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*Iranian J Publ Health, 2005, Vol. 34, No. 1, pp.1-7*

# Frequencies of Mutations in the Connexin 26 Gene (GJB2) in Two Populations of Iran (Tehran and Tabriz)

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## Abstract
While hearing loss has been considered to be a very heterogeneous disorder, mutations in Gap junction beta 2 (GJB2) gene encoding Connexin 26 (Cx26) protein are the major cause of autosomal recessive and sporadic non-syndromic deafness in many populations. In this study, we have investigated the prevalence of the GJB2 gene mutations using nested PCR pre screening strategy and direct sequencing method. Two hundred and seventy two hearing impaired subjects were studied from 210 families obtained from two large cities of Iran (Tehran and Tabriz). Twenty four different genetic variants were identified. Cx26 mutations were found in 53 of the 210 families (25.2%) including T8M, 35delG, W24X, R32H, V37I, E47X, 167delT, delE120, Y136X, R143W, R184P, 235delC and V27I+E114G. Homozygosity and compound heterozygosity for the Cx26 mutations were found in 39 of 210 (18.5%) families. Homozygosity for the 35delG mutation was the most common that causes hearing loss in 28 (13.3%) patients. Six novel variants H16R, E101E, K102Q, G200R, 327delG and G130A were detected in this study. As a conclusion, the present survey revealed that the rate of mutation in Cx26 gene in our area is lower than in Europe; nevertheless, this rate is regarded as a considerable cause of deafness in the cited provinces in Iran.

**Keywords:** *Connexin 26, GJB2, Deafness, Autosomal recessive non syndromic hearing loss, Iran*

## Introduction
Hearing loss is the most frequent sensory disorder that affects 1 in 1000 neonates with more than 60% heredity form (1). About 80% of the hereditary cases are non-syndromic and the major mode of inheritance is autosomal recessive (2). Mutations in the Connexin 26 (GJB2) gene (GJB2; MIM# 121011) at the DFNB1 locus (DFNB1; MIM#220290) on chromosome 13q12 have been shown to be involved in the development of autosomal recessive and sporadic non-syndromic deafness in many populations (3-8). Connexin 26 is a member of the family proteins encodes the gap junction protein that is expressed in a variety of tissues including cochlea. Gap junctions are being served as a major communication system allowing the rapid exchange of electrolytes, second messengers and metabolites between adjacent cells (4). It is also thought that this gap junction plays an

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important role in auditory transduction, by recycling endolymphatic potassium ions (9). Although, a single mutation known as 35delG accounts for the majority of Cx26 mutations in some populations (10-12) but is less frequent or even absent in other ethnic groups. They care other common mutations such as 235delC in the Japanese and Korean (13-15), 167delT in the Ashkenazi Jews (16) and R143W mutation in an African village (17).
In a previous study, 43 autosomal recessive non-syndromic hearing loss subjects from 34 families in Iran were analysed to identify GJB2 gene mutations and 11 different genetic variants were observed. GJB2-related deafness mutations were found in 9 of 34 ARNSHL families (26.5%) and homozygosity in 7 (20.6%) (18). In the present study we have identified the spectrum and prevalence of Connexin 26 mutations using nested PCR pre screening strategy and direct sequencing technique in coding region of the gene.

