutation may be located in the promoter, untranscribed regions or exon one. It is also possible that another gene causing hearing loss is involved in these patients. Further analysis is needed to discover the problem of patients with mono allelic mutations in the coding region of *GJB2*.

## Conclusion

In summary, these data support and extend the previous findings concerning the contributions of *GJB2* to hearing loss, but our results show:

-*GJB2* mutation is increased in the Iranian population because of an increase in consanguine marriages.

-358delGAG mutation may be considered a pathologic mutation but more functional studies are necessary.

## Acknowledgment

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