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Impact of Consanguineous Marriages in *GJB2*-Related Hearing Loss in the Iranian Population: A Report of a Novel Variant

Nejat Mahdieh,[1–3] Bahareh Rabbani,[4,5] Atefeh Shirkavand,[1] Hamideh Bagherian,[1] Zahra Shahab Movahed,[1] Paanti Fouladi,[1] Faezeh Rahiminejad,[1] Masoumeh Masoudifard,[1] Mohammad Taghi Akbari,[3] and Sirous Zeinali[1,6]

Mutations in *GJB2* and *GJB6* genes are the main causes of autosomal recessive nonsyndromic hearing loss (ARNSHL) in many populations. Here, we investigated *GJB2* and *GJB6* mutations in 114 patients from 77 affected ARNSHL families including 54 consanguineous marriages and 23 nonrelative marriages in the Iranian population. Clinical studies and genetic counseling were performed for all families. *GJB2* and *GJB6* genes were directly sequenced. Three known *GJB6* large deletions [del(*GJB6*-D13S1830), del(*GJB6*-D13S1854), and a 920 kb deletion] were also checked by quantification of a common deleted region within the *GJB6* gene. The frequency of consanguinity was 70.13% among the studied families. Biallelic *GJB2* mutations were 16.67% in consanguineous marriages and 4.35% in nonrelative marriages. Mutations found were 35delG, delE120, R127H, M163V, W24X, V37I, G12D, V84A, 313-326del14, and E110K. The latter was a novel variant. Neither point mutation nor a large deletion in the *GJB6* gene was found in the population. Mean frequency of *GJB2* mutations was 17.92%. *GJB2* mutations (and not *GJB6* mutations) are the major causes of hearing loss in Iran. The role of consanguineous marriages is also highlighted in occurrence of *GJB2*-related hearing loss. We suggest that other genes may be involved in the population.

Introduction

Hearing loss (HL) is the most prevalent sensory defect, affecting 1 in 500–650 newborns (Morton and Nance, 2006). Fifty percent to 60% of early onset HL is due to genetic factors (Parving, 1999). Two-thirds of genetic HL can be attributed to nonsyndromic HL. The remaining cases can be traced to a syndrome. Autosomal recessive nonsyndromic hearing loss (ARNSHL) is the most frequent among nonsyndromic hearing impairments, accounting for 75%–80% of early-onset deafness (Tekin *et al*., 2001).

Nearly 140 loci and 55 different genes causing HL have been identified so far (http://hereditaryhearingloss.org). The first locus for ARNSHL, DFNB1, which contains the *GJB2* and *GJB6* genes, is the major cause of ARNSHL in the Caucasian population (Kelsell *et al*., 1997). *GJB2*-related HL has been studied extensively in nearly all of the world's populations (Sobe *et al*., 1999; Abe *et al*., 2000; Hamelmann *et al*., 2001; Bonyadi *et al*., 2009; Mahdieh *et al*., 2010b). The coding region of the gene consisting of 680 bp can simply be sequenced. Despite different distributions of *GJB2* mutations in many subpopulations, a relatively high rate of the mutations has been reported in many populations (Sobe *et al*., 1999; Abe *et al*., 2000; Hamelmann *et al*., 2001). Some particular mutations in this gene have an ethnic-dependent prevalence; for example, the 35delG mutation is the most common in European countries, and the167delT, 235delC, and R143W mutations are the most frequent in the Ashkenazi Jewish, Japanese, and Ghanian populations, respectively (Sobe *et al*., 1999; Abe *et al*., 2000; Hamelmann *et al*., 2001). The 35delG mutation has a gradient of carrier frequency in two continents, that is, from central-northern to southern Europe and from western to eastern Asia (Mahdieh and Rabbani, 2009).

ARNSHL accounts for a large portion of deafness in the Iranian population. The rate of consanguinity is high among the Iranian population (Saadat *et al*., 2004). Here, we investi-

[1]Kawsar Human Genetic Research Center, Tehran, Iran.
[2]Medical Genetic Group, Ilam University of Medical Sciences, Ilam, Iran.
[3]Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
[4]Growth and Development Research Center, Tehran University of Medical Sciences, Tehran, Iran.
[5]Medical Genetic Group, Ghazvin University of Medical Sciences, Ghazvin, Iran.
[6]Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran.

gated *GJB2* mutations in consanguineous and nonconsanguineous marriages in 77 Iranian families with ARNSHL using an allele-specific polymerase chain reaction (PCR) and sequencing procedure as well as *GJB6* large deletions using real-time PCR. In addition, we compared our results with previous studies.

