Identification of Novel PTPRQ and MYO1A Mutations in An Iranian Pedigree with Autosomal Recessive Hearing Loss

Farah Talebi, M.Sc.1, Farideh Ghanbari Mardasi, M.Sc.2*, Javad Mohammadi Asl, Ph.D.3, Saeed Tizno, M.D.4, Marziye Najafvand Zadeh, M.Sc.2

1. Ahvaz Welfare Organization, Ahvaz, Iran
2. Department of ENT, Faculty of Medicine, Guilan University of Medical Sciences, Guilan, Iran
3. Department of Medical Genetics, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
4. Department of ENT, Faculty of Medicine, Guilan University of Medical Sciences, Guilan, Iran

*Corresponding Address: P. O. BOX: 64516-84534, Department of Nursing, Shoushtar Faculty of Medical Sciences, Shoushtar, Iran
Email: ghanbari246@gmail.com

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Abstract
Autosomal recessive non-syndromic hearing loss (ARNSHL) is defined as a genetically heterogeneous disorder. The aim of the present study was to screen for pathogenic variants in an Iranian pedigree with ARNSHL. Next-generation targeted sequencing of 127 deafness genes in the proband detected two novel variants, a homozygous missense variant in PTPRQ (c.2599 T>C, p.Ser867Pro) and a heterozygous missense variant in MYO1A (c.2804 T>C, p.Ile935Thr), both of which were absent in unaffected sibs and two hundred unaffected controls. Our results suggest that the homozygous PTPRQ variant maybe the pathogenic variant for ARNSHL due to the recessive nature of the disorder. Nevertheless, the heterozygous MYO1A may also be involved in this disorder due to the multigenic pattern of ARNSHL. Our data extend the mutation spectrum of PTPRQ and MYO1A, and have important implications for genetic counseling in unaffected sibs of this family. In addition, PTPRQ and MYO1A pathogenic variants have not to date been reported in the Iranian population.

Keywords: Hearing Loss, MYO1A, Novel Variant, PTPRQ

Introduction
Hearing impairment is one of the most common sensorineural disorders in humans, affecting approximately one in 500-1,000 newborns. Hereditary hearing impairments are mainly transmitted in an autosomal dominant or recessive fashion (1) with mitochondrial (2) or X-linked (3) inheritance reported in frequently. Considering the isolated forms, about 80% of hereditary deafness cases manifest as autosomal recessive non-syndromic hearing loss (ARNSHL) (4). To date, more than 100 genes have been implicated in ARNSHL (5).

Molecular diagnosis plays a key role in clinical management, prognosis evaluation and prenatal diagnosis (PND) for ARNSHL families (6). However, the genetic heterogeneity of hearing impairment had undermined genetic diagnosis in most cases until recently. With the advent of next-generation sequencing (NGS) technology, heterogeneous disorders are now open to routine genetic testing and comprehensive genetic analysis. Targeted NGS of the identified deafness genes (a “gene panel” that generally covers the exons and flanking intronic sequences) can provide a basis for a broad first-step study of pathogenic variants in ARNSHL (7). We thus aimed to screen the deafness gene panel in a proband with ARNSHL and of Iranian origin. Herein, we report two novel missense pathogenic variants in PTPRQ and MYO1A, both of which may explain the ARNSHL phenotype in the proband.

Case report
The proband is a 23-year-old Iranian male with a clinical diagnosis of hearing impairment (Fig.1A). No exact complications have been reported during his perinatal period. However, at age of 21 months, his mother suspected hearing loss because of his poor response to sound. He was born from a consanguineous marriage (first cousin unaffected parents). There was no family history of inherited diseases such as ARNSHL or congenital malformations in his pedigree. Two hundred unrelated subjects of Iranian origin with normal hearing were screened for the pathogenic variants as controls. Written informed consent was obtained from all participants according to the guidelines of the Ethics Committee of the Ministry of Health and Medical Education of Iran.

Blood samples were collected from the proband and his parents. Genomic DNA was extracted from blood samples of all participants using the standard salting out method (8). Targeted NGS was carried out by using a custom designed NimbleGen chip capturing 127 hearing impairment genes including but not limited to PTPRQ, GJB6, MYO1A, MYO7A, SLC26A4, and MT-RNR1 (BGI-Clinical Laboratories, China). The genomic
region containing the variant were amplified (primer sequences are available upon request) in 25 μL volumes and 35 cycles: 95°C for one minute, 65°C for 40 seconds and 72°C for one minute and then the polymerase chain reaction (PCR) product was sequenced with direct sanger sequencing carried out with automated DNA sequencer (ABI3130, Applied Biosystems, USA) (validation with a second independent sample of DNA) to confirm presence of potential pathogenic variants in the proband and his parents for segregation analysis.

