Review Article

Heterogeneity of Hereditary Hearing Loss in Iran: a Comprehensive Review

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Abstract

A significant contribution to the causes of hereditary hearing impairment comes from genetic factors. More than 120 genes and 160 loci have been identified to be involved in hearing impairment. Given that consanguine populations are more vulnerable to most inherited diseases, such as hereditary hearing loss (HHL), the genetic picture of HHL among the Iranian population, which consists of at least eight ethnic subgroups with a high rate of intermarriage, is expected to be highly heterogeneous. Using an electronic literature review through various databases such as PubMed, MEDLINE, and Scopus, we review the current picture of HHL in Iran. In this review, we present more than 39 deafness genes reported to cause non-syndromic HHL in Iran, of which the most prevalent causative genes include *GJB2*, *SLC26A4*, *MYO15A*, and *MYO7A*. In addition, we highlight some of the more common genetic causes of syndromic HHL in Iran. These results are of importance for further investigation and elucidation of the molecular basis of HHL in Iran and also for developing a national diagnostic tool tailored to the Iranian context enabling early and efficient diagnosis of hereditary hearing impairment.

Keywords: Consanguinity, hereditary hearing loss, Iran, mutation spectra

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Introduction

Sensorineural hearing loss (SNHL) is the most common birth defect. It affects 1 in every 500 newborns in developed countries and has a prevalence of 3 infants per 1000 population in Iran.[1] Causality is multifactorial with both genetic and environmental factors implicated in its development (Figure 1). In a recent study in which comprehensive clinical genetic testing with targeted genomic enrichment and massively parallel sequencing was completed for 1119 sequentially accrued patients with SNHL, a genetic diagnosis was identified in ~70% of subjects of Middle Eastern descent.[2] This percentage is higher than that reported in other ethnicities, reflecting a higher coefficient of inbreeding in the 'consanguinity belt', a region extending from North Africa through the Middle East to India, thereby enriching populations in this region with multiple recessive diseases, including non-syndromic hearing loss (autosomal recessive non-syndromic hearing loss, ARNSHL).

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Within Iran, the geographical pattern of consanguineous marriages ranges from 15.9% (α: 0.0068) in the northern provinces to 47.0% (α: 0.0216) in the eastern provinces. As the second largest nation in the Middle East with a total of 75.1 million inhabitants (http://www.amar.org.ir/Portals/1/Iran/Atlas_Census_2011), Iran is also amongst the world's most heterogeneous populations.[3] At least seven different ethnic groups are recognized, including Persian (61%), Azeri Turk (16%), Kurd (10%), Lur (6%), Baluch (2%), Arab (2%), and Turkmen and Turkic tribes (2%), with other ethnicities comprising the remaining 1% of the population (http://www.indexmundi.com/iran/demographics_profile.html) (Figure 2). Persians mostly reside in the center of the country, with the other ethnic groups living closer to the borders, where they share cultural roots with neighboring countries. Over the past three decades, numerous genes implicated in ARNSHL have been identified in Iranian families; however, to date, no review has assessed the spectrum of pathogenic variants reported in all known deafness-associated genes in Iran. In this report, we present a comprehensive genetic picture of SNHL in Iran, which in turn provides an excellent opportunity to support the evidence-informed health policy being developed in the country.

Mutation spectra of NSHL in Iran

Monogenic SNHL is an extremely heterogeneous disorder. More than 150 mapped loci and over 95 genes are causally implicated in NSHL, including 58 loci for autosomal dominant genes, 87 loci for autosomal recessive genes, and 6 loci for X-linked genes (http://www.hereditaryhearingloss.org).

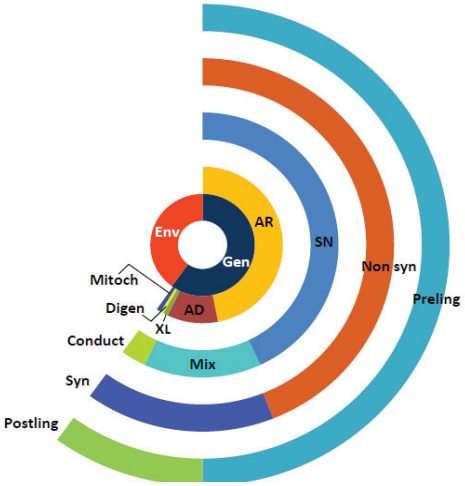

Figure 1. Classification of hereditary hearing loss. Env = environmental, Gen = genetic, AR = autosomal recessive, AD = autosomal dominant, XL = X-Linked, Digen = digenic, Mitoch = mitochondrial, SN = sensorineural; Mix = mixed; Conduct = conductive; Non syn = non-syndromic; Syn = syndromic; Preling = prelingual; Postling = postlingual.

