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ORIGINAL ARTICLE

# Whole exome sequencing identifies novel compound heterozygous pathogenic variants in the *MYO15A* gene leading to autosomal recessive non-syndromic hearing loss

**Akram Sarmadi[1,2] · Samane Nasrniya[1] · Sina Narrei[2] · Zahra Nouri[2] · Hamidreza Abtahi[3] · Mohammad Amin Tabatabaiefar[1,4,5]**

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## Abstract

Autosomal recessive non-syndromic hearing loss (ARNSHL) is a highly heterogeneous disease, for which more than 70 genes have been identified. *MYO15A* mutations have been reported to cause congenital severe-to-profound HL. In this study, we applied the whole exome sequencing (WES) to find the cause of HL in an Iranian family. A proband from an Iranian non-consanguineous family with hearing impaired parents, was examined via WES, after excluding *GJB2* mutations as the most common ARNSHL gene via Sanger sequencing. Co-segregation analysis of the candidate variant was done in the family members. Interpretation of variants was according to the American College of Medical Genetics and Genomics (ACMG) guidelines. WES results showed novel compound heterozygous variants (p.Arg1507Ter and p.Val2815Valfs*10) in the *MYO15A* gene. These two variants, residing in highly conserved regions, were found to be co-segregating in the family and fulfill the criteria of being categorized as pathogenic, according to the ACMG guidelines. Here, we report successful application of WES to identify the molecular pathogenesis of ARNSHL in a patient with ARNSHL, as an example of an extremely heterogeneous disease. In agreement with previous studies, *MYO15A* is regarded to be important in causing HL in Iran.

**Keywords** Compound heterozygous · Hearing loss · Whole exome sequencing · Iran · *MYO15A* · Pathogenic variant

## Introduction

Hereditary hearing loss (HL), as the most frequent neurosensory disorder, affects millions of people all over the world with an estimated 1–2 out of 1000 newborns affected (https://www.gov.uk/guidance/newborn-hearing-screening-programme-overview). Genetic factors are the main reason for HL, with more than 50% of cases having a genetic etiology. Non-syndromic HL (NSHL) account for 70% of all congenital HL with all Mendelian inheritance patterns. However, autosomal recessive non-syndromic hearing loss (ARNSHL) is the most frequent hereditary pattern, for which over 74 genes have been identified (The Hereditary Hearing Loss Homepage, http://hereditaryhearingloss.org/). In Iran, the frequency of congenital HL suggests it as the second most frequent disability following intellectual impairment [1]. Up to now, more than 163 different known genes (http://hereditaryhearingloss.org/), have been identified for NSHL and lots of studies have reported that *GJB2* gene mutations are the major cause of ARNSHL. In Iran, the prevalence of *GJB2* mutations (DFNB1) is variable in different

Akram Sarmadi, and Samane Nasrniya contributed equally to the study.

✉ Mohammad Amin Tabatabaiefar
tabatabaiefar@med.mui.ac.ir

1 Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

2 Genetics Department, Erythron Pathobiology and Genetics lab, Isfahan, Iran

3 Department of Otolaryngology, Al-Zahra Hospital, Isfahan University of Medical Sciences, Isfahan, Iran

4 Pediatric Inherited Diseases Research Center, Research Institute for Primordial Prevention of Noncommunicable Disease, Isfahan University of Medical Sciences, Isfahan 81746-73461, Iran

5 GenTArget Corp (GTAC), Deputy of Research and Technology, Isfahan University of Medical Sciences, Isfahan, Iran

populations, from 38% in the north regions to about 4–8% in the southern regions [2]. Variants of *GJB2*, *SLC26A4*, *MYO15A*, are the most common causes of HL in Iran as well as other parts of the world.

Variants in the *MYO15A* gene (OMIM#602666) are recognized as a common cause of HL, in the third place after *GJB2* and *SLC26A4* variants, in the Iranian population. *MYO15A*, residing at the DFNB3 locus, mapped on chromosome 17p11.2, including 66 exons which encompass 71 Kb of genomic DNA.

The *MYO15A* gene encodes the myosin XVA (Unconventional myosin-XVa) that is a large protein with 3530 amino acids in its longest form (MYO15_HUMAN, Q9UKN7, and NP_057323.3). Myosins are a large family of actin-dependent molecular motors that bind actin and use ATP to generate force for movement along actin filaments [3]. According to the presence or lack of exon 2, there are two alternatively spliced transcripts isoform, classes 1 and 2 respectively. The long isoform of the human myosin XVA consists of: an N-terminal domain (aa 1–1223), an ATPase motor head domain (aa 1224–1899), the neck region contains two light chain-binding motifs (IQ; aa 1909–1942), the tail region contains 2 MyTH4 (myosin tail homology 4) domains (aa 2066–2174 and 3051–3161), 2 FERM (band 4.1, ezrin, radixin, and moesin) domains (aa 2687–2867 and 3217–3497), SH3 (src homology 3) domain (aa 2865–2959), and a C-terminal class I PDZ-binding motif (Fig. 1a) [4].

