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Mutations in the MyTH4 domain of *MYO15A* are a common cause of DFNB3 hearing loss

A. Eliot Shearer, BSc[1,*], **Michael S. Hildebrand, PhD**[1,*], **Jennifer A. Webster, BA**[2], **Kimia Kahrizi, MD**[3], **Khadijeh Jalalvand**[3], **Sanaz Arzhanginy**[3], **William J. Kimberling, PhD**[4], **Dietrich Stephan, PhD**[2,5,6], **Melanie Bahlo, PhD**[7], **Richard J.H. Smith, MD**[1,8], and **Hossein Najmabadi, PhD**[3]

[1]Department of Otolaryngology - Head and Neck Surgery, University of Iowa, Iowa City, IA, USA

[2]Neurogenomics Division, Translational Genomics Research Institute, Phoenix, AZ, USA

[3]Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

[4]Department of Genetics, Boys Town National Research Hospital, Omaha, NE, USA

[5]Arizona Alzheimer's Consortium, Phoenix, AZ, USA

[6]Banner Alzheimer's Institute, Phoenix, AZ, USA

[7]Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

[8]Interdepartmental PhD Program in Genetics, Department of Otolaryngology, University of Iowa, Iowa City, Iowa City, IA, USA

Abstract

Objectives—To use clinical and genetic analyses to determine the mutation causing autosomal recessive non-syndromic hearing loss (ARNSHL) segregating in two consanguineous Iranian families.

Study Design—Family study.

Methods—Members of each family received otologic and audiometric examination for the type and extent of hearing loss. Linkage mapping using Affymetrix 50K GeneChips and STRP analysis localized the hearing loss in both families to the DFNB3 locus. Direct sequencing of the *MYO15A* gene was completed on affected members of both families.

Results—Family L-3165 segregated a novel homozygous missense mutation (c.6371G>A) that results in a p.R2124Q amino acid substitution in the myosin XVa protein. While family L-896 segregated a novel homozygous missense (c.6555C>T) mutation resulting in a p.P2073S amino acid change.

Conclusions—These are the first *MYO15A* mutations reported to cause DFNB3 sensorineural hearing loss in the Iranian population. Like other mutations located in the myosin tail homology 4 (MyTH4) domain, the p.R2124Q and p.P2073S mutations are predicted to disrupt the function of the myosin XVa protein, which is integral to the mechanosensory activity of hair cells in the inner ear.

Corresponding author: Michael Hildebrand Department of Otolaryngology - Head and Neck Surgery University of Iowa 5270 CBRB Building Iowa City, IA 52242, USA Tel: +319 335 6677 Fax: +613 353 5869 michael-hildebrand@uiowa.edu.
*These two authors contributed equally to this work

Keywords

DFNB3; *MYO15A* gene; missense mutation

INTRODUCTION

Sensorineural hearing loss (SNHL) is the most prevalent genetic sensory defect in humans. It is estimated that globally 4 out of every 10,000 children born have profound hearing loss[1]. Non-syndromic SNHL accounts for ~70% of hereditary hearing loss and 80% of SNHL cases have an autosomal recessive mode of inheritance (ARNSHL). To date, 25 genes and 67 loci have been implicated in ARNSHL[2].

The first report of hearing loss at the DFNB3 locus was from an isolated village in Indonesia where 2% of the population was hearing impaired[3]. Subsequently, it was shown that the causative mutation in this village and two unrelated families resided in the *MYO15A* gene[4]. Since these original reports, 24 additional DFNB3-causing mutations have been identified in *MYO15A*, which spans 71 kb on chromosome 17p11.2 and is comprised of 66 exons that encode a 3,530 amino acid protein in its longest form. (Table 1 and Fig.1)[5–8].

The encoded protein is the unconventional myosin XVa. Myosin XVa is unique among unconventional myosins in that it includes a long N-terminal domain (coded by exon 2) that is alternatively spliced to generate distinct class 1 and class 2 protein isoforms[10, 11]. The N-terminal domain is required for normal hearing, as premature stop mutations that result in loss of this domain cause DFNB3 hearing loss[6]. Myosin XVa also contains domains that are conserved within the myosin protein family, including the motor domain, IQ motifs (calmodulin/myosin light chain binding), MyTh4 domains (*M*yosin-*T*ail like *H*omology region 4), FERM motifs (*4*.1 protein, *E*zrin, *R*adixin, and *M*oesin), SH3 domain (*S*rc *H*omology *3*), and the PDZ ligand domain (Fig.1A)[10, 12].

DFNB3-causing mutations have been identified in all domains, with the exception of the IQ domains in the neck region, in families from Pakistan, India, Turkey, Indonesia, Brazil and North America (Table 1). In this study, we have characterized two Iranian ARNSHL families and identified two novel DFNB3-causing mutations in *MYO15A*. These novel missense mutations are both located in the first MyTh4 domain and are predicted to disrupt normal myosin XVa function.

MATERIALS AND METHODS

Family Reports

Family L-3165 is a five-generation consanguineous Iranian family segregating apparent ARNSHL (Fig. 2A). Hearing impaired persons appear in the 4th and 5th generations, consistent with autozygosity by descent. Family L-896 (Fig. 2B) is also a five-generation consanguineous Iranian family segregating apparent ARNSHL. In contrast to family L-3165, the inheritance pattern in this family appears to be pseudo-dominant.

