



Brief Report of Variants Detected in Hereditary Hearing Loss Cases in Iran over a 3-Year Period

*Niloofer BAZAZADEGAN¹, Raheleh VAZEHAN², Mahsa FADAEI², Zohreh FATTAHI¹, Ayda ABOLHASSANI², Elham PARSIMEHR², Zahra KALHOR², Mehrshid FARAJI ZONOOZ², Fatemeh AHANGARI², Shima DEHDAHSI², Farshide SAMIEE³, Payman JAMALI⁴, Haleh HABIBI⁵, Younes NOURIZADEH⁶, Shokouh MAHDAVI⁷, Maryam BEHESHTIAN¹, Ariana KARIMINEJAD², Richard JH SMITH⁸, *Hossein NAJMABADI²*

1. Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
2. Kariminejad-Najmabadi Pathology & Genetics Center, Tehran, Iran
3. Genetic Medical Counseling Center, Qazvin, Iran
4. Shabrood Genetic Counseling Center, Welfare Office, Semnan, Iran
5. Genetic Counseling Center, Family Health Clinic, Mobasher Hospital, Hamedan, Iran
6. Genetic Counseling Center Welfare Organization, Ilam, Iran
7. Welfare Institution Genetic Office, Tehran, Iran
8. Molecular Otolaryngology & Renal Research Laboratories, Department of Otolaryngology-Head and Neck Surgery, University of Iowa Carver College of Medicine, Iowa City, IA, USA

*Corresponding Author: Email: hnajm12@yahoo.com

(Received 10 Apr 2018; accepted 19 Jul 2018)

Abstract

Background: Diagnosis of hereditary hearing loss (HHL) as a heterogeneous disorder is very important especially in countries with high rates of consanguinity where the autosomal recessive pattern of inheritance is prevalent. Techniques such as next-generation sequencing, a comprehensive genetic test using targeted genomic enrichment and massively parallel sequencing (TGE + MPS), have made the diagnosis more cost-effective. The aim of this study was to determine HHL variants with comprehensive genetic testing in our country.

Methods: Fifty *GJB2* negative individuals with HHL were referred to the Kariminejad-Najmabadi Pathology and Genetics Center, Tehran, one of the reference diagnostic genetic laboratories in Iran, during a 3-year period between 2014 and 2017. They were screened with the OtoSCOPE test, the targeted genomic enrichment and massively parallel sequencing (TGE + MPS) platform after a detailed history had been taken along with clinical evaluation.

Results: Among 32 out of 50 *GJB2* negative patients (64%), 34 known pathogenic and novel variants were detected of which 16 (47%) were novel, identified in 10 genes of which the most prevalent were *CDH23*, *MYO7A* and *MYO15A*.

Conclusion: These results provide a foundation from which to make appropriate recommendations for the use of comprehensive genetic testing in the evaluation of Iranian patients with hereditary hearing loss.

Keywords: OtoSCOPE; Hereditary hearing loss; Novel variant; Known variant

Introduction

Genetic causes underlie up to 80% of prelingual hearing loss, one of the most prevalent birth defects (1). To date, more than 150 loci, i.e. about 90 genes, have been reported in non-syndromic hearing loss (<http://www.hereditaryhearingloss.org>).

Iran has a heterogeneous population with a high rate of consanguineous marriages (2). Such populations can be considered to be unique resources of recessive rare genetic disorders. Although HHL is not an uncommon defect, the genetic heterogeneity makes many gene-specific HL types quite rare (3). With regard to the high rate of consanguinity in Iran, which increases the risk of recurrence of autosomal recessive forms of genetic disorders such as deafness, *GJB2* mutations are the most prevalent cause of HL among several genes related to autosomal recessive non-syndromic hearing loss (ARNSHL) (4-6). Other AR genes which are in the high prevalence category are *SLC26A4*, *MYO15A*, *MYO7A*, *CDH23*, and *PCDH15* (7).

