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Genetic Analysis through OtoSeq of Pakistani Families Segregating Prelingual Hearing Loss

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Abstract

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Author Contributions

Mohsin Shahzad, enrollment of families, acquisition, analysis and interpretation of data, drafting the article and figures; **Theru A. Sivakumaran**, acquisition, analysis and interpretation of NGS data, revising the article critically for important intellectual content, final approval; **Tanveer Qaiser**, **Zawar Hussain**, and **Munir A. Bhinder**, ascertainment of Pakistani families, clinical data, acquisition and analysis of linkage data, final approval; **Julie M. Schultz**, acquisition, analysis, interpretation of Sanger sequencing data and revising the article critically for important intellectual content; **Megan Flanagan**, microdroplet PCR based enrichment of the DNA samples, final approval; **Diane Kissell**, microdroplet PCR based enrichment of the DNA samples, final approval; **John H. Greinwald**, helpful discussion and suggestions during the course of this study, revising the article critically for important intellectual content, final approval; **Shaheen N. Khan**, ascertainment of Pakistan families and their clinical data, interpretation of linkage data, final approval; **Sheikh Riazuddin**, conception and design of study in Pakistan, revising the article critically for important intellectual content, final approval; **Thomas B. Friedman**, conception of study, critically revising the article, final approval; **Kejian Zhang**, design of NGS based screening study, review and interpret the NGS results and final approval of the manuscript; **Saima Riazuddin**, conception and design of study, analysis and interpretation of the data, revising the article critically for important intellectual content, final approval; and **Zubair M. Ahmed**, conception and design of study, analysis and interpretation of data, drafting and revising the article critically for important intellectual content, final approval.

Disclosures

Competing interests: OtoSeq is offered as a commercial test through the Molecular Genetics Laboratory at Cincinnati Children's Hospital Medical Center, and Kejian Zhang and Sivakumaran Theru Arumugam are employees of this laboratory.

Objective—To identify the genetic cause of prelingual sensorineural hearing loss in Pakistani families using a next-generation sequencing (NGS)-based mutation screening test named OtoSeq.

Study Design—Prospective Study

Setting—Research laboratory

Subjects and Methods—We used three fluorescently labeled short tandem repeat (STR) markers for each of the known autosomal recessive nonsyndromic (DFNB) and Usher syndrome (USH) locus to perform a linkage analysis of 243 multi-generational Pakistani families segregating prelingual hearing loss. After genotyping, we focused on 34 families with potential linkage to *MYO7A*, *CDH23* and *SLC26A4*. We screened affected individuals from a subset of these families using the OtoSeq platform to identify underlying genetic variants. Sanger sequencing was performed to confirm and study the segregation of mutations in other family members. For novel mutations, normal hearing individuals from ethnically matched backgrounds were also tested.

Results—Hearing loss was found to co-segregate with locus-specific STR markers for *MYO7A* in 32 families, *CDH23* in one family and *SLC26A4* in one family. Using the OtoSeq platform, a microdroplet PCR-based enrichment followed by NGS, we identified mutations in 28 of the 34 families including 11 novel mutations. Sanger sequencing of these mutations showed 100% concordance with NGS data and co-segregation of the mutant alleles with the hearing loss phenotype in the respective families.

Conclusion—Using NGS based platforms like OtoSeq in families segregating hearing loss, will contribute to the identification of common and population specific mutations, early diagnosis, genetic counseling and molecular epidemiology.

Keywords

Hearing loss; Usher syndrome; microdroplet PCR; next generation sequencing; clinical diagnosis; genetic etiology; *PDS*; *MYO7A*; *CDH23*

Introduction

Hereditary deafness is phenotypically and genetically heterogeneous affecting nearly 300 million people worldwide.^{1–3} Clinically, hearing loss is manifested by wide variation in associated symptoms, age of onset, the specific frequencies affected and progressive loss of hearing thresholds.^{1–3} Genetically, it has been estimated that ~1% of all human genes may be implicated in the etiology of hereditary deafness.^{1,4} To date, the molecular genetic etiology for approximately 100 deafness genes have been reported.^{5–8} The major challenges and goals in the field have been to identify all the genes containing mutations leading to deafness and to decipher the functions of the encoded protein in auditory and vestibular pathways. In the last five years, the emergence of genome enrichment arrays followed by massively parallel sequencing (next-generation sequencing, NGS) technologies has accelerated gene discovery for human disorders, including deafness.^{9–18} NGS has also had an important impact on research and clinical diagnosis in many diseases, including cancer,^{9–12,15} intellectual disabilities,^{14,18} mitochondrial disorders,^{19–21} polycystic kidney disease,²² and ciliary disorders,²³ because NGS can simultaneously interrogate a large set of