Myosin XVA protein regulates actin bundle protrusion structure in stereocilia, the mechanosensory sound transducing organelles in the inner ear hair cells and is necessary for elongation and the maintenance of them [5, 6]. Whirlin regulates actin filament elongation by binding to SH3-MYTH4-FERM-domain-containing region of the MYO15A protein and its interaction plays a role in cohesion of stereocilia [7]. Mutations of the cargo binding MyTH4-FERM tandems of the myosin XVA are frequently reported to be associated with HL and is among the most prevalent genetic causes of ARNSHL. The majority of *MYO15A* variants have been reported in patients with severe-to-profound congenital NSHL phenotypes from consanguineous families in the Middle East and Southeast Asia [8].

The recent development of whole exome sequencing (WES) offers an unparalleled opportunity to identify causative mutations in HL, as an extremely heterogeneous disorders. It has the potential of discover new genes and variants [9].

In this study, we reported a non-consanguineous Iranian family with a history of profound sensorineural HL

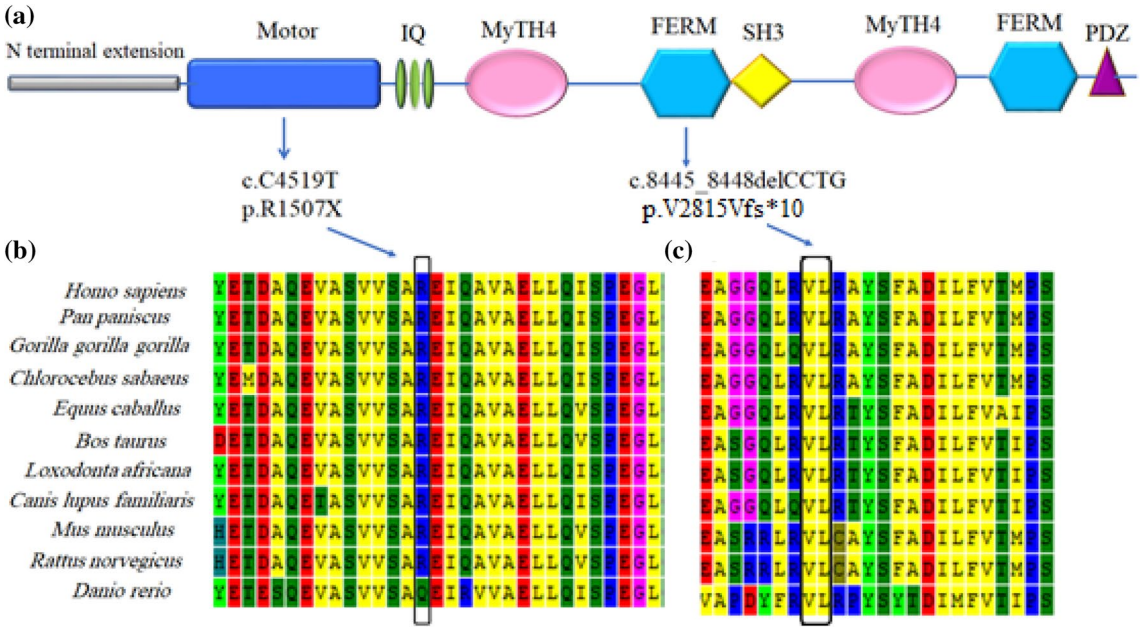



**Fig. 1** Schematic illustration of MYO15A protein domains and two mutations in the highly conserved regions. **a** Schematic illustration of MYO15A protein domains and location of mutations in MYO15A protein domains. **b** Multiple sequence alignment showed that p.R1507X is located in a highly conserved region. **c** Deletion of four bases, CCTG, lead to Valine and Leucine amino acids deletion in highly conserved regions and consequently modify MYO15A protein sequence. Changes in conserved amino acids in parts **b** and **c** are highlighted in black boxes

compatible with ARNSHL. We performed WES to provide molecular diagnosis and clarify the underlying genetic etiology of HL in the family.

## Materials and methods

### Subject and clinical evaluations

A seven-year-old boy (proband in the Fig. 2) with congenital HL from an Iranian non-consanguineous family was ascertained in this study. The parents and a younger brother suffered from bilateral Congenital HL too (the pedigree is shown in Fig. 2). Detailed clinical evaluation of the patient was performed. Family history and audiological tests consist of Play Audiometry, Tympanometry, acoustic stapedial reflex, Transient/Distortion Product Oto Acoustic Emission (TE/DPOAEs), Auditory Brainstem Response (ABR), Auditory Steady State Response (ASSR) and physical examination were ascertained. Written informed consent signed by the parents through interviews.

