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Review Article

Familial and Sporadic GJB2-Related Deafness in Iran: Review of Gene Mutations

**M Hashemzadeh Chaleshtori [1], DD Farhud [2], MA Patton [3]*

[1] Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2] Genetic Clinic, Vallie Asr Sq., 16Keshavarz Blvd. Tehran, Iran
[3] Dept. of Medical Genetics, St Georges Hospital Medical School, University of London, London, UK

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Abstract

Background: Mutations in the GJB2 gene encoding connexin 26 protein, are the main cause for autosomal recessive and sporadic non syndromic hearing loss in many populations. Here, we have taken together and reviewed results from our six previous publications, our unpublished data from ten Iranian provinces, as well as data from two previous mutation reports to provide a comprehensive collection of data for GJB2 mutations and deafness in Iran. **Methods:** In all, 1095 hearing impaired students and their deaf siblings from 890 families in 10 provinces of Iran were studied. The prevalence and type of the GJB2 gene mutations were investigated using nested PCR pre screening strategy and direct sequencing of the coding exon of the gene. **Results:** Altogether 31 different genetic variants were detected from which 17 GJB2 mutations were identified. GJB2 mutations were found in 14.6% of deaf families (18.29% of familial and 12.7% of sporadic cases). We found GJB2 mutations in both alleles in 78% of GJB2 mutations chromosomes. However, 35delG mutation was the most common GJB2 mutation accounting for 74.5% of the mutations in populations studied. **Conclusion:** Our data indicated that a specific combination of GJB2 mutations types and frequencies was presented in different populations of Iran. These results also highlight the importance of GJB2 mutations in development of hearing loss in familial and sporadic deaf families in different parts of the country and can be used as a basis of genetic counseling and clinical guideline in Iran.

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

Introduction

Hearing loss is the most frequent sensory disorder that affects about 4% of people under 45 yr of age containing a broad spectrum of clinical presentations such as congenital or late onset, conductive or sensorineural and syndromic or non-syndromic (1). The incidence of prelingual deafness is about 1 in 1000 neonates of which more than 60% of cases are being inherited (2-4). About 80% of the hereditary deafness cases are non-syndromic and the major mode of inheritance is autosomal recessive (5).

Hearing loss is a very heterogenous disorder and happens due to genetic or environmental causes or both. More than 100 genes may be involved in non syndromic hearing loss. Despite the contribution of several different genes in causing deafness, mutations in the connexin 26 (GJB2) gene have been shown to be involved in the development of autosomal recessive and sporadic non syndromic hearing loss (ARSNSHL) in many populations (6-11). Connexin 26 is a member of 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 (7, 12, 13). It is also thought that gap junctions play an important role in auditory transduction, by recycling endolymphatic potassium ions (13, 14). A single mutation, known as 35delG, accounts

***Corresponding author:** Tel: +98 381 3346692, Fax: +98 381 3330709, E-mail: mchalesh@skums.ac.ir

for the majority of GJB2 mutations in some populations particularly people of European descent (10, 15-17). The 35delG mutation is less frequent or even absent in other ethnic groups. However GJB2 mutations are ethnic specific and other mutations such as 235delC (18-20), 197delT (8, 21) and R143W (22) are common in East Asian, Ashkenazi Jews and African population respectively.

In this study, we reviewed our six previous publications on 890 ARSNSHL families (23-28) and discussed in comparison with 717 autosomal recessive non syndromic hearing loss (ARNSHL) families of other investigators (29, 30). In addition, we have reported our unpublished data from GJB2 mutations in 298 familial and 592 sporadic subjects in ten provinces of Iran.

Materials and Methods

Inclusion criteria for this study, DNA sampling and mutation analysis have been described elsewhere (23, 26). Briefly, subjects to be included in this study had to meet the following criteria: [A] a pedigree structure consistent with autosomal recessive or sporadic pattern [B] both parents have normal hearing [C] one or more deaf children in the family [D] hearing loss in the absence of other clinical features [E] hearing loss was not a result of environmental factors such as infection, trauma, rubella, meningitis, mumps, ototoxic drugs and premature birth.

