International Journal of Pediatric Otorhinolaryngology 76 (2012) 1610–1615

Contents lists available at SciVerse ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

Screening of *OTOF* mutations in Iran: A novel mutation and review

Nejat Mahdieh [a,b,c,g,*], Atefeh Shirkavand [b], Bahareh Rabbani [c], Mustafa Tekin [d], Bahman Akbari [e], Mohammad Taghi Akbari [g], Sirous Zeinali [a,f,**]

[a] Medical Genetic Group, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran
[b] Kawsar's Human Genetics Research Center, Tehran, Iran
[c] Growth and Development Research Center, Tehran University of Medical Sciences, Tehran, Iran
[d] John P Hussman Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, FL, USA
[e] Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[f] Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran
[g] Department of Medical Genetics, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran

ARTICLE INFO

Article history:
Received 15 June 2012
Received in revised form 21 July 2012
Accepted 27 July 2012
Available online 18 August 2012

Keywords:
OTOF
ARNSHL
Hearing loss
Auditory neuropathy
Iranian population
Synaptopathy and deafness

ABSTRACT

Objective: Mutations in *OTOF* have been reported to cause nonsyndromic hearing loss in different populations. The purpose of this study is screening of *OTOF* mutations in Iranian population.
Methods: Thirty-eight consanguineous families affected with autosomal recessive nonsyndromic hearing loss (ARNSHL) and negative for *GJB2* or *GJB6* mutations were screened by autozygosity mapping and Sanger sequencing to find *OTOF* mutations.
Results: A novel homozygous frameshift mutation (c.1981dupG) was found to cause hearing loss in one family and no other *OTOF* variants were detected in the remaining families. The affected individuals were homozygous forp. D661GfsX2 causing defect in long isoform of otoferlin.
Conclusions: We conclude that *OTOF* mutations are not the major cause of ARNSHL in the Iranian population but still may play an important role in HL; therefore evaluation the OTOF gene is of concern.
© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Hearing loss (HL) is the most prevalent sensory deficit in human beings affecting about 1 in 1000 newborns around the world; over 70–80% of them are due to genetic factors. Current knowledge shows that 85–90% of cases with HL are not associated with other clinical symptoms, and are referred to as nonsyndromic hearing loss (NSHL). Approximately the 80% of families in this group follow autosomal recessive inheritance. The autosomal recessive loci are called DFNB followed by a number corresponding to the order that the locus was first described; DFNB1 to DFNB96 have been reported so far (see Hereditary Hearing Loss Homepage at http://webh01.ua.ac.be/hhh/for more details). It has been estimated that 1% of the human genes, i.e. 200–250 genes, are responsible for hereditary HL [1]. More than 55 genes have been identified to cause HL.

Mutations in the *OTOF* gene (named due to its homology to Ferlin (Fer-1)) encoding otoferlin at DFNB9 result in autosomal recessive nonsyndromic hearing loss (ARNSHL) which is usually associated with auditory neuropathy (AN) [2,3]. *OTOF* mutations have been reported in many countries [4–9] and different mutation spectra have been described in these populations. Frequency of *OTOF* mutations appears to be different among ARNSH and DFNB9 associated with AN which accounts for 2–5% of the pathogenesis in these individuals [6,9,10]. The *OTOF* gene, located on chromosome 2p23.1, consists of 48 exons; it encodes several isoforms of otoferlin which has 1997 amino acids in the long isoform consisting of a transmembrane region and six cytoplasmic C2 domains [11]. *Otof* is expressed in the brain, vestibular system, inner and outer hair cells [11,12]. Otoferlin is a member of the ferlin protein family; it may play an important role in vesicle recycling and efficient and linear encoding of low-intensity stimuli at the synapse between inner hair cells (IHCs) and auditory nerve fibers [13,14]. Recently, some point mutations were described to cause plasma membrane's folding [15]. Up to know, more than 80various mutations in *OTOF* have been identified in familial or sporadic NSHL around the world [7,9,10,16]. Here, we report a novel frameshift mutation in an Iranian family from a study of 38 consanguineous ARNSHL patients. A review of the reported

* Corresponding author at: Medical Genetics Lab. of Dr. Zeinali, Kawsar's Human Genetic Research Center, Tehran, Iran. Tel.: +98 2166480780; fax: +98 9121372040.
** Corresponding author at: Biotechnology Research Center, Pasteur Institute of Iran, Pasteur Ave.,13164 Tehran, Iran. Tel.: +98 2166480780; fax: +98 9121372040.
E-mail addresses: nmahdieh@yahoo.com, mahdieh@modares.ac.ir, nmahdieh@gmail.com (N. Mahdieh), zeinali@kawsar.ac.ir (S. Zeinali).

0165-5876/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ijporl.2012.07.030

Table 1
Characteristics of the STR markers and primers used in this study, based on *OTOF* gene location on chr2:26,680,072–26,781,566. These markers are located on downstream (OTOF-SD1 and OTOF-SD2; S and D stand for STR and Downstream, respectively), upstream (OTOF-SU1; U stands for Upstream) and within the gene (OTOF-SI1; I denotes Intragenic).