For consenting persons in each family, audiologic testing was completed to document the degree of hearing loss. The test results for family L-3165 are displayed (Fig.3). Examination by an otolaryngologist and clinical geneticist excluded a syndromic presentation. Ten milliliters of whole blood were obtained as a DNA source. Human research institutional review boards at the Welfare Science and Rehabilitation University and the Iran University of Medical Sciences, Tehran, Iran, and the University of Iowa, Iowa City, Iowa, USA approved all procedures.

SNP Genotyping and Linkage Analysis of Family L-3165

Genomic DNA from individuals I-1, I-2, II-1, II-4, II-5, II-6, II-7, II-8, II-9 in family L-3165 (Fig. 2) was genotyped for 50,000 SNPs using Affymetrix 50K XBA GeneChips at the Translational Genomics Research Institute (TGEN, Phoenix, Arizona). Genotypes were determined using the BRLMM genotyping algorithm[13, 14]. Genotyping data were examined with PEDSTATS[15] for Mendelian inheritance errors, and MERLIN[16] for errors based on inferred double recombination events between tightly linked markers**.**

An autosomal, genome-wide parametric linkage analysis was performed since males and females appeared equally affected. All linkage analysis was performed with MERLIN. Since the pedigree had 18 bits it was analyzed using exact multipoint linkage analysis[16]. A subset of 6432 single nucleotide polymorphisms (SNPs) spaced approximately 0.5 cM apart across the genome and with an average heterozygosity of 0.43 was chosen from the 50K XBA set to satisfy the linkage equilibrium requirements of the Lander-Green algorithm for linkage analysis[17]. The selection and assembly of the data files were performed with an in-house PERL script (linkdatagen.pl, http://bioinf.wehi.edu.au/software). An initial parametric linkage analysis was run assuming a fully penetrant autosomal recessive model with a disease allele frequency of Pr(a)=0.0001 and penetrances of pr(disease|aa)=1=pr(disease|aA), Pr(disease|AA)=0. Haplotypes inferred with Merlin were imported into Haplopainter[18].

STRP analysis of Family L-896

Short tandem repeat (STRP) analysis was completed using markers developed by the Cooperative Human Linkage Center and made available through Research Genetics Incorporated (Invitrogen, Carlsbad, CA) according to a published methodology[1]. Markers with heterozygosity > 50% were selected for this analysis.

PCR and Sequencing

The *MYO15A* gene was amplified using gene-specific primers (Table 2). Amplification reactions were cycled using a standard protocol on a GeneMate Genius thermocycler (ISC BioExpress, UT, USA). Sequencing was completed with BigDye™ v3.1 Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. Sequencing products were read using an ABI 3730s sequencer (Perkin Elmer, Waltham, MA). All sequencing chromatograms were compared to published cDNA sequence; nucleotide changes were detected using Sequencher v4.5 (Gene Code Corporation, Ann Arbor, MI).

Conseq Analysis

Conservation scores for each amino acid in myosin XVa when compared to 50 similar protein sequences was determined using the Conseq program (http://conseq.tau.ac.il/). Any conservation score greater than 1 standard deviation above the mean for the myosin XVa amino acid sequence was regarded as highly conserved.

RESULTS

Linkage Mapping to the *DFNB3* Locus

Linkage analysis of family L-3165 detected one hundred and thirty nine Mendelian (0.24% error) and 465 double recombinant (0.8%) errors in the genome-wide analysis. The highest parametric LOD score of 3.7 was achieved for a region on chromosome 17 (Fig. 4). There were no other regions in the genome where the LOD score exceeded 3. The 1-LOD-drop region spanned approximately 56 cM flanked by markers SNP_A-1671362 and SNP_A-1663708 at chromosomal position 17p13.1-q24.3 (9.9–52 Mb). Haplotype analysis reveals that the critical

region coincides with the region identified in the linkage analysis (Fig.5). STRP analysis revealed DFNB3 was also the likely disease locus for family L-896 (data not shown).

Mutations in MYO15A

The critical region in both families contained the known ARNSHL locus DFNB3. Direct sequencing of the 65 exons of *MYO15A* identified novel homozygous missense mutations. In family 3165 a homozygous c.6371G>A mutation was identified that substitutes a glutamine for an arginine (p.R2124Q) in the first MyTH4 domain (Fig.2A and 6A). In family L-896 a novel homozygous missense mutation (c.6555C>T) was also detected in the first MyTH4 domain that results in substitution of a proline for a serine (p.P2073S) (Fig. 2B and 6B).

The R2124 and P2073 residues are both located in the first MyTH4 domain of the MYO15A protein and are conserved between human and mouse. The substitution of an uncharged, polar glutamine for arginine results in loss of the positive charge on the arginine sidechain. The bulky, non-polar sidechain of proline is replaced with a polar serine sidechain. The c.6371G>A mutation was not observed in 42 (84 chromosomes) Iranian control or 94 (188 chromosomes) CEPH control individuals. The c.6555C>T mutation was not observed in 43 Iranian control (86 chromosomes) or 93 (186 chromosomes) CEPH control individuals.