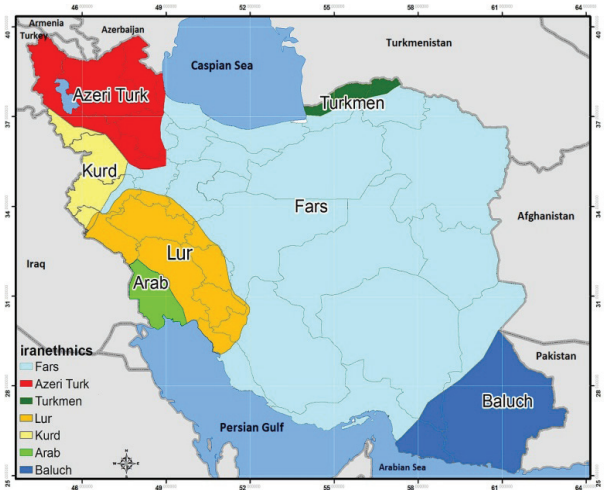

Figure 2. Distribution of ethnic groups in Iran.[4]

Genetic causes of ARNSHL

GJB2

GJB2 encodes the connexin 26 protein (Cx26), a member of the gap-junction protein family that facilitates the transfer of small molecules between cells. By recycling endolymphatic potassium, it plays a critical role in auditory transduction.[5] Mutations in this gene are associated with both ARNSHL and autosomal dominant non-syndromic hearing loss (ADNSHL) at the DFNB1 and DFNA3 loci, respectively. Over 100 variants have been implicated in ARNSHL (http://davinci.crg.es/deafness/index.php), with only a few variants implicated in autosomal dominant hearing loss, most frequently in association with a skin phenotype (Keratitis-Ichthyosis-Deafness (KID) syndrome [OMIM: 148210], Bart-Pumphrey syndrome [OMIM: 149200], Vohwinkel syndrome [OMIM: 124500], and palmoplantar keratoderma (PPK) with deafness [OMIM: 148350]).

Unexpectedly, a single mutation in *GJB2*, c.35delG (p.Gly12Valfs) has been found to cause more than 60% of *GJB2*-related ARNSHL in individuals of Northern European ancestry,[6–8] with other ethnic groups also carrying founder mutations [c.167delT (p.Leu56Argfs) in Ashkenazi Jews, c.235delC (p.Leu79Cysfs) in the East Asian population, and c.427C>T (p.Arg143Trp) in Ghana].

In Iran, the prevalence of *GJB2*-related HL is relatively low (Table 1). Overall, it accounts for only 11% of ARNSHL; however, there is a *GJB2* cline across Iran. In the northwest of the country, the prevalence of *GJB2*-related HL is 38.3% but this percentage drops to 0% in the south,[9] a change that reflects the ethnic footprint of Iran – in northwest Iran, the *GJB2* mutation pattern mimics that of neighboring Turkey (21.4–30%),[10, 11] whereas in the south, it mimics that of Persian Gulf and Arab countries such as Oman (0%).[9,12–14]

Overall, in Iran, the c.35delG variant of *GJB2* is the most commonly identified mutation (homozygous and compound heterozygous in 44% and 33% of individuals with *GJB2*-related HL, respectively)[6,13] and may in fact have originated in an Iranian population.[15] Other mutations also show ethnic-specific enrichment. For example, amongst the Baluchi population, the two most common *GJB2* mutations are c.71G>A, p.Trp24* (80%) and c.380G>A, p.Arg127His (20%).[16] The former likely spread to the Baluchi population from India.[17] Bonyadi *et al.* have also provided evidence that the c.-23+1G>A (IVS1+1G>A) mutation may have arisen in the Iranian Azeri Turkish population, where this allele is found with a prevalence of 4.9% (17/348) in families affected by ARNSHL.[18]

In southern European populations, a large deletion 5′ of *GJB2* that includes a portion of *GJB6* (named Δ(*GJB6*-D13S1830)) is common.[6,19] Interestingly, this deletion has not been reported in northeastern Mexico, China, Turkey or Tunisia.[20–23] Similarly, neither Riazalhosseini *et al*.[24] nor Najmabadi *et al*.[11] have identified Δ(*GJB6*-D13S1830) in Iran, suggesting a recent founder effect for this deletion in populations of western Europe.

