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REVIEW

Genetic causes of nonsyndromic hearing loss in Iran in comparison with other populations

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Hearing loss (HL) is the most prevalent sensory defect affecting 1 in 500 neonates. Genetic factors are involved in half of the cases. The extreme heterogeneity of HL makes it difficult to analyze and determine the accurate genetic causes of the impairment. Up to now, 10 genes, namely, *GJB2, GJB6, SLC26A4, TECTA, PJVK, Col11A2, Myo15A, TMC1, RDX* and microRNA (miR-183), have been studied in an Iranian population. The prevalence of HL in Iran was estimated to be 2–3 times higher than that in other parts of the world. Here, the most common bases of congenital nonsyndromic hearing loss (NSHL) are discussed. We reviewed *GJB2, GJB6* (large deletion), *TECTA, SLC26A4* and *PEJVK* mutations, and studied their frequencies and distributions in different ethnic groups in 1934, 500, 121, 80 and 34 unrelated families throughout Iran, respectively. *GJB2* mutation was the most common factor causing NSHL, with a mean frequency of 18.17% in the Iranian population. The importance of Iran's geographical location in the migration pathway from west to east through the silk route was also highlighted. *SLC26A4* and *TECTA* mutations were the second and third main reasons of HL and accounted for up to 10 and 4% of prelingual HL in Iran, respectively. Mutations in *GJB2*, SLC26, *TECTA* and *PJVK* genes have an important role in HL in Iran and a screening test should be generated for better intervention and diagnosis programs.
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INTRODUCTION

Hearing loss (HL) is a complex disorder and accounts for 1.86 of 1000 newborns worldwide. The frequency of the disorder increases by age, that is, it affects 2.7 of 1000 before the age of 5 years and 3.5 of 1000 in adolescents.[1] Many environmental factors, such as drug exposure, bacterial or viral infections and trauma, can cause HL; however, a significant proportion of cases is due to genetic factors. Different criteria are applied for the classification of HL (Table 1). Considering clinical phenotypes, nonsyndromic hearing loss (NSHL) is responsible for nearly two-third of hereditary congenital cases and the remaining accounts for syndromic forms of HL. The only impairment in the former group is HL, whereas HL is a part of broader clinical symptoms in the syndromic form.

The cochlea is an important part of the inner ear, containing the organ of Corti. Sound vibrations are converted into electrobiochemical impulses in this organ and sent to the brain through the auditory nerve. Numerous genes have been described to be expressed in this pathway, including genes encoding cytoskeleton proteins, gap junction channels, membrane transport proteins, ion channels, regulatory elements and microRNAs (miRNAs).[2–4]

Monogenic inheritance (NSHL) is responsible for 70% of all congenital HL. All Mendelian inheritance patterns have been observed for prelingual HL. Different distributions of each pattern have been delineated in many studies: autosomal recessive (*DFNB* loci) in 80%, autosomal dominant (*DFNA* loci) in 20%, X-linked (*DFN* loci) in 1% and mitochondrial inheritance in 1% of cases.[2–7] Thus, autosomal recessive NSHL (ARNSHL) has a high prevalence among these patterns of inheritance. In traditional communities such as the Middle East, consanguineous or intragroup marriages may even increase the proportion of ARNSHL among all forms of HL. More than 80 loci have been identified for ARNSHL, of which a significant percentage is attributed to mutations in the *DFNB1* locus (especially *GJB2* mutations) in Caucasians.

Iran's population is ~75 million, with HL affecting an estimated 450 000 individuals.[8] In other words, 1 in 166 individuals is affected. Accurate genetic counseling is desired for early intervention of late-onset forms. Significant amounts of health-care expenditures are targeted for its maintenance each year in Iran; hence, preliminary molecular diagnosis would help to reduce the socioeconomical and mental disturbances of the affected individuals and their families.

HL encompasses a broad field of research, including understanding mechanisms underlying the disease, genes involved in different pathways, gene therapy (as a new treatment modality) and molecular diagnosis, for example, preimplantation genetic diagnosis. Considerable assistance in care is achieved through collection of mutations for diagnostic laboratories, clinicians and researchers.[9] Recently, the

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Table 1 Various criteria for the classification of hearing loss

Criterion	*Class*	*Definition and example*
Etiology	Environmental (acquired)	Caused by acquired agents such as viral and bacterial infection (prenatal, for example, CMV, toxoplasmosis, rubella; postnatal, for example, meningitis), hyperbilirubinemia, head trauma, anoxia, noise exposure and ototoxic drugs
	Genetic	Caused by mutations in nuclear and mitochondrial genes
	Idiopathic	Unexplained hearing loss
Age onset	Prelingual hearing loss	Hearing loss occurs before language has been acquired
	Postlingual hearing loss	Hearing loss occurs after the onset of speech
	Presbycusis	Age-related hearing loss
Clinical phenotypes	Syndromic	Deafness is associated with other phenotypes
	Nonsyndromic	Deafness is the only defect
Place of defect	Conductive hearing loss	Caused by a problem conducting sound waves through the outer ear, tympanic membrane or middle ear
	Sensorineural hearing loss	Caused by damage to the inner ear (vestibulocochlear nerve)
	Mixed hearing loss	Caused by a combination of sensorineural and conductive hearing loss
Severity	Mild	Difficulty in hearing of 26–40 dB sounds
	Moderate	41–55 dB
	Moderately severe	56–70 dB
	Severe	71–90 dB
	Profound	>90 dB
Inheritance pattern	Autosomal dominant (AD)	*DFNA* loci (*DFNA1-62*)
	Autosomal recessive (AR)	*DFNB* loci (*DFNB1-85*)
	Sex-linked (XR)	*DFN* loci (*DFN1-8*)
	Mitochondrial	12SrRNA (*MT-RNR1*), $tRNA^{Ser(UCN)}$ (*MT-TS1*)

Abbreviation: CMV, cytomegalovirus

Table 2 Studied genes involved in congenital hearing loss in Iranian population

	Gene properties		*Studied*	*No. of affected*	
Gene	*Location*	*No. of exons*	families	families	*References*
GJB2 (*DFNB1*)	13q11–12	2	1934	387	Najmabadi *et al.*,[24] Hashemzadeh Chaleshtori M *et al.*,[25] Bonyadi *et al.*[26] and Mahdieh *et al.*[27]
GJB6 (*DFNB1*)	13q11–12	6	>500	0	Najmabadi *et al.*,[24] Bonyadi *et al.*[26] and Mahdieh *et al.*[27]
TECTA (*DFNB21*)	11q22–24	23	75	1	Alasti *et al.*[78]
		45	3	77	
		1	1	76	
SLC26A4 (*DFNB4*)	7q31	21	80	8	Kahrizi *et al.*[60]
Pejvakin	2q31.2	7	30	2	Hashemzadeh Chaleshtori *et al.*[69]
		4	4	66	
TMC1 (*DFNB7/11*)	9q13–21	24	10	5	Hilgert *et al.*[2]
COL11A2 (*DFNB53*)	6p21	62	1	1	Chen *et al.*[92]
MYO15A (*DFNB3*)	17p11.2	66	2	2	Shearer *et al.*[104]
RDX (*DFNB24*)	11q23	14	1	1	Shearer *et al.*[101]
miR-183	—	—	576	0	Hildebrand *et al.*[106]

genetic causes of NSHL in Jews, Japanese and Chinese populations have been described.[10–12] In this survey, we emphasized the importance of identifying the genetic bases of NSHL and summarized the published data on the frequency of gene mutations in various loci studied in Iran; we provided counseling criteria for the patients. The distribution of the gene mutations was also shown.

MOLECULAR GENETICS

Many genes and loci pinpoint prelingual HL, including genes encoding cytoskeletal proteins such as myosin VI (*MYO6*), VIIA (*MYO7A*), XV (*MYO15*) and IIIA (*MYO3A*), structural proteins such as α-tectorin (*TECTA*), stereocilin (*STRC*), collagen type 11 (*COL11A2*) and otoancorin (*OTOA*), regulatory elements such as POU3F4 (*POU3F4*), POU4F3 (*POU4F3*) and EYA4 (*EYA4*), ion channel and transport proteins such as connexin-26, -30, -31, -40 proteins and pendrein (*SLC26A4*) and miRNAs.[2,3,4] Clinical diagnostic and research laboratories are widely available for conducting research on these genes. Studies on the characterization of these genes and the impact of the different combination of mutations are still an emerging field. It is widely believed that there are further unknown genes to be recognized.

At present, 10 genes have been examined in the country (Table 2). Whereas some genes have been analyzed in just one family, *GJB2* mutations have been investigated in at least 1934 families throughout Iran.

GJB2 gene structure and mutations

DFNB1 locus for ARNSHL was mapped to chromosome 13q11–12.[13] This locus contains two genes, *GJB2* (MIM 121011, GeneID 2706) and *GJB6* (MIM 604418, GeneID 10804); *GJB2* (gap junction subunit β2) encodes connexin (26 kDa) protein, which has two exons of which exon 1 is untranslated. *GJB2* is expressed as a 2.5-Kb protein, containing 226 amino acids in cochlea-supporting cells, suggesting a potential role in endolymph potassium recycling. This protein is arranged into a four-transmembrane (TM)-spanning fragment (Figure 1). Mutations in this single gene are the main reason of prelingual HL in Caucasians and in many ethnic groups worldwide. Six subunits of connexin proteins are gathered to form a hemiconnexon localizing in the cell membrane. Two hemiconnexons from adjacent cells form a channel to transport small metabolites and ions such as potassium. Connexin-26, expressed in hair cells, is implicated in reclaiming K+ in the cochlear duct. Deterioration of K+ recycling caused by *GJB2* mutations may give rise to HL.[14,15]

As of June 2010, more than 200 different allelic variants in the *GJB2* gene have been ascertained (http://www.hgmd.cf.ac.uk/ac/index.php).

