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 Nursing, Shoushtar Faculty of Medical Sciences, Shoushtar, 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*

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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.

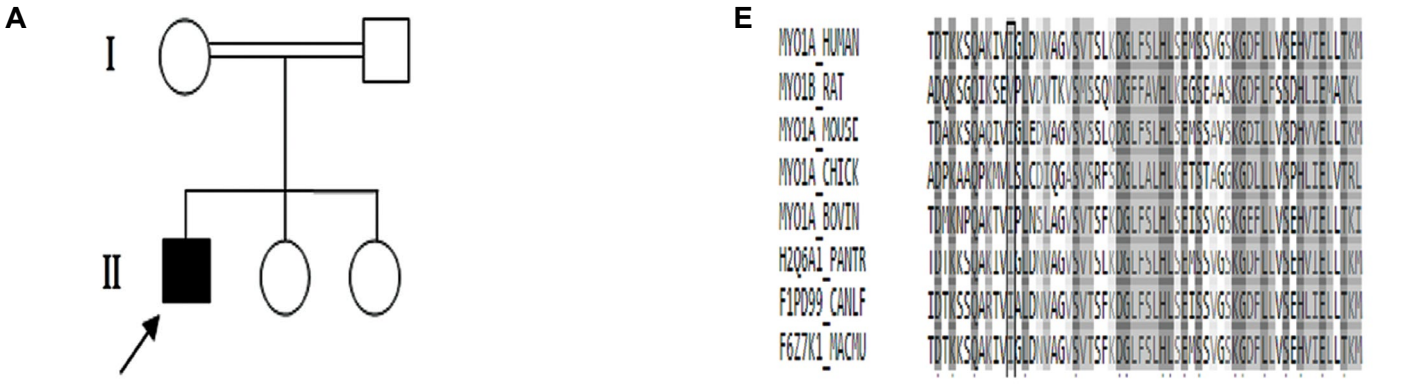

Fig.1: Genetic analysis of the ARNSHL proband. **A.** Pedigree of family with ARNSHL, the proband is denoted in black. Partial sequences of **B.** *PTPRQ,* **C.** *MYO1A* in the proband showing that homozygous mutation (c.2599T>C) in *PTPRQ* and the heterozygous mutation (c.2804 T>C) in *MYO1A,* both 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).

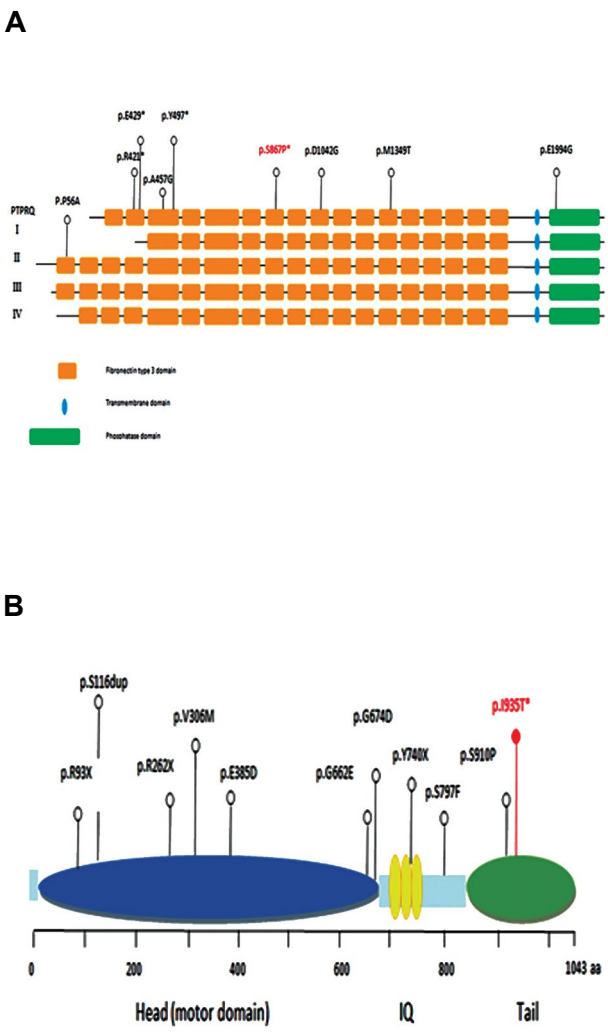

Fig.2: Diagram structure of PTPRQ and Myosin-IA proteins. Schematic of **A.** PTPRQ and **B.** Myosin-IA proteins show the locations of the pathogenic variants in humans. The two novel mutations reported in this study are shown in red font (p.Ser867Pro and p.Ile935Thr).

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	Nonsence	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.A457G in Morocco (12), and p.D1042G and p.E1994G in China (21)] and two in a heterozygous 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

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.