DISCUSSION

The unconventional myosin XVa protein is required for normal auditory function as its mutation leads to SNHL at the DFNB3 locus. Two other unconventional myosin proteins, VI and VIIa, are similarly required for normal auditory function, and their mutation also leads to hearing impairment (reviewed in Brown 2008). Myosins are molecular motor proteins that drive the movement of actin filaments via ATP hydrolysis to facilitate muscle contraction, organelle trafficking, cell movement, cytokinesis, and signal transduction[12].

Hair bundles located at the apex of sensory hair cells in the cochlea are responsible for the mechano-electrical transduction of sound waves in humans. Each hair bundle consists of up to 300 extracellular projections called stereocilia that contain an actin core and are arranged in rows of increasing height, forming a characteristic staircase architecture[19]. Extracellular lateral projections (side-links) hold the stereocilia of hair bundles together while tip-links at the apex of the stereocilia facilitate signal transduction[19, 25]. Myosin XVa immunoreactivity has been observed in stereocilia tips at E18.5 in the mouse, coinciding with formation of the hair bundles of the inner ear. Myosin XVa is located at the stereocilia tips following hair bundle maturation and the amount present is directly proportional to the length of the stereocilia[11, 20].

Homozygous *Shaker-2* (*sh2*) mice are deficient in myosin XVa due to a premature stop codon in the motor domain of the protein[9] (Fig.1). These mice exhibit profound deafness similar to that observed in human DFNB3 patients, in addition to vestibular defects that are not present in human patients. Hair cell stereocilia in *sh2* mice are correctly positioned during development, but are much shorter and do not display the characteristic staircase architecture when compared to those of wild-type mice[9]. Originally, it was hypothesized that the absence of myosin XVa in hair bundles resulted in a defect in mechanosensation[21]. However, the abnormally short stereocilia of deaf *sh2* mice retain mechanosensory activity suggesting that stereocilia length and the architecture of the hair bundle are crucial in maintaining normal hearing function[22].

The *whirler* (*whi*) mouse, which contains a mutation in the *whirlin* gene, shows profound hearing loss similar to that of *shaker* mice[26]. Homozygous *whirler mice* have a deficiency in the whirlin protein and their stereocilia appear similar to those in *shaker* mice: abnormally short with no staircase formation[4, 9]. The tail of myosin XVa, of which the MyTH4 domains

form part, interacts with whirlin, suggesting that these two proteins are partners[20, 21]. The PDZ ligand domain of myosin XVa and two of the three PDZ binding domains of whirlin are required for this interaction. Myosin XVa is the only myosin in mammals known to contain a PDZ domain[21].

Homozygous *whirler* mice (lacking whirlin) have myosin XVa present at their stereocilia tips, while homozygous *shaker* mice (lacking myosin XVa) have neither whirlin nor myosin XVa present at their stereocilia tips[11,21]. Myosin XVa has been observed migrating towards the plus ends of actin filaments at stereocilia tips, confirming myosin XVa activity as a motor protein[21]. These data support the hypothesis that myosin XVa acts as a transport protein and whirlin is its cargo during development of hair bundles in the cochlea.[11, 20, 21] This transport most likely occurs during lengthening and formation of stereocilia staircase morphology, given the localization of myosin XVa during this period[20,21].

Mutations in all but one protein domain of myosin XVa have been shown to be causative of DFNB3 hearing loss. Three missense mutations in the first MyTH4 domain of the protein have been described previously: two homozygous mutations in Indian and Balinese families, and one hemizygous mutation in a family affected by Smith-Magenis syndrome from North America (Table 1). Here we report two further mutations in the first MyTH4 domain and the first DFNB3 mutations segregating in Iranian families. The arginine-to-glutamine and proline-to-serine substitutions result in the loss of positively charged and bulky, non-polar sidechains and replacement with residues containing polar sidechains. These alterations are predicted to negatively affect the function of the myosin XVa protein, perhaps by disrupting its interaction with whirlin.

Given the large number of DFNB3-causing *MYO15A* mutations identified in various populations (Fig.1 and Table 1) and the size of the gene, it would be useful to develop a more targeted screening approach for suspected DFNB3 ARNSHL families. In Table 3 and Figure 1 all known *MYO15A* mutations and the relative conservation of amino acids in each domain of the protein are depicted. A targeted sequencing strategy would focus primarily on the domains with the highest mutation rate and highest percentage of conserved amino acids. The motor domain is the most highly conserved (84.5%) domain and the site of ten mutations. Conversely, the N-terminal domain, which is unique among myosins, contains the least conserved residues and only three mutations. Therefore, sequencing of the motor domain should be a priority.

When the mutations identified in this study are included, the first MyTH4 domain contains a relatively high number of known mutations (5). This domain also contains a high percentage of conserved amino acids relative to other domains of the protein. We therefore recommend prioritizing screening of exons encoding the motor and first MyTH4 domains in ARNSHL families linked to the DFNB3 locus.

