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REVIEW

GJB2-related hearing loss in central Iran: Review of the spectrum and frequency of gene mutations

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Abstract

Mutations in the *GJB2* gene are a main cause of autosomal-recessive nonsyndromic hearing loss (ARNSHL) in many populations. Previous studies have estimated the average frequency of *GJB2* mutations to be ~16% in Iran, but would vary among different ethnic groups. Here, we have taken together and reviewed results from our two previous publications and data from searching other published mutation reports to provide a comprehensive collection of data for *GJB2* mutations and HL in central Iran. In all, 332 unrelated families were included and analyzed for the prevalence and type of the *GJB2* gene mutations. In total, the frequency of *GJB2* mutations was found to be 16% in the central provinces, which is significantly higher than those identified in southern populations of Iran. Also, c.35delG was the most frequent mutation in the related population. The present study suggests that mutations in the *GJB2* gene, especially c.35delG, are important causes of HL in central Iran and can be used as a basis of genetic counseling and clinical guidelines in this region.

KEYWORDS

clinical guidelines, *GJB2*, Iranian population, nonsyndromic hearing loss

1 | INTRODUCTION

Hearing loss (HL) is a complex impairment and accounts for one to two of 1,000 newborns all over the world (http://hearing.screening.nhs.uk/nationalprog). Many environmental agents, such as drug exposure, bacterial or viral infections, and trauma, can cause HL; however, a significant proportion of cases is related to genetic factors. It is estimated that 70% of cases includes nonsyndromic HL (NSHL), where HL is not associated with additional clinical features (Morton, 1991). Although all Mendelian inheritance patterns have been observed for prelingual HL, autosomal-recessive modes of inheritance (ARNSHL) makes up 80% of the NSHL cases (Morton & Nance, 2006). ARNSHL is highly heterogeneous, for which over 100 mapped loci are known to be involved (http://hereditaryhearingloss.org). Nevertheless, a single locus, DFNB1(13q11-12) which contains *GJB2* (NM_004004.5) and *GJB6* (NM_001110219.2) genes, accounts for about 50% of the etiology in many Western populations (Gasparini et al., 2000; Van Laer et al., 2001). *GJB2* encodes the connexin 26 protein (Cx26), which is a type of gap junction protein involved in the inner ear homeostasis of the cochlear fluids, endolymph and perilymph, through recycling of potassium ions (Maeda et al., 2009). The gene has a simple genomic structure consisting of 680 bp can be sequenced simply (Zelante et al., 1997). To date, more than 100 pathogenic mutations in the *GJB2* gene have been identified resulting in ARNSHL (http://davinci.crg.es/deafness). The prevalence of *GJB2* mutations varies among different populations (Azadegan-Dehkordi, Ahmadi, Koohiyan, & Hashemzadeh-Chaleshtori, 2019b; Talbi et al., 2019). In Caucasians, c.35delG is the most common mutation with the carrier frequency as high as 2%–4% (Pandya et al., 2003; Seeman et al., 2005). However, c.235delC and p.Trp24* are the most frequent mutations in the Japanese (Abe, Usami, Shinkawa, Kelley, & Kimberling, 2000; Nishio & Usami, 2015) and Indians (Bhalla, Sharma, Khandelwal, Panda, & Khullar, 2009; RamShankar et al., 2003), respectively. Iran's

population is ~80 million, with HL affecting an estimated more than 450,000 individuals. In other words, one in 166 individuals is affected, which makes it a major public health issue (Mahdieh, Bagherian, Shirkavand, Sharafi, & Zeinali, 2010).

Over the last decade, a series of studies have been conducted on the Iranian population to identify the mutation spectrum and prevalence of *GJB2* mutations (Azadegan-Dehkordi et al., 2019a; Falah et al., 2019; Hashemzadeh-Chaleshtori et al., 2008; Hosseinipour et al., 2005; Koohiyan & Ahmadi, 2019; Mahdieh, Mahmoudi, Ahmadzadeh, & Bakhtiyari, 2016; Sadeghi et al., 2009). The diverse ethnicities, coupled with the high rate of consanguinity rates (38% in average) (Saadat, 2005), tend to change mutation frequencies among ethnic groups (Mahdieh et al., 2011; Sloan-Heggen et al., 2015). Therefore, for accurate and effective genetic counseling, studying certain ethnic groups is of high importance (Babanejad et al., 2012; Bakhchane et al., 2016). In this paper, we summarized the published data on the frequency and profile of the *GJB2* gene mutations in 332 unrelated families from six different provinces, namely, Lorestan, Isfahan, Chaharmahal and Bakhtiari, Markazi, Qom, and Yazd, in the central part of Iran.