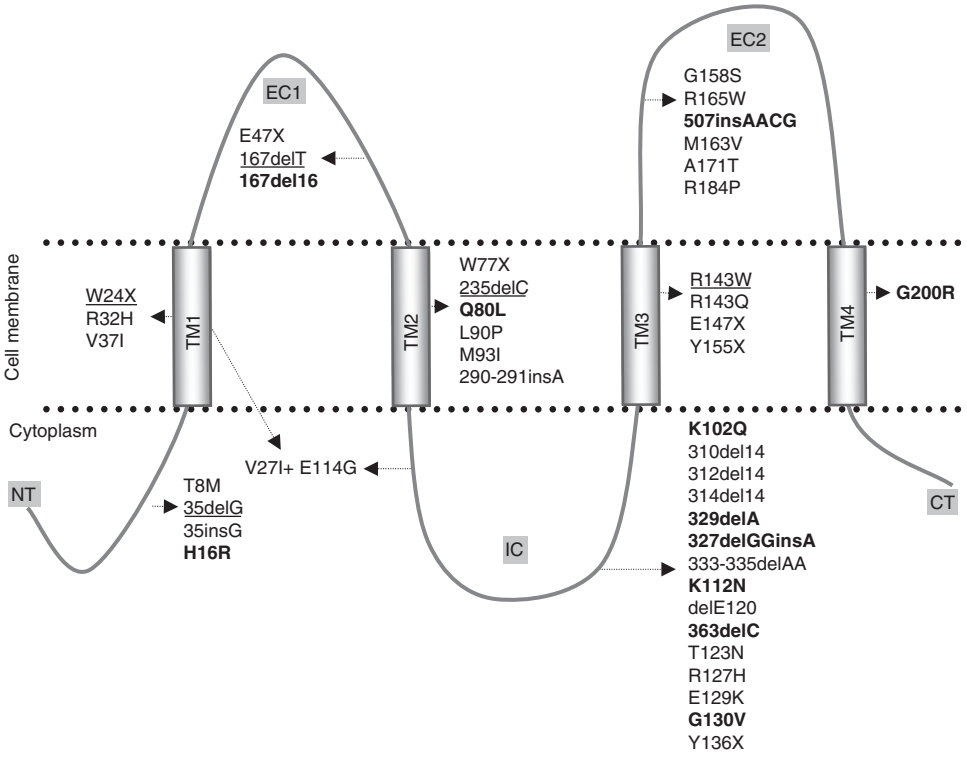

Figure 1 Schematic structure, domains and distribution of mutations of Cx26 protein in Iranian patients. Novel mutations reported for the first time are indicated in bold. The most common mutations in specific populations (35delG, 167delT, 235delC, R143W and W24X mutations in Caucasian, Ashkenazi Jewish, Japanese, Ghanian and Indian populations, respectively) are underlined. 35delG, W24X, 167delT, 235delC and R143W are located on NT, TM1, EC1, TM2 and TM3 domains, respectively. Other depicted mutations have also been reported in other populations. TM1–TM4 denotes transmembrane domains, EC1-2 denotes extracellular domains, IC denotes cytoplasmic domain, NT denotes amino (NH2) terminus and CT denotes carboxyl (COOH) terminus.

Among these variations, one truncating mutation named 35delG (deletion of guanine in position 30–35; rs80338939) is the most prevalent mutation and responsible for up to 70% of *GJB2* variants. This mutation leads to a frameshift change and synthesis of a premature truncated protein. The mean carrier frequencies of the 35delG mutation are 1.89, 1.52, 0.64, 1 and 0.64 for European, American, Asian, Oceanic and African populations, respectively.[16] The 167delT, 235delC, R143W and W24X mutations are the most common mutations in the Ashkenazi Jewish,[17] Japanese,[18] Ghanian[19,20] and Indian populations,[21,22] respectively (Figure 1). Thus, an ethnic-specific pattern is noted for some mutations of this gene,[16,21] which is different from that reported among various cohorts of Iran.

GJB2 mutations could result in NSHL. Genotype–phenotype analyses have not completely established a specific correlation between different combinations of genotypes and clinical features. There are many inter- and even intrafamilial phenotypic variations, ranging from mild to profound HL.[23] However, the role of modifier genes is proposed, which may give rise to more phenotypic variations.

Approximately 50 mutations in the *GJB2* gene have been detailed in deaf Iranian families (Table 3 and Figure 1). At least 1934 affected families have been investigated so far. In a study, *GJB2*-related HL was reported to occur in 16.7% of cases (111 of 664 different nuclear families).[24] In this survey, 35 simplex cases were also analyzed. In 2007, Hashemzadeh Chaleshtori *et al.*,[25] stated that the frequency of *GJB2*-related HL is 19.1% of deaf families (170 of 890 families). In addition, 209 unrelated Azeri patients were reviewed, with a frequency of 28.22% for *GJB2* gene mutations.[26] The mean frequency of *GJB2* mutations among the Iranian deaf groups is ~18.17%, whereas *GJB2* mutations are responsible for 50% of HL in Caucasian populations. We also examined 50 unrelated deaf families (data not shown). Briefly, medical history was taken, and audiometric examinations and pedigree analyses were carried out; genomic DNAs were extracted according to standard methodology. The 35delG homozygous subjects were determined using allele-specific PCR.[27] We sequenced the remaining samples by ABI 3130 sequencer (Applied BioSystems, Foster City, CA, USA).[24,27]

On the basis of these results, it can be concluded that the frequency of *GJB2* mutations decreases gradually both west to east and north to south. A northwest-to-southeast *GJB2*-related HL gradient is suggested across Iran, drawing the migration pathway of the initial founders. The 35delG mutation is the most prevalent mutation in deaf Iranian individuals, accounting for 66.26% of all *GJB2* mutations (466 of 703) and 12.05% of all studied alleles (466 of 3868) (Table 3). As known, this mutation provides 20% of all hereditary HL among American-Caucasian populations.[28] The eight most frequent mutations of the *GJB2* gene, namely, 35delG, R127H, delE120, W24X, V27I+ E114G, R184P, -3170G>A and 235delC, are responsible for 86.34% (607 of 703) of all pathogenic alleles in Iran (Table 3 and Figure 1). The 235delC mutation, which is the most common (up to 80%) among East Asian countries,[18,29–32] makes up for 1.85% (13 of 703) of *GJB2* mutations in Iran. The R127H, delE120 and W24X are the second, third and fourth common mutations, with a sum of 11.52% (81 of 703) of all pathogenic alleles. The R127H mutation is due to a G-to-A transition at codon 127, substituting arginine with histidine. The pathogenic effect of this amino-acid substitution is

Table 3 Frequencies of *GJB2*, *SLC26A4*, *PJVK*, *TECTA* and *TMC1* gene mutations in the previous studies in Iranian populations[2,24–26,60,66,67,69,76–78,113–115]

Allele variants	*No. of alleles (%)*
GJB2 mutations	
Insertion/deletion	
35delG	466 (12.05)
delE120	28 (0.72)
235delC	13 (0.34)
−3170G>A	13 (0.34)
167delT	6 (0.16)
363delC	5 (0.13)
314del14	4 (0.10)
312del14	4 (0.10)
327delGGinsA	3 (0.08)
167del16	2 (0.05)
310del14	2 (0.05)
290–291insA	2 (0.05)
35insG	1 (0.03)
333–335delAA	1 (0.03)
507insAACG	1 (0.03)
329delA	1 (0.03)
Subtotal	552 (14.27)
Substitutions	
R127H	30 (0.78)
W24X	23 (0.6)
V27I+E114G	18 (0.46)
R184P	16 (0.41)
R32H	9 (0.23)
R143W	8 (0.21)
M163V	*6 (0.16)*
−3517G>A	4 (0.10)
IVS1+1G>A	4 (0.10)
Y155X	*3 (0.08)*
H16R	2 (0.05)
Q80L	2 (0.05)
G200R	2 (0.05)
Y136X	2 (0.05)
W77X	2 (0.05)
L90P	2 (0.05)
T8M	2 (0.05)
V37I	2 (0.05)
A171T	*2 (0.05)*
R143Q	1 (0.05)
G12D	1 (0.03)
E47X	1 (0.03)
E147X	1 (0.03)
M93I	1 (0.03)
R165W	1 (0.03)
G158S	1 (0.03)
K112N	1 (0.03)
G130V	1 (0.03)
K102Q	1 (0.03)
T123N	*1 (0.03)*
E129K	*1 (0.03)*
Subtotal	151 (3.9)
Total mutations	703 (18.17)
Polymorphisms	
V153I	93 (2.4)
V27I	26 (0.67)
−3558T>C	21 (0.54)
E114G	3 (0.08)
F146F	2 (0.05)
M34T	1 (0.03)
G160S	1 (0.03)
I69I	1 (0.03)
Total polymorphisms	148 (3.83)
Total studied alleles	3868
SLC26A4 mutations	
G334V	2 (1. 25)
R409H	2 (1. 25)
R79X	2 (1. 25)
T721M	2 (1. 25)
L445W	2 (1. 25)
S448L	2 (1. 25)
1197delT	1 (0.625)
T420I	1 (0.625)
L597S	1 (0.625)
965insA	1 (0.625)
Total alleles	160 (100)
PJVK mutations	
T54I	2 (2.86)
R183W	6 (8.57)
726delT (F242LfsX7)	2 (2.86)
122delA	2 (2.86)
988delG (V330LfsX7)	2 (2.86)
Polymorphisms	
793C>T	3 (4.28)
793C>G	4 (5.71)
874G>A	2 (2.86)
Total alleles	70 (100)
TMC1 mutations	
100C>A (R34X)	2
77611G>A	2
Polymorphisms	
94615A>C	2
Total alleles	10
TECTA mutations	
c.649insC	2 (0.83)
266delT	2 (0.83)
c.5211C>A	2 (0.83)
9.6 kb deletion	2 (0.83)
c.6203–6218del16	2 (0.83)
Total alleles	242 (100)

Novel Variants are shown in bold letters; Unknown Mutations are shown in Italic letters.

controversial and remains to be confirmed. Combined genotypes with variants such as V37I, delE120 and/or R127H could show a variable phenotypic expression modulated by modifier agents.[23,33] Interestingly, R127H is distributed throughout Iran with the same frequency;[25] thus, arginine 127 may be a mutational hotspot. The delE120 (deletion of glutamic acid in position 120) and W24X (tryptophan to stop codon) may arise from ancestral founders in western and southwestern parts of Iran, respectively. However, further investigations are required to clarify this assumption.

