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Original Article (Pages: 17286-17298)

# Frequency of c.35delG Mutation in GJB2 gene in Patients with Autosomal Recessive Non-Syndromic Hearing Loss of Five Ethnic Groups in Golestan, Iran

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***Abstract***

***Background:*** Hereditary Hearing Loss (HL) is one of the most prevalent sensorineural disorders worldwide. Several hundreds of genes have been reported to have associations with this condition. Autosomal Recessive Non-Syndromic Hearing Loss (ARNSHL), with the highest frequency of severe to profound types of deafness, is responsible for the majority of non-syndromic HL. DFNB1 locus containing gap junction beta-2 protein (GJB2) gene is the main reported pathogenic variant in most cases of non-syndromic deafness globally. In the present study, we investigated the allele frequency of c.35delG mutation among families with different ethnicities residing in Golestan province of Iran.

***Methods:*** Audiological assessments, including pure-tone audiometry (PTA), tympanometry, and otoacoustic emission (OAE) tests, have been conducted to include and group subjects. Blood samples have been taken from probands and all their family members; and they have undergone allele-specific polymerase chain reaction (ASPCR) test. Moreover, direct sequencing has been performed to confirm the PCR results.

***Results:*** In our study, 28 out of 128 families with ARNSHL had c.35delG mutation. We observed that 15.4% of subjects had c.35delG+/c.35delG+ genotype, 7.4% had c.35delG+/normal genotype and 77.2% had no c.35delG mutation. The overall allele frequency of c.35delG is 19.1%. Regarding the consanguineous marriage rate, the Sistani ethnic group showed the highest (91%), and Azeris had the lowest rates (55%).

***Conclusion:*** The present work showed that severe forms of ARNSHL are associated with c.35delG homozygous mutation in comparison to other genotypes. We also demonstrated that c.35delG mutation is more prevalent in Turkmen and Fars ethnic groups in Golestan province of Iran.

***Key Words:*** Autosomal recessive nonsyndromic hearing loss (ARNSHL), C.35delG mutation, Connexin 26, GJB2 gene, Hearing loss.

* Please cite this article as: Hajilari M, Sharifinya A, Khosravi T, Kianmehr A, Taziki MH, Khosravi A, Oladnabi M. Frequency of c.35delG Mutation in GJB2 gene in Patients with Autosomal Recessive Non-Syndromic Hearing Loss of Five Ethnic Groups in Golestan, Iran. Int J Pediatr 2023; 11 (01):17286-17298. DOI: **10.22038/ijp.2023.69158.5122**

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Received date: Nov.23,2022; Accepted date: Jan.13,2023

## 1- INTRODUCTION

As the most frequent sensory impairment, hearing loss (HL) affects millions of individuals around the globe. It approximately accounts for 1 in 1000 newborns (http://hearing.screening.nhs.uk/nationalprog). The rate increases significantly to 2.7 and 3.5 when it comes to children before the age of five and adolescents, respectively (1). Moreover, based on NIH data (https://www.nidcd.nih.gov/health/age-related -hearing-loss), one in every three people over 65 has HL, suggesting a gradual age-associated increase. Taking global population aging (https://www.un.org/en/global-issues/ageing) into consideration, it would be likely for HL to be a major health problem in the near future. Although environmental factors like physical damage, ear infections, and drug exposure can cause HL, 60 to 80 percent of cases are due to genetic factors (1,2). These with small hereditary congenital forms are classified into syndromic (part of a condition along with other manifestations) and non-syndromic HL (NSHL). The latter accounts for 70 percent of cases and follows all monogenic inheritance patterns (3). More than 400 HL-related genes have been reported, of which 120 NSHL genes have been identified so far (https://hereditaryhearingloss.org/). The autosomal recessive pattern is responsible for 80 percent of NSHL cases, with over 100 mapped loci, including DFNB1 (13q11-12) locus hosting GJB2 gene (NM_004004.5). Mutations in GJB2 have been reported in different populations and statistically are the main cause of NSHL around the world (4, 5). Nonetheless, the frequency and spectrum of GJB2 mutations are highly variable among different ethnicities and populations (4, 6). Moreover, the lack of genotype-phenotype correlations due to the genetic and clinical heterogeneity is an intriguing challenge that necessitates further studies.

### 1-1. GJB2 Gene Structure and Connexin-26 Function

The DFNB1 locus contains GJB2 (OMIM: 121011) and GBJ6 (OMIM: 604418) genes. GJB2 has two exons, of which only exon 2 has a coding region that encodes a transmembrane protein named Gap Junction Subunit beta2, also known as Connexin-26. Connexin proteins are named after their molecular weights. Thus, Connexin-26 (Cx26) is a 26 KDa protein containing 226 amino acids. As shown in **Fig. 1A**, each Cx26 is a 4-pass transmembrane protein which along with five other Cx26 subunits constructs a hemiconnexon in the plasma membrane of hair cells - sensory receptors in cochlea responsible for auditory signal transduction.

Hemiconnexons of two adjacent hair cells get connected together to form a gap junction responsible for intercellular exchange of small molecules and ions such as K+ (7, 8). Cx26 makes it possible for hair cells to recycle K+ into cochlear duct. Mutations in GJB2 cause K+ recycling impairment. Accumulation of K+ leads to hair cell degeneration which is one of the hypotheses for the NSHL mechanism (9).

### 1-2. GJB2 Mutations

Based on HGMD Professional 2021.4, more than 450 mutations have been reported in the GJB2 gene. Most of them are missense or nonsense mutations; as seen in **Fig. 1B**, GJB2 mutations are accompanied by high phenotype variability in HL patients, suggesting that epigenetic factors such as modifying genes and miRNAs may play key roles. Although different mutations are reported among different populations and ethnicities, c.35delG is the most prevalent pathogenic mutation, accounting for 70 percent of the GJB2 mutations in NSHL cases in Caucasian populations (10, 11). In the present study, we aimed to 1) calculate the frequency of 35delG mutation in families

with ARNSHL that reside in Golestan province and 2) evaluate the genotype-phenotype correlation between c.35delG mutation and other parameters among the subjects.