2 | METHODS

This study includes results from our two previous publications on *GJB2*-related HL in Iran. We also performed a PubMed, InterScience, and ScienceDirect search using search terms "*GJB2* mutations" or "connexin 26," and "Iran." Among the search results, we limited the search to humans who held information on molecular genetics of HL. Studies were included when fulfilling the following three criteria: (1) performance on NSHL subjects, (2) described the ethnicity of tested subjects, and (3) detection by comparable molecular methods. Studies were excluded if HL was a result of environmental factors such as infection, trauma, rubella, meningitis, mumps, ototoxic drugs, and premature birth. Research data including 332 unrelated deaf families from the southern provinces were collected. The frequency and mutation type of 332 deaf families were extracted from relevant studies and categorized, corresponding with geographical boundaries. In silico analyses were also performed by available software tools to predict the pathogenicity of the mutation.

3 | RESULTS

Data from 332 unrelated families were gathered for analysis (Figure 1). The groups studied consisted of 120 families from Yazd Province (36%), 79 families from Chaharmahal and Bakhtiari (24%), 54 families from Lorestan (16%), 40 families from Isfahan (12%), and 40 families from Qom and Markazi (12%). The *GJB2* mutation frequencies of each studied group includes 31.2%, 18.8%, 16.25%, 7.5%, and 6.9% of total studied families ($n = 332$) of Isfahan, Lorestan, Qom and Markazi, Yazd, and Chaharmahal and Bakhtiari provinces, respectively.

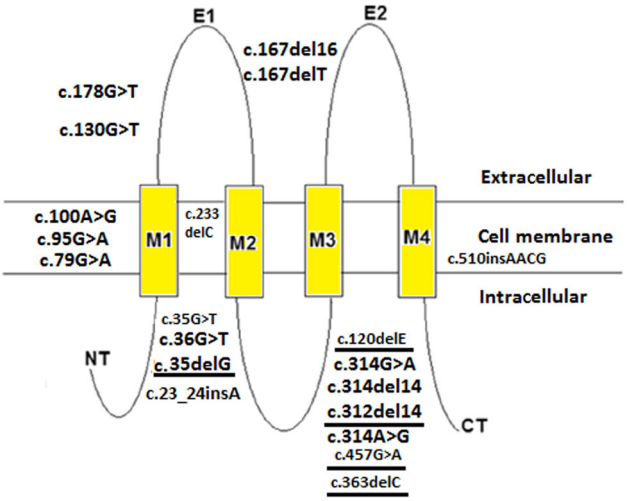

FIGURE 1 Schematic structure, domains and distribution of mutations of the Cx-26 protein in this study. The most common mutations in central Iran (c.35delG, c.312del14, c.457G > A, c.363delC, and c.358_360delGAG) are underlined [Colour figure can be viewed at wileyonlinelibrary.com]

In total, 21 different variants were identified, 14 of which were reported as pathogenic. These include c.35delG, c.23_24insA, c.167delT, c.100A > G, c.163A > G, c.79G > A, c.95G > A, c.233delC, c.35G > T, c.363delc, c.510insCGAA, c.176del16, c.457G > A, c.130T > G, -3170G > A, c.178T > G, c.358_360delGAG, c.312del14, c.314del14, and c.512insAACG. In the studied populations, c.35delG was the most frequent mutation. The highest rate of c.35delG mutation was detected in Isfahan, accounting for 15% of detected mutations, while this rate was 1.6% in Yazd. Figure 2 shows the distribution of the identified mutations in the schematic structure of Cx26. A specific combination of *GJB2* mutation types and frequencies were found in different studied provinces (Table 1). A higher number of *GJB2* mutation diversity (12 types) was observed in Isfahan province while the lowest diversity identified was in Bakhtiari populations (four types).