STR	Primer sequence (5′ – 3′)	Repeated motif	PCR conditions	Location (Hg 19)
OTOF-SI1 (D2S2350)	F: GGTTGAATGAGGAGAAGTATGT R: TAGCATACCATCTGAAATACGA	(CA)22	Tm: 64; 1 min; 30 cycles	chr2:26728160–26728446
OTOF-SD1	F: ATTGGGAGAGCTACATTGCA R: GTTTCTAAGCAGACAGAGATGG	(TC)21 & (CA)8	Tm: 62; 1 min; 30 cycles	chr2:26877557–26877855; 96 kb apart from the gene
OTOF-SU1	F: GTCAAGCTGATTAACGTGTCC R: AGTTTATTGCAGTTACATAGGATG	(TG)19	Tm: 62; 1 min; 30 cycles	chr2:26519551–26519665; 160 kb apart from the gene
OTOF-SD2	F: TCTAGCTTCTACCTCCACATTG R: CAAAGTCCTGTGAGGCAAGT	(TC)18 & (TG)12	Tm: 62; 1 min; 30 cycles	chr2:26948875–26949118; 167 kb apart from the gene

mutations around the world is also presented in this study for comparison.

2. Materials and methods

A total 38 *GJB2*-negative unrelated families affected with ARNSHL were screened in this study. Inclusion criteria were mentioned elsewhere [17]. In this study, the possibility of environmental factors causing HL was ruled out by medical examinations. Pedigree structures in all families were consistent with an autosomal recessive mode of inheritance for the condition. Taking a complete history for each family was followed by pedigree analysis and audiometric and physical examinations. Pure-tone average (PTA) was calculated at 250, 500, 1000, 2000, 4000 and 8000 Hz for all probands.

After obtaining the informed consent, DNA samples were extracted from whole blood cells using a standard method. Del (GJB6-D13S1830), del (GJB6-D13S1854) and a 920 kb deletion were ruled out by the procedure mentioned elsewhere [17–19]. Then, all samples were subjected to haplotype analysis; four short tandem repeat (STR) markers were selected using SERV (http://www.igs.cnrs-mrs.fr/SERV/) software [20]. These markers are located on downstream (OTOF-SD1 and OTOF-SD2; S and D stand for STR and Downstream, respectively), upstream (OTOF-SU1; U stands for Upstream) and within the gene (OTOF-SI1; I denotes Intragenic). PCR was performed for the markers using fluorescence labeled primers; PCR reactions were carried out using a general protocol with some minor modifications; a 25 μl total volume containing 0.2 mM of dNTP (KBC, Iran), 1 unit of Taq-DNA polymerase (KBC, Iran) and 1× PCR buffer (20 mM Tris–HCl pH 8.5, 50 mM KCl), and 0.2 μM of each primer (Metabion, Germany) were added. Sequences of primers and PCR conditions in our study are shown in Table 1. After examining heterozygosity of the STR markers, haplotype analysis was applied for all families and their members. In families where the *OTOF* locus was segregating, all exons of *OTOF* were sequenced directly using an ABI 3130 (Applied BioSystems, ABI, US) sequencer. Chromas v2.33 (Technelysium, TenwantinQld, Australia) software was used to analyze the sequencing results. The sequencing results were aligned to the RefSeq NGNG_009937.1 to find the variants.

A search on the previous studies of *OTOF* mutations was performed in the database for other populations.

3. Results

In only one of 38 families the DFNB9 locus co-segregated with the phenotype (Fig. 1). The homozygous haplotype observed in this family was not present in other families. Direct sequencing of OTOF gene of two affected individuals in this family showed a single base pair duplication at position 1981 (c.1981dupG; p.D661GfsX2). The mutation co-segregated in the entire family. Any other potentially pathogenic variant was not found in the 48 exons or donor or acceptor splice sites of the gene. 1981DupG leads to a frameshift, changing aspartate codon, GAT to glycine GGA, followed by a stop codon at the second position after the changed codon. This frameshift mutation was searched for novelty in dbSNP and HGMD which is not reported. Structure and domains of otoferlin protein is illustrated in Fig. 2. The related mutations of OTOF gene and

