The Promise of Whole-Exome Sequencing in Detecting Novel Variants of the MYO15A Gene in Two Iranian Pedigrees with Non-Syndromic Hearing Loss, A descriptive experimental study

CURRENT STATUS: UNDER REVIEW

BMC Medical Genetics

somayeh khatami
Zanjan University of Medical Sciences

Masomeh Askari
Royan Institute

Fateme Bahreini
Hamedan University of Medical Science

Morteza Hashemzadeh Chaleshtori
shahrekord university of medical science

Samira Asgharzadeh
Shahrekord University of Medical Science

Corresponding Author
Asgharzade2336@gmail.com
ORCiD: https://orcid.org/0000-0001-6992-2941

DOI:
10.21203/rs.2.19322/v1

SUBJECT AREAS
Medical Genetics

KEYWORDS
MYO15A, Whole exome sequencing, First approach, consanguineous
Abstract

**Background:** Clinical genetic diagnosis of non-syndromic hearing loss (NSHL) is quite challenging. With regard to its high heterogeneity as well as large size of some genes, it is also really difficult to detect causative mutations using traditional approaches. One of the recent technologies called whole-exome sequencing (WES) has been thus developed in this domain to remove the limitations of conventional methods.

**Methods:** This study was a report on two unrelated pedigrees with multiple affected cases of hearing loss (HL). Accordingly, clinical evaluations and genetic analysis were performed in both families.

**Results:** The implementation of WES to uncover autosomal recessive non-syndromic hearing loss (ARNSHL) and its related variants was reported in the present study. Two novel variants of MYO15A i.e. c.T6442A:p.W2148R and c.10504dupT:p.C3502Lfs*15 were correspondingly identified and then segregations were confirmed using Sanger sequencing. According to online prediction tools, both identified variants seemed to have damaging effects.

**Conclusion:** This study further supported the effectiveness of WES for genetic diagnosis of ARNSHL as a first approach.

Introduction

Hearing impairment is considered as an etiologically heterogeneous sensory deficiency. In this regard, genetic hearing loss (HL) has been divided into syndromic and non-syndromic types. Considering the high rate of consanguineous marriages in the Middle East, autosomal recessive non-syndromic hearing loss (ARNSHL) is reportedly more prevalent.[1] However; due to the wide variety of pathogenic genes associated with non-syndromic hearing loss (NSHL), including both nuclear and mitochondrial ones, the disease includes diverse patterns of inheritance comprised of autosomal dominant, autosomal recessive, mitochondrial, and X-linked recessive. Disease heterogeneity has been admittedly recognized as the most important challenge in genetic diagnosis of NSHL. Diagnostic approaches which have been relied on conventional methods based on genetic testing of the most common genes, often fail to determine the exact genetic cause of the disease in many countries including Iran.[2] In heterogeneous populations like Iran, the distribution of mutations in the gap
junction beta-2 protein, also known as connexin 26, (i.e. GJB2) gene as a major cause of ARNSHL can be extremely diverse depending on patients’ ethnicities. The prevalence rate of GJB2-related hearing loss (HL) has been reported by 38.3% in northern Iran, but the percentage of such variations has been found rarely in southern regions.[2] Various frequencies of causative mutations, compound heterozygotes, as well as nuclear modifier genes can also render the molecular diagnosis of ARNSHL as a challenge.[2, 3] Moreover, mutation screening in large genes such as MYO15A can be impeded following the use of traditional approaches based on Sanger sequencing.[4] Today, molecular genetic testing on the basis of multi-gene screening such as whole-exome sequencing (WES) are being used instead of traditional diagnostic procedures.[5]

In this study; WES was employed as a first approach to find genetic causes of NSHL in two distinct pedigrees, manifesting an autosomal recessive type of NSHL.