Materials and Methods

A total of 114 patients from 77 families including 54 consanguineous and 23 nonconsanguineous marriages were studied. Inclusion criteria were (1) confirmation of HL by audiologic testing, (2) absence of other clinical symptoms, (3) confirming the autosomal recessive inheritance by pedigree drawing, and (4) existence of two or more affected members within the family. A complete medical history was taken for each family. Pedigree analysis and audiometric and physical examinations were also performed. Pure-tone average was calculated at 250, 500, 1000, 2000, 4000, and 8000 Hz for all probands.

After obtaining informed consent, genomic DNA samples were extracted from WBC using a standard method. The most common mutation, 35delG, was screened by allele-specific PCR (Mahdieh *et al*., 2004). The 35delG homozygous subjects were excluded for further analyses. The remaining samples were sequenced directly using an ABI 3130 (Applied BioSystem, ABI) sequencer. Sequencing primers for Cx26 and Cx30 were Cx26F: 5′AAGTCTCCCTGTTCTGTCCTAG3′, Cx26R: 5′CCTCATCCCTCTCATGCTGTC3′, Cx30F: 5′GTCTGTAATATCACCGTGTCAC3′, and Cx30R: 5′CTCTTCAGGCTACAGAAGGAAC3′. Chromas v2.33 (Technelysium) software was used to analyze the sequencing results.

We checked the del(*GJB6*-D13S1830) large deletion by Gap-PCR and del(*GJB6*-D13S1854) and a 920-kb deletion by real-time PCR (Riazalhosseini *et al*., 2005; Mahdieh *et al*., 2010c).

We also performed a review of previous studies in the Iranian population. We analyzed the number of affected samples, the most common mutation, frequency of *GJB2* mutations, *GJB2*-related percentage of HL, and presence of the del(*GJB6*-D13S1830) large deletion.

Results

The possibility of environmental HL caused by infections, trauma, etc., was excluded by examining medical histories. All pedigree structures in 77 families showed an autosomal recessive pattern of the condition. Audiological tests showed that all subjects had a moderate-to-profound nonsyndromic HL.

Totally, 30 mutant alleles were detected from 154 investigated alleles (Table 1). Nine known mutations and a novel variant were identified in our patients: 35delG, delE120, R127H, M163V, W24X, V37I, G12D, V84A, E110K, and 313-326del14. The E110K variant was not detected in 44 healthy controls, but we could not perform a segregational analysis because of unavailability of DNA samples from the parents. All mutations and polymorphisms are presented in Table 1. Twenty-six percent (20/77) of the families had at least one mutation. Ten families were biallelic, including homozygous and compound heterozygous, for *GJB2* mutations. All genotypes are listed in Table 2. The 35delG mutation appeared in 50% (15/30) of all mutant alleles. This mutation was observed homozygously in five families and heterozygously in three families.

Table 1. *GJB2* Variations and Genotypes in This Study

Variations	*No. of alleles*
Known mutations	
35delG	15 (9.74%)
delE120	3 (1.94%)
R127H	2 (1.3%)
W24X	1 (0.65%)
V37I	1 (0.65%)
G12D	1 (0.65%)
M163V	1 (0.65%)
313-326del14	3 (1.94%)
Unknown mutations	
V84A	2 (1.3%)
Novel variants	
E110K	1 (0.65%)
wt	124 (80.52%)
Total	154

Consanguinity was determined in 70.13% of the cases (54 of 77 families). From consanguineous marriages, nine families were homozygous or compound heterozygous for *GJB2* mutations. One of 23 nonrelative marriages showed homozygous status in *GJB2* gene (Table 2). We also summarized the results of previous studies to compare them with our study (Table 3). Based on these reports, 1961 familial affected patients have been investigated for *GJB2* mutations. The mean frequency of *GJB2* mutation was 17.92% in the Iranian population (Table 3). Del(*GJB6*-D13S1830) was found in none of the studied patients.