More detailed studies on *GJB2* showed new mutations of the gene. So far, 12 novel mutations have been identified in deaf Iranian individuals, which are as follows: 167del16, H16R, Q80L, 507insAACG, K102Q, 329delA, 327delGGinsA, 363deC, G130V, K112N, −3517G>A and G200R (Table 3 and Figure 1). One novel mutation (−3517G>A) is found in regulatory regions. Five of these mutations are located on intracytoplasmic domain 2. *GJB2* polymorphisms are observed in 3.83% (148 of 3868) of deaf patients; V153I, valine to isoleucine substitution, accounts for 62.84% of polymorphic alleles (93 of 148). Gene polymorphisms could alter an amino acid, thereby, causing changes in the properties of the given isoformic protein. Thus, it could be postulated that *GJB2* gene polymorphisms among deaf patients, may in association with other gene polymorphisms, lead to some degree of HL. In other words, specific isoforms of two different proteins, for example, connexin-26 and other proteins such as connexin-30, may not have a functional interaction. However, structural and functional analyses on different polymorphisms of various interacting proteins are needed to confirm this hypothesis.

More recently, researchers have shown that mutations of *GJB2* can function in a digenic manner with the *GJB3* gene.[34] Hence, mutation analysis of this gene should be considered in *GJB2* heterozygotes. Because of the high frequency of *GJB2* mutations in many populations, *GJB2* analysis should be depicted for sensorineural NSHL, although the frequency of *GJB2*-related HL in Iran is lower than that in European countries.

Molecular genetic testing for *GJB2* in Iran includes an amplification refractory mutation system for common mutations, followed by a sequencing procedure.

GJB6 gene

GJB6 (MIM 604418, GeneID 10804), in the vicinity of *GJB2* located on 13q12, encodes a connexin (30 kDa) protein that participates in the formation of hemiconnexon structures in the membrane of hair cells. As in *GJB2*, each connexin protein has four TM segments, two extracellular loops, a cytoplasmic loop formed between the two inner TM segments and the amino and carboxy termini, both facing the cytoplasmic side. Connexin-30 has 261 amino acids having 76% homology to human connexin-26. A few point mutations in the *GJB6* gene have been found to associate with autosomal dominant HL and hidrotic ectodermal dysplasia.[35]

Four *GJB6* large deletions, a 140 kb deletion[36] or a 150 kb deletion,[37] named del(*GJB6*-D13S1830) 342 kb,[38] del(*GJB6*-D13S1854) 232 kb,[39] a 920 kb deletion[40] and del(chr13:19,837,344–19,968,698),[41] have been described to bring about HL in association with a single mutation in the *GJB2* gene. Existence of several breakpoints within this region suggests that other unknown deletions may be responsible for *GJB2* heterozygotes. Up to 50% of *GJB2* heterozygotes have a large deletion around the *GJB6* gene.[42] The del(*GJB6*-D13S1830), the most frequent mutation in Spain,[42] France,[42,43] Argentina,[44] Brazil[45] and the United Kingdom,[42] has not been found in Iranian populations,[24,26,27,46,47] suggesting the existence of point mutations in regulatory elements of the gene, and possibly different breakpoints around the gene and/or another gene(s). Moreover, these deletions have not been reported in Turkey,[48] India,[49] China and Croatia.[50]

Genetic testing for *GJB6* large deletions may be different on the basis of the deletion that is investigated, and includes gap-PCR, quantitative-PCR and DNA sequencing for point mutations.

SLC26A4 gene

Solute carrier gene (*SLC26A4* gene, MIM 605646, GeneID 5172), located on *DFNB4* (chromosome 7q31), encodes pendrin protein, which functions as a transmembrane chloride/iodide transporter in cochlea and in a few other tissues.[51,52] An ∼5 Kb transcript is expressed into 780 amino-acid (86 kDa) protein. The real structure of pendrin has not been defined yet; on the basis of protein structure prediction programs, two models have been proposed for pendrin protein: 12TM and 15TM models.[53,54] This ion transporter also exchanges other anions such as HCO^-, OH^-, I^- or formate.[55] Variations in this gene, as a second prevalent cause of HL, can contribute to both syndromic (Pendred syndrome, PS) and ARNSHL (*DFNB4*).[56,57] PS is associated with severe sensorineural HL and thyroid symptoms ranging from small changes in thyroid size to a large extent goiter. An enlarged vestibular aqueduct determined by a computed tomographic image is also present in PS patients.[58] *DFNB4* and PS explain ∼1–8% of prelingual HL.[59]

Approximately 100 mutations have been designated in the *SLC26A4* gene.[58,60] A total of 80 Iranian families were investigated for mutations in this gene using the autozygosity by descent approach using short tandem repeat markers.[60] Autozygosity by descent of the *SLC26A4* gene was observed in 12 of them. Enlarged vestibular aqueduct and an impaired function of the thyroid were also found in all families.[60] In their work, Kahrizi *et al.* found the following mutations in eight families: T420I, 1197delT, G334V, R409H, T721M, R79X, S448L, L597S, 965insA and L445W. R79X and R409H mutations (Figure 2a) were the most common among other mutations in this population, whereas L236P, T416P, E384G and IVS8+1G>A are the common mutations in other populations that are responsible for 75% of all mutated alleles.[61] The IVS7-2A>G and H732R mutations are more prevalent in East Asia.[62–65] Thus, ethnic specificity of mutations in this gene is also observed and it should be considered in screening programs in Iranian subpopulations. On the basis of preliminary data, *SLC26A4* mutations are responsible for up to 10% of prelingual HL. However, further studies are needed to elucidate the exact frequencies of *SLC26A4* mutations in these subpopulations.

DFNB59 gene

DFNB59 gene (*PJVK*, MIM 610219, GeneID 494513), located on chromosome 2q31.1–q31.3, encodes pejvakin, which functions as a member of the gasdermin protein family in the cochlea.[66] Pejvakin possibly has an important role in the cell signaling of hair cells and sensory neurons.[67,68] The 352-amino-acid pejvakin protein is translated from a 5 kb cDNA.

The gene has seven exons spanning 9.8 kb of the genomic sequence, of which the first exon is noncoding. *PJVK* mutations were clarified as the cause of sensorineural HL for the first time in Iranian families.[66] So far, four mutations (T54I, R183W, 726delT and c.988delG) in *PJVK* gene have been found in Iran (Figure 2b). The frequency of *PJVK* mutations has been reported to be 4 of 60 chromosomes in Iran.[69] Further studies are required to determine other mutations and carrier frequencies of mutations in the *PJVK* gene in our different ethnic groups. Mutations of this gene have been also documented in Turkish and Dutch populations.[70]

TECTA gene

The *TECTA* gene (MIM 602574, GeneID 7007), located on chromosome 11q22–q24, encodes α-tectorin, which is one of the major noncollagenous parts of the tectorial membrane in the inner ear. This membrane as an extracellular matrix covers the neuroepithelium of the cochlea and contacts the stereocilia of hair cells transducing sounds into electrical signals.[71]

The *TECTA* gene encodes a precursor protein of 2155 amino acids, determined to contain 23 exons. Mutations in the *TECTA* gene have