Materials And Methods

**Subjects and Clinical Evaluations**

The study was approved by the Ethics Committee of Shahrekord University of Medical Sciences (IR.SKUMS.REC.1397.008), Iran. Two Iranian families from Hamedan Province with hearing impairments with no additional symptoms were thus studied. Moreover, informed written consent was taken from both families and the proband from each family was further subjected to clinical evaluations of the inner ear accompanied by pure-tone audiometry (PTA).

**Molecular Analysis**

Genomic deoxyribonucleic acid (DNA) was extracted from whole peripheral blood of each study subject utilizing DNA Extraction Kit DNP (Sinacolon, Iran) according to the manufacturer’s instructions. Purity and concentration of DNA samples were further measured via Thermo Scientific NanoDrop 2000c Spectrophotometer.

DNA samples from each pedigree’s proband (Figure 1A, V-4 in family 1; Figure 2A, II-3 in family 2) were then subjected to WES at Macrogen Online Sequencing Order System (Seoul, South Korea) on Genome Analyzer/HiSeq 2000 (Illumina, San Diego, CA, USA, 151-bp paired-end reads). It should be noted that the library had been prepared through SureSelect XT Library Prep Kit (Agilent
Technologies, CA, USA). Data analysis was correspondingly performed using an in-house developed pipeline, adopted from Genome Analysis Tool Kit v3.6 and ANNOVAR software.[6] Homozygous missense, start codon change, splice site, nonsense, stop loss, and indel variants with minor allele frequency <1% were further filtered in dbSNP (version 138), 1000 Genomes Project, Exome Aggregation Consortium, and NHLBI GO Exome Sequencing Project (ESP). Based on autosomal recessive inheritance, the homozygosity region of samples was determined using homozygosity mapping algorithms.

Several online prediction software including MutationTaster2, FATHMM, PANTHER, SIFT, PROVEAN, MetaLR, PolyPhen-2, CADD, and ConSurf were also used to evaluate the pathogenic effect of the variants. Next, the variants were investigated in the Human Gene Mutation Database and the related literature to survey their association with a phenotype and novelty of the variants.

Besides, candidate variant segregation from exome data was evaluated through polymerase chain reaction (PCR)-based Sanger sequencing. Therefore, the following primers were synthesized: 5’-GAACTACATCGTGCAGAAGG-3’ and 5’-CCTATCCAGTCCCACTCACT-3’ for human MYO15A c.T6442A variant and 5’-CCACCATTCGGCCTTCCA-3’ and 5’-CTGCCTCCTCTTAGTGTCCTC-3’ for human MYO15A c.10504dupT variant.

Results
Clinical and molecular findings

The first family pedigree is displayed in Figure 1A. Accordingly, four members of the pedigree including two affected and two unaffected individuals who consented to be included in this study are indicated. In this family, the proband (V: 4) was a 21-year-old woman with congenital HL. No additional abnormal phenotypic features were also observed in the proband, including visual impairments or any limb and facial malformations. The parents were consanguineous and both showed normal hearing. According to the audiogram, the proband is suffering from congenital profound deafness (Figure 1B).

A three-generation pedigree, depicted in Figure 2A, was presented as the second family with ten members, six males and fourteen females, suffering from ARNSHL. The proband was a 25-year-old
male individual, born as the second child of non-sanguineous healthy parents, who had been diagnosed for congenital HL when he was one year old. At the age of 5, he had gone through bilateral cochlear implant surgery based on physical examinations and audiometry testing. Audiogram analysis also confirmed HL to be profound in the proband (II: 3) (Figure 2B).

Considering all limitations in genetic diagnosis of ARNSHL, it was decided to perform WES as a first approach on proband’s genome DNA. Following the filtering step, two novel homozygous variants in MYO15A i.e. c.T6442A:p.W2148R and c.10504dupT:p.C3502Lfs*15 were prioritized in family I and II, respectively. Given that the proband of family I was the offspring of consanguineous parents, WES dataset revealed that MYO15A (c.T6442A) variants had resided in the large homozygous regions on chromosome 17 (Figure 3). All in silico programs also predicted damaging effects of p.W2148R variant. Mutation taster further suggested that c.10504dupT variant had deleterious effects.

