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REVIEW

Studying gap junction beta 2-related deafness in Iranian population

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ABSTRACT

Hearing loss is the most common sensory disorder in humans, from every 1000 births, 1 is affected by severe to profound deafness. Many genes are involved in deafness that *GJB2* gene is one of the most important ones and encodes the connexin 26 proteins. A mutation called delG35 composes most of mutated alleles of connexin 26 and is also the most common cause of congenital sporadic and hereditary deafness. Open Access Journals (DOAJ), Google Scholar, PubMed (NLM), LISTA (EBSCO), and Web of Science have been searched for literature. *GJB2* gene mutations play the most significant role in non-syndromic deafness in Iran. 35delG mutation has a high frequency in most parts of Iran, especially in the North and North-West and is also the most common mutation in GJB2 deaf population of Iran. *GJB2* gene mutations in Iran play less important roles compared with other countries in causing deafness; however, so far, it has been introduced as the gene that plays the most significant role in causing deafness gene in Iran. It seems that many other genes and loci play roles in causing deafness in Iran that requires more studies to be conducted.

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Key words: Deafness - Sensorineural hearing loss - Connexin 26 - Human DFNA3 protein.

According to the World Health Organization, about 360 million (328 million adults and 32 million children) equivalent to 5% of people worldwide have been disabling hearing loss.[1] And it is estimated that between 1 and 5 persons of every 1000 newborns have at least 40 decibels hearing disorder.[2, 3] Deafness is the most common sensory disorder in humans, and 1 of 1000 individuals is affected by severe to profound hearing loss while some degree of hearing loss has been observed in the normal population in 4% of younger than 45 years and 10% of older than 65 years.[4-6] In terms of the severity, deafness is divided to mild (24-40 dB), moderate (41-55 dB), moderately severe (71-90 dB), severe (71-90 dB), and deep (90 dB).[5] In the definition of deafness, a person who is not able to hear the sound of 25 dB in both ears, is affected by hearing loss and people who have hearing defects in the limit of 90 dB or more have been mainly profound hearing loss and often use sign language to communicate.[1] In general, causes of deafness are divided into two categories: congenital and acquired. The congenital causes are the causes which develop hearing impairment at birth or immediately after birth, and their reasons may be hereditary or non-hereditary and included items such as rubella, syphilis, infection, low birth weight, prematurity, jaundice and but the acquired factors are the causes which after birth, and at any age may cause hearing loss and include cases such as ototoxic drugs, otitis media, harsh environmental sounds,

infectious diseases such as measles, meningitis, and finally chronic ear infections.[7] Genetic deafness is accounted for about 50% of the deafness.[8] About 70% of genetic deafness is non-syndromic, and its inheritance pattern is autosomal recessive (80%), autosomal dominant (15%), X-dependent (3%), and mitochondrial (2%) (Figure 1).[9-11] This type of hearing loss manifests with no symptoms or other defects. Statistics show that half of non-syndromic deafness before speaking is hereditary, and more than 80% of these cases are inherited in an autosomal recessive manner.[12-16]

Connexin 26 protein

So far, there are more than 100 loci that are associated with the incidence of hearing loss, it has been found that among which mutations in *DFNB1* locus (13q11-12) alone include 50% of autosomal recessive deafness. In this locus, there are genes of *GJB2* (encoding connexin 26) and *GJB6* (encoding connexin 30).[17] Many genes involve in deafness that *GJB2* gene is one of the most important that encoded connexin 26 protein (Cx26). This gene with a length of 5.5 kb takes place on the long arm of chromosome 13 and has been formed from two exons and 1 intron. Exon 2 is the only sequence of the *GJB2* gene required to produce the connexin 26 protein.[18, 19] mRNA of this gene has about 2.4 kb length and the synthesized proteins contains 226 amino acids.[19, 20] These proteins play important roles in formation of gap junction channels between the cells and allow the transmission of potassium ions and small molecules.[20] Gap junctions are the structures in the adjacent cell membrane of multicellular organisms, and connexin 26 is present in the inner ear cochlea. After stimulation of hair cells, the connections are responsible for returning potassium ions through synapses in the apical of these cells and guard cells and fibroblasts into the endolymph containing high (concentrations of potassium) in the inner cochlea.[21]