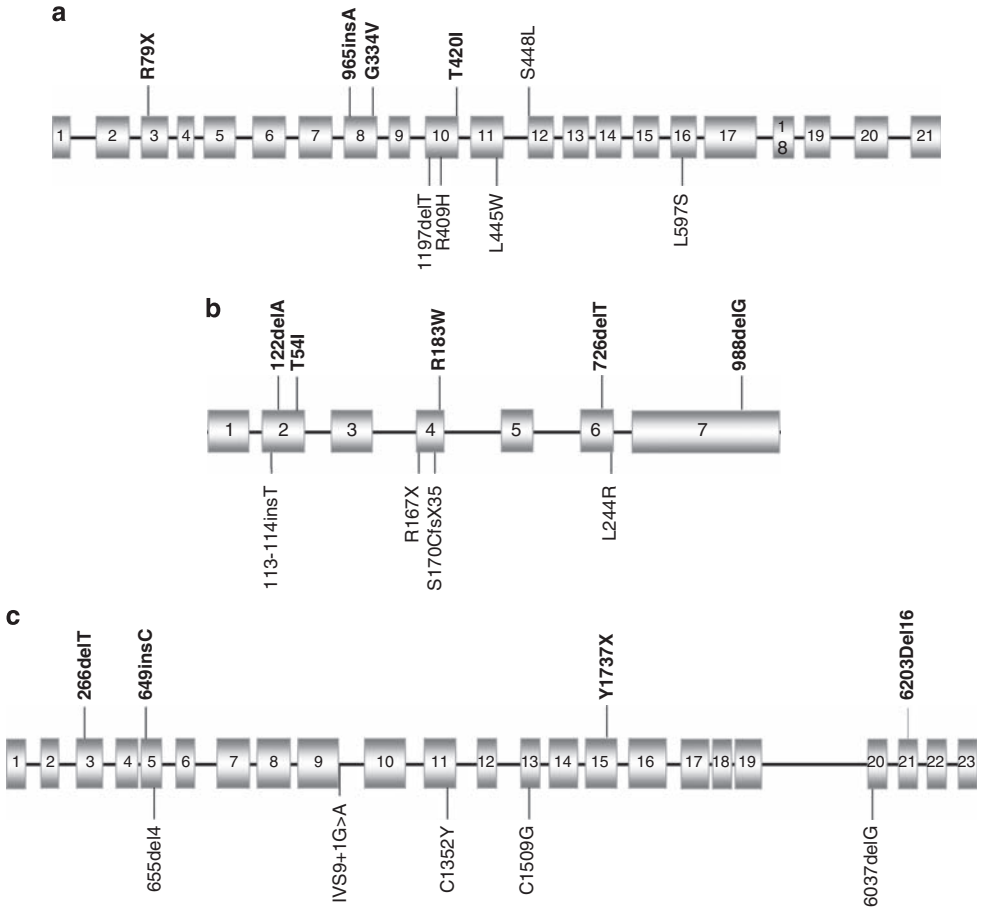

Figure 2 Novel mutations reported for the first time are indicated in bold at the top of the *SLC26A4*, *PJVK* and *TECTA* genes in Iranian patients. (**a**) Schematic representation exons of the *SLC26A4* gene and mutations found in Iranian families. Only mutations reported in the Iranian population are shown in the figure. (**b**) Schematic exons of the *PJVK* gene and distribution of its mutations. Mutations reported in other populations are shown below. (**c**) Schematic exons of the *TECTA* gene and distribution of its mutations. Mutations reported in other populations are shown below.

been found in both autosomal dominant and autosomal recessive HL (*DFNA8/12* and *DFNB21*).[71–74] All presented missense mutations lead to the dominant form, whereas inactivating mutations bring about recessive HL.[75] *TECTA* mutations have been detected in Austrian, Belgian, French and Swedish families.[7] Three studies have been recorded on *TECTA* mutations in Iranian population; totally, *TECTA* mutations were demonstrated in 5 of 121 studied patients.[76–78] The five mutations with the same frequency (2 of 242 alleles for each) reported are as follows: c.649insC, 266delT, c.5211C>A, 9.6 kb deletion and c.6203–6218del16 (Figure 2c).[76–78] Altogether, the *TECTA* gene was involved in 10 of 242 alleles (4.13%) in our population. α-Tectorin has three types of functional domains: entactin-like domain at N-terminus, four von Willebrand type D domains in the central part of the protein and zona pellucida domain at the C-terminal end.[79] Mutations of various parts of α-tectorin lead to HL at different frequencies and lead to specific genotype–phenotype correlations.[71,80–82]

TMC1 gene

Mutations in the transmembrane cochlear-expressed gene 1 (*TMC1*), located on chromosome 9q13–21, can also result in both progressive autosomal dominant and autosomal recessive HL (*DFNA36* and *DFNB7/DFNB11*).[83,84] There are eight vertebrate *TMC* genes on the basis of their sequence homology.[85] This gene (MIM 606706, GeneID 117531) is considered as a transmembrane protein, and its mutations are associated with prelingual and postlingual HL. A total of 24 exons of *TMC1* encode the mRNA. *TMC1* mutations have been found in at least 6% (4 of 65) of *GJB2*-unrelated ARNSHL patients from eastern Turkish populations. Mutation prevalence of the *TMC1* gene is ~5% of *GJB2*-negative ARNSHL in Tunisian, Pakistani and Indian families.[86–88] One mutation, named 100C>T (R34X), is responsible for 47% of all *TMC1* mutations in ARNSHL families.[2] On the basis of the linkage analysis between the mutation and polymorphic markers, it may arise from a common ancestor dating back to 1075–1900 years.[89] This mutation was found only in one family from Iran.[2] Another mutation, 77611 G>A, with the same frequency was also determined in Iranian families.[2]

COL11A2 gene

The *COL11A2* gene (MIM 120290, GeneID 1302) is located on chromosome 6p21.3, encoding the α-2 chain of minor fibrillar collagen XI. Collagens are the major components of bones and cartilages. Several cases of syndromic HL due to mutations of collagens have been reported. Collagen type XI comprises <10% of cartilage collagens. This gene contains at least 62 exons spanning 30.5 Kb.[90] The gene lies within the MHC region and is only 1.1 kb from the retinoid X receptor-β and ~40 kb from DPB2. *COL11A2* mutations can generate both autosomal recessive and progressive autosomal dominant HL (*DFNB53* and *DFNA13*).[91–93] Mutations in this gene have illustrated to cause *DFNA13* HL in one Dutch family.[94]

In one Iranian family, homozygosity for a *COL11A2* mutation, P621T, has also been reported to result in ARNSHL.[92]

Genetic testing includes PCR–restriction fragment length polymorphism for known missense mutations and DNA sequencing.

MYO15A gene

MYO15A (MIM 602666, GeneID 51168), located on 17p11.2, encodes an unconventional myosin XVA (myosin heavy-chain-15A) that functions as a motor molecule moving along actin filaments in the stereocilia of hair cells.[95,96] At least 39 myosin genes, grouped into 12 classes, have been found within the human genome.[97]

It contains 66 exons encoding an 11.9 Kb transcript. Mutations in this large gene can cause autosomal recessive HL (*DFNB3*) and account for 5% of ARNSHL in Pakistan.[7,95,98,99] Two *MYO15A* mutations have also been reported in 66 ARNSHI Turkish families.[100] More recently, two homozygous missense mutations were exemplified in two consanguineous Iranian families; c.6371G>A and c.6555C>T lead to p.R2124Q and p.P2073S amino-acid substitutions.[101]

RDX gene

RDX gene (MIM 179410, GeneID 5962) has been mapped on chromosome 11q23, encoding a cytoskeletal protein named radixin, which possibly functions as linking molecule between actin and plasma membrane.[102]

Mutations in this gene can lead to autosomal recessive HL (*DFNB24*).[103,104] It contains 14 exons. There are two *RDX* psuedogenes in the human genome; a truncated version of the gene named *RDXP2* was mapped to Xp21.3 and another one, *RDXP1*, was mapped on chromosome 11p. A homozygous splice site mutation (c.698+1G>A) in intron 7 of the *RDX* gene has been reported in an Iranian ARNSHL family.[104]

microRNA

HL has been ascribed to variations in miRNA genes.[4] miRNAs binding to their complementary domains in mRNAs affect posttranscriptional repression.[105] Mutations in miR-96 genes belonging to the miR-183 miRNA family in two ARNSHL *DFNA50* families have been determined by Mencía *et al*. Hildebrand *et al*. screened 192 unmapped Iranian ARNSHL families and identified a homozygous c.*95C>A variation in the miR-96/182 binding site in the *RDX* 3′ untranslated region. This nucleotide substitution disrupts the binding of miR-96 and miR-182, and/or creates a new binding site for miR-507 and miR-557. However, c.*95C>A has not been associated with HL in humans.[106] They also studied miR-183 genes in 576 families and found no potential pathogenic variants.

MOLECULAR TESTING FOR SYNDROMIC AND NSHL

Molecular diagnosis of syndromic and NSHL has a significant benefit for affected children and their parents because of prevention of unessential diagnostic tests and undesirable clinical consequences of psychological and socioeconomical effects. Planning for therapy approaches such as cochlear implant also demands an accurate diagnosis.[107]

Attributed to extreme heterogeneity and the relatively small contribution of different genes, it is very expensive and really impractical to perform a comprehensive analysis of all known and unknown genes involving in inherited HL. Syndromic bases can be diagnosed by an accurate clinical evaluation and by molecular tests for the known syndromes; hence, a multidisciplinary team is needed for this goal.

It is generally accepted to screen the mutations of some genes that are more prevalent than others. Two genes, *GJB2* and *GJB6*, are analyzed for patients affected by prelingual NSHL in Iranian genetic laboratories. The procedure of genetic testing includes DNA extraction from peripheral blood cells, buccal cells, a part of blood spots or tissues and mutation analysis by molecular methods such as allele-specific PCR for common mutations and sequencing of the exonic region of the gene.

GBJ2 has only a single coding exon; hence, it is simply sequenced. There are many difficulties in the interpretation of the *GJB2* analysis; first, many families show a heterozygous genotype for the *GJB2* gene, that is, having only a single mutant allele. Second, inter and intrafamilial phenotypic variability caused by even the same mutation complicates the prediction of the degree of HL.

Regarding the phenotypic expression of individuals being PS or enlarged vestibular aqueduct, the homozygosity mapping of the affected is implemented for the *SLLC26A4* gene, which is carried out by short tandem repeat markers.

