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GJB2 Mutations: Passage Through Iran

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Hereditary hearing loss (HHL) is a very common disorder. When inherited in an autosomal recessive manner, it typically presents as an isolated finding. Interestingly and unexpectedly, in spite of extreme heterogeneity, mutations in one gene, *GJB2*, are the most common cause of congenital severe-to-profound deafness in many different populations. In this study, we assessed the contributions made by *GJB2* mutations and chromosome 13 g.1777179_2085947del (the deletion more commonly known as del (*GJB6*-D13S1830) that includes a portion of *GJB6* and is hereafter called Δ(*GJB6*-D13S1830)) to the autosomal recessive non-syndromic deafness (ARNSD) genetic load in Iran. Probands from 664 different nuclear families were investigated. *GJB2*-related deafness was found in 111 families (16.7%). The carrier frequency of the 35delG mutation showed a geographic variation that is supported by studies in neighboring countries. Δ(*GJB6*-D13S1830) was not found. Our prevalence data for *GJB2*-related deafness reveal a geographic pattern that mirrors the south-to-north European gradient and supports a founder effect in southeastern Europe.

KEY WORDS: ***GJB2* mutations; 35delG; Iran; hereditary hearing loss; Δ(*GJB6*-D13S1830)**

INTRODUCTION

Hearing loss is the most common sensory defect in humans. One in 1,000 babies is born with severe-to-profound deafness, and some degree of hearing loss impacts normal communication in 4% of people younger than age 45 years and 10% of people aged 65 years or older [Morton, 1991; Gorlin et al., 1995; Petit, 1996]. Etiology is multifactorial and includes genetic and environmental factors. Estimates suggest that half of prelingual non-syndromic deafness is inherited, and in more than 80% of these cases, the mode of transmission is autosomal recessive [Morton, 1991; Marazita et al., 1993; Gorlin et al., 1995].

In many different populations, mutations in one gene, *GJB2*, are the most important cause of prelingual non-syndromic deafness [Chaib et al., 1994; Maw et al., 1995; Carrasquillo et al., 1997; Denoyelle et al., 1997; Kelsell et al., 1997; Van Camp et al., 1997; Zelante et al., 1997; Morell et al., 1998; Park et al., 2000]. In persons of northern European extraction, one allele variant of *GJB2* predominates—the 35delG mutation [Denoyelle et al., 1997; Kelley et al., 1998; Lench et al., 1998; Green et al., 1999; Gasparini et al., 2000; Lucotte and Mercier, 2001]. This mutation is followed closely by Δ(*GJB6*-D13S1830) in several populations such as the Spanish [Del Castillo et al., 2002, 2003]. In this study, we assessed the frequency of *GJB2* mutations and Δ(*GJB6*-D13S1830) in the Iranian population. *GJB2* and *GJB6* encode connexin 26 and connexin 30, respectively.

MATERIALS AND METHODS

Patients

Probands segregating presumed autosomal recessive non-syndromic deafness (ARNSD) were eligible for inclusion in this study if they met the following inclusion criteria: (1) audiologic testing to confirm hearing loss; (2) absence of other abnormal clinical features that would be consistent with syndromic hearing loss; (3) presence of at least one other family member with hearing loss; (4) an inheritance pattern consistent with autosomal recessive transmission. On consenting persons, audiometric testing and physical examinations were completed, followed by venipuncture to obtain 10 ccs of whole blood as a DNA source. Human Research Institutional Review Boards at the Welfare Science and Rehabilitation University and the Iran University of Medical Sciences, Tehran, Iran, and the University of Iowa, Iowa City, IA approved all procedures.

Genetic Testing

Genetic testing was completed using a tiered approach. The first step was an allele-specific polymerase chain reaction (ASPCR) assay to screen all study participants for the 35delG mutation using previously described primers [Scott et al., 1998a]. No further testing was done on persons homozygous for the 35delG allele variant of *GJB2*, and in this group, the diagnosis of *DFNB1* deafness was made.

