RESEARCH ARTICLE

Identification of a Novel MYO15A Mutation in a Chinese Family with Autosomal Recessive Nonsyndromic Hearing Loss

Hong Xia1,2, Xiangjun Huang1, Yi Guo1,3, Pengzhi Hu4, Guangxiang He5, Xiong Deng1, Hongbo Xu1, Zhijian Yang1, Hao Deng1*

1 Center for Experimental Medicine and Department of Neurology, the Third Xiangya Hospital, Central South University, Changsha, China, 2 Department of Emergency, the Third Xiangya Hospital, Central South University, Changsha, China, 3 Department of Medical Information, Xiangya School of Medicine, Central South University, Changsha, China, 4 Department of Radiology, the Third Xiangya Hospital, Central South University, Changsha, China, 5 Department of Otolaryngology-Head Neck Surgery, the Third Xiangya Hospital, Central South University, Changsha, China

* hdeng008@yahoo.com

Abstract

Autosomal recessive nonsyndromic hearing loss (ARNSHL) is a genetically heterogeneous sensorineural disorder, generally manifested with prelingual hearing loss and absence of other clinical manifestations. The aim of this study is to identify the pathogenic gene in a four-generation consanguineous Chinese family with ARNSHL. A novel homozygous variant, c.9316dupC (p.H3106Pfs*2), in the myoxin XVa gene (MYO15A) was identified by exome sequencing and Sanger sequencing. The homozygous MYO15A c.9316dupC variant co-segregated with the phenotypes in the ARNSHL family and was absent in two hundred normal controls. The variant was predicted to interfere with the formation of the Myosin XVa-whirlin-Eps8 complex at the tip of stereocilia, which is indispensable for stereocilia elongation. Our data suggest that the homozygous MYO15A c.9316dupC variant might be the pathogenic mutation, and exome sequencing is a powerful molecular diagnostic strategy for ARNSHL, an extremely heterogeneous disorder. Our findings extend the mutation spectrum of the MYO15A gene and have important implications for genetic counseling for the family.

Introduction

Congenital or prelingual hearing loss is a common sensorineural disorder, with a prevalence of about one in 500–1,000 at birth, and at least half of the cases are caused by genetic factors [1,2]. At least 70% of the cases manifest with isolated hearing loss without other associated clinical features, which is classified as nonsyndromic deafness [3]. Hereditary hearing loss mainly displays autosomal recessive or autosomal dominant transmission [2], and X-linked [4] or mitochondrial inheritance [5] is occasionally reported. Most of hereditary deafness manifests as autosomal recessive nonsyndromic hearing loss (ARNSHL) [3].
ARNSHL is an extremely heterogeneous disease, generally manifested with congenital or prelingual hearing loss without associated clinical symptoms, though postlingual hearing loss has also been reported [2,6]. The individuals with early-onset deafness often encounter obstacles for linguistic development [7]. Since identification of the gap junction protein beta-2 gene (GJB2) as the disease gene for ARNSHL [8], more than 42 genes have been identified and at least 1,949 pathogenic variants have been reported [9]. Mutations in these genes affect cochlear homeostasis, cellular organization, neuronal transmission, cell growth, differentiation and survival, and tectorial membrane associated proteins [2]. Cochlear implantation has been reported to offer satisfactory auditory performance to patients with severe to profound deafness caused by mutations in the GJB2 gene, the solute carrier family 26 member 4 gene (SLC26A4), the otoferlin gene (OTOF) [10], or the myosin XVa gene (MYO15A) [11]. Genetic diagnosis plays an important role in prognosis evaluation, clinical management, and prenatal diagnosis for ARNSHL families [12].

It is difficult to identify causative mutations using regular Sanger sequencing because of high heterogeneity of ARNSHL. Recently, exome sequencing has been introduced and confirmed as an effective alternative strategy [13]. In this study, a novel homozygous mutation in the MYO15A gene was identified in a Chinese ARNSHL family by exome sequencing.