However, some potential difficulties may occur in establishing a genetic cause for HL in patients; genetic heterogeneity, uncertainty about pathogenicity of the mutation, reduced penetrance of some mutations, phenotypically affected heterozygotes and a negative family history of the patient are the common reasons for such difficulties. Here, affected homozygotes for *GJB2* mutations are ruled out by molecular study. Heterozygote patients for the *GJB2* gene are subjected to analysis to investigate *GJB6* large deletions. *GJB2*-unrelated affected individuals (*GJB2* negatives) are clinically examined in detail. On the basis of previous studies, there are specific clinical indications for the genes to be examined first; for example, if sensorineural HL is accompanied by enlarged vestibular aqueduct and goitrous changes, *SLC26A4* is analyzed. This procedure for the genetic diagnosis of HL would be beneficial to developing countries such as Iran as it is cost-effective and diminishes the fees spent on health practice. Moreover, a multiplex screening system for the analysis of ARNHL by the use of short tandem repeat markers could be an advantageous way to determine the involved genes in these countries; in this approach, affected families should be investigated for the prevalent genes by autozygosity by decent and subsequent sequencing of the related gene. However, many clinical and research laboratories are available for genetic testing around the world. Genetic investigation of HL genes is feasible by microarray and genome-wide scanning.[108,109]

ETHNICITY

Genetic and molecular testing for diagnosis of the major cause of HL should be designed considering the ethnicity of patients, especially in Iran with various cohorts and different cultures. Ethnicity should be considered even for other genetic diseases. The critical and specific position of Iran and the existence of various ethnic groups with different cultures (for example, Persian, Azeri, Gilaki and Mazandarani, Kurd, Lur, Turkmen, Arab, balooch and so on) suggest the high heterogeneity throughout Iran,[23,110] but specific intraethnic traditions such as intragroup marriages may give rise to a high homogeneity in some loci and mutations within groups and not among groups.

GJB2 mutations are responsible for 22.2% of deaf families in northwest Iran, that is, in west and east Azerbaijan, Ardebil and Zanjan; a significant proportion of population in these provinces are Azeri. *GJB2*-related HL has the highest frequency (38%) in north Iran, that is, in Gilan, Mazandaran and Golestan provinces, and the lowest frequency (0–4.3%) of *GJB2* mutations has been observed in south and southeast Iran, that is, in Booshehr, Hormazgan and Sistan and Baloochestan.[24,25] *GJB2*-related HL in western Iran (15.7% including Kermanshah, Kordestan, Hamedan, Lorestan and Ilam) is more than that in central and northeast Iran: 15.3 and 13%, respectively (Figure 3).

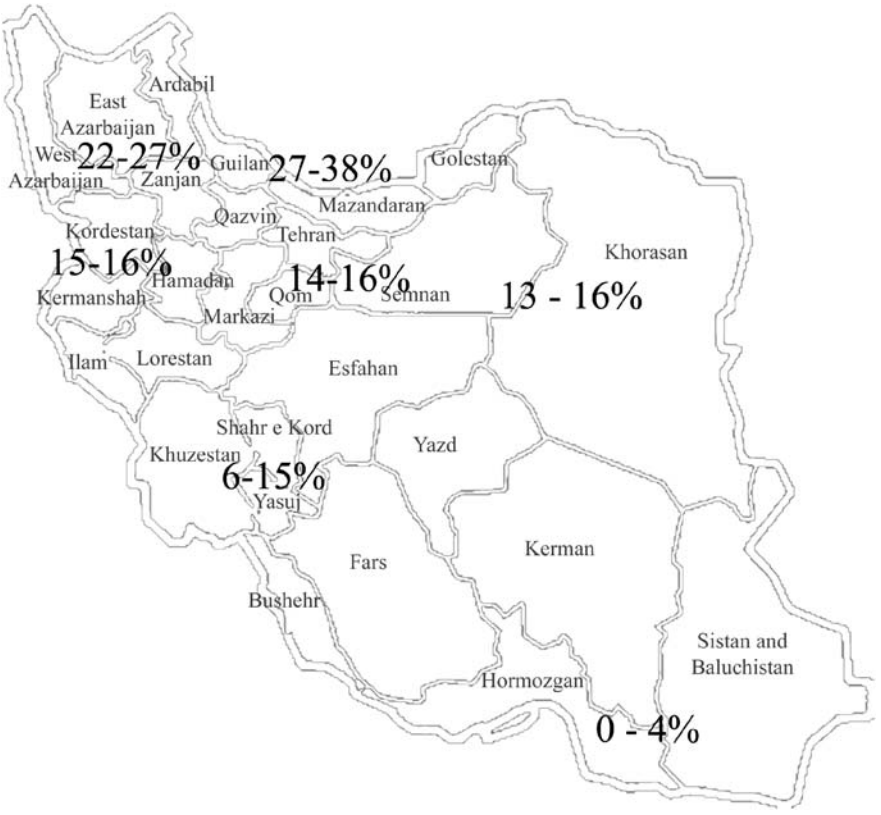

Figure 3 Distribution of *GJB2* mutations in different regions of Iran. Azeri, Lur, Kurd, Arab, Gilaki and Baloochi populations are distributed in the northwest (22–27%), west (15–16%), southwest (6–15%), south (0–4%), north (27–38%) and southeast (0–4%) regions of the country. Data are adapted from references Najmabadi *et al.*,[24] Hashemzadeh Chaleshtori *et al.*,[25] Bonyadi *et al.*[26] and Mahdieh *et al.*[27]

Ethnic background has an important role in the frequencies of *GJB2* mutations; for example, 35delG is the most common mutation that allocates a large proportion of *GJB2* mutations in nearly all populations, especially in Caucasians, as well as 167delT, 235delC and R143W in Ashkenazi Jews, East Asians and Ghana, respectively. The 35delG mutation is also the most common (71.6%) allele of *GJB2* in deaf Iranian patients;[24] its carrier frequency (2.8%) in the Gilan province is even more than that in some Caucasian populations.[111] Further and more comprehensive studies are needed to define other genes and their mutations on the basis of ethnicity in Iran. However, ethnicity should be an important factor for determining the most appropriate type of assay.

GENETIC COUNSELING

HL is a sensory impairment that can be treated by cochlear implant, especially in children before the age of 5 years. Genetic counseling and genetic testing for families with HL can be helpful, effective and appropriate for preimplantation genetic diagnosis, newborn screening, detection of carrier mutations and preventing the recurrence of the condition in affected families.

HL affects 0.22–3.61 per 1000 neonates in the United States,[112] whereas 1 out of 166 individuals suffers from HL. Thus, the frequency of HL in Iran is 1.7–27 higher than in the United States. In addition, this condition may show up higher rates compared with other populations of the world by two to three times.

A complete family history is taken. It includes age of onset of HL, environmental agents that may bring about HL, mother's complications during pregnancy, careful and detailed physical examinations and pedigree taking. For establishing HL, audiological tests are required. Recurrent risk is calculated on the basis of inheritance pattern. Regarding the *GJB2* mutations, 35delG is the most common mutation that is checked first. In Iranian ethnic groups, specific mutations are common, which are investigated in each group; for example, the frequency of the IVS1þ+1G>A mutation and W24X shows a high frequency in Kurdish and Baloochi groups and accounts for 9.4 and 6% of the mutant alleles in these populations, respectively.[23,24,27]

In conclusion, unraveling gene mutations of HL would help to improve health care and affect our treatment of the condition. Executing appropriate screening programs may lead to designing therapeutic and treatment strategies in Iran.

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1 Morton, C. C. & Nance, W. E. Newborn hearing screening–a silent revolution. *N. Engl. J. Med.* **354,** 2151–2164 (2006).
2 Hilgert, N., Alasti, F., Dieltjens, N., Pawlik, B., Wollnik, B. & Uyguner, O. Mutation analysis of TMC1 identifies four new mutations and suggests an additional deafness gene at loci DFNA36 and DFNB7/11. *Clin. Genet.* **74,** 223–232 (2008).
3 Dror, A. A. & Avraham, K. B. Hearing loss: mechanisms revealed by genetics and cell biology. *Annu. Rev. Genet.* **43,** 411–437 (2009).
4 Mencía, A., Modamio-Høybjør, S., Redshaw, N., Morín, M., Mayo-Merino, F., Olavarrieta, L. *et al.* Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss. *Nat. Genet.* **41,** 609–613 (2009).