## 2- MATERIAL AND METHODS

### 2-1. Study Sample

The participants included a total of 272 hearing-impaired individuals (139 males and 133 females) from 128 families of five different ethnicities (Turkmen, Sistani, Fars, Iranian Baluch, and Azeri) residing in Golestan province of Iran.

#### 2-1-1. Inclusion criteria

To include a subject in this study, we have set five criteria:

1) Confirmed hearing loss based on pure-tone audiometry via air and bone conduction.

2) No history of exposure to HL-causing drugs or infections (Exclusion of acquired HL).

3) No observation of other associated signs and symptoms (Exclusion of syndromic HL).

4) Confirmed autosomal recessive inheritance based on pedigree analysis.

5) At least two affected members within the proband's family.

6) Normal hearing in both parents of the proband.

### 2-2. Audiological Assessment

The gold standard for evaluating thresholds in HL patients is Pure-Tone Audiometry (PTA). We performed PTA in a sound booth. Tone thresholds by air and bone conduction were obtained at frequencies 250, 500, 1000, 2000, 4000, and 8000 Hz with up to 120 dB of intensity. Tympanometry is a non-invasive acoustic test for hearing healthcare objectives such as characterization of tympanic membrane and its anomalies, including ventilation tubes. It alters air pressure in the ear canal; then records the relative changes in tympanic membrane's movement. The results are graphically represented on tympanograms. According to Jerger classification (12), which is based on the patterns of curves, tympanograms are divided into three types: Types A, B, and C, as depicted in **Fig. 2**; tympanograms horizontal and vertical axes are represented as the middle ear pressure and compliance, respectively. The latter is displayed by peak height and correlated with external ear volume (ECV). A tympanogram type A (normal tracing) is a teepee-like curve referring to a normal middle ear with the lowest probability of a physio-pathological anomaly. The curve occurs at zero daPa of air pressure with normal ECV. A tympanogram type B (flattened or abnormal tracing) also has a normal ECV; however, the low compliance of the curve is mostly a sign of decreased tympanic membrane mobility, possibly due to otitis media with effusion (OME). Many clinical studies refer to type B as certainly abnormal tracing. A type C tympanogram (negatively shifted tracing) is highly correlated to tympanic membrane retraction. This curve is mostly seen in middle-ear infections such as Acute Otitis media (AOM). Although the probability of hearing impairment related to this curve is clinically considered to be low, further evaluations are recommended. An otoacoustic emission (OAE) is a commonly used method in hearing screening. The OAE test evaluates the cochlea and functional status of hair cells located in inner-ear. In this study, we performed OAE along with tympanometry for every individual. Following PTA, tympanometry, and OAE examinations, the degree of HL severity among subjects was classified into five groups: normal (0–20 dB), mild (25–40 dB), moderate (45–60 dB), severe (65–85 dB), and profound (>85 dB).