Altogether 1095 patients from 890 families with mild to profound sensorineural hearing loss were investigated. The subjects were students of schools for deaf and their sibs between 2 and 35 yr of age (mean 13.8 yr in 10 provinces of the country). Medical history and pedigree information were obtained by a questionnaire. Informed consent was obtained from all subjects or parents of under aged patients.

Mutation detection of the coding region of the GJB2 gene was performed using nested PCR prescreening and subsequent direct sequencing procedure. Genomic DNA was extracted from peripheral blood following the standard protocols. The whole samples were first screened for the predominant GJB2 mutations (35delG) using nested PCR strategy. Subsequently, the negative 35delG samples and samples heterozygous for the 35delG allele were sequenced for the coding region (exon 2) of the gene.

Results

Marriage between close relatives or consanguinity was detected in 71% of the deaf families studied. Of 890 families studied, 298(33.5%) and 592(66.5%) presented with familial and sporadic deafness respectively (Table1).

Altogether, 31 different genetic variants were detected from which 17 GJB2 mutations including IVS1+1G>A, T8M, 35delG, W24X, V27I+E114G, R32H, V37I, E47X, 167delT, W77X, 235delC, L90P, delE120, R127H, Y136X, R143W and R184P were identified in 259 of 1780 chromosomes (14.6%). The allele variants and genotypes identified in various Iranian ARSNSHL families are summarized in Table 2 and 3 (11). GJB2 mutations were found in 109/596 (18.29%) and 150/1184 (12.7%) of familial and sporadic deaf families chromosomes respectively (Table 4). We found GJB2 mutations in both alleles in 202 of 1780 chromosomes (11.35%). A specific combination of GJB2 mutations types and frequencies were found in different populations of Iran (Table 2, 3). The highest frequency of GJB2 mutations (27.6%) were found in Gilan province in the north of the country while the lowest frequency (3.6%) were detected in Sistan va Baloochestan province in the southeast (Fig. 1). Also, a higher GJB2 mutations diversity (11 type) were detected in metropolitan in central but the lower diversity identified in Kordestan (1 type) and Khoozestan (1 type) in the west and south west of the country. We found 1 Unknown Mutations namely M163V (11). Six polymorphisms including -3558T> C, V27I, M34T, E114G, V153I and G160S were also detected in 80 of 1780 chromosomes (4.5%) (11). In addition, an allelic variant namely S86T was detected homozygous

in the whole chromosomes studied (compare to Gene bank accession M86849) (31). Seven novel variants including -3517G> A, H16R, K102Q, 327delGGinsA, 363delC, G130V and G200R were also detected in Tehran, Azarbaijan Sharqi, Gilan and Chaharmahal va Bakhtiar provinces. All novel allelic variants were named following the nomenclature recommendations (32).

However, 35delG was the most prevalent GJB2 mutation accounting for 193 of 259 (74.5%) of the GJB2 mutations chromosomes and 193 of 1780 (10.8%) of all chromosomes studied. The second and third prevalent GJB2 mutations detected in our study was R127H and V27I+E11G with sum of 8.9% of the GJB2 mutations chromosomes.

Table 1: ARNSHL families and genetic relationship between relatives in various Provinces of Iran

Relationship	**Azarbaijan Sharqi**	**Kordestan**	**Chaharmahal va Bakhtiari**	**Khoozestan**	**Gilan**	**Tehran**	**Hormozgan**	**Golestan**	**Khorasan**	**Sistan va Baloochestan**	**Total**
Second degree	-	-	-	-	-	1	-	-	-	2	3
Third degree	36	16	43	45	31	97	40	30	76	42	456
Fourth degree	4	5	5	10	4	20	10	2	8	15	83
Fifth degree	6	4	9	5	5	17	11	2	16	15	90
Non relative	25	26	22	13	47	38	44	21	12	10	258
consanguinity	64.8%	49%	72.2%	82.2%	46%	78%	58.1%	61.8%	89.3%	88.1%	71%
familial	41	16	31	22	14	39	23	26	48	34	298
Sporadic	30	35	48	51	73	134	82	29	64	50	592
Total families	71	51	79	73	87	173	105	55	112	84	890