## Materials and Methods

***Patients*** We have analysed 272 patients from 210 unrelated families, consisting of 139 families from Tehran and 71 families from Tabriz, diagnosed with non-syndromic and autosomal recessive hearing loss (ARNSHL). Their ages were between 2 and 35 years old (mean: 13.3), attending hearing-impaired schools in Tehran and Tabriz. Medical history and pedigree information were obtained by interviewing and filling questionnaires. Consanguinity was observed as 74.8% in both populations under study. There were no syndromic features and the hearing of all parents was normal. All patients had mild to profound sensorineural hearing loss.
***DNA analysis*** DNA was extracted from 10 ml peripheral whole blood using standard salting out procedures. In the first step, all samples were screened for the 35delG mutation using nested PCR strategy. Using a nested PCR procedure, the one base pair deletion of 35delG was screened in 210 families. The entire coding sequence of Cx26 gene (Genbank accesion# M86849) was amplified using primers CX26F5 5'CCTGTGTTGTGTGCATTCGTC3'/CX26R 5 5'CTCATCCCTCTCATGCTGTC3' (782 bp) at an annealing temperature of 59ºC. The amplified product was then diluted and used as a template for a second round of PCR using primers CX26F4 5'CACGCTGCAGACGATCC3'/ CX26R4 5'GGTGGAGTGTTTGTTCAC3'(43 bp) at an annealing temperature of 56º C. The amplified products were then separated by electrophoresis on a 15% polyacrylamide gel (40% 19:1 acrylamide: bisacrylamide) at 35 mA for 5:30 hours and the products were then visualised by silver staining. The 35delG mutations were detected by identification of two separated bands of 43 bp for the wild type and 42 bp for the mutant allele. Then, in order to confirm the results of this study and also for finding other mutations in the GJB2 gene, direct sequencing was used. One set of primers was used to produce the template for sequencing in coding (Genbank accesion# U43932) regions. The primers CX26F5/ CX26R5 (as described above) were used to amplify the entire coding region of the gene. Sequencing was carried out in Sequence Laboratories (SeqLab), Gottingen, Germany, using ABI 3100 and ABI 3700 sequencer.

## Results

In this study we analyzed the coding region of connexion 26 in 210 ARNSHL families from two large cities of Iran (139 families in Tehran and 71 families in Tabriz) using nested PCR and consequent direct sequencing. Thirteen Cx26 mutations including 35delG, T8M, 167delT, R143W, W24X, R32H, V37I, E47X, delE120, Y136X, R184P, 235delC and V27I+ E114G were found in 53 of 210 (25.2%) families (Table 1). We identified 39 of 210 families (18.5%) with homozygous and compound heterozygous for Cx26 mutations, whereas, 14 other families (6.7%) revealed mutation only in

one allele. Twenty eight families (13.3%) were homozygous for the 35delG mutation in two cities. Also, 4 polymorphisms V27I, S86T, R127H and V153I and one unknown mutation M163V were found. S86T polymorphism was observed in all families (100%). Six new variants (H16R, E101E, K102Q, G130A, G200R and 327delG) were also detected (Fig. 1).

In Tehran, with a mixed population, 31(22.3%) families expressed 9 mutations among 139 families. Four novel variants including E101E, K102Q, G200R and 327delG were found in Tehran. Three variants E101E, K102Q and 327delG were heterozygous, which occurred in the second intracellular domain (IC2) of the Cx26 protein. The G200R, containing one homozygous change, occurred in the fourth transmembrane domain (TM4).

Twenty two (31%) families revealed 8 mutations among 71 deaf families from Tabriz. Also two new variants were found in this city including H16R and G130A. Both of these variants were heterozygous, that occurred in the first intracellular domain (IC1) and second intracellular domain (IC2) of the Cx26 protein, respectively.

**Table 1:** Genetic variation in the GJB2 gene identified in Iranian autosomal recessive non-syndromic hearing loss families

| Genotype | Tehran Families n (%) | Tabriz Families n (%) | Total |
|---|---|---|---|
| T8M/V153I | 2(1.4) | - | 2 |
| 35delG/35delG | 17(12.2) | 11(15.5) | 28 |
| 35delG/wt | 2(1.4) | 3(4.2) | 5 |
| 35delG/167delT | 1(0.7) | 1(1.4) | 2 |
| 35delG/R143W | 1(0.7) | - | 1 |
| H16R/R143W | - | 1(1.4) | 1 |
| W24X/wt | 1(0.7) | - | 1 |
| V27I/wt | - | 1(1.4) | 1 |
| V27I+E114G/wt | - | 1(1.4) | 1 |
| R32H/R32H | 1(0.7) | - | 1 |
| V37I/wt | - | 1(1.4) | 1 |
| E47X/wt | 1(0.7) | - | 1 |
| S86T | 139(100) | 71(100) | 210 |
| E101E/wt | 1(0.7) | - | 1 |
| K102Q/wt | 1(0.7) | - | 1 |
| delE120/delE120 | - | 1(1.4) | 1 |
| R127H/wt | - | 2(2.8) | 2 |
| G130A/wt | - | 1(1.4) | 1 |
| Y136X/Y136X | - | 1(1.4) | 1 |
| R143W/R143W | 1(0.7) | - | 1 |
| V153I/wt | 5(3.6) | 2(2.8) | 7 |
| M163V/wt | - | 1(1.4) | 1 |