Materials and Methods

Subjects

A four-generation consanguineous Chinese Han family with ARNSHL was recruited, and four members of the family participated in this study. Bilateral prelingual deafness was observed in the two siblings (IV:1 and IV:2, Fig 1A), who received neither hearing aids nor cochlear implantation in their childhood. However, their parents (III:1 and III:2, Fig 1A) had normal hearing. Two hundred ethnically-matched unrelated subjects (age 29.5±6.5 years) with normal hearing were enrolled as controls. Clinical and audiometric assessments were performed, and peripheral blood samples were collected from all the subjects after obtaining written informed consent from the participants or guardians. The study was approved by the Institutional Review Board of the Third Xiangya Hospital, Central South University, China.

Clinical evaluations

Clinical and audiometric assessments were performed on the subjects of the family in the Third Xiangya Hospital, Changsha. Pure tone audiometry (PTA), tympanometry, acoustic reflex (AR) thresholds, auditory brainstem responses (ABR), transient evoked otoacoustic emission (TEOAE) and distortion product otoacoustic emission (DPOAE) were conducted. Magnetic resonance imaging (MRI) was carried out to exclude congenital inner ear malformations. The hearing level was assessed at 250, 500, 1000, 2000, 4000 and 8000 Hz by PTA, and sorted into normal (<20 dBHL), mild (20–40 dBHL), moderate (41–70 dBHL), severe (71–95 dBHL), and profound (>95 dBHL) deafness [14].

Whole exome sequencing and variant analysis

Genomic DNA was extracted from peripheral blood samples of all the subjects using standard phenol-chloroform extraction method [15]. Exome sequencing was conducted by Novogene Bioinformatics Institute, Beijing, China. At least 1.5 micrograms (µg) of genomic DNA from the proband (IV:2, Fig 1A) was sheared by Covaris sonicators, and was enriched, hybridized, and captured on the Agilent SureSelect Human All Exon V5, following the manufacturers’ procedures. The captured library was sequenced with the Illumina HiSeq 2000 sequencing
instruments. The average sequencing depth of 57.36× provided enough depth to exactly call variants at 97.4% of targeted exome [16].

The clean reads without adapter or debased reads were mapped to the human reference genome (UCSC hg19, http://genome.ucsc.edu/) using Burrows-Wheeler Alignment tool (BWA) [17,18]. Single nucleotide polymorphisms (SNPs) and insertions/deletions were identified by the Sequence Alignment/Map tools (SAMtools) [19], and then Picard was applied to mark duplicate reads. All variants were screened with the SNP database version 142 (dbSNP142), 1000 Genomes Project (version 2014 October), and NHLBI Exome Sequencing Project (ESP) 6500. Functional prediction was carried out by Sorting Intolerant from Tolerant (SIFT) and Polymorphism Phenotyping version 2 (PolyPhen-2). Candidate variants were annotated by the ANNOVAR (Annotate Variation) software [20].

**Direct Sanger sequencing and functional prediction**

Direct Sanger sequencing was performed to confirm potential causative variants in the family with ABI3500 sequencer (Applied Biosystems, Foster City, CA, USA) [21]. Primer sequences for pathogenic variant in the MYO15A gene (NM_016239.3) were designed as follows: 5’-
and 5'-ACTCACTGCTTGGAGCTGGT-3'

MutationTaster was applied to the functional prediction of the MYO15A pathogenic variant [22].

**Results**

**Clinical findings**

Both patients (IV:1 and IV:2, Fig 1A) presented with deafness and dumbness. Bilateral profound sensorineural hearing loss with thresholds over 95 dBHL was revealed by PTA. Type A tympanometric curve was shown by acoustic immittance measurement, and no inner ear anomaly was discovered by MRI in the two patients. The ABR at 97 dB, AR, TEOAE and DPOAE were absent in both ears of the proband (IV:2, Fig 1A) and the right ear of the elder sibling (IV:1, Fig 1A), while the waves I, III and V of ABR were elicited at 80 dB, remarkably elevated acoustic reflex threshold (80–105 dB) was recorded, and low amplitude DPOAE was elicited at 500, 1000 and 4000 Hz in the left ear of the IV:1 patient, which suggested that some residual hearing might exist in the left ear of the IV:1 patient. The clinical information of the ARNSHL family was summarized in Table 1.