genes. For example, OtoSCOPE (Otologic Sequence Capture Of Pathogenic Exons) was a platform developed to examine the exons of 54 known genes associated with human deafness.²⁴ OtoSCOPE uses hybridization-based enrichment of targeted exons, followed by NGS on Illumina and 454 sequencers.²⁴ Additional platforms for the genetic diagnosis of hearing disorders were developed, which varied in the number of targeted exons/genes and NGS analyzers.^{25,26}

Recently, OtoSeq, a microdroplet-based target enrichment²⁷ followed by NGS to screen 24 reported genes associated with autosomal recessive nonsyndromic hearing loss was developed at Cincinnati Children's Hospital Research Foundation.²⁸ Here, we report genetic screening of 34 consanguineous Pakistani families segregating prelingual hearing loss linked to STR markers for *MYO7A*, *CDH23* or *SLC26A4* using OtoSeq. We found 24 mutations in 28 of the 34 families including 11 *MYO7A* alleles not previously reported. Among the 11 novel mutations, seven were missense substitutions, one nonsense, one frameshift and two splice site changes.

MATERIALS AND METHODS

Family ascertainment

The study of Pakistani families was approved by IRBs at the National Centre of Excellence in Molecular Biology (NCEMB), Lahore, Pakistan (FWA00017939), at the National Institutes of Health, USA (Combined Neuroscience IRB; OH-93-N-016) and at the Cincinnati Children's Hospital Research Foundation, USA (2009-0684; 2010-0291). Written informed consent was obtained from adult subjects and parents of minor subjects. We performed medical history interviews to identify possible clinical features of syndromic hearing loss and rule out potential environmental causes. In all families, hearing was evaluated in audiology clinics by pure tone audiometry at octave frequencies with intensities up to 110 dBHL, and vestibular function was evaluated by tandem gait and Romberg testing.²⁹ For eleven available individuals with bi-allelic mutations of *MYO7A*, funduscopic examination was performed by an ophthalmologist to evaluate the individuals for retinitis pigmentosa.

DNA isolation and genotyping in Pakistani families

Genomic DNA was extracted from peripheral blood samples using a standard protocol. Using genomic DNA from affected and unaffected members of 243 Pakistani families segregating autosomal recessive hearing loss, we screened for linkage of the deafness phenotype to short tandem repeat (STR) markers for all of the reported DFNB/USH genes/loci using a previously described protocol.³⁰ In 34 families, the deafness phenotype showed linkage to markers for the *MYO7A*, *CDH23*, or *SLC26A4* genes. DNA samples from these families were screened for mutations using micro-droplet PCR-based target enrichment and NGS.

Target Enrichment and Next-Generation Sequencing (NGS)

Target enrichment and NGS were performed according to the protocol given in the accompanying paper by Sivakumaran and co-authors in this issue.²⁸ Briefly, the coding and

flanking intronic regions of all 24 genes were enriched using microdroplet PCR (RainDanceTechnologies, Inc. Lexington, MA).²⁸ The resulting enriched amplicons were gel purified, subjected to NGS library preparation using a Nextera DNA sample prep kit (Epicenter Biotechnologies, Madison, WI) and sequenced using an IlluminaHiSeq 2000 (Illumina, San Diego, CA). The raw NGS sequence reads were filtered for quality and subjected to an NGS data analysis pipeline.²⁸ The sequence variants observed in the patients were searched against the Human Gene Mutation Database (HGMD), dbSNP and NHBLI Exome Variant Server (EVS). Variants listed as mutations in the HGMD and variants that were either absent or had low minor allele frequency in dbSNP or EVS were evaluated further. All novel missense variants were evaluated using *in silico* prediction programs, Polyphen-2, SNPs3D, Mutation Taster and SIFT.^{31–34}