Table 2: GJB2 genotypes identified in 890 Iranian ARSNSHL families from 10 provinces of Iran

Genotypes	**Tehran**	**Azarbaijan Sharqi**	**Kordestan**	**Chaharmahal va Bakhtiari**	**Khoozestan**	**Gilan**	**Hormozgan**	**Golestan**	**Khorasan**	**Sistan va Baloochestan**	**Total**	**References**
-3558T>C/-3558T>C	6	-	-	-	-	-	-	-	-	-	6	23
-3558T>C/wt	9	-	-	-	-	-	-	-	-	-	9	23
-3517G>A/-3517G>A	1	-	-	-	-	-	-	-	-	-	1	23
-3517G>A/wt	2	-	-	-	-	-	-	-	-	-	2	23
IVS1+1G>A/wt	1	-	-	-	-	-	-	-	-	-	1	23
T8M/ V153I	2	-	-	-	-	-	-	-	-	-	2	25

Table 2: Continued…

35delG/35delG	22	11	6	5	3	18	1	3	11	-	80	23,24,25,26,27,28
35delG/167delT	1	1	-	-	-	-	-	1	-	-	3	25,27
35delG/ R143W	1	-	-	-	-	-	-	-	-	-	1	25
35delG/wt	2	3	3	-	3	11	1	3	3	-	29	24,,25,27,28
H16R/ R143W	-	1	-	-	-	-	-	-	-	-	1	25
W24X/ W24X	-	-	-	-	-	-	1	-	-	1	2	28
W24X/wt	2	-	-	-	-	-	-	-	-	-	2	23,25
V27I/ V27I	-	-	-	-	-	-	1	-	-	-	1	28
V27I/wt	-	1	-	1	-	1	4	1	5	-	13	24,25, 26, 27,28
V27I+ E114G/ V27I+ E114G	-	-	-	-	-	-	-	1	-	1	2	27,28
V27I+ E114G/wt	-	1	-	1	-	1	-	-	3	-	6	24,25,26
R32H/ R32H	1	-	-	-	-	-	-	-	-	-	1	25
M34T/wt	-	-	-	-	-	-	-	-	1	-	1	24
V37I/wt	-	1	-	-	-	-	-	-	-	-	1	25
E47X/wt	1	-	-	-	-	-	-	-	-	-	1	25
W77X/ W77X	-	-	-	-	-	-	-	-	1	-	1	24
235delC/235delC	3	-	-	-	-	-	-	-	1	-	4	23,24,25
L90P/ delE120	-	-	-	-	-	-	-	-	1	-	1	24
K102Q/wt	1	-	-	-	-	-	-	-	-	-	1	25
327delGGinsA/327delG GinsA	-	-	-	-	-	1	-	-	-	-	1	24
327delGGinsA/wt	1	-	-	-	-	-	-	-	-	-	1	25
E114G/wt	-	-	-	-	1	-	-	-	-	-	1	27
delE120/ delE120	-	1	-	-	-	-	-	-	-	-	1	25
363delC/363delC	-	-	-	2	-	-	-	-	-	-	2	26
R127H/wt	1	2	-	1	-	-	4	-	3	2	13	23,24,25,26,28
G130V/wt	-	1	-	-	-	-	-	-	-	-	1	25
Y136X/ Y136X	-	1	-	-	-	-	-	-	-	-	1	25
R143W/R143W	1	-	-	-	-	-	-	-	-	-	1	25
V153I/V153I	1	-	-	-	-	-	1	-	-	1	3	23
V153I/wt	9	2	1	3	-	4	1	2	5	6	33	23,24,25,26,27,28
G160S/wt	-	-	-	-	-	1	-	-	-	-	1	24
M163V/ M163V	1	-	-	-	-	-	-	-	-	-	1	23
M163V/wt	-	1	-	-	-	-	-	-	-	-	1	25
R184P/ R184P	2	1	-	-	-	-	-	-	-	-	3	25
R184P/wt	-	1	-	-	-	-	-	-	-	-	1	25
G200R/ G200R	1	-	-	-	-	-	-	-	-	-	1	25
Total	72	29	10	13	7	37	14	11	34	11	238	