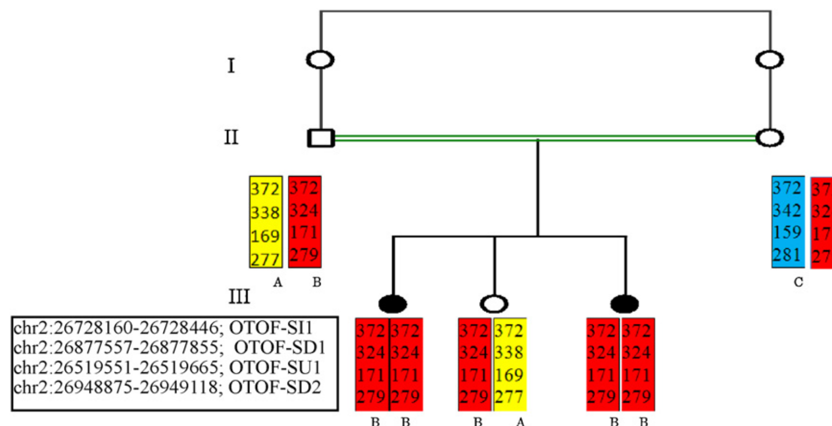

Fig. 1. Haplotype analysis of STR markers of OTOF gene. As shown, 4 markers were selected for homozygosity mapping: one in the OTOF gene (OTOF-SI1), one upstream of OTOF gene (OTOF-SU1) and two of the markers located in the downstream of OTOF gene (OTOF-SD1 and OTOF-SD2). Haplotype of the parents (II generation) is denoted as A/B (highlighted in yellow and red) for II1 (father) and B/C (red and blue) for II2 (mother). The affected III1 carries haplotype B/B (red/red), III2 carries A/B and the other sibling III3 carries B/B which is also affected. Healthy daughter showed a heterozygous haplotype. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of the article.)

Fig. 2. Schematic structure, domains and distribution of mutations of otoferlin protein reported so far. Novel frameshift mutation is underlined. The most common mutations in specific populations (p.Y1497X, p.Q829X, c.2905_2923delins11 and p.E1700Q mutations in the Lebanese, Spanish, Argentina and Taiwanese populations, respectively) are indicated in bold. p.Q829X, p.Y1497X, c.2905_2923delins11 and p.E1700Q are located on I3, C2E, C2D, and C2F domains, respectively. TM denotes transmembrane domains (aa 1964–1984), EC denotes extracellular domains, I1-I5 denotes inter C2 domains [C2A (aa 1–97), C2B (aa 254–352), C2C (aa 417–528), C2D (aa 960–1067), C2E (aa 1493–1592), and C2F (aa 1733–1863)], CC (arrow sign) denotes coiled coil domain (aa 792–821), NT denotes amino (NH2) terminus and CT denotes carboxyl (COOH) terminus. Isoform 1, known as Long isoform is presented here. Isoform 2, known as Short-1 has not residues 1–747 (i.e. C2A, B, C) and 1245–1264, and it differs in aa sequences 1943–1997. Isoform 3 known as Short-2, has not residues 1–690 (i.e. C2A, B, C) and it differs in aa sequences 691–738. Isoform 4 known as: Short-3, has not residues 1–747 and 1245–1264. Isoform 5 differs in aa sequence 1943–1997.
Domain Data extracted from Yasunaga et al. [11] and http://www.uniprot.org/uniprot/Q9HC10.

otoferlin are depicted in this figure. The most common mutations are indicated in specific populations such as Lebanese, Spanish, Argentina and Taiwanese populations named as p.Y1497X, p.Q829X, c.2905_2923delins11 and p.E1700Q mutations, respectively. The mutation found in this study is located in a region between domains C2C and C2D (Fig. 2).

Up to May 2012, 92 OTOF gene mutations have been reported; mutations are summarized based on functional effect of mutation, amino acid changes, the related phenotypes and the population (Table 2). 25% (23/92) are characterized as frameshift mutations, 19 mutations were specified as nonsense mutations, 35 missense mutation, one small deletion, one large deletion, and 13 intronic mutations.

4. Discussion

Mutations in at least 10 genes have been described to cause congenital HL in Iran [19]. To our knowledge, this is the first report about an *OTOF* mutation in this population.

Yasunaga et al., in 1999, identified the first mutation in *OTOF* in a Lebanese family with ARNSHL. Many studies have been performed to demonstrate the role of *OTOF* mutations in ARNSHL among various ethnic cohorts. *OTOF* mutations have been reported to be 5 and 2.3% of ARNSHL among Turkish and Pakistani affected families, respectively [6,8]; DFNB9 is the fifth cause of hereditary HL in both populations. Prevalence between these two populations is expected to be observed in Iran because of geographic location and ethnic and historical connections.

Since the first 19 exons of *OTOF* code for the long isoform of Otoferlin, the p.D661GfsX2 mutations, located at exon 17, is expected to affect the long isoform of the protein which is required for normal hearing. Human ferlin family includes dysferlin, myoferlin, otoferlin and ferl1. These large proteins contain at least four domains named C2 (second-constant sequence) and a C-terminal transmembrane domain (Fig. 2). Otoferlin binds to SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins syntaxin-1 and SNAP25 as well as the voltage-gated Cav1.3 calcium channel. It is essential to endosomal trans-Golgi trafficking [21] and calcium-activated exocytosis of neurotransmitter-containing vesicles at inner hair cells (IHCs) of cochlea [22]. Both sides of otoferlin (N and C termini) can regulate SNAREs complexes [23] and thus both otoferlin fragments, (each containing three C2 domains) can activate Ca+triggered vesicle fusion at synaptic membranes [24]. Any alteration, therefore, in amino acid sequences may affect functions of the protein leading to clinical phenotypes such as HL. Especially frameshift mutations causing a truncated protein have serious impact of the function of protein. As shown in Table 2, the reported mutations distributed in all domains of the otoferlin usually cause profound HL.