The structure of the MyTH4 domain has not been characterized. In other myosins, the MyTH4 domain has been implicated in microtubule binding as well as actin binding to the plasma membrane[24]. Some data suggest that the MyTH4/FERM domains in myosin XVa are required for localization of myosin XVa to stereocilia tips, although their specific function has not been elucidated[25]. The co-localization of myosin XVa and whirlin proteins appears essential to form the transmembrane actin microfilament assembly complex at the stereocilia tips. We speculate that the p.R2124Q and p.P2073S mutations interfere with the interaction between myosin XVa and whirlin, thereby preventing the formation of this complex which appears to be required for normal hearing.

V. CONCLUSION

We have identified novel mutations in the *MYO15A* gene in two Iranian DFNB3 families. These mutations are the first to be described in the Iranian population and both affect the first MyTH4 domain of the myosin XVa protein. The first MyTH4 and motor domains are highly conserved at the protein level and constitute common sites for DFNB3-causing mutations. As such they should be preferentially screened in DFNB3 ARNSHL families. Hearing impairment in a mouse model of DFNB3 has been rescued by supplementation of wild-type *MYO15A*[21]. Given advances in genetic therapies for hearing loss[27], a similar gene replacement strategy could be developed for DFNB3 families like the ones described in this report.

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Abbreviations

MYO15A	myosin 15A gene
MYO15A	myosin 15A protein
ARNSHL	autosomal recessive non-syndromic hearing loss
SNHL	sensorineural hearing loss
MYTH4	myosin tail homology 4 domain

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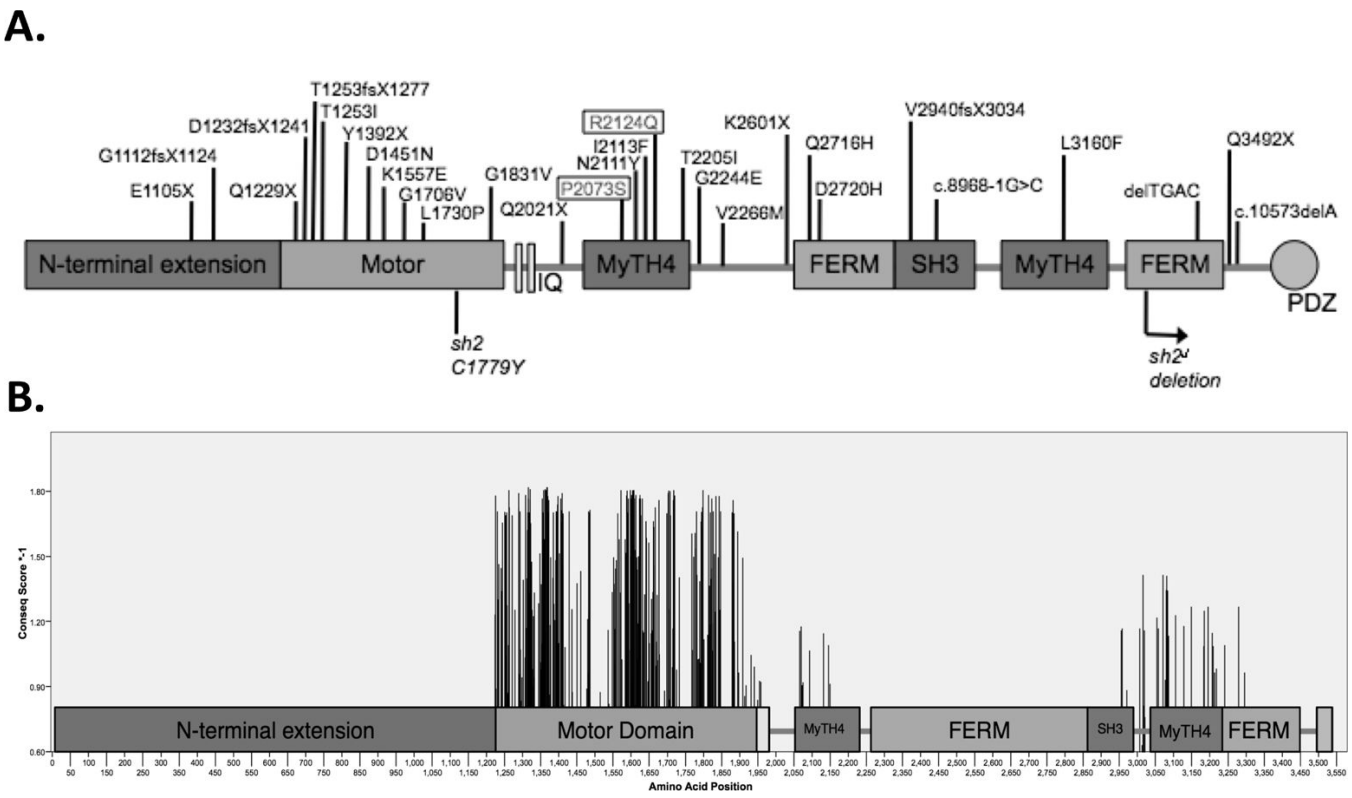

Figure 1.