The frequency of the detected variants was checked in the 1000 genomes database (http://WWW.1000genomes.org/). Next, in silico functional prediction of the missense variants were performed with bioinformatics tools including Sorting Intolerant from Tolerant (SIFT) (9), Polymorphism Phenotyping V2 (PolyPhen2) (10) and Mutation Taster (11).

All genomic data analysis including read alignment, variant calling and novel mutation identification was undertaken by BGI that detected two novel variants in *PTPRQ* and *MYO1A* co-segregating in the family. The variant in exon 17 of *PTPRQ* (c.2599T>C) results in a serine to proline substitution at codon 867 (Ser867Pro) (Fig.1B). The second variant was found in exon 26 of *MYO1A* (c.2804 T>C) (Fig.1C), leading to an isoleucine to threonine substitution (Ile935Thr). Both missense variants were predicted to be pathogenicity the three prediction tools (Table 1). Reported mutations in *PTPRQ* and *MYO1A* are summarized in Table 2, and 3 respectively. Interestingly, no pathogenic variants were identified in the other 125 genes in the proband. The two detected variants were confirmed by sanger sequencing. Both missense variants alter highly evolutionary conserved amino acids (Fig.1D, E). To confirm pathogenicity, presence of the two variants was checked in unaffected individuals in the pedigree. The unaffected parents and one of his sisters (II-3) were heterozygous for the *PTPRQ* variant while the *MYO1A* variant was only identified in the mother in a heterozygous state. Both variants were not detected in the other sister (II-2) and the 200 healthy controls of Iranian origin. Figure 2 shows the locations of these variants.

### Table 1: Results of in silico prediction tools for functional effect of the novel missense mutations

| Gene/Variant | SIFT score | PolyPhen score | Mutation taster |
|--------------|------------|----------------|-----------------|
| ENST00000614701, S867P | 0.007 (DAMAGING) | 0.787 (possibly damaging) | disease causing |
| ENST00000300119, I935T | 0.004 (DAMAGING) | 0.908 (possibly damaging) | disease causing |

### Table 2: Reported mutations in PTPRQ

| Origin | Pathogenic variant | Protein effect | Domain | Exon | Type of mutation | Inheritance pattern | Zygosity |
|--------|--------------------|----------------|--------|------|-----------------|---------------------|----------|
| Palestinian | c.1285C>T | p.Gln429Stop | EC | 9 | Nonsense | AR | Homozygous |
| Dutch | c.1491T>A | p.Tyr497Stop | EC | 10 | Nonsense | AR | Homozygous |
| Moroccan | c.1369A>G | p.Ala457Gly | EC | 10 | Missense | AR | Homozygous |
| Chinese | c.3125A>G | p.Asp1042Gly | EC | 20 | Missense | AR | Homozygous |
| Chinese | c.5981A>G | p.Glu1994Gly | EC | 37 | Missense | AR | Homozygous |
| Japanese | c.166C>G | p.Pro56Ala | EC | 2 | Missense | AR | Compound heterozygous |
| Japanese | c.1261C>T | p.Arg421Stop | EC | 9 | Nonsense | AR | Homozygous |
| Japanese | c.4046T>C | p.Met1349Thr | EC | 25 | Missense | AR | Compound heterozygous |
| Japanese | c.6453+3delA | - | CP | 41 | Splice site | AR | Compound heterozygous |
| Iranian | c.2599T>C | Ser867Pro | EC | 17 | Missense | AR | Homozygous |

CP; Cytoplasmic domain, EC; Extracellular domain, and AR; Autosomal recessive.
Fig. 1: Genetic analysis of the ARNSHL proband. A. Pedigree of family with ARNSHL, the proband is denoted in black. Partial sequences of B. PTPRQ, C. MYO1A in the proband showing that homozygous mutation (c.2599T>C) in PTPRQ and the heterozygous mutation (c.2804 T>C) in MYO1A, both co-segregating with the phenotype. Mutated nucleotides are marked with vertical lines (black). Protein alignment shows conservation of residue D. 867 in PTPRQ, and E. 935 in MYO1A across seven and eight species respectively. These two novel mutations occur at evolutionarily conserved amino acid positions marked with vertical lines (black).

Fig. 2: Diagram structure of PTPRQ and Myosin-IA proteins. Schematic of A. PTPRQ and B. Myosin-IA proteins show the locations of the pathogenic variants in humans. The two novel mutations reported in this study are shown in red font (p.Ser867Pro and p.Ile935Thr).
### Table 3: Reported mutations in MYO1A