The fact that early diagnosis in HHL cases may be helpful in prevention and treatment elucidates the need to find the most efficient and cost-effective way to investigate the genetic causes of HL in every population. Hence, this report represents our experience of investigating 50 *GJB2* negative HL cases using the OtoSCOPE test, which can screen all the genes involved in hearing loss at once.

Materials and Methods

Fifty *GJB2* negative individuals with HHL were referred to the Kariminejad-Najmabadi Pathology and Genetics Center, Tehran, one of the reference diagnostic genetic laboratories in Iran, during a 3-year period between 2014 and 2017. Their detailed history was taken along with a clinical evaluation.

All patients completed consent forms and their family pedigrees were drawn to determine the pattern of inheritance.

Hearing thresholds were measured by pure-tone audiometry following standard protocols (8). The targeted genomic enrichment and massively parallel sequencing (TGE + MPS) platform was updated from v6 to v8 as part of our standard operating procedure, increasing the number of genes screened from 116 to 152, using custom-designed Sure Design capture technology (Agilent Technologies, Santa Clara, CA, USA). All data were filtered and analyzed using a variety of in silico mutation prediction programs including PhyloP, SIFT, LRT, Mutation taster, PolyPhen (HDIV) and GERP (9). Annotated variants were also considered from the Deafness Variation Database (deafnessvariationdatabase.org).

All results were discussed at a multidisciplinary meeting. The variants in each patient were discussed individually and, in the context of unique clinical information, the most comprehensive diagnosis was provided. Positive results were confirmed via Sanger sequencing before reporting.

Results

Overall, 50 individuals with HHL were enrolled. About 30% of probands had prelingual HL with non-syndromic phenotype. After data analysis, 34 HHL variants were detected in 32 out of the 50 individuals (Table 1) while 18 did not show any hearing loss related variants. The causal variant was detected in 24 out of 33 consanguineous cases. Sixteen new variants were detected among all HL related variants in this study. Four patients had retinitis pigmentosa (RP) with HL in three causative genes, *MYO7A*, *CDH23* and *USH2A*, with novel and known variants. Auditory neuropathy was associated with one novel variant in the *OTOF* gene (Table 1).