Discussion

The prevalence of ARNSHL is relatively high in the Iranian population because of the high rate of consanguineous marriages in this population (Saadat *et al*., 2004; Hashemzadeh

Table 2. Genotypes Presented in Consanguineous and Nonconsanguineous Marriages

Relationship	*No. of families*	*Genotypes*	*No. of genotypes*
Second degree	0	0	0
Third degree	38	35delG/35delG	4
		delE120/delE120	1
		V84A/V84A	1
		35delG/E110K	1
		G12D/wt	1
		M163V/wt	1
		R127H/wt	1
		W24X/wt	1
Fourth degree	3	313–326del14/313–326del14	1
Fifth degree	13	35delG/35delG	1
		35delG/wt	2
		V37I/wt	1
Nonrelative	23	35delG/35delG	1
		R127H/wt	1
		delE120/wt	1
		313–326del14/wt	1
Consanguinity	70.13%		20

TABLE 3. COMPARISON OF PREVIOUS STUDIES IN IRANIAN POPULATION

No.	Studied subjects	GJB2-related (bialleles)	All studied alleles	No. of mutant alleles	35delG	235delC	W24X	167delT	GJB6	Ref.
1	298 (familial)	101	596	109	81	0	3	1	ND	Hashemzadeh *et al.* (2007)
	592 (sporadic)		1184	150	112	8	3	2		
2	664 (familial)	111	1328	250	167	0	11	3	0	Najmabadi *et al.* (2005)
	35 (sporadic)	3	70	7	5	0	0	0	0	
3	53 (familial)	9	106	17	10	2	0	0	0	Sadeghi *et al.* (2009)
4	209 (familial)	49	418	118	76	3	4	0	0	Bonyadi *et al.* (2009)
5	33 (ND)	3	66	22	7	0	0	0	ND	Hamid *et al.* (2009)
6	72 (familial)	10	154	30	15	0	1	0	0	Present study
Total	1961	286 (14.58%)	3922	703 (17.92%)	473 (12.1[a], 67.28[b])	13 (0.33[a], 1.86[b])	22 (0.56[a], 3.15[b])	6 (0.15[a], 0.86[b])	0	

[a]Frequency among all alleles.
[b]Frequency among mutant alleles.
ND, not determined.

et al., 2007). A wide spectrum of *GJB2* mutations causing ARNSHL have been reported in Iranian patients (Najmabadi *et al.* 2005; Hashemzadeh *et al.*, 2007; Mahdieh *et al.*, 2010b). Although *GJB2* mutations are the main cause of HL and account for up to 50% of all genetic cases of prelingual HL from western countries, frequency of *GJB2* mutations is relatively low in different subpopulations of Iran and is responsible for only up to 14%–20% in ARNSHL (Table 3). Results showed a prominent role for consanguinity in occurrence of *GJB2*-related HL in the given population.

In our study, *GJB2* mutations were found in 19.48% (30/154) of all alleles. The 35delG mutation was the most common mutation, accounting for up to at least 50% of all mutant alleles. Previous studies have shown that this mutation is the most prevalent one in European counties and Iranian groups (Marlin *et al.*, 2005; Najmabadi *et al.*, 2005; Mahdieh and Rabbani 2009, 2010b).

The genetic basis of HL in Iranian cohorts may be possibly more complex than other groups because of Iran's location along the Silk Road, where there are many distinct (ethnic) groups and a long history of immigration from neighboring nations. In addition, a relatively high rate of familial marriages in some regions of Iran and a diversity of cultures in other parts of the country may lead to greater complexity of this issue (Mahdieh *et al.*, 2005, 2010b; Hashemzadeh *et al.*, 2007). Also, high frequency of *GJB2* mutations or mutational homozygosity in other genes may be the result of consanguineous marriages in particular regions. From 20 families with *GJB2*-related HL in this study, 16 and 4 families had consanguineous and nonrelative marriages, respectively.

Nine of 54 consanguineous marriages (16.67%) were biallelic, whereas 4.35% of the nonrelative marriages (1 of 23 families) had the same pattern. Further, ~13% of the families (7 of 54) with consanguineous marriages had heterozygous genotypes as well as nonrelative families (3 of 23) (Table 2). In other words, the role of consanguinity in homozygous mutations of *GJB2*-related HL could be important, especially in the V48A and delE120 mutations observed in different families (Table 2). The frequency of consanguineous families who had one mutated allele was the same as the nonrelative ones.