In 35delG heterozygotes, DHPLC analysis of the coding sequence of *GJB2* (exon 2) was completed and complemented by direct sequencing if elution profiles were not consistent with the 35delG heterozygote state. Persons were diagnosed with *DFNB1* deafness if a second deafness-causing *GJB2* allele variant was identified in exon 2. In samples in which the elution profile was consistent with only the 35delG carrier state, the non-coding exon of *GJB2* (exon 1) was sequenced and a PCR-based assay was used to screen for Δ(*GJB6*-D13S1830),

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TABLE I. Proband Ascertainment by Geographic Region

Geographic region	Probands	*GJB2*-related deafness
Northwest	90	20 (22.2%)
West	172	27 (15.7%)
Southwest	46	7 (15.2%)
North	47	18 (38.3%)
Central	170	26 (15.3%)
South	10	0
Northeast	31	4 (13%)
Southeast	98	9 (9.2%)
Total	664	111 (16.7%)

as previously described [Del Castillo et al., 2002]. If either of these other mutations was identified, the diagnosis of *DFNB1* deafness was made.

DHPLC screening of exon 2 of *GJB2* was also completed in all persons in whom the 35delG mutation was not detected by the ASPCR. If abnormal elution profiles were observed, the sample was sequenced, and if two deafness-causing allele variants of *GJB2* were identified, the diagnosis of *DFNB1* was made. If only a single deafness-causing allele variant of *GJB2* was identified, we screened the non-coding exon of *GJB2* and for Δ(*GJB6*-D13S1830), as described above [Del Castillo et al., 2003]. The finding of either of these mutations together with a deafness-causing allele variant of exon 2 of *GJB2* rendered the diagnosis of *DFNB1*.

Lastly, we randomly selected and screened 115 study participants who carried two normal *GJB2* alleles for Δ(*GJB6*-D13S1830).

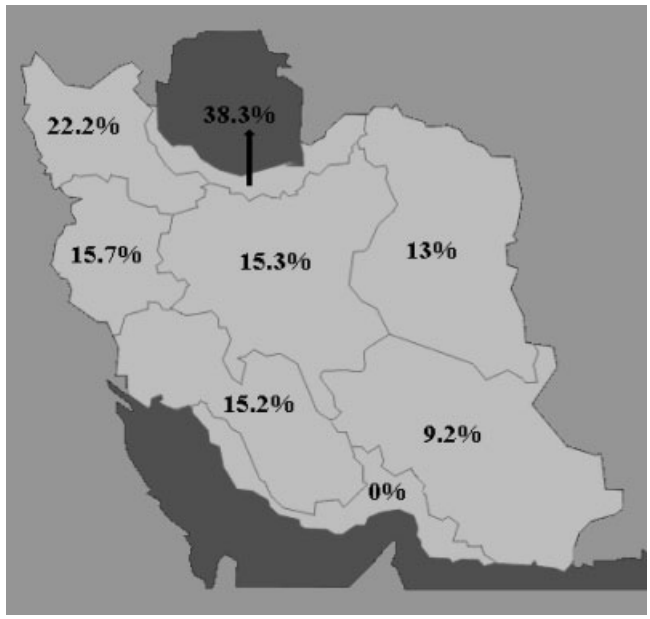

Fig. 1. Iran had been divided into eight regions based on geographic and ethnic considerations (Northwest-West Azerbaijan, East Azerbaijan, Ardebil, and Zanjan; North-Gilan, Mazandaran, and Golestan; Northeast-Khorasan; West-Kordestan, Hamedan, Kermanshah, Lorestan, and Ilam; Central-Tehran, Qazvin, Markazi, Qom, Semnan, Esfahan, and Yazd; Southwest-Khoozestan, Charmahaal, Kohkilooyeh, and Fars; South-Booshehr, and Hormazgan; Southeast-Kerman, Sistan and Baloochestan). Percentages show the frequency of *GJB2*-related deafness.

RESULTS

Patients

Probands from 664 families participated in this study. Study participants were ascertained throughout Iran, which we divided into eight regions based on geographic and racial identity (Table I, Fig. 1). We also completed mutation screening in 35 simplex cases of deafness. Since there were no other deaf persons in these 35 families, the possibility of acquired congenital deafness could not be excluded.