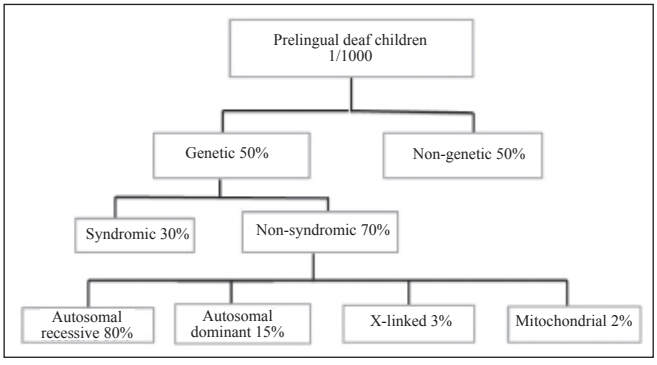

Figure 1.—Frequency of deafness type.

GJB2 gene mutations

In different populations, *GJB2* gene mutation is one of the most important causes of non-syndromic deafness before speaking, and *GJB2* gene mutation has been known as the basis of non-syndromic recessive deafness mutations in DFNB1.[22-29] Numerous studies from around the world have shown that distribution and *GJB2* gene mutations in different geographical areas, and different ethnic groups are different, for example, in the East Asian population 235delG mutation allocated the highest rate of mutation in the *GJB2* gene,[30-33] while mutations among Ashkenazi Jews is 167delT.[27] Reviewing the results of studies in multiple populations showed different percentages of *GJB2* genes causing deafness. In a study in Turkey, the relationship between *GJB2* gene and non-syndromic deafness has been reported with an autosomal recessive inheritance patterns in 21.4% of cases (in 14 families).[34] In another study from the same country on 60 affected families, *GJB2* gene mutations have been reported in 31.7% of cases.[35] According to studies conducted in Iran, the highest mutation rate has been reported in the *GJB2* gene in Gilan and Eastern Azerbaijan provinces, while the lowest rate has been reported from the Sistan-Baluchistan province.[36, 37] In Table I,[38-55] part of the studies reported in the *GJB2* gene associated with hearing loss had been reported in Iran. Accordingly, different mutations within this gene have spread in the various ethnic groups indicating the existence of a founder effect.[56]

Mutations in *GJB6*

A deletion almost as 309 kb with a break point within the coding region of *GJB6* causes deafness in *1DFNB* locus.[57-59] In a multinational and analytical study of the 9 countries, deletion of *GJB6* 5.9 includes about 9.7% of all *1DFNB* alleles in Spain, France, England, Israel and Brazil. In Belgium and Australia, it was 1.3-1.4%, which showed a lower prevalence.[60] Genetic testing for deafness in DFNB1 locus includes screening for *GJB6* genes.

TABLE I.—*Frequency of GJB2 and GJB6 mutations in the previous studies in Iranian populations.*