The study was approved by the Review Board of Isfahan University of Medical Sciences.

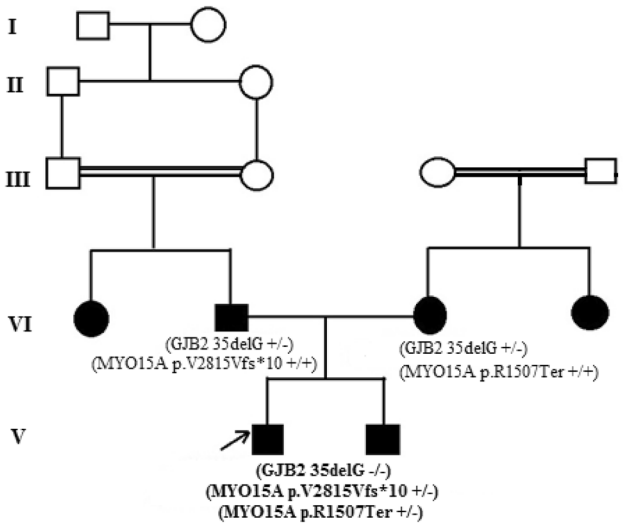



**Fig. 2** The pedigree of the family. The proband (V-1) is marked with an arrow. The co-segregation of GJB2 and MYO15A genes are shown by ±

### Molecular study

#### DNA extraction, *GJB2* sequencing

Genomic DNA was extracted using Prime Prep Genomic DNA Extraction kit from blood (GeNet Bio, South Korea) according to the manufacturer's instruction. Quality and quantity of DNA was assessed by agarose gel and Nanodrop 2000 instrument (Thermo Fisher Scientific Inc, USA), respectively. Sanger sequencing was performed in order to exclude mutations in exon 2 of the *GJB2* as the most common deafness gene. The forward and reverse primers sequences are listed in Table 1 [10].

After making sure there was no mutation in the *GJB2*, the patients' samples were applied for WES.

#### WES and bioinformatics analyses

About 300 ng of genomic DNA from the affected individual was prepared to carry out WES. The Sample was sent to Macrogen (South Korea) and was subjected to WES using the NovaSeq 4000 platform (Illumina, US). The mean depth of coverage was 100X for greater than 92% of targeted regions. These clean reads were then aligned to the reference human genome sequence (hg19) using the Burrows-Wheeler Aligner (BWA) (http://bio-bwa.sourceforge.net/). The GATK Software (https://www.broadinstitute.org/gatk/) was used for base quality re-calibration and variants calling. Single nucleotide polymorphisms (SNPs) and insertions/deletions were identified by the Sequence Alignment/Map tools (SAMtools). Variant filtering was performed based on MAF < 1% in dbSNP version 147, 1000 genomes project phase 3 database, NHLBI GO exome sequencing project (ESP), exome aggregation consortium (ExAC) and Iranome database for missense, nonsense, splicesite, stop loss, start codon change, frame-shift and in-frame indels.

Pathogenic prediction scores were calculated for non-synonymous variants to assess the influence of amino acid substitution on protein structure and function with Polyphen2 (http://genetics.bwh.harvard.edu/pph2), SIFT (http://sift.jcvi.org), MutationTaster (http://www.mutationtaster.org/), and MutationAssessor (http://mutationassessor.org). The American College of Medical Genetics and Genomics (ACMG) guidelines recommend that specific standard terminology is used to describe variants identified in genes that cause Mendelian disorders, including pathogenic, likely pathogenic, uncertain

**Table 1** Primers sequence using for *GJB2* exon 2 and *MYO15A* exon 13 and 46 sequencing

| Gene | Exon number | Forward primer (5′–3′) | Reverse primer (5′–3′) |
|---|---|---|---|
| *GJB2* | 2 | CTCCCTGTTCTGTCCTAGCT | CTCATCCCTCTCATGCTGTC |
| *MYO15A* | 13 | GCCCGGCCCTGTTTTTCATA | TTGTTCGAAGGCCAGATGCC |
| *MYO15A* | 46 | ATCCTCACACGCCCTTCATC | CAGTCTGTATCCCAGTCTCCAG |

significance, likely benign and benign variants. Thus, we used the ACMG guidelines to classify the variants [11].

### Variant confirmation

Direct Sanger sequencing was performed to confirm potential causative variants in the family. Then, co-segregation analysis was performed using exon-specific custom primers to examine segregation of the genotype and HL phenotype among the parents. PCR amplification and sequencing of the fragments were performed using the primers listed in Table 1.

Electropherograms were compared with reference sequence (NM_016239) using SeqMan software version 5.00 © (DNASTAR, Madison, WI, USA). Next, the variants were investigated in the Human Gene Mutation Database (HGMD) and the literature to seek the novelty of the variant or its association with HL.