There is no hotspot for a pathogenic mutation in the OTOF gene (Table 2), but all reported mutations are gathered in two regions with the exception of c.4023 + 2T > C in intron 32, exons 13–30 (53 variants) and exons 35–48 (30 Variants). Four founder mutations have been inferred from the literature; p.Y1497X, p.Q829X, p.A969LfsX30, and p.E1700Q mutations are common among the Lebanese, Spanish, Argentina and Taiwanese populations, respectively [3,7,10,25]. Although p.Q829X mutation has been described

Table 2
OTOF mutations reported to date and associated audiologic phenotypes.

Mutation		Position		Severity of HL	Population	AN	Ref
Nucleotide change	Amino acid change	Genic level	Protein level				
Frameshift							
c.1103_1104delinsC	p.G368AfsX2	Ex13	I	Severe-profound	Pakistan	ND	[6]
c.1180dupG	p.E394GfsX6	Ex13	I	Profound	Spain	–	[10]
c.1236delC	p.E413NfsX9	Ex14	I	Profound	Spain	+	[10]
c.1552-1567del16	p.R518TfsX15	Ex15	C2C	Severe-profound	Brazil	+	[30]
c.1601delC	p.P534QfsX4	Ex16	I	Profound	Austria	–	[10]
c.1609delG	p.V537VfsX1	Ex 16	I	Profound	Italy	+	[31]
c.1651delG	p.E551SfsX5	Ex16	I	Severe-profound	USA	+	[32]
c.1740delC	p.S581PfsX40	Ex 16	I	Severe	Chinese	+	[33]
c.1886dupA	p.P630AfsX5	Ex 17	I	Profound	USA	+	[4]
c.1946-1965del20	p.R649PfsX5	Ex 17	I	Variable	Japan	+	[16]
c.1958delC	p.P653LX13	Ex 17	I	ND	Turkish	ND	[8]
c.1966delC	p.R656GfsX10	Ex 17	I	Profound	Italy	+	[31]
c.1981dupG	**p.D661GfsX2**	**Ex 17**	**I**	**Profound**	**Iran**	**ND**	**Present study**
c.2348delG	p.G783AfsX17	Ex 21	I	Profound	USA, Brazil	+	[4,30]
c.2684_2685delGG	p.G895EfsX106	Ex 24	I	Profound	Spain	–	[10]
c.2732 2735dupAGCT	p.Y913AfsX90	Ex 24	I	Profound	Italy	–	[10]
c.2905_2923delins CTCCGAGCGCA	p.A969LfsX30	Ex 25	C2D	Profound	Argentina, Brazil	+,–	[10,30]
c.2975_2978delAG	p.Q994VfsX6	Ex 25	C2D	Severe	Chinese	+	[33]
c.3704del16	p.D1235AfsX30	Ex30	I	Mild-profound	Italy	+	[34]
c.4467dupC	p.I1490HfsX19	Ex 37	I	ND	Turkey	ND	[8]
c.5011dupT	p.W1671LfsX73	Ex 41	I	Profound	Spain	–	[10]
c.5800dupC	p.L1934PfsX251[a]	Ex 46	I	Profound	Spain, Brazil	+	[10,30]
c.5800dupC	p.L1934PfsX185[b]	Ex 46	I	Profound	Spain	+	[10]
Nonsense							
c.709C>T	p.R237X	Ex 8	I	Severe-profound	UAE	–	[35]
c.1273C>T	p.R425X	Ex14	C2C	Severe-profound	Pakistan	ND	[6]
c.1422T>A	p.Y474X	Ex14	C2C	ND	Japan	+	[16]
c.1607G>>A	p.W536X	Ex16	I	Severe-profound	Pakistan	ND	[6]
c.2122C>>T	p.R708X	Ex 18	I	Profound	Spain, Colombia	–	[5]
c.2239G>>T	p.E747X	Ex 20	I	Profound	Libya	–	[10]
c.2316C>A	p.C772X	Ex 21	I	Profound	Italy	+	[34]
c.2485C>>T	p.Q829X	Ex 22	I	Profound	UK, USA, Spain, Cuba, Colombia, Germany, Argentina, French, Mexico	+,–	[10,25]
c.2649C>A	p.C883X	Ex n 23	I	Profound	Spain	+	[10]
c.2887C>T	p.R963X	Ex 25	C2D	Moderate-profound	UK	–	[36]
c.3400C>T	p.R1134X	Ex 28	I	Profound	Italy, Brazil	–,+	[10,30]
c.4157C>T	p.R577X		I	ND	Palestinian	–	[37]
c.4275G>A	p.W1425X	Ex 36	I	Profound	Spain	+	[5]
c.4351G>T	p.G1451X	Ex 36	I	Profound	Germany	–	[10]
c.4483C>T	p.R1495X	Ex 37	C2E	Profound	Germany	–	[10]
c.4491T>A	p.Y1497X	Ex 37	C2E	Profound	Lebanonese		[3]
c.4809C>A	p.Y1603X	Ex 40	I	Severe-profound	Pakistan	ND	[6]
c.5431A>T	p.K1811X	Ex 44	C2F	Severe-profound	Brazil	+	[30]
c.5466C>G	p.Y1822X	Ex 46	I	Profound	Japan	+	[16]
Missense							
c.765G>C	p.Q255H	Ex9	C2B	Profound	Italy	–	[10]
c.1102G>A	p.G368R	Ex12	C2B	ND	Chinese	+	[9]
c.1194T>A	p.D398E	Ex13	I	Severe	Chinese	+	[33]
c.1469C>A	p.P490Q	Ex15	C2C	Profound	Turkish	–	[38]
c.1544T>C	p.I515T	Ex15	C2C	Temperature sensitive	USA, Turkey	+,–	[4,38]
c.1621G>A	p.G541S	Ex15	C2C	Mild HL	Japan	+	[16]
c.1718T>G	p.L573R	Ex 16	I	Severe-profound	Pakistan	ND	[6]
c.1841G>A	p.G614E	Ex 17	I	Severe-profound	Brazil	+	[30]
c.2180A>G	N727S	Ex18	I	ND	Chinese	+	[9]
c.2381G>A	p.R794H	Ex 21	CC	Severe-profound	USA	–	[4]
c.2465G>T	p.R822W	Ex 22	I	Moderate-profound	UK	–	[36]
c.2891C>A	p.A964E	Ex 25	C2D	Profound	Italy	–	[10]
c.3032T>C	p.L1011P	Ex 26	C2D	Profound	Turkey	+	[39]
c.3239G>C	p.R1080P	Ex 27	I	Severe-profound	Brazil	+	[30]
c.3269C>A	p.A1090E	Ex 27	I	Severe-profound	Pakistan	ND	[6]
c.3413T>C	p.L1138P	Ex 29	I	Profound	Germany, Austria	–	[10]
c.4559G>A	p.R1520Q	Ex 38	C2E	Profound	France	+	[40]
c.4718T>C	p.I1573T	Ex 39	C2E	ND	Turkey	ND	[8]
c.4819C>T	p.R1607W	Ex 40	I	Severe	Chinese	+	[33]
c.4960G>A	p.G1654S	Ex 40	I	Severe-profound	Brazil	+	[30]
c.5026C>T	p.R1676C	Ex 40	I	ND	Chinese	+	[9]
c.5091G>A	p.P1697P	Ex41	I	ND	Japan	+	[16]
c.5098G>C	p.E1700Q	Ex 41	I	Moderate-profound	Taiwanese	+	[7]
c.5197G>A	p.E1733K	Ex 43	C2F	Severe-profound	Pakistan	ND	[6]