Table 1: Detected novel and known variants in 32 patients

Patient ID	Consanguinity	Gene	Nucleotide change	Protein change	Zygosity	Pathogenicity prediction	OtoSCOPE version	Observed features	Reported phenotype (MIM #)	Known/Novel variant [Reference]
D68 567	Yes	<i>MYO7A</i>	c.1708C>T	p.Arg570*	Homozygous	4/5	V6	HL	Deafness, autosomal dominant 11 Deafness [MIM#601317]; autosomal recessive 2 [MIM#600060]; Usher syndrome; type 1B [MIM#276900]	Known variant (10)
D79 212	Yes		c.1708C>T	p.Arg570*	Homozygous	4/5	V7	HL		Known variant (10)
D79 453	No		c.5215C>T	p.Arg1739*	Homozygous	4/5	V7	HL		Known variant (11)
D72 929	Yes		c.3564_3570 del-TGCCCGG	p.Tyr1188*	Homozygous	ND	V6	HL		Known variant (12)
D78 454	Yes		c.5567delG	p.Arg1856Profs*23	Homozygous	ND	V7	HL+CHD		Novel
D82 779	Yes		c.6028G>T	p.Asp2010Tyr	Homozygous	6/6	V7	HL+RP		Novel
D87 273	No		c.75_82delG GCGGTGG / c.3718C>T	p.Ala26Glufs*13 / p.Arg1240Trp	Heterozygous/ Heterozygous	ND/ 5/6	V8	HL+RP		Novel/ Known variant (11)
D63 292	Yes	<i>CDH23</i>	c.3491delG	p.Leu1166Trpfs*11	Homozygous	ND	V6	HL+RP	Deafness; autosomal recessive 12 [MIM#601386]; Usher syndrome; type 1D; Usher syndrome, type 1D/F digenic [MIM#601067]	Novel
D83 195	Yes		c.4562A>G	p.Asn1521Ser	Homozygous	5/6	V7	HL		Known variant (13)
D80 835	Yes		c.2897G>A	p.Arg966His	Homozygous	6/6	V7	HL		Novel
D86 014	No		c.1064C>A	p.Thr355Asn	Homozygous	5/6	V8	HL		Novel
D88 410	Yes		c.5908G>A	p.Glu1970Lys	Homozygous	4/4	V8	HL		Known variant (14)
D79 868	No	<i>MYO15A</i>	c.3956C>G	p.Ser1319Cys	Homozygous	5/5	V7	HL	Deafness, autosomal recessive 3 [MIM#600316]	Novel
D85 556	No		c.3867-1G>A / c.5810G>A	- / p.Arg1937His	Heterozygous/ Heterozygous	3/4 / 3/5	V8	HL		Novel/ Known variant (15)
D86 357	Yes		c.9437A>C	p.His3146Pro	Homozygous	3/5	V8	HL		Novel
D81 653	Yes	<i>USH2A</i>	c.2944_2945insT	p.Cys982Leufs*2	Homozygous	ND	V7	HL+RP	Usher syndrome, type 2A [MIM#276901]	Known variant (16)
D88 377	Yes		c.7501C>T	p.Gln2501*	Homozygous	4/4	V8	HL		Known variant (17)
D69 627	Yes		c.13792C>T	p.Gln4598*	Homozygous	ND	V6	HL		Novel
D86 480	Yes	<i>CDC14A</i>	c.1033C>T	p.Arg345*	Homozygous	2/4	V8	HL	Deafness, autosomal recessive 105 [MIM#616958]	Novel
D87 154	No		c.1126C>T	p.Arg376*	Homozygous	3/4	V8	HL		Known variant (18)
D75 660	Yes	<i>OTOF</i>	c.1981dupG	p.Asp661Glyfs*2	Homozygous	ND	V6	HL	Auditory neuropathy, autosomal recessive, 1; Deafness, autosomal recessive 9 [MIM#601071]	Known variant (19)
D79 455	Yes		c.2680G>A	p.Glu894Lys	Homozygous	6/6	V7	HL		Novel
D85 222	Yes	<i>SLC26A4</i>	c.1226G>A	p.Arg409His	Homozygous	6/6	V8	HL	Deafness, autosomal recessive 4, with enlarged vestibular aqueduct [MIM#600791]; Pendred syndrome [MIM#274600]	Known variant (20)
D87 275	Yes		c.882_883delCA	p.His294GlnfsTer35	Homozygous	ND	V8	HL		Known variant (21)
D79 301	No	<i>PAX3</i>	Deletion of exons 1-4	-	Heterozygous	ND	V7	HL+Heterochromatridis + White forelock	Craniofacial-deafness-hand syndrome [MIM#122880]; Waardenburg syndrome, type 1 [MIM#193500]; type 3 [MIM#148820]	Novel
D84 787	Yes	<i>COL11A2</i>	c.966dupC	p.Thr323Hisfs*19	Homozygous	ND	V7	HL		Deafness, autosomal recessive 53 [MIM#609706]; Deafness, autosomal dominant 13 [MIM#601868]; Otopondylomegapiphysal dysplasia, autosomal dominant [MIM#184840]; Otopondylomegapiphysal dysplasia, autosomal recessive [MIM#215150]
D68 163	No	<i>AIFM1</i>	c.1264C>T	p.Arg422Trp	Hemizygous	3/3	V6	HL	Deafness, X-linked 5 [MIM#300614]	Known variant (22)
D73 555	Yes	<i>TMC1</i>	Duplication of exons 9-12	-	Homozygous	ND	V7	HL		Deafness, autosomal recessive 7 [MIM#600974]; Deafness, autosomal dominant 36 [MIM#606705]
D88 396	Yes	<i>KARS</i>	c.1097G>C	p.Cys366Ser	Homozygous	5/6	V8	HL	Deafness, autosomal recessive 89 [MIM#613916]	Novel
D86 742	Yes	<i>TMPR3</i>	c.1211C>T	p.Pro404Leu	Homozygous	6/6	V8	HL		Deafness, autosomal recessive 8/10 [MIM#601072]
D88 130	Yes	<i>MLAR-VELD2</i>	c.1498C>T	p.Arg500Ter	Homozygous	2/4	V8	HL	Deafness, autosomal recessive 49 [MIM#610153]	Known variant (24)
D73 519	Yes	<i>MITF</i>	c.640C>T	p.Arg214*	Heterozygous	3/4	V7	HL+White forelock		Waardenburg Syndrome type 2A [MIM#193510]; COMMAD syndrome [MIM#617306]; Tietz albinism-deafness syndrome [MIM#103500]; Waardenburg syndrome/ocular albinism, digenic [MIM#103470]