## Results

### Clinical findings

The seven-year-old proband, his parents and younger brother all had congenital bilateral profound NSHL revealing by audiological reports and clinical manifestations. History and physical examination of the patients did not reveal any environmental factor as a cause of HL and confirmed non-syndromic form of HL.

### Molecular findings

Direct sequencing of the coding exon of the *GJB2* gene did not show any mutation. WES revealed compound heterozygous variants in the *MYO15A* gene including a previously reported stop gain variant, c.4519C > T (p.Arg1507Ter), and a novel frameshift variant, c.8445_8448delCCTG (p.Val2815Valfs*10). Both variants are located in highly conserved regions of the protein (Fig. 1b, c). Both were predicted to be deleterious by Mutation Taster as well as several other prediction tools (Table 2) and were also absent from dbSNP version 147, 1000 genomes project phase 3, NHLBI GO ESP, ExAC, Iranome, HGMD and Clinvar databases and the novel variant was not found in the literature, either. The variants were found to be co-segregating with the disease in the family: the stop gain mutation in exon 13 was found in the affected mother homozygously and the 4 nucleotide deletion in the exon 46 was found in the affected father homozygously too. Thus, the proband was compound heterozygous for variants. DNA sequence electropherogram of the family members are shown in the Fig. 3.

According to the ACMG guidelines, the variants could be classified as **pathogenic**.

In summary, **p.Arg1507Ter** variant met the PVS1, PS4, PM1, PM2, PM4 and PP3 criteria and **p.Val2815Valfs*10** variant met the PVS1, PM1, PM2, PM4 and PP3 criteria.

## Discussion

Non-syndromic hearing loss (NSHL) is a highly genetically heterogeneous disorder with more than 70 known causative genes [12]. *MYO15A* variants are documented as one of the most prevalent genetic causes of ARNSHL worldwide [13] and is recognized as a common cause of HL, in the third place after *GJB2* and *SLC26A4* variants in the Iranian population [14, 15].

The DFNB3 was introduced in 1995 for the first time as a locus related to deafness and was refined to chromosome 17p11.2. According to information provided in various databases, so far more than 700 variants have been reported in *MYO15A* from all over the world. However, professional HGMD has indicated that 272 of these variants are associated with disease. They involve a wide spectrum of mutation type include missense/nonsense (176), splicing [16], small deletion [17], small insertion [18], small indel [2], gross deletion [1] and complex [1] mutation (http://www.hgmd.cf.ac.uk/). Initially, most of the variants of *MYO15A* were found in patients with severe-to-profound congenital HL from consanguineous families in the Middle East and Southeast Asia [8]. After the development of high throughput technology in DNA sequencing, the frequency of the gene variants in other regions of the world have increased. The frequency of *MYO15A* gene mutations in Iran is about 5.5–9.6% [2, 15, 19]. Up to now, 45 pathogenic or likely pathogenic mutations have been reported in this gene from the Iranian population, including missense [20], small insertion or deletion (Indel) [8], splice site [6], nonsense [5] and deletion (3–1 inframe and 2 frameshift deletion) variant. The

**Table 2** In silico analysis of identified variants in the *MYO15A* gene in the WES results

| Gene & transcript | Variant & frequency | OMIM ID | Zygosity | CADD Score | SIFT-pred | Polyphen- pred | Inheritance | ACMG classification |
|---|---|---|---|---|---|---|---|---|
| *MYO15A* NM_016239 | c.4519C > T (p.Arg1507Ter) | 600316 DFNB3 | Het | 41 | Deleterious | Damaging | AR | Pathogenic |
| *MYO15A* NM_016239 | c.8445_8448delCCG (p.Val2815Valfs*10) | 600316 DFNB3 | Het | – | – | – | AR | Pathogenic |