SLC26A4

SLC26A4 at the DFNB4 locus encodes an iodide/chloride symporter known as pendrin that mediates the electro-neutral exchange across plasma membranes of Cl^-/HCO_3^- in the inner ear and Cl^-/I^- in the thyroid. Mutations cause either ARNSHL or Pendred syndrome (PS), both of which are characterized by SNHL and enlargement of the vestibular aqueduct (EVA) or Mondini malformation, with an iodine organification deficiency and goiter additionally seen with Pendred syndrome. Over 300 mutations have been identified in *SLC26A4* gene (http://www.hgmd.cf.ac.uk/ac/gene). It is the second leading cause of ARNSHL in many populations, including Iran, where its reported prevalence ranges from 4.8% to 18% (Table 2).[42,43]

MYO15A

Unconventional myosins differ from conventional myosins by virtue of long N-terminal extensions preceding the conserved motor domain. The protein encoded by *MYO15A* serves in intracellular transport and is essential for the organization and maturation of stereociliary hair bundles. Its deficiency results in

Table 1. *GJB2*-related deafness in Iran.

Author/year [Ref]	Mutations %	Method	Sample size in patients (families)	Origin (Country, Ethnic group or Province)
Najmabadi *et al.*/2002[6]	11	ASPCR, SSCP and direct sequencing	168 (83)	Throughout the country
Mahdieh *et al.*/2004 [24]	22	ASPCR, DHPLC, sequencing	229 (86)	Kurd
Najmabadi/2005 [9]	16.7	ASPCR, DHPLC and direct sequencing	(664)	Throughout the country
Riazalhosseini *et al.*/2005 [25]	18.2	ASPCR, DHPLC and direct sequencing	385	Throughout the country
Hashemzadeh Chaleshtori *et al.*/2007 [26]	14.6	Nested PCR and direct sequencing	1095 (890)	10 provinces of Iran
Naghavi et al./2008 [16]	18 (11% Baluchi, 7.2% Sistani)	ASPCR, DHPLC and directed sequencing	100	Sistan and Baluchestan
Bonyadi *et al.*/2009 [27]	28	ARMS-PCR, SSCP and sequencing	(209)	Azeri Turkish
Galehdari *et al.*/2009 [28]	0	Direct sequencing	61	Southwest Iran (Arabian origins)
Hamid *et al.*/2009 [29]	33.3	Direct sequencing	50 (33)	Throughout the country
Motasaddi Zarandy *et al.*/2011 [30]	31	ARMS-PCR	201	Throughout the country
Daneshi *et al.*/2011 [31]	19.9	Nested PCR and direct sequencing	166	Throughout the country
Mahdieh *et al.*/2011 [32]	17.9	Direct sequencing	114 (77)	Throughout the country
Tabatabaiefar *et al.*/2011 [33]	16.2	Direct sequencing and linkage analysis	(37)	Chaharmahal and Bakhtiari, Fars, Gilan, Tehran, Khuzestan, East Azerbaijan, and Kurdistan
Bazazzadegan *et al.*/2012 [34]	16	ARMS-PCR and direct sequencing	2322	Throughout the country
Davarnia *et al.*/2012 [35]	26	ARMS-PCR and direct sequencing	(50)	Ardabil
Bonyadi *et al.*/2014 [36]	31.8	ARMS-PCR (35delG), SSCP, PCR-RFLP (IVS1+1G>A) and direct sequencing	508	Azeri Turkish
Zeinali *et al.*/2015 [37]	19.4	ARMS-PCR and direct sequencing	418	Throughout the country
Mahdieh *et al.*/2015 [38]	14.5	ARMS-PCR and direct sequencing	62	Ilam
Haghighat-Nia *et al.*/2015 [39]	11.8	Direct PCR-sequencing	220	Central Iran

Gene symbol/ Locus	Chromosomal location	Mutation type worldwide				Prevalent variants reported in Iran (Ref)
		Missense/ Nonsense	Splicing	Regulatory	Small del/ins and indels	
GJB2/ DFNB1, DFNA3A	13q11-q12	247*	2*	1*	69*	c.35delG: 6.3-74.5%[26,40]/ c.-23+1G>A: 15.7-16.5%[37,41]/ c.7+1G>A: 3.3%[37]/ c.35G>A: 2.8%, c.358_360delGAG: 2.8% and c.311_324del14: 2.2%[37]

*Ref: Stenson *et al.* (2003), The Human Gene Mutation Database (HGMD®): 2003 Update. Hum Mutat (2003) 21:577-581. PCR = polymerase chain reaction; ASPCR = Allele-specific PCR; SSCP = Single-strand conformation polymorphism; DHPLC = Denaturing high-per formance liquid.