**Exome sequencing**

A total of 19,816,364 pairs of sequenced reads with the average read length of 125 bp were generated by exome sequencing, and 98.76% (19,569,878) of sequenced reads passed the quality assessment and were mapped to 99.81% of the human reference genome [16]. Known variants identified in dbSNP142 with minor allele frequency (MAF) > 1%, 1000 Genomes Project with a frequency of > 0.5%, and NHLBI ESP6500 were filtered out. PolyPhen-2 and SIFT were applied to predict functional effects of non-synonymous SNPs. Subsequently, a homozygous MYO15A c.9316dupC variant was observed in the proband (IV:2, Fig 1A and 1B) and other possible pathogenic mutations for ARNSHL were excluded.

**Identification of pathogenic mutation**

The homozygous MYO15A c.9316dupC variant was confirmed by Sanger sequencing. The same homozygous MYO15A variant was also detected in his affected sibling (IV:1, Fig 1A), and the heterozygous MYO15A c.9316dupC variant was identified in both of his unaffected parents (III:1 and III:2, Fig 1A and 1C). However, the variant was absent in two hundred ethnically-matched unrelated controls (Fig 1D). The homozygous MYO15A c.9316dupC variant, which co-segregated with the phenotype of deafness and dumbness in the family, and was predicted to lead to a shift in the reading frame at amino acid position 3106 and a premature stop codon (p.H3106PfsX2) by MutationTaster [22], might be the disease-causing mutation in the ARNSHL family.

| Subjects | Age | Hearing loss | DPOAE | ABR | AR | MRI | MYO15A c.9316dupC mutation |
|----------|-----|--------------|-------|-----|----|-----|---------------------------|
| III:1    | 58  y | Normal       | Bil (+) | Bil (+) | Bil (+) | Normal | Heterozygous |
| III:2    | 57  y | Normal       | Bil (+) | Bil (+) | Bil (+) | Normal | Heterozygous |
| IV:1     | 32  y | Bil profound | L (A), R (-) | L (A), R (-) | L (A), R (-) | Normal | Homozygous |
| IV:2     | 28  y | Bil profound | Bil (-) | Bil (-) | Bil (-) | Normal | Homozygous |

A, abnormality; ABR, auditory brainstem responses; AR, acoustic reflex; Bil, bilateral; DPOAE, distortion product otoacoustic emissions; L, left; MRI, magnetic resonance imaging; MYO15A, the myosin XVa gene; R, right; y, years; +, presence; -, absence

Table 1. Phenotypes and genotypes of the ARNSHL family.

TGC...
Discussion

In 1995, a disease gene locus (deafness, autosomal recessive 3; \textit{DFNB3}) for ARNSHL was first mapped to chromosome 17p-17q12 by linkage analysis of two large multi-generation families from Bengkala, Bali [23], and then was further refined to chromosome 17p11.2 [24]. In 1998, the homozygous p.N2111Y, p.I2113F and p.K2601C (previously known as p.N890Y, p.I892F and p.K1300C) mutations in the \textit{MYO15A} gene were identified in three unrelated \textit{DFNB3} families [25,26]. A hemizygous p.E2205I mutation of the \textit{MYO15A} gene was also reported to be associated with moderately severe hearing loss in a Smith-Magenis syndrome (del(17)p11.2) patient [27].