4 | DISCUSSION

This study reviews the prevalence and type of the *GJB2* gene mutations by means of a literature review and compares 332 deaf families from six provinces in central Iran. The genetic

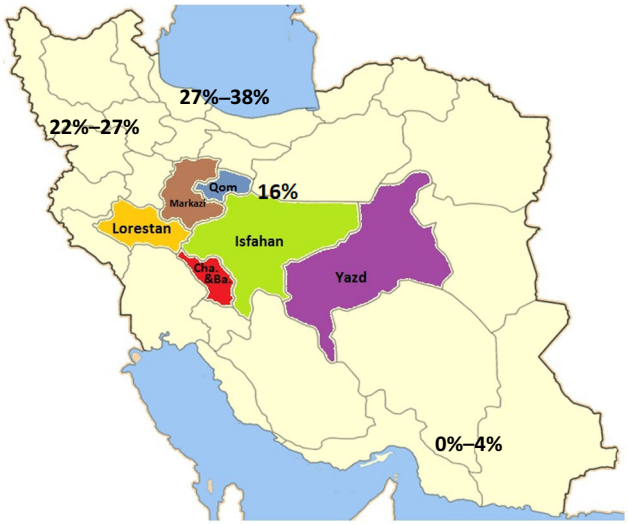

FIGURE 2 The prevalence of *GJB2*-related mutations in different regions of Iran (northwest 22%–27%, north 27%–38%, southeast 0%–4%). Six central provinces (Lorestan, Isfahan, Chaharmahal and Bakhtiari, Markazi, Qom, and Yazd) are shown on the map [Colour figure can be viewed at wileyonlinelibrary.com]

epidemiology of ARNSHL is very different among populations because of founder effects (Davarnia et al., 2012; Laleh et al., 2017; Zarepour et al., 2019). The Iranian population is composed of many different ethnic groups, so it is important to discuss ethnic-specific data (Chaleshtori et al., 2002; Haghighat-Nia et al., 2015; Koohiyan, 2019). Accepting the northwest-to-southeast *GJB2* HL gradient throughout Iran, our data indicates a west-to-east gradient among Iranian populations with a *GJB2* mutations frequency of 31.2% for Isfahan province and 7.5% for Yazd province. The results obtained from other studies have shown that the mutation frequency of *GJB2* varies between 0% and 35% within different parts of Iran (Bazazzadegan et al., 2012). The study performed by Najmabadi et al. (Najmabadi et al., 2005) on 664 ARNSHL families indicated that *GJB2*-related HL accounts for 16.7% in the Iranian population and the c.35delG mutation was the most common *GJB2* mutation (~72% of the identified *GJB2* mutations). In another cohort study, Chaleshtori et al. (Chaleshtori, Farhud, & Patton, 2007) showed the frequency of *GJB2* mutations to be 27.5% in the north and northwest provinces of Iran, while it was less than 4% in the southeast region. The observed northwest-to-southeast *GJB2* HL gradient is further supported by data specific to southeast and northwest Iran, where the populations are related to neighboring Oman and Turkey (Simsek et al., 2001; Yilmaz et al., 2010). Bonyadi et al. (Bonyadi, Esmaeili, Abhari, & Lotfi, 2009) showed that *GJB2* mutations were responsible for about 28% of ARNSHL in Iranian Azeri Turkish patients (northwest Iran) and c.35delG was the most prevalent mutation accounting for 64.5% of *GJB2* mutations, which is similar to the reported results in the Turkish population (Duman, Sirmaci, Cengiz, Ozdag, & Tekin, 2011; Kalay, Caylan, Kremer, de Brouwer, & Karaguzel, 2005). Our results showed that the contribution of *GJB2* mutations to ARNSHL is 7.5% in Yazd Province, which is similar to the presented data from the Baluhi population in southeast Iran. Naghavi et al. (Naghavi et al., 2008) screened 100 ARNSHL families from Sistan and Baluchistan Provinces in southeast Iran for *GJB2* mutations. They reported that *GJB2* mutations were detected in 7% of the ARNSHL families studied.