Table 3: GJB2 allele variants identified in 890 Iranian ARSNSHL families from 10 provinces of Iran (11). The percentage are given in bracket (%)

Allele variants	**Tehran**	**Azarbaijan Sharqi**	**Kordestan**	**Chaharmahal va Bakhtiari**	**Khoozestan**	**Gilan**	**Hormozgan**	**Golestan**	**Khorasan**	**Sistan va Baloochestan**	**Total**
Mutations											
IVS1+1G>A	1(0.3)	-	-	-	-	-	-	-	-	-	1(0.06)
T8M	2(0.6)	-	-	-	-	-	-	-	-	-	2(0.1)
35delG	48(13.9)	26(18.3)	15(14.7)	10(6.4)	9(6.2)	47(27.1)	3(1.5)	10(9.1)	25(11.2)	-	193(10.8)
W24X	2(0.6)	-	-	-	-	-	2(1)	-	-	2(1.2)	6(0.34)
V27I+ E114G	-	1(0.7)	-	1(0.7)	-	1(0.6)	-	2(1.8)	3(1.4)	2(1.2)	10(0.6)
R32H	2(0.6)	-	-	-	-	-	-	-	-	-	2(0.1)
V37I	-	1(0.7)	-	-	-	-	-	-	-	-	1(0.06)
E47X	1(0.3)		-	-	-	-	-	-	-	-	1(0.06)
167delT	1(0.3)	1(0.7)	-	-	-	-	-	1(0.9)	-	-	3(0.17)
W77X	-	-	-	-	-	-	-	-	2(0.9)	-	2(0.1)
235delC	6(1.8)	-	-	-	-	-	-	-	2(0.9)	-	8(0.45)
L90P	-	-	-	-	-	-	-	-	1(0.5)	-	1(0.06)
delE120	-	2(1.4)	-	-	-	-	-	-	1(0.5)	-	3(0.17)
R127H	1(0.3)	2(1.4)	-	1(0.7)	-	-	4(1.9)	-	3(1.4)	2(1.2)	13(0.7)
Y136X	-	2(1.4)	-	-	-	-	-	-	-	-	2(0.1)
R143W	3(0.9)	1(0.7)	-	-	-	-	-	-	-	-	4(0.22)
R184P	4(1.2)	3(2.2)	-	-	-	-	-	-	-	-	7(0.39)
Unknown Mutations											
M163V	2(0.6)	1(0.7)	-	-	-	-	-	-	-	-	3(0.17)
Novel Variants											
-3517G>A	4(1.2)	-	-	-	-	-	-	-	-	-	4(0.22)
H16R	-	1(0.7)		-	-	-	-	-	-	-	1(0.06)
K102Q	1(0.3)	-	-	-	-	-	-	-	-	-	1(0.06)
327delGGinsA	1(0.3)	-	-	-	-	2(1.2)	-	-	-	-	3(0.17)
363delC	-	-	-	4(2.6)	-	-	-	-	-	-	4(0.22)
G130V	-	1(0.7)	-	-	-	-	-	-	-	-	1(0.06)
G200R	2(0.6)	-	-	-	-	-	-	-	-	-	2(0.1)
Polymorphisms											
-3558T>C	21(6.1)	-	-	-	-	-	-	-	-	-	21(1.18)
V27I	-	1(0.7)	-	1(0.7)	-	1(0.6)	6(2.9)	1(0.9)	5(2.3)	-	15(0.84)
M34T	-	-	-	-	-	-	-	-	1(0.5)	-	1(0.06)
E114G	-	-	-	-	1(0.7)	-	-	-	-	-	1(0.06)
V153I	13(3.8)	2(1.4)	1(1)	3(1.9)	-	4(2.3)	3(1.5)	2(1.8)	(2.3%)	8(4.8)	41(2.3)
G160S	-	-	-	-	-	1(0.6)	-	-	-	-	1(0.06)
Total variants	115(33.2)	45(31.7)	16(15.7)	20(12.7)	10(6.8)	56(32.2)	18(8.6)	16(14.5)	48(21.4)	14(8.3)	358(20.1)
Total alleles	46	142	102	158	46	74	10	10	24	68	1780