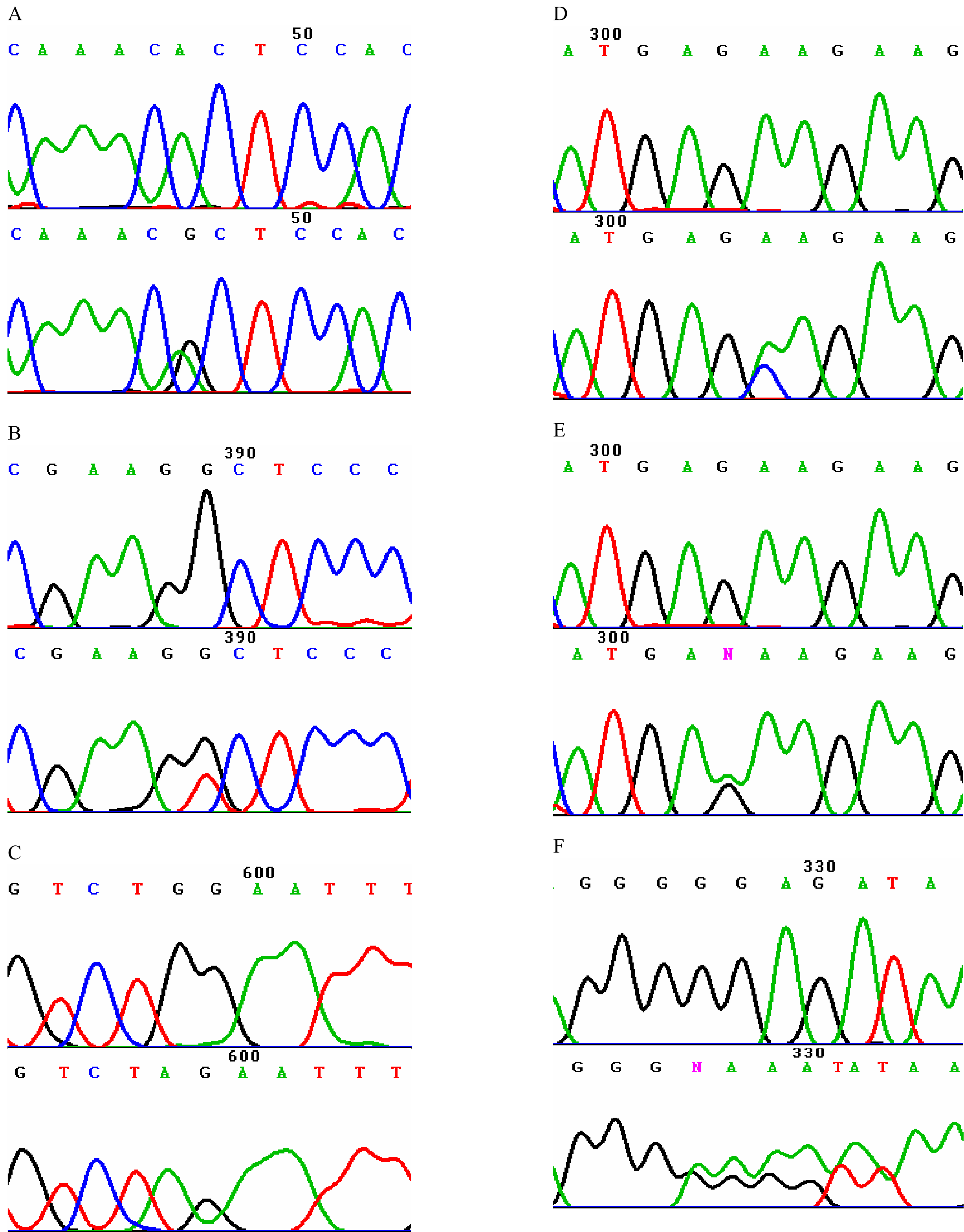


**Fig. 1:** Nucleotide sequences of the novel variants have been identified in Cx26 gene (A:H16R, B:G130A, C:G200R, D:K102Q, E:E101E, F:327delG) in comparison with control group from Iran.

## Discussion

Mutations in connexin 26 gene have been detected in many ethnic populations and are the most common cause of ARNSHL in many populations (4, 14). Mutations in Cx26 are responsible for 50% of autosomal recessive deafness. Thirty-five delG mutation is more frequently observed in Caucasian families from Tunisia, France, New Zealand and UK (10). This mutation is responsible for 10% of all childhood hearing loss and for 20% of all childhood hereditary hearing loss in American Caucasians originated from north and south Europeans (19). Our finding showed a lower rate of mutations in GJB2 (25.2%). This is also lower than those reported from Italy, Spain (49%) and Lebanon (33%) (11, 20). However, the high frequency of the 35delG mutation in white populations possibly is the results of a founder effect rather than a mutational hot spot (5,10,19).

Rate of 35delG mutation in Tehran (22.3%) was lower than that in Tabriz (31%) is the most common in both cities (58.5% of 53 families). Genetic variations in the cited locus may be due to migration during recent decades in Tehran and Tabriz. Since the rate of migration to Tehran is high, we consider it with a mixed population. Tabriz is alike Tehran, where migration occurs frequently.

The genetic variation, 327delG, was found in one of our patients, which observed in Gilan province (21). Upon reviewing this patient questionnaire, we noted that her mother was from Gilan. Therefore, such variation is probably originated from Gilaki nation. However more investigations are warranted to confirm such hypothesis.

In the previous study (18), using a method similar to the present survey (nested PCR pre screening strategy and direct sequencing method), in coding and non-coding regions of the gene, the GJB2-related deafness mutations were found in 7 from 34 families (20.6%) with homozygosity in this gene. In the present investigation we identified 39 from 210 families (18.5%) with homozygous and compound heterozygous mutations. The difference in observed mutation rate might be happened due to ignoring non-coding region analysis in this study.