5 Morton, N. E. Genetic epidemiology of hearing impairment. *Ann. NY. Acad. Sci.* **630,** 16–31 (1991).
6 Cryns, K. & van Camp, G. Deafness genes and their diagnostic applications. *Audiol. Neurootol.* **9,** 2–22 (2004).
7 Finsterer, J. & Fellinger, J. Nuclear and mitochondrial genes mutated in nonsyndromic impaired hearing. *Int. J. Pediatr. Otorhinolaryngol.* **69,** 621–647 (2005).
8 Quds Newspaper. http://www.qudsdaily.com/archive/1388/html/7/1388-07-07/page2.html (2009).
9 Cotton, R. G., Al Aqeel, A. I., Al-Mulla, F., Carrera, P., Claustres, M., Ekong, R. *et al.* Capturing all disease-causing mutations for clinical and research use: toward an effortless system for the Human Variome Project. *Genet. Med.* **11,** 843–849 (2009).
10 Ben-Yosef, T. & Friedman, T. B. The genetic bases for syndromic and nonsyndromic deafness among Jews. *Trends Mol. Med.* **9,** 496–502 (2003).
11 Usami, S., Wagatsuma, M., Fukuoka, H., Suzuki, H., Tsukada, K., Nishio, S. *et al.* The responsible genes in Japanese deafness patients and clinical application using Invader assay. *Acta. Otolaryngol.* **128,** 446–454 (2008).
12 Ouyang, X. M., Yan, D., Yuan, H. J., Pu, D., Du, L. L., Han, D. Y. *et al.* The genetic bases for non-syndromic hearing loss among Chinese. *J. Hum. Genet.* **54,** 131–140 (2009).
13 Guilford, P., Ben Arab, S., Blanchard, S., LeVilliers, J., Weissenbach, J., Belkahia, A. *et al.* A non-syndrome form of neurosensory, recessive deafness maps to the pericentromeric region of chromosome 13q. *Nat. Genet.* **6,** 24–28 (1994).
14 Goodenough, D. A., Goliger, J. A. & Paul, D. L. Connexins, connexons, and intercellular communication. *Annu. Rev. Biochem.* **65,** 475–502 (1996).
15 Tekin, M., Arnos, K. S. & Pandya, A. Advances in hereditary deafness. *Lancet* **358,** 1082–1090 (2001).
16 Mahdieh, N. & Rabbani, B. Statistical study of 35delG mutation of GJB2 gene: a meta-analysis of carrier frequency. *Int. J. Audiol.* **48,** 363–370 (2009).
17 Morell, R. J., Kim, H. J., Hood, L. J., Goforth, L., Friderici, K., Fisher, R. *et al.* Mutations in the connexin 26 gene (GJB2) among Ashkenazi Jews with nonsyndromic recessive deafness. *N. Engl. J. Med.* **339,** 1500–1505 (1998).
18 Kudo, T., Ikeda, K., Kure, S., Matsubara, Y., Oshima, T., Watanabe, K. *et al.* Novel mutations in the connexin 26 gene (GJB2) responsible for childhood deafness in the Japanese population. *Am. J. Med. Genet.* **90,** 141–145 (2000).
19 Brobby, G. W., Muller-Myhsok, B. & Horstmann, R. D. Connexin 26 R143W mutation associated with recessive nonsyndromic sensorineural deafness in Africa. *N. Engl. J. Med.* **338,** 548–549 (1998).
20 Hamelmann, C., Amedofu, G. K., Albrecht, K., Muntau, B., Gelhaus, A., Brobby, G. W. *et al.* Pattern of connexin 26 (GJB2) mutations causing sensorineural hearing impairment in Ghana. *Hum. Mutat.* **18,** 84–85 (2001).
21 Maheshwari, M., Vijaya, R., Ghosh, M., Shastri, S., Kabra, M. & Menon, P. S. Screening of families with autosomal recessive non-syndromic hearing impairment (ARNSHI) for mutations in GJB2 gene: Indian scenario. *Am. J. Med. Genet. A.* **120,** 180–184 (2003).
22 Bouwer, S., Angelicheva, D., Chandler, D., Seeman, P., Tournev, I. & Kalaydjieva, L. Carrier rates of the ancestral Indian W24X mutation in GJB2 in the general Gypsy population and individual subisolates. *Genet. Test.* **11,** 455–458 (2007).
23 Mahdieh, N., Bagherian, H., Shirkavand, A., Sharafi, M. & Zeinali, S. 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**, 1089–1091 (2010).
24 Najmabadi, H., Nishimura, C., Kahrizi, K., Riazalhosseini, Y., Malekpour, M., Daneshi, A. *et al.* GJB2 mutations: passage through Iran. *Am. J. Med. Genet. A* **133A,** 132–137 (2005).
25 Hashemzadeh Chaleshtori, M., Farhud, D. D. & Patton, M. A. Familial and sporadic GJB2-related deafness in Iran: review of gene mutations. *Iran. J. Public Health* **36,** 1–14 (2007).
26 Bonyadi, M., Esmaeili, M., Abhari, M. & Lotfi, A. Mutation analysis of familial GJB2-related deafness in Iranian Azeri, Turkish patients. *Genet. Test. Mol. Biomarkers* **13,** 689–692 (2009).
27 Mahdieh, N., Nishimura, C., Ali-Madadi, K., Riazalhosseini, Y., Yazdan, H., Arzhangi, S. *et al.* The frequency of GJB2 mutations and the (GJB6-D13S1830) deletion as a cause of autosomal recessive non-syndromic deafness in the Kurdish population. *Clin. Genet.* **65,** 506–508 (2004).
28 Kelley, P. M., Harris, D. J., Comer, B. C., Askew, J. W., Fowler, T., Smith, S. D. *et al.* Novel mutations in the connexin26 gene (GJB2) that cause autosomal recessive (DFNB1) hearing loss. *Am. J. Hum. Genet.* **62,** 792–799 (1998).
29 Abe, S., Usami, S., Shinkawa, H., Kelley, P. M. & Kimberling, W. J. Prevalent connexin 26 gene (GJB2) mutations in Japanese. *J. Med. Genet.* **37,** 41–43 (2000).
30 Lee, K. Y., Choi, S. Y., Bae, J. W., Kim, S., Chung, K. W., Drayna, D. *et al.* Molecular analysis of the GJB2, GJB6 and SLC26A4 genes in Korean deafness patients. *Int. J. Pediatr. Otorhinolaryngol.* **72,** 1301–1309 (2008).
31 Dai, P., Yu, F., Han, B., Yuan, Y., Li, Q., Wang, G. *et al.* The prevalence of the 235delC GJB2 mutation in a Chinese deaf population. *Genet. Med.* **9,** 283–289 (2007).
32 Dai, P., Yu, F., Han, B., Liu, X., Wang, G., Li, Q. *et al.* GJB2 mutation spectrum in 2,063 Chinese patients with nonsyndromic hearing impairment. *J. Transl. Med.* **7,** 26 (2009).
33 Roux, A. F., Pallares-Ruiz, N., Vielle, A., Faugère, V., Templin, C., Leprevost, D. *et al.* Molecular epidemiology of DFNB1 deafness in France. *BMC Med. Genet.* **5,** 5 (2004).
34 Liu, X. Z., Yuan, Y., Yan, D., Ding, E. H., Ouyang, X. M., Fei, Y. *et al.* Digenic inheritance of non-syndromic deafness caused by mutations at the gap junction proteins Cx26 and Cx31. *Hum. Genet.* **125,** 53–62 (2009).
35 Lamartine, J., Munhoz Essenfelder, G., Kibar, Z., Lanneluc, I., Callouet, E., Laoudj, D. *et al.* Mutations in GJB6 cause hidrotic ectodermal dysplasia. *Nat. Genet.* **26,** 142–144 (2000).
36 Lerer, I., Sagi, M., Ben-Neriah, Z., Wang, T., Levi, H. & Abeliovich, D. A deletion mutation in GJB6 cooperating with a GJB2 mutation in trans in non-syndromic deafness: A novel founder mutation in Ashkenazi Jews. *Hum. Mutat.* **18,** 460 (2001).