Table 2. *SLC26A4*-related deafness in Iran.

Author/Year [Ref]	Mutations %	Methods	Sample size in patients (families)	Origin (Country, Ethnic group or Province)
Kahrizi *et al.*/2009 [44]	10	Homozygosity mapping and direct sequencing	(80)	Throughout the country
Babanejad *et al.*/2012 [43]	4.8	Homozygosity mapping and direct sequencing	(144)	Throughout the country
Reiisi *et al.*/2014 [45]	~ 7	Linkage analysis and direct sequencing	(30)	West of Iran
Yazdanpanahi *et al.*/2015 [46]	9.1	Linkage sequencing	(121)	Throughout the country
Sloan-Heggen *et al.*/2015 [42]	12.3	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country

Table 3. *MYO15A*-related deafness in Iran.

Author/Year [Ref]	Mutations %	Methods	Sample size in patients (families)	Origin (Country, Ethnic group or Province)
Fattahi *et al.*/2012 [51]	5.7	Homozygosity mapping and direct sequencing	(140)	Throughout the country
Babanejad *et al.*/2012 [43]	4.8	Homozygosity mapping and direct sequencing	(144)	Throughout the country
Sloan-Heggen *et al.*/2015 [42]	9.6	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country

severe-to-profound congenital non-syndromic deafness and was first reported as the underlying cause of deafness endemic to an isolated village in Indonesia.[47]

Now recognized as a common cause of ARNSHL, in 2009, Shearer and colleagues reported the first *MYO15A* mutations causing ARNSHL in the Iranian population,[48] and more recently, Sloan-Heggen and colleagues found that *MYO15A* mutations accounted for 9.6% of the HL in a study of 302 Iranian families affected by ARNSHL (Table 3).[42] This prevalence is similar to that reported in neighboring Pakistan (5%) and Turkey (9.9%).[49,50]

MYO7A

Another unconventional myosin is myosin VIIA, encoded by *MYO7A*. It is expressed in both the ear and the eye, and consistent with this pattern, is associated with NSHL (DFNB2; DFNA11) and Usher syndrome (USH1B). *MYO7A* mutations account for ~5% of ARNSHL in Iran.[42] Interestingly, in a large family affected by ARNSHL caused by homozygosity for c.1184G>A (p.Arg395His), Hildebrand *et al*. noted phenotypic inconsistencies suggesting the existence of genetic modifiers of the DFNB2 phenotype.[52]

Other genes implicated in ARNSHL in Iran

In a large cohort of Iranian families (302) who underwent comprehensive testing for ARNSHL using targeted genomic enrichment and massively parallel sequencing of all genes implicated in NSHL, *CDH23* and *PCDH15* mutations also emerged as common causes of hearing loss, identified in 4.6% and 3% of families, respectively (Table 4).[42]

The extreme heterogeneity of ARNSHL in Iran is illustrated in Table 4. Many of the mutations are novel and present in homozygosity, consistent with the high coefficient of inbreeding and creating an extremely rich spectrum of genetic causes of NSHL that includes *CIB2*, *COL11A2*, *DFNB31*, *MARVELD2*, *TMIE*, *ESPN*, *GPSM2*, *GRXCR1*, *KCNQ1*, *OTOGL*, *RDX*, *PDZD7*, *STRC* and *TRIOBP*, as well as X-linked genes such as *PRPS1* and *POU3F4*.[42,60–62] ADNSHL, such as from DFNA5 mutation, has not been reported as a common cause of HHL in Iran[63]; however, a novel *GJB2* mutation (c.351G>A, p.Asp46Asn) has been identified in two families with ADNSHL from a village in northern Iran.[64]