Domains of the myosin XVa protein. A. Diagram showing the location of human (top) and mouse (bottom) *MYO15A* mutations. The p.R2124Q and p.P2073S mutations are boxed Figure is not to scale. B. Diagram showing the amino acid positions in each domain of myosin XVa (x-axis) and Conseq conservation scores for each residue (y-axis). Any residue with a Conseq score less than 7 (E-value >0) was excluded. Figure is to scale.

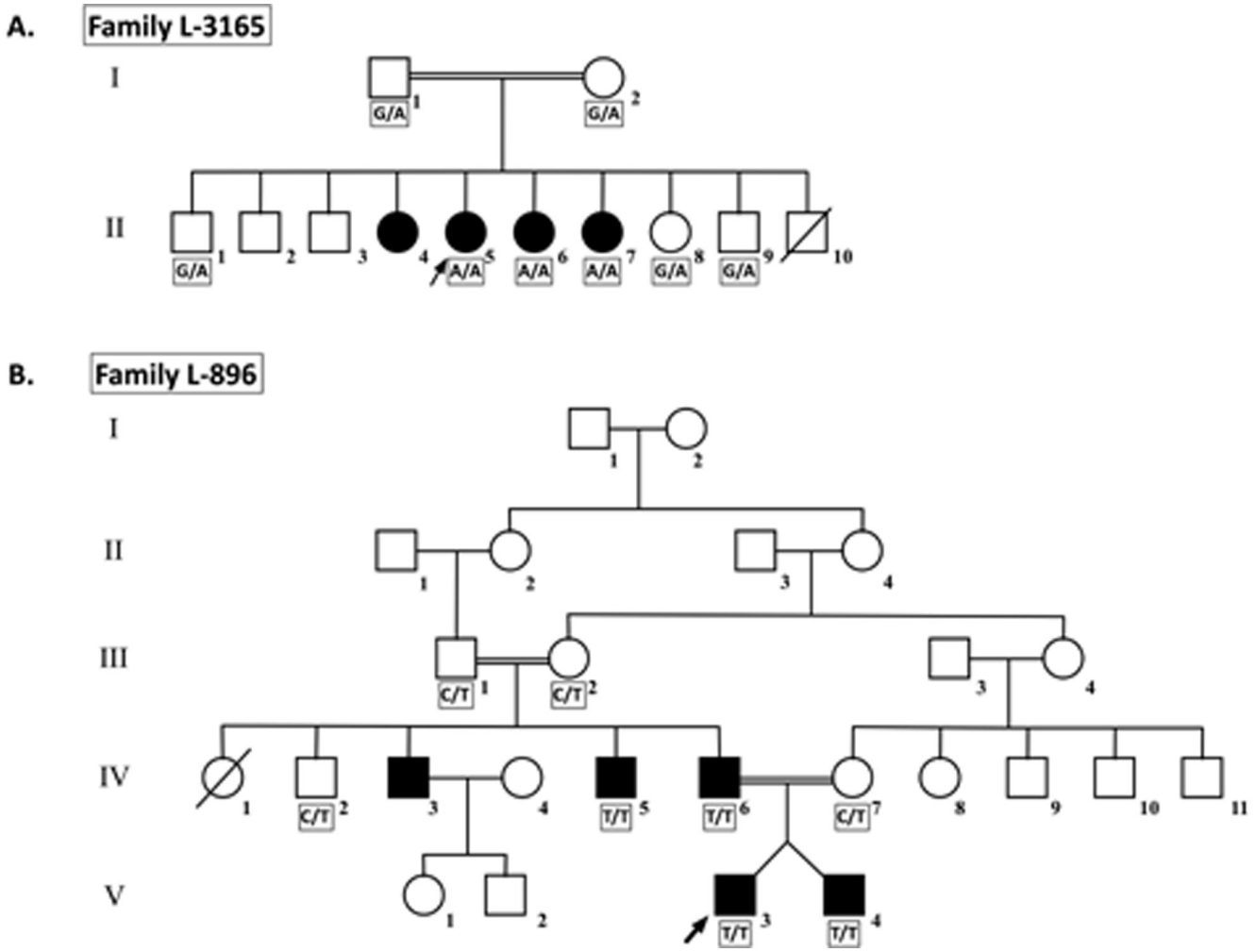

Figure 2.

Pedigrees of the Iranian families affected with DFNB3 ARSNHL A. Family L-3165; Genotypes for individuals carrying the c.6371G>A mutation are shown. B. Family L-896; Genotypes for individuals carrying the c.6555C>T mutation are shown. Open symbols = unaffected; filled symbols = affected; diagonal line = deceased.