37 Pallares-Ruiz, N., Blanchet, P., Mondain, M., Claustres, M. & Roux, A. F. A large deletion including most of GJB6 in recessive non syndromic deafness: a digenic effect? *Eur. J. Hum. Genet.* **10,** 72–76 (2002).
38 del Castillo, I., Villamar, M., Moreno-Pelayo, M A., del Castillo, F. J., Alvarez, A., Tellería, D. *et al.* A deletion involving the connexin 30 gene in nonsyndromic hearing impairment. *N. Engl. J. Med.* **346,** 243–249 (2002).
39 del Castillo, F. J., Rodríguez-Ballesteros, M., Alvarez, A., Hutchin, T., Leonardi, E., de Oliveira, C. A. *et al.* A novel deletion involving the connexin-30 gene, del(GJB6-d13s1854), found in trans with mutations in the GJB2 gene (connexin-26) in subjects with DFNB1 non-syndromic hearing impairment. *J. Med. Genet.* **42,** 588–594 (2005).
40 Feldmann, D., Le Maréchal, C., Jonard, L., Thierry, P., Czajka, C., Couderc, R. *et al.* A new large deletion in the DFNB1 locus causes nonsyndromic hearing loss. *Eur. J. Med. Genet.* **52,** 195–200 (2009).
41 Wilch, E., Azaiez, H., Fisher, R. A., Elfenbein, J., Murgia, A., Birkenhäger, R. *et al.* A novel DFNB1 deletion allele supports the existence of a distant cis-regulatory region that controls GJB2 and GJB6 expression. *Clin. Genet.* **78**, 267–274 (2010).
42 del Castillo, I., Moreno-Pelayo, M. A., del Castillo, F. J., Brownstein, Z., Marlin, S., Adina, Q. *et al.* Prevalence and evolutionary origins of the del(GJB6-D13S1830) mutation in the DFNB1 locus in hearing impaired subjects: a multicenter study. *Am. J. Hum. Genet.* **73,** 1452–1458 (2003).
43 Marlin, S., Feldmann, D., Blons, H., Loundon, N., Rouillon, I., Albert, S. *et al.* GJB2 and GJB6 mutations: genotypic and phenotypic correlations in a large cohort of hearing-impaired patients. *Arch. Otolaryngol. Head Neck Surg.* **131,** 481–487 (2005).
44 Gravina, L. P., Foncuberta, M. E., Prieto, M. E., Garrido, J., Barreiro, C. & Chertkoff, L. Prevalence of DFNB1 mutations in Argentinean children with non-syndromic deafness. Report of a novel mutation in GJB2. *Int. J. Pediatr. Otorhinolaryngol.* **74,** 250–254 (2010).
45 Batissoco, A. C., Abreu-Silva, R. S., Braga, M. C., Lezirovitz, K., Della-Rosa, V., Alfredo, T. Jr. *et al.* Prevalence of GJB2 (connexin-26) and GJB6 (connexin-30) mutations in a cohort of 300 Brazilian hearing-impaired individuals: implications for diagnosis and genetic counseling. *Ear Hear.* **30,** 1–7 (2009).
46 Riazalhosseini, Y., Nishimura, C., Kahrizi, K., Shafeghati, Y., Danseshi, A., Jogataie, M. T. *et al.* Delta (GJB6-D13S1830) is not a common cause of nonsyndromic hearing loss in in the Iranian patients. *Arch. Iran. Med.* **8,** 104–108 (2005).
47 Esmaeili, M., Bonyadi, M. & Nejadkazem, M. Common mutation analysis of GJB2 and GJB6 genes in affected families with autosomal recessive non-syndromic hearing loss from Iran: simultaneous detection of two common mutations (35delG=del(GJB6-D13S1830)) in the DFNB1-related deafness. *Int. J. Pediatr. Otorhinolaryngol.* **71,** 869–873 (2007).
48 Evirgen, N., Solak, M., Dereköy, S., Erdoĉan, M., Yildiz, H., Eser, B. *et al.* Genotyping for Cx26 and Cx30 mutations in cases with congenital hearing loss. *Genet. Test.* **12,** 253–256 (2008).
49 Padma, G., Ramchander, P. V., Nandur, U. V. & Padma, T. GJB2 and GJB6 gene mutations found in Indian probands with congenital hearing impairment. *J. Genet.* **88,** 267–272 (2009).
50 Sansoviæ, I., Knezeviæ, J., Musani, V., Seeman, P., Barisiæ, I. & Paveliæ, J. GJB2 mutations in patients with nonsyndromic hearing loss from Croatia. *Genet. Test. Mol. Biomarkers* **13,** 693–699 (2009).
51 Sheffield, V. C., Kraiem, Z., Beck, J. C., Nishimura, D., Stone, E. M., Salameh, M. *et al.* Pendred syndrome maps to chromosome 7q21-34 and is caused by an intrinsic defect in thyroid iodine organification. *Nat. Genet.* **12,** 424–426 (1996).
52 Borck, G., Roth, C., Martiné, U., Wildhardt, G. & Pohlenz, J. Mutations in the PDS gene in German families with Pendred's syndrome: V138F is a founder mutation. *J. Clin. Endocrinol. Metab.* **88,** 2916–2921 (2003).
53 Gillam, M. P., Sidhaye, A. R., Lee, E. J., Rutishauser, J., Stephan, C. W. & Kopp, P. Functional characterization of pendrin in a polarized cell system. Evidence for pendrin-mediated apical iodide efflux. *J. Biol. Chem.* **279,** 13004–13010 (2004).
54 Dossena, S., Rodighiero, S., Vezzoli, V., Nofziger, C., Salvioni, E., Boccazzi, M. *et al.* Functional characterization of wild-type and mutated pendrin (SLC26A4), the anion transporter involved in Pendred syndrome. *J. Mol. Endocrinol.* **43,** 93–103 (2009).
55 Mount, D. B. & Romero, M. F. The SLC26 gene family of multifunctional anion exchangers. *Pflugers Arch.* **447,** 710–721 (2004).
56 Everett, L. A., Glaser, B., Beck, J. C., Idol, J. R., Buchs, A., Heyman, M. *et al.* Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). *Nat. Genet.* **17,** 411–422 (1997).
57 Li, X. C., Everett, L. A., Lalwani, A. K., Desmukh, D., Friedman, T. B., Green, E. D. *et al.* A mutation in PDS causes non-syndromic recessive deafness. *Nat. Genet.* **18,** 215–217 (1998).
58 Campbell, C., Cucci, R. A., Prasad, S., Green, G. E., Edeal, J. B., Galer, C. E. *et al.* Pendred syndrome, DFNB4, and PDS/SLC26A4 identification of eight novel mutations and possible genotype-phenotype correlations. *Hum. Mutat.* **17,** 403–411 (2001).
59 Smith, R. I. H. & Hone, S. Genetic screening for deafness. *Pediatr. Clin. North Am.* **50,** 315–329 (2003).
60 Kahrizi, K., Mohseni, M., Nishimura, C., Bazazzadegan, N., Fischer, S. M., Dehghani, A. *et al.* Identification of SLC26A4 gene mutations in Iranian families with hereditary hearing impairment. *Eur. J. Pediatr.* **168,** 651–653 (2009).
61 Schrijver, I. Hereditary non-syndromic sensorineural hearing loss: transforming silence to sound. *J. Mol. Diagn.* **6,** 275–284 (2004).
62 Harada, D., Namba, A., Abe, S. & Usami, S Distribution and frequencies of PDS (SLC26A4) mutations in Pendred syndrome and nonsyndromic hearing loss associated with enlarged vestibular aqueduct: a unique spectrum of mutations in Japanese. *Eur. J. Hum. Genet.* **11,** 916–922 (2003).
63 Usami, S., Abe, S., Weston, M. D., Shinkawa, H., Van Camp, G. & Kimberling, W. J. Non-syndromic hearing loss associated with enlarged vestibular aqueduct is caused by PDS mutations. *Hum. Genet.* **104,** 188–192 (1999).