Genetic Testing

In total, 111 of 664 probands (16.7%) were found to have *GJB2* deafness-causing allele variants and were diagnosed with *DFNB1* deafness. In northwest and west Iran, where a

TABLE II. *GJB2* Deafness Genotypes Based on Geographic Regions

Genotypes	NW	W	SW	N	C	S	NE	SE	Iran
310del14/310del14	—	—	—	—	1	—	—	—	1
35delG/35delG	14	16	7	15	16	—	1	1	70
W24X/W24X	—	—	—	—	1	—	—	3	4
delE120/delE120	—	1	—	—	1	—	—	1	3
314del14/314del14	—	1	—	—	1	—	—	—	2
167delT/R184P	—	—	—	—	—	—	1	—	1
R184P/−3170G > A	—	—	—	—	—	—	1	—	1
35delG/−3170G > A	3	3	—	2	2	—	1	—	11
35delG/R143W	—	—	—	—	—	—	—	1	1
35delG/W24X	1	1	—	—	—	—	—	—	2
35delG/R32H	—	—	—	—	1	—	—	—	1
35delG/delE120	1	1	—	—	—	—	—	—	2
R32H/R32H	—	1	—	—	1	—	—	—	2
35delG/IVS1 + 1G > A	—	2	—	—	—	—	—	—	2
R184P/IVS1 + 1G > A	—	1	—	—	—	—	—	—	1
W24X/−3170G > A	—	—	—	1	—	—	—	—	1
312del14/312del14	—	—	—	—	2	—	—	—	2
167delT/167delT	—	—	—	—	—	—	—	1	1
R127H/R127H	—	—	—	—	—	—	—	2	2
Q80L/Q80L	1	—	—	—	—	—	—	—	1
Total	20	27	7	18	26	0	4	9	111

TABLE III. *GJB2* Allele Variants by Geographic Regions

Allele variant	Iran	Northwest	West	Southwest	North	Central	South	Northeast	Southeast
Mutation									
delE120	13 (1%)	3 (1.6%)	5 (1.4%)	—	—	3 (0.9%)	—	—	2 (1.02%)
167delT	3 (0.2%)	—	—	—	—	—	—	1 (1.6%)	2 (1.02%)
R184P	3 (0.2%)	—	1 (0.3%)	—	—	—	—	2 (3.2%)	—
310del14	2 (0.15%)	—	—	—	—	2 (0.6%)	—	—	—
V27I + E114G	3 (0.2%)	—	—	1 (1.1%)	—	2 (0.6%)	—	—	—
R32H	5 (0.37%)	—	2 (0.6%)	—	—	3 (0.9%)	—	—	—
314del14	4 (0.3%)	—	2 (0.6%)	—	—	2 (0.6%)	—	—	—
35delG	167 (12.6%)	35 (19.4%)	42 (12.2%)	15 (16.3%)	32 (34%)	37 (11%)	—	3 (4.8%)	3 (1.5%)
IVS1 + 1G > A	3 (0.2%)	—	3 (0.9%)	—	—	—	—	—	—
−3170G > A	13 (1%)	3 (1.6%)	3 (0.9%)	—	3 (3.2%)	2 (0.6%)	—	2 (3.2%)	—
R127H	11 (0.8%)	—	3 (0.9%)	—	1 (1.1%)	1 (0.3%)	—	—	6 (3.1%)
W24X	11 (0.8%)	1 (0.5%)	1 (0.3%)	—	1 (1.1%)	2 (0.6%)	—	—	6 (3.1%)
R143W	1 (0.07%)	—	—	—	—	—	—	—	1 (0.51%)
E129K	1 (0.07%)	1 (0.5%)	—	—	—	—	—	—	—
312del14	4 (0.3%)	—	—	—	—	4 (1.2%)	—	—	—
M93I	1 (0.07%)	—	—	—	—	—	—	—	1 (0.51%)
Novel									
507insAACG	1 (0.07%)	—	1 (0.3%)	—	—	—	—	—	—
329delA	1 (0.07%)	—	—	—	—	1 (0.3%)	—	—	—
363delC	1 (0.07%)	1 (0.5%)	—	—	—	—	—	—	—
Q80L	2 (0.15%)	2 (1%)	—	—	—	—	—	—	—
Polymorphism									
V153I	36 (2.7%)	2 (1%)	12 (3.5%)	6 (6.5%)	4 (4.2%)	2 (0.6%)	—	1 (1.6%)	9 (4.6%)
V27I	9 (0.7%)	3 (1.6%)	3 (0.9%)	—	—	2 (0.6%)	—	1 (1.6%)	—
V52V	—	—	—	1 (1.1%)	—	—	—	—	—
I69I	1 (0.07%)	—	—	—	—	1 (0.3%)	—	—	—
Total alleles tested	1,328	180	344	92	94	340	20	62	196

relation with other Turk populations exists, we found that 22.2% and 15.7% of probands, respectively, had *GJB2*-related deafness. In north Iran, the percentage of *GJB2*-related deafness was even higher at 38.3%, while in the south and southeast, it was lower (Table I, Fig. 1).