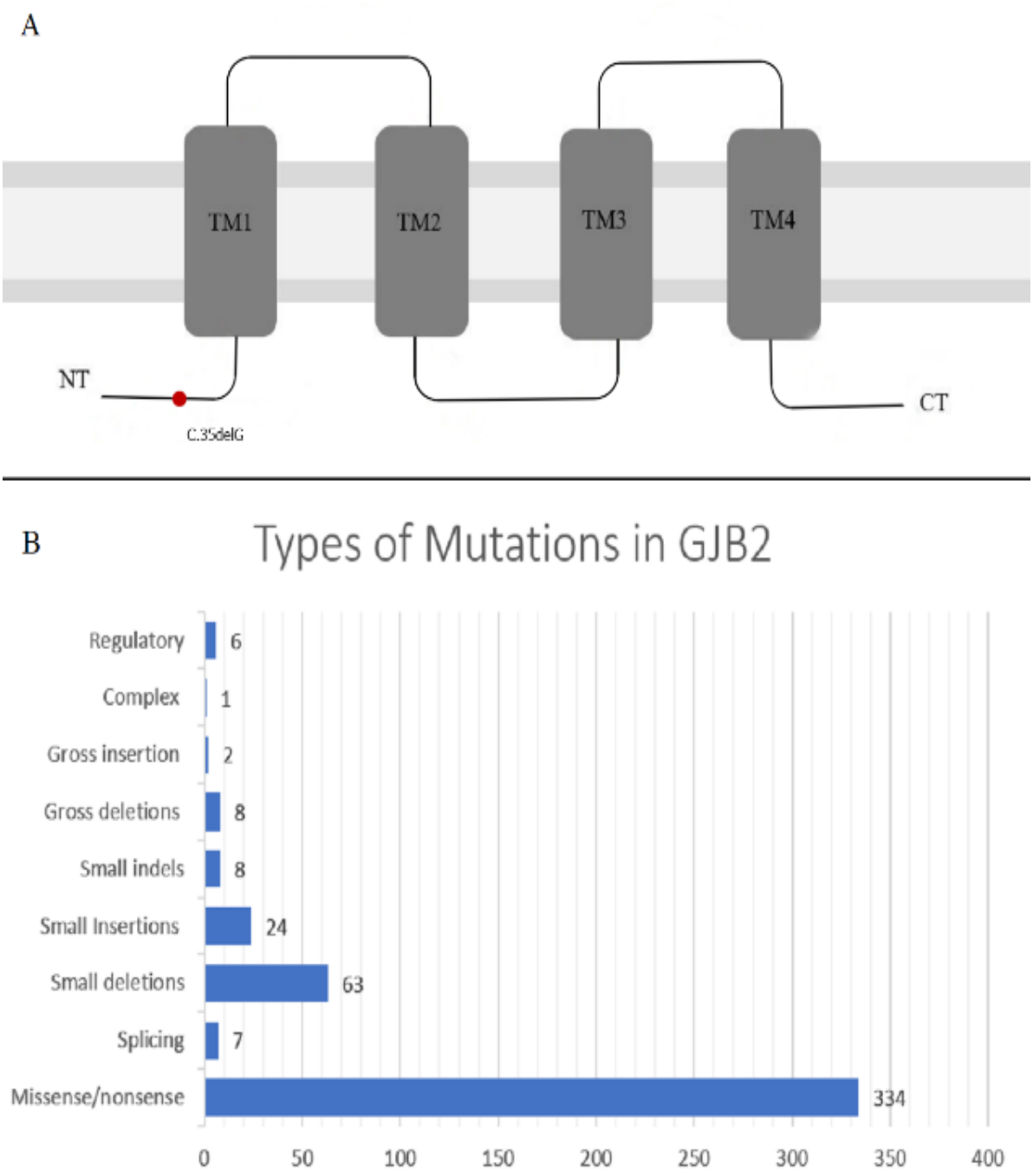


**Fig. 1:** A) Connexin-26 structure. GJB2 gene encodes Connexin-26 (Cx26), a multipass protein crucially expressed in hair cells. Two hundred twenty-six amino acids of Cx26 are arranged in 4 transmembrane units. Mutations in GJB2 result in harmful aggregation of K+ and lead to different kinds of imperfect proteins and possibly different types of HL severity. As depicted above, in the case of c.35delG, the truncated protein has none of the transmembrane domains. B) Types of Gene Mutations in GJB2. Based on the HGMD database, 453 mutations have been identified in the GJB2 gene. These include 334 Missense/nonsense mutations, 7 splicing mutations, 63 small deletions, 24 small insertions, 8 small indels, 8 gross deletions, 2 gross insertions, 1 complex, 6 regulatory mutations.

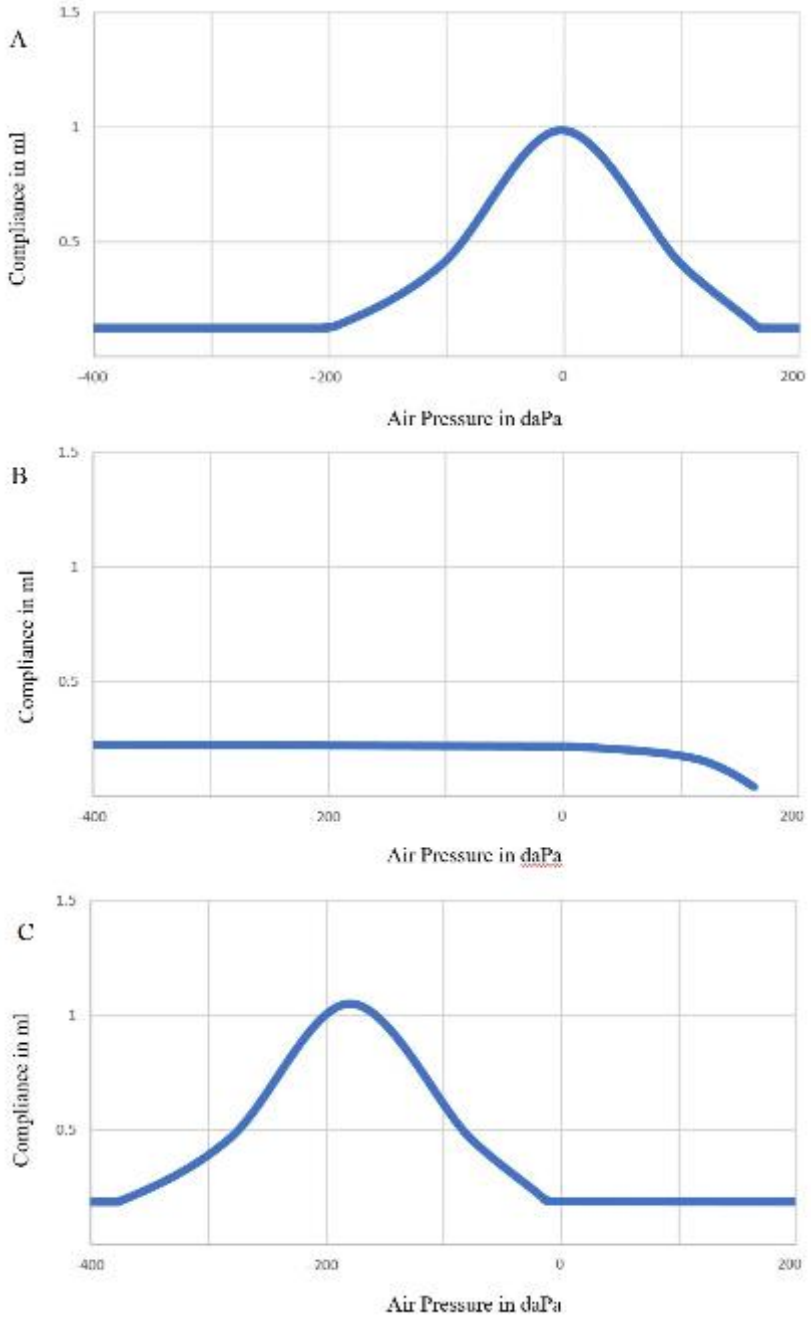


**Fig. 2:** Different types of Tympanometry Curves. Sections A, B, and C showed tympanograms types A, B, and C, respectively.

### 2-3. DNA Mutational Analysis for c.35delG

The most effective technique to analyze the c.35delG mutation as a single nucleotide polymorphism (SNP) is allele-specific polymerase chain reaction (ASPCR). In this regard, we took a 10 ml peripheral blood sample from all family members, including parents, all affected members, and at least one healthy individual. Using Kowsar DNA extraction kit (Cat. No. K1135), we extracted Genomic DNA and measured the concentration of the nucleic acid product using a NanoDrop spectrophotometer. In the next step, using ASPCR, all DNA samples were screened for c.35delG allele

variant. We obtained normal, mutant and reverse primers for ASPCR and also primers for GJB2 exon 2 from a previous study conducted by Scott et al. (13). Two common reverse and forward primers were also used based on another previous study (14). ASPCR procedure was performed using 40ng of genomic DNA sample in an 8.4µL PCR reaction containing 1.25 µL of PCR buffer (100 mmol of tris-hydrochloride, pH 8.8, 500 mmol of potassium chloride, 15 mmol of magnesium chloride, 0.01% wt/vol gelatin); 800 µmol of dNTPs; 25 pmol of each normal, mutant and the common primer; and 15 pmol of each control primer. The products were then loaded on 1.5% agarose gel containing ethidium bromide for electrophoresis. Samples that showed c.35delG+/c.35delG+ genotype in ASPCR evaluation were selected for further molecular confirmation.