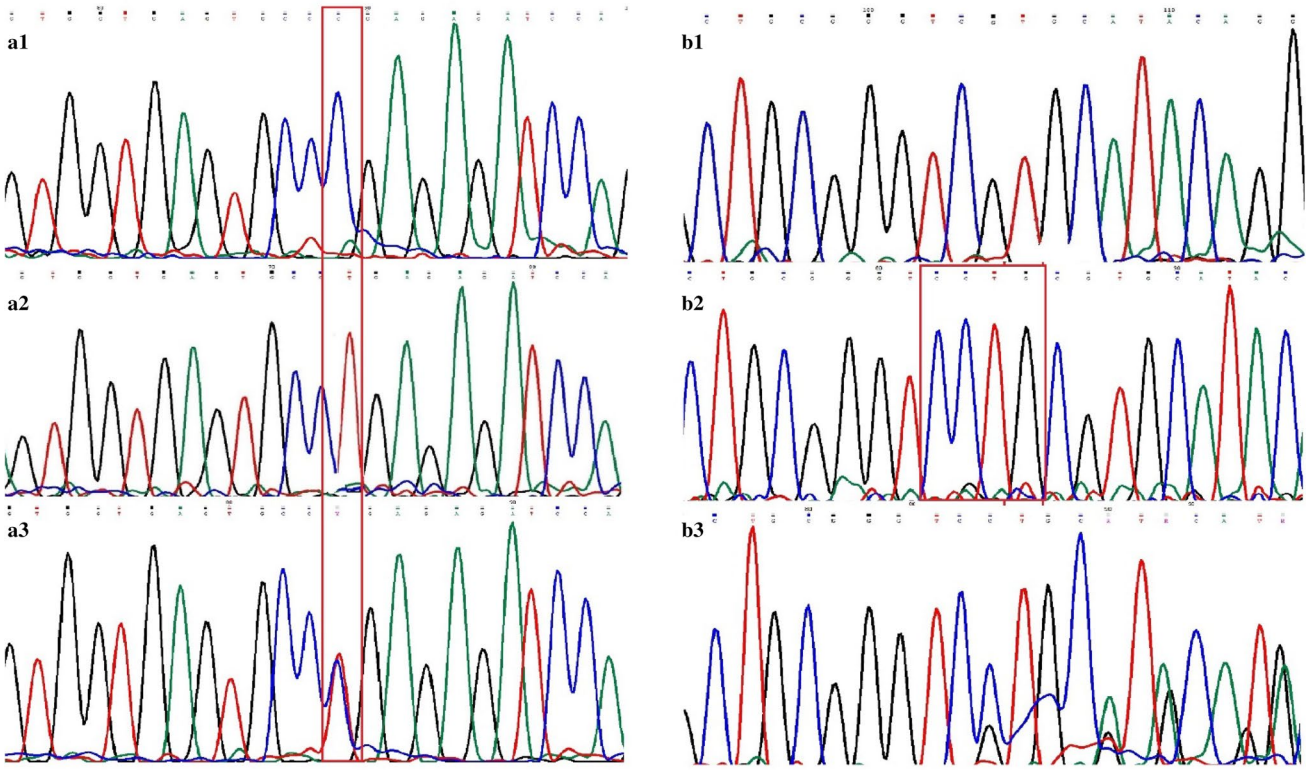
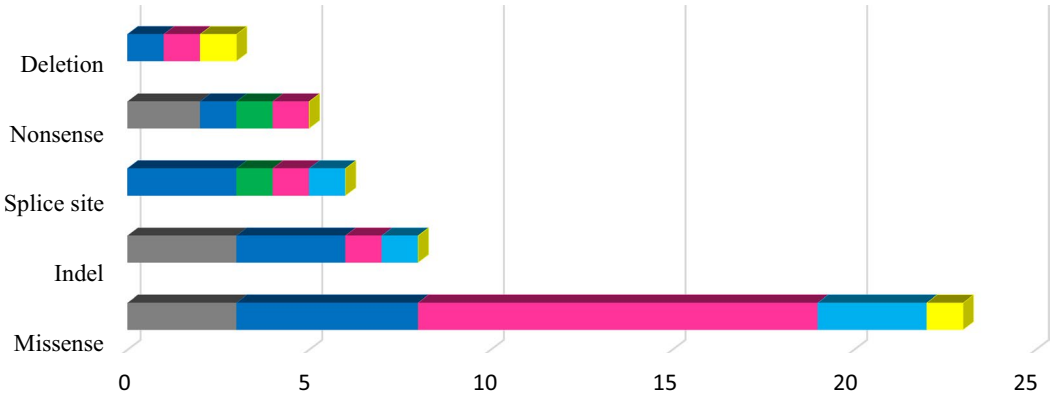



**Fig. 3** The electropherogram of the variants regions sequencing of the family. Sequencing of the stop gain variant (c.4519C > T, p.Arg1507Ter): **a1** father, without this variant homozygously. **a2** mother, with the homozygous variant (c.4519C > T). **a3** the proband, with the heterozygous variant. Sequencing of the frameshift variant (c.8445_8448delCCTG, p.Val2815Valfs*10): **b1** father, with this 4 nucleotide deletion homozygously. **b2** Mother, without this deletion homozygously. **b3** The proband, with the heterozygous variant, because of the deletion of this 4 nucleotides heterozygously, the electropherogram is messy

frequency of these mutations and their location in different domains of the MYO15A protein is shown in Fig. 4. Most of the *MYO15A* variants in the Middle East and South Asia were homozygous, most likely because of the custom of consanguineous marriage in these areas. While, in Southeast Asia and Europe more compound heterozygous variants have been identified [21].

In this study, we provided some lines of evidence for pathogenicity of two found variants. We performed a co-segregation analysis along with computational analysis to improve our diagnostic value.