Table 4: GJB2 allele variants identified in Iranian ARSNSHL families based on familial and sporadic conditions

Allele variants	Familial	Sporadic	Total
Mutations			
IVS1+1G>A	-	1	1(0.06%)
T8M	-	2	2(0.1%)
35delG	81	112	193(10.8%)
W24X	3	3	6(0.34%)
V27I+ E114G	4	6	10(0.6%)
R32H	-	2	2(0.1%)
V37I	1	-	1(0.06%)
E47X	1	-	1(0.06%)
167delT	1	2	3(0.17%)
W77X	2	-	2(0.1%)
235delC	-	8	8(0.45%)
L90P	1	-	1(0.06%)
delE120	3	-	3(0.17%)
R127H	6	7	13(0.7%)
Y136X	2	-	2(0.1%)
R143W	3	1	4(0.22%)
R184P	1	6	7(0.39%)
Unknown Mutations			
M163V	1	2	3(0.17%)
Novel Variants			
-3517G>A	2	2	4(0.22%)
H16R	1	-	1(0.05%)
K102Q	1	-	1(0.06%)
327delGGinsA	2	1	3(0.17%)
363delC	2	2	4(0.22%)
G130V	-	1	1(0.06%)
G200R	-	2	2(0.1%)
Polymorphisms			
-3558T>C	7	14	21(1.18%)
V27I	7	8	15(0.84%)
M34T	-	1	1(0.06%)
E114G	1	-	1(0.06%)
V153I	13	28	41(2.3%)
G160S	-	1	1(0.06%)
Total variants	146(24.5%)	212(17.9%)	358(20.1%)
Total alleles	596	1184	1780

Fig. 1: Frequencies of GJB2 mutations in 10 provinces of Iran. The studied provinces are shown in white colour

Discussion

We have analyzed GJB2 mutations frequencies and spectra in a large cohort of Iranian familial and sporadic non-syndromic patients to assess the role of the above-mentioned gene in causing deafness in Iran. Mutations in GJB2 gene have been detected in many ethnic populations and are the most common cause of autosomal recessive and nonsydromic hearing loss in many populations (7, 19). Our study revealed a rate of 27.6% and 27.5% of GJB2 mutations in ARSNSHL families' chromosomes in Gilan and Azarbaijan Sharqi in the north and North West of the country but a very low rate of 4.3% and 3.6% in Hormozgan and Sistan va Baloochestan in the south and south east of Iran. In contrast with a higher rate of GJB2-related deafness in the north and North West of the country, we found a low rate of GJB2-related deafness (11.8% of deaf families' chromosomes) in Golestan, which is located in the north area similarly. If we ignore the low rate of GJB2-related deafness in Golestan a gradual decrease of GJB2-related deafness is determined as we move from the north and North West to the south and south east of the country. This gradient of GJB2-related deafness is in consistent with other investigators (29) although no infor-

mation relating to GJB2 mutation frequency from Golestan has been documented.

In all, our screening of the coding region of the gene, revealed 18.29% of GJB2 mutations in familial deaf chromosomes which is only slightly higher than a rate of 16.7% that reported from screening of both coding and non coding region by other investigators (29). In addition, the results may vary by the different sample size of ethnic groups to be investigated and method of mutation detection. We also found GJB2 mutations in 12.7% of sporadic deaf chromosomes, which is the first report of GJB2-related sporadic deafness in Iran. The incidence of lower rate of GJB2 mutations in sporadic deaf chromosomes could be due to the inclusion of non genetic deaf families in sporadic group. However, a negative family history does not preclude GJB2 related deafness (33).