Genotypes related to *GJB2*-related deafness are listed in Table II. The most frequent genotype, homozygosity for the 35delG mutation, accounted for 63.1% of *GJB2*-related deafness. Next in frequency was compound heterozygosity for the 35delG mutation and a splice site mutation in exon 1 (−3170G > A), which was found throughout the northern and west regions of Iran. We found two novel mutations, 363delC and Q80L, in a 35delG/363delC compound heterozygote and a Q80L homozygote. Two probands with R127H/V153I were identified from southeast and west Iran.

GJB2 allele variants are shown in Table III. In addition to the 363delC and Q80L, we identified two other novel deafness-causing mutations, 507insAACG and 329delA, although in the carriers of these two mutations a second mutation in the *DFNB1* interval was not identified (507insAACG, west Iran; 363delC and Q80L, northwest Iran; 329delA, central Iran). The most frequent benign polymorphism was V153I, which was carried by nine persons. A single I69I synonymous mutation was identified in a person from central Iran and a V52V synonymous mutation was found in an Arab proband from southwestern Iran. Carrier frequencies for several *GJB2* allele variants among the 553 probands in whom a diagnosis of *DFNB1* deafness could not be made (664−111) are shown in Table IV.

Of the simplex cases, three (8.6%) had *DFNB1* deafness, and of the remaining 32 persons one (3.1%) was a 35delG carrier. This figure is not significantly different from the 1.3% 35delG carrier rate found in the 553 deaf probands with presumed ARNSD ($P = 0.389$), and is similar to that reported in an earlier study on this population (Table V) [Najmabadi et al., 2002].

None of patients screened for Δ(*GJB6*-D13S1830) was shown to carry this deletion.

DISCUSSION

Deafness at the *DFNB1* locus is the most common cause of ARNSD in many countries throughout the world [Zelante et al., 1997; Estivill et al., 1998; Kelley et al., 1998; Morell et al., 1998; Scott et al., 1998]. Typically caused by mutations in

TABLE IV. Carrier Frequencies for Selected *GJB2* Allele Variants Among the 553 Deaf Probands in Whom *DFNB1* Deafness Could not be Diagnosed

Genotypes	Frequency
35delG/wt	1.05% (7)
delE120/wt	0.9% (5)
R127H/wt	0.7% (4)
V27I + E114G/wt	0.2% (1)
507insAACG/wt	0.2% (1)
E129K/wt	0.2% (1)
M93I/wt	0.2% (1)

TABLE V. *GJB2* Genotypes in 35 Simplex Cases of Congenital Deafness

Genotype	Frequency	Etiology of deafness
wt/wt	27 (77%)	Unknown
V153I/wt	2 (5.7%)	Unknown
35delG/35delG	1 (2.85%)	*DFNB1* deafness
35delG/delE120	1 (2.85%)	*DFNB1* deafness
35delG/K112N[a]	1 (2.85%)	*DFNB1* deafness
35delG/wt	1 (2.85%)	Unknown
V27I + E114G/wt	1 (2.85%)	Unknown
V27I/wt	1 (2.85%)	Unknown

[a]K112N, novel allele variant.

GJB2, the deafness is characteristically congenital and stable, varying in severity from moderate to profound [Morton, 1991; Gorlin et al., 1995; Petit, 1996]. One *GJB2* mutation, the 35delG allele variant, is most common in populations of northern European ancestry. The carrier rate for this mutation among Caucasian people ranges from 1% to 3% [Kelley et al., 1998]. In other racial groups, other *GJB2* mutations are more common, as for example, 167delT among Ashkenazi Jews and R143W in Africans [Brobby et al., 1998; Morell et al., 1998; Lerer et al., 2000; Park et al., 2000; Sobe et al., 2000; Hamelmann et al., 2001; Shahin et al., 2002].