Many studies have shown that mutations in *MYO15A* gene lead to severe-to-profound congenital HL. While there are some reports noting that some variants in almost all six domains of the protein are correlated with a milder phenotype, specially pathogenic variants in the N-terminal domain cause milder types of HL than those in other domains of the protein [21, 22]. Mutations in the motor domain, as the largest domain, are more frequent [21]. This domain consists of ATP and actin binding sites, which can produce force for actin filaments movement. Numerous studies have emphasized the importance of motor and tail regions of myosin XVA in the proper structure and function of auditory system. Thus, variants affecting these regions lead to shorter stereocilia associated with HL phenotype [5]. However, the large N-terminal domain is critical for the maintenance of functional stereocilia and its proper function is essential for normal hearing [23]. The stop gain mutation in the motor domain, which is a previously reported variant, p.R1507Ter, results in a truncated non-functional protein missing part of the IQ, both MYTH4, both FERM, SH3 and PZD domains of myosin-XVA. The importance of the FERM and MyTH domains of MYO15A protein is highlighted by the HL suffering individuals who were homozygous for mutations in these domains. Several studies show that the most frequent pathogenic mutations causing HL are located in the motor, FERM, and MyTH4 domains of *MYO15A* gene [18, 24]. An overview of truncated protein mutations in different exons of the *MYO15A* gene is listed in Table 3. Notably, the N-terminal tail, motor domain, and two FERM domains are apparently more vital and mutations in the first several domains are more deleterious for protein functions [24]. The most frequent truncating mutations occur in the giant exon 2 of the protein that codes for the main part of the N-terminal domain. This shows that isoform 1 of the MYO15A protein, which contains exon 2, is critical for normal hearing in




| | Missense | Indel | Splice site | Nonsense | Deletion |
|---|---|---|---|---|---|
| N-terminal | 3 | 3 | 0 | 2 | 0 |
| Motor | 5 | 3 | 3 | 1 | 1 |
| IQ | 0 | 0 | 1 | 1 | 0 |
| MyTH4 | 11 | 1 | 1 | 1 | 1 |
| FERM | 3 | 1 | 1 | 0 | 0 |
| SH3 | 1 | 0 | 0 | 0 | 1 |

**Fig. 4** Spectrum of pathogenic mutations in *MYO15A* gene (DFNB3) in the Iran population. Different types of mutation including missense, Indel, splice site, nonsense and deletion are shown on the vertical axis and different types of domain of the MYO15A protein are shown in different colors

human. The mechanism(s) through which these mutations change the MYO15A function are currently unknown. However, the p.C1797Y mutation within the motor domain was described by *Rehman* et al., as the cause of profound HL due to its role in causing a truncated protein functionally preventing MYO15A from trafficking to its normal location at the tips of stereocilia [12]. Furthermore, most of the mutations resulting in truncating proteins do not lead to a functional protein as the result of the deletion of important downstream domains.

Our study was on a non-consanguineous Iranian family involving the parents and their two sons, suffering from HL. The parents had consulted a genetic counselor before marriage and the only genetic test they were prescribed was sequencing of *GJB2* and *GJB6* genes; because of high frequency of pathogenic mutations in these two genes. Both were heterozygous for *GJB2* 35delG mutation. Thus, there was a 25% chance of having a child with HL. As they were not relative, sequencing of other genes was not prescribed for them. It was necessary, however to recommend prenatal diagnosis for the 35delG variant in the GJB2 gene. After the birth of two hearing impaired children, the family was referred to our lab. The eldest son was selected to be tested using WES. The results reveal that he had two different mutations, a stop gain and a frameshift, in the *MYO15A* gene, causing severe-to-profound HL. As a result, it was found that parents had different homozygous variants in the same gene. In this regard, all offspring of this couple were expected to be compound heterozygous for these two mutations in *MYO15A* gene and to be hearing impaired. Therefore, for this couple, the birth of a hearing impaired child could not have been avoided even through application of preimplantation genetic diagnosis (PGD). However, in case the parents had been recommended WES before their marriage, their genetic etiology would have been determined and they could have made a more informed decision.

Interestingly, however, recent psychological Studies indicate that marital satisfactions in couples who are both deaf are higher than couples one of whom is hearing impaired [25]. In many deaf communities, the members do not consider their HL as a disability. Thus, many of them not only refuse to receive prenatal tests for their fetuses, but also evade having cochlear implants for their hearing impaired children [20].

Revolutionary advances in the field of genomics over recent decades trough comprehensive genetic analysis made it possible to detect the exact genetic cause of HL in most cases. With the great tendency of the deaf-to-deaf intermarriage, genetic testing can certainly help these couples to make the right decision before marriage as well as before pregnancy, with the aim of having a child without HL.