We performed another PCR to amplify the exon 2 of GJB2 gene in these samples. The amplification was confirmed using electrophoresis and visualized with UV light. The final amplified products were sent for direct sequencing, as the gold standard technique, to confirm the mutation's existence, location, and type. The direct sequencing was performed as a quality control for all mutant homozygotes and heterozygotes plus a number of healthy homozygous individuals. Reference sequence (Refseq) was taken from the UCSC genome browser in FASTA format.

## 3- RESULTS

Out of 128 families with at least two members with ARNSHL, c.35delG mutation was absent in 100. Among all subjects in this study, 15.4 percent (42 including 23 males and 19 females) had homozygous c.35delG mutation, 7.4 percent (20 including 9 males and 11 females) had heterozygous c.35delG mutation, and in 77.2 percent there were no observed c.35delG mutant allele. **Fig. 3A, 3B,** and **3C** provided statistical data on age-gender distribution, ethnic groups, and severity of HL among subjects, respectively.

Our results showed that the Sistani ethnic group, with 91 percent, and Azeri ethnic group, with 55 percent, had the highest and lowest consanguineous marriage rates, respectively. Consanguineous marriage was observed in 17 out of 19 families with homozygous c.35delG mutation. Interestingly, among 25 Sistani families, one showed a homozygous c.35delG mutation. Moreover, the incidence of c.35delG mutation in Turkmen and Fars ethnic groups was significantly more than others. Results of all ethnic groups are demonstrated in **Table 1**.

Audiological evaluations showed that out of all 272 individuals who were included in this study, 199 had profound HL, out of which 42 had homozygous c.35delG mutation. No mild and six individuals with moderate HL were observed. **Table 2** shows the audiological findings coupled with c.35delG mutation status of all subjects in our study. We used a direct sequencing method to confirm ASPCR evaluations. The results of direct sequencing were completely matched with ASPCR.

**Fig. 4A** demonstrates the gel electrophoresis bands of three DNA samples with homozygous c.35delG, normal homozygous and heterozygous 35delG mutation (1, 2 and 3, respectively), which are visualized with UV light.

**Fig. 4B** represents three different panels of direct sequencing among the patients. Our results showed that patients with c.35delG homozygous mutation have more severe HL in comparison to c.35delG compound heterozygotes. It is also demonstrated that there is no significant correlation between c.35delG mutation and gender, location of residence, and education level.

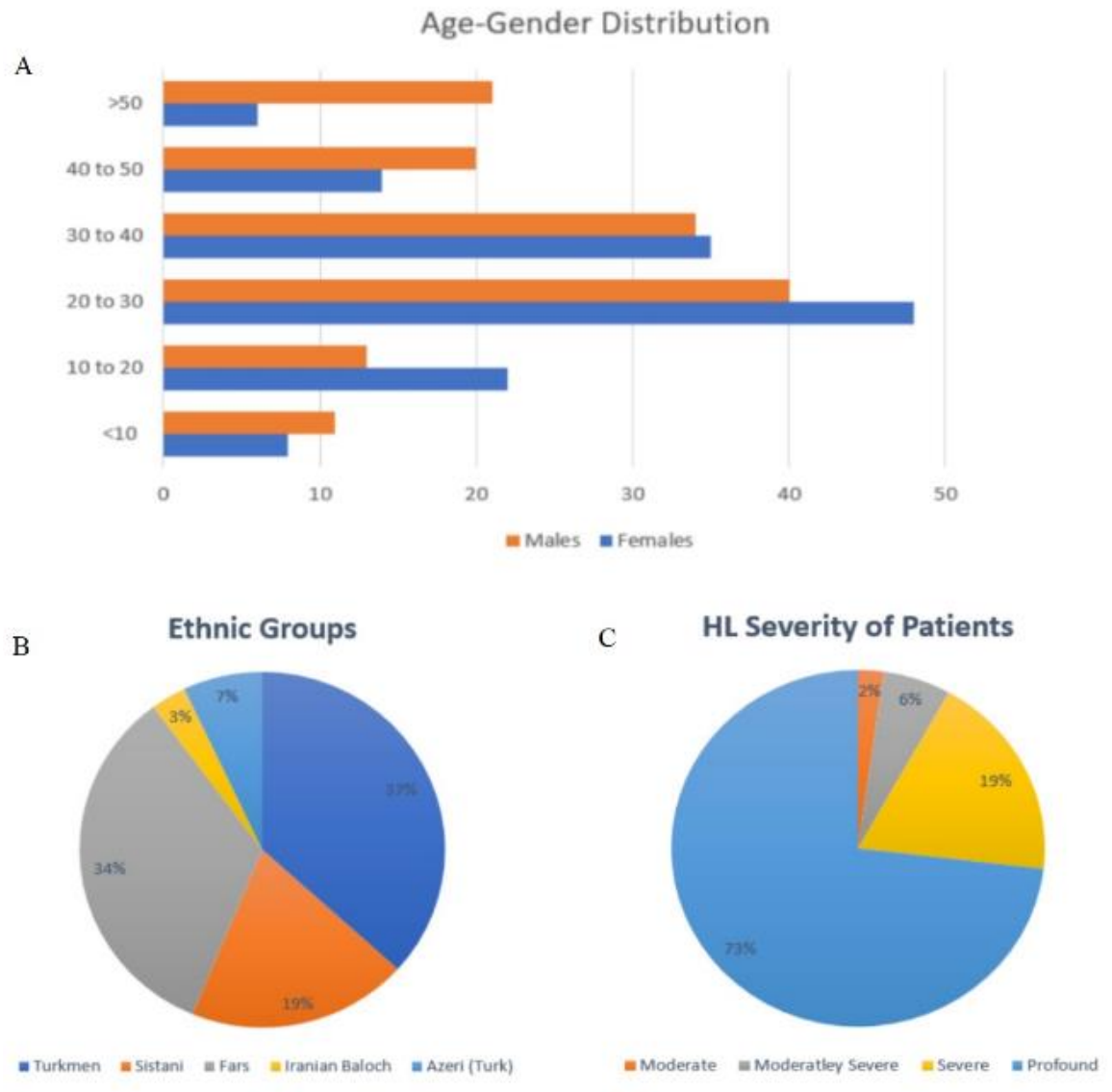


**Fig. 3**: Study Sample Stats. Most of the subjects were in their twenties (A), from Turkmen ethnic group (B), and had profound HL (C).