Mitochondrial causes of genetic hearing loss

Mitochondrial-associated HL accounts for about 1% of prelingual deafness and is characterized by extreme pleiotropy. Individuals carrying the A1555G mutation in *MTRNR1*, for example, can have hearing thresholds in the normal to severe-to-profound range with loss that presents at birth or in late adulthood, suggesting the presence of major modulators of the phenotype.[65] Amongst 152 unrelated families from five Iranian provinces and of four ethnic backgrounds, two (1.3%) segregated the A1555G mutation, consistent with data reported for Caucasian populations.[66] The frequency of two other mtDNA mutations, A3243G and A7445G, was much lower (0.1%).[66]

Genetic causes of syndromic hearing loss

Amongst the most common causes of SHL worldwide are Usher syndrome and Pendred syndrome (Table 5).[67]

Pendred syndrome accounts for 1%–10% of HHL, and in Iran, it appears to be the most common cause of SHL.[44] Most causal genetic variants are identified in *SLC26A4*, with a possible founder mutation (c.965insA) in northwest Iran.[68–70] The latter finding highlights the necessity of screening *SLC26A4* when phenotypes such as an enlarged vestibular aqueduct or goiter are present.[71]

Usher syndrome, with an estimated prevalence of 3.2–6.2 per 100000,[72] is responsible for up to 5% of congenital HL and >50% of deaf-blindness.[73] Twelve genes cause Usher syndrome,[74] with five additionally implicated in NSHL (*MYO7A*, *USH1C*, *CDH23*, *PCDH15*, and *WHRN*). Mutations in *ADGRV1* (*GPR98*) are associated with Usher syndrome 2, with a large deletion recently reported in Iran.[75,76]

Other types of SHL include Brown-Vialetto-Van Laere syndrome (BVVLS),[77] Wolfram syndrome (WFS), also called DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness),[78] and distal renal tubular acidosis (dRTA). Although the precise prevalence of dRTA is not known, it appears to be more common in Iran than in Western countries. A recessive subtype of dRTA associated with anemia is frequently diagnosed in infants and young children who present with progressive hearing loss.[79] In 10 unrelated deaf Iranian families with dRTA, both reported and novel *ATP6V1B1* mutations were identified.[80]

Waardenburg syndrome (WS) is one of the most prevalent forms of ADSHL in Iran. Its estimated prevalence is 1 in 42,000, and it may account for 1%–4% of severe-to-profound HL.[81] In a group of Iranian patients (12 patients/4 unrelated families) with WS1, both reported and novel *PAX3* mutations were found.[82]

Interestingly, a contiguous gene deletion syndrome, loss of *CATSPER2* and *STRC*, which leads to deafness-infertility syndrome (DIS), has been identified in Iranian families.[83,84]

Transition in molecular diagnosis in Iran: From linkage analysis to massively parallel sequencing for gene discovery

Traditional approaches to novel gene discovery for hearing loss have been based on genome-wide linkage analysis to identify deafness loci followed by a variety of methods to fine map the

Table 4. Other genes frequently causing autosomal recessive hearing loss (ARHL) in Iran.