## Conclusion

In conclusion, given the genomic advancement and availability of the related technology, the genetic etiology of HL is being clarified more easily and less costly. Deaf couples

**Table 3** Overview of protein truncated mutations in *MYO15A* gene in different population of the world

| | Variant | Exon number | Phenotype | Reference | Population |
|---|---|---|---|---|---|
| 1 | p.Lys96Glufs*132 | 2 | NSHL | Brownstein [26] | Ashkenazi |
| 2 | p.Arg125Valfs*102 | 2 | NSHL | Brownstein [26] | Ashkenazi |
| 3 | p.Lys140Serfs*304 | 2 | NSHL | – | NA |
| 4 | p.Glu152Glyfs*82 | 2 | profound HL | Vozzi [27] | Qatar |
| 5 | p.Glu179Ter | 2 | profound HL | Park [28] | Korea |
| 6 | p.Glu209Ter | 2 | NSHL | Neveling [29] | Netherland |
| 7 | p.Pro286Serfs*15 | 2 | Severe HL | Zhang [21] | China |
| 8 | p.Tyr289Ter | 2 | Moderate to severe HL | Cengiz [22] | Turkey |
| 9 | p.Tyr349Ter | 2 | NSHL | Miyagawa [30] | Russa |
| 10 | p.Val378Alafs*66 | 2 | NSHL | Sommen [31] | German |
| 11 | p.Tyr380Metfs*64 | 2 | Pre-lingual Progressive HL | Vona [32] | German |
| 12 | p.Tye380Ter | 2 | NSHL | Vona [32] | NA |
| 13 | p.Gln396Argfs*36 | 2 | Moderate to severe HL | Bashir [17] | Pakistan |
| 14 | p.Gln396Prof*38 | 2 | NSHL | Miyagawa [30] | Russa |
| 15 | p.Gln404Ter | 2 | NSHL | Sommen [31] | German |
| 16 | p.Ser779Ter | 2 | NSHL | Moteki [33] | Japan |
| 17 | p.Ser819Ter | 2 | Severe HL | – | NA |
| 18 | p.Pro824Glnfs*39 | 2 | NSHL | https://databases.lovd.nl | NA |
| 19 | p.Pro839Argfs*20 | 2 | NSHL | – | NA |
| 20 | p.Trp920Ter | 2 | NSHL | Sloan-Heggen [15] | Iran |
| 21 | p.Gln1105Ter | 2 | Severe HL | Nal [24] | Pakistan |
| 22 | p.Arg1112Valfs*1124 | 2 | Severe HL | Nal [24] | Pakistan |
| 23 | p.Arg1113Valfs*12 | 2 | Severe HL | Nal [24] | Pakistan |
| 24 | p.Arg1129Ter | 2 | NSHL | Sommen [31] | German |
| 25 | p.Arg1169Ter | 2 | profound HL | Liburd [34] | Pakistan |
| 26 | p.Q1175insAfsX1188 | 2 | severe to profound HL | Li [35] | China |
| 27 | p.Ser1176Valfs*14 | 2 | Severe HL | Zhang [21] | China |
| 28 | p.Gln1229Ter | 3 | profound HL | Liburd [34] | Pakistan |
| 29 | p.Asp1232fsX1241 | 4 | profound HL | Liburd [34] | Pakistan |
| 30 | p.Thr1253fsX1277 | 5 | Severe HL | Nal [24] | Pakistan |
| 31 | p.His1290Alafs*25 | 6 | NSHL | Bai [36] | China |
| 32 | p.Tyr1392Ter | 9 | Severe HL | Nal [24] | Pakistan |
| 33 | p.Gln1425Ter | 10 | Severe HL | Miyagawa [30] | Turkey |
| 34 | **p.Arg1507Ter** | **13** | **NSHL** | https://databases.lovd.nl/ | **NA** |
| 35 | p.Gln1510Ter | 13 | NSHL | Sloan-Heggen [15] | Iran |
| 36 | p.Gln1524Ter | 13 | NSHL | – | NA |
| 37 | p.Tyr1607Ter | 15 | NSHL | Sloan-Heggen [15] | Iran |
| 38 | p.Cys1666Ter | 16 | NSHL | Belguith [37] | Tunisia |
| 39 | P,Gln1669Ter | 16 | NSHL | – | NA |
| 40 | p.Pro1697Alafs*2 | 17 | NSHL | – | NA |
| 41 | p.Gly1706Glnfs*102 | 18 | NSHL | Nal [24] | Pakistan |
| 42 | p.Arg1763Alafs*45 | 19 | NSHL | Neveling [29] | Netherland |
| 43 | p.Ser1776Ter | 19 | NSHL | https://databases.lovd.nl | NA |
| 44 | p.Leu1779Trpfs*18 | 19 | NSHL | Ammar-Khodja [38] | Algerian |
| 45 | p.Phe1807Leufs*6 | 21 | Severe to profound HL | Fattahi [19] | Iran |
| 46 | p.Ala1884Ter | 23 | NSHL | Duman [39] | Turky |
| 47 | P.Arg1898Ter | 23 | Severe HL | Zhang [21] | China |
| 48 | p.Arg1937Thrfs*10 | 23 | Moderate to severe HL | Cengiz [22] | Turkey |
| 49 | p.Tyr1945Ter | 24 | NSHL | Chang [40] | Korea |
| 50 | p.Trp1975Ter | 25 | Severe to profound HL | Fattahi [19] | Iran |