Δ(*GJB6*-D13S1830), a deletion of approximately 309 kb with one breakpoint inside the *GJB6* coding region, also causes deafness at the *DFNB1* locus [Del Castillo et al., 2002; Stevenson et al., 2003; Erbe et al., 2004]. In a recent multinational study, analyzing data from nine countries, Δ(*GJB6*-D13S1830) was shown to account for 5.9%–9.7% of all *DFNB1* alleles in Spain, France, the United Kingdom, Israel, and Brazil. Lower frequencies were found in Belgium and Australia (1.3%–1.4%) [Del Castillo et al., 2003]. Although the frequency of Δ(*GJB6*-D13S1830) in these populations was not high enough to result in a large number of homozygous patients, the authors recommend that genetic testing for deafness at the *DFNB1* locus include screening for Δ(*GJB6*-D13S1830).

In this study, the 35delG mutation was the most common deafness-causing allele variant of *GJB2* we identified, occurring in 71.6% of persons with *DFNB1* deafness. Since the Iranian population is composed of many different ethnic groups, to better analyze our findings, we looked for ethnic-specific biases and compared our results to studies in neighboring countries. In general, the Persian population is mainly in central Iran, the Azeri are in the northwest, and the Gilaki, Mazandarani, and Turkmen are in the north. Kurds and Lurs are in the west region and Arabs are in the south (Table VI).

TABLE VI. Ethnic Groups in Iran and Their Percentage Distributions

Ethnic group	Percent	Percentage distribution
Persian	51	44.6
Azeri	24	22.1
Gilaki and Mazandarani	8	5.4
Kurd	7	15.5
Arab	3	2.3
Lur	2	5
Balooch	2	5
Turkmen	2	0.1
Other	1	—
Total	100	100

We found the highest percentage of *GJB2*-related deafness in the north and northwest regions of Iran (north, 38.3%; northwest, 22.2%) (Table I, Fig. 1). This population is bounded on the north by the Caspian Sea and remains relatively isolated by mountains from other parts of Iran. Interestingly, in Turkey the incidence of *GJB2*-related deafness is similar, reported in one study to be 21.4% in 14 families with ARNSD. Identified allele variants in the Turkish population included the 35delG and 299–300delAT [Bayazit et al., 2003]. In another study from Turkey of 60 families, *GJB2* mutations were found in 31.7% of deaf probands, with the 35delG mutation accounting for 73.6% of all *GJB2* deafness-causing alleles [Uyguner et al., 2003]. The frequency of the 35delG allele in the Turkish deaf has been reported to range from 5% to 53% [Baris et al., 2001; Tekin et al., 2003]. Taken together, these studies and our data suggest that there is a gradual decrease in the frequency of the

TABLE VII. *GJB2* Allele Variants by Ethnicity

Allele variant	Turk	Kurd	Gilaki and Mazandarani	Lur	Persian	Arab	Baloochi	Turkmen	Iran
Mutation									
delE120	4 (1.3%)	3 (1.4%)	—	2 (3%)	4 (0.7%)	—	—	—	13 (1%)
167delT	—	—	—	—	1 (0.17%)	—	2 (3%)	—	3 (0.2%)
R184P	—	1 (0.5%)	—	—	2 (0.34%)	—	—	—	3 (0.2%)
310del14	—	—	—	—	2 (0.34%)	—	—	—	2 (0.15%)
V27I + E114G	1 (0.3%)	—	—	—	2 (0.34%)	—	—	—	3 (0.2%)
R32H	1 (0.3%)	4 (1.9%)	—	—	—	—	—	—	5 (0.4%)
314del14	—	—	—	2 (3%)	2 (0.34%)	—	—	—	4 (0.3%)
35delG	51 (17.3%)	21 (10.2%)	27 (37.5%)	5 (7.6%)	58 (9.8%)	5 (16.7%)	—	—	167 (12.6%)
IVS1 + 1G > A	—	3 (1.4%)	—	—	—	—	—	—	3 (0.2%)
−3170G > A	4 (1.3%)	2 (1%)	2 (2.8%)	—	5 (0.84%)	—	—	—	13 (1%)
R127H	1 (0.3%)	2 (1%)	1 (1.4%)	—	5 (0.84%)	—	2 (3%)	—	11 (0.8%)
W24X	1 (0.3%)	—	1 (1.4%)	1 (1.5%)	4 (0.7%)	—	4 (6%)	—	11 (0.8%)
R143W	—	—	—	—	—	—	—	—	1 (0.07%)
M93I	—	—	—	—	—	—	1 (1.5%)	—	1 (0.07%)
E129K	1 (0.3%)	—	—	—	—	—	—	—	1 (0.07%)
312del14	—	—	—	—	4 (0.7%)	—	—	—	4 (0.3%)
Novel									
329delA	—	—	—	—	1 (0.17%)	—	—	—	1 (0.07%)
363delC	1 (0.3%)	—	—	—	—	—	—	—	1 (0.07%)
507insAACG	—	1 (0.5%)	—	—	—	—	—	—	1 (0.07%)
Q80L	2 (0.7%)	—	—	—	—	—	—	—	2 (0.15%)
Polymorphism									
V27I	6 (2%)	—	—	—	3 (0.5%)	—	—	—	9 (0.7%)
I69I	—	—	—	—	1 (0.17%)	—	—	—	1 (0.07%)
V52V	—	—	—	—	—	1 (3.3%)	—	—	1 (0.07%)
V153I	4 (1.3%)	8 (4%)	3 (4.2%)	5 (7.6%)	12 (2%)	—	4 (6%)	—	36 (2.7%)
Total alleles tested	294	206	72	66	592	30	66	2	1,328