64 Wang, Q. J., Zhao, Y. L., Rao, S. Q., Guo, Y. F., Yuan, H., Zong, L. *et al.* A distinct spectrum of SLC26A4 mutations in patients with enlarged vestibular aqueduct in China. *Clin. Genet.* **72,** 245–254 (2007).
65 Hu, H., Wu, L., Feng, Y., Pan, Q., Long, Z., Li, J. *et al.* Molecular analysis of hearing loss associated with enlarged vestibular aqueduct in the Mainland Chinese: a unique SLC26A4 mutation spectrum. *J. Hum. Genet.* **52,** 492–497 (2007).
66 Delmaghani, S., del Castillo, F. J., Michel, V., Leibovici, M., Aghaie, A., Ron, U. *et al.* Mutations in the gene encoding pejvakin, a newly identified protein of the afferent auditory pathway, cause DFNB59 auditory neuropathy. *Nat. Genet.* **38,** 770–778 (2006).
67 Schwander, M., Sczaniecka, A., Grillet, N., Bailey, J. S., Avenarius, M., Najmabadi, H. *et al.* A forward genetics screen in mice identifies recessive deafness traits and reveals that pejvakin is essential for outer hair cell function. *J. Neurosci.* **27,** 2163–2175 (2007).
68 Ebermann, I., Walger, M., Scholl, H. P., Charbel Issa, P., Lüke, C., Nürnberg, G. *et al.* Truncating mutation of the DFNB59 gene causes cochlear hearing impairment and central vestibular dysfunction. *Hum. Mutat.* **28,** 571–577 (2007).
69 Hashemzadeh Chaleshtori, M., Simpson, M. A., Farrokhi, E., Dolati, M., Hoghooghi Rad, L., Amani Geshnigani, S. & Crosby, A. H. Novel mutations in the pejvakin gene are associated with autosomal recessive non-syndromic hearing loss in Iranian families. *Clin. Genet.* **72,** 261–263 (2007).
70 Collin, R. W., Kalay, E., Oostrik, J., Caylan, R., Wollnik, B., Arslan, S. *et al.* Involvement of DFNB59 mutations in autosomal recessive nonsyndromic hearing impairment. *Hum. Mutat.* **28,** 718–723 (2007).
71 Verhoeven, K., Van Laer, L., Kirschhofer, K., Legan, P. K., Hughes, D. C., Schatteman, I. *et al.* Mutations in the human alpha-tectorin gene cause autosomal dominant nonsyndromic hearing impairment. *Nat. Genet.* **19,** 60–62 (1998).
72 Alloisio, N., Morlé, L., Bozon, M., Godet, J., Verhoeven, K., Van Camp, G. *et al.* Mutation in the zonadhesin-like domain of alpha-tectorin associated with autosomal dominant non-syndromic hearing loss. *Eur. J. Hum. Genet.* **7,** 255–258 (1999).
73 Balciuniene, J., Dahl, N., Jalonen, P., Verhoeven, K., Van Camp, G., Borg, E. *et al.* Alpha-tectorin involvement in hearing disabilities: one gene–two phenotypes. *Hum. Genet.* **105,** 211–216 (1999).
74 Mustapha, M., Weil, D., Chardenoux, S., Elias, S., El-Zir, E., Beckmann, J. S. *et al.* An alpha-tectorin gene defect causes a newly identified autosomal recessive form of sensorineural pre-lingual non-syndromic deafness, DFNB21. *Hum. Mol. Genet.* **8,** 409–412 (1999).
75 Moreno-Pelayo, M. A., Goodyear, R. J., Mencía, A., Modamio-Høybjør, S., Legan, P. K., Olavarrieta, L. *et al.* Characterization of a spontaneous, recessive, missense mutation arising in the Tecta gene. *J. Assoc. Res. Otolaryngol.* **9,** 202–214 (2008).
76 Naz, S., Alasti, F., Mowjoodi, A., Riazuddin, S., Sanati, M. H., Friedman, T. B. *et al.* Distinctive audiometric profile associated with DFNB21 alleles of TECTA. *J. Med. Genet.* **40,** 360–363 (2003).
77 Meyer, N. C., Alasti, F., Nishimura, C. J., Imanirad, P., Kahrizi, K., Riazalhosseini, Y. *et al.* Identification of three novel TECTA mutations in Iranian families with autosomal recessive nonsyndromic hearing impairment at the DFNB21 locus. *Am. J. Med. Genet. A* **143A,** 1623–1629 (2007).
78 Alasti, F., Sanati, M. H., Behrouzifard, A. H., Sadeghi, A., de Brouwer, A. P., Kremer, H. *et al.* A novel TECTA mutation confirms the recognizable phenotype among autosomal recessive hearing impairment families. *Int. J. Pediatr. Otorhinolaryngol.* **72,** 249–255 (2008).
79 Legan, P. K., Rau, A., Keen, J. N. & Richardson, G. P. The mouse tectorins. Modular matrix proteins of the inner ear homologous to components of the sperm-egg adhesion system. *J. Biol. Chem.* **272,** 8791–8801 (1997).
80 Pfister, M., Thiele, H., Van Camp, G., Fransen, E., Apaydin, F., Aydin, O. *et al.* A genotype-phenotype correlation with gender-effect for hearing impairment caused by tecta mutations. *Cell. Physiol. Biochem.* **14,** 369–376 (2004).
81 Plantinga, R. F., de Brouwer, A. P., Huygen, P. L., Kunst, H. P., Kremer, H. & Cremers, C. W. A novel TECTA mutation in a Dutch DFNA8/12 family confirms genotype-phenotype correlation. *J. Assoc. Res. Otolaryngol.* **7,** 173–181 (2006).
82 Collin, R. W., de Heer, A. M., Oostrik, J., Pauw, R. J., Plantinga, R. F., Huygen, P. L. *et al.* Mid-frequency DFNA8/12 hearing loss caused by a synonymous TECTA mutation that affects an exonic splice enhancer. *Eur. J. Hum. Genet.* **16,** 1430–1436 (2008).
83 Greinwald, J. H. Jr., Scott, D. A., Marietta, J. R., Carmi, R., Manaligod, J., Ramesh, A. *et al.* Construction of P1-derived artificial chromosome and yeast artificial chromosome contigs encompassing the DFNB7 and DFNB11 region of chromosome 9q13-21. *Genome Res.* **7,** 879–886 (1997).
84 Kurima, K., Peters, L. M., Yang, Y., Riazuddin, S., Ahmed, Z. M., Naz, S. *et al.* Dominant and recessive deafness caused by mutations of a novel gene, TMC1, required for cochlear hair-cell function. *Nat. Genet.* **30,** 277–284 (2002).
85 Kurima, K., Yang, Y., Sorber, K. & Griffith, A. J. Characterization of the transmembrane channellike (TMC) gene family: functional clues from hearing loss and epidermodysplasia verruciformis. *Genomics* **81,** 300–308 (2003).
86 Kitajiri, S. I., McNamara, R., Makishima, T., Husnain, T., Zafar, A. U., Kittles, R. A. *et al.* Identities, frequencies and origins of TMC1 mutations causing DFNB7/B11 deafness in Pakistan. *Clin. Genet.* **72,** 546–550 (2007).
87 Kalay, E., Karaguzel, A., Caylan, R., Heister, A., Cremers, F. P., Cremers, C. W. *et al.* Four novel TMC1 (DFNB7/DFNB11) mutations in Turkish patients with congenital autosomal recessive nonsyndromic hearing loss. *Hum. Mutat.* **26,** 591 (2005).
88 Tlili, A., Rebeh, I. B., Aifa-Hmani, M., Dhouib, H., Moalla, J., Tlili-Chouchène, J. *et al.* TMC1 but not TMC2 is responsible for autosomal recessive nonsyndromic hearing impairment in Tunisian families. *Audiol. Neurootol.* **13,** 213–218 (2008).
89 Saïd, M. B., Hmani-Aifa, M., Amar, I., Baig, S. M., Mustapha, M., Delmaghani, S. *et al.* High frequency of the p.R34X mutation in the TMC1 gene associated with nonsyndromic hearing loss is due to founder effects. *Genet. Test. Mol. Biomarkers* **14,** 307–311 (2010).
90 Lui, V. C., Ng, L. J., Sat, E. W. & Cheah, K. S. The human alpha 2(XI) collagen gene (COL11A2): completion of coding information, identification of the promoter sequence, and precise localization within the major histocompatibility complex reveal overlap with the KE5 gene. *Genomics* **32,** 401–412 (1996).
91 Vikkula, M., Mariman, E. C., Lui, V. C., Zhidkova, N. I., Tiller, G. E., Goldring, M. B. *et al.* Autosomal dominant and recessive osteochondrodysplasias associated with the COL11A2 locus. *Cell* **80,** 431–437 (1995).
92 Chen, W., Kahrizi, K., Meyer, N. C., Riazalhosseini, Y., Van Camp, G., Najmabadi, H. *et al.* Mutation of COL11A2 causes autosomal recessive non-syndromic hearing loss at the DFNB53 locus. *J. Med. Genet.* **42,** e61 (2005).
93 McGuirt, W. T., Prasad, S. D., Griffith, A. J., Kunst, H. P., Green, G. E., Shpargel, K. B. *et al.* Mutations in COL11A2 cause nonsyndromic hearing loss (DFNA13). *Nat. Genet.* **23,** 413–419 (1999).
94 de Leenheer, E. M., Bosman, A. J., Kunst, H. P., Huygen, P. L. & Cremers, C. W. Audiological characteristics of some affected members of a Dutch DFNA13/COL11A2 family. *Ann. Otol. Rhinol. Laryngol.* **113,** 922–929 (2004).
95 Friedman, T. B., Liang, Y., Weber, J. L., Hinnant, J. T., Barber, T. D., Winata, S. *et al.* A gene for congenital, recessive deafness DFNB3 maps to the pericentromeric region of chromosome 17. *Nat. Genet.* **9,** 86–91 (1995).
96 Wang, A., Liang, Y., Fridell, R. A., Probst, F. J., Wilcox, E. R., Touchman, J. W. *et al.* Association of unconventional myosin MYO15 mutations with human nonsyndromic deafness DFNB3. *Science* **280,** 1447–1451 (1998).
97 Berg, J. S., Powell, B. C. & Cheney, R. E. A millennial myosin census. *Mol. Biol. Cell.* **12,** 780–794 (2001).
98 Friedman, T. B., Hinnant, J. T., Ghosh, M., Boger, E. T., Riazuddin, S., Lupski, J. R. *et al.* DFNB3, spectrum of MYO15A recessive mutant alleles and an emerging genotype-phenotype correlation. *Adv. Otorhinolaryngol.* **61,** 124–130 (2002).
99 Nal, N., Ahmed, Z. M., Erkal, E., Alper, O. M., Lüleci, G., Dinç, O. *et al.* Mutational spectrum of MYO15A: the large N-terminal extension of myosin XVA is required for hearing. *Hum. Mutat.* **28,** 1014–1019 (2007).
100 Kalay, E., Uzumcu, A., Krieger, E., Caylan, R., Uyguner, O., Ulubil-Emiroglu, M. *et al.* MYO15A (DFNB3) mutations in turkish hearing loss families and functional modeling of a novel motor domain mutation. *Am. J. Med. Genet. A* **143A,** 2382–2389 (2007).
101 Shearer, A. E., Hildebrand, M. S., Bromhead, C. J., Kahrizi, K., Webster, J. A., Azadeh, B. *et al.* A novel splice site mutation in the RDX gene causes DFNB24 hearing loss in an Iranian family. *Am. J. Med. Genet. A* **149A,** 555–558 (2009).
102 Hoeflich, K. P. & Ikura, M. Radixin: cytoskeletal adopter and signaling protein. *Int. J. Biochem. Cell Biol.* **36,** 2131–2136 (2004).
103 Khan, S. Y., Ahmed, Z. M., Shabbir, M. I., Kitajiri, S., Kalsoom, S., Tasneem, S. *et al.* Mutations of the RDX gene cause nonsyndromic hearing loss at the DFNB24 locus. *Hum. Mutat.* **28,** 417–423 (2007).
104 Shearer, A. E., Hildebrand, M. S., Webster, J. A., Kahrizi, K., Meyer, N. C., Jalalvand, K. *et al* Mutations in the first MyTH4 domain of MYO15A are a common cause of DFNB3 hearing loss. *Laryngoscope* **119,** 727–733 (2009).
105 He, L. & Hannon, G. J. MicroRNAs: small RNAs with a big role in gene regulation. *Nat. Rev. Genet.* **5,** 522–531 (2004).
106 Hildebrand, M. S., Witmer, P. D., Xu, S., Newton, S. S., Kahrizi, K., Najmabadi, H. *et al.* miRNA mutations are not a common cause of deafness. *Am. J. Med. Genet. A* **152A,** 646–652 (2010).
107 Bitner-Glindzicz, M. Hereditary deafness and phenotyping in Humans. *Br. Med. Bull.* **63,** 73–94 (2002).
108 Choi, S. Y., Kim, Y. E., Ahn, D. B., Kim, T. H., Choi, J. H., Lee, H. R. *et al.* Construction of a DNA chip for screening of genetic hearing loss. *Clin. Exp. Otorhinolaryngol.* **2,** 44–47 (2009).
109 Choi, S. Y., Lee, K. Y., Kim, Y. E., Bae, J. W., Oh, S. K., Kim, S. Y. *et al.* Application of allele-specific primer extension-based microarray for simultaneous multi-gene mutation screening in patients with non-syndromic hearing loss. *Int. J. Mol. Med.* **25,** 315–320 (2010).
110 Mahdieh, N., Tafsiri, E., Karimipour, M. & Akbari, M. T. Heterozygosity and allele frequencies of the two VNTRs (ApoB and D1S80) in Iranian population. *Indian J. Hum. Genet.* **11,** 31–34 (2005).
111 Hashemzadeh Chaleshtori, M., Farrokhi, E., Shahrani, M., Kheiri, S., Dolati, M., Hoghooghi Rad, L. *et al.* High carrier frequency of the GJB2 mutation (35delG) in the north of Iran. *Int. J. Pediatr. Otorhinolaryngol.* **71,** 863–867 (2007).
112 Mehra, S., Eavey, R. D. & Keamy, D. G. The epidemiology of hearing impairment in the United States: Newborns, children, and adolescents. *Otolaryngol. Head Neck Surg.* **140,** 461–472 (2009).
113 Hamid, M., Karimipoor, M., Chaleshtori, M. H. & Akbari, M. T. A novel 355-357delGAG mutation and frequency of connexin-26 (GJB2) mutations in Iranian patients. *J. Genet.* **88,** 359–362 (2009).
114 Sadeghi, A., Sanati, M. H., Alasti, F., Hashemzadeh Chaleshtori, M. & Ataei, M. Mutation analysis of Connexin 26 gene and del in patients with hereditary deafness from two provinces in Iran. *Iran. J. Biotechnol.* **3,** 255–258 (2005).
115 Sadeghi, A., Sanati, M. H., Alasti, F., Hashemzadeh Chaleshtori, M., Mahmoudian, S. & Ataei, M. Contribution of GJB2 mutations and four common DFNB loci in autosomal recessive non-syndromic hearing impairment in Markazi and Qom provinces of Iran. *Iran. J. Biotechnol.* **7,** 108–111 (2009).