Homozygous \textit{MYO15A} mutations cause 6.2\% of ARNSHL in Turkey [3], and mutations in the \textit{MYO15A} gene account for no less than 5\% of autosomal recessive profound hearing loss in Pakistan [27]. To date, at least 86 pathogenic variants of the \textit{MYO15A} gene have been reported in deafness populations [3,11,26–47], which are summarized in Fig 2. The p.D2720H mutation in the \textit{MYO15A} gene is considered as a founder mutation in Pakistan [46], and the p.R1937Tfs*10 and p.S3335Asfs*121 mutations in the \textit{MYO15A} gene were also identified as founder mutations in Turkish population [3]. Most mutations in the \textit{MYO15A} gene are connected with congenital severe to profound sensorineural deafness [27,46], while some patients also display progressive hearing loss [11,36]. Intriguingly, a homozygous p.Y289* mutation in the \textit{MYO15A} gene was associated with maintenance of considerable residual hearing in two Turkish patients [3]. High frequency hearing loss or retention of some hearing at low frequency was also reported in patients with \textit{MYO15A} mutations [11,46].

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{The schematic structure and the mutations of the human myosin XVa. The myosin XVa consists of 3530 amino acids, including an N-terminal extension domain and Motor domain, two light chain binding IQ motifs, two myosin-tail homology 4 (MYTH4) domains and band 4.1/ezrin/radixin/moesin (FERM) domains, a Src-homology-3 (SH3) domain and a C-terminal class I PDZ-ligand domain. The novel \textit{MYO15A} mutation in this study is showed with red box at the bottom of the figure, and previously reported mutations are displayed at the top of the figure. \textit{MYO15A}, the myosin XVa gene.}
\end{figure}
The MYO15A gene contains 66 exons and encodes several alternatively spliced transcripts in the inner ear [25]. The complete transcript consists of 3530 amino acids, including a long N-terminal extension encoded by exon 2, an N-terminal motor domain, two light chain binding IQ motifs, and a tail region containing two myosin-tail homology 4 (MyTH4) domains, two band 4.1/ezrin/radixin/moesin (FERM) domains, a Src-homology-3 (SH3) domain and a C-terminal class I PDZ-ligand domain [25,48]. Myosin XVα protein is mainly expressed in the cuticular plate and stereocilia of the cochlear inner and outer hair cells [25], and is commonly localized at the tips of inner ear sensory cell stereocilia [49]. Myosin XVα is involved in staircase formation of the hair bundle, which is indispensable to sound detecting and head movement [48,49].

Homozygous p.C1779Y mutation in the Myo15 gene, a murine homologue of the human MYO15A gene, cause profound sensorineural deafness, vestibular defects, and extremely short stereocilia on the inner and outer hair cells in shaker-2 mice [50].

In this study, the homozygous c.9316dupC variant in the MYO15A gene was identified in the two affected siblings, but was absent in the unaffected parents and two hundred normal controls. The homozygous c.9316dupC variant in the MYO15A gene co-segregated with the phenotype of deafness in the ARNSHL family and might be the disease-causing mutation. Both affected siblings display bilateral prelingual, profound sensorineural hearing loss, in accordance with most MYO15A-associated ARNSHL phenotypes [3]. Their language acquisitions were hindered by profound prelingual deafness [7], thus they also present with dumbness phenotypes. The audiometric tests of the IV:1 patient implied that the patient might have some residual hearing, consistent with the previous report [46].

The novel c.9316dupC variant in the MYO15A gene, located in the second MyTH4 domain [43], was predicted to result in a shift in the reading frame and a premature stop codon (p. H3106Psf/C) by MutationTaster [22], which leads to a truncated protein missing part of the second MyTH4 domain, the second FERM domain and PDZ-ligand in the tail region of myosin XVα (Fig 2). More than ten mutations have been reported in the second MyTH4 and FERM domain of myosin XVα (summarized in Fig 2). The MyTH4-FERM region is involved in formation of the Myosin XVα-whirlin-Eps8 complex [51] and microtubule binding [52]. Thus, the c.9316dupC variant in the MYO15A gene might interfere with formation of the Myosin XVα-whirlin-Eps8 complex, which is indispensable for stereocilia elongation and sound detecting [48,51].