Sanger Sequencing

For confirmation and segregation analyses, the genomic DNA of each participating member of each family was PCR amplified with primers for exons harboring mutant alleles and sequenced as described.³⁵ Briefly, 15 µl of PCR product was treated with 0.3 U of shrimp alkaline phosphatase and 3 U of exonuclease I at 37°C for 1 hr, followed by incubation at 80°C for 15 min. DNA sequencing reactions contained 3.2 pmol primer, 0.6 µl Big Dye Terminator Ready Reaction Mix (Applied Biosystems, Foster City, CA) and 2 µl 5× dilution buffer (400 mM Tris-HCl, pH 9 and 10 mM MgCl₂) in a 10 µl reaction. The cycling conditions were 96°C for 2 min, followed by 45 cycles of 96°C for 10 s, 55°C for 10 s and 60°C for 4 min. The sequencing reaction products were ethanol precipitated and the pellets were suspended in 10 µl of deionized formamide. Sequences were obtained on an ABI 3730 XL (Applied Biosystems).

RESULTS

Linkage analysis

The study participants comprised 243 families segregating severe-to-profound, prelingual, recessive deafness ascertained through NCMB after the original 557 families reported by Riazuddin *et al.*³⁶ As part of our routine genetic screening protocol, we performed linkage analysis of the NCMB cohort by using short-tandem repeat (STR) markers flanking reported DFNB and USH loci/genes. Through these linkage studies, we identified 34 families co-segregating recessive hearing loss and STR markers linked to *MYO7A* (32 families), *CDH23* (1 family) or *SLC26A4* (1 family) genes (Figures 1 and 2). Further analysis using additional STR markers revealed shared *MYO7A*-linked haplotypes in some families (data not shown).

Mutational analysis

For *MYO7A*, families PKDF896, PKDF1079 and PKDF1080 had the same haplotype for three markers closely linked to *MYO7A* (data not shown). Similarly, families PKDF624, PKDF937, PKDF948 and PKDF960 had the same *MYO7A*-linked haplotypes. Using a micro-droplet PCR-based target enrichment followed by NGS (OtoSeq), we screened one affected individual from each of 29 Pakistani families with unique *MYO7A*, *CDH23* and *SLC26A4* linkage haplotypes (Figures 1 and 2) for 24 known deafness-causing genes.²⁸

Unbiased analysis (blinded to linkage data) of the NGS data revealed potential pathogenic mutations in 23 of 29 samples screened. We confirmed these mutations through Sanger sequencing (Figure 3). Finally, segregation analysis studies revealed potential pathogenic alleles in 28 of 34 Pakistani families (Table 1). None of the novel mutations were detected in 192 chromosomes from normal-hearing participants that were geographically and ethnically matched to study participants with hearing loss.

We identified 22 homozygous mutant alleles of *MYO7A* (Table 1) that segregated among 26 families (Figures 1 and 2). Among the 11 novel mutations, seven were missense substitutions of residues evolutionarily conserved in a variety of myosin VIIA orthologs (Figure 4); these are predicted to have deleterious effects on the function of this unconventional myosin (Table 1). In addition, we identified one new nonsense (p.Arg1967*), one frameshift (p.Asp1638Metfs*7) and two splice site (c.2367+1G>A, c.6354+1G>A) mutations. Assuming that these mutant mRNAs escape degradation by the nonsense-mediated decay pathway, these mutations are predicted to result in truncation of the encoded protein.³⁷

We also identified eleven reported *MYO7A* alleles that segregated in 16 families, including four missense (p.Arg241Cys, p.Arg241His, p.Ala826Thr, p.Gly2163Ser), two nonsense (p.Lys420*, p.Trp838*), three frameshift (p.Glu166Argfs*3, p.Tyr1302Thrfs*96, p.Asp1613Valfs*31) and two splice site mutation (c.2695-9A>G, c.3504-1G>C) (Table 1). Surprisingly, two different homozygous, potentially pathogenic mutations of *MYO7A* (p.Gly434Arg, p.Ala826Thr) were segregating in family PKDF959 (Table 1). The p.Ala826Thr mutation was initially identified in USH1B subjects from Algeria and Morocco.³⁸ We also previously identified the p.Ala826Thr mutation in a large consanguineous Pakistani family.³⁶ Parents of these affected individuals were heterozygous for both alleles and had normal hearing, suggesting two possibilities: 1) Both alleles are present in the *cis*-configuration and parents have one normal copy of *MYO7A*; 2) Alternatively, p.Gly434Arg may be a rare polymorphism, which is in linkage-disequilibrium with the p.Ala826Thr pathogenic allele in family PKDF959. Currently, we do not have lymphoblast cell-derived cDNA samples available from these families and at genomic DNA level these two alleles are ~16Kb apart, which precludes our attempt to determine the *cis*/trans configuration of these two alleles. Intriguingly, no potential pathogenic mutation in the protein coding exons or in the splice junctions of *MYO7A* was detected in six other families with linkage to chromosome 11q13.5 STR markers (data not shown).