Table 2 (*Continued*)

Mutation		Position		Severity of HL	Population	AN	Ref
Nucleotide change	Amino acid change	Genic level	Protein level				
c.5384T>G	p.F1795C	Ex 44	C2F	Profound	Italy	–	[10]
c.5410_5412delGAG	p.E1804del	Ex 44	C2F	Variable	Scotland	+	[41]
c.5473C>G	p.P1825A	Ex 44	C2F	ND	Spain	–	[25]
c.5524G>A	p.D1842N	Ex45	C2F	Moderate	Japan	+	[16]
c.5567G>A	p.R1856Q	Ex 45	C2F	Severe-profound	Pakistan	ND	[6]
c.5785A>C	p.N1929H	Ex n 46	I	Severe-profound	Brazil	+	[30]
c.5815C>T	p.R1939W	Ex 48	I	Severe-profound	Pakistan	ND	[6]
c.5816G>A	p.R1939Q[b]	Ex 48	I	Severe-profound	USA	+	[32]
c.5860_5862delATG	p.I1954del[b]	Ex 48	I	Profound	Spain	+	[5]
c.5960C>G	p.P1987R[b]	Ex 48	EC	Moderate-severe/ severe-profound	USA	+	[32]
c.5992T>C	p.X1988RextX30	Ex48	EC	Moderate	Japan	+	[16]
Small deletion							
c.2295_2297del	p.E766del	Ex 20	I	Severe-profound	Pakistan	ND	[6]
Large deletion							
Del52.8-61.6 kb		Int 18	SS	Profound	Italy	+	[34]
Intronic							
c.711-2A>G	AC	Int8	SS	Severe-profound	India	–	[11]
c.897-2T>A	AC	Int 9	SS	Profound	Italy	+	[34]
IVS9+5G>A	–	Int 9	SS	Profound	Japan	+	[16]
c.2093+1G>T	DS	Int17	SS	Moderate-severe	USA	+	[4]
c.2093-2C>T	AC	Int 17	SS	Mild to profound	Italy	+	[34]
c.2866+1G>A	Splice site	Int24	SS	Severe-profound	Druze/Israel	–	[42]
c.3409-2A>G	Splice site	Int 28	SS	Profound	UK	+	[4]
c.3570+2T>C	Splice site	Int 28	SS	ND	Chinese	+	[9]
c.4023+2T>C	Splice site	Int 32	SS	ND	Chinese	+	[9]
c.4227+1G>T	Splice site	Int 35	SS	Profound	Spain, Argentina	+,–	[10]
c.4362+2T>G	Splice site	Int 36	SS	Profound	Spain	+	[5]
c.4799+1G>C	Splice site	Int 39	SS	Profound	USA	+	[32]
c.5533+1G>A	Splice site	Int 44	SS	Profound	France	+	[40]