The present data show that the prevalence of GJB2-related deafness in Iran is 2-3 times less than that reported from different population with European descent (34-36). Compare to the rate of GJB2-related deafness in the neighboring countries we suggest that this rate of GJB2-related deafness is less than the rate in Turkey (37, 38) in the north west of the country but is higher than that reported in Pakistan (39) in the south east of Iran which is the continuation of north and north west to the south and south east GJB2-related deafness gradient.

A single mutation known as 35delG accounts for the majority of GJB2 mutations in some populations (10, 15-17). 35delG mutation is responsible for 10% of all childhood hearing loss and for 20% of all childhood hereditary hearing loss in American caucasians with northern and south- ern European origin (40). Our finding showed that 35delG is the most prevalent GJB2 mutation accounting for 193 of 259 (74.5%) of the GJB2 mutations chromosomes and 193 of 1780 (10.8%) of all chromosomes studied. The second and third prevalent GJB2 mutations detected in our study was R127H and V27I+ E11G with sum of 8.9% of the GJB2 mutations chromosomes. In the current study, different rates of 35delG mutation were obtained in different ethnic populations. The highest rate of 35delG mutation was detected in Gilan accounting for 47 of 174(27%) of deaf families chromosomes while the lowest rate was identified in Sistan va Baloochestan with 0.0% of deaf families chromosomes. Interestingly, 35delG mutation identified in 47 of 48 (98%) of GJB2 mutations in Gilan. This high rate of allele frequency for the 35delG mutation is approximately the same as most European, North American and meditrranean populatins. In contrast to Gilan, we did not find any 35delG mutation in Sistan va Baloochestan. This lower rate of 35delG mutation has been reported in some populations of Pakistani (41), Omani (31), Japanese (18, 20), Korean (19) and British Asian families (42).

It has been shown that 10-42% of GJB2-related patients have mutations in only one allele (43-45). There are also several reports that a large deletion involving the GJB6 gene namely del (GJB6-D13S1830) exist as a pathogenic mutation in the second allele of these heterozygote GJB2-related patients (45, 46). We identified GJB2 mutations in both alleles in 202 of 259 (78%) of GJB2 mutations chromosomes and in 202 of 1780 deaf chromosomes (11.35%). Except our initial investigation which both exon 1 and 2 of the GJB2 gene were studied in 34 families (23), we didn't examine our samples for genetic alteration in exon 1 of the GJB2 gene. Also no patient was tested for del (GJB6-D13S1830) of GJB6 gene. However more than 500 autosomal recessive non syndromic deaf subjects have been tested for del (GJB6-D13S1830) in Iran but no mutation has been detected (47, 48, 30). The failure of our study to detect the second allele of these heterozygote GJB2-related patients might be due to mutation in non-coding region of the gene that we did not screen. It could be also due to another connexin gene or interaction with other gene product. These heterozygote GJB2-related patients might be a simple carrier and their deafness may have another cause (49).

Apart from genetic and inherited problem, consanguinity is a common pattern of marriage all over the country (50). Our study revealed 71% of consanguinity (double first cousins, first cousins, first cousins once removed and double cousins) in the deaf families studied from which first cousins marriage was the more common with the rate of 56% of overall consanguinity (Table 1). We found also the most common type of consanguineous marriage between first cousins who were the children of two brothers (data not shown). However, the rate of consanguinity determined in this study is relatively high compare to the rate of 37.3% and 38.6% reported for the normal population in Iran (50, 51).

In order to provide a more complete profile of GJB2 mutations in different part of the country, we have combined our data with the results of other studies to provide a comprehensive and detailed GJB2 mutation profile across different regions of Iran (Table 5).