Another finding of this study was the mutation spectrum of the Yazd Province, which was different from those of Iranian population regions. Bazazzadegan et al. screened 120 NSHL families from Yazd Province in central Iran for *GJB2* mutations. They reported that *GJB2* mutations were found in 7.5% of the NSHL families studied. Surprisingly, c.312del14 was the most frequent *GJB2* mutation (56.2% of the identified *GJB2* mutations) in Yazd Province, while c.35delG was identified in only 25% of *GJB2* mutations in this province (Bazazzadegan et al., 2012). However, the Yazdi population is ethnically distinct from the rest of Iran. Besides, 79 unrelated ARNSHL families from Chaharmahal and Bakhtiari Province were reviewed, with a frequency of 6.9% for *GJB2* gene mutations (Chaleshtori et al., 2007). This low rate of the c.35delG mutation has been reported in some populations of the south and southeast of Iran like Hormozgan and Bushehr Provinces (Chaleshtori et al., 2002).

The study performed by Koohiyan et al. (Koohiyan, Hashemzadeh-Chaleshtorib, Salehia, & Abtahic, 2018) on 40 ARNSHL families indicated that *GJB2*-related HL accounts for 31.2% in central Iran. This is about four times the frequency of *GJB2* mutations in Yazd Province. In addition, Bazazzadegan et al. (2012) screened 53 NSHL families from Lorestan Province in central Iran for *GJB2* mutations. They reported that *GJB2* mutations were found in 18.8% of the NSHL families studied. This is near the frequency of *GJB2* mutations in Lorestan Province. On the basis of these results, it can be concluded that the frequency of c.35delG decreases gradually both west to east and north to south (Figure 2), drawing the migration pathway of the initial founders.

In our studied populations, the most common mutation was c.35delG, accounting for 43% of *GJB2* mutations. The c.35delG mutation is found to be the most common mutation in many world populations as well as many countries in the Middle East (Adhikary et al., 2015; Al-Qahtani et al., 2010; Ghasemnejad, Khaniani, Zarei, Farbodnia, & Derakhsahan, 2017; Vozzi et al., 2014; Yoong et al., 2011). The analysis of the geographical distribution of mutations located in the *GJB2* gene showed more allelic heterogeneity in central compared to southern Iran (Esmaeili, Bonyadi, & Nejadkazem, 2007; Mahdieh et al., 2004). The five most frequent mutations of the *GJB2* gene in central Iran, namely, c.35delG, c.312del14, c.457G > A, c.358_360delGAG, and c.363delC are responsible for 56% of all pathogenic alleles in central Iran (Table 1).

TABLE 1 *GJB2* mutations, their frequencies, and in silico analyses in central provinces of Iran

Mutations	No. (%)					Mutation type	Classification	Functional effect	
	Isfahan	Lorestan	Chaharmahal and Bakhtiari	Qom and Markazi	Yazd			Mutation taster	SIFT
c.23_24insA	1 (1.25)	0	0	0	0	Frameshift	NT	Disease causing	Damaging
c.35delG	12 (15)	10 (9.4)	5 (3.1)	7 (8.7)	4 (1.6)	Deletion/ nonsense	T	Disease causing	NA
c.35G > T	1 (1.25)	0	0	0	0	Missense	NT	Disease causing	Damaging
c.95G > A	2 (2.5)	0	0	0	0	Missense	NT	Disease causing	Damaging
c.100A > G	1 (1.25)	0	0	0	0	Missense	NT	Disease causing	Damaging
c.130T > G	1 (1.25)	0	0	0	0	Missense	NT	Disease causing	Damaging
c.163A > G	1 (1.25)	0	0	0	0	Missense	NT	Disease causing	Damaging
c.167delT	0	0	0	0	1 (0.41)	Frameshift	T	Disease causing	NA
c.176del16	0	0	0	2 (2.5)	0	Frameshift	T	Disease causing	NA
c.178T > G	1 (1.25)	0	0	0	0	Missense	NT	Disease causing	Damaging
c.233delC	0	0	0	2 (2.5)	0	Frameshift	T	Disease causing	NA
c.314del14	0	2 (1.8)	0	0	2 (0.83)	Frameshift	T	Disease causing	NA
c.312del14	0	0	0	0	9 (3.7)	Frameshift	T	Disease causing	NA
c.358_360delGAG	2 (2.5)	0	0	0	2 (0.83)	In frame deletion	NT	Disease causing	Damaging
c.363delc	0	0	4 (2.5)	0	0	Frameshift	T	Disease causing	NA
c.510insCGAA	0	1 (0.94)	0	0	0	Frameshift	T	Disease causing	NA
c.512insAACG	0	2 (1.8)	0	0	0	Frameshift	T	Disease causing	NA
c.23+1G > A	0	1 (0.94)	0	0	0	Missense	T	Disease causing	Damaging
c.79G > A	1 (1.25)	0	1 (1.25)	0	0	Missense	NT	Polymorphism	Tolerated
c.314A > G	1 (1.25)	0	0	0	0	Missense	NT	Polymorphism	Tolerated
c.457G > A	1 (1.25)	4 (3.7)	1 (1.25)	2 (2.5)	0	Missense	NT	Polymorphism	Tolerated
Normal	55	86	147	67	222				
Total	80	106	158	80	240				