Conclusion
The homozygous c.9316dupC variant in the MYO15A gene was the pathogenic mutation in our ARNSHL family. Our study demonstrated that exome sequencing is a powerful molecular diagnostic strategy for ARNSHL, an extremely heterogeneous genetic disorder. Our findings extend the mutation spectrum of the MYO15A gene, and have implication in genetic counseling for the ARNSHL family.

Acknowledgments
The authors are grateful to the participating individuals and investigators for their co-operation and efforts in collecting clinical and genetic information and DNA specimens.

Author Contributions
Conceived and designed the experiments: HD HX. Performed the experiments: HD HX HBX XD ZJY. Analyzed the data: HD HX XJH YG PZH HBX. Contributed reagents/materials/analysis tools: HD HX XJH YG PZH GXH. Wrote the paper: HD HX.
References

1. Mahdieh N, Shirkavand A, Rabbanib B, Tekin M, Akbari B, Akbari MT, et al. Screening of OTOF mutations in Iran: a novel mutation and review. Int J Pediatr Otorhinolaryngol. 2012; 76: 1610–1615. doi:10.1016/j.ijporl.2012.07.030 PMID: 22906306

2. Duman D, Tekin M. Autosomal recessive nonsyndromic deafness genes: a review. Front Biosci (Landmark Ed). 2012; 17: 2213–2236.

3. Cengiz FB, Duman D, Sirmaci A, Tokgoz-Yılmaz S, Erbek S, Ozturkmen-Akay H, et al. Recurrent and private MYO15A mutations are associated with deafness in the Turkish population. Genet Test Mol Biomarkers. 2010; 14: 543–550. doi:10.1089/gtmb.2010.0039 PMID: 20642360

4. Stanton SG, Griffin A, Stockley TL, Brown C, Young TL, Benteau T, et al. X-linked hearing loss: two gene mutation examples provide generalizable implications for clinical care. Am J Audiol. 2014; 23: 190–200. doi:10.1044/2014_AJA-13-0040 PMID: 24687041

5. 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; 133A: 27–30. PMID:15637703

6. Hoefsloot LH, Feenstra I, Kunst HP, Kremer H. Genotype phenotype correlations for hearing impairment: approaches to management. Clinical Genetics. 2014; 85: 514–523. doi:10.1111/cge.12339 PMID: 24547994

7. Dror AA, Avraham KB. Hearing impairment: a panoply of genes and functions. Neuron. 2010; 68: 293–308. doi:10.1016/j.neuron.2010.10.011 PMID: 20959396

8. Kelsell DP, Dunlop J, Stevens HP, Lench NJ, Liang JN, Parry G, et al. Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. Nature. 1997; 387: 80–83. PMID: 9139825

9. Shearer AE, Epsteiner RW, Booth KT, Ephraim SS, Gurrola JN, Simpson A, et al. Utilizing ethnic-specific differences in minor allele frequency to re categorize reported pathogenic deafness variants. American Journal of Human Genetics. 2014; 95: 445–453. doi:10.1016/j.ajhg.2014.09.001 PMID: 25262649

10. Wu CC, Liu TC, Wang SH, Hsu CJ, Wu CM. Genetic characteristics in children with cochlear implants and the corresponding auditory performance. Laryngoscope. 2011; 121: 1287–1293. doi:10.1002/lary.21751 PMID: 21557232

11. Miyagawa M, Nishio SY, Hattori M, Moteki H, Kobayashi Y, Sato H, et al. Mutations in the MYO15A Gene Are a Significant Cause of Nonsyndromic Hearing Loss: Massively Parallel DNA Sequencing-Based Analysis. Ann Otol Rhinol Laryngol. 2015; 124: 158S–168S. doi:10.1177/0003489415575058 PMID: 25792667