**Table-1:** Number of families, their genotypes, and consanguineous marriage rates are represented for each ethnic group. (-/-= normal/normal, +/+ = c.35delG+/c.35delG+, +/- = c.35delG/normal)

| Ethnic Groups | Number of family | +/+ | +/- | -/- | Con. Marriage (%) |
|---|---|---|---|---|---|
| Turkmen | 47 | 8 | 5 | 34 | 70 |
| Sistani | 25 | 1 | 0 | 24 | 91 |
| (Fars) | 43 | 7 | 4 | 32 | 60 |
| Iranian Balochs | 4 | 1 | 0 | 3 | 60 |
| Azeris (Tork) | 9 | 2 | 0 | 7 | 55 |
| Total | 128 | 19 | 9 | 100 | - |

**Table-2:** Severity of Hl among subjects with different genotypes.

| Severity of HL | c. 35delG | | | Total |
|---|---|---|---|---|
| | N/N | M/M | M/N | |
| Mild (26-40 dB) | 0 | 0 | 0 | 0 |
| Moderate (41-55 dB) | 6 | 0 | 0 | 6 |
| Moderately Severe (56-70 dB) | 14 | 0 | 2 | 16 |
| Severe (71-90 dB) | 44 | 2 | 5 | 51 |
| Profound (> 90dB) | 146 | 40 | 13 | 199 |
| Total | 210 | 42 | 20 | 272 |

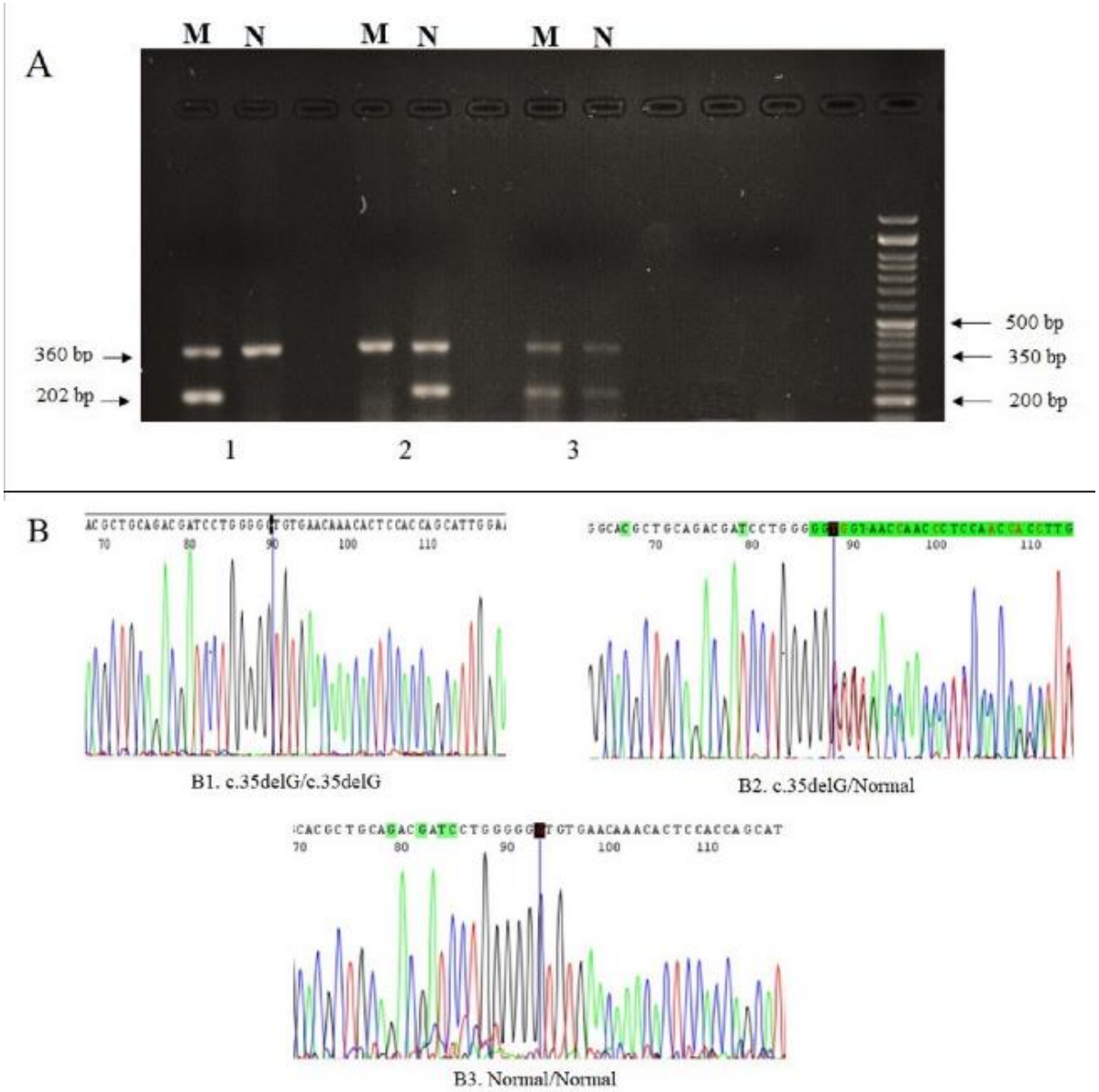


**Fig. 4:** A) Gel Electrophoresis Results. The 360-bp normal control bands in all samples show the proper PCR performance. Samples 1, 2 and 3 represents c.35delG+/ c.35delG+, normal/normal and c.35delG+/normal genotypes, respectively. B) Direct Sequencing Chromatograms. Panel B1, B2 and B3 demonstrate the sequences of three subjects with c.35delG$^{+}$/c.35delG$^{+}$ genotype, c.35delG$^{+}$/- genotype and -/- genotype, respectively.