Study (year)	Types of hearing loss	Location	N. participants and methods	Main results
Bazazzadegan *et al.* (2012) [38]	NSHL	Iranian population	2322 deaf probands of diverse ethnicities. Direct PCR-sequencing	In total, 374 (16%) families segregated GJB2-related deafness caused by 45 different mutations and 5 novel variants; c.35deIG mutation was most commonly identified and accounts for similar to 65% of the *GJB2* mutations
Bonyadi *et al.* (2009) [39]	ARNSHL	Azeri Turkish patients	Probands from 209 different nuclear families. Direct PCR-sequencing	*GJB2* mutations were found in 28% of the patients. Among these patients, 44 families had 35delG mutation
Chaleshtori *et al.* (2008) [40]	NSHL	Gilan in north and Khorasan in east of Iran	38 subjects (5 families and 2 individuals) ARMS nested PCR PCR/RFLP	The *GJB2* allelic variants including 363delC, 327delGGinsA, H16R and G200R have been co segregated in 5 families and are not found in control subjects
Chaleshtori *et al.* (2007) [41]	ARSNSHL	Iranian population	1095 hearing impaired students and their deaf siblings from 890 families in 10 provinces of Iran. Nested PCR and direct sequencing	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)
Chaleshtori *et al.* (2007) [42]	Unaffected unrelated Iranian subjects	Iranian population	550 unaffected unrelated subjects from 4 provinces of Iran. Nested PCR	found a high carrier frequency of 2.8% in Gilan province in the north of Iran. The overall 35delG carrier frequency was found to be 1.25% in the populations studied
Davarnia *et al.* (2012) [43]	ARNSHL	Azeri patients	Fifty families ARMS PCR	Thirteen families demonstrated alteration in the Cx26 (26%). The 35delG mutation was the most common one, accounting for 69.2% (9 out of 13 families)
Esmaeili *et al.* (2007) [44]	ARNSHL	Iranian population	133 Iranian deaf patients. Multiplex allele-specific PCR	The frequency of 35delG was about 18.5%, however, del(GJB6-D13S1830) was not found in the studied patients
Falah *et al.* (2011) [45]	NSHL	Iranian population	100 Iranian deaf patients. PCR, direct sequencing	Eight known mutations plus one novel (358delGAG) were found in 25% of study group. The 35delG mutation (64%) constituted the majority of GJB2 mutations
Galehdari *et al.* (2009) [46]	ARNSHL	Arabian origins	61 deaf patients and 26 control subjects PCR direct sequencing	None of the analyzed samples revealed deafness-associated mutation
Hashemi *et al.* (2012) [47]	NSHL	South Iranian	50 patients PCR direct sequencing	Mutations were detected in 15 out of 50 patients (30%). Eight different mutations were identified; six of them were previously identified (35delG, V27I M34V, V153I, A149T, V198M). The remaining two alleles, L28I and N169T, were novel variants
Kashef *et al.* (2015) [48]	ARNSHL	Iranian population	103 patients PCR-based direct sequencing	identified the second mutant allele in splice site of exon-1 of GJB2 which is known as IVS1+1G > a in 17 probands. no found mutation in promoter region of GJB2. It emphasizes to approach exon1 of GJB2 in case of ARNSHL genetic diagnosis
Mahdieh *et al.* (2010) [49]	ARNSHL	Iranian population	114 patients. directly sequenced	Mutations found were 35delG, delE120, R127H, M163V, W24X, V37I, G12D, V84A, 313-326del14, and E110K. Mean frequency of GJB2 mutations was 17.92%. GJB2 mutations (and not GJB6 mutations)
Mahdieh *et al.* (2010) [50]	NSHL	Iranian population	9 heterozygous families SYBR green-based PCR	Not detect any deletion in the GJB6 gene using this method
Najmabadi *et al.* (2020) [51]	ARNSHL	Iranian population	168 persons from 83 families. ASPCR, SSCP	GJB2-related deafness was diagnosed in 9 families, the carrier frequency of the 35delG allele in this population was approximately 1% (1/83)

(*To be continued*)

TABLE I.—*Frequency of GJB2 and GJB6 mutations in the previous studies in Iranian populations (continues).*