Some various hypotheses regarding heterozygous individuals have been discussed. The digenic inheritance was primarily proposed by different laboratories (del Castillo *et al.*, 2002; Stevenson *et al.*, 2003). The supporting evidence for the digenic inheritance was the double heterozygotes of *GJB6* and *GJB2*. But further studies have shown that *GJB6* large deletions could abolish the expression of both *GJB2* and *GJB6* in *cis* with the deletions (Rodriguez-Paris and Schrijver, 2009; Wilch *et al.*, 2010). In addition, the possibility of digenic inheritance of *GJB2* with other genes encoding connexins, for example, Cx31, has been reported (Liu *et al.*, 2009). However, these individuals may be *GJB2* heterozygotes that mutation in other genes may be the reason of their pathogenicity.

G to A at position 328, E110K, leads to substitution of glutamic acid to lysine at position 110 located in the intracellular domain. Glutamic acid has carboxyl groups in its side chains, whereas lysine is a basic amino acid; in addition, this variant was not detected in healthy samples. Hence, E110K may act as a mutation. Functional studies, however, is required to clarify its effect.

Amino acid substitutional mutations including R127H, V37I, G12D, V84A, E110K, and M163V were relatively common (9 of

30 mutant alleles) in our patients. The R127H and V37I mutations have been reported in other populations (Marlin *et al*., 2005; Najmabadi *et al*., 2005). The M163V mutation has been described in the Iranian population. Small deletions (35delG, delE120, and 313–326del14) have been reported previously in our population. delE120 is common in the Turkish and Kurdish populations and shows a high degree of phenotypic variability in affected patients (Uyguner *et al*., 2003; Mahdieh *et al*., 2004, Hişmi *et al.* 2006, 2010a; Tekin *et al*., 2005; Bonyadi *et al*., 2009).

35delG mutation accounts for 10% of all childhood HL and 20% of all childhood hereditary HL in American Caucasians (Kelley *et al*., 1998). Mean frequency of this mutation is 12.1% of all investigated alleles in Iran. 167delT, 235delC, and W24X are the most common mutations in Ashkenazi Jewish, Japanese populations, and Ghana, respectively (Sobe *et al*., 1999; Abe *et al*., 2000; Hamelmann *et al*., 2001); also, these mutations are responsible for only 0.15%, 0.33%, and 0.56% of alleles in the Iranian population, respectively (Table 3).

Recently, other researchers have reported four large deletions in *GJB6* gene in families carrying one mutation of the *GJB2* gene (del Castillo *et al*., 2002, 2005; Feldmann *et al*., 2009), that is, mutations of two genes in the form of a double heterozygote give rise to HL. del(*GJB6*-D13S1830) has not been identified yet in our subpopulations (Mahdieh *et al*., 2004; Riazalhosseini *et al*., 2005; Bonyadi *et al*., 2009), although it may account for up to 10% of ARNSHL in some ethnic groups (del Castillo *et al*., 2003). Other deletions may be involved in *GJB2* heterozygote families, and further investigations are required to identify possible deletions of *GJB6* gene in our patients. Ten of 20 (50%) families had only one mutation in the *GJB2* gene. High frequency of heterozygosity (50%) in our study and previous studies of Iranian patients could not support the digenic form of nonsyndromic HL that has been previously described. Another possibility is that other unknown genes may also be involved in this condition. To date, Δ(*GJB6*-D13S1830) has not been found in any of the Iranian patients (Table 3); thus, single mutations in the *GJB2* gene may be associated with other unknown genes in the form of digenic inheritance. However, further studies are required to identify these genes.

In conclusion, *GJB2* mutations account for 14.58% of the ARNSHL cases. However, mutations in this gene are important in ARNSHL, especially in the case of consanguineous marriages, which should be considered in genetic counseling, screening, and healthcare programs.

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No competing financial interests exist.

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Address correspondence to:
Sirous Zeinali, Ph.D.
Associate Professor of Human Genetics
Biotechnology Research Center
Pasteur Institute of Iran
Pasteur Ave.
Tehran 13164
Iran

E-mail: zeinali@kawsar.ir

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