In the USH1D family (PKDF475; Figure 1), we identified a homozygous transition mutation (c.8530C>T) that resulted in a substitution of serine for proline at position 2844 (p.Pro2844Ser) in the 27th ectodomain (Table 1). The p.Pro2844Ser mutation was recently reported to be associated with type 1 Usher syndrome segregating in another Pakistani family.³⁹ In the PDS family (PKDF609; Figure 1), we identified a homozygous transversion mutation (c.716T>A) in exon 6 of *SLC26A4* that results in a substitution of valine by aspartic acid (p.Val239Asp) in the encoded protein, Pendrin (Table 1).

Intriguingly, in four families (PKDF609, PKDF771, PKDF788, PKDF948), the pathogenic alleles identified through OtoSeq could not explain the deafness phenotype shared by all

affected individuals in the family (Figures 1 and 2). In these families, one or two affected individuals (indicated by grey filled symbols in Figures 1 and 2) were either heterozygous for the identified mutation or were homozygous for the wild type allele. Segregation analysis using chromosome 11q13.5-linked STR markers also revealed non-segregation of *MYO7A* mutation harboring haplotype in these individuals.

Clinical evaluation—Hearing evaluation using pure tone audiometry revealed severe to profound or profound degree of hearing loss in the affected individuals of families segregating *MYO7A*, *CDH23* or *PDS* mutations. Although not rigorously tested using posturography and a rotary chair, Romberg and tandem gait tests suggested impaired vestibular function among the affected individuals homozygous for *MYO7A* and *CDH23* mutations. Only affected individuals from five (marked by * in Table 1) families segregating *MYO7A* alleles were available for ophthalmologic examination. Fundusoscopic examination of the two oldest affected individuals from these families revealed signs of retinitis pigmentosa along with narrowing of retinal blood vessels, bone spicules and waxy appearance of optic disk. The severity of retinitis pigmentosa ranged from mild to severe loss of vision. Based on the clinical presentation, these families were classified as segregating Usher syndrome type I. For family PKDF609 two affected individuals with homozygous *PDS* mutation were examined by a local physician and had palpable goiters.

DISCUSSION

Screening of 243 Pakistani families with STR markers for known DFNB/USH loci, revealed evidence of linkage to markers for *USH1B/DFNB2*, *USH1D/DFNB12* and *PDS/DFNB4* loci in 34 families. Because the genes mutated at the *USH1B/DFNB2* (*MYO7A*),⁴⁰ *USH1D/DFNB12* (*CDH23*),^{35,41} and *PDS/DFNB4* (*SLC26A4*)⁴² loci were included in OtoSeq, we used OtoSeq to uncover mutations of these genes in these 34 families. Genetic screening using OtoSeq revealed 24 mutations (Table 1). As anticipated from linkage analysis studies, 22 of the identified alleles were present in the *MYO7A* gene. These include 11 missense, three nonsense and eight predicted frameshift alleles. The allelic heterogeneity for *MYO7A* in Pakistan is similar to that reported for *MYO7A* in the United States and United Kingdom.^{38,43–45} Our results indicate that *MYO7A* mutations are a significant cause of recessive hearing loss in Pakistan and in many other populations.^{38,43–47} Taken together with our earlier result,³⁶ *MYO7A* mutations account for 6.25% (50 of 800 families; 95% CI: 4.77 – 8.15%) of hearing loss in our NCEMB cohort of Pakistani families segregating prelingual hearing loss.

As of January 2013, 260 different mutant alleles of *MYO7A* have been reported and are listed in the Human Gene Mutation Database (<http://www.hgmd.cf.ac.uk/ac/gene.php?gene=MYO7A>). Although some of these are recurrent variants,^{36,45,48–51} most are private and distributed across the gene. In our study, only two mutations (c.721C>T and c.1258A>T) of *MYO7A* were found in multiple families (Table 1). Together with our earlier result,³⁶ we observed a distinct spectrum of *MYO7A* mutations that did not include alleles prevalent in other populations, such as p.Arg666* (United Kingdom),⁴⁵ p.Arg241Gly (Italy),⁴⁶ p.Gln821* (Spain),⁵² and p.Cys31* (Denmark).⁵³ The three most common alleles (c.496delG, c.721C>T, c.1258A>T) only accounted for ~24% of the cases of deafness in the

NCEMB study population and thus hierarchical mutation screening would not be a cost-effective approach for molecular diagnosis.