Najmabadi and coworkers (22) studied 168 autosomal recessive nonsyndromic deafness from 83 families in Iran, using allele-specific PCR for screening the 35delG and pre screening method of single strand conformational polymorphism (SSCP) followed by sequencing to screen the other variants. GJB2-related deafness was diagnosed in 9 families (11%) with homozygous and compound heterozygous. Moreover, they found 9 different genetic variants. However, we detected 24 different genetic variants and found 39 (18.5%) of 210 families with homozygous and compound heterozygous for Cx26 mutations. This difference might be due to lower SSCP analysis efficiency and/or selected areas where the rate of mutation was lower than our.

In our survey, S86T polymorphism was observed in all cases. Similar observation was previously reported in Gilan and Khorasan provinces (18). Thus, it seems that such polymorphism exists in all populations of Iran.

In conclusion, the role of GJB2 gene mutations in autosomal recessive and sporadic non-syndromic hearing loss in Iran may be lower than that in other populations, studied so far (10, 11, 19). The rate of consanguinity in this study was relatively high (74.8%), compared to the rate of 37.3% consanguinity reported before (23). Such a high rate may be due to the consanguinity or ascertainment among our deaf population. Since populations in Iran are composed of several ethnic groups, more works on the ethnic populations are warranted.

## Acknowledgments

We would like to thank the Ministry of Education and Training of the Islamic Republic of Iran, Department of Exceptional Children and

all the individuals, families, students and schools for hearing-impaired in Tehran and Tabriz for their contribution to this study. The Department of Exceptional Children, Zafar Avenue, Tehran, Iran, supported this study.

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