HL, Hearing loss; CHD, Congenital heart defect; RP, Retinitis pigmentosa; MIM, Mendelian Inheritance in Man; ND, Not determined

Variants with pathogenicity score were checked using a maximum of six computational methods (PhyloP, SIFT, LRT, Mutation taster, PolyPhenHDIV and GERP) to study conservation of missense variants and functional significance.

Discussion

In the present study, 50 individuals with HHL were studied. Because of the heterogeneity and the role of different loci and genes in HHL, an affordable technique was required to minimize the cost and time needed for diagnosis. OtoSCOPE was chosen for detection of causative variants related to hearing loss as it can screen all the genes involved in hearing loss at once. After evaluating with this test, 16 known variants were detected in 16 individuals in whom four showed retinitis pigmentosa (RP) and hearing loss with homozygous and compound heterozygous variants in *MYO7A*, *CDH23* and *USH2A* genes.

Twelve genes are known to cause Usher syndrome (26). In this study, only three causative genes, *MYO7A*, *CDH23* and *USH2A* with novel and known variants contributed to both RP and hearing loss. Two of these genes, *MYO7A* and *CDH23*, are among five genes involved in neurosensory hearing loss (26). Mutations in the *MYO15A* gene were seen in three affected individuals. Deficiency in the protein encoded by the *MYO15A* gene results in severe to profound congenital non-syndromic hearing loss (27).

Our patients with *MYO15A* gene mutations also had severe to profound phenotype. The first *MYO15A* mutations causing ARNSHL was reported in the Iranian population and believed this mutation to be a common cause of ARNSHL (28). Recently, *MYO15A* mutations accounted for 9.6% of HL in a study on 302 Iranian families affected by ARNSHL (3). In our study, 3/50 affected individuals had *MYO15A* gene mutations in which four novel and known variants were detected. This is very similar to other findings (29). One novel variant in the *USH2A* gene was detected in one individual, and one novel and one known variant were also detected in the *PAX3* and *MITF* genes, respectively, in two persons with Waardenburg syndrome and profound HL. Waardenburg syndrome is one of the most

prevalent forms of autosomal dominant syndromic hearing loss (ADSHL) in Iran. It may account for 1%-4% of severe-to-profound HL (30). Recently, *PAX3* mutations were reported in a group of Iranian patients with this syndrome (31). In our study *MYO7A*, *CDH23*, *MYO15A* and *USH2A* genes were the most prevalent genes with known and novel variants. Other genes with a high rate of mutations were *CDC14A*, *OTOF* and *SLC26A4*.

In our clinical diagnostic laboratory, we were able to diagnose a genetic cause of deafness in 32 out of 50 persons (64%). This rate ranged from 10% to 83% in several small cohort studies (32). This perhaps reflects the higher coefficient of inbreeding in our population, as in other populations with Middle Eastern ethnicity, where the diagnostic rate is higher (72%) (33). Other patients had no hearing loss related mutations, perhaps indicating the presence of other rare causative genes identified with future whole exome or whole-genome sequencing.