12. Ghid M, Choi BY. Strategy for the customized mass screening of genetic sensorineural hearing loss in koreans. Korean J Audiol. 2014; 18: 45–49. doi:10.7874/kja.2014.18.2.057 PMID: 25279224

13. Diaz-Horta O, Duman D, Foster JN, Sirmaci A, Gonzalez M, Mahdieh N, et al. Whole-exome sequencing efficiently detects rare mutations in autosomal recessive nonsyndromic hearing loss. PLoS One. 2012; 7: e50628. doi:10.1371/journal.pone.0050628 PMID: 23226338

14. Bae SH, Baek JI, Lee JD, Song MH, Kwon TJ, Oh SK, et al. Genetic analysis of auditory neuropathy spectrum disorder in the Korean population. Gene. 2013; 522: 65–69. doi:10.1016/j.gene.2013.02.057 PMID: 23562982

15. Zheng W, Deng X, Liang H, Song Z, Gao K, Yang Y, et al. Genetic analysis of the fused in sarcoma gene in Chinese Han patients with essential tremor. Neurobiology of Aging. 2013; 34; 2073–2078.

16. Yuan L, Wu S, Xu H, Xiao J, Yang Z, Xia H, et al. Identification of a novel PHEX mutation in a Chinese family with X-linked hypophosphatemic rickets using exome sequencing. Biological Chemistry. 2015; 396: 27–33. doi:10.1515/hsz-2014-0187 PMID: 25060345

17. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009; 25: 1754–1760. doi:10.1093/bioinformatics/btp324 PMID: 19451168

18. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, et al. The human genome browser at UCSC. Genome Research. 2002; 12: 966–1006. PMID: 12045153

19. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009; 25: 2078–2079. doi:10.1093/bioinformatics/btp352 PMID: 19505943

20. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Research. 2010; 38: e164. doi:10.1093/nar/gkq603 PMID: 20601685