35delG mutation and in *GJB2*-related deafness in general as we move from the northwest to south and east through the Persian Gulf countries (Table VII).

This observed northwest-to-southeast *GJB2* deafness gradient is further supported by data specific to southeast Iran where the population shares close ethnic ties to neighboring Pakistan. The incidence of *GJB2*-related deafness in these regions is similar (Table I, Fig. 2). In southeast Iran, we found that *GJB2*-related deafness accounted for 9.2% of the ARNSD genetic load and in Pakistan, in a study of 27 families with presumed ARNSD, only one family had *GJB2*-related deafness (3.7%) [Brown et al., 1996]. In the southern part of Iran, where the population is mainly Arab, *GJB2*-related deafness was not found. Studies of *GJB2*-related deafness in Arab populations in Oman also have identified no deafness-causing mutations and complete absence of the 35delG and 167delT mutations [Simsek et al., 2001a,b].

The northwest-to-southeast *GJB2* deafness gradient through the Persian Gulf countries mirrors the south-to-north European gradient identified through a meta-analysis of European countries [Gasparini et al., 2000; Lucotte and Mercier, 2001; Van Laer et al., 2001; Rothrock et al., 2003], and reflects the historical importance of southern Europe and the eastern Mediterranean as regions of diversity through population movement, wars, and migrations. Data from Greece, for example, are consistent with this hypothesis, as *GJB2*-related deafness accounts for one-third of ARNSD [Antoniadi et al., 2000; Iliades et al., 2002].

Our data highlight the importance that clinicians and geneticists must pay to the general pattern of *GJB2*-related deafness across the world. It is important to be aware of the diverse influences on ARNSD in discrete populations. These differences will affect the relative mutation load specific genes make to ARNSD in different countries.

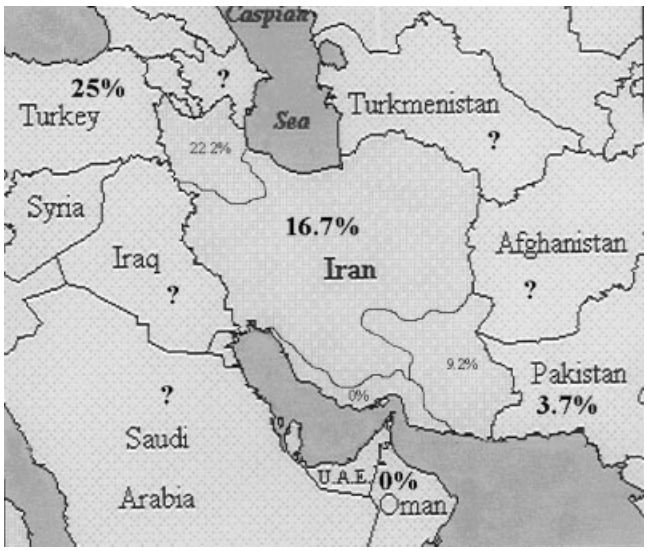

Fig. 2. *GJB2*-related deafness in neighboring countries showing subdivisions in Iran for comparison. Turkey has an average of 25% *GJB2*-related deafness and the juxtaposed region in Iran has 22.2%. Next to Pakistan, with 3.7% *GJB2*-related deafness, we find 9.2% *GJB2*-related deafness, and in south Iran as in Oman, there is no *GJB2*-related deafness. Numbers printed larger and in bold format show whole country percentages, and numbers written in light and smaller fonts show regional data within Iran.

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