Moreover, the first conservative amino acid alternation (p.C3502L) predicted to be pathogenic using online softwares (Table 1).

Additionally, the results of Sanger sequencing confirmed the presence of p.W2148R variant in MYO15A gene in the proband and other affected members who were studied, but the unaffected sister (V.5) and her father were found heterozygous for the mutation (Figure 4A).

The findings of Sanger sequencing also revealed co-segregation of c.10504dupT variant in the second family (Figure 4B). The affected proband was thus homozygous, whereas his unaffected sister (II:5) and his father (I:1) were heterozygous for this locus and his unaffected brother (II:2) lacked the variant.

It should be noted that these two variants were absent in 50 ethnically-matched control cases.

**Discussion**

Based on WES data as well as segregation and genotype-phenotype correlational study, mutations in MYO15A were identified as the causes of NSHL in the first and second families examined.

MYO15A, a new branch of the myosin superfamily protein-coding gene, has thus a role in stereo cilia formation.[7] This gene is considered as the third leading cause of ARNSHL in many populations,[8] including Iran, with a reportedly prevalence rate ranged from 4.8-9.6%.[9-11] Mutations in this gene
are accordingly associated with severe-to-profound HL. Screening of 66 coding exons through Sanger sequencing is expensive and more time-consuming. On the contrary, high-throughput techniques can save time and money[12].

MYO15A is a different form of myosins with long N-terminal extensions preceding the conserved motor domain[13]. The novel c.T6442A: p variant is located in the first domain of myosin tail homology4 (MTH4) and is also conserved between different species (data not shown). The substitution of a highly conserved (Table 1) hydrophobic amino acid non-polar tryptophan for arginine can thus lead to loss of the positive charge on the arginine side-chain.

The c.T6442A:p.W2148R variant in the arginine side-chain of MYO15A can accordingly alter protein structure and interfere with actin binding to the plasma membrane as well as microtubule binding. In this respect, interaction of MyTH4 domain and PDZ domain of whirlin proteins seems essential for formation of a complex of actin microfilaments at the stereo cilia tips. Moreover, interaction of MYO15A and EPS8 can have similar activities in acting binding of cochlear hair cell stereo cilia. [14, 15]

In the second studied family, WES could successfully detect a novel homozygous insertion variant i.e. c.10504dupT:p.C3502Lfs*15 in MYO15A gene, co-segregated with the disease within the pedigree. This variant between the second FERM and PDZ domains could also lead to a reading frame shift at position 10504 and a stop codon (p.C3502Lfs*15) with truncation and translation of mRNA resulting in lack of its conserved amino acids at C-terminal (data not shown).

Today, the topic of oligogenic inheritance traits has made a big wave in diagnostic medicine; since, in many monogenic diseases, it represents that there is not just one gene affected phenotype which causes new challenges in diagnosing these diseases and it can be more complicated for diseases with heterogenic pathology in which many genes are involved [16].

Conclusion
It was concluded that accurate mutational analysis of MYO15A, as a third cause of HL in Iran is of utmost importance for genetic counseling and even prenatal diagnosis.[17] As stated before, traditional approaches are not affordable for clinical applications. The present study confirmed that
WES is a reliable first approach for screening of MYO15A variants in patients with hearing impairments.

Declarations

**Ethics approval and consent to participate:** Sharekord University of Medical Sciences Ethics Committee (IR.SKUMS.REC.1397.008).

**Consent for publication:** Informed consent for publication was obtained.

**Availability of data and materials:** Dr. Samira Asgharzadeh had full access to the study and take full responsibility for the integrity and accuracy of data analysis.