**Table 3** (continued)

| | Variant | Exon number | Phenotype | Reference | Population |
|---|---|---|---|---|---|
| 51 | p.Gln2021Ter | 27 | Severe HL | Nal [24] | Pakistan |
| 52 | p.Gln2034Ter | 27 | NSHL | – | NA |
| 53 | p.Ala2104Cysfs*19 | 29 | NSHL | Yang [41] | China |
| 54 | p.Ala2153Profs*100 | 29 | Severe HL | Miyagawa [30] | Japan |
| 55 | p.Cys2163Alafs*90 | 29 | NSHL | – | NA |
| 56 | p.Gln2197Ter | 30 | NSHL | – | NA |
| 57 | p.Val2238Glyfs*92 | 31 | NSHL | Baux [16] | French |
| 58 | p.Arg2298Ter | 32 | NSHL | Sloan-Heggen [15] | Iran |
| 59 | p.Gln2315Glufs*11 | 32 | NSHL | – | NA |
| 60 | p.Asp2375Valfs*41 | 34 | Pre-lingual Progressive HL | Vona [32] | German |
| 61 | p.Asp2403fs*2414 | 35 | NSHL | Shahin [42] | Bethlehem |
| 62 | p.Pro2409Glnfs*8 | 35 | NSHL | – | NA |
| 63 | p.Gln2571Hisfs*35 | 39 | Profound HL | Zhang [21] | China |
| 64 | p.Gln2574Ter | 39 | Profound HL | Zarepour [43] | Iran |
| 65 | p.Lys2601Ter | 40 | NSHL | Wang [44] | India |
| 66 | p.Ser2661Ter | 42 | NSHL | Duman [39] | Turky |
| 67 | p.Asn2678Ter | 42 | Profound HL | Zhang [21] | China |
| 68 | p.Leu2693Cysfs*45 | 42 | NSHL | Zhang [21] | China |
| 69 | p.Gln2812Serfs*16 | 46 | NSHL | Baux [16] | French |
| 70 | **p.Val2815Valfs*10** | **46** | **Profound NSHL** | **This study** | **Iran** |
| 71 | p.Tyr2819Ter | 47 | NSHL | Sloan-Heggen [15] | Iran |
| 72 | p.Arg2903Ter | 48 | NSHL | Baux [16] | French |
| 73 | p.Trp2923Ter | 49 | NSHL | Woo [45] | China |
| 74 | p.Trp2931Glyfs*103 | 50 | NSHL | Zhang [21] | China |
| 75 | p.Val2940fs*3034 | 50 | Severe HL | Nal [24] | Pakistan |
| 76 | p.Gly2941Valfs*94 | 50 | Severe HL | Nal [24] | Pakistan |
| 78 | p.Arg2967ProfsTer33 | 50 | NSHL | Budde [46] | Egyptian |
| 79 | p.Arg2967Ter | 50 | NSHL | Budde [46] | Egyptian |
| 80 | p.His3106Profs*2 | 55 | NSHL | Xia [47] | China |
| 81 | p.Arg3134Ter | 56 | moderate to profound NSHL | Schrauwen [48] | Belgium |
| 82 | p.Leu3204Cysfs*17 | 57 | NSHL | Akbariazar [49] | Iran |
| 83 | p.Gln3264Ter | 60 | Severe HL | Chang [40] | Korea |
| 84 | p.Asp3320Thrfs*2 | 62 | NSHL | Lezirovitz [50] | Brazil |
| 85 | p.Ser3335Alafs121 | 62 | NSHL | Duman [39] | Turky |
| 86 | p.Tyr3475Ter | 64 | NSHL | Baux [16] | French |
| 87 | p.Gln3492Ter | 65 | Severe HL | Nal [24] | Pakistan |
| 88 | p.Ser3525Alafs*29 | 65 | NSHL | Lezirovitz [50] | Brazil |
| 89 | p.Ser3525Glnfs*79 | 65 | NSHL | – | NA |

should be subjected to genetic counseling both before marriage and before pregnancy and a step-by-step follow-up should be made to avoid the birth of hearing impaired children.

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**Data availability** The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

**Ethical approval** The study was approved by the Review Board of Isfahan University of Medical Sciences (Grant nos. 295176, 396133 and 394805).

**Informed consent** Written informed consent was obtained from all of the participants in the study and a written consent to participate was obtained from the parents of the patient (younger than the age of 16). Written informed consent for publication of clinical details and clinical images was obtained from the all of the participants and from the parents the participant under the age of 18.

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