Conclusion

Platforms such as OtoSCOPE, providing comprehensive genetic screening for deafness, will allow clinicians to improve patient care by providing prognostic information, and in cases with both RP and hearing loss, offer families preventative strategies to minimize the rate of progression of retinitis pigmentosa.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

We would like to thank all the families included in this study for their time and generosity in supplying personal data.

Conflict of interest

The authors declare that there is no conflict of interests.

References

1. Smith R, Green G, Camp G (1999). Hereditary hearing loss and deafness overview. <https://www.medschool.lsuhs.edu/pediatrics/docs/Deafness%20and%20Hereditary%20Hearing%20Loss%20Overview.pdf>. Retrieved March, 13 1999.
2. Saadat M, Ansari-Lari M and Farhud D (2004). Short report consanguineous marriage in Iran. *Ann Hum Biol*, 31 (2): 263-269.
3. Sloan-Heggen CM, Babanejad M et al (2015). Characterising the spectrum of autosomal recessive hereditary hearing loss in Iran. *J Med Genet*, 52 (12): 823-829.
4. Bazazzadegan N, Nikzat N, Fattahi Z et al (2012). The spectrum of GJB2 mutations in the Iranian population with non-syndromic hearing loss—a twelve year study. *Int J pediatr Otorhinolaryngol*, 76 (8): 1164-1174.
5. Hashemzadeh Chaleshtori M, Farhud DD et al (2008). Molecular Pathology of 6 Novel GJB2 Allelic Variants Detected in Familial and Sporadic Iranian Non Syndromic Hearing Loss Cases. *Iran J Public Health*, 37(3):9-18.
6. Hashemzadeh Chaleshtori M, Montazer Zohour M et al (2006). Autosomal Recessive and Sporadic Non Syndromic Hearing Loss and the Incidence of Cx26 Mutations in a Province of Iran. *Iran J Public Health*, 35(1):88-91.
7. Beheshtian Maryam, Babanejad Moigan, Azaiez Hela et al (2016). Heterogeneity of Hereditary Hearing Loss in Iran: a Comprehensive Review. *Arch Iran Med*, 19 (10): 720-728.
8. Smith Rjh Fau - Shearer AE, Shearer Ae Fau - Hildebrand MS, Hildebrand Ms Fau - Van Camp G and Van Camp G (2014). Deafness and Hereditary Hearing Loss Overview BII - GeneReviews(R).
9. Shearer AE, DeLuca AP, Hildebrand MS et al (2010). Comprehensive genetic testing for hereditary hearing loss using massively parallel sequencing. *Proc Natl Acad Sci USA*, 107 (49): 21104-21109.
10. Yoshimura H, Iwasaki S, Nishio S-y et al (2014). Massively parallel DNA sequencing facilitates diagnosis of patients with Usher syndrome type 1. *PLoS One*, 9(3): e90688.
11. Cremers FP, Kimberling WJ, Külm M et al (2007). Development of a genotyping microarray for Usher syndrome. *J Med Genet*, 44 (2): 153-160.
12. Sommen M, Schrauwen I, Vandeweyer G et al (2016). DNA diagnostics of hereditary hearing loss: a targeted resequencing approach combined with a mutation classification system. *Hum Mutat*, 37 (8): 812-819.
13. Seco CZ, Wesdorp M, Feenstra I et al (2017). The diagnostic yield of whole-exome sequencing targeting a gene panel for hearing impairment in The Netherlands. *Eur J Hum Genet*, 25 (3): 308-314.
14. Sloan-Heggen CM, Bierer AO, Shearer AE et al (2016). Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. *Hum Genet*, 135 (4): 441-450.
15. Fattahi Z, Shearer AE, Babanejad M et al (2012). Screening for MYO15A gene mutations in autosomal recessive nonsyndromic, GJB2 negative Iranian deaf population. *Am J Med Genet A*, 158 (8): 1857-1864.
16. Stabej PLQ, Saihan Z, Rangesh N et al (2012). Comprehensive sequence analysis of nine Usher syndrome genes in the UK National Collaborative Usher Study. *J Med Genet*, 49 (1): 27-36.
17. Pierrache LH, Hartel BP, Van Wijk E et al (2016). Visual prognosis in USH2A-associated retinitis pigmentosa is worse for patients with Usher syndrome type IIa than for those with nonsyndromic Retinitis Pigmentosa. *Ophthalmology*, 123 (5): 1151-1160.
18. Delmaghani S, Aghaie A, Bouyacoub Y et al (2016). Mutations in CDC14A, encoding a protein phosphatase involved in hair cell ciliogenesis, cause autosomal-recessive severe to profound deafness. *Am J Hum Genet*, 98 (6): 1266-1270.
19. Mahdieh N, Shirkavand A, Rabbani B et al (2012). Screening of OTOF mutations in Iran: a novel mutation and review. *Int J Pediatr Otorhinolaryngol*, 76 (11): 1610-1615.
20. Van Hauwe P, Everett LA, Coucke P et al (1998). Two frequent missense mutations in Pendred syndrome. *Hum Mol Genet*, 7 (7): 1099-1104.
21. Yazdanpanahi N, Tabatabaiefar MA, Farrokhi E et al (2013). Compound heterozygosity for two novel SLC26A4 mutations in a large