References

1. Duman D, Tekin M. Autosomal recessive nonsyndromic deafness genes: a review. Front Biosci (Landmark Ed). 2012; 17: 2213-2236.
2. Cengiz FB, Duman D, Sırmacı A, Tokgöz-Yilmaz S, Erbek S, Öztürkmen-Akay H, et al. Recurrent and private MYO15A mutations are associated with deafness in the Turkish population. Genet Test Mol Biomarkers. 2010; 14(4): 543-550.
3. Wang Q, Li R, Zhao H, Peters JL, Liu Q, Yang L, et al. Clinical and molecular characterization of a Chinese patient with auditory neuropathy associated with mitochondrial 12S rRNA T1095C mutation. Am J Med Genet A. 2005; 133(1): 27-30.
4. 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; 158A(10): 2485-2492.
5. Taghizadeh SH, Kazeminezhad SR, Sefidgar SA, Yazdanpanahi N, Tabatabaeifar MA, Yousefi A, et al. Investigation of LRTOMT gene (locus DFNB63) mutations in Iranian patients with autosomal recessive non-syndromic hearing loss. Int J Mol Cell Med. 2013; 2(1): 41-45.
6. Chang MY, Choi BY. Strategy for the customized mass screening of genetic sensorineural hearing loss in koreans. Korean J Audiol. 2014; 18(2): 45-49.
7. Rehm HL. Disease-targeted sequencing: a cornerstone in the clinic. Nat Rev Genet. 2013; 14(4): 295-300.
8. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988; 16(3): 1215.
9. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009; 4(7): 1073-1081.
10. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010; 7(4): 248-249.
11. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods. 2014; 11(4): 361-362.
12. Schraders M, Oostrik J, Huygen PL, Strom TM, van Wijk E, Kunst HP, et al. Mutations in PTPRQ are a cause of autosomal-recessive nonsyndromic hearing impairment DFNB84 and associated with vestibular dysfunction. Am J Hum Genet. 2010; 86(4): 604-610.
13. Shahin H, Rahil M, Abu Rayan A, Avraham KB, King MC, Kanaan M, et al. Nonsense mutation of the stereociliar membrane protein gene PTPRQ in human hearing loss DFNB84. J Med Genet. 2010; 47(9): 643-645.
14. Oganesian A, Poot M, Daum G, Coats SA, Wright MB, Seifert RA, et al. Protein tyrosine phosphatase RQ is a phosphatidylinositol phosphatase that can regulate cell survival and proliferation. Proc Natl Acad Sci USA. 2003; 100(13): 7563-7568.
15. Takenawa T, Itoh T. Phosphoinositides, key molecules for regulation of actin cytoskeletal organization and membrane traffic from the plasma membrane. Biochim Biophys Acta. 2001; 1533(3): 190-206.
16. Goodyear RJ, Legan PK, Wright MB, Marcotti W, Oganesian A, Coats SA, et al. A receptor-like inositol lipid phosphatase is required for the maturation of developing cochlear hair bundles. J Neurosci. 2003; 23(27): 9208-9219.
17. Chi-Rosso G, Gotwals PJ, Yang J, Ling L, Jiang K, Chao B, et al. Fibronectin type III repeats mediate RGD-independent adhesion and signaling through activated beta1 integrins. J Biol Chem. 1997; 272 (50): 31447-31452.
18. Napper CE, Drickamer K, Taylor ME. Collagen binding by the mannose receptor mediated through the fibronectin type II domain. Biochem J. 2006; 395(3): 579-586.
19. Weber P, Zimmermann DR, Winterhalter KH, Vaughan L. Tenascin-C binds heparin by its fibronectin type III domain five. J Biol Chem. 1995; 270(9): 4619-4623.
20. Sakuma N, Moteki H, Azaiez H, Booth KT, Takahashi M, Arai Y, et al. Novel PTPRQ mutations identified in three congenital hearing loss patients with various types of hearing loss. Ann Otol Rhinol Laryngol. 2015; 124(1): 184S-192S.
21. Gao X, Su Y, Chen YL, Han MY, Yuan YY, Xu JC, et al. Identification of two novel compound heterozygous PTPRQ mutations associated with autosomal recessive hearing loss in a chinese family. PLoS One. 2015; 10(4): e0124757.
22. Hasson T, Skowron JF, Gilbert DJ, Avraham KB, Perry WL, Bement WM, et al. Mapping of unconventional myosins in mouse and human. Genomics. 1996; 36(3): 431-439.
23. Tyska MJ, Mooseker MS. MYO1A (brush border myosin I) dynamics in the brush border of LLC-PK1-CL4 cells. Biophys J. 2002; 82(4): 1869-1883.
24. Eisenberger T, Di Donato N, Baig SM, Neuhaus C, Beyer A, Decker E, et al. Targeted and genomewide NGS data disqualify mutations in MYO1A, the "DFNA48 gene", as a cause of deafness. Hum Mutat. 2014; 35(5): 565-570.
25. Donaudy F, Ferrara A, Esposito L, Hertzano R, Ben-David O, Bell RE, et al. Multiple mutations of MYO1A, a cochlear-expressed gene, in sensorineural hearing loss. Am J Hum Genet. 2003; 72(6): 1571-1577.
26. Nambiar R, McConnell RE, Tyska MJ. Myosin motor function: the ins and outs of actin-based membrane protrusions. Cell Mol Life Sci. 2010; 67(8): 1239-1254.