Study (year)	Types of hearing loss	Location	N. participants and methods	Main results
Onsori *et al.* (2014) [52]	ARNSHL	Marand, Iran	A patient, 40-year-old Iranian PCR products and direct sequencing	A novel heterozygous -T to -C transition at codon 202 (TGC→CGC) of the GJB2 gene in a patient, 40-year-old Iranian woman, which replaces a cysteine with an arginine residue (C202R)
Peyvandi *et al.* (2011) [53]	NSHL	Baqiyatallah Hospital, Tehran, Iran	42 children PCR amplification and direct sequencing	6 patients (14.3%) with the 35delG mutation on the Cx26 gene, two homozygotes and four heterozygotes
Rezaei *et al.* (2011) [54]	NSHL	Iranian population	100 unrelated healthy individuals. Genotypes of the STR markers were determined using PCR	Among the 42 possible haplotypes examined, four haplotypes showed relatively high frequencies
Zeinali *et al.* (2015) [55]	ARNSHL	Iranian population	418 Iranian individuals. ARMS-PCR	Among 418 investigated cases, a total of 81 patients (similar to 19.4%) with biallelic pathogenic mutations in the GJB2 gene and 13 cases with only one pathogenic mutant allele were identified. The total allele frequencies of the two most frequent mutations, c.35delG and c.-23+1G > A, among mutated alleles were found to be around 59% and 15.7%, respectively

NSHL: non-syndromic hearing loss; PCR-RFLP: PCR-restriction fragment length polymorphism; ARNSHL: autosomal recessive non-syndromic hearing loss; ARMS-PCR: tetra-primer amplification refractory mutation system-polymerase chain.

In a study in Iran, after reviewing 1605 subjects affected from non-syndromic autosomal recessive hearing loss, 227 patients (14.1%) demonstrated GJB2 and GJB6 deafness as 0% that have allocated small percentage of this frequency compared with many countries and it seems that other genetic positions are involved in the creation of this type of hearing loss.[27, 61-64] It seems that, in Iran, *GJB6* mutation does not cause deafness (Table I).

Common mutations of 35delG

There is a mutation called delG35 that first was reported by Zlant *et al.* in 1997, it formed the majority of the mutant alleles of connexin 26 and is also the most common cause of congenital sporadic and hereditary deafness. DelG35 mutation is responsible for 10% of deafness in children and 20% of hereditary hear loss in Caucasian children in America and southern Europe.[7, 65] There are six guanine nucleotide repeats in 30 to 35 position of *GJB2* gene, that deletion of any nucleotide in this area causes 35delG or 30delG mutations.[16] The major cause of congenital sporadic and hereditary deafness in whites' populations is 35delG mutation. The frequencies of carriers of this mutation have been reported in northern Europe as 1.26% and 1.96% in southern Europe.[56, 66, 67] In studies conducted in northern Europe, 35delG has been the most common mutation in this area.[24, 61, 68-71] This mutation and deletion (*GJB6*-D12S1830) have been studied in most populations such as Spain.[57, 60] 35delG allele frequency in the Iran-neighboring Turkish population has been reported between 5-53%.[72, 73] In recent surveys, frequency of this mutation has been estimated as 18% in Azerbaijani population.[44]

Based on the review conducted by Chaleshtori *et al*., delG35 mutation is the most common mutation of the connexin 26 gene mutations in Iran. As well as the rate of this mutation among carriers was 1.8% that Gilan province had the highest prevalence as 2.8%.[42] 35delG has been observed in most of the world's populations with different prevalence and frequency of this mutation different between different ethnic groups of Iran has been demonstrated in Table II.[21, 41, 43, 74-76]

Discussion

Over the past few decades, the prevalence of acquired hearing loss compared with genetic hearing loss has been reduced, and a relative growth can be seen in the population of children with genetic hearing loss. The frequency of genetic hearing loss in Asian countries such as Iran has been higher than most of the Eu-