## 4- DISCUSSION AND CONCLUSION

From an epidemiological point of view, due to different ethnic backgrounds in geographical regions, hearing loss can occur differently even within a country. Founder effect also casts a heavy shadow on this diversity. The majority of reported GJB2 mutations are focused on exon 2 coding regions, although some studies have found other HL-related mutations in noncoding regions. For instance, Denoyelle et al. discovered c.-23+1G>A mutation in exon 1 (15). Further investigations by Angeli et al. revealed that c.-23+1G>A affects splicing process of GJB2 transcript (16). Another study also discovered new variants in noncoding regions associated with HL (17). These findings require further evaluations to fill the gaps in our understanding of GJB2 role in HL. Besides c.35delG, other frequent variants are more population specific. c.167delT in Ashkenazi Jews (18), c.235delC in Japanese (19) , c.71G>A in Indians and Pakistanis (20), and IVS1+1G>A in Mongolians (21) have been reported in different populations worldwide. The high incidence of c.35delG mutation in European, North American, Middle Eastern, and African populations is likely to result from a founder effect instead of a mutational hotspot at the locus (22, 23). Moreover, c.35delG mutation hasn't been observed in South and East Asian populations, suggesting that didn't exist in the common ancestor of all modern humans. Instead, it might have appeared around 12000 years ago in central Asia (24). Najmabadi et al. also reported that GJB2 mutations, including c.35delG variant, went through migration from Iran to European populations and then to North America (25). A cohort study by Amorini et al. showed that eastern Sicily population in Italy might be the founder of c.35delG. They also suggested that the high frequency of this mutation could be a possible heterozygosity advantage (26). Iran hosts many different ethnicities, each having a unique genetic background. Therefore, population-based genetic studies in the country should consider ethnic groups as a strong directional factor (27). Autosomal recessive conditions, like ARNSHL, are significantly more probable to appear in families with consanguineous marriage. Saadat et al.'s study has shown that 38.6 percent of Iranian families prefer consanguineous marriages, with 27.9 percent of the first cousin marriage rate (28). With different types of reported HL, Golestan province acts as a small-scale Iran itself, since many migration flows have been destined for the region (29). The region also has approximately 65 percent consanguineous marriage rate (30). Reviewing recent works on GJB2 mutations in Iran (31), graphically depicted in **Fig. 5**, has revealed that the incidence of GJB2-related HL has gradually decreased from northwest to the southeast of the country.

The gradient could be the footsteps of the founders. Moreover, previous studies on GJB2-related HL in Iran's northern populations have shown that c.35delG has the most frequency among all GJB2 mutations (58.4 percent) (32). The frequency is much lower in southern regions of the country. The frequency of c.35delG mutation decreases from north to south and west to east of Iran, following the aforementioned pattern of other GJB2 mutations (10). In our study, allele frequency of c.35delG mutation has been investigated in 272 ARNSHL patients from Golestan province. Our results showed that the mutation frequency is 19.1%. Two other studies have been conducted, so far, on GJB2 mutations in this province. A 12-year study conducted by Bazazzadegan et al. on a total number of 2322 individuals from all 31 provinces of Iran has discovered c.35delG mutation responsible for 65 percent of GJB2 mutations. They studied 30 non-syndromic hearing impaired subjects from Golestan

province, out of which 17 had c.35delG variant (33). In another study, Hosseinipour et al. investigated 179 ARNSHL families in three provinces of Iran, out of which 55 resided in Golestan province. They found ten c.35delG variants in Golestan province, which is about 9 percent of all variants in the study (34). Compared to previous studies in this province, our sample is significantly more diverse and contains more subjects, making our results more statistically reliable.