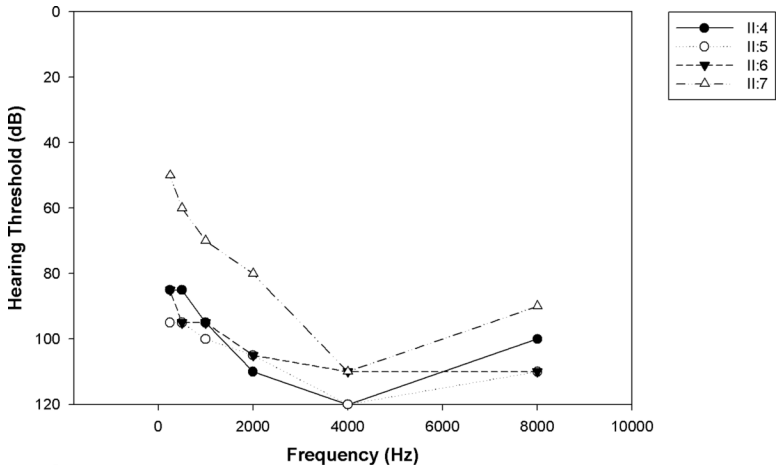

Figure 3.
Audiograms of affected members of the family L-3165. All affected individuals displayed severe-to-profound bilateral SNHL.

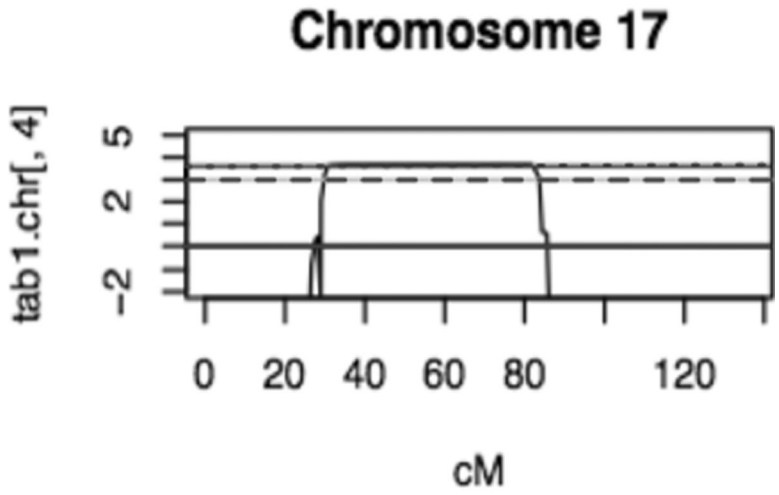

Figure 4.
Linkage Mapping of family L-3165. Results from chromosome 17 showing significant LOD score of 3.7 (dotted line). LOD scores of 1 (solid line) and 3 (dashed line) are also indicated

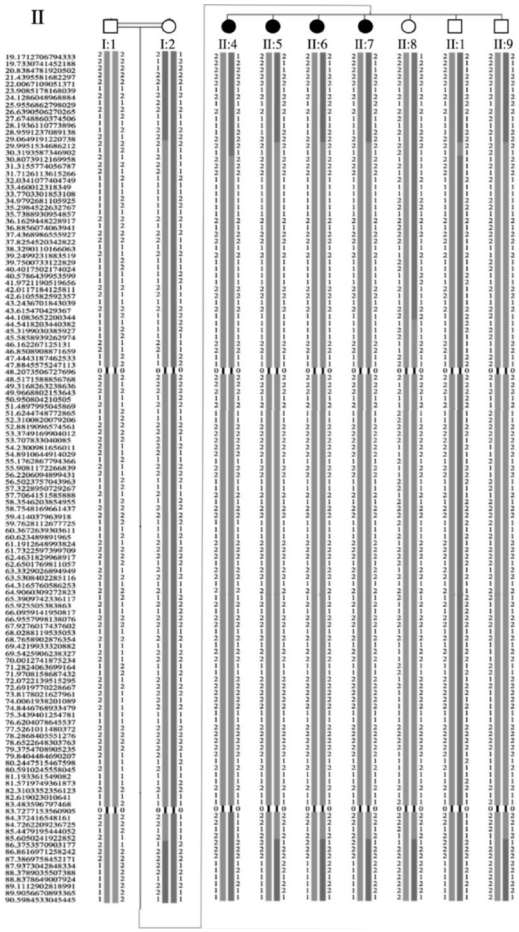

Figure 5.
Haplotype analysis of family L-3165. Affymetrix SNP markers on chromosome 17 are shown.

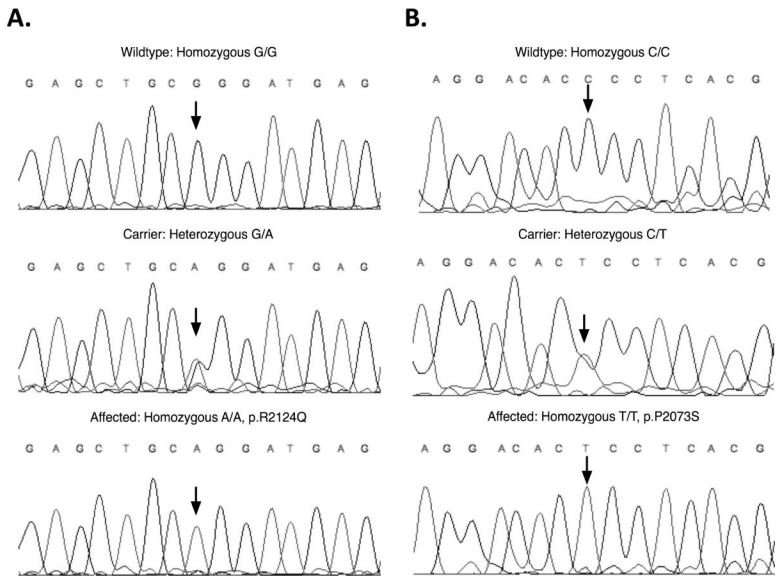

Figure 6.

