

## Report of a New Mutation and Frequency of Connexin 26 gene (GJB2) Mutations in Patients from Three Provinces of Iran

*A Hosseinipour<sup>1</sup>, M Hashemzadeh Chaleshtori<sup>2,3</sup>, R Sasanfar<sup>1,\*</sup>, DD Farhud<sup>2</sup>, A Tolooi<sup>1</sup>  
M Doulati<sup>4</sup>, L Hoghooghi Rad<sup>4</sup>, M Montazer zohour<sup>2</sup>, M Ghadami<sup>1</sup>*

<sup>1</sup>*Dept. of Exceptional Children, Ministry of Education and Training, Tehran, Iran*

<sup>2</sup>*Dept. of Human Genetic, School of Public Health, Tehran University of Medical Sciences, Iran*

<sup>3</sup>*Dept. of Biochemistry and Genetics, Medical School, Shahrekord University of Medical Sciences, Iran*

<sup>4</sup>*Dept. of Biology, School of Basic Sciences, Science and Research Campus, Tehran Islamic Azad University, Iran*

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### Abstract

Autosomal recessive and sporadic non-syndromic hearing loss (ARSNSHL) is the major form of hereditary deafness. Mutations in the GJB2 gene encoding the gap-junction protein Connexin 26 have been identified to be highly associated with ARSNSHL. In this study we have analyzed 196 deaf subjects from 179 families having one or more deaf children in 3 provinces of Iran, including Kordestan, Khuzestan and Golestan. The nested PCR prescreening strategy and direct sequencing technique were used to detect the mutations in coding exon of the gene. Altogether 3 GJB2 recessive mutations including 35delG, 167delT and V27I+E114G, were identified in 23 of 179 families (12.8%). Fourteen of 179 families were observed to have GJB2 mutation in both alleles (7.8%). A novel variant (R159H) also was found in a deaf family from Khuzestan. Four polymorphisms V27I, E114G, S86T and V153 I also were detected in 7 families. A polymorphism (S86T) was seen in the whole population studied. Our data indicated that the rate of connexin 26 mutations is different in this three Iranian population and is lower than the high frequency of 35delG (26%) reported from Gilan province in the north of Iran.

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

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### Introduction

Hearing loss is the most common sensory deficit and it is estimated that more than 70 million people are suffering from this problem in the world. Hearing loss affects 1 infant per 1000 born with 60 % of inherited cases (1, 2).

Autosomal recessive non-syndromic hearing loss is the cause of 80% of hereditary deafness cases from which 30-40 percent are associated with mutation in the Connexin 26 gene (GJB2) (3).

More than 60 mutations have been identified in GJB2 gene, with predominant 35delG mutation responsible for 30-60 percent of Connexin 26 mutations in white Populations (4-7).

In this study we have investigated the spectrum and prevalence of Connexin 26 mutations in 196 ARSNSHL subjects from 179 families in three different Iranian populations (Kordestan, Khuzestan and Golestan).

The nested PCR prescreening strategy and direct sequencing technique were used to detect the mutations in coding exon of the gene.

### Materials and Methods

A total of 196 deaf individuals of 179 families from 3 different provinces (Golestan in north, Kordestan in west and Khuzestan in south) of Iran, were investigated. These are 3 originally different populations including Turkmans in

Golestan, Kords in Kordestan and Arabs in Khuzestan. The patients were students of hearing-impaired schools and their siblings between ages of 4 to 27 (mean 14.3 y). The patients analyzed in this study were mostly related (%90 consanguinity). The medical history and pedigree information were collected by a questionnaire. Both parents of each patient had normal hearing with one or more affected children in family. All patients had mild to profound sensorineural hearing loss. All the families were informed and consents were obtained.

DNA was extracted from 5 ml of peripheral blood following the standard procedures. Pre-screening of 35delG and sequencing of Connexin 26 coding exon were performed as previously described (8).

The entire coding sequence of cx26 gene (Genbank accession # M86849) was amplified using primers CX148F2 5`CCTGTGTTGTGTGCATTCGTC3`/CX929R3 CTCATCCCTCTCATGCTGTC3`5` (43 bp) at an annealing temperature by electrophoresis on a 15% polyacrylamide gel (40% 19:1 acrylamide: bisacrylamide) at 35 mA for 2:30 hours and the products were detected by identification of two separate bands of 43 bp for the wild type and 42 bp for the mutant allele. Two sets of primers were used to produce the template for sequencing in both coding and non-coding (Genbank accession #

U43932) regions. The first pair of primers CX148F2 / CX929R3 (as described above) was used to amplify the entire coding region of the gene. The second pair of primer CX1197F1 5`AGGCGGGCGCTCGGGGTAAC3`/CX1679RI5`TCCCCGCGCCAGGTTCTCTG3` (483 bp) was used to amplify the non-coding, the flanking donor splicing site and upstream region to the gene at annealing temperature of the gene in both directions. In addition, two internal primers CX586R2 5`CTTCGATGCGGACCTTCTGG3` and CX482F3 5`TGGCCTACCGGAGACATGAG3` were used for sequencing of the coding region. Sequencing was carried out using an ABI Big Dye Terminator on an ABI 377 automated sequencer (8).