| Origin | Pathogenic variant | Protein effect | Exon | Domain | Type of mutation | Inheritance pattern | Zygosity |
|--------|-------------------|----------------|------|--------|------------------|---------------------|----------|
| Italian | 277C/T            | R93X           | 3    | Myosin motor | Nonsense         | AD                  | Heterozygous |
| Italian | 349-350A          | 349-350insCTT  | 4    | Myosin motor | Insertion        | AD                  | Heterozygous |
| Italian | 916G/A            | V306M          | 10   | Myosin motor | Missence         | AD                  | Heterozygous |
| Italian | 1155G/T           | E385D          | 12   | Myosin motor | Missence         | AD                  | Heterozygous |
| Italian | 1985G/A           | G662E          | 18   | Myosin motor | Missence         | AD                  | Heterozygous |
| Italian | 2021G/A           | G674D          | 18   | Myosin motor | Missence         | AD                  | Heterozygous |
| Italian | 2390C/T           | S797F          | 22   | -       | Missence         | AD                  | Heterozygous |
| Italian | 2728T/C           | S910P          | 25   | TH1    | Missence         | AD                  | Heterozygous |
| Pakistani | c.784C>T       | p.Arg262*      | 10   | Myosin motor | nonsense        | AD                  | Heterozygous |
| German    | c.2220T>G         | p.Tyr740*      | 21   | IQ 2   | nonsense         | AD                  | Heterozygous |
| Iranian   | c.2804T>C         | I935T          | 26   | TH1    | Missence         | AR/ compound heterozygous | Heterozygous |

TH1; Class I myosin tail homology, AD; Autosomal dominant, and AR; Autosomal recessive.

**Discussion**

Here we report two novel missense variants in *PTPRQ* and *MYO1A* in an Iranian family displaying hearing loss. Protein Tyrosine Phosphatase, Receptor Type Q (*PTPRQ*) is a stereociliar membrane protein, composed of three domains which include an extracellular domain (containing 18 fibronectin III repeats), a membrane spanning domain (trans membrane domain) and a cytoplasmic domain (phosphatase domain) (12-14). It plays key roles in cell shape changes, regulation of actin filament organization and formation of stereocilia in hair cells of the inner ear (15) with its loss or malfunction resulting in shaft connector malformation of hair cell stereocilia (16).

The novel homozygous *PTPRQ* variant detected in the proband is located in the *fibronectin type III-9* domain (extracellular domain). This extracellular domain is able to bind ligands including extracellular proteins, collagen and heparin as well as ligands on the cell (17-19). The wild-type residue is polar while the mutant residue is non-polar, thus likely to affect *PTPRQ* interactions with ligands.

Additionally, this is the first *PTPRQ* variant found in an Iranian population. To date, 9 variants in *PTPRQ* have been reported. All *PTPRQ* variants previously reported were detected in prelinguistic or congenital hearing loss patients (20). The proband in this study had congenital hearing loss, consistent with previous reports. Of the 9 reported *PTPRQ* variants, five were missense variants in the extracellular (EC) domain of which three were found in a homozygous state [p.P56A and p.M1349T in Japan (20)]. Three were also nonsense variants in the EC domain that were found in homozygous [p.Q429X in Palestine (13) and p.Y497X in Holland (112)] or heterozygous [p.R421X in Japan (20)] state. The ninth variant was a heterozygous splice site variant (c.6453+3delA) detected in a Japanese family (20).

We also identified a novel heterozygous variant in *MYO1A* as a potentially causative variant of congenital ARNSHL in the proband. *MYO1A* encodes Myosin-IA, a protein with 1043 amino acids, belonging to the myosin super family (22, 23). *MYO1A* contains three core domains, an N-terminal motor domain, a central neck region made up of IQ motifs and a tail region. *MYO1A* functions as an actin-based molecular motor and is implicated in directing the movement of organelles along the actin filaments (24).

Variants within this gene have been reported to cause ARNSHL (25). To date, 10 recessive variants in *MYO1A* have been shown to be associated with ARNSHL in patients of Italian, German and Pakistani descent. However, variants in *MYO1A* have not to date been reported in the Iranian population.

The c.2804 T>C variant located in the *C-terminal tail homology-1 (TH1) domain, which is responsible for membrane binding* (26). Therefore, missense variants that alter a nonpolar aliphatic amino acid to polar amino acids with a hydroxyl group may modify the interaction of the tail domain with membranous compartments and alter its movement. Therefore this novel variant is likely to negatively affect the function of the TH1 domain. ARNSHL has an autosomal recessive inheritance pattern.

**Table 3: Reported mutations in MYO1A**
and since neither parents nor the proband are homozygous, it is unlikely to be causal in this case. However, this variant might cause pathogenicity in case another variant is acquired in future generations and result in compound heterozygosity.

Our findings confirm that two novel variants in PTPRQ and MYO1A may be causative of ARNSHL in a consanguineous Iranian family. In conclusion, by using NGS in this study, we show that this method can be useful for detecting rare causative genetic variants in ARNSHL patients, such as those detected in MYO1A and PTPRQ.

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Author’s Contributions

F.T.; Study conception and design. F.Gh.M.; Acquisition of data, analysis and interpretation of data, drafting of manuscript, critical revision. S.T., M.N.Z., J.M.A.; Analysis and interpretation of data. All authors read and approved the final manuscript.

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