Mutations in the *MYO15A* gene. Chromatograms showing the c.6371G>A mutation found in family L-3165 (A) and c..6555C>T mutation found in family L-896 (B).

Table 1

DFNB3-causing *MYO15A* mutations.

Exon (domain)	Amino Acid Change	Mutation Type	Origin of Family (#)	Reference
Exon 2 (N-terminal)	E1105X	nonsense	Pakistan (1)	6
Exon 2 (N-terminal)	G1112fsX1124	deletion frameshift	Pakistan (1)	6
Exon 3 (motor)	Q1229X	Nonsense	Pakistan (1)	5
Intron 4	D1232fsX1241	splice donor site	Pakistan (1)	5
Intron 4	T1253fsX1277	splice donor site	Pakistan (1)	6
Exon 5 (motor)	T1253I	missense	India (1)	6
Exon 10 (motor)	Y1392X	nonsense	Pakistan (1)	6
Exon 12 (motor)	D1451N	missense	India (1)	6
Exon 15 (motor)	K1557E	missense	Pakistan (1)	6
Exon 18 (motor)	G1706V	missense	Pakistan (1)	6
Exon 19 (motor)	L1730P	missense	Pakistan (1)	6
Exon 21 (motor)	G1831V	missense	Turkey (1)	7
Exon 28	Q2021X	nonsense	Pakistan (1)	6
Exon 30 (MyTH4 a)	N2111Y	missense	India (1)	4
Exon 30 (MyTH4 a)	I2113F	missense	Bali (1)	4
Exon 30 (MyTH4 a)	R2124Q	missense	Iran (1)	This study
Exon 30 (MyTH4 a)	P2073S	missense	Iran (1)	This study
Exon 31 (MyTH4 a)	T2205I	missense*	North America (1)	5
Exon 32	G2244E	missense	Pakistan (1)	6
Exon 33	V2266M	missense	Pakistan (1), Turkey (1)	6
Exon 41	K2601X	nonsense	India (1)	4
Exon 44 (FERM a)	Q2716H	missense	Pakistan (1)	5
Exon 45 (FERM a)	D2720H	missense	Pakistan (4)	6
Exon 51 (SH3)	V2940fsX3034	frameshift	Pakistan (1)	6
Intron 50	-	splice donor site	Turkey (1)	7
Exon 57 (MyTH4 b)	L3160F	missense	Pakistan (1)	6
Exon 62 (FERM b)	-	deletion frameshift**	Brazil (1)	8
Exon 65	Q3492X	nonsense	Pakistan (1)	6
Exon 66 (PDZ ligand)	-	deletion frameshift	Brazil (1)	8

* Mutation was found in a patient hemizygous at the DFNB3 locus with Smith-Magenis Syndrome

** Mutation was found in a hemizygous individual.

Table 2

Oligonucleotides used for amplification of the *MYO15A* gene.

Exon	Forward Primer (5'–3')	Reverse Primer (5'–3')
2	MULTIPLE – AVAILABLE UPON REQUEST	
3	ATG ACC AAG CCA GGG GTC	CTC TGG CTG GGA GGG TG
4	GAC CCA TGC CAG AAC CAG	AGA AAT CTG TGC GTC CCA CC
5	ATC TGT CCG GAT GGA AAC AG	TCT GAC TCA TGG CTC AGG TG
6--7	GGG AGG TGT GGG AGC TTA G	TCG GGA GTA CAT GAG GTG TG
8	TCC TGG AGA GAG TGG TGG TC	CTA GGA CAG GCC TTT GGA TG
9--10	GGG TGT CCC CAG CTA TGC	TAT CTG TAC CTC CCA CCC CG
11	GTT CTC ATC TGC AGC CCA CT	AAA CTC ACC CTC CCC AAA TC
12	CAA CTC AGG CCA CCA CAC TA	AAA ACA GGA ACA AGT GAT ATG TGC
13	GAC TAC TGG CAT GAG CCA CA	TGA CCC AGG GAC AGA GAG AG
14--15	GCT TTC CGG AGG CAG AG	GAG GGA GGC GAG ACC TTG
16	AGG GAA GGT AGG GGC AAA	CTG TCT CCA AGG AGG TCC AC
17	ATT CAA CAT GGG AGG GAG G	TGA GGA CAT GAG GCT GAG AG
18	ATA GTG AGG TTG CCA CCA GG	TCT CCA ACA GCT AGC AGC AC
19	TCC CTC CTA GGA TAG ACA GAG AG	AAG GCA GGC TGG GTG TG
20	TTC CTC CTC ATT TCG GTC TC	CAA GGT CAC ACA GCA TGG G
21–22	TTC CTC CTC ATT TCG GTC TC	CAA GGT CAC ACA GCA TGG G
23	TAG CAG ACA CCT CGG GTA GG	GAC TCA GTA GTT GTG GAC CCC
24	CTT AGT CCA GCC TCC TGG C	TTC AGG CGT GAC CTC TCC
25	AGG GCC TCT CTA CCT TTT GG	CTA AGT GCC CTT TCC CCT TC
26--28	GTG CCG GTC GTC ACC TC	CCC AGG GCA AGG ACA ATG
29	CAC AGA GCA GTG GGT CCA G	CTC ATG GCC CAG TTT CAG G
30	GGG GAC TGG AAG GAA CAA C	CTT TAA GAC CCT GCC TTG GG
31	CAG CCC TCA GCC CCA AG	ACT GGG CCC TGC TGA CTC
32	GCA CAG CCA AAC TGG ACT C	CCT TCT GCC TGG GAG TGG
33	TCT GTT CAT GTT TAG GGT CTG G	CTC AGC CTG TCC CAG CAG
34--35	GGA GAA AGC CAC TGA ATA CCA G	GAG AAG CTC TCA GGT CAC CC
36--38	AGT GTC AGG TGC CTG TTG C	TCC TCT TTA CAG CTT GTG TCT CC
39--40	TCT GGA GTC CCA GAG AGC AG	GGG CCA TGA TGG ACA CTC
41--42	ATG TGA TGG GAA AGG GAG AC	CTG TGC CCA CAG ACT TCC TC
43	ACT CTA GCC TGG GGG ACA AC	CCC AAG TCC TAG ACC CTC CT
44	CCC AGG AGG ACA GAA AAA GG	GGG AGG GGG AGA TTC AAT AA
45	AGT ATA GTC CAG CCT GGG TCC	CTG GCT GTG CCT CTG ACT G
46	TGG CCA TCT CAT CCA TTT CT	CAC AGC TAG GAG CTG CAC AC
47	GAA CCA GCT GGA CAC ACA GA	AAA TGG GTT TGC TTC AAT GG
48	GGG CAG GAC AGG ATC AGA AG	AGG GAG ATC CCT GTT GCT G
49--50	CTA GGC CTC TGG GAG TGG	CAC CAC GAG TGG GTG AAA C