## Results

One hundred and ninety six ARSNSHL individuals from 179 families in 3 provinces of Iran (Kordestan, Golestan and Khuzestan) were investigated.

Twenty one of 179 deaf families (11.7%) were found to have 35delG mutation. The detected mutations were then confirmed by sequencing of the appropriate exon. In order to determine GJB2 mutations other than 35delG, sequencing of the whole coding region of the gene were carried out and altogether 8 different variants were detected (Table 1).

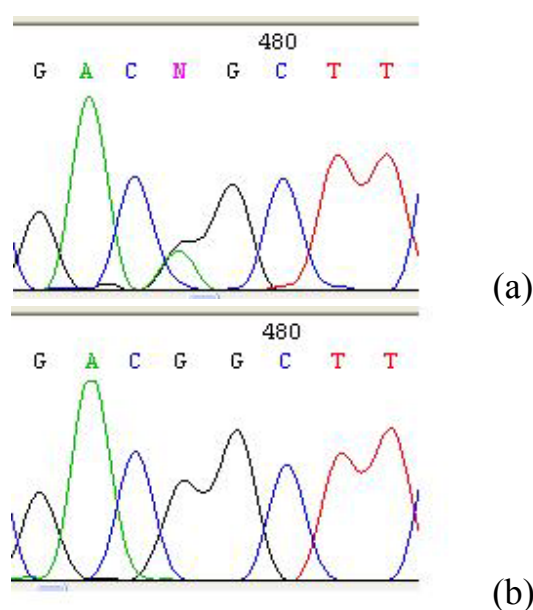
**Table 1:** Cx26 genetic variants identified in Iranian ARSNSHL families (compare to Genbank accession # M86849)

Genotype	Golestan families	Kordestan families	Khuzestan families	Total
	No (%)	No (%)	No (%)	
35delG/35delG	3 (5.5)	6 (11.8)	3 (4.1)	12
35delG/wt	3 (5.5)	3 (5.9)	3 (4.1)	9
35delG /167delT	1 (1.8)	0 (0.0)	0 (0.0)	1
V27I/E114G	1 (1.8)	0 (0.0)	0 (0.0)	1
V27I/wt	1 (1.8)	0 (0.0)	0 (0.0)	1
E114G/wt	0 (0.0)	0 (0.0)	1 (1.4)	1
V153I/wt	2 (3.6)	1 (2)	0 (0.0)	3
R159H/wt	0 (0.0)	0 (0.0)	1 (1.4)	1
S86T/S86T	55 (100)	51 (100)	73 (100)	179

Three GJB2 resesicive mutations including 35 delG, 167delT and V27 I+E114 G were detected in 23 of 179 families (12.8%). In addition 4 polymorphisms V27I, S86T, E114G and V153I were determined. S86T was found in 100% of our cases.

A novel variant R159H was found in Arab family from Khuzestan (Fig.1).

The most common mutation was 35delG in 21 out of 23 deaf families with GJB2 mutation (91%).



**Fig. 1:** Nucleotide sequence of the novel variant (R159 H) (a) compared to control (b)

Three different deaf populations including Turkmen in Golestan province, Kords in Kord-estan province and Arabs in Khuzestan province were studied.

Fifty five deaf families (mean age 15.5 years) from Golestan province in north of Iran were analysed for mutations of Cx26.

Three different GJB2 mutations including 35 delG, 167delT and V27I+ E114G were found in 8 of 55 deaf families (14.5%). In addition, fifty one deaf families (mean age 11.8 y) from Kord-estan province in west of Iran were investigated for Cx26 mutations. Only one GJB2 mutation

35 delG, was identified in 9 of 51 deaf families (17.6%).

We also determined the Cx26 mutations of seventy three deaf families (mean age 15.8 y) from Khuzestan province in south of Iran. Only one GJB2 mutation, 35delG, was found in 6 of 73 deaf families (8.2%).

## Discussion

Only 14 out of 179 families were observed to have mutations in both alleles (7.8%). Thirty-five delG was the most common mutation containing 21 of 23 GJB2 mutations (91%). While the predominant mutation was 35delG in the population studied but the frequency of this mutation was very low in comparison to the high frequency of 35delG (26%) reported from Gilan province in the north of Iran (9).

A novel variant (R159H) also was found in this study which occurs in the second extracellular domain (E2) of the Cx26 protein.

As the novel variant happened in heterozygous style, it is difficult to talk about the pathogenicity of the mutation.

The rate of Cx26 mutations in this study (12.8% of deaf families) is lower than European, North American and Mediterranean populations (4-7). Regarding a low rate of Cx26 mutation in this study we would expect the contributions of other genes to cause autosomal recessive non-syndromic hearing loss. Iran is a big country with several racial, cultural, social and geographical situation.

We have several neighboring country and people of the border provinces are expose to the mixing via migrations and marriages.

In agreement with another study (8) we found that spectrum and frequencies of Cx26 mutation and polymorphisms may vary in each ethnic group or populations.

Finally, identification of spectra and frequencies of GJB2 mutations in different populations could be very helpful to easily detection and control of Cx26 associated deafness.

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