**Competing interests:** All authors declare no conflict of interest.

**Funding:** Sharekord University of Medical Sciences research grant No. 2685.

**Author Contributions:** Somayeh Khatami, data analysis, wrote the manuscript, answered Reviewers comments, proved manuscript final version; Masomeh Askari, data analysis, performed Experiments, technical editing and revising the manuscript; Fatemeh Bahreini, performed Experiments, technical editing and revising the manuscript; Morteza Hashemzadeh Chaleshtori, prepared reagents/tools, technical editing and revising the manuscript, proved manuscript final version; Samira Asgharzade, study design, validated data, wrote the manuscript, proved manuscript final version.

**Acknowledgements:** We sincerely thank the patients and their families for participating in this study.

**Abbreviations**

**AR:** Autosomal recessive

**NSHL:** Non-syndromic hearing loss

**WES:** Whole exome sequencing

**References**

1. Najmabadi H, Kahrizi K. Genetics of non-syndromic hearing loss in the Middle East. Int J Pediatr Otorhinolaryngol. 2014;78(12):2026-36.

2. Beheshtian M, Babanejad M, Azaiez H, Bazazzadegan N, Kolbe D, Sloan-Heggen C, et al. Heterogeneity of hereditary hearing loss in Iran: a comprehensive review. Arch
3. Khatami S, Rokni-Zadeh H, Mohsen-Pour N, Biglari A, Changi-Ashtiani M, Shahrooei M, et al. Whole exome sequencing identifies both nuclear and mitochondrial variations in an Iranian family with non-syndromic hearing loss. Mitochondrion. 2019;46:321-5.

4. Woo H-M, Park H-J, Baek J-I, Park M-H, Kim U-K, Sagong B, et al. Whole-exome sequencing identifies MYO15A mutations as a cause of autosomal recessive nonsyndromic hearing loss in Korean families. BMC Med Genet. 2013;14(1):72.

5. Seco CZ, Wesdorp M, Feenstra I, Pfundt R, Hehir-Kwa JY, Lelieveld SH, et al. The diagnostic yield of whole-exome sequencing targeting a gene panel for hearing impairment in The Netherlands. Eur J Hum Genet. 2017;25(3):308.

6. Pourreza MR, Mohammadi H, Sadeghian L, Asgharzadeh S, Sehhati M, Tabatabaiefar MA. Applying Two Different Bioinformatic Approaches to Discover Novel Genes Associated with Hereditary Hearing Loss via Whole-Exome Sequencing: ENDEAVOUR and HomozygosityMapper. Advanced Biomedical Research. 2018;7.

7. Rędowicz MJ. Myosins and deafness. J Muscle Res Cell Motil. 1999;20(3):241-8.

8. Rehman AU, Bird JE, Faridi R, Shahzad M, Shah S, Lee K, et al. Mutational spectrum of MYO15A and the molecular mechanisms of DFNB3 human deafness. Hum Mutat. 2016;37(10):991-1003.

9. Fattahi Z, Shearer AE, Babanejad M, Bazazzadegan N, Almadani SN, Nikzat N, et al. Screening for MYO15A gene mutations in autosomal recessive nonsyndromic, GJB2 negative Iranian deaf population. Am J Med Genet A. 2012;158(8):1857-64.

10. Babanejad M, Fattahi Z, Bazazzadegan N, Nishimura C, Meyer N, Nikzat N, et al. A comprehensive study to determine heterogeneity of autosomal recessive nonsyndromic hearing loss in Iran. Am J Med Genet A. 2012;158(10):2485-92.

11. Sloan-Heggen CM, Babanejad M, Beheshtian M, Simpson AC, Booth KT, Ardalani F, et
al. Characterising the spectrum of autosomal recessive hereditary hearing loss in Iran. J Med Genet. 2015;52(12):823-9.

12. Zhang J, Guan J, Wang H, Yin L, Wang D, Zhao L, et al. Genotype-phenotype correlation analysis of MYO15A variants in autosomal recessive non-syndromic hearing loss. BMC Med Genet. 2019;20(1):60.