Exon	Forward Primer (5'–3')	Reverse Primer (5'–3')
51	CCC CTT AGT CAC AAG ACA AGA C	TTA TCC CCA CTC GCC TCA C
52	CTA GGG GTT CGC TTG TCA GT	AGT GGG GCC TCC GAG ACT
53	TGT GAG GCT CAT TTC AGT GC	AGG GTG CTG AGA ATC AGA GG
54--55	TGT GTC CCC TTT CTG TTC TG	TGA TAG ATG GGG AAA CTG AAC C
56	GTG CCC ACC CTG TTC TTA TG	CCT CCT GGA GCA TGG ACA C
57	TCT CAG CTC AAT CCC AGG AG	TCC ACC CAG TCC CCA AG
58	ATG GGG GAG TAA ATG CCT TC	GGC TTG TGT CTC CCA TTC AT
59	CAG GAG ACA AGG GCT GTC C	CTG GAG CCT GGG CTG TC
60	AGA AGG ACA GAG GTC AAG CC	AAA TCT GGG TGG AGG GC
61	AAG CTG TGT CCC AGA ACA GG	ACA GGG CCT GAA TCA TGA AC
62	TGA GAG GGC AGG GTT GC	CAT GCA TGT CCC CAG GTC
63	ACA GTG AGG ATT GCC TGA GC	TAC CCA TCC TCC ATG ACC AC
64	AGC CCA GAG AAG CTA TGC AG	AGG CTC AGA GGA GGG AAG AG
65	TGG TTG AGA CTA TCC TCG CC	GAC CTG ACC TAT CTT GGA GCC
66	CAA GGT AAG AGC TGG GGA AG	TTG ATC CTG AGA GGT TCA GTG

Table 3

Characteristics of myosin XVa domains

Region:	Domain:	Amino Acids	Number of amino acids in domain	Encoded by:	Number ofexons in domain	Number of known mutations in domain	Mutations per exon	%of highly conserved amino acids
Head	N-terminal extension	1–1230	1231	Exons 2–3	2	2	1.00	1.2
	Motor Domain	1231–1942	712	Exons 3–24	22	10	0.45	84.5
Neck	IQ domains	1943–1970	28	Exons 24–25	2	0	0.00	0.9
	Other domain	1971–2059	89	Exons 25–28	4	1	0.25	0.0
Tail	MyTH4 (a)	2060–2230	171	Exons 29–31	3	5	1.67	3.2
	Other domain	2231–2255	25	Exons 32–42	11	3	0.27	0.0
	FERM (a)	2256–2857	602	Exons 43–48	6	2	0.33	0.0
	SH3	2858–2989	132	Exons 49–51	3	2	0.67	1.2
	Other domain	2990–3028	39	Exon 52	0	0	0.00	1.8
	MyTH4 (b)	3029–3230	202	Exons 53–59	7	1	0.14	6.5
	FERM(b)	3231–3450	220	Exons 59–64	6	1	0.17	0.9
	Other domain	3451–3497	47	Exon 65	1	1	1.00	0.0
	PDZ ligand	3498–3531	34	Exon 66	1	1	1.00	0.0