SS: splicing site; EC: extracellular; CC: coiled coil domain; AS: acceptor site; DS: donor site; ND: not determined; Ex: exon; Int: intron.
[a] Isoform with exon 47.
[b] Isoform without exon 47.

to be frequent in Spanish patients, it is observed among the most of European patients with the probably same frequency; thus, glutamine829 may be a mutational hotspot. Stop codon (nonsense and frameshift variants inserting a stop codon) mutations leading to absence of otoferlin in cochlea may cause inadequate performance of the IHCs. Most of the reported mutations (42 of 92 variants) are stop codon ones that the majority cause severe-profound ARNSHL (Table 2). c.1981dupG is a frameshift mutation terminating immediately to a stop codon, p.D661GfsX2.

There is a great discrepancy in the frequency of the mutations among different ethnic populations; for example, 9.2% of deaf families showed *OTOF* mutations in a study by Varga et al., 2006 [4] while this gene is involved in 2.3% of Pakistani deaf families [6]. It means that an ethnic-specific pattern is highlighted for some mutations of this gene. Although differences in ethnic background may be a plausible explanation, some inclusion criteria such as selection of deaf families with auditory neuropathy as Varga et al. and Chiu et al. did, could not be ignored. Iran is positioned at the critical and specific region and various ethnic groups with different cultures (for example, Persian, Azeri, Gilaki and Mazandarani, Kurd, Lur, Turkmen, Arab, Balooch, etc.) are living in this country; hence a high heterogeneity throughout Iran is predicted [17–19,26–29]. On the other hand, specific traditions such as intragroup marriages may lead to a high homogeneity in some loci and mutations within groups and not across the groups. In conclusion, based on these preliminary data, *OTOF* mutations may not be a major cause of ARNSHL in the Iranian population. However, further studies are required in different subgroups living in Iran in order to better assess the distribution of deafness genes in different groups.

Conflict of interests

None.

Acknowledgements

We are thankful to families participating in this study. We also appreciate staffs of Kawsar Institute of Human Genetics.

References

[1] J. Finsterer, J. Fellinger, Nuclear and mitochondrial genes mutated in nonsyndromic impaired hearing, Int. J. Pediatr. Otorhinolaryngol. 69 (5) (2005) 621–647.
[2] H. Chaib, C. Place, N. Salem, S. Chardenoux, C. Vincent, J. Weissenbach, et al., A gene responsible for a sensorineural nonsyndromic recessive deafness maps to chromosome 2p22-23, Hum. Mol. Genet. 5 (1) (1996) 155–158.
[3] S. Yasunaga, M. Grati, M. Cohen-Salmon, A. El-Amraoui, M. Mustapha, N. Salem, et al., A mutation in OTOF, encoding otoferlin, a FER-1-like protein, causes DFNB9, a nonsyndromic form of deafness, Nat. Genet. 21 (4) (1999) 363–369.
[4] R. Varga, M.R. Avenarius, P.M. Kelley, B.J. Keats, C.I. Berlin, L.J. Hood, et al., OTOF mutations revealed by genetic analysis of hearing loss families including a potential temperature sensitive auditory neuropathy allele, J. Med. Genet. 43 (7) (2006) 576–581.
[5] M. Rodriguez-Ballesteros, F.J. del Castillo, Y. Martin, M.A. Moreno-Pelayo, C. Morera, F. Prieto, et al., Auditory neuropathy in patients carrying mutations in the otoferlin gene (OTOF), Hum. Mutat. 22 (6) (2003) 451–456.
[6] B.Y. Choi, Z.M. Ahmed, S. Riazuddin, M.A. Bhinder, M. Shahzad, T. Husnain, et al., Identities and frequencies of mutations of the otoferlin gene (OTOF) causing DFNB9 deafness in Pakistan, Clin. Genet. 75 (3) (2009) 237–243.
[7] Y.H. Chiu, C.C. Wu, Y.C. Lu, P.J. Chen, W.Y. Lee, A.Y. Liu, et al., Mutations in the OTOF gene in Taiwanese patients with auditory neuropathy, Audiol. Neurootol. 15 (6) (2010) 364–374.
[8] D. Duman, A. Sirmaci, F.B. Cengiz, H. Ozdag, M. Tekin, Screening of 38 genes identifies mutations in 62% of families with nonsyndromic deafness in Turkey, Genet. Test. Mol. Biomarkers 15 (1–2) (2011) 29–33.
[9] J. Wang, Y.Y. Fan, S.J. Wang, P.F. Liang, J.L. Wang, J.H. Qiu, Variants of OTOF and PJVK genes in Chinese patients with auditory neuropathy spectrum disorder, PLoS One 6 (9) (2011) e24000.
[10] M. Rodriguez-Ballesteros, R. Reynoso, M. Olarte, M. Villamar, C. Morera, R. Santarelli, et al., A multicenter study on the prevalence and spectrum of mutations in the otoferlin gene (OTOF) in subjects with nonsyndromic hearing impairment and auditory neuropathy, Hum. Mutat. 29 (6) (2008) 823–831.
[11] S. Yasunaga, M. Grati, S. Chardenoux, T.N. Smith, T.B. Friedman, A.K. Lalwani, et al., OTOF encodes multiple long and short isoforms: genetic evidence that the long ones underlie recessive deafness DFNB9, Am. J. Hum. Genet. 67 (3) (2000) 591–600.