TABLE II.—*Frequency of 35delG mutation in Iran classified by province.*

Original province of the population	Frequency of 35delG mutation	Study (year)
Gilan	27.1%	Chaleshtori *et al.* (2004) [74]
Isfahan	22.5%	Rezaei *et al.* (2010) [21]
East Azerbaijan	18.3%	Bonyady *et al.* (2009) [75] Davarnia *et al.* (2012)[43]
Kurdistan	14.7%	Chaleshtori *et al.* (2007) [41]
Tehran	13.9%	Chaleshtori *et al.* (2007) [41]
Razavi Khorasan	11.2%	Chaleshtori *et al.* (2004) [74]
Golestan	9.1%	Chaleshtori *et al.* (2007) [41]
Chaharmahal and Bakhtiari	6.4%	Chaleshtori *et al.* (2007) [41]
Khuzestan Province	6.2%	Chaleshtori *et al.* (2007) [41]
Kerman	2.3%	Bazaz-zadegan *et al.* (2005) [76]
Hormozgan	1.5%	Chaleshtori *et al.* (2007) [41]
Sistan and Baluchestan	0%	Chaleshtori *et al.* (2007) [41]

ropean and American countries due to the high rate of consanguineous marriage, and on the other hand, acquired hearing loss have decreased in the European and American countries because it reduces environmental factors of hearing loss. The information obtained from genetic tests can play an important role in major decisions, including decisions on treatment such as the use of hearing aids or cochlear implants and genetic counseling before marriage and before pregnancy (as well as during pregnancy). Therefore, acknowledgments of different factors affecting non-syndromic hearing loss in distinct populations is important. The differences influence specific gene's mutations that cause autosomal recessive non-syndromic hearing loss in different countries. According to the results of research conducted in Iran by an average 70% of deaf people are consequences of consanguineous marriages, hence active genetic counseling and preventive barriers and cultural development, and consanguinity marriage has been very effective. On the other hand, control on the number of offspring in deaf families is another case of culture development and genetic counseling. Inter group marriages in rural areas and small and closed populations are the other main factor increasing the incidence of homozygosity and genetic diseases, including deafness. Therefore, it is recommended that inter group marriages, especially in small villages and small population groups with a history of autosomal recessive diseases must be avoided. Given that the Iranian population is a mix of different tribes, new results can be found by examining them, which ultimately helps to genetic counseling, control, and treatment of this patient group. Various articles and reports from all over the world reveal that *GJB2* gene mutations are different in various geographical regions and ethnic groups. 35delG mutation in European and American white population, 167delT mutation in Ashkenazi Jews, and 235delC mutation in East Asia have the highest incidence. Additionally, 35delG mutation is common to most parts of the world. The frequencies of *GJB2* gene mutations were investigated in several provinces of Iran. The results indicated that these mutations are common in Iranian population and most genotype has been associated with 35delG mutation (Table II).

Conclusions

Iran's population consists of ethnic groups, including Fars in central Iran, Azeri in north-west, Gilaki and Mazandaran in the north, Kurds, Lors, and Arabs in the west and the south of the country. The highest percentage of GJB2-related hearing loss is observed in northern Iran. According to the obtained information, the frequency observed for the *GJB2*-related hearing loss from North-West to South-East (where the population has ethnically a relationship with Pakistan), is reduced. In South-Eastern Iran, *GJB2*-related hearing loss includes 6.8% of non-syndromic autosomal recessive hearing loss cases while in a study in Pakistan on 27 families with autosomal recessive non-syndromic hearing loss, only one family (3.7%) showed *GJB2*-related hearing loss.[77] *GJB2*-related hearing loss has not been found in the most Southern part of the country where the Arab population lives. Studies of *GJB2* gene dependent hearing loss on the Arab race in Oman showed no mutation and mutations of 35delG and 167delT were not detected.[78, 79] The slope of *GJB2*-related hearing loss from the Northwest toward the Southeast and also to provinces of the Persian Gulf and Strait of Hormuz has been revealed hearing loss slope from South to North Europe, which has been obtained from studies in European countries.[16, 69, 71, 80] Although *GJB2* gene mutations played no significant role in Iran like other countries, but so far it has been introduced as the most significant gene mutation causing hearing loss in Iran. It seems that many other genes and loci play roles in development of hearing loss in Iran that requires more studies to be conducted.

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