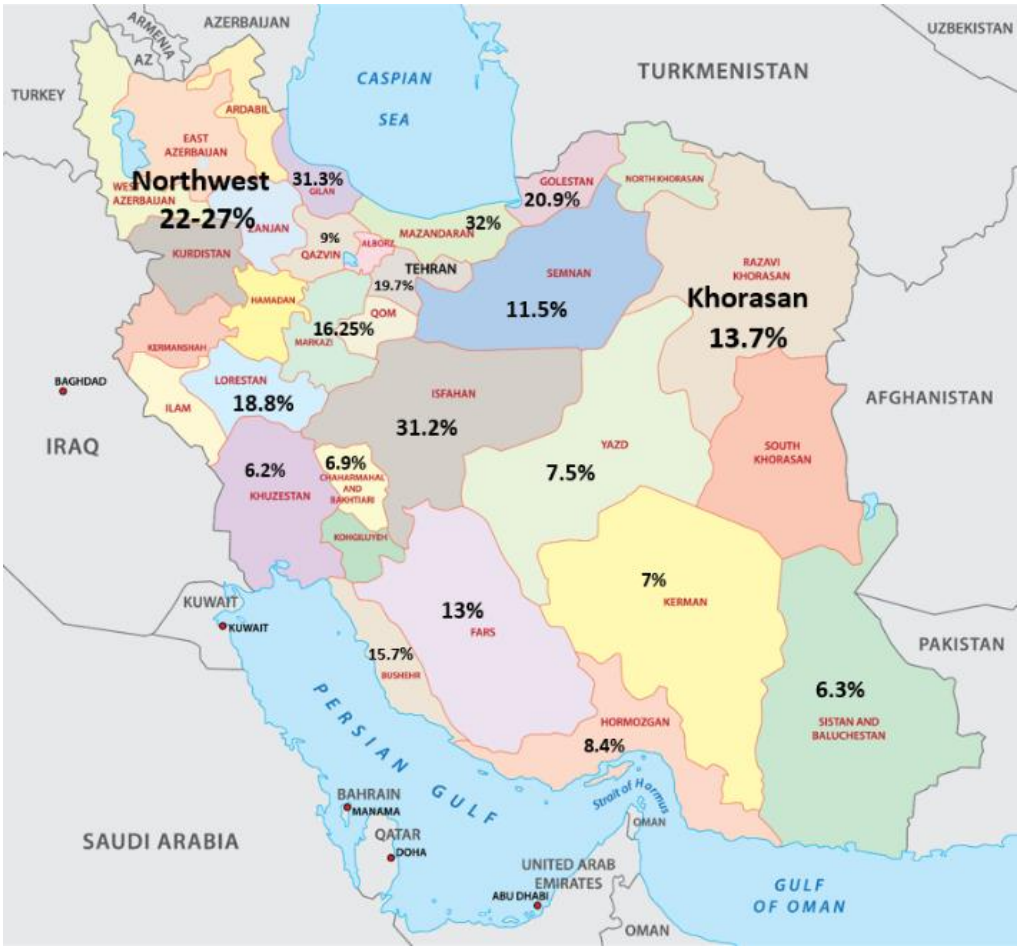


**Fig. 5:** Prevalence of GJB2 mutations throughout the country. The percentage decreases from northwestern regions to southeastern regions, maybe as a result of founders' migration.

### 4-1. Limitations of the Study

There are tens of reported mutations in GJB2. Other deafness-related genes also play significant roles. Therefore, it is likely that there are other families in our cohort whose hearing loss is not a result of c.35delG or even GJB2. Since the c.35delG represents the most common genetic HL variant, evaluating this mutation was the most cost- and time-effective approach to screen the cohort and perform genetic counseling for the families. However, it made our data limited.

## 5- ETHICAL CONSIDERATIONS

The study protocol was ethically confirmed by the ethics committee of Golestan University of Medical Sciences (Confirmation Number:

IR.GOUMS.REC.1397.343). Written informed consent had been obtained from all participants (or their parents), before the study started.

## 6- CONFLICT OF INTEREST

None.

## 7- ACKNOWLEDGMENTS

We thank all the patients and their families for taking part in this study.

## 8- FUNDING

This study was financially supported by the Golestan University of Medical Sciences (Grant Number: 110482).

## 9- REFERENCES

1. Morton CC, Nance WE. Newborn hearing screening—a silent revolution. New England Journal of Medicine. 2006; 354(20):2151-64.

2. Shearer AE, Hildebrand MS, Smith RJ. Hereditary hearing loss and deafness overview. GeneReviews [Internet]. 2017.

3. Mahdieh N, Rabbani B, Wiley S, Akbari MT, Zeinali S. Genetic causes of nonsyndromic hearing loss in Iran in comparison with other populations. Journal of human genetics. 2010; 55(10):639-48.

4. Chan DK, Chang KW. GJB2-associated hearing loss: Systematic review of worldwide prevalence, genotype, and auditory phenotype. The Laryngoscope. 2014; 124(2):E34-E53.

5. Hilgert N, Smith RJ, Van Camp G. Forty-six genes causing nonsyndromic hearing impairment: which ones should be analyzed in DNA diagnostics? Mutation Research/Reviews in Mutation Research. 2009; 681(2-3):189-96.

6. Kenneson A, Van Naarden Braun K, Boyle C. GJB2 (connexin 26) variants and nonsyndromic sensorineural hearing loss: a HuGE review. Genetics in Medicine. 2002; 4(4):258-74.

7. Goodenough DA, Goliger JA, Paul DL. Connexins, connexons, and intercellular communication. Annual review of biochemistry. 1996; 65(1):475-502.

8. Tekin M, Arnos KS, Pandya A. Advances in hereditary deafness. The Lancet. 2001; 358(9287):1082-90.

9. Zhao H-B. Hypothesis of K+-recycling defect is not a primary deafness mechanism for Cx26 (GJB2) deficiency. Frontiers in molecular neuroscience. 2017; 10:162.

10. Azadegan-Dehkordi F, Ahmadi R, Koohiyan M, Hashemzadeh-Chaleshtori M. Update of spectrum c. 35delG and c.-23+ 1G> A mutations on the GJB2 gene in individuals with autosomal recessive nonsyndromic hearing loss. Annals of human genetics. 2019; 83(1):1-10.

11. Gasparini P, Rabionet R, Barbujani G, Melchionda S, Petersen M, Brøndum-Nielsen K, Metspalu A, Oitmaa E, Pisano M, Fortina P, Zelante L, Estivill X. High carrier frequency of the 35delG deafness mutation in European populations. European Journal of Human Genetics. 2000; 8(1):19-23.

12. Jerger J. Clinical experience with impedance audiometry. Archives of otolaryngology. 1970; 92(4):311-24.

13. Scott D, Kraft M, Carmi R, Ramesh A, Elbedour K, Yairi Y, Srisailapathy CR, Rosengren SS, Markham AF, Mueller RF, Lench NJ, Camp GV, Smith RJ, Sheffield VC. Identification of mutations in the connexin 26 gene that cause autosomal recessive nonsyndromic hearing loss. Human mutation. 1998; 11(5):387-94.

14. Angeli S, Utrera R, Dib S, Chiossone E, Naranjo C, Henríquez O, Porta M. GJB2 gene mutations in childhood deafness. Acta oto-laryngologica. 2000; 120(2):133-6.

15. Denoyelle F, Marlin S, Weil D, Moatti L, Chauvin P, Garabédian É-N, Petit C.

Clinical features of the prevalent form of childhood deafness, DFNB1, due to a connexin-26 gene defect: implications for genetic counseling. The Lancet. 1999; 353(9161):1298-303.

16. Angeli SI. Phenotype/genotype correlations in a DFNB1 cohort with ethnic diversity. The Laryngoscope. 2008; 118(11):2014-23.