[12] N. Schug, C. Braig, U. Zimmermann, J. Engel, H. Winter, P. Ruth, et al., Differential expression of otoferlin in brain, vestibular system, immature and mature cochlea of the rat, Eur. J. Neurosci. 24 (12) (2006) 3372–3380.

[13] I. Roux, S. Safieddine, R. Nouvian, M. Grati, M.C. Simmler, A. Bahloul, et al., Otoferlin, defective in a human deafness form, is essential for exocytosis at the auditory ribbon synapse, Cell 127 (2) (2006) 277–289.

[14] D. Dulon, S. Safieddine, S.M. Jones, C. Petit, Otoferlin is critical for a highly sensitive and linear calcium-dependent exocytosis at vestibular hair cell ribbon synapses, J. Neurosci. 29 (34) (2009) 10474–10487.

[15] P. Heidrych, U. Zimmermann, A. Bress, C.M. Pusch, P. Ruth, M. Pfister, et al., Rab8b GTPase, a protein transport regulator, is an interacting partner of otoferlin, defective in a human autosomal recessive deafness form, Hum. Mol. Genet. 17 (23) (2008) 3814–3821.

[16] T. Matsunaga, H. Mutai, S. Kunishima, K. Namba, N. Morimoto, Y. Shinjo, et al., A prevalent founder mutation and genotype–phenotype correlations of OTOF in Japanese patients with auditory neuropathy, Clin. Genet. (2012).

[17] N. Mahdieh, B. Rabbani, A. Shirkavand, H. Bagherian, Z.S. Movahed, P. Fouladi, et al., Impact of consanguineous marriages in GJB2-related hearing loss in the Iranian population: a report of a novel variant, Genet. Test. Mol. Biomarkers 15 (7–8) (2011) 489–493.

[18] N. Mahdieh, C. Nishimura, K. Ali-Madadi, Y. Riazalhosseini, H. Yazdan, S. Arzhangi, et al., The frequency of GJB2 mutations and the Delta (GJB6-D13S1830) deletion as a cause of autosomal recessive non-syndromic deafness in the Kurdish population, Clin. Genet. 65 (6) (2004) 506–508.

[19] N. Mahdieh, M. Raeisi, A. Shirkavand, H. Bagherian, M.T. Akbari, S. Zeinali, Investigation of GJB6 large deletions in Iranian patients using quantitative real-time PCR, Clin. Lab. 56 (9–10) (2010) 467–471.

[20] M. Legendre, N. Pochet, T. Pak, K.J. Verstrepen, Sequence-based estimation of minisatellite and microsatellite repeat variability, Genome Res. 17 (12) (2007) 1787–1796.

[21] M. Zak, M. Pfister, N. Blin, The otoferlin interactome in neurosensory hair cells: significance for synaptic vesicle release and trans-Golgi network (Review), Int. J. Mol. Med. 28 (3) (2011) 311–314.

[22] N.A. Ramakrishnan, M.J. Drescher, D.G. Drescher, Direct interaction of otoferlin with syntaxin 1A, SNAP-25, and the L-type voltage-gated calcium channel Cav1.3, J. Biol. Chem. 284 (3) (2009) 1364–1372.

[23] C.P. Johnson, E.R. Chapman, Otoferlin is a calcium sensor that directly regulates SNARE-mediated membrane fusion, J. Cell. Biol. 191 (1) (2010) 187–197.

[24] A. Lek, F.J. Evesson, R.B. Sutton, K.N. North, S.T. Cooper, Ferlins: regulators of vesicle fusion for auditory neurotransmission, receptor trafficking and membrane repair, Traffic 13 (2) (2011) 185–194.

[25] V. Migliosi, S. Modamio-Hoybjor, M.A. Moreno-Pelayo, M. Rodriguez-Ballesteros, M. Villamar, D. Telleria, et al., Q829X, a novel mutation in the gene encoding otoferlin (OTOF), is frequently found in Spanish patients with prelingual non-syndromic hearing loss, J. Med. Genet. 39 (7) (2002) 502–506.