21. Guo Y, Yang H, Deng X, Song Z, Yang Z, Xiong W, et al. Genetic analysis of the S100B gene in Chinese patients with Parkinson disease. Neuroscience Letters. 2013; 555: 134–136. doi:10.1016/j.neulet.2013.09.037 PMID: 24076007
22. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods. 2014; 11: 361–362. doi:10.1038/nmeth.2890 PMID: 24681721
23. Friedman TB, Liang Y, Weber JL, Hinnant JT, Barber TD, Winata S, et al. A gene for congenital, recessive deafness DFNB3 maps to the pericentromeric region of chromosome 17. Nature Genetics. 1995; 9: 86–91. PMID: 7704031
24. Liang Y, Wang A, Probst FJ, Arhya IN, Barber TD, Chen KS, et al. Genetic mapping refines DFNB3 to 17p11.2, suggests multiple alleles of DFNB3, and supports homology to the mouse model shaker-2. American Journal of Human Genetics. 1998; 62: 904–915. PMID: 9529344
25. Liang Y, Wang A, Belyantseva IA, Anderson DW, Probst FJ, Barber TD, et al. Characterization of the human and mouse unconventional myosin XV genes responsible for hereditary deafness DFNB3 and shaker 2. Genomics. 1999; 61: 243–258. PMID: 10552926
26. Wang A, Liang Y, Fridell RA, Probst FJ, Wilcox ER, Touchman JW, et al. Association of unconventional myosin MYO15 mutations with human nonsyndromic deafness DFNB3. Science. 1998; 280: 1447–1451. PMID: 9603736
27. Liburd N, Ghosh M, Riazuddin S, Naz S, Khan S, Ahmed Z, et al. Novel mutations of MYO15A associated with profound deafness in consanguineous families and moderatly severe hearing loss in a patient with Smith-Magenis syndrome. Human Genetics. 2001; 109: 535–541. PMID: 11735029
28. Gu X, Guo L, Ji H, Sun S, Chai R, Wang L, et al. Genetic testing for sporadic hearing loss using targeted massively parallel sequencing identifies 10 novel mutations. Clinical Genetics. 2015; 87: 588–593. doi: 10.1111/cge.12431 PMID: 24853665
29. Shafique S, Siddiqi S, Schraders M, Oostrik J, Ayub H, Bilal A, et al. Genetic spectrum of autosomal recessive non-syndromic hearing loss in Pakistani families. PLoS One. 2014; 9: e100146. doi: 10.1371/journal.pone.0100146 PMID: 24949729
30. Brownstein Z, Abu-Rayyan A, Kartunkel-Doron D, Sirigu S, Davidov B, Shohat M, et al. Novel myosin mutations for hereditary hearing loss revealed by targeted genomic capture and massively parallel sequencing. European Journal of Human Genetics. 2014; 22: 768–775. doi: 10.1038/ejhg.2013.232 PMID: 24105371
31. Riahi Z, Bonnet C, Zainine R, Louha M, Bouyacoub Y, Laroussi N, et al. Whole exome sequencing identifies new causative mutations in Tunisian families with non-syndromic deafness. PLoS One. 2014; 9: e99797. doi: 10.1371/journal.pone.0099797 PMID: 24266666
32. Gao X, Zhu QY, Song YS, Wang GJ, Yuan YY, Xin F, et al. Novel compound heterozygous mutations in the MYO15A gene in autosomal recessive hearing loss identified by whole-exome sequencing. J Transl Med. 2013; 11: 284. doi: 10.1186/1479-5876-11-284 PMID: 24206587
33. Miyagawa M, Naito T, Nishio SY, Kamatani N, Usami S. Targeted exon sequencing successfully discovers rare causative genes and clarifies the molecular epidemiology of Japanese deafness patients. PLoS One. 2013; 8: e71381. doi: 10.1371/journal.pone.0071381 PMID: 23967202
34. Woo HM, Park HJ, Baek JI, Park MH, Kim UK, 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: 72. doi: 10.1186/1471-2350-14-72 PMID: 23865914
35. Yang T, Wei X, Chai Y, Li L, Wu H. Genetic etiology study of the non-syndromic deafness in Chinese Hans by targeted next-generation sequencing. Orphanet J Rare Dis. 2013; 8: 85. doi: 10.1186/1750-1172-8-85 PMID: 23767834
36. Miyagawa M, Nishio SY, Ikeda T, Fukushima K, Usami S. Massively parallel DNA sequencing successfully identifies new causative mutations in deafness genes in patients with cochlear implantation and EAS. PLoS One. 2013; 8: e75793. doi: 10.1371/journal.pone.0075793 PMID: 24130743
37. Fattahi Z, Shearer AE, Babanejad M, Bazazzadegan N, Almadani SN, Nikzat N, et al. Screening for MYO15A gene mutations in autosomal recessive nonsyndromic deafness, GJB2 negative Iranian deaf population. Am J Med Genet A. 2012; 158A: 1857–1864. doi: 10.1002/ajmg.a.34411 PMID: 22736430
38. Bashir R, Fatima A, Naz S. Prioritized sequencing of the second exon of MYO15A reveals a new mutation segregating in a Pakistani family with severe hearing loss. Eur J Med Genet. 2012; 55: 99–102. doi: 10.1016/j.ejmg.2011.12.003 PMID: 22245518
39. Brownstein Z, Friedman LM, Shahin H, Onon-Karmi V, Kol N, Abu RA, et al. Targeted genomic capture and massively parallel sequencing to identify genes for hereditary hearing loss in Middle Eastern families. Genome Biol. 2011; 12: R89. doi: 10.1186/gb-2011-12-9-r89 PMID: 21917145
40. Duman D, Sirmaci A, Cengiz FB, Ozdag H, Tekin M. Screening of 38 genes identifies mutations in 62% of families with nonsyndromic deafness in Turkey. Genet Test Mol Biomarkers. 2011; 15: 29–33. doi: 10.1089/gtmb.2010.0120 PMID: 21117948
41. Imtiaz F, Taibah K, Ramzan K, Bin-Khamis G, Kennedy S, Al-Mubarak B, et al. A comprehensive introduction to the genetic basis of non-syndromic hearing loss in the Saudi Arabian population. BMC Med Genet. 2011; 12: 91. doi: 10.1186/1471-2350-12-91 PMID: 21726435