17. Yuan Y, Yu F, Wang G, Huang S, Yu R, Zhang X, Huang D, Han D, Dai P. Prevalence of the GJB2 IVS1+ 1G> A mutation in Chinese hearing loss patients with monoallelic pathogenic mutation in the coding region of GJB2. Journal of translational medicine. 2010; 8(1):1-7.

18. Morell RJ, Kim HJ, Hood LJ, Goforth L, Friderici K, Fisher R, Camp GV, Berlin CI, Oddoux C, Ostrer H, Keats B, Friedman TB. Mutations in the connexin 26 gene (GJB2) among Ashkenazi Jews with nonsyndromic recessive deafness. New England Journal of Medicine. 1998; 339(21):1500-5.

19. Nishio S-y, Usami S-i. Deafness gene variations in a 1120 nonsyndromic hearing loss cohort: molecular epidemiology and deafness mutation spectrum of patients in Japan. Annals of Otology, Rhinology & Laryngology. 2015; 124(1_suppl):49S-60S.

20. Salman M, Bashir R, Imtiaz A, Maqsood A, Mujtaba G, Iqbal M, Naz S. Mutations of GJB2 encoding connexin 26 contribute to non-syndromic moderate and severe hearing loss in Pakistan. European Archives of Oto-Rhino-Laryngology. 2015; 272(8):2071-5.

21. Tekin M, Xia XJ, Erdenetungalag R, Cengiz FB, White TW, Radnaabazar J, Dangaasuren B, Tastan H, Nance WE, Pandya A. GJB2 mutations in Mongolia: complex alleles, low frequency, and reduced fitness of the deaf. Annals of human genetics. 2010; 74(2):155-64.

22. Banjara H, Mungutwar V, Swarnkar N, Patra P. Detection of connexin 26 GENE (GJB2) mutations in cases of congenital non syndromic deafness. Indian Journal of Otolaryngology and Head & Neck Surgery. 2016; 68(2):248-53.

23. Zoidl G, Dermietzel R. Gap junctions in inherited human disease. Pflügers Archiv-European Journal of Physiology. 2010; 460(2):451-66.

24 Dzhemileva L, Posukh O, Barashkov N, Fedorova S, Teryutin F, Akhmetova V, Khidiyatova I, Khusainova R, Lobov S, Khusnutdinova E. Haplotype diversity and reconstruction of ancestral haplotype associated with the c. 35delG mutation in the GJB2 (Cx26) gene among the Volga-Ural populations of Russia. Acta Naturae (англоязычная версия). 2011; 3(3 (10)):52-63.

25. Najmabadi H, Nishimura C, Kahrizi K, Riazalhosseini Y, Malekpour M, Daneshi Farhadi M, Mohseni M, Mahdieh N, Ebrahimi A, Bazazzadegan N, Naghavi A, Avenarius M, Arzhangi S, Smith RJH. GJB2 mutations: passage through Iran. American Journal of Medical Genetics Part A. 2005; 133(2):132-7.

26. Amorini M, Romeo P, Bruno R, Galletti F, Di Bella C, Longo P, Briuglia S, Salpietro C, Rigoli L. Prevalence of deafness-associated connexin-26 (GJB2) and connexin-30 (GJB6) pathogenic alleles in a large patient cohort from Eastern Sicily. Annals of Human Genetics. 2015; 79(5):341-9.

27. Fattahi Z, Beheshtian M, Mohseni M, Poustchi H, Sellars E, Nezhadi SH, Amini A, Arzhangi S, Jalalvand K, Jamali P, Mohammadi Z, Davarnia B, Nikuei P, Oladnabi M, Mohammadzadeh A, Zohrehvand E, Nejatizadeh A, Shekari M, Bagherzadeh M, Shamsi-Gooshki E, Börno S, Timmermann B, Haghdoost A, Najafipour R, Khorram Khorshid HR, Kahrizi K, Malekzadeh R, Akbari MR, Najmabadi H. Iranome: A catalog of

genomic variations in the Iranian population. Human mutation. 2019; 40(11):1968-84.

28. Saadat M, Ansari-Lari M, Farhud D. Short report consanguineous marriage in Iran. Annals of human biology. 2004; 31(2):263-9.

29. Oladnabi M, Hasheminasab Gorji E, Mohammadi M, Lotfi S, Handlebar J, Dastaviz F, Bagheri A. Hereditary and Non-hereditary Pattern of Deafness in Golestan Province, Iran. Journal of Mazandaran University of Medical Sciences. 2021; 31(199):174-8.

30. Hajilari M, Oladnabi M, Kianmehr A, Taziki MH, Zamiri Abdollahi F. Hereditary hearing loss and consanguinity in Turkmen population of Iran: a retrospective study. International Journal of Pediatrics. 2019; 7(11):10323-34.

31. Koohiyan M, Koohian F, Azadegan-Dehkordi F. GJB2-related hearing loss in central Iran: Review of the spectrum and frequency of gene mutations. Annals of Human Genetics. 2020; 84(2):107-13.

32. Koohiyan M, Azadegan-Dehkordi F, Koohian F, Hashemzadeh-Chaleshtori M. Genetics of hearing loss in north Iran population: An update of spectrum and frequency of GJB2 mutations. Journal of Audiology & Otology. 2019; 23(4):175.

33. Bazazzadegan N, Nikzat N, Fattahi Z, Nishimura C, Meyer N, Sahraian S, Jamali P, Babanejad M, Kashef A, Yazdan H, Sabbagh Kermani F, Taghdiri M, Azadeh B, Mojahedi F, Khoshaeen A, Habibi H, Reyhanifar F, Nouri N, Smith RJH, Kahrizi K, Najmabadi H. The spectrum of GJB2 mutations in the Iranian population with non-syndromic hearing loss—a twelve year study. International journal of pediatric otorhinolaryngology. 2012; 76(8):1164-74.

34. Hosseinipour A, Chaleshtori MH, Sasanfar R, Farhud D, Tolooi A, Doulati M, et al. Report of a new mutation and frequency of connexin 26 gene (GJB2) mutations in patients from three provinces of Iran. Iranian Journal of Public Health. 2005; 34(1):47-50.