Table 5: Overview of types and frequencies of GJB2 allelic variants in different regions of Iran (The percentage are given in bracket). NW: northwest, W: west, SW: southwest, N: north, C: central, S: south, NE: northeast, SE: southeast

Allele variants	NW	W	SW	N	C	S	NE	SE	Total	References
Mutations										
IVS1+1G>A	-	3(0.7)	-	-	1(0.1)	-	-	-	4(0.1)	23,29
-3170G>A	3(0.9)	3(0.7)	-	3(0.8)	2(0.3)	-	2(0.7)	-	13(0.4)	29
T8M	-	-	-	-	2(0.3)	-	-	-	2(0.06)	25
35delG	61(18.9)	57(12.8)	34(8.6)	89(23.5)	95(11.6)	3(1.3)	28(9.8)	3(0.8)	370(11.5)	23,24,25,26, 27,28,29,30
W24X	1(0.3)	1(0.2)	-	1(0.3)	5(0.6)	2(0.9)	-	8(2.2)	18(0.6)	23,25,28,29, 30
V27I+ E114G	1(0.3)	-	2(0.5)	1(0.3)	2(0.3)	-	3(1)	2(0.5)	11(0.3)	24,25,26,27, 28,29
R32H	-	2(0.4)	-	-	5(0.6)	-	-	-	7(0.2)	25,29
V37I	1(0.3)	-	-	-	-	-	-	-	1(0.03)	25
E47X	-	-	-	-	1(0.1)	-	-	-	1(0.03)	25
167delT	1(0.3)	-	-	1(0.3)	1(0.1)	-	1(0.3)	2(0.5)	6(0.2)	25,29
176del16	-	-	-	-	2(0.3)	-	-	-	2(0.06)	30
W77X	-	-	-	-	-	-	2(0.7)	-	2(0.06)	24
235delC	-	-	-	-	8(1)	-	2(0.7)	-	10(0.3)	23,24,25,30
L90P	-	-	-	-	1(0.1)	-	1(0.3)	-	2(0.06)	24,30
M93I	-	-	-	-	-	-	-	1(0.3)	1(0.03)	29
310del14	-	-	-	-	2(0.3)	-	-	-	2(0.06)	29
312del14	-	-	-	-	4(0.5)	-	-	-	4(0.1)	29
314del14	-	2(0.4)	-	-	2(0.3)	-	-	-	4(0.1)	29
delE120	5(1.5)	5(1.1)	-	-	3(0.4)	-	1(0.3)	2(0.5)	16(0.5)	24,25,29
R127H	2(0.6)	3(0.7)	1(0.3)	1(0.3)	2(0.3)	4(1.7)	3(1)	8(2.2)	24(0.7)	23,24,25,26, 28,29
Y136X	2(0.6)	-	-	-	-	-	-	-	2(0.06)	25
R143W	1(0.3)	-	-	-	3(0.4)	-	-	1(0.3)	5(0.2)	25,29
R184P	3(0.9)	1(0.2)	-	-	4(0.5)	-	2(0.7)	-	10(0.3)	25,29
Unknown Mutations										
E129K	1(0.3)	-	-	-	-	-	-	-	1(0.03)	29
M163V	1(0.3)	-	-	-	2(0.3)	-	-	-	3(0.09)	23, 25
A171T	-	-	-	-	1(0.1)	-	-	-	1(0.03)	30
Novel Variants										
-3517G>A	-	-	-	-	4(0.5)	-	-	-	4(0.1)	23