[26] H. Najmabadi, C. Nishimura, K. Kahrizi, Y. Riazalhosseini, M. Malekpour, A. Daneshi, et al., GJB2 mutations: passage through Iran, Am J. Med. Genet. A 133A (2) (2005) 132–137.

[27] N. Mahdieh, E. Tafsiri, M. Karimipour, M. Akbari, Heterozygosity and allele frequencies of the two VNTRs (ApoB and D1S80) in Iranian population, Indian J. Hum. Genet. 1 (2005) 31–34.

[28] N. Mahdieh, H. Bagherian, A. Shirkavand, M. Sharafi, S. Zeinali, High level of intrafamilial phenotypic variability of non-syndromic hearing loss in a Lur family due to delE120 mutation in GJB2 gene, Int. J. Pediatr. Otorhinolaryngol. 74 (9) (2010) 1089–1091.

[29] N. Mahdieh, B. Rabbani, S. Wiley, M.T. Akbari, S. Zeinali, Genetic causes of nonsyndromic hearing loss in Iran in comparison with other populations, J. Hum. Genet. 55 (10) (2010) 639–648.

[30] J. Romanos, L. Kimura, M.L. Favero, F.A. Izarra, M.T. de Mello Auricchio, A.C. Batissoco, et al., Novel OTOF mutations in Brazilian patients with auditory neuropathy, J. Hum. Genet. 54 (7) (2009) 382–385.

[31] R. Santarelli, I. Del Castillo, M. Rodriguez-Ballesteros, P. Scimemi, E. Cama, E. Arslan, et al., Abnormal cochlear potentials from deaf patients with mutations in the otoferlin gene, J. Assoc. Res. Otolaryngol. 10 (4) (2009) 545–556.

[32] R. Varga, P.M. Kelley, B.J. Keats, A. Starr, S.M. Leal, E. Cohn, et al., Non-syndromic recessive auditory neuropathy is the result of mutations in the otoferlin (OTOF) gene, J. Med. Genet. 40 (1) (2003) 45–50.

[33] D.Y. Wang, Y.C. Wang, D. Weil, Y.L. Zhao, S.Q. Rao, L. Zong, et al., Screening mutations of OTOF gene in Chinese patients with auditory neuropathy, including a familial case of temperature-sensitive auditory neuropathy, BMC, Med. Genet. 11 (2010) 79.

[34] C. Zadro, A. Ciorba, A. Fabris, M. Morgutti, P. Trevisi, P. Gasparini, et al., Five new OTOF gene mutations and auditory neuropathy, Int. J. Pediatr. Otorhinolaryngol. 74 (5) (2010) 494–498.

[35] M.J. Houseman, A.P. Jackson, L.I. Al-Gazali, R.A. Badin, E. Roberts, R.F. Mueller, A novel mutation in a family with non-syndromic sensorineural hearing loss that disrupts the newly characterised OTOF long isoforms, J. Med. Genet. 38 (8) (2001) E25.

[36] T. Hutchin, N.N. Coy, H. Conlon, E. Telford, K. Bromelow, D. Blaydon, et al., Assessment of the genetic causes of recessive childhood non-syndromic deafness in the UK—implications for genetic testing, Clin. Genet. 68 (6) (2005) 506–512.

[37] H. Shahin, T. Walsh, A.A. Rayyan, M.K. Lee, J. Higgins, D. Dickel, et al., Five novel loci for inherited hearing loss mapped by SNP-based homozygosity profiles in Palestinian families, Eur. J. Hum. Genet. 18 (4) (2010) 407–413.

[38] F. Mirghomizadeh, M. Pfister, F. Apaydin, C. Petit, S. Kupka, C.M. Pusch, et al., Substitutions in the conserved C2C domain of otoferlin cause DFNB9, a form of nonsyndromic autosomal recessive deafness, Neurobiol. Dis. 10 (2) (2002) 157–164.

[39] M. Tekin, D. Akcayoz, A. Incesulu, A novel missense mutation in a C2 domain of OTOF results in autosomal recessive auditory neuropathy, Am. J. Med. Genet. A 138 (1) (2005) 6–10.

[40] I. Rouillon, A. Marcolla, I. Roux, S. Marlin, D. Feldmann, R. Couderc, et al., Results of cochlear implantation in two children with mutations in the OTOF gene, Int. J. Pediatr. Otorhinolaryngol. 70 (4) (2006) 689–696.

[41] S. Marlin, D. Feldmann, Y. Nguyen, I. Rouillon, N. Loundon, L. Jonard, et al., Temperature-sensitive auditory neuropathy associated with an otoferlin mutation: Deafening fever! Biochem. Biophys. Res. Commun. 394 (3) (2010) 737–742.

[42] A. Adato, L. Raskin, C. Petit, B. Bonne-Tamir, Deafness heterogeneity in a Druze isolate from the Middle East: novel OTOF and PDS mutations, low prevalence of GJB2 35delG mutation and indication for a new DFNB locus, Eur. J. Hum. Genet. 8 (6) (2000) 437–442.