Gene	Locus	Author/Year [Ref]	Mutations %	Method	Sample size in patients (families)	Origin (Country, Ethnic group or Province)
TECTA	DFNB21	Meyer *et al.*/2007 [53]	6.7	Homozygosity mapping and direct sequencing	(45)	Throughout the country
		Babanejad *et al.*/2012 [43]	2.7	Homozygosity mapping and direct sequencing	(144)	Throughout the country
		Alasti *et al.*/2008 [54]	1.3	Genotyping and sequencing	(75)	Throughout the country
		Sloan-Heggen *et al.*/2015 [42]	1.3	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
TMC1*	DFNB7/11	Babanejad *et al.*/2012 [43]	2	Homozygosity mapping and direct sequencing	(144)	Throughout the country
		Davoudi-Dehaghani *et al.*/2013 [55]	2.2	Homozygosity mapping and direct sequencing	159 (54)	Throughout the country
		Sloan-Heggen *et al.*/2015 [42]	2	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
ILDR1	DFNB42	Babanejad *et al.*/2012 [43]	2.8	Homozygosity mapping and direct sequencing	(144)	Throughout the country
		Sloan-Heggen *et al.*/2015 [42]	2	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
LRTOMT	DFNB63	Taghizadeh *et al.*/2013 [56]	0	PCR- SSCP and direct sequencing	157	East Azarbaijan, Kurdistan, Gilan and Golestan
		Babanejad *et al.*/2012 [43]	1.4	Homozygosity mapping and direct sequencing	(144)	Throughout the country
		Sloan-Heggen *et al.*/2015 [42]	1.3	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
OTOF	DFNB9	Mahdieh *et al.*/2012 [57]	2.6	Autozygosity mapping and direct sequencing	(38 *GJB2* or *GJB6*-negative)	Throughout the country
		Babanejad *et al.*/2012 [43]	0.7	Homozygosity mapping and direct sequencing	(144)	Throughout the country
		Sloan-Heggen *et al.*/2015 [42]	1	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
CDH23**	DFNB12	Sloan-Heggen *et al.*/2015 [42]	4.6	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
PCDH15**	DFNB23	Sloan-Heggen *et al.*/2015 [42]	3	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
PJVK	DFNB59	Babanejad *et al.*/2012 [43]	1.4	Homozygosity mapping and direct sequencing	(144)	Throughout the country
		Sloan-Heggen *et al.*/2015 [42]	2	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
		Hashemzadeh Chaleshtori *et al.*/2007 [58]	~6.7	Direct sequencing	(30 *GJB2*-negative)	Chaharmahal and Bakhtiari, Gilan, Khuzestan, East Azerbaijan, Kurdistan and Tehran
USH2A**	USH2A	Sloan-Heggen *et al.*/2015 [42]	2.3	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
OTOA**	DFNB22	Sloan-Heggen *et al.*/2015 [42]	2	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
CABP2**	DFNB93	Sloan-Heggen *et al.*/2015 [42]	1.7	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
TMPRSS3**	DFNB8/ 10	Sloan-Heggen *et al.*/2015 [42]	1.7	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
GIPC3**	DFNB15/72/95	Sloan-Heggen *et al.*/2015 [42]	1.3	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
ADGRV1**	_	Sloan-Heggen *et al.*/2015 [42]	1	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
LHFPL5**	DFNB66/67	Sloan-Heggen *et al.*/2015 [42]	1	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
MYO6**	DFNB37	Sloan-Heggen *et al.*/2015 [42]	1	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
PTPRQ**	DFNB84	Sloan-Heggen *et al.*/2015 [42]	1	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country
USH1C**	DFNB18	Sloan-Heggen *et al.*/2015 [42]	1	Custom targeted genomic enrichment (TGE) panel	(302 *GJB2*-negative)	Throughout the country

*A potential genetic modifier effect has been reported.[59] **Indicates the first report of the gene as causative in the Iranian population.

Table 5. General delineation of syndromic hearing loss reported in Iran.

•Phenotype	Gene symbols
• **Pendred syndrome** • Phenotypic variety of hearing loss with or without other findings such as goiter	*SLC26A4*, *FOXI1*, *KCNJ10*
• **Usher syndrome** • Combination of hearing defects, vision impairments and intermittent vestibular dysfunction	*MYO7A*, *USH1C*, *CDH23*, *PCDH15*, *SANS*, *CIB2*, *USH2A*, *VLGR1*, *WHRN*, *CLRN1*, *PDZD7*, *GPR98*
• **Brown-Vialetto-Van Laere syndrome** • Hearing loss and a variety of cranial nerve palsies	*SLC52A3*, *SLC52A2*
• **Wolfram syndrome** • Neuroendocrine degenerative disorders including diabetes insipidus, early-onset diabetes mellitus, optic atrophy and deafness	*WFS1*, *CISD2*
• **Distal renal tubular acidosis** • A disorder of impaired net acid secretion by the distal tubule characterized by hyperchloremic metabolic acidosis with progressive and irreversible deafness in some cases	*ATP6B1*, *ATP6V0A4*, *SLC4A1*
• **Waardenburg syndrome** • Hearing impairement with minor defects in structures accruing from the neural crest, such as pigmentation anomalies of hair, skin and eyes	*PAX3*, *MITF*, *SNAI2*, *EDNRB*, *EDN3*, *SOX10*
• **Jervell and Lange-Nielsen syndrome** • Long QT syndrome (see this term) characterized by congenital profound bilateral sensorineural hearing loss	*KCNQ1*, *KCNE1*

locus and screen the identified candidate genes. In families segregating ARNSHL, the power of homozygosity mapping was exploited, identifying shared regions of homozygosity within or across families.[85] Nevertheless, the required work and time investment were often substantial and measured in years. It is now possible to circumvent many of these steps and in suitable families, after mutations in known SNHL genes have been excluded, whole exome sequencing can be performed using appropriate filtering metrics to expeditiously map (if needed) and identify novel deafness-causing genes (Figure 3).