13. Anderson DW, Probst FJ, Belyantseva IA, Fridell RA, Beyer L, Martin DM, et al. The motor and tail regions of myosin XV are critical for normal structure and function of auditory and vestibular hair cells. Hum Mol Genet. 2000;9(12):1729-38.

14. Belyantseva IA, Boger ET, Naz S, Frolenkov GI, Sellers JR, Ahmed ZM, et al. Myosin-XVa is required for tip localization of whirlin and differential elongation of hair-cell stereocilia. Nat Cell Biol. 2005;7(2):148.

15. Behlouli A, Bonnet C, Abdi S, Bouaita A, Lelli A, Hardelin J-P, et al. EPS8, encoding an actin-binding protein of cochlear hair cell stereocilia, is a new causal gene for autosomal recessive profound deafness. Orphanet J Rare Dis. 2014;9(1):55.

16. Badano JL, Katsanis N. Beyond Mendel: an evolving view of human genetic disease transmission. Nature Rev Genet. 2002;3(10):779-89.

17. Zarepour N, Koohiyan M, Taghipour-Sheshdeh A, Nemati-Zargaran F, Saki N, Mohammadi-Asl J, et al. Identification and Clinical Implications of a Novel MYO15A Variant in a Consanguineous Iranian Family by Targeted Exome Sequencing. Audiol Neurotol. 2019;24(1):25-31.

Table
Table1: In Silico and Bioinformatics Analysis of the Variants.
| variant | c.T6442A:p.W2148R | c.10504dupT:p.C3502Lfs*15 |
|---------|-------------------|---------------------------|
| Locus   | DFNB3             | DFNB3                     |
| dbSNP rsID | Novel          | Novel                      |
| ConSurf score | 8               | 8                          |
| MutationTaster2 | Disease causing | Disease causing          |
| SIFT    | Damaging          | deleterious                |
| Polyphen2 | Probably damaging | Probably damaging         |
| FATHMM  | Damaging          | Damaging                   |
| PROVEAN | Deleterious       | Deleterious                |
| MetaLR  | Damaging          | Not down                   |
| CADD_phred | 24.3          | Not down                   |
| PANTHER | Probably damaging | Probably damaging         |
| Segregates in the family | Yes | Yes                      |

**Figures**

Figure 1

Pedigree and the proband audiogram. (A) Pedigree of family 1 having autosomal recessive form of NSHL disease is drawn. Hearing impaired individuals are illustrated by black-filled symbols. The proband (V: 4), for whom whole exome Sequencing has been carried out is indicated by arrow. (B) Audiogram for pure tone audiometry (PTA) of the proband with profound hearing loss in both ears.
Figure 2

: Pedigree and the proband audiogram. (A) Three-generation pedigree of family 2 having autosomal recessive form of NSHL disease is drawn. Hearing impaired individuals are illustrated by black-filled symbols. The proband (II: 3), for whom whole exome Sequencing has been carried out is indicated by arrow. (B) Audiogram for pure tone audiometry (PTA) of the proband with profound hearing loss in both ears.

Figure 3

Homozygosity region in the proband (V: 4) of pedigree. Coordinate homozygosity region on chromosome 17 in proband [17927849-18239689].
Identification of c.T6442A:p.W2148R and c.10504dupT:p.C3502Lfs*15 variants in impaired- and normal-hearing individuals of the studied families. A Partial sequence chromatograms of MYO15A gene from two unaffected (IV.2 and V.5) and affected individuals (V: 3 and V: 4) illustrate T to A transition at position 6442. B) Partial sequence chromatograms of MYO15A gene containing (c.10504dupT:p.C3502Lfs*15) variant in proband (II: 3), his father (I: 1) and his siblings (II: 2 and II:5) illustrates insertion of T as indicated.