42. Belguith H, Ala-Hmani M, Dhouib H, Said MB, Mosrati MA, Lahmar I, et al. Screening of the DFNB3 locus: identification of three novel mutations of MYO15A associated with hearing loss and further suggestion for two distinctive genes on this locus. Genet Test Mol Biomarkers. 2009; 13: 147–151. doi: 10.1089/gtmb.2008.0077 PMID: 19309289

43. Shearer AE, Hildebrand MS, Webster JA, Kahrizi K, Meyer NC, Jalalvand K, et al. Mutations in the first MyTH4 domain of MYO15A are a common cause of DFNB3 hearing loss. Laryngoscope. 2009; 119: 727–733. doi: 10.1002/lary.20116 PMID: 19274735

44. Lezirovitz K, Pardono E, de Mello AM, de Carvalho ESF, Lopes JJ, Abreu-Silva RS, et al. Unexpected genetic heterogeneity in a large consanguineous Brazilian pedigree presenting deafness. European Journal of Human Genetics. 2008; 16: 89–96. PMID: 17851452

45. Kalay E, Uzumcu A, Krieger E, Caylan R, Uyguner O, Ulubil-Emiroglu M, et al. MYO15A (DFNB3) mutations in Turkish hearing loss families and functional modeling of a novel motor domain mutation. Am J Med Genet A. 2007; 143A: 2382–2389. PMID: 17853461

46. Nal N, Ahmed ZM, Erkal E, Alper OM, Luleci G, Dinc O, et al. Mutational spectrum of MYO15A: the large N-terminal extension of myosin XVA is required for hearing. Human Mutation. 2007; 28: 1014–1019. PMID: 17546645

47. Shahin H, Walsh T, Rayyan AA, Lee MK, Higgins J, Dickel D, et al. Five novel loci for inherited hearing loss mapped by SNP-based homozygosity profiles in Palestinian families. European Journal of Human Genetics. 2010; 18: 407–413. doi: 10.1038/ejhg.2009.190 PMID: 19888295

48. Belyantseva IA, Boger ET, Naz S, Frolokov GI, Sellers JR, Ahmed ZM, et al. Myosin-XVa is required for tip localization of whirlin and differential elongation of hair-cell stereocilia. Nature Cell Biology. 2005; 7: 148–156. PMID: 15654330

49. Belyantseva IA, Boger ET, Friedman TB. Myosin XVa localizes to the tips of inner ear sensory cell stereocilia and is essential for staircase formation of the hair bundle. Proc Natl Acad Sci U S A. 2003; 100: 13958–13963. PMID: 14610277

50. Probst FJ, Fridell RA, Raphael Y, Saunders TL, Wang A, Liang Y, et al. Correction of deafness in shaker-2 mice by an unconventional myosin in a BAC transgene. Science. 1998; 280: 1444–1447. PMID: 9603735

51. Manor U, Disanza A, Grati M, Andrade L, Lin H, Di Fiore PP, et al. Regulation of stereocilia length by myosin XVa and whirlin depends on the actin-regulatory protein Eps8. Current Biology. 2011; 21: 167–172. doi: 10.1016/j.cub.2010.12.046 PMID: 21236676

52. Weber KL, Sokac AM, Berg JS, Cheney RE, Bement WM. A microtubule-binding myosin required for nuclear anchoring and spindle assembly. Nature. 2004; 431: 325–329. PMID: 15372037