The genetic diagnoses of NSHL traditionally focused on a few genes such as *GJB2* using very limited but cost-effective techniques that included allele-specific PCR (ASPCR), amplification-refractory mutation system PCR (ARMS PCR) and single strand conformational polymorphism analysis (SSCP), as well as Sanger sequencing[6] (Table 1). Unfortunately, the diagnostic rate was exceedingly low (basically only *GJB2*-positive cases), and more widespread adoption of these methods was precluded by the large number of genes that needed to be screened and the cumulative total expense and time required.

New technologies have drastically changed this approach and

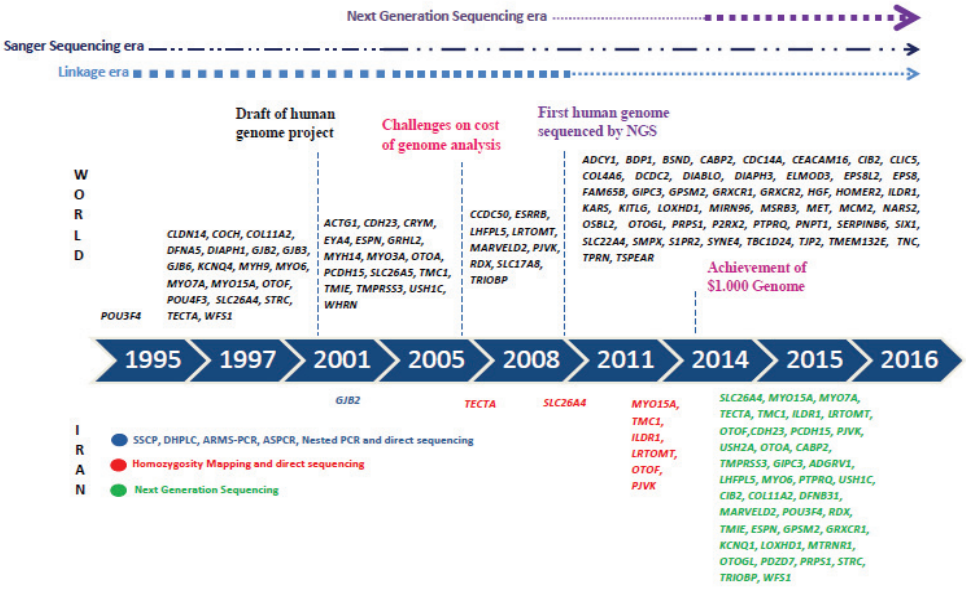

Figure 3. Method discovery timeline for autosomal nonsyndromic hereditary hearing loss[85] [***Source:*** http://hereditaryhearingloss.org/ (Accessed June 2016)].

have made comprehensive genetic testing using targeted genomic enrichment and massively parallel sequencing the preferred and most cost-effective test in the clinical evaluation of deafness after an audiogram. The power and the necessity of using targeted genomic enrichment and massively parallel sequencing were recently demonstrated in a screening of 302 *GJB2*-negative Iranian families from 12 different ethnic groups in which 179 deafness-causing variants, including 110 novel single nucleotide or small insertion-deletion variants, were identified in 40 genes (genetic diagnosis 68%).[42]

Messages for the Iranian healthcare system

Because of the high burden of deafness, the second most frequently occurring disability in Iran,[43] HHL prevention should have a specific focus in the comprehensive national program for non-communicable diseases control. Dedicated facilities for cost-effective genetic testing with appropriate complementary counseling services, including reproductive risk assessment and public education programs, are essential to link families who need this care with the potential benefits to be derived from genetic testing for carrier detection, prenatal diagnosis (PND), preimplantation genetic diagnosis (PGD), and genetic screening.

Based on the data presented in this paper, a cost-effective genetic testing method should focus on targeted genomic enrichment and massively parallel sequencing of all genes implicated in syndromic and non-syndromic hearing loss in Iran. This program can be effectively implemented by empowering the family physician to serve as a gate-keeper, provide family awareness, and refer identified families to special teams of specialists with focused training on hereditary hearing loss. The cost-effective and easy-to-access genetic testing strategy envisioned would require support through appropriate funding investment by the Ministry of Health and Medical Education.

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