Note. T, truncated protein; NT, nontruncated protein; NA: not available.

The c.35delG mutation, which is the most common (up to 85%) among northern regions (Chaleshtori et al., 2007), makes up for 43% of *GJB2* mutations in the central population. The c.312del14, c.457G > A, and c.358_360delGAG are the second, third, and fourth most common mutations, with a total of 10.2%, 9.1%, and 4.5% of all pathogenic alleles. The c.312del14 mutation, a frameshift variant, leads to the formation of a premature stop codon at amino acid position 109, which results in a truncated protein with probably no functional properties. In silico analyses are consistent with

the pathogenicity of the mutation (Table 1). The c.312del14 mutation is the rare mutation in Iranian populations (Hashemi, Ashraf, Saboori, Azarpira, & Darai, 2012), but this mutation shows a high frequency in Yazd Province (accounts for 56% of the mutant alleles), because of founder effects. Yazdi populations are isolated with cultural, lingual, religious, and geographical barriers from other parts of Iran. *V153I*, a missense mutation is the result of c.457G > A transition, changing a TGG codon for a valine residue to a TGA codon for isoleucine, which probably leads to a nonfunctional protein (Rabionet, Gasparini, & Estivill, 2000). The pathogenic effect of this amino acid substitution is controversial and remains to be confirmed.

More recently, researchers have shown that mutations of *GJB2* can function in a digenic manner with the *GJB3* or *GJB6* genes (Rodriguez-Paris & Schrijver, 2009). Hence, mutation analysis of this gene should be considered in *GJB2* heterozygotes (Kooshavar et al., 2013; Lin et al., 2001). Because of the high frequency of *GJB2* mutations in many populations, *GJB2* analysis should be depicted for HL, although the frequency of *GJB2*-related HL in Iran is lower than that of European countries (Gasparini et al., 2000; Lucotte, 2007). Hashemzadeh-Chaleshtori et al. (2008) showed that more than 40% of patients were heterozygous carriers in south and southeast Iran (Chaleshtori et al., 2002). Further investigation is needed to detect the genetic cause of HL in patients with monoallelic *GJB2* mutations (Shahin et al., 2002; Walid, Bassel, Ali, & Moassass, 2017).

5 | CONCLUSION

The critical and specific position of Iran and the existence of various ethnic groups with different cultures suggest the high heterogeneity throughout Iran, but specific intraethnic traditions such as intragroup marriages may give rise to a high homogeneity in some loci and mutations within groups. *GJB2* mutations are responsible for ∼16% of deaf families in central Iran, which is less than that in northwest Iran (22%–27%), showing a migration pathway from west to east through the silk route. Regarding the *GJB2* mutations, c.35delG is the most common mutation that is tested first. In studied populations, specific mutations are common, which are detected in each group; for example, the frequency of c.312del14 shows a high rate in Yazd Province, accounting for 56% of the mutant alleles. This study highlights the importance of establishing incidence based on the local population of specific and common *GJB2* mutations in designing screening strategies.

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CONFLICT OF INTEREST

No potential conflicts of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Mahbobeh Koohiyan performed data analysis, wrote the manuscript, revised the manuscript, answered reviewer comments, and approved the final version; Farideh Koohian and Fatemeh Azadegan-Dehkordi designed the study, validated data, and performed data analysis.

STATEMENT OF ETHICS

The authors have no ethical conflicts to disclose.

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