- Iranian pedigree with Pendred syndrome. *Clin Exp Otorhinolaryngol*, 6 (4): 201-208.
22. Zong L, Guan J, Ealy M et al (2015). Mutations in apoptosis-inducing factor cause X-linked recessive auditory neuropathy spectrum disorder. *J Med Genet*, 52(8):523-31.
 23. Masmoudi S, Antonarakis SE, Schwede T et al (2001). Novel missense mutations of TMPRSS3 in two consanguineous Tunisian families with non-syndromic autosomal recessive deafness. *Hum Mut*, 18 (2): 101-108.
 24. Riazuddin S, Ahmed ZM, Fanning AS et al (2006). Tricellulin is a tight-junction protein necessary for hearing. *Am J Hum Genet*, 79 (6): 1040-1051.
 25. Nobukuni Y, Watanabe A, Takeda K et al (1996). Analyses of loss-of-function mutations of the MITF gene suggest that haploinsufficiency is a cause of Waardenburg syndrome type 2A. *Am J Human Genet*, 59 (1): 76-83.
 26. Lenarduzzi S, Voizzi D, Morgan A et al (2015). Usher syndrome: an effective sequencing approach to establish a genetic and clinical diagnosis. *Hear Res*, 320 18-23.
 27. Friedman TB, Liang Y, Weber JL et al (1995). A gene for congenital, recessive deafness DFNB3 maps to the pericentromeric region of chromosome 17. *Nat Genet*, 9 (1): 86-91.
 28. Shearer AE, Hildebrand MS, Webster JA et al (2009). Mutations in the first MyTH4 domain of MYO15A are a common cause of DFNB3 hearing loss. *Laryngoscope*, 119 (4): 727-733.
 29. Babanejad M, Fattahi Z, Bazazzadegan N et al (2012). A comprehensive study to determine heterogeneity of autosomal recessive nonsyndromic hearing loss in Iran. *Am J Med Genet A*, 158 (10): 2485-2492.
 30. Read AP, Newton VE (1997). Waardenburg syndrome. *J Med Genet*, 34 (8): 656-665.
 31. Jalilian N, Tabatabaiefar MA, Farhadi M et al (2015). Molecular and clinical characterization of Waardenburg syndrome type I in an Iranian cohort with two novel PAX3 mutations. *Gene*, 574 (2): 302-307.
 32. Shearer AE, Smith RJ (2015). Massively parallel sequencing for genetic diagnosis of hearing loss: the new standard of care. *Otolaryngol Head Neck Surg*, 153 (2): 175-182.
 33. Najmabadi H, Kahrizi K (2014). Genetics of non-syndromic hearing loss in the Middle East. *Int J Pediatr Otorhinolaryngol*, 78 (12): 2026-2036.