By contrast, the p.Val239Asp allele of *SLC26A4* segregating in family PKDF609 is a recurrent mutation and has been identified previously in 17 other Pakistani PDS families. We previously demonstrated that three alleles of *SLC26A4* (p.Ser90Leu, p.Val239Asp and p.Gln446Arg) account for ~66% of cases of DFNB4/PDS deafness in the NCEMB study population.⁵⁴ Thus, hierarchical mutation screening using a tetra-primer ARMS (amplification refractory mutation system) assay would have been a cost-effective approach for molecular diagnosis in the PKDF609 family.⁵⁴

In six of the potential *MYO7A* (chromosome 11q13.5)-linked families, we did not observe mutations in the coding exons or splice junctions of *MYO7A*. There are at least three possible reasons for our failure to detect *MYO7A* mutations in these families. First, our assay is designed to only screen the coding exons and the flanking 20 bp that includes the donor and acceptor splice junctions. Hence, mutations in *cis*-acting regulatory or splice elements of *MYO7A* that are necessary for *MYO7A* expression in the inner ear and retina would not be detected. Second, the OtoSeq platform has limited resolution in detecting insertions (up to 22 nucleotides),²⁸ and may have missed complicated mutations like large insertions. Third, the hearing loss segregating in these six families may be spuriously linked to markers at 11q13.5. However, for four of these families the linkage scores for markers of chromosome 11q13.5 were statistically significant (>3.5). Fourth, there may be an additional gene on chromosome 11q13.5 in which mutant alleles cause hearing loss. There are examples of closely linked deafness genes and overlapping deafness loci in humans.^{55–57}

CONCLUSION

Identification of 24 mutations, including 11 novel alleles, in 28 Pakistani families emphasizes the genetic heterogeneity of this disorder. Our findings, along with the results of previous studies,^{35,36,39,54,58–61} suggest that besides *HGF*, *CIB2* and *PDS*, mutant alleles of *MYO7A* and *CDH23* are among the major causes of prelingual recessive hearing loss within the Pakistani population.

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Figure 1.

Pakistani families with recurrent mutations. Asterisks indicate subjects that contributed DNA samples. Black symbols represent affected individuals homozygous for the identified mutations. Grey filled symbols represent deaf individuals not due to *MYO7A* or *PDS* mutations.

Figure 2.

Pakistani families co-segregating recessive deafness and novel mutations in *MYO7A*. Double lines indicate consanguineous marriages. Grey filled symbol in family PKDF788 represents an individual with hearing impairment not associated with the *MYO7A* mutation.

Figure 3.

Sanger sequence chromatograms of novel *MYO7A* mutations in an affected individual and a wild type sequence in a control individual. Arrows in the homozygous mutant chromatograms indicate the location of the mutant nucleotide (red).

	p.His133Pro	p.Arg395Cys	p.Gly434Arg	p.Leu452Pro	p.Arg1723Thr	p.Pro1726Leu	p.Lys2118Asn
Human	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F R P P P K H T	- F E V K Q T T	
Bushbaby	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F R P P P K H T	- F E V K Q T T	
Marmoset	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- G R V P R P G R A T	- F E V K Q T T	
Guinea Pig	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F R P P P K H T	- F E V K Q T T	
Mouse	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F R P P P K H T	- F E V K Q T T	
Rat	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F R S P P K H T	- F E V K Q T T	
Cat	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F X X X X X X X	- F E V K Q T T	
Cow	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F R P P P K H T	- F E V K Q T T	
Dolphin	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F R P P P K H T	- F E V K Q T T	
Ferret	- - - - -	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F R P P P K H T	- F E V K Q T T	
Opossum	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F R P P P K H T	- F E V K Q T T	
Anole Lizard	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F R P P P K H T	- F E V K Q T T	
Chicken	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F R P P P K H T	- F E V K Q T T	
Cod	M P P H I F A	- L D V R D A F	- R S I G L L D	- X X X X X X X	- D Y F X X X X X X X	- F E V K Q T T	
Coelacanth	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F R P P P K H T	- L F L Q Q T T	
Stickleback	M P P H I F A	- L D V R D A F	- R S I G L L D	- F P C C L F	- D Y F R P P P K H T	- F E V K Q T T	
Tilapia	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F R P P P K H T	- F E V K Q T T	
Zebrafish	M P P H I F A	- L D V R D A F	- R S I G L L D	- F E Q L C I N	- D Y F R P P P K H T	- F E V K Q T T	
C. intestinalis	L P P H I F A	- N D V K D A F	- Y S M G L L D	- F E Q L C I N	- D F F R A P I P Q T	- F E V R Q T T	
Fruitfly	L P P H I F A	- V D V R D A F	- N A I G V L D	- F E Q F C I N	- D H F R L P P K R T	- F E V K Q T T	