Table 5: Continued…

H16R	1(0.3)	-	-	-	-	-	-	-	1(0.03)	25
Q80L	2(0.6)	-	-	-	-	-	-	-	2(0.06)	29
K102Q	-	-	-	-	1(0.1)	-	-	-	1(0.03)	25
327delGGinsA	-	-	-	2(0.5)	1(0.1)	-	-	-	3(0.09)	24,25
329delA	-	-	-	-	1(0.1)	-	-	-	1(0.03)	29
363delC	1(0.3)	-	4(1)	-	-	-	-	-	5(0.2)	26,29
G130V	1(0.3)	-	-	-	-	-	-	-	1(0.03)	25
507insAAGG	-	1(0.2)	-	-	-	-	-	-	1(0.03)	29
G200R	-	-	-	-	2(0.3)	-	-	-	2(0.06)	25
Polymorphisms										
-3558T>C	-	-	-	-	21(2.7)	-	-	-	21(0.7)	23
V27I	4(1.2)	3(0.7)	1(0.3)	4(1)	2(0.3)	6(2.6)	6(2.1)	-	26(0.8)	24,25,26,27, 28,29
M34T	-	-	-	-	-	-	1(0.3)	-	1(0.03)	24
V52V	-	-	1(0.3)	-	-	-	-	-	1(0.03)	29
I69I	-	-	-	-	1(0.1)	-	-	-	1(0.03)	29
E114G	-	-	1(0.3)	2(0.5)	-	-	-	-	3(0.09)	23
V153I	4(1.2)	13(2.9)	9(2.3)	10(2.6)	18(2.3)	3(1.3)	6(2.1)	17(4.7)	80(2.5)	23,24,25,26, 27,29,30,28
G160S	-	-	-	1(0.3)	-	-	-	-	1(0.03)	24
Total variants	96(29.8)	94(21)	53(13.4)	115(30.4)	198(25)	18(7.8)	58(20.3)	44(12.1)	676(21)	
Total alleles	322	446	396	378	792	230	286	364	3214	

Here, a total of 1607 probands including 890 probands of this study, 664 probands of Najmabadi and coworkers and 53 probands of Sadeghi and coworkers were reviewed (23-30). As Najmabadi and coworkers have reported their results within geographical regions, to be able to include their data we divided Iran into eight Geographical regions similarly. All data were collected within 8 regions including northwest: Azarbaijan Gharbi, Azarbaijan Sharqi, Ardebil, and Zanjan; north: Gilan, Mazandaran and Golestan; norteast: Khorasan; west: Kordestan, Hamedan, Kermanshah, Lorestan and Ilam; central: Tehran, Qazvin, Markazi, Qom, Semnan, Esfahan and Yazd; southwest: Khoozestan, Chaharmahal va Bakhtiari, Kohgilooyeh va Boirahmad and Fars; south: Booshehr and Hormozgan; southeast: Kerman and Sistan va Baloochestan (29).

This review revealed a specific combination of GJB2 mutations types and frequencies in different regions of Iran. Forty four different genetic variants were detected from which 23 GJB2 mutation were identified in 517 of 3214 chromosomes (16.1%) of ARSNSHL families. GJB2 mutations were found in 367/2030 (18.1%) and 150/1184 (12.7%) of familial and sporadic deaf families chromosomes respectively all over the country (Table 5). These data showed that 35 delG is the most prevalent GJB2 mutation accounting for 370 of 517 (71.6%) of the GJB2 mutations chromosomes and 370 of 3214 (11.51%) of all chromosomes reviewed. The R127H, W24X, delE120, -3170G> A and V27I+E114G were the following common mutations respectively. In all, 10 novel variants including- 3517G> A, H16R, Q80L, K102Q, 327delGGinsA, 329 delA, 363delC, G130V, 507insAAGG and G200R were detected in central, northwest, north, west and southwest Iran.

The high rate of GJB2 variants diversity could be reflect of the co-existence of several different ethnic populations and immigrations to big cities during last century in Iran. In addition, historical background like occurrence of different wars with foreign nations, immigrations, and location of the route of the Silk Road could support this diversity. In contrast with the high diversity of overall country, we found very low rate of diversity in some populations such as Korde-

stan, Gilan, Khoozestan and Chaharmahal va Bakhtiari who are probably isolated with cultural, lingual, religious or geographical barriers.
In conclusion, due to high GJB2 allelic diversity, low contribution of non-coding region of the gene in causing deafness (23, 29) and small size of the gene, we would recommend a 2-step procedure to detect the GJB2 mutation. In the first step direct sequencing of coding region of the gene and in the second step direct sequencing of the non-coding region of the gene in case of finding a heterozygote GJB2 mutation or no GJB2 mutation. These data highlight the importance of GJB2 mutations in development of hearing loss in familial and sporadic deaf families in different parts of the country and are of great importance for providing a basis for genetic counseling and clinical guidelines.

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