Figure 4.

ClustalW multiple sequence alignments of myosin VIIA. Amino acid residues p.His133, p.Arg395, p.Gly434, p.Leu452, p.Arg1723, p.Pro1726 and p.Lys2118 of human myosin VIIA are conserved among orthologs. Non-contiguous portions are separated by dotted lines.

Table 1

Mutant alleles found in twenty-eight Pakistani families with prelingual SNHL

Family	Gene	Mutation [#]	Effect on protein	Domain	Allele frequency	Reference
PKDF475 [*]	CDH23	c.8530C>T	p.Pro2844Ser	EC27		39
PKDF820	MYO7A	c.398A>C	p.His133Pro	Motor	0/188	this study
PKDF764	MYO7A	c.496delG	p.Glu166Argfs [*] 3	Motor		36
PKDF896 [*] PKDF1079 PKDF1080	MYO7A	c.721C>T	p.Arg241Cys	Motor		48
PKDF514	MYO7A	c.722G>A	P.Arg241His	Motor		51
PKDF930	MYO7A	c.1183C>T	p.Arg395Cys	Motor	0/182	this study
PKDF624 PKDF937 PKDF948 PKDF960	MYO7A	c.1258A>T	p.Lys420 [*]	Motor	0/188	62
PKDF959	MYO7A	c.1300G>C c.2476G>A	p.Gly434Arg p.Ala826Thr	Motor IQ	0/188	this study; 38
PKDF803 [*]	MYO7A	c.1355T>C	p.Leu452Pro	Motor	0/182	this study
PKDF947	MYO7A	c.2367+1G>A	aberrant splicing [§]	IQ	0/176	this study
PKDF362	MYO7A	c.2513G>A	p.Trp838 [*]	IQ		50
PKDF771 [*]	MYO7A	c.2695-9A>G	aberrant splicing	CC	0/178	63
PKDF843	MYO7A	c.3504-1G>C	aberrant splicing [*]	MyTH4		45
PKDF410	MYO7A	c.3904delT	p.Tyr1302Thrfs [*] 96	FERM		49
PKDF1074	MYO7A	c.4838delA	p.Asp1613Valfs [*] 31	SH3		36
PKDF332	MYO7A	c.4911_4912insAT	p.Asp1638Metfs [*] 7	SH3	0/176	this study
PKDF860	MYO7A	c.5168G>C	p.Arg1723Thr	MyTH4	0/176	this study
PKDF760	MYO7A	c.5177C>T	p.Pro1726Leu	MyTH4	0/176	this study
PKDF788 [*]	MYO7A	c.5899C>T	p.Arg1967 [*]	FERM	0/190	this study
PKDF434	MYO7A	c.6354G>C	p.Lys2118Asn	FERM	0/184	this study
PKDF546	MYO7A	c.6354+1G>A	aberrant splicing [§]	FERM	0/184	this study

Family	Gene	Mutation#	Effect on protein	Domain	Allele frequency	Reference
PKDF423	<i>MYO7A</i>	c.6487G>A	p.Gly2163Ser	FERM		53
PKDF609	<i>PDS</i>	c.716T>A	p.Val239Asp	-		59

* Vestibular, auditory and fundoscopic evaluations revealed Usher syndrome type I phenotype segregating in these families. Affected individuals of other families homozygous for *MYO7A* mutations had vestibular dysfunction, evaluated through Ronberg and tandem gait tests, but no ophthalmology data is available from these families.

Novel mutations are in bold and were predicted pathogenic by Polyphen-2, SNPs3D, MutationTaster and SIFT prediction algorithms.

§ Predicted by the Human Splicing Finder program (<http://www.umd.be/HSF/>). EC: ectodomain; CC: coiled coil region.